MINISTRY OF AGRICULTURE, TRADE, FORESTRY AND WATER MANAGEMENT
Veterinary Directorate

Technical Assistance for the Control and Eradication of Classical Swine Fever (CSF) and Rabies in Serbia

CRIS No: 226-870

Strategic operational multi-annual action plan for eradication, control and monitoring of CSF including plan for non-vaccination eradication of CSF in Serbia

April 2012

Project funded by the European Union

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MINISTRY OF AGRICULTURE, TRADE, FORESTRY AND WATER MANAGEMENT
Veterinary Directorate

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Final DRAFT

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October 2011

1. Project Title

Technical Assistance for the Control and Eradication of Classical Swine Fever (CSF) and Rabies

2. Details

<table>
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<th>Agriculture</th>
<th>Partner Institution / Beneficiary</th>
<th>Ministry of Agriculture, Forestry and Water Management / Veterinary Directorate</th>
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<td>Project No.:</td>
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<td>11 July 2012</td>
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List of Abbreviations

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<table>
<thead>
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<th>Abbreviation</th>
<th>Definition</th>
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</thead>
<tbody>
<tr>
<td>AH</td>
<td>Animal health</td>
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<tr>
<td>AW</td>
<td>Animal Welfare</td>
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<tr>
<td>BIP</td>
<td>Border Inspection Post</td>
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<tr>
<td>CCA</td>
<td>Central competent Authority</td>
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<tr>
<td>CSF</td>
<td>Classical Swine Fever</td>
</tr>
<tr>
<td>CD</td>
<td>Computer Database</td>
</tr>
<tr>
<td>CVO</td>
<td>Chief Veterinary Officer (Director of the Veterinary Directorate)</td>
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<tr>
<td>DB</td>
<td>Data base</td>
</tr>
<tr>
<td>DG SANCO</td>
<td>Directorate General for Health and Consumers – European Commission</td>
</tr>
<tr>
<td>EC</td>
<td>European Commission</td>
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<tr>
<td>EFSA</td>
<td>European Food Safety Agency</td>
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<tr>
<td>EU</td>
<td>European Union</td>
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<tr>
<td>FAO</td>
<td>Food and Agriculture Organization</td>
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<tr>
<td>FVO</td>
<td>Food and Veterinary Office</td>
</tr>
<tr>
<td>GIS</td>
<td>Geographical Information System</td>
</tr>
<tr>
<td>I&amp;R</td>
<td>Animal Identification and Registration</td>
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<tr>
<td>ISO</td>
<td>International Organization for Standardization</td>
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<tr>
<td>IPA</td>
<td>Instrument for Pre-Accession Assistance</td>
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<tr>
<td>IT</td>
<td>Information Technology</td>
</tr>
<tr>
<td>LIMS</td>
<td>Laboratory Management Information System</td>
</tr>
<tr>
<td>MS</td>
<td>Member States</td>
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<tr>
<td>MAFWM</td>
<td>Ministry of Agriculture, Forestry and Water Management</td>
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<tr>
<td>OIE</td>
<td>World Animal Health Organization (Office International des Epizooties)</td>
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<tr>
<td>ORV</td>
<td>Oral Rabies Vaccination</td>
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<tr>
<td>OIE</td>
<td>World Animal Health Organization</td>
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<tr>
<td>PDA</td>
<td>Personal Digital Assistant (handheld computer)</td>
</tr>
<tr>
<td>PRAG</td>
<td>Practical guide to contract procedures for EC external actions</td>
</tr>
<tr>
<td>PVS</td>
<td>OIE Tool for the Evaluation of Performance of Veterinary Services</td>
</tr>
<tr>
<td>RASFF</td>
<td>Rapid Alert System for Food and Feed</td>
</tr>
<tr>
<td>SOP</td>
<td>Standard Operating Procedure</td>
</tr>
<tr>
<td>SPS</td>
<td>Sanitary and Phytosanitary Agreement</td>
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<tr>
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<td>Training needs assessment</td>
</tr>
<tr>
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<td>Terms of Reference</td>
</tr>
<tr>
<td>TS</td>
<td>Technical Specifications</td>
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<td>VD</td>
<td>Veterinary Directorate</td>
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<tr>
<td>VD-MAFWM</td>
<td>Veterinary Directorate of the Serbian Ministry of Agriculture, Forestry and Water Management</td>
</tr>
<tr>
<td>VetUP</td>
<td>Veterinary Information System</td>
</tr>
<tr>
<td>VIMS</td>
<td>Veterinary Information Management System</td>
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<tr>
<td>WHO</td>
<td>World Health Organisation</td>
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<td>WTO</td>
<td>World Trade Organisation</td>
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INTRODUCTION

Preventive vaccination against CSF has been implemented in Serbia for years. The mandatory vaccination of pigs against CSF has been funded from the government (MAFVM-Veterinary directorate) budget since 2006. In the same year the last big CSF epidemic occurred. After that annual CSF vaccination programmes proved to be successful and no cases of CSF were reported from October 2007 to November 2010. In November 2010 two CSF outbreaks have been confirmed in Sremska Mitrovica. Information on CSF outbreaks over the last six years have been presented in the Table 1.

The MAFWM is considering phasing out of preventive CSF vaccination, taking into account that this is one of the preconditions to be met in order to Serbia is able to certify pigs and pig meat for export to the EU.

<table>
<thead>
<tr>
<th>Year</th>
<th>Outbreak</th>
<th>Diseased</th>
<th>Died</th>
<th>Killed</th>
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<tr>
<td>2006</td>
<td>401</td>
<td>4,735</td>
<td>1,564</td>
<td>6,198</td>
</tr>
<tr>
<td>2007</td>
<td>21</td>
<td>650</td>
<td>1,342</td>
<td>5,217</td>
</tr>
<tr>
<td>2008</td>
<td>0</td>
<td>0</td>
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<td>2009</td>
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<td>2010</td>
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<td>202</td>
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<td>2011</td>
<td>0</td>
<td>0</td>
<td>0</td>
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</table>

*Source: Veterinary Directorate*

1. Mandate

This project is part of a general programme initiated by DG SANCO: the IPA project “Western Balkan programme for eradication of rabies/classical swine fever” managed by DG ENLARG with implementation sub delegated to DG SANCO. The project encompasses the setting up and execution of surveillance; coordination and exchange of information on the situation and evolution of CSF and rabies programmes in the Western Balkans countries; and regional and international cooperation between the involved national veterinary services.

Pre-described strategy to control Classical Swine Fever outbreaks does not exist. In order to effectively control the disease, each country should have a complete plan of actions, financial and human resources to implement it under the particular conditions prevailing in the country.

The Project is assisting the Veterinary Directorate of the MAFWM in control and eradication of Classical swine fever (CSF) and rabies in Serbia in compliance with EU standards. According to the ToR the project partners should assess the epidemiological situation and prepare multi annual action plan containing disease control and eradication measures and protocol for disease surveillance.
2. CSF Strategy and strategy objectives

2.1 The main objective

The main objective is to minimise the CSF threat and risk in feral and domestic pigs in Serbia, through progressive control and eradication, as well as to prevent new CSF virus introduction. This will allow for stabilisation of national pig production and trade, rehabilitation of exports of pigs and pig meat, increase consumer confidence in food safety and improve the livelihoods of all pig sector stakeholders including rural people.

2.2 Specific short and long term objectives

The Strategic Operational Multi-annual Action Plan for Eradication Control and Monitoring of CSF will be implemented over two time frames: short term and long term.

- **The short term objectives are:**
  - to stop preventive CSF vaccination;
  - to reduce the risk of new CSF outbreaks deploying preventive biosecurity measures as well as conventional control and eradication methods (e.g. stamping out, compensation payments, registration and control of pig movements and, when appropriate emergency vaccination according to the national legislation and CSF contingency plan, application of "Compartmentalisation" concept when possible as well as constant high level of CSF awareness).

- **The long-term objectives are:**
  - to achieve and maintain CSF disease free status without vaccination;
  - to assure implementation of preventive biosecurity measures in all pig holdings in Serbia;
  - to assure constant communication on CSF risks and preventive measures with all stakeholders;
  - to assure sustainable and competitive pig production and trade on international markets.

To fulfill both short and long term objectives it is necessary to restructure the pig sector in Serbia in terms of improving biosecurity in pig production, so ensuring implementation of CSF preventive measures and introduction of cost sharing scheme for compensation payments.

3. Rationale

The rationale for developing and implementing a national strategic plan for changing the CSF control policy with the final objective being the eradication of CSF infection in Serbia is multiple. Key reasons are:
CSF is a highly infectious and dynamically evolving disease that spreads rapidly and widely across countries and continents.

CSF is often trans-boundary in nature, with the potential to cause epidemics with devastating consequences for pig industry of the affected country.

CSF often emerges and spreads rapidly as a consequence of globalizing markets.

CSF maybe spread widely and quickly by feral pigs.

CSF impacts on the livelihoods of rural people and may substantially impact national economy.

CSF threatens national and international trade and places the pig sector at risk. CSF outbreaks sometimes are beyond the scope and resources of a single country to control or eradicate CSF infection.

4. Pillars of the Strategy

- **Prevention**: Activities to ensure CSF prevention measures are identified and undertaken including adoption of preventive biosecurity measures in pig production sector.

- **Responsibilities**: Responsibilities to all levels of government, industry, segments of society and individuals are identified.

- **Communication**: CSF policy change is communicated to all stakeholders (e.g. individuals, communities and governments, recognising that they all share the responsibility to prevent occurrence of CSF and limit the spread of infection and to eradicate the disease).

- **Surveillance**: CSF surveillance systems at national and international provide for continuous "situational awareness" and ensure prompt reaction in case of CSF outbreak.

- **Preparedness for control and eradication**: Actions to combat and limit the spread of the CSF infection as well as to eradicate the CSF infection are identified and elaborated in CSF contingency plan. This means that in case of an CSF outbreak stamping out policy is applied, sampling in a holding when a pigs are killed, post-epidemic sampling and screening before lifting restriction in protection and surveillance zones as well as sampling and screening in relation to repopulation. Emergency vaccination may be considered as a control option according to the criteria for its implementation as stipulated in the national legislation. The most effective way to protect country is to keep the infection beyond the borders of Serbia. It is recognised that if the neighbouring countries are not implementing similar measures the disease can be easily re-introduced in the country. Therefore, control and eradication of CSF at regional level is a more realistic outcome if all countries in the region have the same approach.

- **Maintenance of CSF disease/infection freedom**: CSF awareness, preventive measures as well as surveillance system is continuously in place to maintain CSF free status without preventive vaccination in Serbia. Regarding people’s awareness, the change is from “raising awareness” to “maintaining awareness”.

*Project funded by the European Union*
5. Background

5.1 Purpose of this document

This document was prepared in accordance with the Serbian Veterinary Legislation, transposing Council Directive 2001/89 and introducing Community measures for the control and eradication of CSF. It sets out the disease control principles that have been approved by the Veterinary Directorate of the MAFWM and pig industries and provides a road map and time frames for short, medium and long term priority activities, to be supported by the Serbian Government for future preparedness and response to CSF outbreaks.

This control strategy was prepared in consultation between VD at the MAFWM (hereafter referred to as Government), veterinary profession, and organisations representing pig producers and processors. We have sought to make this strategy both effective in achieving its disease control objectives and practical to operate on the ground.

The framework of the proposed Strategy will increase the capability of all stakeholders to respond during an outbreak of CSF. This will significantly reduce the socio-economic impact the disease.

5.2 Strategic fit

The strategy is consistent with key policy principles:

- The EU Animal Health and Welfare Strategy's principle “prevention is better than cure”.
- Responsibility and cost sharing schemes of subsidies and compensation payments require close working between Government and industry in developing control measures, having in mind that the disease has no human health implications, but potential of severe economic trade impacts on industry.
- Adoption of the World Organisation for Animal Health (OIE) recommendations on control measures, compliance with EU legislation and international obligations to trading partners.
- Takes account of ongoing obligations for the welfare of animals.
- Takes account of wildlife management policies.
- Supports the Serbian Government’s disease contingency plan.

Government is required to ensure a response to CSF is consistent with EU legislation and OIE disease control principles. These include responsibilities to mitigate the risk of spread to other member states or third countries. The response aims to quickly assess and close disease risk pathways to other pigs, either domestic or those living in the wild.

6. Strategic control framework

6.1 Disease control planning on socio-economic impact analysis

Economic, political and social issues have a significant impact on the choice and implementation of disease prevention, control and eradication strategies. Analysis of the costs and benefits of different approaches supports informed choice of control strategies, because it can provide guidance on whether a proposed strategy is economically viable, the potential source of finance, the risks of non-compliance with regulations and the best means to provide exit strategies for producers and processors, who cannot afford to comply with

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measures that are more stringent. It is important that such an analysis take account of indirect as well as direct impacts on a wide range of stakeholders. Full analysis of economic and social effects of control measures under emergencies will not be possible and therefore veterinary authorities in drafting possible control strategies should assess these in advance of outbreaks based on experiences from other countries.

6.2 Disease management principles

The strategy recognises that control and eradication of CSF cannot be viewed as only a responsibility of the Veterinary Directorate of the MAFWM and that the country must have an integrated national system of plans of all government levels and all non-government sectors to address the threat. Therefore, it should be guided by the following principles:

- Government will use all instruments of national power to address the threat of the disease.
- Districts will have credible preparedness plans (Local CSF contingency plan) for rapid response to outbreaks within their jurisdictions.
- Private sector and Non-governmental organizations (NGOs), like pig breeders, hunting associations, animal welfare organizations etc. will play a significant role in the preparedness and implementation of the control and eradication measures.
- Owners of backyard pigs should be informed about the risk of infection and the protective measures.
- The regional and global partnerships will be leveraged to address the threat.

Managing CSF is primarily about managing the risk. CSF is not normally present in the country although occasionally some outbreaks occur and are quickly contained and eradicated and therefore risks can be managed in two ways:

- reducing the likelihood of an outbreak of CSF by putting in place measures to prevent an incursion of CSF in Serbia or in disease free areas of the country and to detect it if it should occur;
- being prepared for rapid response in order to reduce the impact of an incursion of CSF if it occurs.

6.3 Emergency preparedness planning

Emergency preparedness planning is essential and aims to develop capacities for early warning and rapid response to every CSF outbreak so that introductions of CSF infection are not allowed to overwhelm the capacity to respond. This requires preparation, in advance, of both national and local levels of CSF contingency plans as well as operational manual containing guidelines and protocols for implementation.

7. Compartmentalisation and zoning

Compartmentalisation and zoning are concepts which could be applied when complete eradication cannot be achieved in a country in the short to medium-term. These concepts are developed particularly in order to allow for international trade following the OIE guidelines. Compartmentalisation have been used by integrated large scale producers for many years to
keep livestock free from specific infections and diseases. However they are also applicable to domestic marketing to provide assurance to consumers that products is derived from disease-free sources.

Compartmentalisation relies on demonstration of CSF infection freedom within pig production sector. Potentially, it could also be applied to a situation where CSF virus is present in feral pigs, but is proved not to be circulating in domestic pigs. However, in the absence of mandatory application of biosecurity for licensing purposes, it is likely to require an interest of pig industry and extremely competent national authority to be able to demonstrate separation of domestic pigs from wild boars sufficiently securely to confidently maintain compartment freedom. Zoning relates to demonstration of disease freedom on a geographic basis.

This requires that the Veterinary directorate exert control at the region/zone level equivalent or superior to that at the national level. Fundamental to its application is the Veterinary directorate’s control over the compartment and the free exchange of information necessary to convince importing countries that the risk of disease introduction from trade is minimised. Therefore, the procedures for establishing trade based upon the compartmentalisation concept should be similar to those practised for regionalisation/zoning. All disease control approaches require the combined contributions of Veterinary directorate and individual producers. Compartmentalisation requires a higher relative investment of resources per unit of animal production by the producers and the veterinary budget than zoning or national disease control programs.

8. Gaining and maintenance of the disease freedom

To gain CSF free status, without vaccination, measures stipulated in the CSF Ordinance and Diagnostic manual must be fully implemented. CSF surveillance must be conducted permanently in order to collect information that will provide for confirmation that there is no CSF virus circulation in pig population (domestic pigs, wild boar or both) the country.

9. Measures to prevent the CSF virus introduction and spread

Minimum biosecurity measures must be laid down in the national legislation and implemented in order to prevent introduction and spread of CSF virus in both domestic and wild pig population.

10. Disease notification

Every suspect case, as assessed according to CSF Diagnostic manual, and confirmed case of CSF must be notified to the competent authority as provided in national legislation.

Every confirmed case of CSF must be notified to the OIE, neighboring countries and EU.

11. Communication and awareness

Public awareness and support for CSF disease control activities is of crucial importance. Pig keepers, other stakeholders in the industry and the general public need to understand why early reporting, culling of infected animals, movement control and market controls are necessary to gain their support.
Education of pig farming community, pig dealers, transporters, veterinarians, hunters, on the actual risks of transmission of CSF virus to domestic pigs and wild boar, is one of the key objectives.

When implementing surveillance programmes in wildlife full regard shall be paid to the requirements of the legislation on protection and conservation of all wild species. Close communication and co-operation between epidemiologists, wildlife specialists and the competent authority for Nature conservation should be ensured when designing and implementing CSF surveillance in wild boar.

Communication strategy must be an essential part if the national CSF contingency plan. In this context, the communication objectives are to:

- inform about the ways in which one can prevent the arrival of the epidemic on Serbian territory;
- prepare the country for managing the risk of mass CSF epidemic;
- help manage the crisis and maintain the organisation of society during an epidemic;
- sustain trust and credibility;
- prepare for the end of the crisis and the resumption of normal activities.

To reach this goal, communication must be three-fold:

- informative communication relative to the CSF situation and the state of preparation;
- pedagogical communication on behaviour: the disease risks, prevention and management;
- communication to elicit trust in the authorities: following the measures taken.

Following the country adopted communication procedure, it is expected that the challenge for information and training actions includes:

- spread out the information on the risk of CSF to all stakeholders;
- prior sensitization and by providing appropriate, transparent and coherent information in order to create favourable conditions for the management of a crisis in the case of an epidemic of CSF;
- facilitate neighbourhood solidarity and individual responsibility in the face of risk.

12. Restructuring of the pig sector

Restructuring the pig sector is an important element to guard against the damaging effects of CSF, but is also one of the most complicated interventions to be undertaken requiring understanding of the whole socio-economic system. Restructuring requires different approaches at different pig sector levels in the country, by virtue of the differences in their infrastructures, marketing characteristics, backyard versus commercial pig production, and socio-economic impact. Therefore the restructuring should be seen as a long-term gradual process, affecting the various segments of the sector in different ways and at different rates. It is an inevitable that the process is to be clearly regulated. Socio-economic impact of restructuring the pig sector must be considered. It is expected that the Government policy supports the sector by:

- Improving animal health services at village level by means of organising community based early warning networks, utilising the local veterinarians;
- Increasing farmers’ general awareness through simple biosecurity guidelines on control of CSF;
- Providing access to credit as a tool for rehabilitation in addition to direct compensation by the government;
- Developing farmers’ groups and/or associations to help improve awareness and dissemination of information.
The fast payment of a realistic level of compensation is an absolute pre-requisite to gain community support for disease reporting and implementing control and eradication programmes in the village pig and small-scale producer scenarios.

13. Implementation of good biosecurity practices

According to the international recommendations a biosecurity manual must be laid down in the national legislation and applied to guarantee the protection and mitigation of CSF risks. Legal framework must be fully in place before implementation of concept of compartmentalisation and OIE structured framework for the application and recognition of compartments within country (in order to allow for a trade without barriers). Factors defining compartment should be laid down in the national legislation as well as related industrie’s responsibility.

Categorisation of pig farms based on the level of their biosecurity should be carried out by using predefined criteria. Restructuring of the pig sector in respect of improving bio-security and introduction of new cost sharing scheme for compensation payments of pig owners affected by the disease and subsidy payments for restocking of farm or for upgrading biosecurity has already been recognised by the Veterinary directorate.

14. Compensation payments

Measures in case of CSF involve culling of all pigs in the infected holding. For that reason the most common practice to ensure the cooperation of pig owners is to compensate animals that are culled for the CSF eradication purposes.

Compensation schemes have been already established in the national veterinary law.

15. Implementation of the multi annual action plan for control and eradication of CSF in Serbia

This multi annual action plan will be applied on the whole territory of the Republic of Serbia taking into consideration that:

- Currently there is no firm evidence that CSF field virus is not circulating in Serbia’s pig population, in particular in small family farms (type backyard holdings) and in wild boars,
- Biosecurity needs to be improved at family farms (type backyard holdings),
- In some municipalities pigs are raised on pastures; where direct or indirect contact with wild boar cannot be excluded,
- During the hunting season 2009/2010, 14 out of 499 wild boar tested were found seropositive and in hunting season 2010/2011, 66 out of 1374 wild boars tested were found seropositive. Seropositive wild boars were found in 19 epidemiological units situated in North-west and Central part of the country,
- According to the results obtained from genotyping of CSF virus isolates collected in 2010 it can be said the virus circulating during the 2010 CSF epidemic is homologous to the CSF viruses circulating in the country from 2006 onwards.

Basic elements of this multi annual action plan are:

- Active clinical monitoring of pigs for CSF, including a targeted sampling and testing scheme, according to the CSF Diagnostic manual;
Enforcement of the ban of prophylactic vaccination against CSF of the domestic pig population;
− Rapid and effective application of the control and eradication measures;
− Epidemiological analysis to assess CSF situation in the country.

When implementing the program the Veterinary directorate will also take into consideration:
− The different types of pig holdings in Serbia: commercial farms and backyard holdings;
− The results from the phylogenetic analysis of the isolated strains and the geographical distribution of the disease/infection;
− The distribution and dynamic of wild boar population and related geographical distribution and density of free ranged domestic pigs;
− Registration of pig farms and animal identification system in place, the electronic registration of movements of pigs, and use of electronic database to allow for tracing purposes;
− Preparedness to implement control/eradication measures in case of CSF outbreak.

16. Duties and responsabilities for implementation of the plan

**Veterinary Directorate** (VD) at the Ministry of Agriculture Forestry Trade and Water Management:
− Central Competent Authority for the control of infectious notifiable diseases, interacts with the institutions involved in the multi annual control and eradication plan for CSF and reports to the EC, OIE and other international organisations.

**Animal Health Department** at the Veterinary Directorate headquarters:
− Elaborates the CSF control strategy and implements the multi annual control and eradication plan for CSF,
− Coordinates actions of all authorities involved in the programme,
− Collects information and prepares reports on the outcome of the programme.

**Animal health officials** at the District Veterinary Inspection Services:
− Enforce the programme on both, the domestic and wild boar population at district level, monitor the actions of all authorities involved at local level and report to the VD
− Supervise the rendering plants and take samples from dead pigs suspicious of CSF or killed for reasons of CSF eradication.

**Veterinary officials** at the District Veterinary Inspection Services in charge of meat inspection:
− Carry out ante and post mortem inspection in accordance with the rules laid down by Council Regulation (EC) 854/2004, Article 5 and Annex I, in addition carry out the documentary and identity checks in accordance with Serbian Veterinary Legislation,
− Ensure health marking of the meat in accordance with Serbian Veterinary Legislation, (which is harmonized with the EU veterinary legislation),
− Carry sampling of pigs for CSF according to the approved by the VD sampling scheme, and ensure the traceability of the samples back to the farm of origin,
− Notify the VD in case of suspicion of CSF and forward corresponding samples to
the NRL in Belgrade,
- Check the cleaning and disinfection of means of pig transports in accordance with the provisions laid down by Serbian Veterinary Legislation.

**Veterinary Stations** should:

- in case of any suspicion of CSF take samples in accordance with the CSF Diagnostic manual;
- carry out the documentary and identity checks in accordance with the Serbian Veterinary Legislation in the context of movement of pigs inside Serbia;
- ensure the traceability of the samples back to the farm of origin;
- record results of clinical surveillance in VETUP traceability database system;
- monitor the improvement and enforcement of biosecurity measures at the farms, including cleaning and disinfection procedures based on the principles laid down in CSF Ordinance (transposing Council Directive 2001/89/EC, Article 12 and Annex II),
- enforce the rules on holding registration, pig identification and movement control, including closing of pigs and imposing restrictions on movement of pigs;
- record and analyse the results of CSF surveillance of domestic pigs and wild boar.

**Hunting Directorate**:

- Cooperates with the Veterinary directorate and other institutions on planning and implementation CSF surveillance of wild boar.

**District Hunting Inspections**:

- Provide carcasses or samples from wild boar (shot at hunting, fallen animals or crashed in car accidents) for inspection and sampling to the Vet.Institute.

**Hunter's Associations**:

- Provides carcasses or samples from wild boars (shot at hunting, fallen animals or crashed in car accidents) for inspection to the Veterinary Institutes.

**National Reference Laboratory (NRL) for CSF in Belgrade**:

- Test the samples collected from both, domestic pigs and wild boars for CSF, record test results in the database system and reports to the VD,
- Carry out confirmation tests on samples with doubtful test results sent by the Regional Laboratories,
- Monitors the procedures at the Regional Laboratories and organises ring-tests at national level;
- Forwards virus isolates to the CRL for CSF, Hanover and takes part in ring-tests.

**The Regional CSF Laboratories**

- Perform CSF serology (ELISA antibody testing) on samples from domestic pigs collected according to the sampling plans, record test results in the database system and report to the VD, and
- Forward positive and doubtful samples to the NRL in Belgrade. The positive samples will be tested with differential virus neutralisation test.

**CRL for CSF at Hanover**:

- Undertakes genotyping of virus isolates forward by the NRL, Belgrade,
− Provides the NRL with test materials on request from the Serbian side,
− Organises ring tests in which the NRL Belgrade will participate.

**Veterinary Public Health Laboratories in Serbia:**

− Forward samples from wild boar -received in the context of *trichinella* examination- to the NRL for CSF, ensure the traceability of the sample and include all the data necessary for the monitoring of CSF in wild boar.

**Veterinary Faculty in Belgrade:**

− Involved in the scientific and epidemiological analysis of the CSF control programme,
− Provide training of veterinarians on CSF.

**Ministry of Internal Affairs:**

− Assisting in the administrative and security measures in case of suspicion or confirmation of CSF.

**Farmers’ Union and the Pig Breeders’ Associations:**

− Inform their members about the CSF control programme and support the VD to perform active surveillance.

**17. Key measures for implementation of the plan**

Categorisation of pig holdings in respect of biosecurity and degree of risk of CSF is needed in the context of CSF control and eradication as well as development of new compensation payment mechanism based on cost sharing responsibility between the pig owners and the Government.

**18. Plan for phasing out vaccination against CSF**

The occurrence of classical swine fever in Serbia during 2010 and current epidemiological situation as well as preliminary results of the serological survey on vaccinated pig herds, lead to amendments of the plan on phasing out of CSF vaccination.

The plan takes into consideration the whole productive pig system, domestic and wild pigs, their role and all the possible links in the occurrence and spreading of the disease. The peculiar farms’ attitude and size as well as different risk factors play an important role in the epidemiology of CSF. As a consequence, phases are defined to progressively implement the preventive and control measures and to largely identify targets and activities, including biosecurity measures, animal movement control and traceability, targeting the pig production system.

Veterinary policy is both a cross-sector and cross-border responsibility considering:

− the importance of technical cooperation between the different actors involved and of strengthening a biosecurity policy on animal production - at farm, animal transport and slaughterhouse level;
− the availability of the appropriate tools for animal health surveillance and control – in cooperation with neighbouring countries and international organisations – including training, the identification and tracing the movements of animals and products thereof.
18.1 Objectives

In order for Serbia to comply with the relevant provisions of acquis communautaire one of the main objective in the strategy for controlling CSF is to discontinue the vaccination. In this regard, the Plan of actions and measures to gradually phase out CSF preventive vaccination has been proposed.

Based on risk factors and the present epidemiological situation, the following is considered:

a) the gradual cease of the vaccination in those farms where the risk of introduction of the virus can be rule out and the disease control measures can be enforceable. The commercial farms represent the first sector of pig production where the vaccination could cease. Area approach /cease of vaccination on all holdings in a specified ares/ may also be considered;

b) to establish the proper conditions to put in force the provisions dealing with the prevention, control and eradication of the disease. This includes also the need to categorize the farms not only on the basis of biosecurity level but also on the basis of production purpose of the herd (fattening or breeding). It is particularly important for the small size farms that can give rise to uncontrolled movement of piglets, facilitating the spread of the CSF infection. At national or local level the system has to foresee well defined procedures for registration and control of the pigs movement;

c) to set up and implement an effective national information system which would be used to manage CSF surveillance as well as control and eradication activities.

18.2 Legal basis

The Law on Veterinary Matters empowers the veterinary authorities to take measures against contagious animal diseases.


Serbian veterinary authorities have drafted national CSF contingency plan and the operational manual to allow for rapid and efficient control and eradication of CSF.

18.3 Pig breeding system

The pig breeding and trading systems in Serbia are influenced, mainly, by the farm’ size and the related productive activity.

The pig breeding system varies as follows:

**Big commercial farms:**

a) These are usually closed cycle farms. They have their own breeding material; different categories of pigs are not kept together; sows are artificially inseminated, piglets are grown for fattening and in the end of th productive process they are sent to the slaughterhouse, almost the 95% of fatteners, whereas the 5% can be sold out like a breeding animals to the small farmers. If they have to restock their breeding material they get animals from the other big farms with pure breeds (if possible owned by the same owner) or from foreign pig breeders. Germany, Denmark, Holland and Canada are the more common trading partners.
b) There are open cycle farms (up to 500 pigs) that are buying weaned piglets from farmers for fattening. They are usually buying on the small farms, not on the livestock markets.

**Small farms:**

a) Backyard, small family herds with few sows (from 1 to 5 mostly) are usually kept, they are usually inseminated artificially or naturally. One boar is usually serving one village but it can be borrowed to the neighbouring village too, and it is then transported with a truck or tractor. The different categories of pigs are kept together.

b) Piglets are fattened for their own consumption, or they can be fattened and then sold on a livestock market to the slaughterhouse. Piglets can be sold after weaning for fattening, usually in the same district.

18.4 Pig trading system

**Big commercial farms** are sending their pigs to a slaughterhouse, usually very few in the nearby, but it depends on the economical circumstances. Big commercial farms can sell their pigs like breeding animals for smaller farms and backyard holdings, but usually a small number of animals.

**Small farms** trade with piglets or fatteners on a livestock market, or the trading can be done on the farm or from owner to owner. This ownership change must be notified to veterinarian (no matter the location).

There are 20 livestock markets, operating in the country (about one per district). There are also pig dealers who are buying pigs on markets and from farmers, transporting them all together and selling them for finishing or to the slaughterhouse.

18.5 Risk Factors

The risk factors linked to the persistence/spread of CSF in Serbia are mainly represented by:

a) **Free ranging pig groups.** This factor is one of the most relevant because strongly associated to other factors. These pigs actually may have free access to dump where contaminated waste food could be thrown or can have contact with infected wild boar.

b) **Geographical risk.** Outbreaks occurred in territories bordering with other countries. It is well known that some of the neighbours reported CSF outbreaks in pigs or cases in wild boar.

c) **Illegal movement of pigs.** The owners of pigs kept in open space and backyard are used to smuggle or trade few animals with other pig keepers of different villages/places. Sometimes not authorized movement can involve large groups of pigs and middle/large size herds. If infected pigs are included in these groups of animals, the disease can spread undetected.

d) **Swill feeding of pigs.** Especially in the backyards is common practice to feed pigs with the waste cooking food and, if contaminated, that represent a good CSF virus vehicle. It mostly happen when safe procedure to inactivate the virus is not applied.

e) **Biosecurity level.** The lacking of biosecurity procedures application may represent a common risk to spread CSF infection.

f) **Undetected cluster of virus pockets.** This could be the risk raised in vaccinated population due to pigs that are not properly vaccinated or not vaccinated at all. In
such cases few animals could maintain the virus at low level in the environment and it can remain undetectable especially if it is a low virulent virus.

19. Defining phases and activities

Based on the analysis of the local breeding system and the risk factors identified during the preliminary investigations carried out by the consultant, the program will be implemented gradually depending on type of a pig holding according to the level of risk. Five phases are foreseen as follows:

**Phase 1: Establishment of legal framework and improvement of biosecurity in pig production and interruption of vaccination in wild boar**

**A) Establishment of legal framework for improvement of bio-security in pig commercial holdings and development of manual on criteria and guidelines to assess biosecurity level in pig commercial holdings**

In this phase, the legal framework for implementation of biosecurity measures will be established. Biosecurity of pig holdings of the commercial sector will be analysed (holding by holding) by the local veterinary service and the shortcomings will be corrected according to pre-defined criteria and guidelines described in a manual issued by the Veterinary directorate.

**B) Interruption of CSF vaccination in wild boars in fenced hunting grounds**

In the Republic of Serbia there are about 13-16 fenced wild boar hunting ground in which the wild boar density ranges from 3 to 127/km². According to the Republic of Serbia legislation in each one of these fenced hunting grounds wild boars have to be vaccinated by injection, using the C strain. It means that wild boars had to be individually caught. Vaccinated wild boars were usually ear tagged but it does not exist a unique and approved system to register vaccinated wild boars. The vaccinated wild boars can also be sold and released – eventually – for restocking purposes in other hunting grounds (fenced or not). At the best knowledge, there no official data describing the possible movement and multiple flows of these vaccinated wild boars. The number of vaccinated animals is not available.

In a framework of any surveillance strategy the presence of:

1) a partially immune population whose antibodies cannot be distinguished when compared to the ones derived from the contact with the wild virus;

2) a number of vaccinated animals that can be sold/released everywhere in Country;

makes very difficult any technical evaluation of the epidemiological situation of CSF in the national wild boar population and thus hamper the adoption of sounding strategy for CSF management in the wild boar population.

On the other hand the presence of a small fraction of wild boar vaccinated population certainly does not prevent the possible endemic persistence of the virus outside fences. It must be underlined that the virus fade out when the herd immunity is very high and less than one susceptible animal/km² remains in the environment. Finally according to the official Serbian data the overall wild boar density is estimated to be less than 0.5 wild boar/km². If so the wild boar population should be already under the threshold density allowing the endemic persistence of the virus.

This means that, due to the actual declared densities, the wild boar population of Serbia cannot acts as the epidemiological reservoir of CSF in the Country. At present it not possible verify how these wild boar population size estimated are correct and robust.

Due to the above mentioned reasons it is highly recommended to suspend immediately the vaccination of the wild boars in fenced hunting ground with the MLV-C strain.
C) Assessment (estimation) of CSF vaccination coverage

Systematic preventive vaccination against CSF has been applied on the whole territory of Serbia for years. Survey of CSF vaccination coverage in 2010 has been designed and implemented by the Veterinary Authority in Serbia (VAS). According to the survey design two clusters of animals where the CSF vaccination coverage was to be assessed had been identified: cluster I: backyard holdings and small farms; and cluster II: medium size farms (semi intensive production) and large industrial farms. CSF vaccination coverage has been assessed at the cluster level. Assuming no bias has been introduced into the results due to a selection process of study units or sampling errors (e.g. selection error, non-response error, and measurement error), results of the study indicate no significant difference in CSF vaccination coverage between two clusters (vaccination coverage ranging from 62% to 78% in Cluster I; vaccination coverage ranging from 61% to 73% in Cluster II). It must be pointed out that the national data on CSF vaccination coverage in 2010, as described above, represent averages and can hide wide variation at the local level. Furthermore, the above mentioned data may indicate that the true CSF vaccination coverage could be much lower than the expected “administrative coverage” calculated by so called administrative method (e.g. dividing the number of doses applied to pigs in 2010 by the number of pigs that should be vaccinated).

The difference between the true and the expected “administrative” CSF vaccination coverage, might be explained by performance of the local veterinary service (e.g. implementation of recommended vaccination protocols). Considering the area responsibility (municipality or part of the municipality) of the local veterinary service a certain degree of variability in vaccination coverage might also be expected at the municipality level. CSF outbreaks in 2010 also support the presence of variability in vaccination coverage at municipality level (despite the same obligatory vaccination policy was to be implemented all over the country). In order to assure that no CSF clinical disease is present as well as to reduce the level of possible CSF virus circulation up to a maximum, it is necessary to achieve CSF vaccination coverage of at least 80% on the whole territory of the country.

Additional CSF vaccination coverage survey will be implemented in order to collect up-to-date information to allow for establishment of the areas in the country where the vaccine coverage assures no or an extremely limited CSF virus circulation in domestic pigs.

D) Training and awareness campaigns for all the stakeholders that contribute to the prevention, early warning, control and eradication of CSF

Training programme was planned to introduce key elements of the strategy to control and eradicate CSF to veterinarians, hunters and pig farmers., as defined by the Veterinary directorate and supported by the EU project, The training activities have already been initiated and in progress.

It is estimated that the first phase will last for 12 months.
Phase 2: interruption of vaccination and surveillance in domestic pigs in the lowest risk holdings (fatteners and breeders – see Annex 1, 2.1 to 2.4 of the Strategy)

Area approach e.g. to cease of vaccination on all holdings in a specified area, taking into account biosecurity level of pig holdings in the area as well as the country’s priorities (e.g. trade) may be implemented.

Another option is sectoral approach e.g. commercial holdings, in which no animals from outside are introduced and a very limited number of animals is sold to other holdings, are considered the holdings at lowest risk of becoming infected and of transmitting the infection to others and they may be the first category of farms to phase out vaccination.

The Veterinary directorate, in cooperation with all relevant stakeholders will decide on an area eligible to stop CSF preventive vaccination on all holdings as well as to prepare a list of eligible farms for phasing out the vaccination when sectoral approach is chosen. Pig commercial holdings must be selected on the basis of implementation of biosecurity measures and assuming that stipulated biosecurity regime is permanently in place and effective. Permanent and effective implementation of strict biosecurity regime in pig production is of paramount importance when area approach to cease CSF preventive vaccination is implemented.

After cease of CSF vaccination a preliminary surveillance (e.g. 6 months period) shall start. The surveillance will include:

- official control of implementation of stipulated bio-security measures in place;
- periodic clinical examination (monthly) according to the CSF Diagnostic manual;
- laboratory testing of fallen stock according to the CSF Diagnostic manual;
- evaluation of production parameters (mortality rate and growing index);
- serological testing of non-vaccinated animals, according to the CSF Diagnostic manual, at a slaughterhouse, once per production cycle;
- virological testing if seropositive pigs found at a herd level as well as additional investigation according to the CSF Diagnostic manual.

At the end of the preliminary surveillance period, also vaccination of breeding animals will be ceased.

It is expected that the Phase 2 will start after the Phase 1 is completed. The estimated time of duration of the Phase 2 is 12 months.

In the first 6 months the preliminary surveillance and cessation of vaccination in fattening pig holdings the vaccination on holdings with breeding pigs will be discontinued and surveillance will be performed for further 6 months to monitor the possible spread of infection.

Additional information on Phase 2 is provided in Table 3 of the Annex I.

Phase 3: Interruption of vaccination and surveillance in all types of commercial holdings (mid to large size – see Annex I, 3.1 to 3.3 of the Strategy)

In order to identify eligible farms for the cessation of vaccination in all pig commercial holdings the following activities will be implemented:

- evaluation of implementation of stipulated biosecurity measures;
- periodic clinical examination (monthly);
Technical Assistance for the Control and Eradication of Classical Swine Fever (CSF) and Rabies in Serbia

- laboratory testing of fallen stock according to the CSF Diagnostic manual;
- evaluation of production parameters (mortality rate to be checked in the Herd book);
- virological testing if seropositive pigs found at a herd level as well as additional investigation according to the CSF Diagnostic manual.

Based on the results of the above mentioned surveillance, a list of uninfected holdings where vaccination may be discontinued will be prepared. In the listed holdings, vaccination will immediately cease, while holdings where virus circulation has been detected will be subjected to control measures in compliance with the provisions of the contingency plan.

After ceasing of vaccination on the above mentioned holdings, surveillance with an aim of detection of any previously undetected virus circulation must consist of:

- official control of implementation of stipulated bio-security measures in place (risk based or a random process to be applied);
- periodic clinical examination (monthly);
- laboratory testing of fallen stock according to the CSF Diagnostic manual;
- evaluation of production parameters (mortality rate to be checked in the Herd book);
- virological testing if seropositive pigs found at a herd level as well as additional investigation according to the CSF Diagnostic manual.

The duration of this phase is foreseen one year.

**Phase 4: Evaluation of results of phasing out of vaccination in commercial holdings and planning of future activities for small-size and backyard holdings**

During the last six months of implementation of the Plan, data collected in commercial herds will be analyzed together with specific surveillance data collected in small-size and backyard herds, to evaluate the epidemiological situation of non-commercial herds and to plan future activities in these herds.

Specific surveillance activities to be performed in small-sized and backyard herds:

- animal movement tracing off commercial herds (electronic registration of every pig movement is in place);
- laboratory testing of fallen stock according to the CSF Diagnostic manual;
- official control of implementation of stipulated minimum bio-security;
- measures in place (risk based or a random process to be applied)
- evaluation of production parameters (mortality rate to be checked in the Herd book);
- virological testing if seropositive pigs found at a herd level as well as additional investigation according to the CSF Diagnostic manual.

**Phase 5: Additional measures to be adopted based on assessment of current CSF situation in wild boar in the country or the region**

This activity is an integral part of CSF surveillance of wild boar.

*Project funded by the European Union*
20. Aims of CSF surveillance in wild boar

Surveillance program for CSF in wild boar must consider:

a) The epidemiological situation of the disease in the country;
b) The density of wild boar population and their geographical distribution;
c) Risk factors that might allow the introduction and persistence of the infection in the environment.

Consequently, a surveillance program in the wild boar population should be aimed in:

1) Detection the introduction of the virus in a free area;
2) Detection the presence of the infection in unknown disease status areas;
3) Estimation of the infection level in CSF infected areas;
4) Understanding the role played by wild boars in the epidemiology of the CSF in the country.

20.1 Surveillance strategies

Surveillance strategy includes:

a) Active (targeted) surveillance:
   i. in the areas where there is no evidence of CSF infection in wild boar, the aim is to detect the presence of CSF infection at 5% level with 95% CI. This aim can be achieved by sampling blood of a defined number of wild boar per sampling unit;
   ii. in the areas where there is direct or indirect evidence of CSF infection the aim may be to estimate the level of CSF infection with 95% CI; This aim can be achieved by sampling of blood and tissues of certain number of wild boar per sampling unit.

b) Passive (general) surveillance: To increase the probability to detect CSF virus the system assuring sampling and laboratory investigation of wild boar found dead, those shot after showing some clinical symptoms or wild boar died due to car accidents, etc. (corresponding to definition of CSF suspect case) will be out in place, in order to rule out CSF according to the CSF Diagnostic manual.

Additional information on CSF surveillance of wild boar is reported in the Annex 3 of the Strategy.

21. Control measures

In the framework of phasing-out CSF vaccination, rapid response and the management of outbreaks, according to the provisions of national legislation protocols established in CSF contingency plan, are essential for a successful control and eradication of CSF.

Prevention is also of paramount importance and can be achieved:

- through effective communication between veterinary authorities,
- veterinary practitioners and pig farmers,
- effective disease reporting and animal identification system,
- a strict import control of live pigs, fresh and cured meat,
- prohibition of feeding pigs with waste food and virological, and
- serological surveillance in both domestic and wild pigs.

Legal provisions should be established to ensure the necessary preparedness to effectively tackle the emergency situations related to one or more outbreaks of CSF, in particular by drawing up plans and setting up control centres and expert groups for CSF.

### 21.1 Performance indicators, deliverables, milestones and management of the project

**Performance indicators** of the project will be:

- Total number and change in percentage of detected non-conformity in biosecurity and percent of non-conformity corrected per month;
- Total number of holdings belonging to the target category of risk for the ongoing phase and monthly percentage of such holdings recruited in the project;
- Monthly percentage of the number of fattening holdings and animals ceasing vaccination;
- Monthly percentage of the number of breeding holding and animals ceasing vaccination.

**Deliverables:**

D1: pre-defined criteria and guidelines on the evaluation of biosecurity - prepared by the veterinary directorate - expected time of delivery: 2 months after the start of the project;

D2: report on the biosecurity of the pig holdings of the commercial sector (holding by holding) - prepared by the Veterinary directorate - expected time of delivery: end of Phase 1;

D3: list on eligible farms for Phase 2 - prepared by the veterinary directorate and the relevant stakeholders - expected time of delivery: 1 month after the start of phase 2;

D4: contingency plan (national and local) - prepared by the Veterinary directorate - expected time of delivery: end of Phase 1;

D5: report on the serological testing at slaughter of unvaccinated fattening animals (activities and results) including data on the virological follow up of any positive result to serological tests - prepared by the veterinary directorate - expected time of delivery: 6 months after the start of Phase 2;

D6: report on the periodic clinical examination of the animals in the farms (activities and results - prepared by the veterinary directorate - expected time of delivery: end of Phase 2;

D7: report of the laboratory control of fallen stock (activities and results - prepared by the veterinary directorate - expected time of delivery: end of Phase 2;

D8: report on the random sample of fattening pigs for each production unit serologically tested at slaughter for Phase 2 - prepared by the veterinary directorate - expected time of delivery: end of Phase 2;

D9: list of eligible farms for phase 3 - prepared by the veterinary directorate and the relevant stakeholders - expected time of delivery: 1 month after the start of Phase 3;

D10: report on the serological testing at slaughter of unvaccinated fattening animals (activities and results) of Phase 3, including data on the virological follow up of any positive result to serological tests - prepared by the Veterinary directorate - expected time of delivery: 6 months after the start of Phase 3;
D11: report on the periodic clinical examination of the animals in the farms included in Phase 3 (activities and results - prepared by the veterinary directorate - expected time of delivery: end of Phase 3;

D12: report on the laboratory control of fallen stock in holdings included in Phase 3 (activities and results - prepared by the veterinary directorate - expected time of delivery: end of Phase 3;

D13: report on the random sample of fattening pigs for each production unit serologically tested at slaughter for Phase 3 - prepared by the veterinary directorate - expected time of delivery: end of Phase 3;

D14: report on the suspect cases detected by the early warning system and by the clinical inspections and confirmatory testing of high-risk groups of animals.

D15: report on the laboratory control of fallen stock (activities and assessments of results - prepared by the Veterinary directorate).

Deliverables D2, D3, from D5 to D9 and from D11 to D15 can be constituted by the access to a fully updated IT information system.

Milestones are foreseen at the end of Phase 1, at mid-term and at the end and of Phases 2, 3 and 4 when main deliverables are foreseen and the passage to the subsequent phase will require a review of the activities performed as well as their effectiveness. At each milestone, a meeting involving all stakeholders is foreseen.

The management of the project will be performed by the competent authority trough the monitoring of the above indicators, deliverables and related timing (Annex 1, Table 3).

## 21.2 Costs

Costs are considered for the implementation of the plan activities and based on the assumption that no outbreaks will occur during the project period and its implementation.

Costs are summarized in table 1, which gives an overall picture of the approximate funding needed phase by phase.

<table>
<thead>
<tr>
<th>Phase</th>
<th>Activity</th>
<th>Number</th>
<th>Unit of measurement</th>
<th>Individual cost</th>
<th>Total cost (€)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase 1</td>
<td>Biosecurity inspections</td>
<td>12</td>
<td>work weeks</td>
<td>1500</td>
<td>18,000.00</td>
</tr>
<tr>
<td>Phase 2</td>
<td>Contingency manual</td>
<td>4</td>
<td>work weeks</td>
<td>1500</td>
<td>6,000.00</td>
</tr>
<tr>
<td></td>
<td>Clinical visits</td>
<td>1500</td>
<td>work days</td>
<td>272.73</td>
<td>409,095.00</td>
</tr>
<tr>
<td></td>
<td>Lab control of fallen stock</td>
<td>2000</td>
<td>PCR test</td>
<td>10</td>
<td>20,000.00</td>
</tr>
<tr>
<td></td>
<td>Sample of slaughtered pigs</td>
<td>37500</td>
<td>ELISA test</td>
<td>2</td>
<td>75,000.00</td>
</tr>
<tr>
<td>Phase 3</td>
<td>Contingency manual (updating)</td>
<td>1</td>
<td>work weeks</td>
<td>1500</td>
<td>1,500.00</td>
</tr>
<tr>
<td></td>
<td>Clinical visits</td>
<td>1500</td>
<td>work days</td>
<td>272.73</td>
<td>409,095.00</td>
</tr>
<tr>
<td></td>
<td>Lab control of fallen stock</td>
<td>2000</td>
<td>PCR test</td>
<td>10</td>
<td>20,000.00</td>
</tr>
<tr>
<td></td>
<td>Sample of slaughtered pigs</td>
<td>37500</td>
<td>ELISA test</td>
<td>2</td>
<td>75,000.00</td>
</tr>
<tr>
<td>Phase 4</td>
<td>Lab control of fallen stock backyard herds</td>
<td>50</td>
<td>PCR test</td>
<td>10</td>
<td>550.00</td>
</tr>
</tbody>
</table>
### Table 2: estimated costs of the plan

<table>
<thead>
<tr>
<th>Lab control of slaughtered pigs from selected backyard herds</th>
<th>400 PCR test</th>
<th>10</th>
<th>4,400.00</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>1,038,640.00</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The strategy can only bring about real change if everyone involved in animal health works together and with all interested operators. The plan provides strategic guidance on the appropriate level of animal health protection and on priorities for action and communication. It is important to follow up the strategy's progress and how best to deliver planned outcomes in clarity and transparency when communicating with veterinarians, farmers and other operators involved. It's also considered necessary to increase awareness of operators through information and training. Communication plan for all stakeholders need to be developed on a yearly basis.

Communication plan should help to ensure effective and user-friendly communications with stakeholders to better explain the importance of implementation of bio-security measures on farms, identification and registration of animals and their movements and rapid response to a suspect or confirmed case of CSF. Results achieved by policy actions in respect with of CSF policy change is also subject to regular communication to all stakeholders.

### 23. Preparedness of operators

Farmers and veterinarians play an integral role in implementation of preventive biosecurity measures at farm level as well as to cooperate with competent authorities and rapidly react in case of CSF suspicion or confirmation of the disease.

Biosecurity means taking steps to ensure that good hygiene practices are in place and are implemented by everyone who comes onto farm. Effective biosecurity measures reduce the likelihood of a disease outbreak that could have serious negative repercussions. Good biosecurity also helps decrease disease treatment costs, improve farm efficiency and protect neighbouring farms and the countryside.

Biosecurity can make the difference between health and disease. This is why farmers who are genuinely concerned with the health, welfare and productivity of their animals take biosecurity seriously as they know. Economic reasons for disease prevention are also an important issue for farmers.

There is no 'one size fits all' programme for biosecurity, but general strategies can be applied to all animals. These can then be supplemented by additional measures adapted to the species and the husbandry system in use.

Successful on-farm biosecurity measures must address a wide variety of factors including: isolation of new animals brought to the farm and of sick animals; controlling the movement of people, animals and equipment; correct use of feed; and procedures for cleaning and disinfecting facilities, procedures for disposal of carcases as well as a good insect and rodent control programme.

However, biosecurity is more than a simple set of practices: it is most effective when it is taken as an attitude to daily tasks and management decisions. By keeping in mind how best to prevent the spread of disease in day-to-day routine can best help to maximise the biosecurity on farm.
Veterinarians, both official and private practitioners, play an important role in the eradication and prevention of diseases. In addition, they are responsible for offering expertise and knowledge in a local situation, translating theory into practical solutions and actions.

For the above reasons, a training plan must be delivered to improve knowledge of all operators, veterinarians, farmers, dealers, transporters, workers at slaughterhouses, regarding basic principles on CSF epidemiology and biosecurity as well as to improve preparedness to rapid response in case of disease outbreak.

24. Information to stakeholders

Regular and informed communication on CSF risks to stakeholders is also of paramount importance, as an incorrect public perception of the risk may force the decision makers to take unjustified or disproportionate measures in case of CSF crisis.

All stakeholders must be regularly up-to-date on CSF control and eradication strategy in place. Multiple communication actions and related responsibilities, promotion of awareness and participation as well as communication of results of implementation of the Strategy should be coordinated by the Veterinary directorate,
## ANNEX I - Activities preceding or following the cease of CSF vaccination

Table 3: Summary table of activities preceding or following the cease of CSF vaccination

<table>
<thead>
<tr>
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*Project funded by the European Union*
ANNEX II - Classical Swine Fever (CSF)

The virus

Classical swine fever virus (CSFV), bovine viral diarrhoea virus (BVDV), and border disease (BDV) belong to the genus Pestivirus of the Flaviviridae family (Becher et al., 1999). They are small, enveloped, positive-single strand RNA viruses and are made up of a single open reading frame (ORF) flanked by a 3' and 5' untranslated region (UTR), the latter contains conserved regions implicated in the translational events (Fletcher and Jackson, 2002; Sizova et al., 1998).

In contrast to CSF and BDV, BVDV can be divided into two biotypes, cyto-pathogenic (CP) and non cyto-pathogenic (NCP) according to their cyto-pathogenic in cell culture. Their genome of about 12.5 to 16.5 kb encodes for a single poly-protein (Meyers et al., 1989): NH2- (Npro-C-ERNS-E1-E2-p7-NS2/3-NS4A-NS4B-NS5A-NS5B)-COOH, which is co and post-translational converted in 12 mature proteins by a combination of virus and host cell proteases (Rumenapf et al., 1993) The virion is made up by 4 structural proteins (C, ERNS, E1 and E2) which are encoded at the 5prime end of the genome. Although the exact virion structure is up until now not known in detail, it consists out a spherical nucleo-capsid and coat, which is composed of numerous proteins C while the surface is made out of ERNS, E1 and E2 in homo-dimeric (ERNS, E2) or hetero-dimeric (E1E2) form (Konig et al., 1995; Thiel et al., 1991; Weiland et al., 1992; Weiland et al., 1990; Weiland et al., 1999). In contrast to E1 and E2, ERNS has no trans-membrane spanning domain and its attachment to the virion is rather tenuous and currently not well known. Whereas the structure and function of some of the envelope proteins have been studied in some detail, the 8 non-structural proteins including an N-terminal protein (Npro), p7, the non-structural proteins (NS) 2, 3, 4A, 4B, 5A and finally 5B, are less characterized. Little is known about mechanisms of viral RNA replication or packaging, and how viral particles are assembled. Virions are released from the host cell by exocytosis, usually without morphological cell damage.

The survival and inactivation of CSFV was recently reviewed (Edwards, 2000). Despite its envelope, CSFV is known to survive for prolonged periods in a favorable environment, cool, moist, protein rich as found in meat. The increased stability in low temperatures, even at low pH (pH4), and in protein rich environments is important as they are encountered during storage. For example; pH values of semi membranous and longissimus dorsi muscle post mortem ranges from 6.17 to 6.71. During the commercial production of pork and pork products, the time and temperature of storage seldom allow the pH to fall below 5.7 (Farez and Morley, 1997) and provide therefore ideal surviving conditions. Survival rates up to 4.5 years for frozen meat have been reported (Edgar, 1949). Treatments, as curing and smoking on the other hand, have little effect on the survivability of the virus. The most important factor is the temperature, duration and height, applied during the processing stage (Edwards, 2000). Survival rates in processed meat products of for example 90 days in salami (Savi et al., 1972) and 126 days in Iberian loins (Mebus et al., 1993) have been reported.

Thermal and pH stability can vary depending on the strains but the inactivation of the virus is most dependant of the medium containing the virus, it is therefore difficult to give guidelines for the survival of CSFV in the environment. Although it has been demonstrated that CSFV in cell culture loses its infectivity after 10 min. at 60°C, it can survive up to 30 min. at 68°C in defibrinated blood. The virus is relatively stable in a range of pH 5-10, but the inactivation process under pH5 is dependent on the temperature (Depner et al., 1992). As enveloped virus, CSF virus is inactivated by organic solvents (ether or chloroform) and by detergents Sodium hydroxide at 2 % concentration is still considered most suitable to disinfect
contaminated premises, but in liquid manure the CSFV can survive for 2 weeks at 20°C and more than 6 weeks at 4°C (Haas et al., 1995).

**Antigenic and genetic typing**

Even though, CSFV is a very stable RNA virus (Vanderhallen et al., 1999), a recent study (Heet al., 2007) indicated that recombination between strains is possible. Differences have been shown depending on the source of the isolates using first a panel of monoclonal antibodies (Edwards et al., 1991). Two panels of monoclonal antibodies, directed against E2 and ERNS glycol-proteins allowed the definition of 21 antigenic types (Kosmidou et al., 1995). A standardized protocol was further designed to type new CSFV isolates, including the genomic fragment to be sequenced, the algorithms for the design of the phylo-genetic trees and the nomenclature of the genetic groups. Three regions of the viral genome were usually evaluated, the 3’ end of the polymerase gene (NS5B), 150 nt of the 5’NTR and 190 nt of the E2 encoding gene.

As several genetic data are available for the E2 glycoprotein gene giving a reliable classification, it is currently most frequently used for genetic typing. The nomenclature of the genetic groups (Lowings et al., 1996) was adapted to fit additional groups from Asia, dividing CSFV in three groups with three or four subgroups: 1.1, 1.2, 1.3, 2.1, 2.2, 2.3, 3.1, 3.2, 3.3, 3.4 (Paton et al., 2000a). The phylogenetic analyses performed during the last decade have demonstrated a link between genotype and geographical origin (Bartak and Greiserwilke, 2000; Stadejek et al., 1997; Vilcek, 1997; Vilcek and Belak, 1996, 1997). Since the beginning of the 1990’s, most of the viruses isolated from the outbreaks that occurred in Western Europe, belonged to the group 2, when isolates of the group 1 were still circulating in South America (Frias-Lepoureaux and Greiser-Wilke, 2002) or Russia (Vlasova et al., 2003). Viruses belonging to the group 3 seem to be confined within Asia (Parchariyanon et al., 2000). Moreover cross protection exists between the different genogroups (e.g. the C-strain based vaccines have been widely used in Asia and Europe to protect the pig against CSFV) The Community Reference Laboratory for CSF in Hannover has developed a computerized database (http://viro08.tiho-hannover.de/eg/csf) where several of the known sequences of isolates of worldwide distribution are registered (Greiser-Wilke et al., 2000b). Although many outbreaks have been reported to OIE, the sequences of isolates from these outbreaks are not still available. This database is a very useful tool to identify the possible sources for new outbreak occurring in previously non infected area (Greiser-Wilke et al., 2000a; Sandvik, 2000, Dreier et al., 2007). In pigs, pestivirus isolates are usually Classical Swine Fever virus.

The terms Bovine Viral Diarrhoea Virus (BVDV) and Border Disease Virus (BDV) are used to indicate that the virus was diagnosed as the cause of infection in either cattle or sheep although these two viruses cannot be differentiated morphologically or structurally from each other (Laude, 1979). The first report of natural infection of swine with BVDV came from Australia in 1964, but BVDV was not isolated from a naturally infected pig until 1973 (Fernelius et al., 1973). However BVDV and BDV can be isolated from naturally infected pigs (Carbrey et al., 1976; Terpstra and Wensvoort, 1988). Moreover, it has been demonstrated through cross neutralization tests and tests using monoclonal antibodies (Wensvoort, 1989; Leforban et al., 1990) that, in the past, BVD virus may have been isolated from pig but mislabelled as CSF virus on the basis of tests using polyclonal antibodies only.

As previously described, cross-species transmission within the *Artiodactyla* have been reported for BVDV as well as BDV. Currently, the genus *Pestivirus* comprises the four approved species BVDV-1, BVDV-2, CSF and BDV and one tentative fifth species represented by a single strain (H138) isolated from a giraffe in Kenya more than 30 years ago (Becher et al., 1999), but recent phylogenetic and antigenic analysis have lead the same authors to propose to split BDV group in 4 other subgroups, BDV-1 for the classical sheep.
isolates, BDV-2 for the mainly sheep isolates related to the previous strain V60 isolated from reindeer, BDV-3 for the ovine Gifhorn isolate that differs significantly from all previously described pestiviruses including BDV (Becher et al., 2003) as well as BDV-4 isolates observed in samples of diseased Chamoix (Thabti et al., 2005; ValdazoGonzalez et al., 2006).

In addition, beside the giraffe strain a further new group of atypical pestiviruses was described in 2004 with the “HoBi” strain isolated from a batch of fetal calf serum being the first member (Schirrmieier et al., 2004). There is now some evidence, that this kind of newly found pestiviruses is common in cattle in some countries in South America and Asia (Greiser-Wilke et al., 2007; Kirkland et al., 2007; Kreutz et al., 2000).

CSFV Virulence

According to Mittelholzer et al., (2000), no significant, qualitative or quantitative differences were found between studied strains of different virulence when either RNA replication or protein synthesis were investigated, even if the ratio of cell-associated virus versus secreted virus proved to be considerably lower for the highly virulent strains when compared to nonvirulent or moderately virulent strains. Mutagenesis studies, performed on the CSFV genome, have identified several regions which are associated with virus virulence although the underlying molecular mechanism remains unknown. Insertion of 19 amino acids into the carboxyl terminus of the E1 region of Brescia resulted in attenuation of the virus and a reduced viremia, spreading to the different tissues and viral shedding (Risatti et al., 2005b). Similar studies, in which genetic regions of different CSFV strains have been exchanged or mutated, resulted in the link between virulence in swine and the E2 region (Risatti et al., 2005a). Three different regions in the E2 have been identified as virulence determinants: glycosylation site at position 805 (Risatti et al., 2007b); a region between 805 and 837 (Risatti et al., 2006) and a stretch of 12 amino acids substitutions in the carboxyl terminus (882 to 1032) (Risatti et al., 2007a). van Gennip et al., (2004) also identified a determinant in E2 (position 710) but a decrease in virulence was only found in conjunction with mutations in the ERNS region (position 276, 476 and 477). Similar to E2, glycosylation sites (position 269) in the ERNS have been found to have an influence on virulence in swine (Sainz et al., 2008). Abrogation of the RNAse activity of ERNS by mutating codons 297 and 346 of the ERNS protein resulted in a changed virulence of the virus (Meyers et al., 1999). In addition the structural proteins, a virulence determinant has also been identified in one of the non-structural proteins, namely Npro (Mayer et al., 2004), using Npro deletion mutants. So far, no reliable in vitro parameters correlating with the virulence of a CSFV strain in pig has been found. Nevertheless, the question of the virulence is of main importance in the field. Highly virulent strains spread very efficiently within a naïve population but are “easy” to detect as they give a lot of clinical symptoms and are often lethal for the pigs. Conversely, an outbreak due to a moderate virulent strain will be difficult to recognise as the clinical symptoms are mild and in some cases, the pigs can recover (Durand et al.,2008). Theses phenomenons have been seen in the last 1990 years with the strains involved in the European outbreaks. Moreover with a low or moderate strain, some pigs can become persistently infected and can spread virus for a longer time (Moennig et al., 2003). Knowing the virulence of a strain involved in a pig outbreak could help in the prediction or modelisation of the spreading and therefore can help to choose the most appropriate control measures to be applied.

Clinical signs

Historically, different levels of virulence have been reported from per acute, acute, chronic or prenatal forms of CSF. The virulence of a strain is difficult to establish as the same isolate can induce different signs depending on the age (younger age animals are more...
susceptible), the breed, the health status and immune status of the inoculated pigs (Depner et al., 1997; Floegel- Niesmann et al., 2003; Moennig et al., 2003).

**Domestic pigs**

Piglets develop more evident clinical signs than the adults do. The constant symptom is the hyperthermia (Davila et al., 2003; Floegel-Niesmann et al., 2003), usually superior to 40°C, but in adults it can be lower (39.5°C). The first usual signs in acute form are anaemia, lethargy, conjunctivitis, respiratory signs and constipation followed by diarrhoea (Cariolet et al., 2008). During a chronic course of the disease, the issue is generally fatal. After displaying at first similar clinical signs as in an acute form, the pigs survived for two to three months but normally not more. They display non specific signs as fever, diarrhoea, wasting, anorexia, and disorders.

In pregnant sows, CSFV is able to cross the placenta of and infect the foetuses during all the stages of pregnancy. Depending on the virulence of the strain and the time of gestation, the infection can result in abortion and stillbirths in early pregnancy and can lead to the birth of persistently viraemic piglets if infection occurs during the first 50-70 days of gestation. These piglets seem normal at birth but rapidly waste or display congenital tremor (Vannier et al., 1981). This course of infection was reported as “late onset CSF” (van Oirschot and Terpstra, 1977). These animals shed a lot of virus for several months and are very dangerous reservoirs and sources of infection.

In adult domestic boars, experimental infection with the CSFV virus has no evident effect on libido and ejaculate parameters of adult boars, (Wehrend et al, 2006). The clinical course was mild in the boars with an increase in body temperature, but never above 39.9°C and a transient anaemia. The libido remained good, and the quality of semen collected in from three boars was always in the range of the minimum requirements for sperm that is used for artificial insemination. In another experiment carried out by Floegel et al. (2000), four young boars were infected with a CSF field virus strain and semen was collected at least every other day after infection. The course of CSF infection was mild but clinically detectable during the second week of infection. CSF virus was isolated from semen of two animals during the pyrexia phase and from the epididymis but not from the testes. Since CSF virus shedding via semen could be proven, it was concluded that the disease may also be transmitted by artificial insemination boars may thus be of special epidemiological relevance for the dissemination of the CSF virus as clinical symptoms are mild.

**Feral pigs or wild boars**

In general, most clinical and pathological signs described for domestic pigs are also observed after infection of wild boar with CSFV (Kaden, 1998; Kaden et al., 1999, Kaden et al., 2001a, Kaden et al., 2004, Kaden et al., 2005, Koenig et al., 2007a). In postnatal infections, lesions are generally caused by widespread thrombosis or endothelial damage, inducing haemorrhagic diathesis and petechiati. However, due to the pigmentation alterations of the skin are difficult to detect.

After experimental CSFV infection in a pregnant wild boar and two wild boar weaners, the clinical, pathological and haematological findings noted in the young wild boars were comparable to those in domestic weaner pigs inoculated with the same virus isolate (Depner et al., 1995a). Both weaners showed the acute haemorrhagic form of CSF, one animal died 18 days post inoculation and the second one had to be euthanized when moribund two days later.

The wild boar sow did not show any signs of illness p. i. but seroconversion was noticed.
Twenty-eight days after infection birth was given to six clinically healthy offspring. One of the newborn proved to be viraemic until death at 39 days of age. Except for poor growth no other symptoms were noticed in this piglet. The non-viraemic litter mates remained healthy, although they had close contact to the persistently infected piglet. High titres of neutralizing antibodies against CSFV were measured in the serum samples of these offspring. All findings were more or less in accordance with observations previously made in domestic pigs when infected with CSFV around 85 to 90 days of gestation. The wild boar was calculated to have been inoculated at about 87 to 92 days of gestation.

A classical swine fever virus (CSFV) field isolate originating from wild boar was investigated for its virulence in domestic pigs and wild boar. Three weaner pigs and two wild boars (yearlings) were intranasally inoculated with the isolate "Spante" and tested for clinical, virological, hematological and serological findings until day 31 post infection (p. i.). One day p.i. the piglets were put in contact to three sentinel pigs. During a period of 31 days, neither the domestic pigs nor the wild boars showed clinical signs specific for CSF. Two infected weaner pigs became transiently viraemic, transmitted CSFV in nasal secretions, showed a slight leucopenia and reacted serologically positive. The contact infection resulted in a viremia in two sentinel piglets on day 30. Only one contact animal developed antibodies. None of the wild boars became viraemic, excreted CSFV in nasal secretions or developed antibodies (Kaden et al., 2006a; Kaden et al., 2000b).

Maternal antibodies can partially protect the wild boar piglets, in an area where the virus has already spread. Instead of an acute and fatal course, the disease is transient, as it was shown during an experimental study conducted to investigate the clinical course of classical swine fever (CSF) in wild boar piglets partially protected by maternal antibodies. Five healthy wild boar piglets with a low serum titre of colostral antibodies against CSF virus were challenged with virulent CSF virus at the age of three months. Apart of reduced food intake and diarrhea no major clinical symptoms were noticed after challenge. These signs were seen during the second and third week of infection, after which the piglets recovered completely. CSF virus was re-isolated from blood samples taken on day 12 and day 19 post challenge. No CSF virus was isolated from blood samples taken later on and from the organ material taken at post mortem examinations no CSF virus could be isolated anymore. It was concluded that the presence of maternal antibodies influences the clinical course of CSF in terms that the outcome is rather transient than lethal. Such wild boar could play a crucial role in the spread of CSF virus and might contribute to the maintenance of long lasting epizootics (Depner et al., 2000). Even if experimental infection in domestic or wild pigs gives similar disease, it is more difficult to identify classical swine fever in the wild as found dead animals are the main alert sign. These carcasses cannot be found easily as they are most of the time eaten by other animals or hidden by high grass during the summer. At post mortem examination, the most frequent gross lesions seen are on the skin: round lesions similar to scabies, and ulcers on the intestine (Chenoufi et al., 2006).

Pathogenesis

CSFV is known to be immunosuppressive (Summerfield et al., 2001a), however neutralizing antibodies appear usually after one to two weeks post infection in recovering pigs. In addition, a specific response of CD8+ killer T-cells was described starting after the first days of CSFV infection (Pauly et al., 1995). Recently, different teams have attempted to understand the mechanisms of the CSFV–host interactions that lead up to the innate immune response evasion and delay the onset of acquired immunity and produce its pathogenic effects. As with other pesti-viruses, CSFV grows in cell culture without any cytopathogenic effect, preventing the antiviral effect of INF α and apoptosis (Ruggli et al., 2003). Even if the majority of pesti-viruses are non-cytopathic in vitro, some BVD viruses from mucosal disease cases or some CSFV strains are also cyto-pathogenic in vitro, and this cyto-pathogenicity of BVDV is correlated with a higher expression of the nonstructural protein.
NS3, which is generated by processing of a fusion protein termed NS2-3 (Kümmerer and Meyers, 2000; Zhang et al., 2003).

Since CSFV is non-cytopathic in vitro, it has been suspected that the serious lesions seen in vivo were linked to immune-pathological damages. The usual entry site is the oronasal route, the first site of virus replication are the tonsils. Then the virus spread to the regional lymph nodes, before reaching, via the peripheral blood, the bone marrow, visceral lymph nodes and lymphoid structures linked to the small intestine, and spleen. The spread of the virus within the pig is usually completed in less than 6 days. During infection, severe changes occur in the bone marrow and in the circulating white cell population, suggesting an indirect cytopathic effect induced in non infected cells either by a soluble factor, or by cell to cell contact (Summerfield et al., 2001b). Interestingly, CSFV replicates in monocytes–macrophages and vascular endothelial cells in pigs. Leukopenia, in particular lymphopenia, is a characteristic early event during CSF (Susa et al., 1992). The leukopenia involved leukocyte sub-populations in a disparate manner, with B-lymphocytes, helper T-cells and cytotoxic T-cells being the most severely affected. Depletion of lymphocyte sub-populations occurs shortly before or at the time virus can be detected by RT-PCR in the serum. The pathogenic mechanism therein would involve indirect virus-host interactions, probably originating from the site of primary infection, rather than a direct effect of the virus or viral protein. Furthermore, these characteristics offer an explanation for the retardation of the cellular and humoral immune response observed during classical swine fever (Summerfield et al., 2001a). ERNS at high concentrations has been pointed out as an apoptosis inducer (Bruschke et al., 1997) on lymphocytes in vitro, but its implication has been under discussion since addition of infected cells supernatant did not induce apoptosis in target cells. The interactions between both viruses and the monocytemacrophage-system result in the release of mediator molecules, which are important for the further progression of the disease. The changes in the haemostatic balance are thought to be caused by pro-inflammatory and antiviral factors, inducing the thrombocytopenia and the mechanisms of the hemorrhages, which are characteristic in the infection (Knoetig et al., 1999). The production of inflammatory cytokines by infected endothelial cells could play a role in the immunosuppression, as well facilitating virus dissemination by attracting monocyctic cells (Bensaude et al., 2004). The question of the CSFV presentation by dendritic cells has been recently studied leading to the observations that CSFV can replicate in dendritic cells (DCs). CFSV could use these highly migrating cells as a vehicle to different sites in the body, especially to lymphoid tissues (Jamin et al., 2008). However, the interaction between CSFV infected DCs and lymphocytes is not sufficient to induce the lymphocyte depletion, without another interaction with the particular environment of the lymphoid follicles (Carrasco et al., 2004). In clinically diseased pigs, CSFV and CSFV RNA can be normally detected from day 2 to 4 onwards (Davila et al., 2003). Duration of viremia depends on the clinical situation and is very short in subclinical infections e.g. of sows (1 to 2 days) or can be very long lasting e.g. during chronic or persistent infection.

**Immunology and vaccination**

Little is known about the immune response of wild boar against CSF. However, as wild boar and domestic pigs are the same species (Sus scrofa) it can be assumed that they have analogous immune response. Neutralising antibodies can be detected around 12 to 14 days after virus inoculation (Table 3). It was shown that nearly the complete induction of neutralising activity depends on the envelope protein E2 (de Smit et al., 2001a, Reimann et al., 2003, Voigt et al., 2007). However, non neutralising antibodies are also developed against the envelope protein ERNS and the nonstructural protein NS3 (Rau et al., 2006). In contrast, detection of NS3-antibodies as well as one of the ERNS-ELISAs is panpesti-virus specific. (Beaudeau et al., 2001, Mars and Van Maanen, 2005)
Concerning cellular immune responses versus CSFV, cytotoxic killer cells were described (Pauly et al., 1995, Piriou et al., 2003) and epitopes for CD4-specific as well CD8-specific stimulation were defined (Armengol et al., 2002). In contrast, the role of both natural killer cells and innate immunity in CSFV infection remains unclear (Suradhat et al., 2005). In recent studies, it was demonstrated that the innate immunity modulating function of Npro is not relevant for the virulence of CSFV (Nicolas Ruggli, poster at the GfV meeting, Heidelberg 2008).

Both CSFV-specific neutralising activity and specific killer cell activity are most important for an effective immune response. However, every part on itself has also the potential to protect pigs from a lethal CSFV-infection. It was demonstrated that E2-subunit vaccines can protect pigs on the basis of high titer of neutralising antibodies (Bouma et al., 1999) while experiments with related pestiviruses or chimeric constructs were efficient without detectable neutralising activity (Reimann et al., 2003; Beer et al., 2007, Voigt et al., 2007). However, the combination of both cellular immunity and neutralising antibody response is obviously crucial for an optimized immunity allowing fast and complete protection with a kind of “sterile immunity”.

Types of vaccines for the potential use of emergency vaccination

The following description about classical CSFV-vaccines is based on the report of SCAHAW in 2003 (“Diagnostic Techniques and Vaccines for Foot-and-Mouth Disease, Classical Swine Fever, Avian Influenza and some other important OIE List A Diseases”). In addition, a recent OIE review article (Blome et al., 2006) can be used as a reference for further information. There are, in general, only two relevant types of CSFV-vaccines on the market: live attenuated (modified live vaccines = MLV) and E2 subunit (marker or DIVA) vaccines (E2subV). While the MLV are licensed or authorised by national authorities, E2SubV was registered by the EMEA. For the moment there is one E2subV commercially available.

Live attenuated/modified live vaccines (MLV)

Classical live vaccines are used both in wild boar and domestic pigs worldwide, and are based on different attenuated virus strains. The most widely used vaccine strain is the so-called “Chinese (C)-strain”, but there is some confusion about the origin of the C-strain and there are several C-strains with different histories. Most, if not all, C-strains have been attenuated by hundreds of serial passages in rabbits (Aynaud, 1988). Other vaccine strains are the Japanese GPE-negative strain, the Thiverval strain, and the Mexican PAV strains (EC, 2003; Blome et al., 2006). C-strain-based vaccines are also used for oral immunization of wild boar with vaccine carrying baits (Kaden et al., 2001a, 2001b, 2001c). In Germany, C-strain baits were used in several federal states like Mecklenburg-Western Pommerania, Rhineland Palatinate and North-Rhine-Westphalia (Kaden et al., 2002, 2003, 2004a, 2005).

E2 subunit marker vaccines (E2subV)

During the development of marker vaccines it became clear that the E2-glycoprotein in a purified form was capable of inducing a protective immunity (Rümenapf et al., 1991; Van Zijl et al., 1991; Hulst et al., 1993; Konig et al., 1995; Van Rijn et al., 1996; Peeters et al., 1997). This finding was the basis for the development of an E2 subunit vaccine that contains as antigen only the E2 glycoprotein of CSFV. The recombinant E2 glycoprotein is produced in cultures of insect cells infected with the baculovirus vector (Hulst et al., 1993). Pigs vaccinated with a sub-unit marker vaccine only develop antibodies against the E2 glycoprotein whereas pigs that have been naturally infected develop antibodies against
different viral proteins (e.g. E2, ERNS, NS3). Consequently, it is possible to distinguish between an infected and a vaccinated pig by means of an ELISA test that detects antibodies only against the ERNS glycoproteins upon infection (Moormann et al., 2000). Two differential diagnostic ERNS antibody ELISA tests (ERNS-antibody ELISAs) are commercially available (SCAHAW, 2003, Blome et al., 2006.

**Efficacy of vaccines for emergency vaccination**

The efficacy of vaccines against CSFV is evaluated after challenge infection with a virulent CSFV strain using the following parameters: clinical score, body temperature, viremia, virus shedding and infection of in “contact animals”. Highly efficacious vaccines are able to induce a so-called “sterile immunity” resulting in a complete block of viral replication upon challenge. In general, most MLV (e.g. C-strain vaccines) are reported as highly efficacious after a single oral or parenteral vaccine application and the onset of protection starts a few days after vaccination. In contrast, E2sub are described as most efficacious after booster injection and onset of immunity was not before several weeks post vaccination. Also, vertical and horizontal spread of challenge virus was described in E2subV vaccinated pigs upon challenge (SCAHAW, 2003; Blome et al., 2006). It was shown that after oral application, MLV are highly efficacious both in domestic pigs and wild boar (Kaden and Lange, 2001; Kaden et al., 2001a; Kaden et al., 2000a).

**Efficacy of the live attenuated/modified live vaccines (MLV)**

Two of the main factors that determine the efficacy of CSFV MLV (Modified live virus vaccine) are the virus strain used and the virus titre. Potency of CSF MLV is tested according to the European Pharmacopoeia in immunization/challenge experiments (European Pharmacopoeia 2008). The recommended challenge infection is carried out 14 days post vaccination and gives the opportunity of a good differentiation between vaccines with diverse potencies. To evaluate the potency of CSF vaccines for emergency usage, even earlier challenge infections are conceivable. In addition, the tonsils of the infected animals should be examined for the presence of challenge virus (Biront and Leunen, 1988). It was also reported that MLV should contain at least 100 PD50 to prevent carriers (Leunen and Strobbe, 1977). A report using an oronasal challenge one week after vaccination demonstrated protection with a MLV containing 160 PD50 (Biront and Leunen, 1988).

The C-strain has been found to be highly efficacious inducing a virtual complete protection against the challenge infections. From around 2 to 4 days after vaccination, challenged pigs did not show any clinical signs nor replication of challenge virus, measured by shedding in oral swabs or by detection of viraemia. This protection has also been demonstrated to last more than a year, probably even lifelong (Biront et al., 1987; Aynaud, 1988; Terpstra et al., 1990; Kaden and Lange 2001, Kaden et al., 2008, Dewulf, 2002 a). As with many modified live vaccines, maternal antibodies interfere with the induction of vaccination immunity: the higher the maternal antibody titre at vaccination the stronger the interference (Vandeputte et al., 2001, Ooi, IPVS 2008). The reported results of good protection were also confirmed by using PCR for CSFV detection in vaccinated and challenged animals (Beer et al., unpublished data). A neutralizing antibody titre of 1/64 or higher is considered as protective against a CSFV infection (Terpstra and Wensvoort, 1988). However, it is not always the case as demonstrated by (Kaden et al.,2006b). The presence of maternally derived antibodies (MDAs) has important implications in any eradication/control strategy. With CSF they can reduce the clinical signs while viremia may still occur (Depner et al., 2000). MDAs usually have disappeared within 3 months of birth (Kaden and Lange, 2004a; Soos et al.,2001) but low levels of MDAs have been also detected for longer periods (Depner et al., 1995a, Müller
et al., 2005). Wild boar piglets, before the age of 3 months do not consume the vaccine baits (Brauer et al., 2006).

With regard to emergency vaccination, it is of relevance how early virus excretion in vaccinated pigs is reduced or prevented and so how early pigs become immune to CSFV infection. These effects will result in reduction or prevention of transmission of challenge virus, which can be examined in so-called transmission experiments (Bouma et al., 2000). It has been found that the C-strain is able to block transmission of virulent challenge virus to vaccinated in-contact pigs from at least 2 to 7 days after vaccination (de Smit et al., 2001b; Dewulf et al., 2003; Dewulf et al., 2002b; Kaden et al., 2001; Kaden et al., 2008), and possibly earlier since no infection was detected in a transmission experiment where vaccinated pigs were in contact with infected pigs at the day of vaccination. (Koenen et al., unpublished observations, Dewulf et al., 2002b). Efficacious CSFV vaccines must also prevent congenital infections with field virus, since these may result in a variety of abnormalities in the foetuses. From an eradication point of view, the most insidious is the birth of persistently infected, immunotolerant piglets that are healthy and survive for months while continuously shedding virus (van Oirschot and Terpstra, 1977). Data on this efficacy aspect of the C-strain are now available. It was shown that pigs orally immunized with Cstrain (Riemser Arzneimittel AG) were completely protected from transplacental infection. In addition, vaccine virus was not detected in any of the piglets from immunized sows (Kaden et al., 2008). Even though there are no published data, field observations over many years indicate that transplacental infection is blocked after intramuscular vaccination (Ooi IPVS 2008; Kaden et al., 2008; SCAHAW 2003). Very recent data show that C-strain RNA is detectable in tonsil samples during at least for 42 days post vaccination (Koenig et al., 2007a), but no infectious virus could be isolated. Concerning the protection from virus persistence in lymphatic organs (tonsils, lymph nodes, spleen), it was demonstrated that infectious virus was not detected after challenge infection and conventional PCR results were also in most cases negative (Kaden et al., 2008; Beer et al., unpublished data,Table 2).

**Efficacy of the E2 subunit marker vaccine (E2subV)**

The E2 subunit vaccine was demonstrated to protect specific pathogen free (SPF) piglets against the clinical course of the disease two weeks after double vaccination or 6 weeks after a single vaccination (Hulst et al., 1993: Konig et al., 1995; Van Rijn et al., 1996; Peeters et al., 1997). More recently, it was demonstrated that, with 32 micrograms E2 in a wateroilwater adjuvant, a protective immunity was conferred as early as 21 days after a single vaccination (Bouma et al., 1999). However, in order to prevent or minimise the spread of the virus in case of an outbreak, the efficacy of the vaccine should be assessed for its ability to stop replication and shedding (van Oirschot, 1999). With one E2subV, that is no longer available, it could be demonstrated that horizontal transmission within the vaccinated group was prevented 10 days after a single vaccination (Bouma et al., 2000). In similar experiments in which conventional piglets and a recent field isolate as challenge virus were used and which were performed in several reference laboratories, it was shown that even 21 days post vaccination a limited transmission was possible (Utenthal et al., 2001). In another experiment where SPF pigs were infected 21 days post vaccination and subsequently brought into contact with susceptible piglets, the vaccinated piglets infected the susceptible piglets by shedding the virus in one group out of eight (Bouma et al., 1999). In addition, it has been shown that virus infection by contact was delayed, but not prevented in twice vaccinated pigs (Dewulf et al., 2000). In experiments evaluating the vertical transmission of the virus, also variable results were obtained. Some reports describe that a double or even a single vaccination of pregnant sows was capable of preventing transplacental infection when using the strain Zoelen, a subtype 2 CSFV strain (de Smit et al., 2000b) or the homologous Brescia strain (Ahrens et al., 2000) as challenge virus. On the other hand, a study conducted by the EU reference laboratories, showed that in pregnant sows, at 2 weeks post E2subV
vaccination and challenged with the recent CSFV field isolate “Paderborn”, a subtype 2 CSFV strain, transplacental infection occurred in 100% of the cases (Depner et al., 2001). Transplacental infection occurred in 5 out of the 12 sows challenged after a double vaccination (Dewulf et al., 2001). Form both studies it was concluded that challenge with a heterologous field virus in pregnant gilts that had received a double vaccination with an E2subV marker vaccine, resulted in clinical protection but neither horizontal nor vertical transmission of the CSF virus were prevented.

A recent comparative study with an E2subV marker vaccine and a C-strain vaccine used for emergency vaccination against CSF demonstrated that, in a vaccinated population, the conventional C-strain vaccine prevents horizontal virus transmission from the day of vaccination and that the E2 sub-unit vaccine can prevent virus transmission after an interval of 14 days (Dewulf et al., 2003). Finally it has to be mentioned that even if there is a very early onset of immunity using MLV, vaccination in CSF infected animals does not positively influence the course and outcome of the infection (Kaden, 1983; Glaner et al., 1984; Leopoldt und Tesmer, 1985; Kaden und Glaner, 1987). MLV should be administered orally about 4 days, and intramuscularly about 2 days before challenge infection, while E2SubV should be administered about 14 to 21 days before challenge to reach protection levels blocking spread of infection. Most of the E2SubV experiments described above used a single vaccination. Nevertheless, the producer of the vaccine advises a primary vaccination schedule of 2 doses, with a 4-week interval.

Safety

In general, safety issues are more often discussed for MLVs while subunit vaccines are normally accepted as innocuous. However, not only for E2subV but also for most of the CSFV MLV only very few cases with side effects were reported.

### Safety of the live attenuated/modified live vaccines (MLV)

Early studies reported that C-strain vaccine virus can pass the placental barrier of pregnant sows but does not seem to produce any abnormality in infected foetuses (Bran et al., 1971; Tesmer et al., 1973) However, a recent study demonstrated the safety of a current C-strain vaccine (C-strain Riems) also for pregnant animals since infection of fetuses was not observed (Kaden et al. 2008). The Thiverval strain appeared to be safe, even in immunosuppressed pigs (Biront and Leunen, 1988, Suradhmat et al., 2006). More recently, Soos et al., 2001 reported that, upon oral or intramuscular administration, neither significant clinical signs, nor CSFV-associated pathology nor adverse effects were detected during pregnancy. Finally, the absence of leucopenia after vaccination was also demonstrated (Swangard et al., 1969 Koenig et al., 2007a). Although Terpstra and Tielen (1976) noticed that C virus spreading was possible under normal field conditions, these results have not
been confirmed by recent data. Furthermore, no evidence for vaccine virus presence in nasal secretion or in faeces was found in domestic animals pigs (Kaden et al., 2004).

No increase of virulence was reported up to now, but, in most cases, the regaining of virulence was tested in piglets only and not in pregnant sows. The C-strain was not isolated from pigs for longer than 1 to 24 weeks (Terpstra, 1978; Lorena et al., 2001, Kaden et al., 2004). However, recent real-time PCR data demonstrated the presence of C-strain RNA in the tonsils for at least 42 days post intramuscular vaccination, but no infectious vaccine virus could be isolated (Koenig et al., 2007a).

Concerning the contamination of MLV with other viruses, the recommendations of the European Pharmacopeia are followed with special emphasis on possible contamination with other pestiviruses. Contamination of a C-strain vaccine batch with another pestivirus has been reported by Wensvoort and Terpstra in 1988. However, new molecular detection techniques now allow the easy and sensitive detection of contamination viruses, especially pesti-viruses. Therefore, the risk has become very low to negligible (Hoffmann et al., 2005, 2006; McGoldrick et al., 1998, 1999; Deregt et al., 2006).

Safety of the E2 subunit marker vaccines (E2subV)

The E2 subunit vaccines have the general safety advantages of inactivated vaccines and are indeed highly safe, apart from a possible local tissue reaction at the injection site (Bouma et al., 1999; Lipowski et al., 2000; Depner et al., 2001.

Differentiation of infected from vaccinated animals (DIVA)

Serological DIVA or marker tests are only available for the E2subV. The test of choice is blocking ELISAs for the detection of ERNS-specific antibodies (Beer et al., 2007). In contrast, vaccination with MLV gives an antibody pattern similar to that of wild type CSFV infection. Nevertheless, real-time PCR detection of CSFV genomes can be used as “genetic DIVA” differentiating CSFV-genome-positive animals from CSFV-genome-negative animals (Beer et al., 2007).

Genetic DIVA is a very useful technique for the early differentiation of non vaccinated -infected and vaccinated - infected animals. While for antibodies 21 to 35 days are needed until the detection limit is reached, the genome investigation by real-time RT-PCR is possible after 2 to 5 days post infection, however, long term status evaluation is depending on serological screening techniques, since the CSFV-genomes are eliminated early after infection, especially in MLV-vaccinated pigs (1 to 60 days depending on the samples materials), and CSFVspecific antibody titres are persisting for month or even years.

Administration of vaccine in the field

Domestic pigs

The E2subV have to be administered by injection. MLVs can be given as well orally as parenterally. However, parenteral injection is the method of choice also for the MLV’s since onset of immunity is reported to be established several days sooner. It has to be mentioned that parenteral application of MLVs was used in Romania also for the immunization of backyard pigs.
Wild boars

Vaccination of wild boar can only be performed with MLVs and by oral application with baits. The possibility to lyophilize C-strain before putting it into the baits and thereby providing additional stability to the vaccine (Faust et al., 2007), further supports vaccination strategies in the wild. However, the bait uptake by younger animals is problematic. Although new smaller baits have been developed, they are still not picked up by animals younger than 3 months (FP6 “CSFVACCINE &WILD BOAR” annual report). The latter indicates that vaccination with baits before that age is probably not possible. In order to follow and study oral uptake of the baits, iophenoxic acid has been successfully used as biomarker (Cowled et al., 2008).

Other candidate vaccines

The different types of future vaccines are reviewed by Dong et al. (2006), Beer et al.(2007), in a report from a previous EC working group (SCAHAW, 2003) as well as in an OIE publication (Blome et al., 2005). Most important candidates are shown in Table 2 (Beer et al., 2007). In summary, all studies concluded that chimeric pestivirus constructs are the most promising second generation candidates for a modified live CSF DIVA vaccine with the potential to combine the efficacy of MLV with the marker properties of E2subV (Dong et al., 2006, Beer et al., 2007). However, registered products will not be available in the next 3 years.
Diagnosis

The clinical signs of CSF are extremely variable and may be confused with many other diseases. Clinical signs can therefore only lead to a clinical suspicion of the disease and any suspicion of CSFV has to be confirmed by laboratory diagnosis. Laboratory diagnosis relies on either agent detection (detecting either viral proteins or genome) or antibody detection. The choice of the laboratory tests used for diagnostic investigation depends mainly on the goal (i.e. surveillance vs. confirmation of suspicions), but also on the infrastructure and experience of a laboratory. The technical annexes of EU legislation as well as the OIE Manual of Standards for Diagnostic Tests and Vaccines provide useful details on the laboratory procedures for diagnosis of CSF. Recent reviews give additional information on most of the tests (Blome et al., 2006; Greiser-Wilke et al., 2007).

Agent detection
Depending on the virulence of the strain, and the tests and samples used, virus can already be detected from 24 hours after an infection. Animals that die from the infection will usually be viraemic until the time of death, whether this is during the acute phase, or after going through a chronic infection that may last up to several months. Immunotolerant pigs are also viraemic during their whole life, which may last up to nine months. Pigs that recover from the infection are usually only viraemic for a short period, from only a few days up to two weeks, after which the virus is no longer detectable in the blood.

**Virus isolation (VI)**

Virus isolation (VI) is based on the incubation of sample material on susceptible cell cultures of porcine origin. If infectious CSF virus is present in the sample, it will replicate in the cells to an amount that can be detected, by immunostaining of the infected cells with conjugated antibodies. Classical swine fever specific antibodies are required to differentiate between CSFV and other pestiviruses.

Suitable samples for isolation of CSF virus from live pigs are leukocytes, plasma or whole blood obtained from non-coagulated blood samples. Suitable tissue samples include tonsil, kidney, spleen ileum and different lymph nodes. Virus isolation is best suited for the investigation of samples from small numbers of animals rather than mass surveillance. The virus isolation procedure is labour intensive and requires at least three days before results are available. Two further cell culture passages may be necessary to detect lower amounts of virus in the sample. This may lead to an investigation time of more than 10 days before a final result is obtained. Samples that suffer from autolysis can be cytotoxic to the cell culture and consequently have limited value.

Virus isolation is still considered the gold standard, even though by now the PCR is recognized as a more sensitive test (Depner et al., 2006a; Depner et al., 2007a). The sensitivity of the VI is usually thought to be high, and in experimental infections, up to 95% sensitivity is reported (Dewulf et al., 2004). However, an evaluation of the VI during the 1997/98 outbreak in the Netherlands, showed that the diagnostic sensitivity of the VI on tonsils in the field was only approximately 77%, which was comparable to the sensitivity of the FAT (Bouma et al., 2001). The sensitivity of the VI on blood samples may also be hampered by the presence of antibodies, although no quantitative data, especially from the field, is available on this.

A positive VI is proof for the presence of infectious virus and any animal, tissue or blood sample being VI positive is assumed to be infectious to other pigs. A negative VI on the other hand does not mean that infectious virus is absent (McKercher et al., 1987; Panina et al., 1992; Mebus et al., 1993, Haegeman et al., 2006).

**RT-PCR**

Reverse transcriptase polymerase chain reaction (RT-PCR) is based on the amplification and subsequent detection of genome fragments. Small fragments of viral RNA are transcribed into DNA fragments during an RT-step, which are subsequently amplified by PCR to detectable quantities. Detection of amplicons is possible by gel electrophoresis, but nowadays mainly real-time RT-PCR’s are being used. These PCR’s use either SYBR green to detect amplicons, or, for enhanced specificity, hydrolysis of hybridization probes (Liu et al., 1991; Roehe and Woodward, 1991; Katz et al., 1993; Diaz et al., 1998; McGoldrick et al., 1998; Aguero et al., 2004; Belak, 2005).
A wide variety of samples are suitable for the PCR, but mainly whole blood samples and tissue samples will be used for the diagnosis of CSF. Beside whole blood, also serum, plasma or isolated leucocytes can be used. Tissue samples of preference are the same as for VI: tonsil, spleen, ileum, lymph nodes. Kidney samples may be less suitable.

Due to its high sensitivity, and the amplification of huge amounts of amplicons, the RT-PCR is also very sensitive to contamination or cross-contamination of samples, reagents or other materials. Separate rooms should be used for separate steps in the PCR diagnostics, for instance pre-treatment of samples, preparing buffers and stock-reagents, RNA-isolation, and RT-PCR. Strict protocols should be in place with respect to movement of people, materials and samples between these rooms, or between these rooms and other rooms in the laboratory.

Furthermore, retesting or independent confirmation of positive samples is always an option for doubtful results. For the same reasons, the real-time RT-PCR (rRT-PCR) requires appropriate laboratory equipment and skilled staff. For both RNA isolation and RT-PCR fully robotized solutions are available nowadays. An RT-PCR can be performed within several hours, but for high-throughput 24-48 hours between receiving samples and sending out results is more realistic. Using approved commercial kits can be useful as usually the reagents are ready for use, reducing the risk of contamination and saving time to perform the assay. Experiences within the FLI during the AIV and BTV outbreaks showed, that testing of up to 800 PCRs per day is possible in one laboratory using automated RNA extraction systems. It has been reported that pooling up to ten samples did not decrease the rRT-PCR sensitivity (Depner et al., 2006; Le Dimna et al., 2008). Pooling of up to 10 samples would therefore lead to a maximum theoretic testing capacity of about 4000 to 8000 pig samples per day in a fully equipped laboratory with trained staff. In case of positive results for a pool, each of the ten samples has to be tested individually, limiting by the way the number of samples tested per day. However, the effect of the pooling on the diagnostic sensitivity of the PCR may be decreased when borderline positive samples are pooled (e.g. screening in vaccinated populations). Pooling strategies therefore need to be evaluated in depth before deciding the sort and the number of samples that can be pooled.

RT-PCR has been found to be the most sensitive method for detection of CSFV (Dewulf et al., 2004; Handel et al., 2004; Depner et al., 2006a; Depner et al., 2007a, Le Dimna et al., 2008) In carcasses from wild boar it is the method of choice, especially if the material is subjected to autolysis and virus is either inactivated or virus isolation is not possible any more due to cytotoxicity of the sample. With the RT-PCR, viral genome can be detected for a long time in certain tissue samples from animals that are fully recovered from an infection. In tonsils from pigs recovered after an infection, viral genome was detectable for at least 9 weeks (Loeffen et al., 2005). An RT-PCR positive result does not necessarily mean that infectious virus particles are present (Dewulf et al., 2005; Haegeman et al., 2006). This situation is also described for other viruses. rRT-PCR is also highly specific up to 100% (Hoffman et al., 2005; Depner et al., 2006; Le Potier et al., 2006b, Le Dimna et al., 2008) especially if specific probes are being used.

Hybridization probes may be slightly more specific than hydrolysis probes, as the latter may be subject to non-specific degradation during high cycle numbers and therefore cause very weak-positive or doubtful results (Ciglenecki et al., 2008). In general it can be said that from an RT-PCR negative result it can be concluded with a very high confidence that the tested animal or tissue sample is not infectious to other pigs, while on the other hand a sample that is RT-PCR positive, is not necessarily infectious. (Dewulf et al., 2005; Haegeman et al., 2006, Le Potier et al., 2006b).

Depending on the vaccine, and the sample to be tested, rRT-PCR can also be used as a DIVA test ('genetic' DIVA, Beer et al., 2007). If the vaccine does not contain any genome (i.e. E2-subunit vaccines) or if the vaccine has deletions or substitutions on the primer sites (i.e. deletion mutants or chimaeric vaccines), an rRT-PCR positive result would be proof for an
infection with field virus (Koenig et al., 2007a). Newly developed C-strain specific real-time RT-PCRs (Leifer et al., submitted) can be used to test vaccinated animals for the presence of MLV, but in case of a positive result, infections with wild type virus can still not be ruled out.

More importantly are therefore PCR’s that are specific for wild type virus (Li et al., 2007, Zhao et al., 2008) that can be used to detect or rule out wild type virus infections, independent of the vaccination status of the animal.

**Immunohistochemistry (IFT)**

The immunofluorescence test (IFT) or fluorescent antibody test (FAT) is based on the detection of viral proteins with FITC-conjugated antibodies (Robertson et al., 1965). The immunoperoxidase test (IPT) is based on the detection of viral proteins with HRP-conjugated antibodies. In the past both tests had been very often used for the confirmation of secondary outbreaks. For the confirmation of primary cases IFT and IPT must be supported by other direct tests (Wensvoort et al., 1986; De Smit et al., 1999, 2000b). The test can only be carried out post-mortem and the organs of preference are the tonsil, spleen, kidney, ileum, and several lymph nodes. From these organs, cryosections are cut for staining. A smear of bone marrow cells might also be used, for instance in case of feral pigs, if organs are not available or are subjected to autolysis.

The test is relatively easy to perform, but requires experienced staff because interpretation of staining is not fully objective. Furthermore a cryostat is needed to cut the cryosections. The test can be performed within few hours. However, for testing larger amounts of samples (100-200 per day may be realistic), 24-48 hours between receiving samples and sending out results is more realistic.

The IFT/FAT is often considered as less sensitive than VI, but an evaluation of the FAT and VI during the 1997/98 CSF outbreak in the Netherlands showed that in the field, the sensitivity of both tests on tonsils was almost equal (75%), (Bouma et al., 2001). This test should just be performed by experienced staff. The quality of the reagents should be controlled for the success of the test.

The specificity of the test depends on the antiserum used. If polyclonal sera are used, positive samples need to be confirmed in a second test, especially to differentiate between CSFV and other pestiviruses. With monoclonal antibodies, the test is, however, highly specific (99.97% according to Bouma et al., 2001). Due to the introduction and implementation of the RT-PCR in many diagnostic labs, this test is not very commonly used anymore.

**Antigen ELISA**

The antigen ELISA is based on the detection of viral proteins, binding to antibodies in an ELISA plate (Shannon et al., 1993; Depner et al., 1995b). The test is easy to perform and is relatively cheap and fast. However, a low sensitivity (from 39% on wild boar samples, according to Depner et al., 2006, to 74.7% on experimental infected pigs according to Dewulf et al., 2004) has been described as this test needs a high virus charge to detect positively. Its use has to be restricted to very recent infection when the vireamia is high. The specificity of this test was also considered as low as cross reaction to others pestivirus were often recorded (EU Diagnostic manual for Classical Swine Fever diagnosis, technical part, 3rd draft, June 2007). These intrinsic properties compared to most of the other diagnostic tests, especially RT-PCR, makes it not anymore the first choice for sensitive CSF detection (Dewulf et al., 2004; Depner et al., 2006; Depner et al., 2007). With the availability of the other tests, the use of the antigen ELISA is being increasingly discouraged. Nevertheless, the recent
panpesti ERNS-antigen capture ELISA kit commercialised for BVDV could be also a useful tool for CSFV detection, since first data showed a higher sensitivity and specificity than the classical CSFV-antigen-capture ELISAs (Beer, pers. communication).

**Sequence analysis**

Between 1970 and until the late 1990s, Germany was struck by several severe and less severe epidemics of CSF (Fritzemeier et al., 2000; Moennig and Plagemann, 1992; Wachendörfer et al., 1978). Since the Institute of Virology became European Reference Laboratory for CSF almost 30 years ago (Council Directive 80/217/EEC and Council Decisions 81/859/EEC), the virus isolates involved were collected and stored. The idea was to keep them to solve the many open questions concerning the virus, of which many still remain without conclusive answers.

One aim was to find methods that would allow distinguishing isolates from individual outbreaks. This was a significant issue, because such information would be an invaluable tool for epidemiologists to trace primary and secondary outbreaks. First success was achieved using monoclonal antibodies against viral proteins for differentiating between Pestiviruses (Greiser-Wilke et al., 1990; Paton et al., 1995; Wensvoort et al., 1989). In addition, mabs were successfully used for typing CSF virus isolates and other Pestiviruses (Kosmidou et al., 1995; Paton et al., 1995). This method is work-intensive and was found to be closely correlated to the availability of the mabs. At that time, technological advances led to the implementation of the polymerase chain reaction (PCR) in most laboratories, and automated DNA sequencing became practicable and affordable. It was then realized that isolates from individual outbreaks could be discriminated by genetic typing. For this, several different regions of the viral genome were used, and it was recognized that genetic typing had to be harmonised to ensure that results from different laboratories are comparable. Therefore, the three most widely used genomic fragments were evaluated, namely fragments of the 3’ end of the polymerase gene (NS5B), (Bjorklund et al., 1999; Lowings et al., 1994), 150 nt of the 5’NTR (Greiser-Wilke et al., 1998; Hofmann et al., 1994; Stadejek et al., 1996) and a fragment (190 nt) of the gene coding for the E2 glycoprotein (Arce et al., 1999; Lowings et al., 1996). A standardised protocol was designed for typing new CSF virus isolates, fixing the three genomic fragments to be used, the algorithms for calculation of the phylogenetic trees, and the nomenclature of the genetic groups (Lowings et al., 1996; Paton et al., 2000a). The CSF viruses were divided into three groups with three or four subgroups each, namely 1.1-1.3, 2.1-2.3, and 3.1-3.4 (Paton et al., 2000a). Geographical distribution of the subgroups has been reviewed previously (Frias-Lepoureau and Greiser-Wilke 2002; Moennig et al., 2003). At the same time, it was decided to store the available epidemiological data (host, year of isolation, country and region) and the nucleotide sequences of the three genomic fragments in a CSF virus database, which was to be accessible online (http://viro08.tihohannover.de/eg/csf). It is held at the European Community Reference Laboratory for CSF in Hannover, Germany, and it was designed to aid genetic typing of new CSF virus isolates (Greiser-Wilke et al., 2000b). Phylogenetic analyses performed in different parts of the world confirmed that CSF virus isolates that differ by genetic typing seem to be characteristic for certain geographic regions (Bartak and Greiser-Wilke, 2000; Blacksell et al., 2005; Chen et al., 2008; Arce et al., 2005; Kamakawa et al., 2006; Li et al., 2006; Pereda et al., 2005; Sabogal et al., 2006; Stadejek et al., 1997; Vlcek et al., 1997).

Extensive use of the database and an increasing number of records, including isolates with identical sequences from related outbreaks in different regions, made it difficult for the user to select a standard dataset for genotyping new isolates. As a consequence, the database was supplemented with a module for searching for identical sequences, performing the alignment with a standard set of sequences, and calculating and graphically displaying the Neighbor-Joining phylogenetic tree (Dreier et al., 2007).
Antibody detection

In classical swine fever virus infected pigs, antibodies are usually detectable in serum samples from one to three weeks after infection. In pigs that have recovered from the disease, protective neutralising antibodies can be detected for several years or even for their lifetime. Antibodies are also sporadically detectable in the terminal stage of lethally diseased animals. In some pigs with chronic form of classical swine fever, antibodies may be detectable for a few days at the end of the first month post-infection (Liess et al., 1976b). Pigs infected in utero may be immunotolerant against the homologue classical swine fever virus and produce no specific antibodies (Terpstra, 1987). However, maternal antibodies can be detected during the first weeks of life. The half-life of maternal antibodies against several viruses in nonviraemic healthy piglets can vary from approximately 8 days, found for CSF (Vandeputte et al., 2001), 12 days for swine influenza (Loeffen et al., 2003), 3 weeks for porcine parvo and foot-and-mouth disease (Francis and Black, 1984; Fenati et al., 2008), or more than 8 weeks for Aujeszky's disease depending on the level of maternal antibodies in the colostrums (Bouma et al., 1997). According to Kaden and Lange (2004) and Müller et al. (2005), the maternal derived antibodies were not detectable after three months after experimental oral immunisation of young female wild boars suggesting a quite high half life value. Half life values of maternal antibodies seem to be determined mainly by the increase in blood volume anyway (Francis and Black, 1984). Because domestic pigs grow much faster than wild boar, this would explain why maternal antibodies in wild boar can be detected much longer than in domestic pigs.

E2-ELISA

Several ELISA techniques using specific monoclonal antibodies have been developed, mainly: competitive or blocking ELISA and non-competitive ELISA's (Wensvoort et al., 1988, Moser et al., 1996; Colijn et al., 1997; Clavijo et al., 2001). The competitive or blocking ELISA is usually based on monoclonal antibodies. If the serum sample contains antibodies to classical swine fever virus, the binding of a selected peroxidase-conjugated monoclonal antibody to virus antigen will be inhibited resulting in a reduced signal.

In general only serum samples will be used in ELISA’s. Although meat juice can also be being used for several other infections, including Salmonella and Aujeszky’s disease (Nielsen et al., 1998; De Lange et al., 2003), some studies carried on antibodies detection from muscular exsudates were not successful (Utenthal and Le Potier, personal communications), probably because the CSF ELISA kits are not sensitive enough. Moreover, CSF ELISA kits are blocking ELISAs where the use of meat juice is really hopeless as any reaction will be blocked by meat juice. ELISA’s are relatively easy to perform, with minimum demands of facilities and personnel. ELISA’s can be fully robotized and automated for high throughput and most can be performed within several hours. However, for high-throughput testing 24-48 hours between receiving samples and sending out results is more realistic. The sensitivity of the E2-ELISA is in general comparable to that of the virus neutralization test (VNT), although the latter is more sensitive in samples obtained within 3 weeks after infection. If no antibodies can be detected in infected pigs, it is usually because they are chronically infected, with a persistent viraemia. The specificity is usually also high, in the range of 98 to >99.5%. Part of the specificity problems may be caused by infections with other pestiviruses. Some aspecific reactions can occur when the quality of the serum is not sufficient. These quality problems are more frequent for wild boars sera even if the quality of the blood sampled by hunters has really been improved for the five last years (Le Potier, pers. communication).

Detection of antibodies does not necessarily mean that the animal is infectious. On the contrary, in most cases where antibodies are present, infectious virus will no longer be
detectable. The E2-ELISA can be used as a DIVA test for vaccines that do not contain the E2 of CSFV. Such vaccines can either have the E2 replaced by that of another pestivirus (Van Gennip et al., 2000; De Smit et al., 2001a) or have it deleted (Van Gennip et al., 2002).

**ERNS-ELISA**

The ERNS-ELISA is based on the same principle as the E2-ELISA’s, but instead detects antibodies against the ERNS -protein. The ERNS -ELISA’s were developed as companion tests for the E2-subunit vaccine (Van Rijn et al., 1999). Two commercially available ERNS -ELISA’s, A and B, were evaluated in a large EU-trial in the late 1990’s (Floegel-Niesmann, 2001). At that time one of the ELISA lacked sensitivity, while the other one was deemed not to be specific enough. A new evaluation by the EU Community reference laboratory in 2003, together with 15 national reference laboratories from the EU, concluded that an improved version of one of the tests (A) was suitable as a DIVA test in combination with the E2-subunit vaccine (Commission Decision 2003/859/EC, Blome et al., 2006).

The sensitivity of the ERNS -ELISA A is in general somewhat lower than that of E2-ELISA’s. Furthermore, it is not CSF-specific, but detects also antibodies against other pestiviruses. For a population where non-CSF pestivirus infections occur, the test is therefore less useful. While this test is developed in combination with the E2-subunit vaccine, it can be used as a DIVA test with any vaccine that does not contain ERNS, including live deletion mutants (Widjojoatmodjo et al., 2000). For chimaeric vaccines, that contain ERNS from a non-CSF pestivirus (Van Gennip et al., 2000; Reimann et al., 2004), the test can, however, not be used as a DIVA test. In these cases the ERNS -test B could be used, as it is CSF-specific, but this test lacks sensitivity (Floegel-Niesmann, 2001).

**Virus neutralisation test (VNT)**

The virus neutralisation test (VNT) is carried out by incubating serum samples in several twofold dilutions with a known amount of virus together with a susceptible cell culture. In the absence of neutralizing antibodies, these cells will get infected and virus replication will take place to detectable amounts of virus. In the presence of neutralizing antibodies, the virus will be neutralized and no virus will grow. Detection of virus is usually done with an immuno cytochemical method (IFT/IPT).

The VNT is a laborious and time-consuming test. Furthermore, because virus is replicated, hygiene and containment procedures should be in place. Requirements for facilities, but also personnel are therefore much higher than for an ELISA. The VNT is considered to be the gold standard of antibody detection. It is regarded as the most sensitive antibody test, but cross-neutralizing antibodies against non-CSF pestiviruses will readily be detected as well. To solve this problem, the VNT for CSFV antibodies is usually carried out in parallel with a VNT for BVDV antibodies and sometimes also a VNT for BDV antibodies. The VNT for the detection of antibodies against BVDV and BDV follows the same principles mentioned above for CSFV. If the CSF-titre is equal to or higher than the BVDV/BDV-titre, the presence of CSF antibodies is confirmed. This procedure results in a highly specific test, but this will be at the expense of the sensitivity. CSF infections in the presence of BVD antibodies will result in false-negative test results (Wieringa-Jelsma et al., 2006).

The procedures of choice for CSFV diagnostic are summarised in Table 3. Few published papers did really estimate specificity or sensitivity of the conventional tests that were used for years. More recently, in studies of the different RT-PCR or rRT-PCR, a comparison was done
with the well-established Virus isolation (gold standard) or with other antigen detection methods (FAT, Ag ELISA).

Figure 1 in Dewulf et al., (2004), shows the usual period of detection after an infection depending on the diagnostic method used, in comparison to the VI in whole blood. The usual procedure to diagnose the presence of CSFV is done in two steps as described in Figure 1. The first test used for herd screening is a method known to be sensitive as rRT-PCR for viral genome detection or E2-ELISA for antibodies detection. Any positive sample is consequently again analysed with a different method as Virus isolation or Virus neutralisation test to check the specificity of the result. Therefore, the combination of the two tests gives a very high specificity, probably close to 100%.

<table>
<thead>
<tr>
<th>Test</th>
<th>Sample type</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Feasibility</th>
<th>p.t.d.</th>
<th>Disadvantages</th>
<th>Advantages</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFT/ IPT</td>
<td>Organ cryostat sections</td>
<td>Medium (75%)*</td>
<td>High with Mabs (99.9%*) to 70%**</td>
<td>Medium to High</td>
<td>Post mortem 4.5</td>
<td>Equipment Expense</td>
<td>Short time</td>
<td>OIE, 2004; *Bouma et al., 2001; *Dewulf et al., 2004</td>
</tr>
<tr>
<td>antigen ELISA</td>
<td>Serum Plasmatic Blood</td>
<td>Low (59%** to 74.7%*** )</td>
<td>Low Cross reaction with pesteivirus</td>
<td>High</td>
<td>7-12</td>
<td>Specificity</td>
<td>Short time Automated systems</td>
<td>Degueur et al., 1995b; *Dewulf et al., 2004; **Dewulf et al., 2004; ***Degueur et al., 2007</td>
</tr>
<tr>
<td></td>
<td>Homogenate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Semi-solid Diagnostic Serum recovery</td>
<td>Molecular Epidemiology</td>
<td></td>
</tr>
<tr>
<td>Virus isolation (VI)</td>
<td>Leukocytes plasma whole blood organs</td>
<td>Medium (77%* to 88-95%**)</td>
<td>High (100%*** )</td>
<td>Medium</td>
<td>5</td>
<td>Time consuming; Cell culture facilities; Automated sample; Up to 10 days for results</td>
<td>Strain recovery, Useful for genetic typing, Molecular Epidemiology</td>
<td>Patou et al., 2000; Aguero et al., 2004; Birlik, 2005</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>Blood organ serum</td>
<td>High (99%)</td>
<td>High (99%)</td>
<td>High</td>
<td>3-5</td>
<td>Detection of infectious virus; The need of skilled staff; Contamination; Stingent quality control</td>
<td>Results after few hours; Useful for genetic typing, Molecular Epidemiology; Suitable for carcasses</td>
<td>OIE, 2004; *Bouma et al., 2001; **Dewulf et al., 2004; ***Koening et al., 2007a</td>
</tr>
<tr>
<td>Real Time</td>
<td>RT-PCR (RTPCR)</td>
<td>Blood organ serum</td>
<td>Very high (100%)</td>
<td>High (99.9%-100%)</td>
<td>High</td>
<td>2</td>
<td>Detection of infectious virus; The need of skilled staff; Stingent quality control; Cost</td>
<td>Results after few hours; Quantitative results; Automated equipment DIVA; Suitable for carcasses</td>
</tr>
<tr>
<td>Ambody ELISA</td>
<td>Serum</td>
<td>High (98.5%)</td>
<td>Medium to High (98% - 99.5%)</td>
<td>High</td>
<td>12-21</td>
<td>Screening test; Quantitative results; Cross-reactivity resulting in false positive or doubtful</td>
<td>Fast Automated systems DIVA</td>
<td>Colins et al., 1997; Langedijk et al., 2001</td>
</tr>
<tr>
<td>VNT**</td>
<td>Serum</td>
<td>High (98%)</td>
<td>Low/High (99.9%)</td>
<td>Medium</td>
<td>12.14</td>
<td>Cross-neutralising antibodies; Time consuming; Quantitative; Differential diagnosis</td>
<td></td>
<td>Liess et al., 1976b</td>
</tr>
</tbody>
</table>

Project funded by the European Union
CSF SURVEILLANCE IN DOMESTIC PIGS

Introduction

Identifying the primary source of CSF infection is difficult and not always possible in spite of intense epidemiological research. In the 1997/98 outbreak in The Netherlands, Elbers et al., (1999) assumed that a transport vehicle could have introduced the virus from Germany. In the 2000 outbreak in England, CSF might have been introduced via infected meat or meat products by people using footpaths that ran past pigs paddocks (Gibbens et al., 2000). On the other hand, in countries were CSF is endemic among wild boar (e.g. Germany) neither trade nor people were significantly sources for infection. Rather the potential sources in Germany are either (1) direct or indirect contact with infected wild boar, or (2) contaminated meat from infected wild boar, or (3) illegal swill feeding (Figure 2.; Teuffert et al., 1997; Kaden et al., 1998; Fritzemeier et al., 2000). It is possible that the same risk factors may apply to other countries with endemic CSF in wild boar population as well.

The consequences of CSF outbreaks depend on the control measures and on the number of infected herds at the end of the high-risk period (HRP) (Klinkenberg et al., 2005). The overall HRP (see below for further explanations) is the time between of introduction of CSFV and the time when all measures are considered to be effective. Thus a long HRP will obviously increase the risk of virus transmission (Horst et al., 1998). Hence, an effective surveillance programmes should aim to keep the HRP as short as possible (Stegeman et al., 2000; Terpstra and De Smit, 2000; Klinkenberg et al., 2005). As shown in Table 4, the HRP of the last CSF outbreaks in domestic pigs in Europe were all approximately 4 to 8 weeks in their length. After introduction of the CSF virus, the disease can spread relatively slowly and some
of the European outbreaks last over one year with significant amount of samples tested (Elbers et al., 1999; Fritzemeier et al., 2000; Stegemann, et al., 2000; Mintiens et al., 2001).

Primary outbreaks (n = 111)

Secondary outbreaks (n = 249)

High Risk Period (HRP)

In theory, the overall HRP can be defined by two different time periods. (1) HRP-1 is defined as the period between the introduction of CSFV into a region and the first detection of infection. The length of HRP-1 depends on (a) the awareness, skill and motivation of farmers, veterinary practitioners and laboratory capabilities and (b) the virulence of the virus strains involved (Engel et al., 2005). (2) HRP-2 is defined as the time between the first animal being detected as infected with CSFV and the establishment of measures to prevent virus spreading (e.g. culling; establishment of restriction zones) (Elbers et al., 1999).

A long HRP-1 may be increased by the nonspecific clinical signs of CSF in its early stages. The individual incubation time usually is about 5 to 7 days (Moennig et al., 2003), on the other hand the herd incubation time is about 4 to 8 weeks. HRP-1 is influenced by both incubation times. A long herd incubation time and hence HRP-1 may be facilitated by virus strains of low virulence, which lead to vague or even absent typical clinical signs (Koenen et al., 1996; Wensvoort and Terpstra, 1985), which are difficult to be detected by the farmer. Furthermore, there are several diseases that should be considered in differential diagnoses which can mask the identification of CSFV. These might include porcine reproductive and respiratory syndrome (PRRS) and porcine dermatitis and nephropathy syndrome (PDNS) (Moennig et al., 2003) as well as postweaning multisystemic wasting syndrome (PMWS). In some cases, increased mortality has been attributed to porcine circovirus type 2 (PCV-2), and haemorrhagic lesions were attributed to septicaemic salmonellosis (Allepuz et al., 2007). On the other hand, the diagnostic value of both gross pathology (Elbers et al., 2003; Elbers et al., 2004) and routine serological surveillance (Crauwels et al., 1999) for the detection of CSF is limited. Hence, tracing of contact herds and clinical examination combined with carefully targeted virological testing of suspicious animals is likely to be the most important measure to immediately uncover secondary outbreaks (Fritzemeier et al., 2000). Certain surveillance measures have also an effect on the progress of disease control measures. As an example, the late detection of the first CSF infection in an area and the structure of pig farming can affect the HRP. The eradication campaigns can be hampered by the reduction of sensitivity of clinical inspections during an active outbreak in an area with high livestock density (Pluimers et al., 1999). Despite systematic epidemiological investigations, gathering precise information on HRP-1 from CSF outbreaks is difficult (Elbers et al., 1999).
Detection of CSF in herds

In practice, clinical detection of CSF may be difficult. The average time from infection until confirmation is estimated to be four weeks in finisher farms and five weeks for sows (Bergevoet et al., 2007). Sometimes months may elapse before CSF outbreaks are correctly diagnosed in extreme cases and reported to the authorities (Engel et al., 2005). A number of factors contribute to this situation and thorough knowledge and analysis may facilitate earlier detection of CSF (Stegeman et al., 1999; Klinkenberg et al., 2003; Bergevoet et al., 2007).

Infection of individual animals

Infection of pigs usually occurs via the oral-nasal route. Approximately 4-6 days p.i. animals become viraemic and develop high fever (Dahle et al., 1991; Dewulf et al., 2004). In parallel animals become infective since virus is detectable in saliva and other excretions. Depending on the age of the animals and viral virulence, clinical symptoms vary from quite uncharacteristic to typical signs, i.e. petechiae and high mortality. The variety of clinical not always indicative of CSF, makes it unlikely that the disease is correctly diagnosed in a herd with only a few sick animals at the beginning of the outbreak. Therefore it takes a “herd incubation time” (Karsten et al., 2005) before CSF becomes visible on a farm.

Infection in herds

Spread of CSF in a farm is a very complex process depending on individual incubation time, age of the pigs (Klinkenberg et al., 2002) contacts between animals, units and buildings as well as transmission by people (Raulo and Lyytikäinen, 2007). Several attempts have been made to quantify intra-herd spread of CSF. After experimental infection of gilts it was observed that contact animals became viraemic only 18-21 days p.i (Dewulf et al., 2001). Depending on the number of initially infected animals in a herd and contact opportunities between animals and groups of animals, it may take at least three weeks and more until a substantial number of pigs is diseased. With increasing number of sick pigs, chances for detection of CSF in a herd improve. Based on the CSF outbreaks in the Netherlands in the years 1997/98 Stegeman et al. (1999) confirmed the slow spread of the virus in a herd. Stegeman et al. (1999) calculated a basic reproduction ratio of infection R0=2.8 for breeding pigs. Fritzemeier et al. (2000) have analysed retrospectively 270 outbreaks in Germany between 1993 and 1995. More than two thirds (71%) of the outbreaks were discovered due to clinical signs in the herd. Later Elbers et al. (2002) performed a similar retrospective study and quantified clinical signs as a diagnostic tool for the detection of CSF. These findings imply that the farmer or the veterinarian was alarmed by clinical signs only when they were evident and present in a larger number of pigs.

This may be the result of the education of veterinarians and farmers on CSF which traditionally describe the disease as peracute that should not be missed clinically. Only then pigs or blood samples were sent in for laboratory diagnosis. In the German study another 20% of the infected herds were identified by epidemiological tracing on and back. They were examined because contacts to CSF virus infected herds were evident, and in some cases pigs displaying clinical signs were already found at that time. However, in none of the latter cases clinical signs had been associated with CSF, nor had CSF been considered as a possible cause of disease. This confirms that in practice a few animals’ sick with CSF are usually overlooked, particularly in large holdings (Depner et al., 2007).
Lack of education and awareness

Despite some occasional CSF epidemics in Europe the infection has become rare during the last 20 years, and many countries and regions have not experienced outbreaks for a couple of decades. Thus there is a low awareness among farmers and veterinarians, and most often this is associated with a lack of knowledge about fundamental facts concerning CSF. The consequence has often been the late diagnosis of CSF outbreaks, in particular primary outbreaks. It is an important task of veterinary and agricultural colleges to promote in depth knowledge on dangerous notifiable diseases. In addition continuing education programmes should provide periodical updates for all stakeholders, and other factors facilitating the introduction of CSF must be minimised (Westergaard, 2008).

Low level of notification and submission of samples for CSF exclusion diagnosis

Whenever the official suspicion of CSF on a farm is raised a number of precautionary measures must be taken according to European and national legislations. This might be the reason for the reluctance of farmers and veterinarians to raise official suspicion, even when severe losses have already occurred on a farm and clinical signs indicate that there might be an outbreak of CSF. This attitude in combination with a limited knowledge and awareness had often led to a delay of notification of a CSF outbreak, contributing to the duration of the "high risk period" before the detection of primary outbreaks.

Monitoring and surveillance systems (MOSS)

Over the past decades, emerging and re-emerging diseases, combined with an intensified trade in animals and animal products have augmented the need of a vigilant and effective disease control. Disease monitoring and disease surveillance, allowing for a timely detection of changes in the prevalence of infectious diseases and the fast installation of control measures are thus of increasing importance to veterinary authorities and policy makers. Recently a possible technical solution for the problem was presented by the working group of Elbers (Crauwels et al., 2001).

An expert system including available knowledge, experience concerning CSF and its differential diagnosis has been established. Veterinarians visiting pig farms are connected to the system via handheld computers. Relevant information is entered by the veterinarian during the visit, and the system will react with appropriate advice to the veterinarian including sampling and diagnostic measures, e.g. to exclude CSF as a cause of diagnosed clinical disorders. This system together with production and mortality data (e.g. automatically provided by the rendering plants) could become at least on the veterinary practice side a technical countermeasure against lack of specific knowledge and awareness.

Passive and active data collection

For this section, the two activities monitoring and surveillance will be addressed by the widely accepted term MOSS (Monitoring and Surveillance System) (Doherr and Audigé, 2001; Stärk,1996). Depending on the methods used for data collection in the frame-work of a MOSS, one can classify the approach as being passive or active (Doherr and Audigé, 2001; Salman, 2003). Passive and active in this sense reflects the role of veterinary authorities for the program under consideration.
Passive data collection is based on the routine reporting of cases and events suspected of being caused by the investigated disease in the whole animal population. In the case of classical swine fever clinical symptoms, an elevated fatality in pig herds or routine post-mortem findings raised on abattoirs are examples for such trigger elements, which call for further investigation of the underlying cause (Elbers et al., 2002; Stärk et al., 2006). The advantage of cost-efficiency due to the use of existing networks (animal owners, veterinary practitioners, routine meat inspection on abattoirs) has to be weighed against possible shortcomings in reporting speed and quality. In general, passive MOSS tend to underestimate the true prevalence of disease (Doherr and Audigé, 2001; Salman et al., 2003; Klinkenberg et al., 2005). The degree of underestimation is dependent on the factors mentioned above and is difficult to assess.

Active data collection, in the framework of an active MOSS, follows a predefined sampling scheme, which gives more control to the investigator. Thus, studies can be designed in respect to the type of disease investigated, and to the exact objectives of the study, respectively. While surmounting some of the mentioned weaknesses of passive MOSS systems, the active approach is more costly, as sampling capacity and diagnostic screening have to be set up and initiated specifically for the particular program. The decision if this increased effort is counterbalanced by benefits in terms of e.g. an earlier or more reliable detection of an outbreak depends on factors inherent to the disease (e.g. contagiousness, socio-economic impact, animal welfare), and on the prevailing disease status in the respective area (Doherr and Audigé, 2001; Salman et al., 2003). In general, active surveillance systems may be better suitable than passive surveillance to estimate prevalences of a disease present in a population, but will hardly be suitable for the early detection of newly introduced diseases in a population (Crauwels et al., 1999). The sample size required to attain an adequate level of statistic confidence may render the complete system unfeasible regarding cost and diagnostic capacities (Cameron and Baldock, 1998a; Doherr and Audigé, 2001; Ziller et al., 2002; Martin et al., 2007a). This issue is important especially when the attempt is to detect a very low prevalence of disease or prove its absence, respectively. Furthermore, small herds may present a problem in surveillance for infectious animal diseases. The typical levels of within-herd design prevalence are not directly applicable. Therefore, the probability of detecting small herds cannot be improved by choosing a larger sample size within the herd (Greiner and Dekker, 2005). The probability of detection of infectious diseases in a country with a large number of small herds is further biased if the disease is limited to herds with a smaller herd size by e.g. lower bio security measures and monitoring efforts.

**Targeted or risk-based surveillance**

The terms ‘targeted’ or ‘risk-based surveillance’ imply that the sampling scheme aims at concentrating investigation efforts on specific animal population, according to the estimated probability, or risk, of these being affected by the disease. Provided that the risk factors were correctly identified and weighted, targeted surveillance yields a higher sensitivity and predictive value positive for a given sample volume than can be expected from randomly sampling across the whole population (Doherr and Audigé, 2001; Stärk et al., 2006).

**Surveillance to ensure freedom from disease**

If the objective of the MOSS is to ensure the “freedom of disease” for an area, defined as disease prevalence under a predefined threshold, different multi-stage sampling strategies may be considered to optimize the cost-benefit ratio of the survey. Basically, after randomly selecting holdings to be included in the survey in the first stage, the sampling process to determine the disease status within these holdings characterizes the strategy (Cameron and...
Baldock, 1998a; Cameron and Baldock, 1998b; Doherr and Audigé, 2001; Ziller et al., 2002; Martin et al., 2007a; Martin et al., 2007b):

a) Cluster-sample: all animals within the selected herds are tested;
b) Individual sample: the within-herd sample sizes are calculated individually for each herd, respecting herd parameters;
c) Limited sample: the same, pre-defined number of animals is tested in all selected herds

Depending on the statistical power needed, the distribution of herd sizes and the financial or logistical capacities of the survey, one of these strategies may be selected as the most suitable for the situation at hand (Ziller et al., 2002).

To achieve the primary objective of keeping the high-risk period as short as possible, surveys exclusively aimed at detecting infected animals / herds by means of randomly distributed serological or virological screening seem insufficient (Crauwels et al., 1999; de Vos et al., 2003; Klinkenberg et al., 2005). Consequently, pre warning programs, allowing to identify and assess the risk of introduction, and to respond adequately, should be considered. If a heightened risk is determined, early warning programs specifically targeting herds exposed to this risk can be enacted to ensure a timely detection of a possible introduction of the virus (Brouwer-Middelesch et al., 2008). To back-up such a system, routinely performed inspections of pig holdings, randomly distributed diagnostic sampling and slaughterhouse inspections may act as a safety net to ensure that no introduction of the virus was missed by the early warning program (Klinkenberg et al., 2005; Stärk et al., 2006; Brouwer-Middelesch et al., 2008). It is important, that MOSS including early warning systems are in place in countries that for decades have been free from the CSF and might consider themselves to be out of the risk. Nevertheless it should be recognized that the approaches mentioned above for disease freedom were applied to confined animals. None of the above approaches were demonstrated for their practicality in free ranging animals such as wild boars.

**Evaluation of MOSS**

When evaluating the quality of a MOSS, one has to bear in mind that each element of the system contributes to its overall performance. The initial detection of cases or events suggesting the occurrence of disease can be characterized by the sensitivity and specificity of the applied diagnostic measures (see Table 5). Apart from the efficiency of diagnosis of individual cases, the sampling strategy in terms of sample volume and distribution over space, time and population strata, as well as the methodology of collation, analysis and communication of generated data have to be considered (Doherr and Audigé, 2001; Salman, 2003; Buehler et al., 2004; Dato et al., 2004; Klinkenberg et al., 2005; Feliziani et al., 2005). Table 5 summarizes the requirements (type of sampling, design prevalence, and confidence) mentioned in the manual 2002/106/EC split by both the reason for sampling and type of holding. On this basis, the following parameters were calculated:

1. Conservative sample size was calculated without considering sensitivity and specificity of the available tests.
2. Corrected sample size, considering sensitivity and specificity of the test as well as the combined sensitivity and specificity in the case of fever measurement and rRT-PCR.
3. Influence of herd size on the corrected sample size on the basis of 50 and 1000 animals/farm respectively.

The corrected sample size in Table 5 is designed according to the following assumptions:

1. It is based on the test hypothesis of freedom of disease. This means it is assumed a priori probability that the number of true positives is zero i.e. no infection in the area.
2. It is designed in such a way that the number of positive test results should be zero as well, independently from any test properties.
(3) It is optimized in such a way that it provides at least one true positive test result as soon as the true prevalence is higher than the design prevalence. However, the apparent prevalence is a result of the addition of true and false positives. This leads to the apparent contradiction that a test with higher sensitivity and specificity might require a higher sample size than a test with lower specificity (as happens e.g. with serology vs. rRT-PCR).

Table 5. Evaluation of sampling protocols Left: Sampling protocols as mentioned in 2002/106/EC, (type of holding, type of sampling, design prevalence, and confidence level of at least 95%). Right: Evaluation of the sampling protocols (conservative sample size, sensitivity, specificity and corrected sample size)

<table>
<thead>
<tr>
<th>Reason</th>
<th>Type of holding</th>
<th>Type of sampling</th>
<th>Design prevalence</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Corrected sample size****</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A) Suspected holdings</td>
<td>Fattening pigs</td>
<td>FRT measurement + confirmation</td>
<td>10</td>
<td>95</td>
<td>99.9*** (99.9)</td>
<td>21 (22)</td>
</tr>
<tr>
<td></td>
<td>Breeding pigs</td>
<td>rRT-PCR</td>
<td>5</td>
<td>95</td>
<td>98</td>
<td>45 (65)</td>
</tr>
<tr>
<td></td>
<td>Semen collection centre</td>
<td></td>
<td>all animals</td>
<td>all animals</td>
<td>all animals</td>
<td>36 (43)</td>
</tr>
<tr>
<td>(B) Culling of confirmed cases</td>
<td>Fattening pigs</td>
<td>ELISA + VNT (rRT-PCR)</td>
<td>10</td>
<td>95</td>
<td>98</td>
<td>21 (22)</td>
</tr>
<tr>
<td></td>
<td>Breeding pigs</td>
<td></td>
<td>5</td>
<td>95</td>
<td>98</td>
<td>36 (43)</td>
</tr>
<tr>
<td></td>
<td>Semen collection centre</td>
<td></td>
<td>all animals</td>
<td>all animals</td>
<td>all animals</td>
<td>36 (43)</td>
</tr>
<tr>
<td>(C) Preventive culling</td>
<td>Fattening pigs</td>
<td>ELISA + VNT (rRT-PCR)</td>
<td>10</td>
<td>95</td>
<td>98</td>
<td>21 (22)</td>
</tr>
<tr>
<td></td>
<td>Breeding pigs</td>
<td></td>
<td>5</td>
<td>95</td>
<td>98</td>
<td>36 (43)</td>
</tr>
<tr>
<td></td>
<td>Semen collection centre</td>
<td></td>
<td>all animals</td>
<td>all animals</td>
<td>all animals</td>
<td>36 (43)</td>
</tr>
<tr>
<td>(D.2) Movement of pigs to another holding</td>
<td>Fattening pigs</td>
<td>FRT measurement + confirmation</td>
<td>10</td>
<td>95</td>
<td>99.9*** (99.9)</td>
<td>20 (22)</td>
</tr>
<tr>
<td></td>
<td>Breeding pigs</td>
<td>rRT-PCR</td>
<td>5</td>
<td>95</td>
<td>99.9*** (99.9)</td>
<td>45 (65)</td>
</tr>
<tr>
<td></td>
<td>Semen collection centre</td>
<td></td>
<td>all animals</td>
<td>all animals</td>
<td>all animals</td>
<td>36 (43)</td>
</tr>
<tr>
<td>(D.3 + D.4) Movement of pigs for slaughter</td>
<td>Fattening pigs</td>
<td>FRT measurement + confirmation</td>
<td>20</td>
<td>95</td>
<td>99.9*** (99.9)</td>
<td>14 (16)</td>
</tr>
<tr>
<td></td>
<td>Breeding pigs</td>
<td>rRT-PCR</td>
<td>5</td>
<td>95</td>
<td>99.9*** (99.9)</td>
<td>45 (103)</td>
</tr>
<tr>
<td></td>
<td>Semen collection centre</td>
<td></td>
<td>all animals</td>
<td>all animals</td>
<td>all animals</td>
<td>36 (43)</td>
</tr>
<tr>
<td>(E) Re-population of farms</td>
<td>Sentinel pigs + Breeding pigs</td>
<td>ELISA + VNT (rRT-PCR)</td>
<td>20</td>
<td>95</td>
<td>99.9*** (99.9)</td>
<td>12 (13)</td>
</tr>
<tr>
<td></td>
<td>Complete farm</td>
<td></td>
<td>10</td>
<td>95</td>
<td>99.9*** (99.9)</td>
<td>21 (22)</td>
</tr>
<tr>
<td>(F) Lifting protection zone</td>
<td>Fattening pigs</td>
<td>ELISA + VNT (rRT-PCR)</td>
<td>10</td>
<td>95</td>
<td>99.9*** (99.9)</td>
<td>21 (22)</td>
</tr>
<tr>
<td></td>
<td>Breeding pigs</td>
<td></td>
<td>5</td>
<td>95</td>
<td>99.9*** (99.9)</td>
<td>36 (43)</td>
</tr>
</tbody>
</table>

Project funded by the European Union
Technical Assistance for the Control and Eradication of Classical Swine Fever (CSF) and Rabies in Serbia

Project funded by the European Union

* Sample size calculations based on the tables of Cannon and Roe (1982) using the value for infinite population size without correcting for sensitivity and specificity as conservative upper limit

** Combined sensitivity (Ses) and specificity (Sps) of both methods fever measurement and rRT-PCR only on febrile animals were calculated using the following equations (Thrusfield, 2005). In practice, only febrile animals (test positive) are selected and subsequently tested with rRT-PCR. The sensitivity of the rRT-PCR testing of febrile animals was assumed to be 99.9% Ses = Se1 * Se2 Sps = Spe1 + Sp2 – (Spe1 * Sp2)

*** Sensitivity and specificity from Elbers et al. (2002)

**** Sample size calculations taking account for sensitivity and specificity of the test as well as herd size were done in a software written by FLI. The results were cross-checked with FreeCalc Software version 2 (Cameron and Baldock, 1998a) in cases FreeCalc determined the sample size using a threshold of 1. The theoretical base is a natural extension of the hypergeometric probability function by the parameters sensitivity and specificity into the probability product space. Because sensitivity, specificity, and the given prevalence are stochastic independent thus it is simple to derive a product probability function.

Therefore, it yields for exact k observed test-positive counts:

\[ f(k) = \sum_{i=\max(0,n-K)}^{\min(NI,0)} \binom{NI}{i} \binom{NN}{n-i} \left( \sum_{j=0}^{\min(k,NI+NN)} \binom{n}{j} \cdot (1-Se)^{k-j} \cdot (1-Sp)^{j-k} \cdot Se^{j} \cdot (1-Se)^{k-j} \cdot (1-Sp)^{j-k} \cdot (Sp)^{n-j-k-j} \right) \]

Whereas \( k \in \{0,\ldots, n\} \) and the five parameters

- NI = number of reality diseased subjects in the whole monitored population,
- NN = number of reality not diseased subjects in the whole monitored population,
- n = sample size,
- Se = sensitivity of the used test T,
- Sp = specificity of the used test T.

The true prevalence is given by \( p = \frac{NI}{NI + NN} \)

The results of Table 5 lead to the following assumptions:
(1) Raise in the body temperature is only a valuable tool if it is combined with subsequent rRTPCR on febrile pigs. It has to be taken into account that (a) vaccinated pigs usually do not develop fever even if they are infected and that (b) the prevalence of infected pigs in vaccinated premises is very low.

(2) The combination of serology and rRT-PCR (as it is required e.g. to move pigs to the slaughter house) does not require any changing in the conservative sample size (see tables of Cannon and Roe, 1982 using the value for infinite population size without correcting for sensitivity and specificity as conservative upper limit) in order to detect either a 5 or 10% design prevalence. However, if the combination of fever measurement and rRT-PCR is applied, the corrected sample size shows to be higher.

(3) In case of very low prevalences (as e.g. in vaccinated populations or begin of infection), the sample size increases significantly. This means that the resources for sampling and testing equally rise in a disproportional way.

(4) Herd size has crucial influence on the sampling size. Particularly in the case of low prevalences (e.g. 1 %) and relatively small herd sizes (e.g. 50 animals) even testing the whole herd with the given test properties and design prevalence does not allow attesting freedom of disease (Cameron and Baldock, 1998a; Greiner and Dekker, 2005). Nonetheless, if the epidemiological situation in the surrounding area and/or repeated testing is considered as well, more concrete conclusions concerning freedom of disease in the region might be drawn.

**Simulation of the efficiency of monitoring systems**

In order to demonstrate the effect of different herd size distribution as well as different prevalence on animal and herd level on design prevalence mentioned in Diagnostic Manual approved by Commission Decision 2002/106/EC a simulation study was conducted (see 3.4.1; Greiner and Dekker, 2005). The software allows for simulating a specified monitoring system within a definite population. It estimates the probability of successfully recognizing an existing infection at a certain time point.

The following parameters are needed for the calculations (the examples are specifically focused on the ToR):

1. **Simulation parameters:**
   - Number of simulation-cycles (= 1000)

2. **Population parameters:**
   - Number of herds in each herd-size category (herd size randomly distributed within the class; each class breaks is based on the recommendations of Huirne and Windhorst (2003) data from a region. In Germany with medium pig density; 639 herds and 67,707 pigs; i.e. on average 106 pigs/herd), in Romania with low pig density; 10,344 herds and 167,790 pigs; i.e. on average 16 pigs/herd)

3. **Disease parameters:**
   - Prevalence on herd-level and the prevalence within herds, (= 1 % infected herds, i.e. in the given example of Germany an average of 6 to 7 infected herds, and a 1 or 25 % within-herd prevalence)

4. **Monitoring parameters:**
   - All herds were tested (adoption to the ToR) and two different within-herd sampling strategies were applied (Ziller et al., 2002):
     - Sampling of the entire animals in the herd (cluster sampling) in order to describe the influence of test properties without considering the effect of sampling.
     - Number of animals sampled based on the conservative sample size for 5 and 10 % individual design prevalence in the herd in order to move fattening and breeding pigs for slaughter (2002/106/EC) (limited sampling; see Table 5).

5. **Test parameters:**

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*Project funded by the European Union*
Sensitivity and specificity of the entire diagnostic procedure (= rRT-PCR as an example; Se = 98.5; Sp = 99.9; (Table 5).

In each simulation run the program marks infected individuals and chooses an appropriate sample according to the input parameters. Thereafter it stores the decision whether the disease is recognized by the monitoring or not, and in addition the numbers of false and true positive and negative test-results.

By means of a simulated exemplary population structure and disease distribution, the study demonstrates (see Table 6) with illustrative numbers the crucial statements mentioned in The combination of limited sampling (i.e. the same, pre-defined number of animals is tested in all selected herds), conservative sample size and the given population structure allows even at the average of 1000 simulation cycles the detection of CSF with a within-herd prevalence of 1 % using a designed prevalence of 5 and 10 %. Because of the low average herd size and high number of herds in Romania (as an example for countries with a higher number of backyard pigs), the sample size, false and true test-results increases significantly using the same disease, monitoring and test parameters. This was also mentioned by Bergevoet et al. (2007).
Ecology of wild boar

Distribution and population size

Wild boars and pigs are species *Suis scrofa*

Wild boar and domestic pig are members of the same species *Suis scrofa* and share thus the same susceptibility to pathogens. Wild boars are native wild mammals in Europe in rare occasions they can mate with the domestic pig, and produce fertile cross-bred. Theoretically domestic pigs can also become feral as it occurs in the USA but this situation is no more observed in Europe and will be thus not treated in the present document. This report is thus only concerned with uncontrolled populations of free-ranging wild boar.

Wild boar population in Serbia

Wild boars are widespread in the Country, although in limited geographical areas and at a very low density. According to the available data, the density of wild boar population in Serbia ranges from 0.2/km² to a maximum of 1.38/km². It is not clear whether this density refers to the whole territory or to the agricultural-forest lands only. There are some 300 hunting grounds in the Country and their surface ranges from 2000 to 100.000 hectares. Most of hunting grounds are managed by public enterprises, which have their own employees. In Serbia two Hunting Associations operate. There are four National Parks in which hunting is allowed. Five hunting grounds are managed directly by the Army.

Each Hunting ground implements a 10 years management plan, which foresees a stability of the wild boar population density and size. A specific Inspectorate of the Ministry of Forest is in charge to organize these censuses.

Census of wild boars in Serbia is carried out by volunteers. Wild boars are driven to the advantage points and counted. Data obtained by this census method are validated by the local hunting enterprise employees thorough direct observation of animals and other indirect sings of presence (agricultural damages etc.). Demographic data are not collected or utilized to validate population size estimates.

As a result of this census totally about 20.000 wild boars are estimated for the whole Serbia. This number is quite low in comparison to the usual densities recorded in western European countries that experienced the CSF (Germany, France, and Italy) where densities can easily reach 4-6 animals for Km².

Hunting bag is a specific function of the census since the number of wild boars to be shot is decided on hunting bag bases. As in many parts of Central Europe, wild boar populations are considered to double each year, so that the hunting bag tends to be half of the censused/estimated population and thus to maintain a stable wild boar population. Serbia tried to apply the same strategy. However the number of wild boars officially hunted during the previous years, are quite below the threshold of 10.000 (approximately 7.000). If so the
wild boar population is likely to have increased during the past 10 years even if this increasing is not visible from the official figures.

Beside the 300 hunting grounds, there are 16 fenced hunting areas in Serbia with approximately 2935 animals in these fenced areas, where each year the young animals are vaccinated against CSF with vaccines prepared with C Strain. Animals are caught using specific traps and then injected with vaccine. The number of vaccinated animals is not clear. There is not post vaccination monitoring to follow up the vaccination coverage neither at population level (percentage of immunized animals) nor at the individual level (Ab quantitative presence). Until recently, some of those animals were used for restocking of other hunting areas. At present this management tool is not practised any more, due to the low effectiveness of restocking and the problem of releasing antibody positive animals in areas where vaccination is not performed. The vaccination in the fenced areas is performed twice a year: in spring (April-May) and in Autumn (November-December).

In the fenced areas hunting is mainly done by driving whereas in the other hunting grounds by individual selection of the animal to be shot and the hunter is accompanied by one employee of the enterprise owning the hunting rights.

In the forest a large number of foxes and jackals are present together with a very limited number of brown bears and wolves.

**Wild boar population are expanding**

Wild boar is a ubiquitous specie that populates most of the European forests, even in wetlands or mountainous areas (Baubet, 1998; Acevedo et al., 2006). The size and range of European populations have critically increased over the last 30 years, possibly due to changes in the practice of hunting, to the expansion of single-crop farming and to climate warming; This development of wild boar population had increased also the risk of maintaining diseases in the wild and the risk of inter-transmission between wild boar and pigs (particularly in open-air farm) or other species including livestock and Man (Hars et al., 2004; Acedevo et al., 2006).

**How to estimate the number of wild boars?**

Due to their nocturnal behaviour and forested habitat, there is no simple way to estimate accurately the population size. The only validated method to estimate the number of wild boar is to practice capture-mark-recapture on small areas during at least 2-3 years, which is time consuming, costly, not available forthwith, and not adapted to the monitoring of large areas (Hebeisen 2007). Alternatively in large areas (>100km²) the hunting bag is considered as a relative index of the population size or density (and the method for estimation in some MS – Annex A, figure 5); but this maybe highly biased depending on the local hunting pressure. When hunting pressure has been estimated in some reference sites raw approximation of population size maybe proposed: for example in the North-East of France and Northern Italy hunting pressure is assumed to be c.a. 0.45-0.50 (Monaco et al., 2003; ONCFS, 2004), so that the population is evaluated as double of hunting bag number.. The structure of hunting bags and the consequent implications for CSF surveillance in wild boar will be discussed.

**Social and spatial structure of wild boar populations**
Wild boar are socially structured

Wild boar is a highly social species. According to the teeth eruption, individuals may be classified into 4 age classes: less than 6 months, so called “piglets”, 6 to 14 months, so called “juveniles”, 14 to 24 months so called “sub adults”, and up to 24 months so called “adults” (Matschke, 1967; Monaco et al., 2003; ONCFS, 2004). Females, piglets and juveniles live inside cohesive social groups comprised of females and their offspring of the current year. Females may leave or enter the group when becoming subadults; subadult’s males unavoidably leave the matriarchal group and often disperse less than 10km from their native area (ONCFS 2004). This social structure is considered as stable (Kaminski et al., 2005; Heibeisen, 2007), and due to this social structure contacts are supposed more likely intra than inter-groups. Due to the polygynous mating system of the species males are at risk to transmit infection between groups during rutting. Then, the artificial feeding of wild boar is widely practiced in Europe; which may favour transmission by generating the aggregation of different social groups (Vicente et al., 2005). Not all MS countries used collected demography data to updated animals that can be shot in spite of plans to reduce the wild boar population size.

Wild boars are territorial

Matriarchal social groups are known to live on a diurnal home-range that may vary from 150 to more than 2000 ha (~500ha in average); adult males are roaming around matriarchal groups and often inhabit over larger areas (1000-2000ha in average) (Baubet, 1998; Fisher et al., 2004, Keuling et al., 2008a; Sodeikat and Pohlmeyer, 2003). Home-range area may vary according to food availability, landscape structure and hunting practice, anyway wild boar is mostly a sedentary species with a short native-dispersal distance (<10Km). Exceptionally, some longdistance movement may occur, particularly when big dogs are used in drive hunt (Maillard and Fournier, 1995; Brandt et al., 2005). The use of space is driven by the availability of food and resting places so that contacts and thus CSF transmission occur mainly in forested areas. Fenced motorways constitute barriers that may be sporadically crossed by wild boar, especially across bridges (Vassant et al., 1993; Vignon et al., 2002; Dobias and Gleich, 2007); the probability wild boar crosses motorways might increase during drive hunt (Vassant et al., 1993; Vignon et al., 2002).

Wild boar population dynamics

Births

Basically most of reproducing females are more than one year old, piglets are always nonbreeding individuals and 30% to more than 60% juvenile females may reproduce depending on food availability (Monaco et al., 2003; ONCFS, 2004; Servanty, 2007; Gethöffer et al., 2007; Cellina 2008). Wild boar sows produce in average 4 to 7 piglets per year depending on their age, their body mass and food availability (Monaco et al., 2003; Servanty, 2007; Gethöffer et al., 2007). The number of wild boar generally doubles and may even triple when exceptional oak mast production occurs (Servanty, 2007). A considerable cause for increased wild boar populations may be the improvement of food supply by agricultural crops. For example maize is the most important item of the vegetarian food category consumed by wild boars (Schley and Roper, 2003).
The peak of births occurs mainly in March and April but may occur earlier when an important oak mast production occurs (Mauget, 1982; Dardaillon, 1988; ONCFS, 2004; Hohmann, 2005). Artificial feeding has not a demonstrated effect on reproduction, except in very poor environment. Births may be distributed from January to September depending on the place, the year and the age structure of the population. European wild boar populations show a prolonged mating and delivering seasons often occurring for several months (January-September). When natural food availability is high the farrowing period tends to become larger (Servanty, 2007). Such wide distribution of births may participate in the persistence of CSF because birth provide new susceptible during a large part of the year.

Natural survival, hunting and turnover

Among all age classes the natural survival is around 0.7-0.8/year (ONCFS, 2004; Focardi et al., in press; Toigo et al., 2008; Hebeisen, 2007); but a part of natural survival wild boar are often intensively hunted: the probability to be shot during hunting may reach more than 0.5 in intensively exploited population (ONCFS, 2004), which generates an important turnover of the population (a new generation every 2.2 years even less than 2 years) and favour a large sample size into wild population (sampling aspects will be detailed in the paragraph dedicated to CSF surveillance in wild boar). As a consequence “herd immunity” is expected to quickly decrease in infected and non-vaccinated populations, which may participate to the re-emergence of infection. intensively hunted: the probability to be shot during hunting may reach more than 0.5 in intensively exploited population (ONCFS, 2004), which generates an important turnover of the population (a new generation every 2.2 years even less than 2 years) and favour a large sample size into wild population (sampling aspects will be detailed in the paragraph dedicated to CSF surveillance in wild boar). As a consequence “herd immunity” is expected to quickly decrease in infected and non-vaccinated populations, which may participate to the re-emergence of infection.

Epidemiology of CSF in wild boars

The role of the wild boar in the classical swine fever (CSF) problematic is primarily of epidemiological interest since wild boar are regarded as a reservoir for CSF virus and as possible source of infection for domestic pigs. Therefore the main aims of controlling CSF in wild boar are to reduce the risk of transmission of the disease to domestic pigs, to prevent it becoming endemic, or to reduce the duration of the endemic phase, and finally to eradicate the disease in wild boar. These goals may be achieved by several measures including hunting as an attempted to reduce the wild boar population and/or vaccination of wild boar to increase the overall immunity of the population.

Principally, CSF virus can persist in a wild boar population only when there is a viraemic animal which transmits the virus to at least one further susceptible wild boar (R>1). When analysing the epidemiology of CSF in wild boar the following three interacting complexes have to be considered: (i) the biology of the wild boar population (e.g. age structure of the population, reproduction rate, carrying capacity of the habitat, etc.), (ii) the disease biology (e.g. course of the infection, immunity, mortality, virulence of the virus, etc.) and (iii) the human interference (e.g. feeding, hunting, vaccination, agriculture). However, monitoring and understanding a disease in an open ecosystem is rather a complex exercise because several parameters of interest e.g. the population structure and dynamics, the population size or the herd immunity remain unknown or can only be roughly estimated due to permanent changes within the population.

While the disease will fade out in small wild boar populations (between 1 000 and 1 500) it may become endemic in larger populations (>2 000) and may persist for several years in
areas with a high wild boar density. The persistence of CSF depends on epidemiological and ecological factors such as the proportion of animals that recover from infection, the occurrence of chronic infections, as well as the social structure and size of the population. Wild boar obviously cannot be managed like domestic pigs, i.e. using exhaustive culling or a conventional vaccination strategy, as individual handling is impossible, and wild boar populations are highly dynamic (i.e. producing new susceptible animals). However, hunting and vaccination can be used to stop transmission by reducing the number of susceptible animals, though inadequate hunting or inappropriate vaccine strategies may reinforce CSF persistence.

Council Directive 2001/89/EC of 23 October 2001 on Community measures for the control of classical swine fever introduces minimum Community measures for the control of the disease. It lays down the measures to be taken in the event of a CSF outbreak. Those measures include plans by Member States for the eradication of CSF from a wild boar population and emergency vaccination of wild boar under certain conditions. Monitoring and sampling procedures in areas where CSF occurs in wild boar are set out in the Diagnostic manual for CSF (Commission Decision 2002/106/EC2). The objective of this chapter is to provide guidance to the VD as regards different options for controlling the disease, including vaccination of wild boar and hunting measures. The guidelines are based on:

- the requirements of Articles 15 and 16 of Directive 2001/89/EC;
- Chapter IV, (H) of the Annex of Commission Decision 2002/106
- The EFSA Scientific Opinion of the Panel on AHAW on a request from Commission on ‘Control and eradication of Classic Swine Fever in wild boar’.

Current distribution of CSF in wild boar (Europe)

The first attempt to map CSF at the European level was provided in review papers at the beginning of the 2000s (Laddomada, 2000; Artois et al., 2002). Over the last five years (2003-2007), CSF has been reported in the EU in Germany, France, Luxembourg, Belgium, Slovakia, Romania, Bulgaria and a lot of other European states such as the Balkan states and Russia. The surveillance efforts implemented by each country can affect the detailed knowledge on outbreaks in wild boar. Regardless, CSF appears yet as widespread among wild boar populations of the European continent. Outbreaks seem to be clustered according to subpopulations, depending on landscape constraints such as the presence of forests and physical barriers (motorways and rivers for example) as observed in Germany and France (Figure 3).

Origin of infection in wild boar

The origin of infection is generally difficult to determine and control in wild populations. Direct contact between wild boar and pigs may occur in very particular situation when semiwild or back-yard pigs are sharing the same territory with wild boar: for example this was likely in Sardinia and in Romania (Laddomada et al., 1994). Then indirect transmission, mainly caused by the release of contaminated meat product in the environment is likely to have been the cause of disease emergence in many areas (Aubert et al., 1994; Artois et al., 2002). More frequently, at least in Western Europe over the last 10 years, outbreaks seemed to reemerge and spread from endemically infected areas; the isolation of previously isolated strains give support to this hypothesis: for example the Uelzen-like strain isolated in Vosges and Palatinate in the 2000’s was very similar to the one isolated 10 years before in the same area (Louguet et al., 2005).
Risk factors

The probability of being infected is higher in young animals that are found dead, especially when disease is emerging, i.e. the first year of outbreak (Kern et al., 1999; Rossi et al., 2005a; Roic et al., 2007; von Rüden et al., 2008). These observations suggest a lethal effect of the virus with higher susceptibility to this virus among young wild boar (Kaden et al., 2006b). Piglets are supposed to be the main reservoir of infection given they have a higher probability to be infected, they represent the more abundant and susceptible (not immune) class in wild boar population, and because some are likely to be permanently infected (Depner et al., 1995a; Kern et al., 1999; von Rüden et al., 2008). Both juveniles and adult individuals might then be long term virus shedders (chronic infections well known from experiments, but not demonstrated in nature) and facilitate the infection chain. Furthermore, subadult and adult individual having more social interactions during dispersal or during rutting may play an important role in the persistence of the virus by ensuring the transmission between social groups (Rossi et al., 2005a; Rossi et al., in press).

How many susceptible wild boars are needed for starting the infection?

- Italy-Suisse = 0.58/km²
- MWP (Germany) = 0.97/km²
- Sardinia = 0.8/km²
- Luxembourg = 1.12/km²

Some risk factors linked with the presence of CSF in wild boars are relatively known and even if there is a lacking of specific studies in Serbia it is possible to make prediction.

One of the most important risk factor for the presence of CSF virus is represented by the wild boar population size and/or density. According to this risk factor the most likely area in which the virus should/might be present is the area bordering Croatia and Hungary (Sombor, Apatin, Odzaci) in which the wild boar density is the highest of Serbia.

High wild boar densities are registered in the fenced hunting grounds. In these areas the density of wild boars is the highest registered in the Country. All animals should be vaccinated but since only caught animals are vaccinated it is not possible understand the level of herd immunity and thus the risk of these high density areas.

A possible risk factor is represented by infected free ranging or back yard pigs. When domestic animals belonging to the low biosecurity sector are sympatric with wild boars the so called ping pong exchange of the virus that will promote the environmental long lasting of the virus.

How many wild boars are needed for maintaining the infection in population?

<table>
<thead>
<tr>
<th>Area</th>
<th>R0</th>
<th>Nt</th>
<th>CCS/year</th>
<th>CCS/long persistence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Germany</td>
<td>6,3</td>
<td>0.58/km²</td>
<td>1800</td>
<td>4000-6000</td>
</tr>
<tr>
<td>Italy-Suisse</td>
<td>4,7</td>
<td>0.97/km²</td>
<td>1000</td>
<td>3000-4000</td>
</tr>
<tr>
<td>Luxembourg</td>
<td>14,5</td>
<td>1.12/km²</td>
<td>2300</td>
<td>6000-8000</td>
</tr>
</tbody>
</table>
The density (> 1-2 Km2), the size (>4-6000) and the geographical distribution (>1000 Km2) of the infected wild boar population are all compatible with a long lasting of the virus and vaccination could be considered.

### Disease evolution observed in past outbreaks

#### Geographical dissemination

The disease does not seem highly contagious as the spreading is generally slow; this is possibly due to the strain moderate virulence and to the sedentary and social behaviour of wild boar (Artois et al., 2002). Nevertheless CSF spreading seems quite unavoidable over forested and connected habitat, i.e. continuous forests, whatever the density of wild boar (Rossi et al., 2005a). The occurrence of open field may slow and even stop disease front, possibly due to a decrease of wild boar density among non-forested areas and a consecutive decrease of contacts between animals (Rossi et al., 2005a). Barriers such as the fenced motorways and large rivers lakes and low density areas seem able to stop disease spreading (Schnyder et al., 2002; Rossi et al., 2005b).

#### Epidemic phase and persistence

Until the 1990’s CSF has been considered as a self-limiting disease in the wild, fading out after the infection has spread through the whole population (Nettles et al., 1989; Hone et al., 1992). But the long-term monitoring of CSF performed in the 1990’s and 2000’s have demonstrated that the virus may persist for years in wild populations (Kern et al., 1999; Laddomada 2000; Artois 2002, Rossi et al., 2005a).

- At first, infection dynamics behave as epidemic (epidemic or invasion phase): while the disease is spreading geographically, at a local level such as the municipality, the proportion of infected increases (the year of disease emergence) to a peak and then decreases; the proportion of immune animal increasing afterwards (Rossi et al., 2005b).
- Then the dynamic of infection may enter a second and more complex phase (endemic/persistence phase) when disease persists from year to year between generations. During that 2nd phase the proportion of infected decreases slowly until it fades out or is not detected. In parallel the host populations quickly compensated the mortality induced by the epizootic and the seroprevalence (of non vaccinated populations) rapidly decreases due to the turnover of the population (Laddomada et al., 1994; Rossi et al., 2005b). Due to the lack of time resolution of the data collected by the EFSA questionnaire, it was not possible to cross validate the decrease in seroprevalence. In some cases apparent disease re-emergence is supposed to be rather related to a secondary epidemic following a silent phase with continued persistence but incidence rates very low making the disease easily overlooked by systematic sampling (Rossi et al., 2005a).

The mechanisms for persistence are difficult to observe in the field. One obvious feature is the correlation between persistence and the population size, i.e. not only density but also the dimension of the population driven by the forested habitat (Figure 4) (Rossi et al., 2005a). In particular population under 1500-2000 wild boar seem to have been infected less than one year and have experienced only an epidemic phase, while above this number persistence occurred over several years (Figure 4, Rossi et al., 2005a). On the contrary there are no field data regarding the possible susceptible wild boar density at which the infection fade out through a density dependent mechanism. o At first, infection dynamics behave as epidemic...
(epidemic or invasion phase): while the disease is spreading geographically, at a local level such as the municipality, the proportion of infected increases (the year of disease emergence) to a peak and then decreases; the proportion of immune animal increasing afterwards (Rossi et al., 2005b).

Mechanisms of transmission and of persistence

What are the supposed mechanisms of transmission?
The social and spatial structure of wild boar populations requires both within group and between group transmissions. The strong within group rate of contact will increase any infection spread whereas the rate of contact between different groups is limited. In such context the infection is likely to spread faster within group rather than between groups. Thus the survival of the infection is mainly linked to the between groups rate of contact. Within social groups, the virus is transmitted by direct and indirect contact, especially between piglets. Between social groups indirect contacts with contaminated excretions and carcasses might contribute to the spread of CSF, as the virus survives in the environment under certain conditions for several days or even weeks (Edwards, 2000; Ribbens et al., 2004a,b; Dewulf et al., 2002b). Transmission between groups during the rutting season can be due to direct contacts of male dispersers or at establishment of new social groups (Kaden, 1999b).

What are the supposed mechanisms of persistence?

Definition of long-term persistence

Understanding the reasons why CSF might persist in natural populations will be important to plan and judge control effort. Long-term persistence is defined as an endemic, recurrent infection within a closed, spatially restricted population. Following introduction the CSFV successively spreads through the area covered by this population removing large proportions of susceptibles (epidemic phase). Although the spread-through might take some time depending on the extent of the area this is not persistence (see MVP time-lines). Long-term persistence of CSF will be observed if, further on, certain mechanisms allow for the re-infection of new born susceptibles in former affected parts of the area (endemic phase). Hence, long-term persistence of CSF must be qualified by time after introduction in conjunction with the extent of the affected population or when recurrent outbreaks are observed inside parts of the areas that already had been affected.

Analysis of the mechanisms

The common rationale of the explanations is building a bridge in time or over distance from the primary outbreak to new born susceptibles. By such linkage consecutive outbreaks are possible without external introductions allowing the virus to survive during the annual break of the natality (in general observed during October-December). Proposed mechanisms relate to host and virus characteristics (Kramer-Schadt et al., 2007):

- Regarding the host: be it a large number of individuals via spatially extent populations (Artois et al., 2002; Rossi et al., 2005a) or high local density associated with a large number of susceptible individuals at a local level (Guberti et al., 1998), having a long birth season, providing fully susceptible individuals after disappearance.
of maternal antibodies, will enhance the probability of virus transmission between generations.

- Regarding the **virus**: the dominance of moderate outcomes of infections ("moderate virulence", Meyers and Thiel 1996), prenatally infected offspring *i.e.* late-onset (Kern et al., 1999), or piglets partially protected by maternal derived antibodies (Depner et al., 2000) are supposed to favour long-term persistence.

Kramer-Schadt et al. (2008) revealed by a formal system analysis the dominance of two mechanisms: the moderate virulence hypothesis; and the extent of the area inhabited by the infected population. All the three other hypotheses were found of limited value to generally explain persistence (Kramer-Schadt et al., 2008).

- The **moderate virulence hypothesis** refers to a higher proportion of transient infections, few acute but rather chronic infections lasting longer than 4 weeks before dying. Such mild outbreaks resulted most often in long-term persistence (Kramer-Schadt et al., 2008). The mild outcome might be due to a combination of virus characteristics and host population conditions. In detail, mild outbreaks cause more transient courses which occur more frequent in older age classes, and hence a sufficient survival though immunity is guaranteed for the reproductive pool. The remaining proportion of individual infections will run lethal but again the assumed mild outbreak relates to few sudden deaths but some chronic courses. It is assumed, that chronics might be infectious for months these rare cases can bridge the temporal gap between last peak of infection and the new generation. This assumption is based on limited experimental data in domestic pigs and wild boars (Depner et al., 1995a).

- The second most important hypothesis was **extent of the population** which allows the persistence of the virus in some part of a large area even though the persistence is not achieved at a local level (Bolker and Grenfell 1996; Rossi et al., 2005a; Figure 4). The mechanism behind this explanation is that of repeated chance: the larger a population stretches the more often rare chance events could happen (such as long-term shedding chronics that bridge time until reproduction sets on). Possibly, the social structure interacts also with the dynamics of CSF in large populations by enhancing the probability of virus persistence, some groups remaining susceptible to the virus and allowing the persistence of CSF transmission (Kramer-Schadt, 2007).
EU procedure for collection of data on CSF in wild boar

In order to reply to the first ToR of the mandate, the working group (WG) decided to search for data on the EU wild boar population, recent/current CSF outbreaks and control measures applied, including vaccination and hunting practises. It was proposed to collect that data through a questionnaire to be distributed to all MS and also to extract that information from the CSF EU database. Data from published articles and from experts’ experience were also included whenever necessary.

Control measures applied to CSF in wild boars

Different degrees of CSF control may be required in wild populations depending on the CSF status of a country/region, on the pig and wild boar population, and on the pig trade.

Prevention of disease emergence and spread among wild populations

First of all, to limit the spreading of wild outbreak looks an essential aim, especially for counties/regions that are not yet infected and were the wild boar population is large enough to allow long-term persistence. To prevent disease emergence through the contacts (direct or meat) between pig and wild boars may be attempted through few tools: the education of hunters and farmers regarding swill feeding in forest and evisceration, the control of swill feeding in forest, electric fences for open-air farming that will avoid physical contacts between wild and domestic animals. Then to stop the natural spread of the disease among wild populations is a more complicated issue that may be attempted using a preventive vaccination and/or measures that may limit animal movements and aggregation: hunting.
restrictions, close game pathway crossing barriers, limit the use of feeding grounds (out of vaccination periods).

**Reduction of the risk of transmission from wild boar to the domestic pig**

To prevent inter-transmission between pig and wild boars may be attempted through the education of hunters and farmers regarding swill feeding in farms, the control of swill feeding in pig farms, the systematic control of wild boar carcasses in infected areas, the compulsory use of electric fences for open-air farming that will avoid physical contacts between wild and domestic animals. In Germany it has been observed that about 60% of outbreaks registered in domestic pigs are secondary outbreaks derived from endemic persistence of CSF in sympatric wild boar populations (Fritzemeier et al., 2000). The first step to lower the risk of transmission of the CSF virus from wild boar to domestic pigs is to ensure the biosecurity level.

In infected areas biosecurity procedures should be addressed in preventing the possible CSF virus spread through infected hunted wild boars:

- Cadavers should be collected in individual separate bags in order to avoid contamination of uninfected cadavers through infected blood;
- Individual animals should be dressed in specific premises (previously designated) and offal should be carefully collected and eliminated safely. Offal should never discharge in the hunting ground;
- Designated premises should be furnished with tap water and electricity. Freezers are also needed for the storage of the dressed carcasses;
- Until the negative laboratory test is obtained, animals should not be removed from the designated premises;
- The number of cars and persons allowed to enter in the yard and/or inside the premises should be reduced as much as possible. Cars should be disinfected and persons should use PPE to avoid contamination.

Together with the above biosecurity measures other preventive actions can be taken in organising the hunting:

- Local hunters only should be allowed to hunt in infected areas; in any case wild boar meat should never be permitted to be transported outside the infected areas since contamination is likely to occur due to the hunting system and habits.
- Cars should be used in paved roads only; only one designated car should transport hunted animals.
- Wild boar carcasses retrieved in the forest/ground should be removed and conveniently destroyed;
- Feeding animals should be forbidden.
- In the infected areas hunters should receive a refreshment course before any hunting season; in particular hunters employed in the pig production chain should be strongly advised on the risk of CSF transmission to pig farms.
- Poaching should be strongly reduced when present.

Then, every control measure that will decrease the proportion of infected wild boar will also decrease the risk of transmission to the domestic pig. This may be achieved *a priori* using both vaccination and hunting measures to decrease the number of susceptible wild boar in the population. The eradication of disease in wild boar is not necessarily required for the protection of the domestic pig depending on the segregation of both populations and efficacy of swill feeding control.
Technical Assistance for the Control and Eradication of Classical Swine Fever (CSF) and Rabies in Serbia

General provisions in case of suspicion and confirmation of CSF in wild boars

Veterinary Directorate has to prepare a written plan of measures to eradicate CSF from a defined infected area after confirmation of a primary case. The plan must contain information on monitoring measures to be enforced after a period of at least 12 months has elapsed from the date of the last confirmed case. These monitoring measures must be maintained for at least 12 months.

Appropriate control and eradication measures have to be decided and applied in an infected area. These may include suspension of hunting and a ban in feeding wild boar. All wild boar shot or found dead in the defined infected area have to be inspected by an official veterinarian and examined for CSF in accordance with the diagnostic manual. Parts not intended for human consumption and carcasses of all animals found positive have to be processed under official supervision.

In accordance with Article 4(1)(a)(v) of Regulation (EC) No 1774/2002 of the European Parliament and of the Council of 3 October 2002 laying down health rules concerning animal byproducts not intended for human consumption, all body parts, including hides and skins, of wild animals, when suspected of being infected with diseases, are classified as Category 1 material.

Such material is to be disposed of or processed in accordance with Article 4(2) of that Regulation. Accordingly, viscera and other parts of wild boar shot or found dead in the areas listed in the Annex to Decision 2008/855/EC, and suspected of being infected with classical swine fever, are to be disposed of or processed in accordance with Article 4(2) of Regulation (EC) No 1774/2002.

Control and eradication of CSF in wild boar populations

Given that the disease in wild boar is a threat for the domestic pigs, eradication of wild reservoir is a declared objective in the EU and we will particularly focus on this ultimate degree of control. To eradicate infection may be theoretically achieved by decreasing the number of susceptible individuals in the population under a threshold level that decrease the probability of the virus to survive.

Both vaccination and hunting limit the spread of the infection through a pure density dependent mechanism. Reducing the number of susceptible wild boar also will reduce the probability that an infected individual will come in contact with a susceptible one. Hunting promotes this mechanism through a direct reduction of immune and susceptible animals whereas vaccination will reduce susceptible individuals only.

In practice eradication of CSF in wild populations is the more complicated control issue because wild animals cannot be managed as domestic pigs by exhaustive culling and vaccination, because population dynamics is complex and reacting to the hunting pressure, and because intrinsic factors drive the persistence of CSF that are the occurrence of mild infections and the dimension of the population.

The applicable tools are:
- **hunting** that theoretically allow to modify the population size, its growth rate and age structure, and that practically is by itself an important socio-economic issue practiced by amateurs.
- **oral vaccination** that allow to reach and maintain maximum herd immunity, but that cannot be exhaustive or homogeneous in the wild and is performed by hunters on feeding grounds.
Hunting

Given that CSF virus transmission theoretically depends on the number of susceptible wild boar, and that hunting can reduce the population (after births) by half per year, one could draw the conclusion that hunting is a simple and direct way to manage the number of animals and eradicate CSF. However, there is little evidence that hunting is an efficient disease management tool. This may be because hunting has a complex effect on population dynamics, depending on the age and sex of the animals targeted. Below are the theoretical effects of two possible targeted hunting scenarios:

Targeting mainly young wild boar (under a year old) is assumed to decrease temporarily the number of susceptible animals. However, harvesting juveniles may leave enough breeding females to maintain a high birth rate, yielding susceptible animals that enable CSF to persist. It has been shown that even if hunting rules are implemented, the result remains far from the goal of reducing the number of juveniles by 85%; the figure achieved was usually closer to 50%.

Alternatively, targeting breeding females would decrease the population long-term. However, it might temporarily increase the turnover of the population, providing ideal conditions for CSF to spread further. This may be particularly critical in dense populations that ‘react’ by flexibly increasing their breeding capacity (density-dependence). Thus, the use of targeted hunting to control CSF is not a simple issue and may even generate the opposite effect.

Intensified, non-discriminatory hunting has never been shown to be efficient in controlling or eradicating CSF, other than in very small and geographically isolated wild boar populations. The main problem is understanding the complex population dynamics of wild boar groups, and devising hunting schemes that are practical, while achieving the desired result from the point of view of CSF epidemiology as regards high-risk animals. Hunting alone is not sufficient to cut the virus transmission chain; it may indeed favour perpetuation of the virus. To summarise, focusing hunting on high-risk classes by age (juvenile) or sex (breeding female) has not proved feasible. Targeting the immune or less susceptible subpopulation by removing adult wild boars (especially if combined with vaccination measures) did not accomplish the aim of fully eradicating the disease either.

The low efficiency of hunting for CSF control is mainly due to:
- increase in turnover;
- non-achievable hunting intensity required in field situations;
- short impact rather than sustainable effects;
- different (even sometimes counteracting) purposes of hunting and disease control, though
- animals need to be hunted for sampling purposes.

There is insufficient scientific knowledge to assess the effect of hunting on the spread of CSF, but according to the above model (simulation of a CSF epidemic in a wild boar population and possible outcomes regarding vaccination):
- absent of hunting doesn’t produce significant changes in virus persistence or spread;
- normal hunting (reaching 45% of the population) also does not produce significant changes in virus persistence or spread;
- a small increase in hunting rates (<60 %) can promote virus persistence and spread;
- very high, impractical, hunting rates > 70-80 % would reduce virus spread significantly, but result in local extinction of wild boar.
It is worth mention that a simple reduction of the population size is not the definitive goal for the eradication program; a specific level of depopulation is needed to reach the wild boar threshold density at which the infections fade out. Usually the threshold density is well below to the actual densities recorded for the wild boar in most of European Countries. Several authors hypothesized that hunting may enhance wild boar movements and the geographical spreading of CSF (Laddomada, 2000; Artois et al., 2002; Schnyder et al.; 2002). Anyway this hypothesis has not yet been fully demonstrated because no study has studied specifically the effect of hunting on disease spread. What is observed in some circumstances is that drive hunt may enlarge the home-range (not in every occasion; Keuling, 2008b) of wild boar and may favour their transit across motorways.

In huge forested areas (green corridors) with no barriers or open fields stopping of drive hunts did not prevent the spreading of the virus (Rossi et al., 2005b; Pol et al., 2008). Restriction of hunting had been implemented in the field in small areas relatively isolated by physical barriers such as the Ticino region (Switzerland, part of the outbreak starting from Varese) and more recently in the Thionville region (France, part of the outbreak starting from Eifel) with some evidence of success (Schnyder et al., 2002; Pol et al., 2008). Until now there are not recognised hunting methods able to prevent the possible spread of wild boars. Hunting approaches have never been considered in a large scale system to prevent the movement of infected wild boars from a country to another one through borders (Alban et al.2005).

**Vaccination of wild boars**

- When vaccination is worth to be planned and done?
- How vaccination prevents the spread of the infection?
- How is conducted?
- When to stop it?

If the infected wild boar population is proven to represent the TRUE epidemiological reservoir of the virus and the density (> 1-2 Km²), the size (>4-6000) and the geographical distribution (>1000 Km²) of the infected wild boar population are all compatible with a long lasting of the virus , vaccination could be considered.

Vaccination is an important tool to control the spread and intensity of infection under certain circumstances. In combination with immunity generated by the circulation of field virus, vaccination decreases virus circulation, and may eliminate the virus in a given area. However vaccination alone, when not supported by other measures, may also fail to reach the desired results.

Areas in which vaccination is to be carried out should be defined according to the landscape structure (e.g., forested areas, motorways, rivers, lakes) and wild boar spatial distribution and connectivity, rather than relying on administrative boundaries. Vaccination strategies also have to strictly define the epidemiological and sampling units.

**The vaccination process increases population immunity progressively:** maximum immunity is only reached after three double campaigns. Thereafter, a continuous vaccination scheme is required to maintain population immunity. By maintaining a high level of immunity, a vaccination scheme limits the intensity of infection and the risk of transmission to the domestic pig.

In the field, the average proportion of immune animals is often up to 60 %, but immunity is much lower in animals less than a year old, as piglets under the age of six months do not eat the vaccine baits currently on the market. The low immunity observed in 3-12 month old wild boar might partially explain the persistence of wild-type virus in vaccinated populations.
present, vaccination is based on the delivery of baits by hand. This needs strong, long-term mobilisation of hunters, as well as thorough preparation and training. It requires an interdisciplinary approach involving hunters, wildlife biologists and veterinarians.

The vaccination scheme applied since the 2000s has been empirically improved to maximize the immunity of the population. At present, there is a definitive vaccination strategy. This consists of at least two repeated vaccinations, using at least 30-50 baits per 1 km² of forest. The baits are delivered by double vaccination three times a year: in spring, summer and autumn. Double vaccination consists of two campaigns, with an interval of about four weeks between them. The schedule aims to maximise the individual antibody titre, and to reach young wild boar that do not eat regular baits before the age of at least 4.5 months. The current recommendation is to administer on average 40 baits in each of two vaccination places per km². But given the absence of a reliable estimate of the number of wild boar and the rate of bait uptake, the number of baits delivered in the field in any given place cannot be adapted to the number of wild boar with any accuracy.

**Vaccination has to be continued for at least a year after the last detection of a CSFV positive animal.**

A single, isolated vaccination campaign cannot increase population immunity enough to control CSF. Furthermore, theoretical approaches suggest that a one-off vaccination campaign would even aggravate the persistence of CSF. It is important to take into account that C-strain vaccinated animals cannot be differentiated serologically from infected animals. That is why long-term virological monitoring during and after vaccination programmes is required. Given the difficulty of surveillance, particularly in vaccinated areas with the C-Strain (in the absence of conventional-DIVA or bio-marker) the only way to ensure an area is disease-free is to monitor both the virus and antibodies during subsequent hunting seasons.

After a vaccination campaign, PCR positive animals can be diagnosed. These animals might be positive due either to vaccine virus or field virus. They can be cross-checked and their status clarified with a discriminatory PCR for wild-type CSF virus (genetic DIVA – discriminatory PCR) as recommended by the EU Reference Laboratory for CSF. In a simulation model of a CSF epidemic in a wild boar population the following characteristics regarding vaccination were seen:

- Vaccination mainly prevents the spread of infection into neighbouring vaccinated areas (by promoting population immunity in disease-free areas);
- It promotes long-term eradication through progressively reducing the ability of the virus to spread to neighbouring areas;
- It always reduces the epidemic peak (number of infected animals/time); endemic evolution of infection may occur when only a low rate of vaccination is achieved;
- Vaccination of about 20% of susceptible animals results in an increased probability of endemic stability (there is a low incidence of the infection spreading in neighbouring patches);
- Campaigns should achieve a minimum target of 40% of susceptible animals;
- If 60% of susceptible animals are vaccinated, this may lead to the eradication of the infection.

According to the model, assuming that vaccination starts 150 days after the virus is introduced, an optimal vaccination scheme should aim to immunise at least 40% of the susceptible animals, ideally to be achieved within the first round of vaccination.

Several countries introduced oral vaccination of wild boars. The vaccine used is attenuated C-strain in liquid form (Chenut et al., 1999) and is incorporated into smelly baits that are attractive for wild boar (Kaden et al., 2000a). In clinical studies on wild boar this vaccine has been shown to induce high titres of neutralizing antibodies and to make animals immune 1-2 weeks after ingestion of baits (Kaden and Lange, 2001). Field trials as well as the broad use

Baits are distributed either by hand or by airplane (Kaden et al., 2000a). Distribution by hand is performed by hunters on feeding grounds. The baits are buried in order to avoid their consumption by non-target species and to maintain them at fresh temperatures (Rossi et al., 2006). Additional bait distribution by aircraft has been applied in Germany to improve group immunity, but this method has not been generalized (Kaden et al., 2001).

Other methods such as the use of eggs, have been also described to deliver C-strain vaccine (Guberti, pers. communication).

Feeding grounds are required to perform oral vaccination in wild boar (Kaden et al., 2000; Kaden et al., 2001). The method has a varying efficacy according season and presence of alternative food sources (Rossi et al., 2006). There is evidence that the aggregation generated by food or water resources may enhance the transmission of pathogens such as M. bovis or Aujeszky’s virus (Vicente et al., 2005). However, so far the effect of feeding grounds on CSF dynamics was not studied.

Besides oral vaccination of infected areas in some field trials an immunisation cordon surrounding or bordering the infected area were established. The concept of the so-called “cordon sanitaire” is to build up a vaccination barrier in a non-infected area to stop the further spread of disease in unaffected territories (Kaden et al., 2002). An immunisation cordon surrounding an infected area with a depth up to 25 km was first applied in Mecklenburg-Western Pomerania, Germany. Furthermore, the border area of Germany (Rhineland-Palatinate) to the infected area in France (Vosges) is still vaccinated until today despite the absence of CSFV positive cases since November 2004 (Commission Decision 2006/805/EC).

In the latter region the establishment of the cordon is encouraged by reducing the restrictions regarding domestic pigs. But the crucial point of every “cordon sanitaire” is the unknown exact distribution and geographical spread of wildlife diseases in the primarily defined infected area, which can result in an infection of the cordon and beyond (Kaden et al., 2002).

The main limitation of oral vaccination in wild boar relates to bait consumption in youngest age classes. Recent experiments demonstrated that even smaller and spherical baits are not taken up by animals younger than 3 months (FP6 project “CSFVACCINE &WILD BOAR” annual report). Therefore, the direct impact of oral vaccination is restricted to animals older than 3 months; however, due to the transfer of colostral immunity, vaccination of older wild boar has an indirect effect on the immune status of the offspring.

The evaluation of the measure is complicated because there is no marker of vaccination with MLVs that enables differentiation of vaccinated and “naturally” immunized individuals. Therefore, when evaluating seroprevalence after the completion of oral immunisation seropositive animals may be carrying antibodies resulting either from vaccination, infection or maternal immunity (Kaden et al., 2006a). Hence ascertain of final success in an orally vaccinated population is rather impossible as it is difficult to monitor the infection at very low prevalence level. Since the 90’s oral vaccination as been implemented, especially in Germany were the strategy has been adjusted over time (Kaden et al.,2000a; Kaden et al.,2001b; Kaden et al.,2004a). In particular the number of vaccination campaigns and their spacing had been adjusted regarding experimental results performed on wild boar (Kaden et al., 2004a).

Since the 2000’s Germany, Luxembourg and France had implemented the same baits and methodology developed by V. Kaden:

The baits are delivered by double vaccination three times a year: in spring, summer and autumn. Double vaccination consists of two campaigns at an interval of approx. four weeks
(Kaden et al., 2003). The schedule aims to maximize the individual antibody titre (Kaden et al., 2004a) and to reach young wild boar that are not eating regular baits before at least 4.5 months (Brauer et al., 2006). A density of 2 vaccination places per km² is recommended were 20 to 40 baits are delivered each time (Kaden et al., 2001b; von Rüden et al., 2008). Unfortunately, as the number of wild boar and the uptake rate are unknown this procedure cannot be adjusted to increase herd immunity. Vaccination has to be continued for at least one year after last detection of a CSFV-positive animal (Kaden et al., 2005). Based on the improved immunisation procedure higher seroprevalence rates were achieved in young animals (von Rüden et al., 2008).

Oral vaccination using the C-strain as been demonstrated to be fully protective at the individual level in facilities (Chenut et al., 1999; Kaden et al., 2006b) and the elimination of CSF from large areas repeatedly happened simultaneously with the intensive application of oral vaccination of wild boars.

Finally a number of different field studies (Table 8) in line with oral vaccination demonstrated an increase in sero-prevalence in all age classes (even if piglets are less often reached), demonstrated fast reduction of virus detections, and failed to demonstrate continued virus circulation after off set of vaccination. Thus there is strong evidence to suggest the efficacy of oral vaccination as measure to control and obviously also to eradicate the disease (e.g. Von Rüden, 2008).

Due to the fact that the antibodies to the vaccine are indistinguishable from those associated with exposure to the virus and the low incidence and sampling sensitivity in endemic situations a definitive prove of the vaccination efficacy in eradicating CSF in wild boar population is still lacking. Moreover vaccination procedures were adjusted several times also in the same areas according to a trial and error approach. Ring vaccination has been also unsuccessfully adopted. At present a definitive vaccination strategy has been adopted and it consists of at least two repeated vaccinations using at least 30-50 baits for 100 hectares of forest.

Data collected on the field suggest that rarely (if ever) vaccination is able to reduce the number of susceptible animals to the threshold density that will bring to an immediate eradication, of the virus, possibly because of the very low baits intake of piglets. The main effect of vaccination is to maintain a high level of herd immunity even when the reduced virus incidence will naturally induce a decrease of the herd immunity. The maintaining of a high level of immunity will favour the eradication of the virus. In such a view vaccination can be considered as one of the possible available tools to control-eradicate the infection.

In the field more often long-term application with several campaigns has foregone eradication. Sometimes the sero-prevalence was detected as high as 60% or more but the virus persisted for years (Kaden et al., 2001b; Rossi et al., 2006) Indeed, as shown in Germany, factors such as the density of the wild boar population, the size of the infected area, the characteristics of the biotype, and the vaccination procedure used and the practical implementation have crucially influenced the sero-conversion rates and the duration of the eradication process (Kaden et al., 2006a,b). Insights gained from the simulation model suggest rather high proportion of protected animals needed to guarantee the final eradication of CSF in wild boars (see the following graphs). Comparison to observed sero-conversion figures from the field show that such levels are difficult to reach. The limitation may be due to the heterogeneity of transmission and vaccination in the population (Rossi et al., in preparation). Thus experience from the field might limit efficacy of vaccination predominantly to the control of the disease (i.e. preventing spread out of an affected area) rather than to its direct eradication. The plausible is that vaccination takes advantage of the separation of wild boars into social groups, and thus the virus spreads easily only inside the group but not between groups (R0 inside group higher than R0 between groups). Vaccinating whole groups at the feeding places then reduces the time-span the virus might survive before it must jump to the next group.
Vaccination allows maintaining a high level of immunity. Especially in animals older than one year the immune proportion reaches 75-90% after one year of vaccination (3 campaigns). On the contrary often less than 30-50% of young wild boars are found immune even after several years (Louguet et al., 2005; Rossi et al., 2006; von Rüden et al., 2008). One explanation for the significantly lower seroprevalence in young wild boars is the insufficient bait consumption due to baits that are quite big and firm (Kern et al., 1999; Brauer et al., 2006; Rossi et al., 2006). Given that small baits experimentally did not solve the problem (FP6 project “CSFVACCINE & WILD BOAR” annual report) and are either not on market, the current means to maximize the vaccination efficacy in young animals is to plan campaigns when animals are at least 6 months, i.e. October-November. Other technical problems then arise such as the competition between vaccine-baits and oak mast production (Rossi et al., 2006).

The results of oral vaccination campaigns are ambiguous. Double vaccination twice a year seemed to stop further virus spread, but it took a long time to achieve a complete disease eradication (see Table 8) (SANCO 10257/2003). Double vaccination three times a year worked much more rapid (see Table 8). Preventive vaccination, especially when performed in low populated areas, looks more efficient than vaccination of yet infected areas (Rossi et al., in preparation); then vaccination seems to have prevented the spreading of infection in some circumstances during recent outbreaks (Staubach and Koenen, pers. communication); it thus seems that vaccination performed in free areas located around outbreaks bring a relative protection (“cordon sanitaire”); this assumption has however to be confirmed using a quantitative approach and taking into account different population structure (Proceedings, ESVV, Uppsala, 2008).

In some vaccinated areas eradication seems to have been achieved, for example in the Brandenburg and Lower Saxony regions (Germany) (Kaden et al., 2001b; von Rüden et al., 2007). But in some case it seems that disease may persist in vaccinated areas like in the present Vosges (France) outbreak started in 2003 (Rossi et al., 2006) or re-emerge like in Eifel region in 2005 (Germany) (Kaden et Depner, pers. communication). Finally there is no simple way to assess in the field the effectiveness of vaccination (versus a non-vaccination scenario) to perform eradication and limit outbreak duration; this assesement has to be performed using models reproducing different scenarios.

### Barriers reinforcement to prevent animal movements

Barriers such as open land, lakes and fenced motorways seem to behave as efficient barriers to CSF spreading (Artois et al., 2002), even if wildlife movement across such barriers cannot be controlled completely. The reinforcement of barriers may be implemented by simple measures such as closing of wildlife pathways (when these do not conflict with road traffic and security), and to limit drive hunts with dogs around the possible pathways (Louguet et al., 2005). In practice it will be always impossible to control any movement of wildlife, but the efficiency and the efficacy of control in both infected and vaccinated areas may benefit of them.

### Simulation of a CSF epidemic in a wild boar population and the possible outcomes of different control measures (hunting vs vaccination or both simultaneous)

To simulate the CSF epidemic in a wild boar population and the possible outcomes of different control measures (hunting vs vaccination or both simultaneous) a continuous metapopulation compartmental model based on the patches approach described by Hanski e
Gilpin (1997) can be used. Each of the 18 patches represents a homogeneous and independent unit of 130 wild boars related to the others by bilateral links. In each patch the wild boar population has own dynamic (recruitment rate, natural, hunting mortalities, fertility and fecundity rates).

The model runs under the following assumptions:
- MSEIR architecture (M=maternal immunized; S=susceptible, E=latent; I=infectious; R=recovered) (Anderson and May, 1991; Hethcote, 2000) with two age-classes: 0-4 months and > 4 months (Figure 6);
- Inter-patch migration density dependent and limited to > 4 months-old animals (Massei and Genov, 2000);
- Intra-patch virus transmission modelled as true-mass action (frequency dependent) (McCallum, 2000). Inter-patch virus spread dependent by latent (E) or infectious (I) animals migration (Arino et al., 2005);
- Logistic growth (Wilson and Bossert, 1974) with both natality and newborn survival dependent on wild boar density (Focardi et al., 1996);
- Age independent coefficient of transmission ($\beta$) (Rossi et al., 2005);
- Seasonal variation in natality and hunting rates (Fenati and Armaroli, 2004).

Implemented versions of the model consider also long virus shedders (immunotollerants and chronic infectious) described in wild boar by Depner et al., 1994. Discrete and stochastic simulation were performed, the latter using Monte Carlo methods based on 1000 replicates. All the parameters, their variability and the distribution followed by each parameter variability included in the stochastic model are listed and described in Annex B. The descriptive model has been validated comparing the model data (expected) with the observed, field smoothed data (goodness of fit). The stochastic model has been validated using the Weighted Root Mean Square Error (WRMSE) (Vesely, 2006). The procedure estimates the WRMSE of the model and two extreme values from the observed data: worst case (WC) e optimised value (OV). The best fit is obtained when WRMSE is near to OV and ranges between OV and WC (OV< WRMSE<WC). If WRMSE is different from OV but remains within the established range (OV-WC) the fit has to be considered good. Finally, the stochastic model outputs obtained running model with different population size were compared with the regression data described by Rossi et al. (2005a) (see Figure 4 in 5.1.4.2) using the test of parallelism. The model has been validated since the two regression lines (the one obtained by the model and the one derived from field data) show no significant differences in both slope and elevation. The model parameters, model validation, sensitivity analysis, metapopulation equations and model references are attached in Annex B.

The counteractive effect for ranges of practical hunting intensities was confirmed and in particular both low and high level of hunting (low: 25% to 35%; high about 60%) will favour the endemic evolution of the virus through density dependent mechanism. Density dependent mechanisms are intended as those demographic and epidemiological outcomes that are strictly dependent on host density. In such framework the main relevant density dependent mechanism is the increasing of sow fertility and fecundity when the whole wild boar population size is decreased. This is mainly due to the fact that female fertility and fecundity is more weight than age dependent. When the population size is decreased the female wild boar are likely to grow weight (food abundance and availability) and then any population size density control will promptly promote an increased – compensative – recruitment. This highly instable dynamic are more enhanced when hunting is coupled with oral vaccination. The following graphs are presented in order to better elucidate model results. In particular the first Figure (7) represents the basic, common situation in which the usual hunting rate observed in the MS is applied (45% of the wild boar population is hunted each year). In each three sub components are present.

The left component (A) shows the sero prevalence over time, the second (B) shows the virus prevalence over time while the third component (C) shows the virus pattern in each one of the modelled metapopulations. The model results can be summarised as follow:
- Absence of hunting doesn’t produce significant changes in virus persistence or spread
- Only high rates > 70-80% could reduce significantly the virus persistence and spread (but such hunting rate is likely to promote also the local extinction of the wild boar population)
- Low rates (< 45% as default value) reduce slightly the virus persistence but increase the epidemic peak (number of infected);
- Small increase in hunting rates (=60%) can promote virus persistence and spread.

Afterwards the simulation model was run including vaccination. In the simulation model vaccination is applied only to susceptible animals, i.e. individuals without antibodies due to natural infection.

Simulation of the infection without vaccination (basic situation with 45% yearly hunting rate); (A: seroprevalence; B: Viro prevalence; C: duration of the infection)

**Model without vaccination**

Some conclusion on vaccination efficacy can be summarised for a model without vaccination:
- Vaccination is a sensible tool for eradication
- Rarely vaccination in itself can eradicate the infection inside the outbreak
  
From the model results some conclusion on vaccination efficacy can be summarised:
- Vaccination is a sensible tool for eradication
- Rarely vaccination in itself can eradicate the infection inside the outbreak
- Primarily, vaccination prevents the spread of the infection in neighbouring patches (promoting herd immunity in free areas);
- Effectiveness of vaccination increase for each trial ;
- Vaccination always reduces the epidemic peak;
- Endemic evolution of infection could occur when a low rate of vaccination is achieved in small areas also;
- Vaccination of about 20% of susceptible animals results in an increased probability of endemic stability (the infection can spread in neighbouring patches with low incidence);
- Considering the common infection and population parameters a minimum target of 40% of vaccinated animals should be achieved (40% of susceptible animals);
- 60% of vaccinated animals will always eradicate the infection

According to the model outputs an optimal vaccination scheme can be also proposed:
- Vaccination should start around at 150 days after virus introduction;
- Vaccination should immunise at least 40% of the still susceptible animals and possibly during the first trial
- Hunting can be permitted but the hunting rate should not exceed 40-45%/year-1 (excluding <4 months age class) no hunting increasing or decreasing in respect to the usual rates.

**Heterogeneity as a factor**

As previously stated very often large wild boar populations are infected. Even if the management of the infection is standardized and applied equally in each patch of the environment stochastic effects are likely to be observed. It is worth mention that both the number of baits and of the feeding places are set a priori and they do not consider properly
the local wild boar density; hunting success is strongly affected by several local effect (from density to forest coverage, etc). These factors can increase the instability of the virus/host/intervention interface resulting in high probable stochastic variability in the final results of the eradication when both hunting and vaccination are utilised. To verify the possible intermingling effects of the local variability on the whole system, the previously described model was run through a Monte Carlo simulation. Model stochastic implementation was based on the introduction of a certain degree of random variability of 8 parameters that were chosen for their high sensitivity or literature discordance of their estimates.

For the majority of the 8 parameters the information concerning their variability was poorly known or defined in such case, randomness based on a uniform distribution was performed. For the survival rate parameter of the long shedding individuals (both chronics and immunotollerants) the Weibull distribution has been chosen. Stochastic models show low probability of endemic evolution (0.6%) when acute infection was considered (basic model) that increase to 10% when chronic long shedding and immunotollerant were included in the model.

It is worth to underline that the effect of stochastic variability allows virus persistence after 5.5 years, in case of virus introduction, in 10% of the model runs. This finding confirms that the combined stochastic effects of few field variables can easily lead to an – un-foreseen – endemic evolution of the virus (Figure 15).

- Primarily, vaccination prevents the spread of the infection in neighbouring patches (promoting herd immunity in free areas);
- Effectiveness of vaccination increase for each trial ;
- Vaccination always reduces the epidemic peak;
- Endemic evolution of infection could occur when a low rate of vaccination is achieved in small areas also;
- Vaccination of about 20% of susceptible animals results in an increased probability of endemic stability (the infection can spread in neighbouring patches with low incidence);
- Considering the common infection and population parameters a minimum target of 40% of vaccinated animals should be achieved (40% of susceptible animals);
- 60% of vaccinated animals will always eradicate the infection.

According to the model outputs an optimal vaccination scheme can be also proposed:
- Vaccination should start around at 150 days after virus introduction;
- Vaccination should immunise at least 40% of the still susceptible animals and possibly during the first trial
- Hunting can be permitted but the hunting rate should not exceed 40-45%/year-1 (excluding <4 months age class) no hunting increasing or decreasing in respect to the usual rates.

**Surveillance and monitoring of CSF in wild boars**

Surveillance for diseases as indicated above can be defined as an ongoing systematic use of routinely collected disease data to provide information which leads to action being taken to manage a disease in a country, e.g. on- or offset of control relative to case detection (following OIE (2007)). The aim of CSF surveillance is the detection of cases and to take some action to control or eradicate the disease as soon as possible. Hence the logical source of information is to target sub-population of high risk to be infected including the previously infected host individuals.

Monitoring is the systematic quality assurance of control treatments or intervention strategies. Note that in contrast to this understanding some guidelines use the notion
‘monitoring of control’ to describe a mixture of both the ongoing disease surveillance during activated control and the performance evaluation of control measures (Commission Decision 2002/106/EC). A well known example for control quality assurance relates to oral mass vaccination against rabies in foxes where the performance of vaccination was measured via seroprevalence or bait uptake (Commission Decision 2002/106/EC). The same approach is not possible in wild boar vaccination programs. Adding tetracycline to CSF vaccine baits is not allowed since wild boar meat is consumed by hunters. The aim of quantitative monitoring of control programs is to assess the efficacy of applied measures. The logical source of information is found in the noninfected sub-population. The information is of interest only during active control.

In surveillance activities, sampling can be addressed to identify indicator animals or to the individuals composing the hunting bag. Indicator animals in wild population are those individuals that for any reason have a high probability to be positive with respect to the target of the surveillance. This includes animals killed due to clinical symptoms or suspicious behavior, found dead, or being involved in human exposure. For diseases that cause mortality or morbidity, the sample source is by definition focused on the diseased individuals, thereby intrinsically focusing the sampling in area and time. Individuals composing the hunting bag are those individuals potentially less likely to have the disease (i.e. not an indicator animal). These are for example animals sampled from regular hunting activity, specific sampling hunts or sampled alive (e.g. structured or non-random selection (OIE, 2004). This sample source is statistically designed to be representative for the healthy population (i.e. susceptible or protected/treated) on large spatial and temporal scales.

The final goal of sampling and monitoring wild boar population for CSF is always to ensure the health status of the domestic pig population with secondary aim is to determine the CSF status through the presence of the virus in the wild boar populations and to address all the actions needed to reduce and/or avoid the spread of the virus from wildlife to domestic pigs.

The active sampling of wild boar for CSF is obviously difficult. Where CSF has been confirmed in the country in the previous three years, a passive surveillance system should be put in place, with the aim of early detecting of reoccurrence of the virus in wild boar. Hunters and gamekeepers should be instructed to report the finding of all dead wild boars to the competent authority. Any carcass that is found should be declared to the authority, which should take samples and carry out laboratory tests according to its evaluation of the epidemiological situation.

Hunting is the sole practical system to obtain samples for active monitoring of vaccination and disease freedom, but the normal aim of hunting is obviously not disease control. Consequently, the sample size is not controlled by the authorities and rarely fits the aims of an epidemiological survey (i.e. detect at least one viral positive animal, or estimate serological prevalence).

In the case of high-risk situations, passive surveillance should be complemented by active serological surveillance (additional hunting). Ideally, the sample size should be large enough to detect 5% (with 95 % CI) of seroprevalence per time and per spatial unit. Sampling activities should be intensified and repeated at least twice a year. CSF may spreads along green corridors, and some physical barriers seem efficient in stopping its spread. Therefore landscape structure (forested areas, motorways, rivers, lakes…etc) influences contacts among wild boar from different populations and has to be taken into account in defining infected and monitoring areas, rather than relying on administrative boundaries. If biologically meaningful borders are not available to determine infected areas, interpretation of data may become difficult.

Repeated sampling over several hunting seasons will increase the probability of detecting persistent cycling of the infection/virus. The surveillance strategy and evaluation of results...
should always consider the epidemiological situation/development of the infection and vaccination status.

Correct estimation of the viral and seroprevalence, however, is of paramount importance to understand the pattern of CSF infection and to validate interventions.

Two main sampling strategies can be applied in large areas:

1) The most reliable (to derive epidemiological conclusions) is to divide the whole infected area into several small areas. Sample size is then calculated for each small area, and findings are inferred from small areas;
2) The whole infected area is surveyed, sample size is calculated in relation to the entire area, and findings are inferred accordingly.

If population size and prevalence estimates are not available, the calculation of sample sizes should assume 5 % of prevalence and a confidence level at 95%.

The sample size in C-strain vaccinated areas should be calculated to assess the stability (or the increasing) of population immunity at the desired level of seroprevalence or its expected prevalence variation (i.e. before and after any intervention).

Area-specific data about the wild boar population structure, hunting regime, or disease history can contribute to the sensitivity of a surveillance system, thereby yielding better estimates.

**Aims and strategies**

**Aims of CSF surveillance in wild boar**

A surveillance program for CSF in wild boar must be aimed considering the epidemiological situation of the Country and in particular to obtain the following information:

d) The epidemiological situation and the disease status of the Country (CSF status)
e) The susceptible species present in the Country and their geographical distribution;
f) The main risk factor that can allow the presence of the infection in the environment

According to the above mentioned points a surveillance scheme in the wild boar population of Serbia should be aimed in:

5) Detecting the introduction of the virus in a free area
6) Detecting the presence of the infection in unknown disease status areas
7) Understanding the role played by the wild boars in the epidemiology of the infection if and when the virus has been found.

**Surveillance strategies**

As a first step a CSF free wild boar population should be denied. It does not exist an official definition (legal) but from the epidemiological point of view a CSF free wild boar population might be:
Technical Assistance for the Control and Eradication of Classical Swine Fever (CSF) and Rabies in Serbia

a) A population where the presence of immune animals is below a detectable level; the threshold of detectability must be coherent with the classic epidemiology of the infection (expected sero-prevalence = 5%)

b) A population where CSF virus has not been detected for at least one two years proving that sample intensity has been sufficient to find at least one positive )1%<expected prevalence of the virus)

Two main assumptions have to be accepted when designing a surveillance aimed in detecting the presence of the infection in a wild boar population (is the wild boar population of Serbia infected of CSF?).

a) Animals are not vaccinated so that presence of antibodies will reveal a previous contact with the CSF virus.

b) CSF virus is endemic (or largely distributed) in the wild boar population, in particular the infection IS NOT sporadic;

c) When introduced in a free population the CSF virus will always show a certain degree of lethality in all age classes.

To maximize the probability to detect the infection both active and passive surveillances should be in place.

In the framework of the active surveillance antibodies presence should be the main target of the investigation. Antibodies due to the infection are long lasting (usually their life is higher than the life span of wild boars in hunting grounds) and their presence mean that the wild boar has come in contact with the virus. The direct search of the virus through sampling is possible but the sampling intensity must be very high since, especially in endemic areas, the expected prevalence of the virus in time unity (one day) is very low (<1%).

The passive surveillance is mainly based on testing found dead wild boars or animals shot after showing some clinical symptoms. Due to the difficulties to retrieve dead animals or recognize clinical signs in the wild it is worth to test all animals belonging to these categories. The sole use of passive surveillance in the early detection is largely justified by the fact that lethality rate of CSF in a virgin population is higher in respect to any sampling method (hunting, catching etc.). The odds ratio between lethality and any other sampling method demonstrates the introduction of the virus can be easily found with passive surveillance only.

To have a passive surveillance strategy in place a suspected case definition in wild boar is needed.

Wild boars suspected case: any wild boar found dead or shot/killed because of clinical signs.

Each one of the animals belonging to the suspect case definition should be laboratory tested to exclude the presence of the CSF virus (and possibly the one responsible for ASF also). In real world term it is more important to exclude the presence of CSF virus rather than to identify the cause of mortality. This approach will strongly reduce the FIRST HIGH RISK PERIOD and thus the spread of the infection in the population.

**Monitoring in case of suspicion and confirmation of CSF in wild boars**

Serological and virological monitoring has to be performed. The size and the geographical area of the target population to be sampled should be defined in advance to establish the

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number of samples to be taken. Sample size must be established as a function of the estimated number of living animals.

If data on population density and size are not available, the geographical area within which to sample must be identified, taking into account the continuous presence of wild boar and the presence of natural or artificial barriers that effectively prevent the animals moving freely. If there are no such barriers, or in case of large areas, identifying sampling areas of not more than 200 km², with an established population of about 400 to 1000 wild boar, is recommended.

The minimum number of animals to be sampled within a defined sampling area must allow detection of 5% seroprevalence with 95% confidence. To achieve this, at least 59 animals must be sampled in each area identified.

In areas with a small population of wild boar, due to the difficulties of surveillance as set out above, the sampling plan should be adapted based on epidemiological advice their proposed surveillance plan in wild boar to the local conditions.

It is also recommended that:

- in areas where hunting pressure is higher and regularly occurs, or selective hunting is carried out as a disease control measure, about half the sampled animals should be aged between three months and one year, 35% should be one to two years old, and 15% over two years old;
- in areas where hunting pressure is very low or absent, at least 32 animals should be sampled for each of the three age classes;
- sampling should be performed over a short period, preferably not more than one month;
- the age of sampled animals should be identified according to their teeth.

When virological monitoring on shot animals is deemed necessary, it must be primarily carried out on animals three months to one year old. All samples to be sent to a laboratory must be accompanied by the questionnaire referred to in Article 16(3)(1) of Directive 2001/89/EC.

**Monitoring after oral vaccination**

After completing oral immunisation, the age class of wild boar that should be examined serologically to detect a new or re-emerging infection depends on the season in which vaccination was completed and the length of time since completion.

In the second year after an oral immunisation campaign, piglets younger than six months might still have maternal antibodies, and boars older than 12 (or 18) months probably still have vaccination antibodies. Hence, a wild boar population is CSF-free if the antibody prevalence in the age class 6-12 (or 18) months is below a certain detection level (i.e. <5%, 95% CI).

In the third and subsequent years after oral vaccination, animals aged 6 to 24 months should be free from CSFV antibodies. Animals older than three years will probably be serologically positive due to vaccination, and animals <6 months might have maternal antibodies.

After the end of the vaccination campaign the following monitoring plan is proposed:

- 1st year after vaccination (0-12 months after completion of the campaign): no serological monitoring, focus on virological testing;
- 2nd year after vaccination (13-24 months after completion of the campaign): serological monitoring of wild boar piglets (6–12 months of age);
Technical Assistance for the Control and Eradication of Classical Swine Fever (CSF) and Rabies in Serbia

- 3rd – 5th year after vaccination (25-60 months): year after vaccination: serological monitoring of piglets and young wild boar (6–24 months of age).

Minimum number of samples per district (or metapopulation) each year: 59 (5 % prevalence with 95% confidence) 5 years after completion of an oral immunisation campaign the wild boar population is likely to be replaced thoroughly by naive animals. Therefore, the population should be considered as fully susceptible again. It has to be kept in mind that antibodies due to vaccination can still be detected if serologically examined animals are older than 60 months.

In addition to serological examinations, **virological tests** should be conducted in all age classes. However, emphasis has to be put on **piglets**, on all **diseased wild boar** and on **animals found dead**. If CSF is suspected, all shot wild boar within a radius of 3 to 5 km have to be examined virologically for at least one month.

**Monitoring and surveillance tools applied in wild boar populations with results observed in the field**

**Source of samples**

There are few possible sources for collecting wild boar samples and the most important id the hunting activity. Hunting season last for all animals from 1\textsuperscript{st} July to 31 December. For males the hunting season begins the 1 February, for females 1\textsuperscript{st} of January and for young animals on 1\textsuperscript{st} February.

Each year a number of about 7.000 heads are hunted in the whole Country. At present no data are available regarding the age classes and gender of the hunted animals and also the fertility and fecundity of the hunted females appears not to be registered.

Another source of samples could be the animals caught in the fenced hunting areas for vaccination purposes. These animals could be samples (blood).

The main sources for the detection of virus or antibodies are sera, tonsils and spleen. Non-invasive samples such as faeces do not necessarily contain enough virus material for detection. Furthermore, the existing diagnostic assays including PCR for virus detection in feces are still limited.

The majority of the samples taken, regardless of their type, are often bad quality as compared to those obtained from domestic animals. This is mainly due to the following facts:

- the main source of sampling is hunting activity;
- hunted animals are very often stressed particularly when the dog drive system is used; in this case haemolysis is a common finding;
- the amount of time elapsed between the hunting success (shot animal) and the sample taking can be long. Usually, the hunted animals are carried to the hunting premise after they have been shot. Not until then the animals are dressed before samples are taken.
- often samples are delivered to the laboratory not before one day after the hunting.

During this time samples are often preserved in a rudimental way (e.g. during winter just indoor). Due to the circumstances under which hunting and dressing occurs, the cross contamination risk is high.
Passive surveillance

Passive surveillance must be in place all year long and in all the Country irrespective of the type of management of the area (hunting grounds, protected areas, National Parks etc.). Each animal that fits the case definition must be examined in order to exclude the presence of CSF (PCR and eventual confirmation). According to the official data provided by the Country Competent Authorities in Serbia there are 20,000 wild boars. Assuming an annual mortality ranging from 3% to 6% (literature) a minimal number of 600-1200 individuals will die yearly. An acceptable level of awareness amongst game wardens, timber men, hunters, will permit to have reported about 10% of these total figures. So that a number of 60-120 samples, widespread in the whole country, is expected and dispatched to the laboratories for CSF test. The above figures should be considered as a good indicator of the level of passive surveillance of the Country.

The role of passive surveillance is to early detect the introduction of the virus in CSF free alternatively, to increase the probability to detect the virus through the sampling of target animals (dead individuals) in infected areas.

Active surveillance

The main role of active surveillance is to demonstrate the presence of CSF virus, or antibodies, at a certain, predetermined, level of detectability (1% and 5% with 95% C.L.). Active surveillance is based on sampling both blood and tissues (spleen and mainly) with a specific sampling intensity and in a specific sampling units.

Due to the above mentioned risk factor the Country can be divided in four different major areas, each one having specific a different risks.

a) Areas in which the wild boar density is registered as high and neighbouring countries reported CSF or vaccinated against CSF;

b) Areas in which past surveys have revealed positive individuals (both sero or virological positive);

c) The fenced hunting areas in which the population density is artificially high

d) The remaining part of the Country

Sampling unit

- The sampling unit is NOT the wild boar population as a whole;
- The sampling unit is the small population (wild boar metapopulation), able to maintain the CSF virus for a certain period of time, usually one year;
- In the European ecological conditions the sampling unit should be identified in a group of about 1000-2000 animals living in an area of about 200-400 km². Can be elastic of course, but not too much;
- Hunting ground do not have characteristics of a sampling unit, and even more: if you fragmented the number of sampling units you have to increase a lot the number of samples.
Example:

Involved Area: 5196 km²
Population: 32000 wild boars
Dens: 6.1 wild boars/ km²
Sampling unit= the whole population
297 samples will detect at least one positive individuals if the infection is prevalent >= 1% (95%LC)
Sampling unit = 17 metapopulations
273 samples in each metapopulation will detect at least one positive individuals if the infection is prevalent >= 1%(95%LC)
297 vs 4641 samples

Theoretically samples should be taken in the same moment. A diluted sampling (6 months time) will reduce dramatically the probability of detecting the infection even if the number of samples is “theoretically” adequate.

Sample size and sampling techniques

A real census of the wild boar population very rarely is available. Moreover, available data on wild boar population size are often underestimated (Zanardi et al.,2003). Currently, the hunting bag when available represents the basic data by which sample size is calculated and very rarely a predetermined sample size is calculated. Official data regarding wild boar population density are often inconsistent when compared with the actual annual hunting data; hunting data often indicate that the wild boar population size is larger than expecteted/foreseen. In the best circumstances the whole (or a high proportion of) hunted population is sampled.

Samples are often taken in an opportunistic system (i.e. first or last shot animals of the day). Samples are generally taken directly by hunters who are responsible for filling the form accompanying each individual sample. Form requests relevant information such as date, locality, hunter name, age and gender of the sampled animals and other information that can vary according to the general strategy applied country by country.

It is worth mentioning that hunting activities have very different purposes than to collect samples. Hunting is practiced purely as a hobby (at least in the EU) and the definitive aim of hunters is to maintain viable, dense populations in order to assure a future increasing of the bags. Moreover there are also technical limitations to using hunting as a primary source of sampling. Hunting is limited in both space (e.g. national parks) protected areas where

<table>
<thead>
<tr>
<th>Area</th>
<th>Wild boar /sqkm</th>
<th>% Hunted wild boars</th>
<th>Hunt. Bag /sqkm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.34</td>
<td>70.53</td>
<td>3.05</td>
</tr>
<tr>
<td>2</td>
<td>0.72</td>
<td>34</td>
<td>0.24</td>
</tr>
<tr>
<td>4</td>
<td>0.82</td>
<td>55</td>
<td>0.45</td>
</tr>
<tr>
<td>5</td>
<td>9.72</td>
<td>43</td>
<td>4.2</td>
</tr>
<tr>
<td>6</td>
<td>11.42</td>
<td>45.9</td>
<td>5.24</td>
</tr>
<tr>
<td>7</td>
<td>1.52</td>
<td>38.8</td>
<td>0.6</td>
</tr>
<tr>
<td>8</td>
<td>0.89</td>
<td>75.3</td>
<td>0.67</td>
</tr>
<tr>
<td>9</td>
<td>#</td>
<td>#</td>
<td>#</td>
</tr>
<tr>
<td>10</td>
<td>0.94</td>
<td>96</td>
<td>0.9</td>
</tr>
<tr>
<td>11</td>
<td>0.49</td>
<td>74.5</td>
<td>0.36</td>
</tr>
<tr>
<td>12</td>
<td>0.000246</td>
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<td>#</td>
</tr>
</tbody>
</table>
hunting is forbidden and time (usually the wild boar hunting season is limited to winter). The hunting bag rarely reflects the real age and gender structure of the hunted population and finally each individual hunter will have his own approach in choosing the hunting area, the animals to shot and the day in which to hunt. Such a large heterogeneity poses severe limitation in using hunting bags as the primary source of samples. Anyway there are no alternative option since any other method to obtain samples is extremely expensive, will rarely reach the same sampling intensity and will also have the same types of limitations. Thus, it is worthwhile to estimate the potential error in estimating the CSF infection rate and early detection in wild boars using practical samples from hunting bags. Issues for estimation of the prevalence of this type of sampling have been addressed elsewhere (Duncan et al., 2008).

Table 11. Proportion of hunting bag sampled for virus and antibody detection (Source: EFSA Questionnaire)

<table>
<thead>
<tr>
<th>Area</th>
<th>Hunt. Bag/sqkm</th>
<th>Viro sample sqkm</th>
<th>% Hunt. bag sampled</th>
<th>Sero sample sqkm</th>
<th>% Hunt. bag sero sampled</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.05</td>
<td>0.245</td>
<td>8.03</td>
<td>0.1</td>
<td>3.28</td>
</tr>
<tr>
<td>2</td>
<td>0.24</td>
<td>0.018</td>
<td>7.50</td>
<td>0.0036</td>
<td>1.50</td>
</tr>
<tr>
<td>4</td>
<td>0.45</td>
<td>#</td>
<td>#</td>
<td>0.0041</td>
<td>0.91</td>
</tr>
<tr>
<td>5</td>
<td>4.2</td>
<td>3.23</td>
<td>76.90</td>
<td>2.79</td>
<td>66.43</td>
</tr>
<tr>
<td>6</td>
<td>5.24</td>
<td>5.18</td>
<td>98.85</td>
<td>4.62</td>
<td>88.17</td>
</tr>
<tr>
<td>7</td>
<td>0.6</td>
<td>0.144</td>
<td>24.00</td>
<td>#</td>
<td>#</td>
</tr>
<tr>
<td>8</td>
<td>0.67</td>
<td>0.0024</td>
<td>0.36</td>
<td>0.0008</td>
<td>0.12</td>
</tr>
<tr>
<td>9</td>
<td>#</td>
<td>0.078</td>
<td>#</td>
<td>0.044</td>
<td>#</td>
</tr>
<tr>
<td>10</td>
<td>0.9</td>
<td>0.83</td>
<td>92.22</td>
<td>0.77</td>
<td>85.56</td>
</tr>
<tr>
<td>11</td>
<td>0.36</td>
<td>0.172</td>
<td>47.78</td>
<td>0.148</td>
<td>41.11</td>
</tr>
<tr>
<td>12</td>
<td>#</td>
<td>#</td>
<td>#</td>
<td>#</td>
<td>#</td>
</tr>
</tbody>
</table>

Investigating the presence of infection

CSF surveillance in wild boars in peace time

At a large scale the MS do not have any strictly defined approach for the early detection of the virus in free areas. Even if one the most important points to avoid further spread of the infection in both wildlife and domestic animals is the very prompt detection of the virus. Moreover a clear definition of CSF suspected case in wild boars is still lacking Some specific countries, being or feeling at risk of the disease, design and implement a surveillance strategy aimed in detecting CSF in wild boars (e.g. Belgium, the Netherlands) by considering the aim of the survey, host population density and spatial distribution (Mintiens et al., 2005). Serological investigation is mainly used since it can detect past exposure to the virus and requires smaller sample sizes (i.e., expected high seroprevalence). Any isolated strain of CSF virus retains a certain degree of lethality in wild boar also and the resulting population mortality is quite high at least at the onset of the infection when it spreads in a fully susceptible and naive population. This high mortality indicated that any early detection activity should be based on a strict passive surveillance. Primary outbreaks in wild boar have been often detected through post mortem examination of retrieved dead infected animals. Also in an infected area the odd ratio of viral positive found dead individuals vs alive sampled animals is 4.66 (95% C.I. 2.09-10.42) (Rossi et al., 2005a; in Germany data suggest even an Odds Ratio of 55 (95%-CI: 43-72) in non vaccinated and up to 200 (166-244 in vaccinated populations (Thulke et al., in press.. Thus in areas and in wild boar population considered at high risks any early detection system should be primarily addressed in retrieving and
examining dead individuals and excluding CSF as a routine (see concept of situation-based surveillance; Thulke et al., in press.)

In potential disease free areas, serological surveys represent a possible tool to detect – indirectly – the infection. Serology is cheap easy to perform and high number of samples can be processed in a short time. Since natural antibodies last for long time (lifelong) both past and on going infections are easily detected. Once the epidemiological and the sampling units are correctly identified, the sampling intensity should be designed to find at least one positive individual in a population with an expected prevalence of 5% and a 95% confidence. Together with a passive surveillance, serology could be used in well known situation considered at high risk (Artois et al., 2002). In these potential disease free areas a virological survey could be performed but both passive and serological surveillance more easily will reveal the presence of the infection than a virological survey on healthy animals. Finally, to detect at least one viral positive animal with an expected low level of prevalence and with an acceptable level of confidence will require a so large sample size that rarely an efficient virological survey will be achieved. It is therefore logical to focus on serological surveys and then attempt to isolate the virus from those areas where serological positive animals are found.

**Determination of infected area**

Many approaches have been applied in order to exactly define the boundaries of the infected area. In the past, infected areas were designed according to the domestic pig legislation (3 km radius). However, as defined in EU legislation (Council Directive 2001/89/EC, Art. 15(a), 16.1, 16.3; Commission Decision 2002/106/EC, Annex, Chapter IV, H), infected areas are designed taking into account the ecological characteristics of the environment and in particular the presence of ecological barriers both natural (rivers) or artificial (highways) and the wild boar continuous spatial distribution. The EU legislation also introduced the concept of metapopulation in order to limit the infected area to the correspondent infected wild boar metapopulation. Unfortunately, in several European countries the wild boar spatial distribution is large enough and the possible presence of metapopulations is rarely known. Thus the resulting infected areas tend to have a corresponding large boundary. Because maintaining high level of quarantine and restriction measures in such large areas is costly in terms of both wild boar control and in limiting pig trade it is a common policy to limit the extension of the boundaries of the infected areas according to a trade off consistent with a cost benefit evaluation. Furthermore, often the infected areas are enlarged due to the lack of knowledge regarding the spatial spread of the infection, the distribution of the infected metapopulation and the hunting seasonal monitoring of the infection. This process constitutes a limiting point in the control/eradication of the infection since the applied control measures are taken later in respect to the real spread of the infection in a determined area. Finally each country applies a specific policy and strategy in order to survey CSF free areas neighbouring to the infected one(s). Unfortunately the relationship between the host density/spatial distribution and the geographical spread of the virus is not yet fully understood. In Rhineland Palatinate the annual spreading of the virus was estimated to approximate 24 km (Irsch, pers. communication) but the possible variables explaining the observed spread were not identified.

**Investigation/surveying by virus and antibody detection**

The actual sampling system is based on an opportunistic approach mainly focussed on hunted animals (% of hunted animals in EU data base with respect to any other sources). The sample size is not designed to detect certain – prefixed – level of actual prevalence (design prevalence), either through viral isolation or seroprevalence, with a certain level of...
confidence. It does however recognize that the number of positive animals for viral isolation is always low compared to the number of the sero positive animals. Nevertheless sample size does not reflect the difference between these two estimations. Results derived from the questionnaire indicated that the applied sample size is rather the same, and irrespective of the different possible aims of sampling (i.e., to estimate the actual viral prevalence or seroprevalence). During the last few years the MS CSF surveys in wild boars results are reported stratified by age and gender. The findings from this survey have improved the possibility of a better understanding of the evolution of the infection. Animals in the 6-12 months age class are targeted in order to demonstrate the absence of virus and antibodies. Antibodies absence from this age class should confirm the absence of the infection from the infected area (no virus circulation during the past 6 months). Unfortunately the application of this simple and robust epidemiological approach is limited due to the inadequate sample size and the prolonged sampling activities. The sample size composed by 6-12 months aged animals is very rarely sufficient to demonstrate the absence of antibodies at the desired prevalence detection and confidence level; moreover the prolonged time period during which samples are taken will further reduce the efficacy of the strategy.

Often the boundary of the infected areas becomes so large that a large wild boar population is expected. In such circumstances the density, the size, and the spatial distribution of the whole infected wild boar population can be composed of several sub populations. Each one of these sub-populations is expected to have different micro-epidemiological characteristics for maintaining the virus for long period of time and in particular to be large enough to represent a possible independent, local, population patch able to maintain CSF virus in the environment. In this case two alternative strategy options can be applied. The whole infected area is surveyed, sampling size is calculated for the entire area and the reported findings refer to the whole area. Alternatively the whole infected area is split into several smaller areas; sample size is calculated for each subarea’s area sampling intensity and results refer to each of the subareas. The second option will reflect the actual micro-epidemiological characters of the disease but it is more intensive and costly than the first option.

<table>
<thead>
<tr>
<th>Assessing Seroprevalence the immune status of the populations</th>
<th>To estimate the population immunity</th>
<th>Sampling a representative proportion of the population. The age stratified sample increase the knowledge of the epidemiological situation</th>
<th>Age stratified sampling needs a huge amount of samples otherwise prevalence estimate is not precise (large variation of the estimated prevalence). Since antibodies are lifelong the timing of sampling will affect the whole result in a minor way.</th>
<th>97</th>
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<tbody>
<tr>
<td>Assessing viroprevalence If the virus is present in the population</td>
<td>To verify the status of the population (infected or not), endemic evolution</td>
<td>Sampling a representative proportion of the population. The age stratified sample increase the knowledge of the epidemiological situation</td>
<td>Due to the difficulty in obtaining the exact sample size virus can be easily undetected. Age stratified sampling needs a huge amount of samples difficult to obtain. Time required to collect samples (time diluted sampling) further reduce the efficacy of this sampling.</td>
<td>865</td>
</tr>
</tbody>
</table>
What is the uniqueness in C-strain vaccinated areas?

Vaccinated areas (at present C-strain only) are considered infected. The applied sampling approach and scheme is the same applied in infected areas. As a result, in most vaccinated areas, all the hunted animals are tested. Unfortunately, the use of serological tests is quite limited due to the fact that antibodies due to vaccination are indistinguishable from those due to the wild virus.

In vaccinated areas, one of the main goals of the sampling is to demonstrate vaccination efficacy. Efficiency is measured in terms of sero prevalence in the whole vaccinated population often irrespective of gender and age classes. Very often statistical tests are used in order to demonstrate vaccination efficacy. In such circumstances, the power of the test should be accurately addressed in order to highlight biologically relevant differences only.

Finally, a common strategy to demonstrate freedom from the infection is to test animals aged 6-12 months of age. Usually, this age class contains more virus positive animals than other age classes. Therefore, the absence of the virus in this age class will indicate the probable absence of virus circulation in the infected population. It is of paramount importance to underline that the sampling size required to demonstrate that this age class is free from the virus is extremely high, also considering that the corresponding expected prevalence must be set at a very low level (≤ 1%). Moreover, since the sampling is diluted in time (usually during the whole hunting season), the efficacy of sampling, even if an adequate sample size is reached, will reduce dramatically the efficacy of the survey.

It is worth mentioning that many of the limitations presented above actually can be prevented in vaccinated areas if and when a marker vaccine will be available also for field vaccination in wild boar populations.

Estimation of Prevalence, Incidence and Spread of the infection

Prevalence data are usually calculated by combining all the available data for each administrative area. Alternatively, prevalence data are presented according to the infection status of the areas (infected, bordering, etc.) and according to a certain period of time (usually represented by the hunting seasons or certain calendar steps i.e. month, year). Virological data are often presented as incidence data. When an age-stratified serological sampling is available, some attempt to calculate the force of infection has been done.

One of the main uncertainties in determining the prevalence of the infection is exact identification of the infected population to be sampled (sampling unit) by both space and time. Such uncertainty may lead to sampling areas that are too small or too large and thus resulting in overestimate or underestimate the real prevalence of the infection and a possible failure in exactly identify other infected areas.

Another critical issue is the time duration during which samples are taken so that the cumulative number of samples can meet the desired sample size whereas, when considered in short time (weeks or months), the sample size might be too low to meet the aim of the survey.

A correct estimation of the viral and seroprevalences, however, is of paramount importance to understand the CSF infection evolution and to validate interventions and can be useful also to estimate other epidemiological parameters worth to be considered when interventions are programmed (i.e. force of infection, R0, etc.). To estimate such epidemiological parameters, the results of both virological and serological tests at individual level must be available. To calculate the exact sample size needed to estimate seroprevalence in natural condition (no vaccination) and when no previous data are available, expected prevalence should be fixed at 50%. This type of assumption will ensure adequate sample size to estimate the prevalence level in the specified area. An alternative strategy could be represented by the exact calculation of the sample size in order to detect a certain level of prevalence variation (i.e.
before and after any intervention). The sampling size should be based on the beta error (power of the test) and the expected variation in prevalence. The confidence level of any sampling should never be accepted when below 95%. Usually CSF virus in wild boar population has a very low prevalence (<5%) and thus to detect it a large sample size is needed and also the time length of the sampling activities should be short. Hence, the above options will not allow reliable estimates of the prevalence of the virus.

**Demonstration of freedom of CSF**

Details concerning freedom of disease are presented above. Most of these principles are applied to wild boar population as well. However there are some specific differences to wild population. Currently, a wild boar area is considered free of CSF when virological tests have been negative for a certain period of time. Negative virological data are often coupled with the serological test result. Nevertheless, a more precise definition of a CSF free wild boar population is still lacking and should be substantiated. Possible definitions are the following:

a) A wild boar population is CSF free when all tested samples are negative for virus detection and the antibody prevalence is below a certain level of detection (i.e. <5%, 95% CI); alternatively, since antibodies are life-long, the above mentioned definition could be applied only to animals within the age class 6 to 12 months. This would exclude (according to established level) virus circulation during the past 12 months.

b) A wild boar population is CSF free when all tested samples are negative for virus detection and the presence of disease indicated by virus prevalence is below a certain level of detection (i.e. <1%, 95% CI); possibly the sampled animals should belong to the high risk age classes.

c) After completing oral immunisation, the age class which should be examined serologically to detect a new or re-emergence of infection depends on the season in which the vaccination was stopped and the period of time elapsed since completion of vaccination (Kaden et al., 2006a). Two years after finishing oral immunisation, boars younger than six months might still have maternal antibodies and boars older than 12 (or 18) months probably still have vaccination antibodies. Hence, a wild boar population is CSF free if the antibody prevalence in the age class 6-12 (or 18) months is below a certain detection level (i.e. <5%, 95% CI). In the third and following years after finishing oral vaccination at least the animals aged 6 to 24 months should be free from CSFV antibodies. In turn, animals older than three years will probably be serologically positive due to vaccination and animals <6 months might have maternal antibodies.

Once agreed on any definition the sampling size should be calculated accordingly and could be large for b). Possibly a new technique to calculate the required sample size that includes time and sampling intensity factors should be developed. In the field it is not always possible to achieve the required sample intensity in relatively short time (possibly in a point time), so that one of the main assumption of the sample size calculation is violated. A new, robust and validated system should be developed in order to estimate virus or antibodies presence (or the errors in detecting them) using time prolonged sampling intensities (Martin et al., 2007a and 2007b).

Currently, the only way to provide sampling frame for providing evidence of declaration of disease freedom is to calculate a sample size and conducting simulation exercises such as presented in section 3 (see tables 5 and 6). This type of computation, however, uses the assumption that the animals or herds are randomly selected from the target population. In wild boar population, as any other free ranging wild animal species the actual population size is unknown. The particular methodology used by the hunters such as solitary stalking, hunting in groups and hunting with or without dogs will likely effect the number of killed
animals and thus introduce a selective bias. Hence the performance of the CSF MOSS based on hunted animals is not well known and difficult to quantify using the available empirical studies.

The non-random sampling by hunters was simulated with a quantitative model aiming at an assessment of the capacity of the procedure to detect presence of low-prevalent CSF infection through diagnostic examination of the collected. In this section we propose one of the possible modelling approaches that may be used to answer the above question. The aim of the model is to show how the hunting system and the wild boar distribution will affect the capacity of the surveillance system.

**The sensitivity of the sampling system for CSF monitoring in wild boars**

The overall sensitivity of structured sampling systems based on hunted animals is not well known in case of CSF in wild boar and difficult to quantify given sparse empirical studies. Nevertheless hunting based surveys are required to monitor CSF when the mortality event of the epidemic has passed and subsequently only few virus positive animals could be expected (e.g. disease fade out in the infected area; or virus intrusion into a vaccinated population). Standard epidemiological calculations of sample design might be applied to ensure a survey that is sensitive to detect the disease, with a-priori defined certainty, whenever it is prevalent beyond a design level.

However, the standard calculations assume uniform and random distribution the wild boars, of the infection, and of the samples collected by hunting. In the context of CSF in wild boars all three conditions might be violated: Indeed, in the field the spatial distribution of the wild boar population is often unknown but known to vary by density and size. The survey design often targets the overall mean disease prevalence; however, CSF prevalence is known to differ spatially as any contagious infection does. The sampling for diagnostic testing is based on hunted animals, however, hunting is known to be not random neither the disease is.

To what extend do such natural complexities impede the sensitivity of the survey system? Or, to what extend is the sensitivity of surveillances systems impeded by the violation of the assumed uniformity of distributions of wild boars, infection, and sampling. The impeded survey was simulated with a model to assess the resulting sensitivity of sampling systems that monitor low prevalent CSF based on samples provided by hunting.

**The efficacy of surveillance and control measures in wild boars: summary and discussion**

**Monitoring and surveillance systems (MOSS)**

The efficiency of monitoring and surveillance systems (MOSS) has to be evaluated with regard to the changing epidemiological situation (Kramer-Schadt et al., 2009). Basically, there are two main tasks:

1. Task 1. Driving and determining the optimal control decisions. The efficacy of available surveillance is to determine and drive decisions on the optimal control actions specifically during onset and off-set of an outbreak in a certain area (i.e. efficacious to speed-up detection; efficacious to determine affected area, efficacious to follow up the spatialtemporal spread; efficacious to demonstrate termination of an outbreak);
2. Task 2. Controlling the quality and performance of the specified control measures. The efficacy of available surveillance activities is to assure quality and performance of control actions taken.

**Efficacy to solve Task 1 (i.e. driving control decisions):**

In principle the information gathered for this report indicates that CSF surveillance, potentially, should be efficacious in solving Task 1, based on:

- The existing sound laboratory basis for confirmation of the disease from field samples with the recent diagnostic methods (i.e. rRT-PCR).
- The emerging CSF outbreak which is related to a mortality event that would provide a long term warning system based on virological data. The system can be extended by surveying the once the infected area based on targeted sampling and hunting activities.
- The scientific knowledge to design sampling issues where this is necessary, and to provide statistical evidence on the termination of an outbreak given that vaccination has stopped.
- The existing MOSS for the particular situation of areas where classical swine fever is suspected to occur or has been confirmed in wild boar (2002/106/EC).

However, the survey of MS indicated difficulties in identifying a consistent scheme of MOSS that are applied across all MS. In addition, some of the applied strategies in the individual MS appear lacking the focus on the local disease situation under consideration.

**Efficacy to solve task 2 (i.e. monitoring quality of intervention and control success):**

The information gathered for this report demonstrates very clearly that surveillance activities are less efficacious in solving Task 2, particularly, when vaccination is applied. The monitoring of success of oral vaccination and the ability to demonstrate disease freedom after a CSF outbreak have been limited due to biological and practical issues:

- The missing ability to differentiate antibodies as a result of natural infection by field virus or from oral vaccination.
- The difficulties to prove freedom from disease without access to the full host population, as usual in wildlife.
- The difficulty to even investigate potential freedom from disease during continued vaccination.

In summary there are two issues that hamper efficacy of the CSF surveillance:

- Lack of a harmonised and complete MOSS for CSF in wild boars which is logically consistent for all disease situations and on the long run capable to be used by all MS.
- Lack of full set of techniques to permanently monitor control performance in vaccinated areas without an operable DIVA vaccine.

"Safety" of fresh meat from CSF field virus derived from emergency vaccinated domestic pigs

General part

An assessment is needed on the safety of fresh meat from pigs vaccinated during an emergency vaccination using 'conventional' live attenuated or marker vaccines after an outbreak of CSF in domestic pigs. There is always the possibility, that CSFV-infected pigs are not recognised and that they are slaughtered during the applied control strategy. Consequently infected meat may go into trade. By definition "unrecognised" pigs are not registered as infected. Despite numerous outbreaks of CSFV that have occurred in Member
States in recent years there are no scientifically sound figures available about the absence of CSFV in fresh meat after the implementation of the non-vaccination strategy according to EU legislation.

It is generally believed that the current control of CSF in domestic pigs without the use of vaccination is the gold standard in terms of safety. Emergency vaccination-to-live was never practiced until 2005 and consequently there are limited available data to assess its potential impact on the spread of the virus. Such vaccination-to-live campaigns that have been implemented in Romania in the last few years have so far not produced sufficient data that can be used to answer the question for safety of the fresh meat derived from vaccinated animals. Consequently, simulation modelling was employed to address the question on the safety of fresh meat as a consequence of a CSF outbreak control strategies with and without vaccination.

Modelling to support control planning is directed at understanding the consequences of the available control tools and scenarios. Identification of misperceptions as well as the shift of intuition towards knowledge is the dominant benefit. A prerequisite of the model-based risk assessment is the identification of established or alternative control processes. Subsequently, the implementation of conceptual models and risk quantification in a simulation tool will allow for experimental evaluation of consistency and logical consequences.

Conceptually, infected pigs could only go into the meat production chain after lifting of the measures taken to eradicate the disease, i.e. after completion of all clinical and laboratory investigations. (Council Directive 2001/89/EC; alternative proposals see Depner et al., 2005). According to legislations such final investigation, hereafter “the final screening”, is supposed to be done 30 days after the very last case detection (Council Directive 2001/89/EC). If all tests of the final screening of the candidate zone score negative after this time, then an end of outbreak is declared and (vaccinated) animals from the area can be slaughtered.

In addition to errors in handling or storage of the vaccine non-compliance in administration or individual pig related factors might also reduce overall efficacy of emergency vaccination. In an ideal application of the emergency vaccination concept, such non-compliances are usually not considered; however, in the field they might have a certain influence (Terpstra and Wensvoort, 1987).

If it is assumed that the emergency vaccination procedure is perfectly practised, then two events must happen before an infected animal might be slaughtered and fresh meat from infected pigs is produced: (1) an infected herd has to escape clinical diagnosis (“hazard herds”) before the final screening starts and (2) this herd is not detected during the final screening due to sample selection or false negative laboratory diagnostic results. Risk assessments have to disentangle both aspects, i.e. failure to detect the disease and errors during final screening. See concepts used in the current text below.

In non-vaccinated herds, infection would lead to an epidemic multiplication of infected animals (see Bergevoet, 2007; Klinkenberg, 2003). Hence, time span between infection and final diagnostic screening determines within herd prevalence at screening, and hence also the risk of false negative final screening results.

In vaccinated herds the time period between vaccination and infection, or infection and subsequent vaccination is crucial, i.e. CSFV may infect herds as long as the animals are not fully protected after administration (“infected before protection”) or herds may become infected before vaccination. In contrast to non-vaccinated herds, the epidemic multiplication in these cases will be slow or even stop when most vaccinated pigs become protected. Thus outbreaks in vaccinated herds are markedly limited in terms of the number of affected animals, virus spread, and signs of disease. Therefore, “infected and vaccinated” herds have a lesser chance to be detected during the time of restriction compared to non-vaccinated herds, where already 70% were found based on clinical signs. During the final screening
procedure that precedes the lifting of restrictions again the very small number of infected animals in “infected and vaccinated” herds again will limit the chance to diagnose the outbreak in such herds. Since the final screening procedure, according to the current legislation, takes place not earlier than 30 days after detection of the last outbreak, the number of virus-positive animals in vaccinated infected herds will be even smaller, because infected animals are either recovered or dead (Bergevoet et al., 2007; Beer et al., 2006).

In the following two sections the related risk for fresh meat will be assessed and appropriate diagnostic procedures to avoid this risk will be evaluated.

**Schemes applied to detect field virus in fresh meat**

**Monitoring at lift-up**

In this section only the monitoring at final screening is considered. In general, the more monitoring is implemented the lower is the risk of missing herds that contain virus- or antibody-positive animals during this procedure.

The efficiency of the monitoring is directly related to the organs sampled, sample number and sensitivity and specificity of the diagnostic systems used. Following the determination of an appropriate control strategy fine-tuning of screening protocols has to be done (see for example sample selection strategies proposed by Bergevoet et al., 2007).

All schemes should use real-time RT-PCR for virus detection and ELISA-systems for antibody investigation (see 9.2.2). The general sampling system for final screening has the aim of detecting a threshold prevalence of e.g., 5% at 95% confidence while covering the herd structure, e.g. by sampling each pen of the holding. This design is necessary because CSF occurs clustered in structured holding, instead of being homogeneously distributed. Bergevoet et al. (2007) simulated the investigation of e.g., 60 samples for farms of up to 600 animals. In larger herds 10% of the animals are sampled, by taking at least one sample per pen. Such practically oriented screening might be purposeful for identification of infected animals in a post-vaccination area.

Targeted sampling of animals with signs of disease, e.g., fever will enhance monitoring efficiencies. In particular the identification of chronically infected animals with characteristic clinical signs will be facilitated.

Without testing all animals, the risk for fresh meat due to the chance of missing infected animals (sample selection) can not be completely avoided. In case of local emergency vaccination the animals of concern, i.e. animals either containing virus or having antibodies against field virus, are expected to be very rare. Thus practical threshold prevalence levels would seldom be met in these herds. Hence, without testing all animals, the complete detection of all herds of concern would be to certain extent a random event (Bergevoet et al., 2007). Therefore, and facilitated by the availability of new diagnostic test methods for the detection of CSFV at least a considerable increase of sample number in comparasion with non vaccinated herds, should be considered for implementation in vaccinated herds. But when ever it is practical the testing up to full size of herds under screening will be beneficial.

**Diagnostics**

In summary of what has been described in 2.6, CSFV can be detected in blood samples during the viremic phase. Wild type CSFV-infected pigs are viremic for several days and...
shed virus for up to 3 weeks (see Annex D on viraemia). In addition, the prolonged CSFV detection in tonsils is possible (PCR+ and VI-). In analogy it was shown that PCR is positive for longer periods after infection than VI. Following the viremic phase, CSFV-specific antibodies can be detected using all established antibody detection tests including DIVA ELISA.

There are chronically infected animals which shed CSFV for more than 28 days and extremes are reported up to 120 days. The detection of these animals during final screening is very important to improve safety of meat from emergency vaccinated herds. Luckily, these animals are showing obvious clinical signs making them a prominent target for targeted diagnostics during the final screening.

Due to its sensitivity rRT-PCR has been shown to be a very suitable method for the mass screening of pigs for CSFV. A high throughput and the possibility for automation and pooling samples make it an economical alternative to VI (Depner et al. 2006a, Depner et al., 2007a). Experience has shown that E2-blocking-antibody ELISAs are the best tools for detection of CSF-specific antibodies. In case the marker vaccine is used, the ERNS -antibody-ELISA has to be considered as diagnostic tool in vaccinated herds.

With these tools the detection of a CSF infection is practical from 2 to 5 days post infection with rRT-PCR, and from dpi 14 to 21 onwards with E2-ELISAs. ERNS -antibodies are often not detectable before 21 to 35 dpi. CSF antibodies persist for several years.

The gold standards “virus isolation” and “neutralisation test” are considered as confirmation assays. Nevertheless, positive PCR results do not necessarily mean, that the animal carries infectious CSFV. The actual infectious potential of a sample can only be assessed using virus isolation in susceptible cell cultures or animal inoculation (Table 14). Individual animals tested negative with rRT-PCR in blood can be excluded as source of infectious fresh meat. In non-vaccinated animals, however, this negative test result is valid for only a very short time. Animals may register negative in the very early stages of infection or they may contract infection right after testing. For MLV vaccinated animals the negative test result is valid up to live long. In conclusion, animals that are correctly vaccinated and tested negative in rRT-PCR sufficiently late after administration have to be classified as “zero risk” animals for fresh meat.
Vaccination

As already described before, the more effective a vaccine is, the better protection can be achieved from a possible carrier status and meat contamination. Two types of vaccines are available for emergency vaccination: MLV and E2subV. While MLV is highly efficacious, E2subV is somewhat less efficacious but has the advantage of DIVA properties. The risk from meat of vaccinated and infected animals depends on the type of vaccine used, the field virus strain and the time between vaccination and field infection. Early infections bear a higher risk of viremia, especially for E2subV vaccinated pigs.

If in the field the vaccination is not properly administered, some animals will not be properly vaccinated and hence not becoming protected. Therefore infections in such animals must be detected by the standard surveillance measures or during final screening. If however, a naive pig is MLV vaccinated against CSF, it will be fully protected against infection with CSF virus. Therefore, fresh meat from vaccinated pigs that were tested and PCR-negative sufficiently late after administration has to be classified as "zero risk" material.

In conclusion, every pig not properly vaccinated during an emergency procedure in the field will set back the effective efficiency given for the applied vaccine. In order to reduce the risk for fresh meat vaccine administration procedure has to be as perfect as possible to avoid any non-compliance at the best. Data exist only for preventive campaigns from the eighties and the existing early generation of tools for diagnosis and treatment: There, retrospective analysis estimated non-compliances up to 10% on the individual level in routine vaccination programs of whole populations. This level is most likely markedly lower for localised emergency application and with regard to the improved tools Experiences with other diseases like bluetongue (BT) or avian influenza (AI) suggest much lower non-compliance rates of <5% under more controlled conditions (German field trials for BT and AI; Beer, pers. communication). In order to further reduce the overall risk for fresh meat efficient biosecurity measures have to be implemented throughout the vaccination process, e.g., veterinarians moving from farm to farm, use of sterile instruments. When using MLV the critical period for

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infection is short because of the rapid onset of protection, thus the danger of cross infections is relatively low compared to E2SubV. For the use of latter biosafety measures during a vaccination campaign have to be as strict as for farm visits of non-vaccinated units in the protection zone.

**Interpretation and discussion**

Emergency vaccination is a valuable additional option for the control of a CSF outbreak situation. Both types of vaccines (MLV and E2subV) have to be taken into consideration, and the diagnostic systems have to be adapted to the selected vaccine type. However, independently from the vaccine type, testing of herds in an outbreak region for CSFV by using real-time RTPCR assays is a basic requirement for the detection of circulating virus. In contrast, marker serology is more or less restricted to final screening in E2subV-vaccinated farms and sensitive E2-serology to non-vaccinated animals (e.g. breeding animals).

In order to minimize the risk of CSFV infectious fresh meat, CSFV rRT-PCR positive pigs should be identified and destructed before slaughter. No sampling schemes and testing procedures are evaluated to be applied to detect field virus in fresh meat of vaccinated and slaughtered pigs following an emergency vaccination campaign. However, the protective effect of the described and available vaccines minimizes the number of viremic animals due to a block or reduction of transmission, and in an ideal assumption, no test procedures are needed since no CSF-virus-positive animals exist at the time point of slaughter. However, due to the potential multifactorial interactions, the vaccination effects have to be calculated and predicted using models in comparison to the conventional culling strategy (see below).

Furthermore, monitoring measures might be able to reduce the risk of slaughtering pigs potentially carrying CSFV. But effective monitoring systems are difficult to define: As a first prerequisite, all monitoring efforts should be concentrated on animals before slaughtering, since detection of CSFV and CSFV-antibodies in carcasses at the slaughterhouse is neither well investigated nor standardized (sampling, methods etc.) and detection of a positive animal at the slaughterhouse would have severe effects on further slaughtering processes. Therefore, different monitoring schemes are suggested, but field data or experiences are limited. In addition, a census test (testing all animals) is theoretically superior, however, for practical reasons, only spot tests are feasible at the moment. Nevertheless, it can be summarized that two different testing and sampling schemes should be combined: (1) an obligatory, strictly targeted sampling, testing all animals with any suspicious clinical signs by using real-time RT-PCR. These samples would also allow to detect almost all chronically infected animals, and (2) “spot testing” by using an optimized sample number to detect a certain CSFV prevalence.

Here, we want to mention a sample number of 60 for all herds with less than 600 animals, and 10% of the animals for larger farms. Samples should be from all (epidemiological) units and pens. Nevertheless, it has to be taken into consideration, that low prevalences (e.g. < 2%) will not be reliably detected with any of the spot test methods. With the availability of highly sensitive diagnostic methods for the detection of CSFV with a negligible risk of false negatives, a considerable increase of the sample number up to full size or census tests should be considered for implementation. This is even more important when contingency plans rely exclusively on final screening test diagnostics to guarantee safety.
ANNEX IV - Glossary

Age classes: for the purpose of this report four age classes of wild boar were distinguished: 0-6 months, 6 months-1 year, 1 year-2 year >2 years.

Backyard pigs: Domesticated swine that are maintained in small scale operation either of home consumptions or for limited trade.

Basic reproduction ratio of infection (R0): average number of secondary cases due to the introduction of one primary case.

Control zone: This term defines an area around a detected outbreak herd that is subject to control measures: either pre-emptive culling, or emergency vaccination. It typically may extend to 1km or 3km, respectively.

Emergency vaccination: vaccination to control infectious animal diseases that might be implemented in a protective (vaccination-to-live) or a suppressive (vaccination-to-kill) way:
- Protective vaccination (vaccination-to-live) means that vaccinated animals are allowed to live out their normal economic lives and their meat is marketed.
- Suppressive vaccination (vaccination-to-kill) means that animals around an infected farm are vaccinated to reduce the spread of infection and eventually are destructed.

Feral pigs: pigs that are raised in free environment throughout their life without any direct dependence from human beings. However, to be consistent with the terminology used in EU legislation, the notion "feral pig" is used to address feral wild boar.

Free ranging pigs: Pigs that are allowed to range free temporally or all the time their life cycle.

Herd incubation time: time elapsed between the infection of the first individual in a herd and detection of clinical disease in the herd.

Infected before protection (ibp): At the herd level the term characterises units that are vaccinated closely after introduction of the infection, or that contract infection after vaccination but before all animals became protected. On the animal level vaccination of an already infected animal will not change the course of the disease. Therefore infection before protection refers only to an infection after vaccination. The time window of individual susceptibility depends on the type and performance of the vaccine.

Infected herd: In the current report the concept of “infected” refers to any herd that contracted an infection and is not yet detected. Is used to cover all stages of a CSF infection, i.e. animals being in incubation, VI and/or rRT-PCR positive (field virus), as well as only antibody-positive. Particularly vaccinated herds may be “infected” without harbouring virus any more.

Intervention zone: The area around the control zone that is subject to standstill (e.g. 10km).

Final screening for lift-up: The diagnostic procedure that precedes a lift-up decision. Usually after 30 days (Directive 2001/89/EC) final screening starts and restrictions are completely lifted when results are negative. Often the lift-up, in practice, comprises the whole intervention zone although some sub-regions may have been much longer without newly detected outbreaks. The rationale of the lift-up time is to ensure that sufficient time elapses for the detection of all infected non-vaccinated herds. In case of vaccinated herds accidentally infected animals are expected to have recovered or died.

Furthermore, in the Council Directive of 16 December 2002 laying down the animal health rules governing the production, processing, distribution and introduction of products of animal origin for human consumption 2002/99/EC a further important point is mentioned: ‘All stages of the production, processing and distribution’ means any stage from and including the primary production of a food of animal origin, up to and including its storage, transport, sale or supply to the final consumer.

Metapopulations: subpopulations with limited contacts with other subpopulations.

Overall High risk period (HRP): defined by two different time periods: (1) HRP-1, the period between the introduction of CSFV into a region and the first detection of infection and (2) HRP-2, the time between the first animal being detected as infected with CSFV and the establishment of measures.

Wild boar: the wild boar and the domestic pig are members of the same species Sus scrofa. Wild boar is native wild mammals in Europe but they can mate with domestic pigs, so fertile cross-bred pigs exist. Domestic pigs can also become feral. This report is concerned with uncontrolled populations of pigs in the wild, principally wild boar.

ANNEX V - REFERENCES


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