The EFSA Journal **(2009) 933 1-15**

SCIENTIFIC OPINION

Animal health safety of fresh meat derived from pigs vaccinated against Classic Swine Fever[1]

Scientific opinion of the Panel on Animal Health and Welfare

(Question No EFSA-Q-2008-427)

Adopted on 12 December 2008

This opinion, published on 3 July 2009, replaces the earlier version published on 30 January 2009[2].

PANEL MEMBERS

Albert Osterhaus, Anette Bøtner, Bo Algers, Christine Müller-Graf, Daniel Guemene, David B. Morton, Dirk U. Pfeiffer, Donald M. Broom, Frank Koenen, Harry J. Blokhuis, J. Michael Sharp, Jörg Hartung, Mariano Domingo, Martin Wierup, Matthias Greiner, Mo Salman, Moez Sanaa, Patrizia Costa, Philippe Vannier and Ron Roberts.

[1] For citation purposes: Scientific Opinion of the Panel on AHAW on a request from Commission on "Animal health safety of fresh meat derived from pigs vaccinated against Classic Swine Fever". *The EFSA Journal* (2009) 933, 1-16

[2] The year of the Question No EFSA-Q-2008-427 has been corrected from 2007 to 2008. No further changes have been introduced in the opinion or its annexes. To avoid confusion, the original version of the opinion has been removed from the website, but is available on request as is a version showing all the changes made

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SUMMARY

Classical swine fever (CSF) is a disease that has been causing major socio-economic damages in the EU during the last decades. Although considerable progress has been made in the eradication and prevention of the disease, the threat for an epidemic still exists.

The measures to control and eradicate CSF are laid down in Community legislation (Council Directive 2001/89/EC and Commission Decision 2002/106/EC) and are based on stamping-out when CSF is confirmed on pig holdings. Emergency vaccination with 'conventional' live attenuated vaccine or marker vaccine can be used as an additional tool to control and eradicate the disease.

In order to support and to improve the control and eradication measures as regards CSF in domestic pigs, EFSA was requested by the Commission to provide scientific advice on the safety of fresh meat (freedom from field virus) derived from vaccinated pigs. Specifically, two terms of reference were given: a) what is the risk that wild-type CSF virus is present in fresh meat obtained from pigs vaccinated in an emergency situation during an outbreak?; and b) what are the sampling schemes and testing procedures needed to detect field virus in fresh meat derived from such vaccinated pigs?

Two types of vaccines are currently authorised in the EU for CSF:

- A modified live vaccine (MLV, C-strain), which is safe and efficacious, inducing early protection;

- An E2 subunit vaccine (E2subV, marker), safe, allowing serological DIVA, but with lower efficacy than MLV.

The usefulness of emergency vaccination in order to limit disease spread and avoid excessive culling and economic damages remains to be evaluated.

Very limited data related to the presence of CSFV in fresh meat have been generated after the implementation of a non-vaccination strategy. Therefore, a simulation modelling approach was developed to assess the risk of emergency vaccination on the safety of meat compared to the current control of CSF in domestic pigs without vaccination.

CSFV is relatively stable in fresh meat and resists the maturation process in meat. Depending on the strain virulence, the age and breed, infected pigs die rapidly or may recover or can develop chronic infections. Viraemia is variable in duration and degree but it is always linked to the spread of virus to other body tissues including skeletal muscles (meat).

The risk scenario includes two events that must happen before an infected animal is slaughtered and infected meat released: an infected herd has to escape clinical diagnosis before lifting of restrictions and during the final screening such a herd is not detected due to sample selection or false negative laboratory tests.

Three scenarios were evaluated through the model:

1. Stamping-out of CSF detected as infected herds, standstill and pre-emptive culling.
2. Stamping-out of CSF detected as infected herds, standstill and emergency vaccination assuming a rapid protection.
3. Same as 2 but assuming a slower protection and DIVA property.

It was concluded from the model that none of the strategies could reduce the risk to absolute zero. Lifting of restrictions can only occur after a certain time span (longer than the viraemic

period of infected animals) after the last outbreak, thus the number of virus-positive animals in vaccinated infected herds at lifting will be very small.

The model indicated that there is a lower risk of virus in fresh meat after emergency vaccination around outbreaks compared to the conventional strategy including pre-emptive culling, provided that control measures are adjusted to the applied vaccine and test systems.

Adjustments may for instance include size of vaccination area, sampling schemes and timing of lift-up of restrictions. Any non-compliance of control measures will increase the risk of infectious animals remaining after lift-up. Chronically infected pigs are a potential hazard for fresh meat. However, in the risk assessment it was assumed that chronically infected pigs will be identified either by clinical signs and/or laboratory tests.

Clinical diagnosis is unreliable and laboratory confirmation is needed. Highly sensitive and specific diagnostic assays are available to diagnose CSF. Agent detection tests are more suitable during early stages of infection. A positive rRT-PCR diagnosis indicates that an animal has been infected with the wild type or MLV virus but it is not necessarly still infectious. Antibody detection tests are mainly suitable for monitoring and surveillance purposes, but not for early diagnosis of suspect cases.

Concerning the efficiency of the monitoring scheme, it is directly related to the diagnostic systems applied (organ sample, sensitivity, specificity) and to the number of samples taken. In case of selecting samples for rRT-PCR based on fever measurement in vaccinated animals, the sample size should be corrected. In terms of overall efficiency, rRT-PCR for virus detection and ELISA-systems for antibody investigation are the tests of choice. Vaccinated animals, that at an appropriate lift-up time are tested rRT-PCR negative for the wild type virus, can be considered as “zero risk” animals. If only a few animals become infected in a herd, which is possible especially when vaccination is applied, sampling and testing only a proportion of the animals may result in not detecting such an outbreak.

Key words: classical swine fever, meat, rRT-PCR, surveillance, control, monitoring, emergency vaccination, vaccination-to-live, standstill, pre-emptive culling, lifting of restrictions, modelling.

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GLOSSARY

- 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 commercialised.
  - Suppressive vaccination (vaccination-to-kill, or vaccination-to-die) means that animals around an infected farm are vaccinated to reduce the spread of infection and eventually are destructed.
- 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.
- 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 rt-RTPCR positive (field virus), as well as only antibody-positive. Particularly vaccinated herds may be "infected" without harbouring virus any more.
- 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.
- Intervention zone: The area around the control zone that is subject to standstill (e.g. 10km).
- 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.
- Meat: as referred in the Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin: *1.1. 'Meat' means edible parts of the animals referred to in points 1.2 to 1.8, including blood.* 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.*

ABREVIATIONS

- AHAW: Animal Health and Animal Welfare
- Commission: European Commision
- CSF: Classical swine fever
- CSFV: Classical swine fever virus
- DIVA: differentiation infected from vaccinated animals
- EFSA: European Food Safety Authority
- EMEA: European Medicines Agency
- HRP – high risk period
- IFAH: International Federation for Animal Health
- MLV: modified live vaccine
- MS: Member States
- OIE: Office International des Epizooties (World organization for Animal health)
- rRT-PCR: real-time RT-PCR
- RT-PCR: Reverse transcriptase polymerase chain reaction
- SCAHAW: Scientific Committee on Animal Health and Animal Welfare
- VI: Virus isolation

BACKGROUND AS PROVIDED BY THE COMMISSION

Classical swine fever (CSF) is one of the diseases that has caused major socio-economic damages in the EU during the last decades. Although during the last years considerable progress has been made in the eradication and prevention of the disease, the threat for an epidemic still exists. The main reasons are that CSF virus is still present in feral pigs of some Member States (MSs) and that the virus is endemic in the Balkan region, including the MSs Bulgaria and Romania. Control measures are in place for those areas within the EU but this situation remains a constant threat for new outbreaks in the domestic pig population.

The measures to control and eradicate CSF are laid down in Community legislation (Council Directive 2001/89/EC and Commission Decision 2002/106/EC). The main measures consist of:

– Eradication measures, based on stamping-out in case CSF is suspected and confirmed on pig holdings. Emergency vaccination with 'conventional' live attenuated vaccine or marker vaccine can be used as an additional tool to eradicate the disease.

– Control measures in case of the presence of CSF in feral pigs. Emergency vaccination with baits containing a live attenuated vaccine can also been used as an additional tool to control the disease.

Two previous opinions of the former Scientific Committee on Animal Health and Animal Welfare (SCAHAW) laid down in following reports are relevant for the above strategy:

– Report of the Scientific Committee on Animal Health and Animal Welfare on Classical Swine Fever in Wild Boar, Adopted 10 August 1999;

– Report of the Scientific Committee on Animal Health and Animal Welfare on Diagnostic Techniques and Vaccines for Foot-and-Mouth Disease, Classical Swine Fever, Avian Influenza and some other important OIE List A Diseases, adopted 24-25th April 2003.

Oral vaccination of feral pigs has been used by several MSs (DE, FR, LU, SK) as an additional tool to control the disease and was assumed to have been mostly beneficial. Emergency vaccination of domestic pigs after an outbreak has not been used in the EU, except transitionally at the moment in Romania. One of the main reasons for this is that fresh meat from vaccinated animals as a generic rule (a derogation is possible in case of vaccination with a marker vaccine) cannot be traded.

Scientific progress has been made since in diagnostic tools and experiences have been gained in the implementation of the control and eradication measures.

Two issues however remain critical in regard to the control and eradication of CSF:

(1) The efficacy of the control and eradication in infected feral pig populations with or without additional use of emergency vaccination.

(2) The usefulness of emergency vaccination with 'conventional' live attenuated or marker vaccine after an outbreak in domestic pigs in order to limit excessive killings of pigs and the destruction of products and limit the economic damages.

In order to support the Commission and the Member States in improving the control and eradication measures as regards classical swine fever in feral and domestic pigs, scientific advice from EFSA would be required in this area.

TERMS OF REFERENCE AS PROVIDED BY THE COMMISSION

In view of the above, and in accordance with Article 29 of Regulation (EC) N° 178/2002, the Commission asks EFSA:

– To provide an opinion on the efficacy of the available surveillance, hunting and vaccination measures to control and eradicate CSF in feral pig populations, considering the possible use of new diagnostic tests and vaccines;

– To provide an opinion on the safety of fresh meat derived from vaccinated pigs for animal health, both from marker and conventional vaccines, taking into account the different control, eradication and surveillance measures required, including the use of new tools and techniques, such as the RT-PCR.

a) What is the risk that wild type virus is present in fresh meat obtained from pigs vaccinated in an emergency situation during an outbreak?

b) What are the sampling schemes and testing procedures needed to be applied to detect field virus in fresh meat derived from pigs vaccinated following an emergency vaccination during an outbreak? Pig vaccination status considers both marker and conventional vaccines.

ACKNOWLEDGEMENTS

The European Food Safety Authority wishes to thank the members of the Working Group, chaired by the panel member Frank Koenen, for the preparation of the Scientific Report, which has been used as the basis for the two Scientific Opinions related to CSF[3]: Michael Sharp, Mo Salman, Moez Sanaa, Christoph Staubach, Claudio Stroe, Domenico Rutili, Hans Thulke, Marie-Frédérique Le Potier, Martin Beer, Maurice Pensaert (EMEA), Sophie Rossi, Vittorio Guberti, Willie Loeffen, Volker Moennig.

The European Food Safety Authority wishes to thank to all the persons and networks that contributed to the elaboration, distribution and collection of data on wild boar (CSF, population, hunting and vaccination): EFSA's SCO and AMU units, EFSA's focal points, National agencies for veterinary medicines (throught EMEA) and IFAH.

The European Food Safety Authority wishes also to thank DG RES for sharing data and the model concerning CSF in wild boar generated in a FP6 program (FP6-5015599-CSFVACCINE&WILDBOAR).

In accordance with the provisions of Article 30 of the Regulation 178/2002 and Article 59 of Regulation 726/2004, the European Food Safety Agency (EFSA) involved the European Medicines Agency (EMEA) in the preparation of this report. The Panel for Animal Health and Welfare (AHAW) of the EFSA is very grateful for the constructive input from EMEA in this report.

Scientific co-ordination for this mandate from the AHAW unit: Sandra Correia Rodeia, Milen Georgiev and Per Have.

[3] Scientific Opinion of the Panel on AHAW on a request from Commission on "Control and eradication of Classic Swine Fever in wild boar". *The EFSA Journal* (2009) 932, 1-18 and on "Animal health safety of fresh meat derived from pigs vaccinated against Classic Swine Fever". *The EFSA Journal* (2009) 933, 1-16

ASSESSMENT

1. CLASSICAL SWINE FEVER

1.1. Conclusions

- CSFV is relatively stable in fresh meat and resists maturation.
- Clinical signs are highly variable and depend on the strain virulence, the breed and age.
- Differential clinical diagnosis is unreliable and laboratory confirmation is needed.
- Duration of viraemia may last from a few days in subclinically infected (usually older) animals to several months in chronically and persistently infected animals (late onset CSF, transplacental infection).
- Most data on viraemia have been generated in experimentally infected pigs using virus isolation on cell culture. Most experiments were not designed to determine the duration of viraemia.
- rRT-PCR is more sensitive to detect viraemia than VI.
- Viraemia is always indicative for the spread of virus to other body tissues including skeletal muscles (meat).

1.2. Recommendations for future research

- Basic research is important and a prerequisite for further developments of rational intervention strategies (vaccines, anti-virals) and diagnostic methods. Viral protein functions, pathogenesis and host responses are still unclear.
- Identification of reliable in vitro parameters for assessing the virulence of CSFV in its natural porcine host.
- Identification of viral virulence factors that act as virulence factors per se, to the point that their presence or absence will definitively render the virus virulent or not.
- Understanding the mechanisms of the virus-host relations is needed to develop more targeted intervention strategies (vaccines or anti-virals), especially concerning the first hours after infection when the virus starts to circumvent the host immune defence.
- Understanding the mechanism of the early protection induced by live attenuated vaccines like C strain.
- New experiments should be conducted in order to evaluate the duration of viraemia using rRT-PCR.

2. VACCINE

2.1. Conclusions

2.1.1. Modified live virus vaccines (MLV)

- MLV are highly efficacious and safe, inducing early protection (1 to 4 days post vaccination), and can be intramuscularly as well as orally administered.
- Carcasses of animals vaccinated with a MLV and infected later than 4 days post vaccination have a negligible risk to carry infectious CSFV.
- MLV are detectable in the blood of vaccinated animals for a maximum of 14 days by virus isolation and PCR and in tonsils for at least least 42 days by PCR.
- Presently available MLV vaccines do not allow serological DIVA.
- MLV-vaccinated animals can be tested for wild type CSFV by genetic DIVA – (discriminatory PCR).
- Natural immunity and protection after vaccination with MLV completely block viremia and transplacental transmission upon challenge infection with CSF field virus.

2.1.2. E2 subunit recombinant vaccine (E2subV)

- E2subV are fully safe. They are administered parenterally and can not be used for oral immunization.
- Data on E2subV efficacy have provided variable results. Some studies showed that upon challenge a partial block of viraemia can be reached at 21 days after a single vaccination. It is assumed that after 14 days the challenge virus transmission is significantly reduced. However sterile immunity cannot be guaranteed.
- When an infection occurs within 14 days after E2subV vaccination, viraemia cannot be completely blocked.
- E2subV allow DIVA using ERNS-antibody detection and rRT-PCR.
- E2subV is licensed for administration of two shots but, based on published data, one shot is envisaged for emergency vaccinations.

  Note: most of the available information is based in one shot application.

2.1.3. Future vaccines

- Chimeric pestiviruses (MLV with DIVA properties) are the most promising next generation marker vaccine prototypes.

2.2. Recomendations

- The level of the requirements for the current MLV used on domestic pigs and wild boar should be harmonised according to EU standards.
- In domestic pigs, testing of blood samples by PCR should not take place until at least 14 days after MLV vaccination.

- Vaccination of CSFV infected animals does not positively influence the course or the outcome of the infection.

2.3. Recomendations for future research

- Research should be performed for development of additional PCRs differentiating C-strain from wild type CSFV and for developing new techniques to demonstrate of the presence of full-length CSFV genomes.
- The novel marker vaccine strategies should be further evaluated and the most promising strategies should be further promoted (e.g. concerning licensing).

3. DIAGNOSIS

3.1. Conclusions

- In general highly sensitive and specific diagnostic assays are available to diagnose CSF.
- rRT-PCR is currently the most suitable diagnostic tool.
- A positive PCR diagnosis indicates that an animal has been in contact with CSFV, not necessarily that it is infectious at the time of detection.
- Because of the high sensitivity of rRT-PCR, blood samples can be pooled up to 10 samples without decreasing the diagnostic sensitivity of the test.
- Chronically infected animals can be detected before the time of slaughter and it is very unlikely that they will reach slaughter weight.
- All agent detection tests are mainly suitable to detect infected animals during early stages of infection.
- Antibody detection tests are mainly suitable for monitoring and surveillance purposes, but not for diagnosis of suspicions.
- There are currently no confirmation tests available for DIVA diagnostic tests relying on the detection of Erns antibodies.

3.2. Recommendations

- rRT-PCR should be used for CSFV detection during an emergency situation.
- Antibody detection should be used for monitoring as well as for additional herd testing in an emergency situation.
- Additional research is needed to determine the significance of positive PCR test results as for all rRT-PCRs.
- The sensitivity or specificity of the currently available Erns antibodies tests should be improved.
- Further research should be developed in order to allow a better differentiation between animals with CSF positive serology and animals with non-CSF pestivirus serology in particular for double infections.

3.3. Recommendations for future research

- Further research should be done on the validity of pooling samples for the rRT-PCR under different circumstances.

4. CSF MONITORING

4.1. Conclusions

- The occurrence of CSFV infection in herds depends on (1) individual incubation time, (2) age of the pigs (3) contact between animals, units and buildings as well as (4) contact via persons (5) awareness of CSF diagnosis and clinical signs.
- If the currently used virological and serological tests are used in combination with the current sampling strategy it is possible to attest freedom of disease for the given designed prevalence and confidence level in the diagnostic manual. Nevertheless, in case of combined fever measurement and rRT-PCR, the corrected sample size should be increased.
- Attesting freedom of CSFV in areas with a large proportion of small herds is difficult solely by diagnostic testing given non-perfect test properties. However, this will not affect the safety of meat.

4.2. Recommendations

- The high risk period (HRP) should be kept as short as possible by detecting the presence of CSFV as soon as possible in order to stop the spread of the virus. This may be reached by:
    - Raising awareness and specific knowledge among farmers and veterinarians,
    - Enhancing diagnostic procedures,
    - Reinforcing education and information,
    - Implementation of expert systems on the detection of infectious diseases.
- Official guidelines for the laboratory differential diagnostic clarification of unspecific clinical signs in pigs with febrile disease without prejudice could facilitate a voluntary or mandatory exclusion diagnosis of CSF. This would lower the threshold to submit samples for laboratory diagnosis.

5. "SAFETY" OF FRESH MEAT FROM CSF FIELD VIRUS DERIVED FROM EMERGENCY VACCINATED DOMESTIC PIGS

5.1. What is the risk that wild type virus is present in fresh meat obtained from pigs vaccinated in an emergency situation during an outbreak?

5.1.1. Conclusions

- Models were used in order to fill the gaps in the available knowledge which are due to limited experience with emergency vaccination and the complexity of the question.
- None of the considered strategies can reduce the risk in the intervention area to absolute zero.
- Because lifting of restrictions is foreseen to be taken place a certain time span after detection of the last outbreak, the number of virus-positive animals in vaccinated infected herds will be very small since then infected animals are either recovered or dead.
- Modelling indicates that there is a lower risk for the occurrence of infectious virus in fresh meat after emergency vaccination strategy compared to the conventional strategy using a non vaccination policy and pre-emptive culling, provided the eradication strategy is adjusted to the applied vaccine, test systems and to level of compliance. Adjustments may for instance include size of vaccination area, sampling schemes and timing of lift-up of any prohibitions, etc.
- Chronically infected pigs are a potential hazard for fresh meat. However, in the risk assessment it was assumed that chronically infected pigs will be identified either by clinical signs and/or laboratory tests.
- Any lower compliance of the application of control methods will increase the risk for infectious animals remaining after lift-up.
- The properly designed and implemented emergency vaccination strategy together with the targeted search of chronically infected animals in vaccinated herds during final screening is expected to cause a lower risk for fresh meat than the conventional non-vaccination strategy.

5.1.2. Recommendations

- Eradication strategies should be evaluated before being used in the field, e.g. in stochastic models. Specific characteristics of the proposed strategy are the input for such models. Based on the outcome of such models, certain measures may need to be adjusted (e.g. vaccination radius, testing schemes, time of lift-up, etc).
- The issue of sufficient and well trained human resources to control an outbreak in particular in high density areas with vaccination should be subject of further analysis.

5.1.3. Recommendations for future research

- The impact of the level of compliance on the performance and safety of emergency control strategies should be urgently investigated.

5.2. What are the sampling schemes and testing procedures needed to be applied to detect field virus in fresh meat derived from pigs vaccinated following an emergency vaccination during an outbreak? Pig vaccination status considers both marker and conventional vaccines.

5.2.1. Conclusions

- The efficiency of the monitoring scheme is directly related to the diagnostic systems applied (organ sample, sensitivity, specificity) and the number of samples taken.
- In terms of overall efficiency real-time rRT-PCR for virus detection and ELISA-systems for antibody investigation (see part "diagnostics") are the tests of choice.
- In case only a few animals become infected in a herd, which is possible especially when vaccination is applied, sampling and testing only a proportion of the animals present may result in not detecting such an outbreak.
- Final screening in MLV vaccinated herds is possible only with rRT-PCR.
- Vaccinated animals that at an appropriate lift up time are tested rRT-PCR negative have to be classified as "zero risk" animals.

5.2.2. Recommendations

- Targeted sampling, i.e. animals with signs of disease like fever, will enhance monitoring efficiency.
- Practically oriented screening schemes are needed for identification of infected animals in a post-vaccination area.
- The requirement for the need of full herd testing should be considered when planning final screening schemes. It would be beneficial to test each animal in herds that are being screened.

5.2.3. Recommendations for future research

- Further evaluation of emergency vaccination needs an aligned evaluation context for all candidate vaccines (efficacy, safety, time till protection).
- The role of animals infected before protection should be the focus of future research. Here especially the possibility of chronic infections in vaccinated pigs should be investigated.

Annex to The EFSA Journal **(2008) 932, 1-18 and 933, 1-16**

SCIENTIFIC REPORT

Control and eradication of Classic Swine Fever in wild boar[1] and

Animal health safety of fresh meat derived from pigs vaccinated against Classic Swine Fever[2]

Scientific opinions of the Panel on Animal Health and Welfare

(Question No EFSA-Q-2007-200)

(Question No EFSA-Q-2008-427)

Adopted on 12 December 2008

This report, annexed to these opinions, published on 3 July 2009, replaces the earlier version published on 30 January 2009[3].

PANEL MEMBERS

Albert Osterhaus, Anette Bøtner, Bo Algers, Christine Müller-Graf, Daniel Guemene, David B. Morton, Dirk U. Pfeiffer, Donald M. Broom, Frank Koenen, Harry J. Blokhuis, J. Michael Sharp, Jörg Hartung, Mariano Domingo, Martin Wierup, Matthias Greiner, Mo Salman, Moez Sanaa, Patrizia Costa, Philippe Vannier and Ron Roberts.

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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.
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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.

- Meat: as referred in the Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin: *1.1. 'Meat' means edible parts of the animals referred to in points 1.2 to 1.8, including blood.* 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 are 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.

ABREVIATIONS

- AHAW: Animal Health and Animal Welfare
- BDV: Border disease virus
- BVDV: Bovine viral diarrhea virus
- CI: Confidence Interval
- Commission: European Commision
- CP: Cytopathogenic
- CSF: Classical swine fever
- CSFV: Classical swine fever virus
- Cull: 1km pre-emptive culling (Scenario description)
- DIVA: differentiation infected from vaccinated animals
- E2Vac14: as Vac14 but all tests in non-vaccinated herds are performed with E2-ELISA instead of rRT-PCR (Scenario description)
- EFSA: European Food Safety Authority
- EMEA: European Medicines Agency
- HRP: High risk period
- Ibp: Infected before protection
- IFAH: International Federation for Animal Health
- MLV: modified live vaccine

- MOSS: Monitoring and surveillance systems
- MS: Member States
- MSEIR: M=maternal immunized; S=Susceptible, E=latent; I= Infectious; R=recovered
- NCP: Non cytopathogenic
- NVT: Neutralisation Virus Test
- OIE: Office International des Epizooties (World organization for Animal health)
- R0: Basic reproduction ratio of infection
- RT-PCR: Reverse transcriptase polymerase chain reaction
- rRT-PCR: real-time RT-PCR
- SCAHAW: former Commission "Scientific Committee on Animal Health and Animal Welfare"
- SEIR: S=Susceptible, E=latent; I= Infectious; R=recovered
- Vac14: 3km vaccination with the slow protective DIVA-vaccine (Scenario description)
- Vac4: 3km vaccination with the fast protective vaccine (Scenario description)
- VI: Virus isolation
- WRMSE: Weighted root mean square error

BACKGROUND AS PROVIDED BY THE COMMISSION

Classical swine fever (CSF) is one of the diseases that have caused major socio-economic damages in the EU during the last decades. Although during the last years considerable progress has been made in the eradication and prevention of the disease, the threat for an epidemic still exists. The main reasons are that CSF virus is still present in feral pigs of some Member States (MS) and that the virus is endemic in the Balkan region, including the MS Bulgaria and Romania. Control measures are in place for those areas within the EU but this situation remains a constant threat for new outbreaks in the domestic pig population.

The measures to control and eradicate CSF are laid down in Community legislation (Council Directive 2001/89/EC and Commission Decision 2002/106/EC). The main measures consist of:

– Eradication measures, based on stamping-out in case CSF is suspected and confirmed on pig holdings. Emergency vaccination with 'conventional' live attenuated vaccine or marker vaccine can be used as an additional tool to eradicate the disease.

– Control measures in case of the presence of CSF in feral pigs. Emergency vaccination with baits containing a live attenuated vaccine can also been used as an additional tool to control the disease.

Two previous opinions of the former Scientific Committee on Animal Health and Animal Welfare (SCAHAW) laid down in following reports are relevant for the above strategy:

– Report of the Scientific Committee on Animal Health and Animal Welfare on Classical Swine Fever in Wild Boar, Adopted 10 August 1999;

– Report of the Scientific Committee on Animal Health and Animal Welfare on Diagnostic Techniques and Vaccines for Foot-and-Mouth Disease, Classical Swine Fever, Avian Influenza and some other important OIE List A Diseases, adopted 24-25th April 2003.

Oral vaccination of feral pigs has been used by several MS (DE, FR, LU, SK) as an additional tool to control the disease and was assumed to have been mostly beneficial. Emergency vaccination of domestic pigs after an outbreak has not been used in the EU, except transitionally at the moment in Romania. One of the main reasons for this is that fresh meat from vaccinated animals as a generic rule (a derogation is possible in case of vaccination with a marker vaccine) cannot be traded.

Scientific progress has been made since in diagnostic tools and experiences have been gained in the implementation of the control and eradication measures.

Two issues however remain critical in regard to the control and eradication of CSF:

(1) The efficacy of the control and eradication in infected feral pig populations with or without additional use of emergency vaccination.

(2) The usefulness of emergency vaccination with 'conventional' live attenuated or marker vaccine after an outbreak in domestic pigs in order to limit excessive killings of pigs and the destruction of products and limit the economic damages.

In order to support the Commission and the Member States in improving the control and eradication measures as regards classical swine fever in feral and domestic pigs, scientific advice from EFSA would be required in this area.

TERMS OF REFERENCE AS PROVIDED BY THE COMMISSION

In view of the above, and in accordance with Article 29 of Regulation (EC) N° 178/2002, the Commission asks EFSA:

– To provide an opinion on the efficacy of the available surveillance, hunting and vaccination measures to control and eradicate CSF in feral pig populations, considering the possible use of new diagnostic tests and vaccines;

– To provide an opinion on the safety of fresh meat derived from vaccinated pigs for animal health, both from marker and conventional vaccines, taking into account the different control, eradication and surveillance measures required, including the use of new tools and techniques, such as the RT-PCR.

a) What is the risk that wild type virus is present in fresh meat obtained from pigs vaccinated in an emergency situation during an outbreak?

b) What are the sampling schemes and testing procedures needed to be applied to detect field virus in fresh meat derived from pigs vaccinated following an emergency vaccination during an outbreak? Pig vaccination status considers both marker and conventional vaccines.

APPROACH

In order to reply to Commission's request for scientific opinion on Classical Swine Fever a group of experts was invited to draft this scientific report that supported the AHAW Panel to draft an Opinion with conclusions and recommendations. Different types of data were collected in order to reply to the mandate. The approach to reply to each of the ToR was different and in some cases involved different expertise areas/ experts. Therefore, it was decided by the AHAW Panel to split the work according to the two ToR into two scientific opinions, keeping however the present report common to both opinions.

ACKNOWLEDGEMENTS

The European Food Safety Authority wishes to thank the members of the Working Group, chaired by the panel member Frank Koenen, for the preparation of this Scientific Report, which has been used as the basis for the two Scientific Opinions related to CSF[4]: Michael Sharp, Mo Salman, Moez Sanaa, Christoph Staubach, Claudio Stroe, Domenico Rutili, Hans Thulke, Marie-Frédérique Le Potier, Martin Beer, Maurice Pensaert (EMEA), Sophie Rossi, Vittorio Guberti, Willie Loeffen, Volker Moennig.

The European Food Safety Authority wishes to thank to all the persons and networks that contributed to the elaboration, distribution and collection of data on wild boar (CSF, population, hunting and vaccination): EFSA's SCO and AMU units, EFSA's focal points, National agencies for veterinary medicines (throught EMEA) and IFAH.

The European Food Safety Authority wishes also to thank DG Research for sharing data and the model concerning CSF in wild boar generated in a FP6 program (FP6-5015599-CSFVACCINE&WILDBOAR).

In accordance with the provisions of Article 30 of the Regulation 178/2002 and Article 59 of Regulation 726/2004, the European Food Safety Agency (EFSA) involved the European Medicines Agency (EMEA) in the preparation of this report. The Panel for Animal Health and Welfare (AHAW) of the EFSA is very grateful for the constructive input from EMEA in this report.

Scientific co-ordination for this mandate from the AHAW unit: Sandra Correia Rodeia, Milen Georgiev and Per Have.

[4] Scientific Opinion of the Panel on AHAW on a request from Commission on "Control and eradication of Classic Swine Fever in wild boar". *The EFSA Journal* (2009) 932, 1-18 and on "Animal health safety of fresh meat derived from pigs vaccinated against Classic Swine Fever". *The EFSA Journal* (2009) 933, 1-16

ASSESSMENT

1. INTRODUCTION

The measures to control and eradicate CSF are laid down in Community legislation (Council Directive 2001/89/EC and Commission Decision 2002/106/EC). The main measures consist of:

- Eradication measures, based on stamping-out in case CSF is confirmed on pig holdings. Emergency vaccination with live attenuated vaccine or marker vaccine can be used as an additional tool to eradicate the disease.
- Control measures in case of the presence of CSF in feral pigs. Preventive vaccination with baits containing a live attenuated vaccine can also been used as an additional tool to control the disease.

Although new developments in diagnostic tools continue to emerge and experience accumulates in the implementation of control and eradication measures, two issues remain critical in regard to the control and eradication of CSF:

- The efficacy of the control and eradication in infected feral pig populations with or without additional use of preventive vaccination.
- The usefulness and risk of emergency vaccination with live attenuated or marker vaccine after an outbreak in domestic pigs in order to limit excessive killing of pigs and destruction of products and limit the economic damages.

In order to support the Commission and the Member States in improving the control and eradication measures as regards classical swine fever in feral and domestic pigs, scientific advice from EFSA was asked on these two issues.

Emergency vaccination to control infectious animal diseases may be implemented either in a protective (vaccination-to-live) or a suppressive (vaccination-to-kill) way. Protective vaccination means that vaccinated animals are kept to the end of a normal production cycle and their meat eventually marketed. Suppressive vaccination means that animals around an infected farm are vaccinated to reduce the spread of infection and to gain time but that they will eventually be destroyed.

Based on the terms of reference in the mandate a set of questionnaires were developed to collect specific information considered relevant in responding to the mandate. Questionnaires on CSF vaccines were distributed to EFSA's Focal Points, CSF NRL networks, EMEA and IFAH. Questionnaires on hunting practices and CSF vaccination of wild boar were distributed to EFSA's Focal Points and to CSF NRL networks. After agreement from participating member states data were also extracted from the EU CSF wild boar data base.

The review on CSF carried out by a consortium in fulfilment of an EFSA art. 36 grant (CFP/EFSA/AHAW/2007/02) provided additional information to this opinion, in particular concerning vaccination, viraemia, epidemiology and diagnostics. The scientific knowledge and modelling concerning CSF in wild boar and the development of live marker vaccines and diagnostics generated in a FP6 program (FP6-5015599-CSFVACCINE&WILDBOAR) was also integrated in the report.

To complement the information gathered from the questionnaires and in order to fill gaps in available knowledge due to limited experience with emergency vaccination, modelling was applied for several scenarios covering vaccination and surveillance:

- In order to evaluate the sampling schemes a software written by FLI (Riems, Germany) is used. The results were cross-checked with FreeCalc Software version 2 (Cameron and Baldock, 1998a).
- For the evaluation of the efficacy of CSF control in wild boar a continuous metapopulation compartmental model based on the approach described by Hanski e Gilpin (1997) and developed in the framework of the FP6-5015599-CSFVACCINE&WILDBOAR was applied.
- For the demonstration of freedom from CSF in wild boar populations a spatial simulation model was applied to illustrate the importance of non-uniformity or clustering of the wild boar population, sampling intensity (i.e. hunting), disease distribution and sensitivity and specificity of diagnostic methods.
- For the question on risk of field virus in vaccinated pigs the assessment was based on a simulation model developed to simulate CSF outbreaks in geographic landscapes with pig holdings (Thulke et al., 2007).

2. CLASSICAL SWINE FEVER

2.1. The virus

Classical swine fever virus (CSFV), bovine viral diarrhea 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, cytopathogenic (CP) and non cytopathogenic (NCP) according to their cytopathogenicity in cell culture. Their genome of about 12.5 to 16.5 kb encodes for a single polyprotein (Meyers et al., 1989) : NH2-(N^{pro}-C-E^{RNS}-E1-E2-p7-NS2/3-NS4A-NS4B-NS5A-NS5B)-COOH, which is co and post-translationally 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, E^{RNS}, 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 nucleocapsid and coat, which is composed of numerous proteins C while the surface is made out of E^{RNS}, E1 and E2 in homodimeric (E^{RNS}, E2) or heterodimeric (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, E^{RNS} has no transmembrane 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 nonstructural proteins including an N-terminal proteinase (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, CSFV 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).

2.1.1. Antigenic and genetic typing:

Even though, CSFV is a very stable RNA virus (Vanderhallen et al., 1999), a recent study (He et 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 E^{RNS} glycoproteins 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 phylogenetic 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-Lepoureau 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 (Schirrmeier 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.

2.1.2. 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 non-virulent 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 E^{RNS} region (position 276, 476 and 477). Similar to E2, glycosylation sites (position 269) in the E^{RNS} have been found to have an influence on virulence in swine (Sainz et al., 2008). Abrogation of the RNAse activity of E^{RNS} by mutating codons 297 and 346 of the E^{RNS} 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.

2.2. Clinical signs

Historically, different levels of virulence have been reported from peracute, 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).

2.2.1. Domestic pigs

Piglets develop more evident clinical signs than the adults. 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 anorexia, lethargy, conjunctivitis, respiratory signs and constipation followed by diarrhea (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, diarrhea, 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 anorexia. 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 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 pyrexic 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 Insemination boars may thus be of special epidemiological relevance for the dissemination of the CSF virus as clinical symptoms are mild.

2.2.2. Feral pigs or wild boars

In general, most clinical and pathological signs described for domestic pigs are also observed after infection of wildboar 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 petechiation. 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 boars 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 diarrhoea 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).

2.3. 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 wityh other pestiviruses, 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 pestiviruses are non-cytopathic in vitro, some BVD viruses from mucosal disease cases or some CSFV strains are also cytopathogenic in vitro, and this cytopathogenicity 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 noncytopathic in vitro, it has been suspected that the serious lesions seen *in vivo* were linked to immunopathological 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). E^{RNS} 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 monocyte-macrophage-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 monocytic 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. Further details of stages of infection are given in chapter 3.

2.4. 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 proteine E^{RNS} and the non-structural protein NS3 (Rau et al., 2006). In contrast, detection of NS3-antibodies as well as one of the E^{RNS}-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-specifc 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 titers 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”.

2.4.1. Types of vaccines for the potential use of emergency vaccination

The following description about classical CSFV-vaccines is based on the previous 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.

See Table 1 based on the data received by EFSA Questionnaire.

2.4.1.1. Live attenuated/modified live (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).

2.4.1.2. 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, E^{RNS}, 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 E^{RNS} glycoproteins upon infection (Moormann et al., 2000). Two differential diagnostic E^{RNS} antibody ELISA tests (E^{RNS}-antibody ELISAs) are commercially available (SCAHAW, 2003, Blome et al., 2006.

2.4.2. Efficacy

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).

Table 1. Classical swine fever vaccines registered or authorised in Europe[5]

Pig Type	Vaccine type	DIVA[6]	Strain	Comercial name	Producer	Registred or authorized
wild boar	MLV[7] oral	No	C - strain[8]	RIEMSER Schweinepest - oral vakzine	Riemser Arzneimittel AG	Bulgaria, France, Germany, Latvia, Luxembourg, Romania, Slovakia,
				SUICINPEST	Istituto Zooprofilattico Sperimentale Perugia	Italy
			Thiverval strain IP-77	PESTIVAC M	SNI Pasteur SA Bucharest	Romania
domestic pigs	MLV parenteral	No	C - strain	RIEMSER[9] Schweinepestvakzine	RiemserArzneimittel AG	Germany
				SUICINPEST	IZS Perugia	Italy
				PESTIFFA	Merial, France	Belgium, Netherlands, Spain
				CZV cepa china	CZ Veterinaria S.A.Spain	Spain
				PORKIRIN	Laboratorios Ovejero S.A.	
			Thiverval	COGLAPEST	CEVA-Phylaxia Co. Ltd.	Hungary, France
			Thiverval strain IP-77	PESTIVAC	SNI Pasteur SA Bucharest	Romania
			Thiverval strain RP/ 93	ROMPESTIVAC	Romvac Company S.A.	Romania
	E2 subunit	Yes	n.a. [10]	PORCILIS PESTI	Schering Plough-Intervet	EU level

[5] data received by EFSA questionnaire
[6] DIVA differentiation infected from vaccinated animals
[7] MLV: modified live virus vaccine
[8] C-strain: Chinese strain
[9] current vaccine in the EU vaccine bank
[10] n.a. origin of strain is not available

2.4.2.1. Live attenuated/modified live (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 CSFV 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 (Bironi and Leunen, 1988). It was also reported that MLV should contain at least 100 PD_{50} to prevent carriers (Leunen and Strobbe, 1977). A report using an oronasal challenge one week after vaccination demonstrated protection with a MLV containing 160 PD_{50} (Bironi 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 (Bironi 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 C-strain (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).

2.4.2.2. 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 water-oilwater 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 (Uttenthal 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.

2.4.3. 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.

2.4.3.1. Live attenuated/modified live (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 (Bironf and Leunen, 1988, Suradhat 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 pestiviruses. Therefore, the risk has become very low to negligible (Hoffmann et al., 2005, 2006; McGoldrick et al., 1998, 1999; Deregt et al., 2006).

2.4.3.2. 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

2.4.4. Differentiation of infected from vaccinated animals

Serological DIVA or marker tests are only available for the E2subV. The test of choice is blocking ELISAs for the detection of E^{RNS}-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 p.i. 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 CSFV-specific antibody titres are persisting for month or even years.

2.4.5. Administration of vaccine in the field

2.4.5.1. 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.

2.4.5.2. Wild boar

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).

2.5. Future 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 e t al., 2007). However, registered products will not be available in the next 3 years.

Table 2. Different types of candidates for CSFV DIVA vaccines.

Type of CSFV vaccine	Examples	Marker principle for serology	References
CSFV peptide vaccines	-Single peptides or a mixture of multiple peptides from antigenic domains BC or A of CSFV envelope Protein E2	-Detection of ERNS- or NS3-antibodies specific for CSVF or pestiviruses, e.g. using blocking ELISAs -Detection of CSFV-E2-domain-specific antibodies using immunogenic peptides, not present in the vaccine	Dong et al., 2002, 2005, 2006, Dong et Chen 2006a, 2006b, Liu et al., 2006
DNA vaccines	-Immunization with expression plasmids with complete or partial CSFV-E2-encoding sequences -E2-expressing plasmids, additionally encoding	-Detection of ERNS S- or NS3-antibodies specific for CSVF or pestiviruses, e.g. using blocking ELISAs	Andrew et al., 2000, Yu et al., 2001, Nobiron et al., 2003, Ganges et al., 2005, Liang et al., 2005, Wienhold et al.,2005, Andrew et al., 2006
Viral vector vaccines	-Expression of E2 (complete or partial), integrated into the genome of other viruses (viral vectors), e.g.:vaccinia virus, pseudorabies virus,adenovirus, parapoxvirus.	-Detection of ERNS or NS3-antibodies specific for CSVF or pestiviruses, e.g. using blocking ELISAs	Konig et al., 1995; van Zijl et al., 1991; Hooft van Iddekinge et al., 1996; Mulder et al., 1994; Peeters et al., 1997; Hammond et al., 2000, 2001, 2003; Hahn et al., 2001;
Chimeric pestiviruses	-CSFV-E2-encoding sequences are inserted into a BVDV backbone -BVDV or BDV sequences are inserted into a CSFV backbone	-Detection of ERNS - antibodies specific for CSFV, e.g. with a blocking ELISA -Detection of E2-antibodies specific for CSFV, e.g. with a blocking ELISA	De Smit et al., 2001b; van Gennip et al., 2002; Reimann et al.,2004, Koenig et al.,2007a, 2007b;
Trans-complemented replicons	-Packaged replicons with a deletion in the ERNS-encoding region -Packaged replicons with a deletion in the E2-encoding region	-Detection of ERNS-antibodies specific for CSVF or pesti¬viruses, e.g. using blocking ELISAs -Detection of E2-antibodies specific for CSFV, e.g. with a blocking ELISA	Widjojoatmodjo et al., 2000; Maurer e tal., 2005; Frey e tal. 2006

For all types of CSFV vaccines also a genetic DIVA principle can be used to differentiate vaccinated from infected animals during an outbreak situation (modified from Beer et al., 2007)

2.6. 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)

2.6.1. 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.

2.6.1.1. 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).

2.6.1.2. 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 theoric 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.

2.6.1.3. 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 HRPO-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.

2.6.1.4. 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 E^{RNS}-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).

2.6.1.5. 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.tiho-hannover.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; Vilcek 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).

2.6.2. 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 non-viraemic 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.

2.6.2.1. 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 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 (Uttenthal and Le Potier, personnal 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 viraemie. 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 occurr 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).

2.6.2.2. E^{RNS}-ELISA

The E^{RNS}-ELISA is based on the same principle as the E2-ELISA's, but instead detects antibodies against the E^{RNS} -protein. The E^{RNS} -ELISA's were developed as companion tests for the E2-subunit vaccine (Van Rijn et al., 1999). Two commercially available E^{RNS} -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 (Commision Decision 2003/859/EC, Blome et al.,2006).

The sensitivity of the E^{RNS} -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 E^{RNS}, including live deletion mutants (Widjojoatmodjo et al., 2000). For chimaeric vaccines, that contain E^{RNS} 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 E^{RNS} -test B could be used, as it is CSF-specific, but this test lacks sensitivity (Floegel-Niesmann, 2001).

2.6.2.3. Virus neutralisation test (VNT)

The virus neutralisation test (VNT) is carried out by incubating serum samples in several two-fold 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 comparasion 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 3. Procedures of choice for CSFV diagnosis

Test	Sample type	Sensitivity	Specificity	Feasibility	p.i.d.[11]	Disadvantages	Advantages	Reference [12]
IFT/ IPT	Organ cryostat sections	Medium (75%)*	High with Mabs (99,9%* to 76%**)	Medium to High	Post mortem 4-5	Equipment Experience	Short time	OIE, 2004; *Bouma et al., 2001; *Dewulf et al., 2004.
antigen ELISA	Serum Plasma Blood Homogenate	Low (39%** to 74,7%***)	Low Cross reaction with pestivirus	High	7-12	Specificity Sensitivity Not for individual diagnosis	Short time Automated systems Cost	Depner et al., 1995b; **Dewulf et al., 2004; ***Depner et al., 2007.
Virus isolation (VI) *	Leukocytes plasma whole blood organs	Medium (77%* to 88-95%**)	High (100%***)	Medium	5	Time consuming, Cost Cell culture facilities Autolysed sample Up 10 days for results	Strain recovery Useful for genetic typing/ molecular epidemiology Antigenic typing	OIE, 2004; *Bouma et al., 2001; **Dewulf et al., 2004; ***Koenig et al., 2007a.
RT-PCR	Blood organ serum	High (99%)	High (99%)	High	3-5	Detection of uninfectious virus The need of skilled staff Contaminations Stringent quality control	Results after few hours Useful for genetic typing/ molecular epidemiology Suitable for carcasses	Paton et al., 2000; Aguero et al., 2004; Belak , 2005.
Real Time RT-PCR (rRT-PCR)	Blood organ serum	Very high (100%)	High (99,9%-100%)	High	2	Detection of uninfectious virus The need of skilled staff Stringent quality control Cost	Results after few hours Quantitative results Automated equipment DIVA Suitable for carcasses	Depner et al., 2007; Zhao et al., 2008; Le Potier et al., 2006; Hoffmann, et al., 2005.
Antibody ELISA	Serum	High (98,5%)	Medium to High (98% - 99,5%)	High	12-21	Screening test Qualitative results Cross reactivity resulting in false positive or doubtful	Fast Automated systems DIVA	Colijn et al., 1997; Langedijk et al., 2001.
VNT[13]	Serum	High (98%)	Low/High (99,9%)	Medium	12.14	Cross-neutralising antibodies Time consuming	Quantitative Differential diagnosis	Liess et al., 1976b.

[11] p.i.d. = Post infection time detection (in days)
[12] EU Diagnostic manual for Classical Swine Fever diagnosis, technical part, 3rd draft, June 2007 http://viro08.tiho-hannover.de/eg/index.html (quoted for all tests)
[13] Gold standard

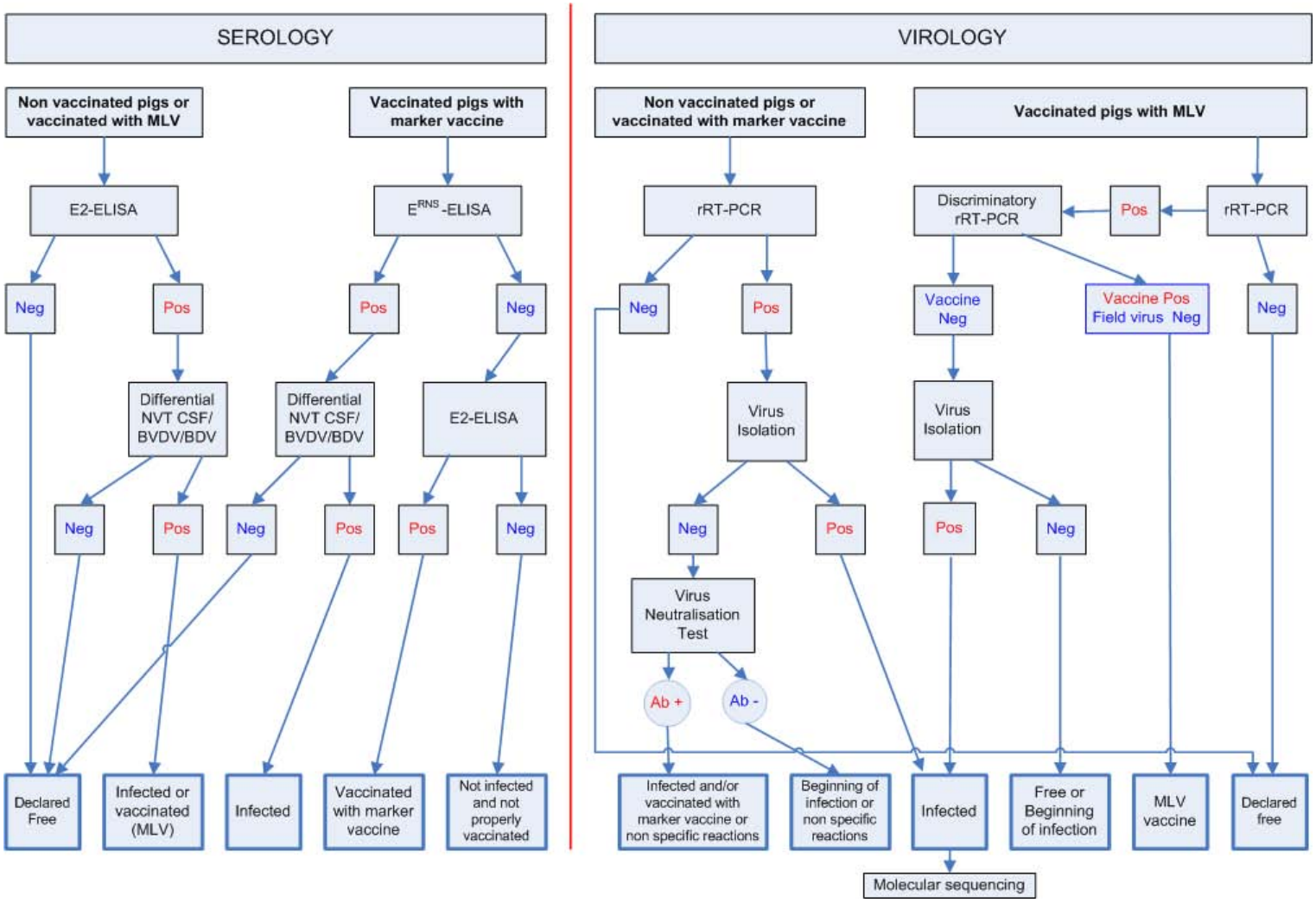


Figure 1. Example of two steps diagnostic procedures for lift-up surveillance routinely used by some CSF NRL

3. CSF MONITORING IN DOMESTIC PIGS

3.1. 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 (see Annex A, Figure 6).

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).

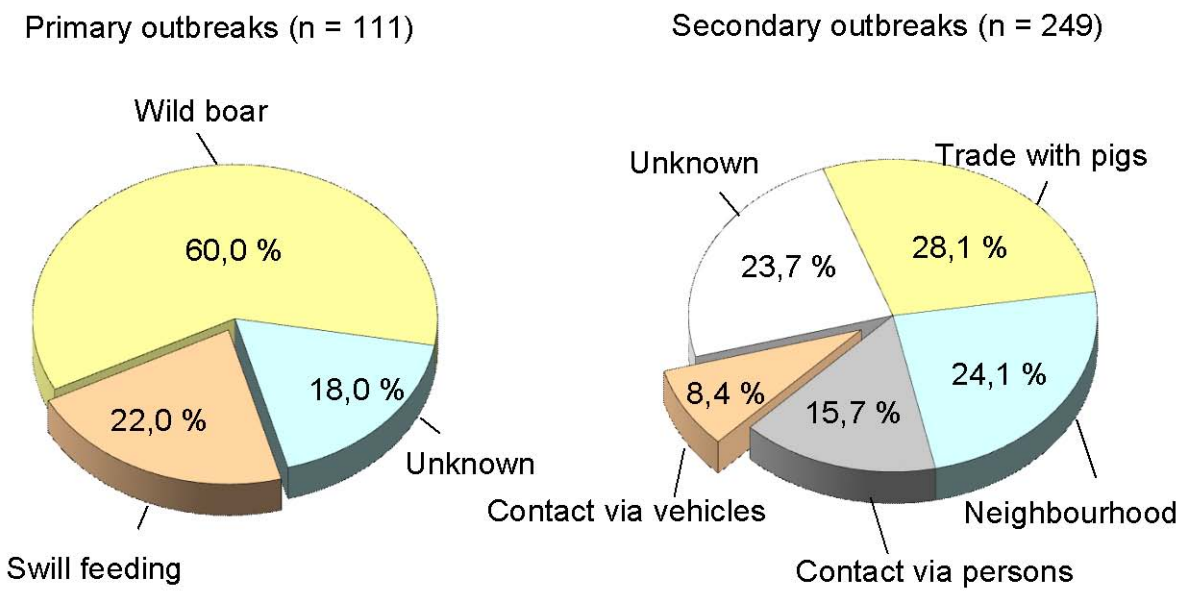


Figure 2. Sources and suspected sources of CSF outbreaks among domestic pigs between 1993 and 02.07.2008 in Germany (updated Teuffert et al., 1997; Fritzemeier et al., 2000)

Table 4. Characteristics for several classical swine fever epidemics in Europe

Country	Year	No. of farms at risk [Eurostat] (year)	No. of outbreaks (SANCO/ 10257/ 2003 –Rev. 8)	Duration of the outbreak (months)	Estimated HRP (weeks)	Source
Belgium	1993/94	15,070 (1993)	52	10	3	Koenen et al. (1996); Vanthemsche (1996)
Belgium	1997	11,630 (1997)	8	2	2	Mintiens et al. (2001)
Germany	1997	141,450 (2000)	23	4	8	Standing veterinary committee, 1997 (Report on outbreaks in Germany); Fritzemeier et al. (2000)
Germany	2006	107,508 (2007)	8	3	10	Standing veterinary committee, 2006 (Report on outbreaks in Germany)
Spain	1997/98	138,990 (1997)	99	17	9	Standing veterinary committee, 1997 (Report on outbreaks in Spain); Greiser-Wilke et al. (2000a)
Spain	2001/02	180,630 (2000)	49	12	7 (Lleida) 4 (Barcelona)	Allepuz et al. (2007)
The Netherlands	1992	26,880 (1993)	8	5	6	Terpstra et al. (1992)
The Netherlands	1997/98	21,010 (1997)	429*	13	6	Elbers et al. (1999)
United Kingdom	1986	32,900 (1986**)	10	3	4	Williams and Matthews (1988)
United Kingdom	2000	11,190 (2000)	16	4	8	Gibbens et al. (2000); Sharpe et al. (2001)

* In the 1997/98 CSF-outbreak in The Netherlands, the negligence towards hygienic measures together with the numerous human and animal contacts of large breeding herds in the 5-6 weeks of high risk caused the spread of virus to at least 36 herds before CSF was first diagnosed (Stegeman et al., 1999).

** MAFF BSE inquiry (www.bseinquiry.gov.uk)

3.2. 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).

3.3. 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).

3.3.1. 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 (see chapters above). The variety of clinical signs, 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.

3.3.2. 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).

3.3.3. 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).

3.3.4. 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.

3.4. 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.

3.4.1. 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 countervailed 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.

3.4.2. 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).

3.4.3. 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.

3.4.4. 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. Evaluation of sampling protocols

Left: Sampling protocols as mentioned in 2002/106/EC, Chapter IV (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)

Reason	Type of holding	Type of sampling	Design prevalence	Conservative sample size	Sensitivity	Specificity	Corrected sample size****	
							50 animals	1000 animals
Suspected holdings (A)	Fattening pigs	Fever measurement + confirmation rRT-PCR	10	29	86.8** (86.9***)	99,9** (9.8***)	26	33
	Breeding pigs		5	59			45	65
	Semen collection centre		all animals	all animals			all animals	all animals
	Fattening pigs	ELISA + VNT (rRT-PCR)	10	29	95 (98.5)	98 (99.9)	21 (22)	25 (29)
	Breeding pigs		5	59			36 (39)	43 (57)
	Semen collection centre		all animals	all animals			all animals	all animals
Culling of confirmed cases (B)	All types	ELISA + VNT (rRT-PCR)	10	29			21 (22)	25 (29)
Preventive culling (C)	Fattening pigs	ELISA + VNT (rRT-PCR)	10	29			21 (22)	25 (29)
	Breeding pigs		5	59			36 (39)	43 (57)
	Semen collection centre		all animals	all animals			all animals	all animals

Reason	Type of holding	Type of sampling	Design prevalence	Conservative sample size	Sensitivity	Specificity	Corrected sample size****	
							50 animals	1000 animals
Movement of pigs to another holding (D.2)	Fattening pigs	Fever measurement + confirmation rRT-PCR	10	29	86,8** (86.9***)	99,9** (9.8***)	26	33
	Breeding pigs		5	59			45	65
	Semen collection centre		all animals	all animals			all animals	all animals
Movement of pigs for slaughter (D.3 + D.4)	Fattening pigs	Fever measurement + confirmation rRT-PCR	20	14	86,8** (86.9***)	99,9** (9.8***)	14	16
	Breeding pigs		5	59			45	103
	Fattening pigs	ELISA + VNT and rRT-PCR	10	29	95 and 98.5	98 and 99.9	21 and 22	25 and 29
	Breeding pigs		5	59			36 and 39	43 and 57
Re-population of farms (E)	Sentinel pigs + Breeding pigs	ELISA + VNT (rRT-PCR)	20	14	95 (98.5)	98 (99.9)	12 (12)	13 (14)
	Complete farm		10	29			21 (22)	25 (29)
Lifting protection zone (F)	Fattening pigs	ELISA + VNT (rRT-PCR)	10	29			21 (22)	25 (29)
	Breeding pigs		5	59			36 (39)	43 (57)
	Semen collection centre		all animals	all animals			all animals	all animals

Reason	Type of holding	Type of sampling	Design prevalence	Conservative sample size	Sensitivity	Specificity	Corrected sample size****	
							50 animals	1000 animals
Lifting surveillance zone (G)	Fattening pigs	ELISA + VNT (rRT-PCR)	10	29			21 (22)	25 (29)
	Breeding pigs		5	59			36 (39)	43 (57)
	Semen collection centre		all animals	all animals			all animals	all animals
Calculation of sample sizes for low prevalences not considered in the diagnostic manual (e.g. 1 % design prevalence)		Fever measurement + confirmation rRT-PCR	1	299	86,8** (86.9***)	99,9** (9.8***)	all animals (still 4 % prevalence possible; i.e. 2 animals)	274
		ELISA + VNT			95	98	all animals (still 2 % prevalence possible i.e. 1 animal)	100
		rRT-PCR			98.5	99.9	all animals (still 2 % prevalence possible; i.e.1 animal)	244

* 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 (Se_s) and specificity (Sp_s) 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 % (see chapter 2.8)

$Se_s = Se_1 * Se_2$

$Sp_s = Spe_1 + Sp_2 – (Spe_1 * Sp_2)$

*** 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. So it yields for exact k observed test-positive counts

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

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 $p = \frac{NI}{NI+NN}$

Approximation formulas are not used and therefore all scopes of the parameter bounded to reach valid results for their approvable intervals (Abramowitz and Stegun, 1972). The construction and calculation of one side confidence intervals is given by the standard way.

Table 5 summarizes the requirements (type of sampling, design prevalence, and confidence) mentioned in the manual 2002/106/EC (Chapter IV) 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).

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 rRT-PCR 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 (see chapter 9).

(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.

3.4.5. 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

(Chapter IV) 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):

– Simulation parameters:
  - Number of simulation-cycles (= 1000)
– 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

    1. in Germany with medium pig density; 639 herds and 67,707 pigs; i.e. on average 106 pigs/herd)
    2. in Romania with low pig density; 10,344 herds and 167,790 pigs; i.e. on average 16 pigs/herd)
– 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)
– 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).
– Test parameters:
  - Sensitivity and specificity of the entire diagnostic procedure (= rRT-PCR as an example; Se = 98.5; Sp = 99.9; (Table 5)

Table 6. Exemplary results applying the simulation model on data from Germany and Romania using rRT-PCR according to the above mentioned parameters

Germany - 639 herds and 67,707 pigs and

Romania -10,344 herds and 167,790 pigs

Scenarios	Mean test-results using 10% individual design prevalence (limited sampling; n = 29)					Mean test-results using 5% individual design prevalence (limited sampling; n = 59)					All animals (cluster sampling)				
	Average sample size	TP	FP*	FN	TN	Average sample size	TP	FP*	FN	TN	Sample size	TP	FP*	FN	TN
Scenario 1 (Germany) – high within-herd prevalence of 25 %	9,228.95	27.14	9.09	0.41	9,192.31	14,334.01	40.20	14.17	0.64	14,279.00	67,707	194.61	67.61	2.88	67,441.90
Scenario 2 (Germany) – low within-herd prevalence of 1 %	9,361.01	1.74	9.54	0.07	9,349.66	14,244.99	6.54	13.97	0.11	14,224.37	67,707	12.81	67.56	0.18	67,620.45
Scenario 3 (Romania) – high within-herd prevalence of 25 %	101,533.94	291.9	101.13	4.45	101,136.46	141,353.00	391.93	141.27	5.98	140,813.82	167,790	452.16	167.18	7.08	167,163.58
Scenario 4 (Romania) – low within-herd prevalence of 1 %	101,606.50	93.83	101.67	1.48	101,409.52	140,379,01	100.21	140.92	1.51	140,136.37	167,790	102.88	167.34	1.59	167,518.19

TP: true positive; FP: false positive; FN: false negative; TN: true negative

* in the MOSS framework false positive samples (FP) will be clarified by further tests

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 chapter 3.4.4. 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).

4. ECOLOGY OF WILD BOAR

4.1. Distribution and population size

4.1.1. Wild boar and pig are *Sus scrofa*

Wild boar and domestic pig are members of the same species *Sus 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.

4.1.2. 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).

4.1.3. How to estimate the number of wild boar?

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 chapter 7.

4.2. Social and spatial structure of populations

4.2.1. 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 (Annex A, figure 6).

4.2.2. 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 matriarcal 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 long-distance 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 (see chapter 5). 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).

4.3. Population dynamics

4.3.1. Births

Basically most of reproducing females are more than one year old, piglets are always non-breeding 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 (see chapter 5).

4.3.2. 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 (see chapter 5).

5. EPIDEMIOLOGY OF CSF IN WILD BOAR

5.1. Descriptive Epidemiology

5.1.1. Current distribution of CSF in wild boar (Europe)

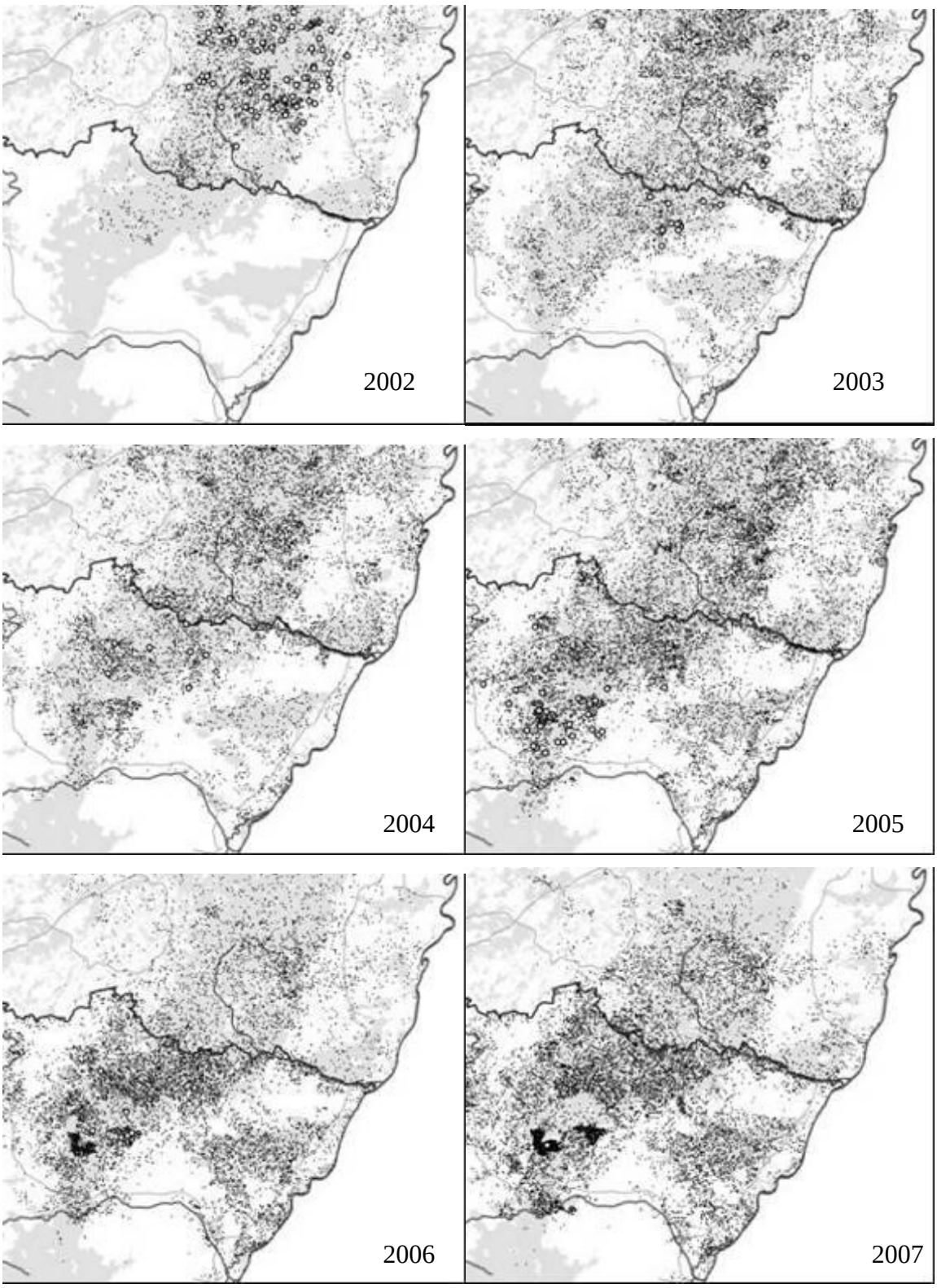
The first attempt to map CSF at the European level was provided in review papers at the beginning of the 2000s (Laddomada, 2000; Artois et al., 2002). Over the last five years (2003-2007), CSF has been reported in the EU in Germany, France, Luxembourg, Belgium, Slovakia, Romania, Bulgaria and a lot of other European states such as the Balkan states and Russia (Table 7). 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).

Table 7. Infection and vaccination status of wild boar and domestic pigs reported by European countries from 2003 up to 2007

(Sources: DG SANCO_document 10257/2003/Rev-8, EU reference laboratory for CSF and EFSA Questionnaire)

Year	Country	wild boar infection	wild boar vaccination	industrial pigs infection	industrial pigs vaccination	backyard infection	backyard vaccination
2003	Germany	yes	yes	yes	no	no back yard	no back yard
2003	Italy	no	no	yes	no	no back yard	no back yard
2003	Luxembourg	yes	yes	yes	no	no back yard	no back yard
2003	Slovak Republic	yes	no	yes	no	no back yard	no back yard
2003	France	yes	no	no	no	no back yard	no back yard
2003	Albania	unknown	no	no	no	yes	yes
2003	Croatia	unknown	unknown	no	yes	yes	yes
2003	Macedonia	yes	no	unknown	unknown	yes	yes
2003	Moldavia	unknown	unknown	no	yes	no	yes
2003	Montenegro	unknown	no	yes	yes	yes	yes
2003	Bulgaria	unknown	no	yes	yes	yes	yes
2003	Romania	yes	yes	no	no	yes	yes
2003	Serbia	unknown	no	unknown	unknown	yes	yes
2003	Bosnia and Herzegovina	unknown	no	unknown	yes	yes	yes
2004	Slovak Republic	yes	no	yes	no	no back yard	no back yard
2004	Germany	yes	yes	no	no	no back yard	no back yard
2004	Luxembourg	no	yes	no	no	no back yard	no back yard
2004	France	yes	yes	no	no	no back yard	no back yard
2004	Bulgaria	yes	no	yes	yes	yes	yes
2004	Croatia	unknown	no	no	yes	no	yes
2004	Montenegro	unknown	no	yes	yes	yes	yes
2004	Moldavia	unknown	no	no	yes	no	yes
2004	Romania	yes	yes	no	no	yes	no
2004	Macedonia	yes	no	unknown	unknown	yes	yes
2004	Serbia	unknown	no	unknown	unknown	yes	yes
2004	Bosnia and Herzegovina	unknown	no	unknown	yes	yes	yes
2005	Slovakia	no	yes	yes	no	no back yard	no back yard

Year	Country	wild boar infection	wild boar vaccination	industrial pigs infection	industrial pigs vaccination	backyard infection	backyard vaccination
2005	Germany	yes	yes	no	no	no back yard	no back yard
2005	France	yes	yes	no	no	no back yard	no back yard
2005	Bulgaria	yes	yes	no	yes	no	yes
2005	Moldavia	unknown	no	no	yes	no	yes
2005	Croatia	unknown	no	no	no	no	no
2005	Romania	yes	yes	no	no	yes	no
2005	Serbia	unknown	no	unknown	unknown	yes	yes
2005	Bosnia and Herzegovina	unknown	no	no	yes	yes	yes
2006	Bulgaria	no	yes	yes	no	yes	no
2006	Germany	yes	yes	yes	no	no back yard	no back yard
2006	Romania	yes	no	no	yes	yes	yes
2006	France	yes	yes	no	no	no back yard	no back yard
2006	Slovakia	yes	yes	no	no	no back yard	no back yard
2006	Croatia	unknown	no	yes	no	yes	no
2006	Moldavia	unknown	unknown	no	yes	no	yes
2006	Montenegro	unknown	no	yes	yes	yes	yes
2006	Macedonia	yes	no	unknown	unknown	yes	yes
2006	Kyrgyzstan	unknown	no	unknown	unknown	yes	yes
2006	Serbia	unknown	no	unknown	unknown	yes	yes
2006	Bosnia and Herzegovina	unknown	no	unknown	yes	yes	yes
2007	Romania	yes	no	yes	yes	yes	yes
2007	Bulgaria	no	yes	yes	no	yes	no
2007	Germany	yes	yes	no	no	no back yard	no back yard
2007	France	yes	yes	no	no	no back yard	no back yard
2007	Croatia	yes	no	yes	no	yes	no
2007	Moldavia	unknown	unknown	no	yes	no	yes
2007	Montenegro	unknown	no	no	yes	yes	yes
2007	Macedonia	yes	no	unknown	unknown	yes	yes
2007	Serbia	unknown	no	unknown	unknown	yes	yes
2007	Bosnia and Herzegovina	unknown	no	unknown	yes	yes	yes
2007	Slovakia	unknown	yes	unknown	unknown	unknown	unknown



In evidence the effect of forests (green corridors), rivers and roads virological positive cases as black circles with white center, virological negative cases as black dots. Motorways are in light-grey color, forested areas as light-grey patches, and rivers in as grey lines and administrative borders in dark-grey color

Figure 3. Investigations of CSF in wild boar from 2002 up to 2007 in Germany (Palatinate) and France (Vosges du Nord) (source: EU data base)

5.1.2. 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 semi-wild 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 re-emerge 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).

5.1.3. Risk factors

The probability to be 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. Furthemore, subadult and adult individual having more social interactions during dispersal or during rutting (see chapter 4) may play an important role in the persistence of the virus by ensuring the transmission between social groups (see § 5.2.1) (Rossi et al., 2005a; Rossi et al., in press).

5.1.4. Disease evolution observed in past outbreaks

5.1.4.1. 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 (see chapter 4) (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; Figure 13).

5.1.4.2. 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.

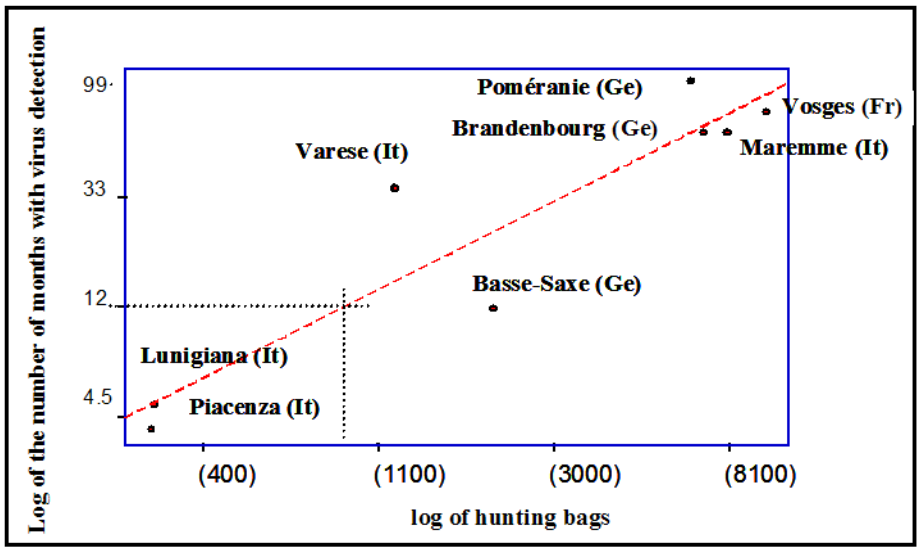


Figure 4. Correlation observed during past outbreak (1990-2002) between population size estimated by the hunting bags and the persistence of outbreak (source: Rossi et al., 2005a).

5.2. Mechanisms of transmission and of persistence

5.2.1. 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).

5.2.2. What are the supposed mechanisms of persistence?

5.2.2.1. 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.

5.2.2.2. 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).

5.3. Procedure followed for the collection of data on CSF in wild boar in EU*:

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. See Annex A, section 1.

6. CONTROL MEASURES APPLIED TO CSF IN WILD BOAR

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.

6.1. 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 coutries/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).

6.2. 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 simpatric 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:

1) Cadavers should be collected in individual separate bags in order to avoid contamination of uninfected cadavers trough infected blood;

2) 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.

3) Designated premises should be furnished with tap water and electricity. Freezers are also needed for the storage of the dressed carcasses;

4) Until the negative laboratory test is obtained, animals should not be removed from the designated premises;

5) 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:

a) 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.

b) Cars should be used in paved roads only; only one designated car should transport hunted animals.

c) Wild boar carcasses retrieved in the forest/ground should be removed and conveniently destroyed;

d) Feeding animals should be forbidden

e) 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.

f) 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.

6.3. 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 (see chapter 5).

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.

6.3.1. Hunting

Given that transmission is supposed to depend on the number of susceptible wild boar and that hunting is able to reduce the population size (after births) to half per year (see chapter 4), hunting may be considered as a simple and direct way to manage the number of wild boar and reach CSF eradication. Anyway there is few evidence that hunting may have been an efficient management tool (Rossi et al., 2005a; von Rüden et al., 2008), some authors even hypothezised that hunting may have aggravated disease persistence (Laddomada, 2000; Artois et al., 2002). This may arise because hunting generates a complex effect on population dynamics depending the age and sex classes that are targeted (Servanty, 2007). We can propose for example two scenarios:

- Targeting hunting (most of time) on young wild boar is assumed to decrease temporarily the number of susceptible, but given this harvesting is mainly reaching juveniles the number of breeding females may remain high enough to maintain a high birth rate (Servanty, 2007) and produce again a number of susceptible that will allow persistence (Kramer-Schadt et al., 2007). In Germany for example, it has been shown that despite of implementing hunting rules, the hunting bag remained far from the goal of a juvenile reduction of 85%, with only up to 50% young wild boar less than 1 year shot (von Rüden et al., 2008).
- Alternatively targeting breeding females would decreased the population size on the long-term (Servanty, 2007; Hebeisen, 2007; Bieber and Ruf, 2005) however it may temporarily increase the turnover of the population providing ideal conditions for the further spread of CSF; this may be particularly critical in dense populations that "react" by flexible increase of their breeding capacity (density-dependence) and thus the use of hunting for CSF control is not a simple issue and may even generate counter-effects (Guberti et al., 1998; Choisy and Rohani, 2006).

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 (see Table 10)

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. See chapter 4.

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).

6.3.2. Vaccination

Several countries (Table 7) 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 of C-strain baits in Germany and France support the positive effect of vaccination on controlling CSF outbreaks in wild boar populations (Kaden, 1998, Kaden et al., 2001b, 2002, 2003, 2004a, 2005).

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 (chapter 7).

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 (see chapter 7) 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 quantitive 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 assessement has to be performed using models reproducing different scenarios.

Table 8. Virus detection and typing as well as control measures regarding CSF in wild boar in Germany

Federal state	**Date of the first case**	**Date of the last case**	**Genetic virus type**	**Time of last vaccination Campaign**	Stop of restriction Lift of restriction foloowing last outbreak
Baden-Wuerttemberg	30.09.1998	19.11.1999	2.3 Uelzen	Oct. 2001	31.12.2002
Brandenburg	14.03.1995	26.04.2000	2.3 Güstrow	Apr. 2001	31.12.2002
Mecklenburg-Western Pommerania	01.03.1993	21.07.2000	2.3 Güstrow 2.3 Rostock 2.3 Spante	June 2002	31.12.2002
Saxony-Anhalt	12.10.1999	19.09.2000	2.3 Uelzen	Nov. 2001	31.12.2002
Saarland	26.01.2001	13.06.2002	2.3 Rostock	Autumn 2003	06/2004
Lower Saxony	12/1999	13.06.2002	2.3 Uelzen	Spring 2004	12/2004
Northrhine-Westphalia					
a1)	22.04.2002	14.10.2002	2.3 Rostock	Spring 2004	09/2004
a2)	07.10.2005	04.05.2007	2.3 Rostock	ongoing	
Rhineland-Palatinate					
b1) Eifelregion	05.01.1999	24.03.2003	2.3 Rostock	Autumn 2004	03/2005
b2) North-Eifel	23.12.2005	11.07.2007	2.3 Rostock	ongoing	
c1) Palatinate	1993	02/1995	2.3 Uelzen	no vaccination	01/1996
c2) Palatinate	23.10.1998	12.11.2004	2.3 Uelzen	ongoing	06/2005

Table 9. Experiences made in European countries with different vaccination procedures against CSF

Date	Area	Strategy	Vaccination procedure	Conclusion
(a) 1993-1995	Lower Saxony, Germany	oral vaccination with modified live vaccine based on the C-strain (Chinese) of CSFV	Double vaccination at an interval of 14 or 28 days twice a year	After the third immunization period no virus was detected. >50% of young boars (≤ 1 year) did not feed on vaccine baits nor became immunized. Piglets showed the lowest antibody prevalence (20-25%) (Kaden et al., 2000; 2002)
(b) Several periods	North-Western Pomerania, Germany	oral vaccination with modified live vaccine based on the C-strain of CSFV	Double vaccination at an interval of 14 or 28 days twice a year; since 2002 double vaccination three times per year	Eradication of CSF in the wild boar population was achieved after many years (Kaden et al., 2006). Repeated immunization campaigns were introduced in 1998 (Kaden et al., 2004).
(c) 1995-1997	Brandenburg, Germany		Single vaccination twice a year (only in autumn 1996 double vaccination at an interval of 14 days)	Eradication of the disease. Total of six vaccination campaigns. CSFV prevalence decreased from 4.65% in 1995 to 0.58% in December 1997. After the third immunisation campaign seroconversion in adults 45%, in yearlings 35%, in pigs 18-28 kg 22.3%, and in piglets 11.4%(Kern and Lahrmann, 2000).
(d) 1998	South of Switzerland	Increased hunting, targeting especially the young age classes		Successful CSF eradication, leading to the conclusion that in naturally confined regions (e.g. mountainous terrain), CSF outbreaks might be self-limiting (Schnyder et al., 2002). Subsequent analyses of wild boar revealed a mean seroprevalence of 0.2% (Leuenberger, 2004) and of 0% (Köppel et al., 2007) respectively.
(e) 1999-2001	Baden-Wuerttemberg, Germany	Oral vaccination with an attenuated type C vaccine	Double vaccination three times per year	Eradication of the disease within a relatively short time. CSFV was not detected beyond the second immunization campaign. Seroprevalence prior to immunization: 38%; 2001: 72% (Kaden et al.,2003); 2002: 25%; 2003: 8.5%. >50% of the piglets were seropositive. Wavelike courses of seroprevalences of yearlings and adults (Kaden et al., 2005).
(f) Jan 1999-Febr 2002	Eifel region, Rhineland-Palatinate, Germany	Increased hunting of young wild boar and hygiene measures		The goal to increase hunting of piglets was not reached for a number of reasons ranging from financial to ethical considerations (von Rüden et al., 2008). Conventional control measures could not prevent CSF from becoming endemic in the wild boar population of the Eifel region. After 3 years (2002) when no improvement of the epidemiological situation was in sight oral immunisation was introduced (von Rüden et al., 2008).
(g)	Eifel region,	oral vaccination with	Double vaccination	Seroprevalence rose fast to 69% and 76% in yearlings and adults

Febr 2002-Oct 2004	Rhineland-Palatinate, Germany	modified live vaccine based on the C-strain (Chinese) of CSFV	with a 4-week interval three times per year (spring, summer, autumn) (Kaden and Lange, 2001). Average of two feeding places per km² of hunting area. Depending on the estimated population density 30-40 vaccine baits per feeding place and vaccination campaign.	respectively and remained stable throughout the duration of the campaign. In piglets only 43% seroprevalence. Vaccination decreased significantly elimination of CSFV. Last virus-positive pig was found 13 months after start of o.i. (von Rüden et al., 2008).
(h) 2002-2003	Luxembourg	oral vaccination with modified live vaccine based on the C-strain of CSFV	Double vaccination with a 4-week interval three times per year	Successful CSF eradication within months (Brauer et al., 2006) (SANCO 10257/2003)
(i) 2004-2008 (on going)	France	oral vaccination with modified live vaccine based on the C-strain of CSFV	Double vaccination with a 4-week interval three times per year	Virus eradication program still on going
2005 - on going)	Slovakia	oral vaccination with modified live vaccine based on the C-strain of CSFV	Double vaccination with a 4-week interval three times per year	Virus eradication program still on going
2006 - on going	Bulgaria	oral vaccination with modified live vaccine based on the C-strain of CSFV	Double vaccination 2 times per year	Virus eradication program still on going

6.3.3. 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.

6.4. 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) was run. Each of the 18 patches represents a homogeneous and independent unit of 130 wild boars related to the others by bilateral links (see Figure 5). In each patch the wild boar population has is own dynamic (recruitment rate, natural, hunting mortalities, fertility and fecundity rates). The model runs under the following assumptions:

a. 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 > 4months) (Figure 6);

b. Inter-patch migration density dependent and limited to > 4 months-old animals (Massei and Genov, 2000);

c. 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);

d. Logistic growth (Wilson and Bossert, 1974) with both natality and newborn survival dependent on wild boar density (Focardi et al.,1996);

e. Age independent coefficient of transmission (β) (Rossi et al., 2005);

f. 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 (Table 1 and 2).

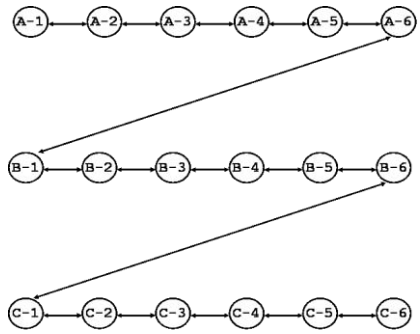
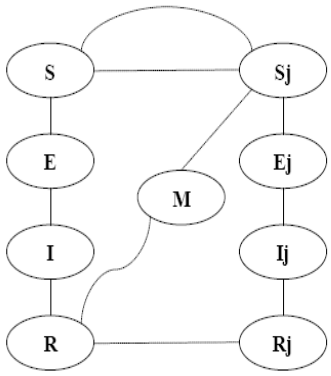


Figure 5. Metapopulation framework - continuous metapopulation compartmental model based on 18 patches (homogeneous and independent unit of 130 wild boars) related to the others by bilateral links (based on Hanski e Gilpin, 1997)



(M=maternal immunized; S=susceptible, E=latent; I= infectious; R=recovered)
Ages classe: 0-4 months (j) and more than 4 months

Figure 6. MSEIR architecture with a patch structure with two age-classes (Anderson and May, 1991; Hethcote, 2000)

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 (section 1).

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

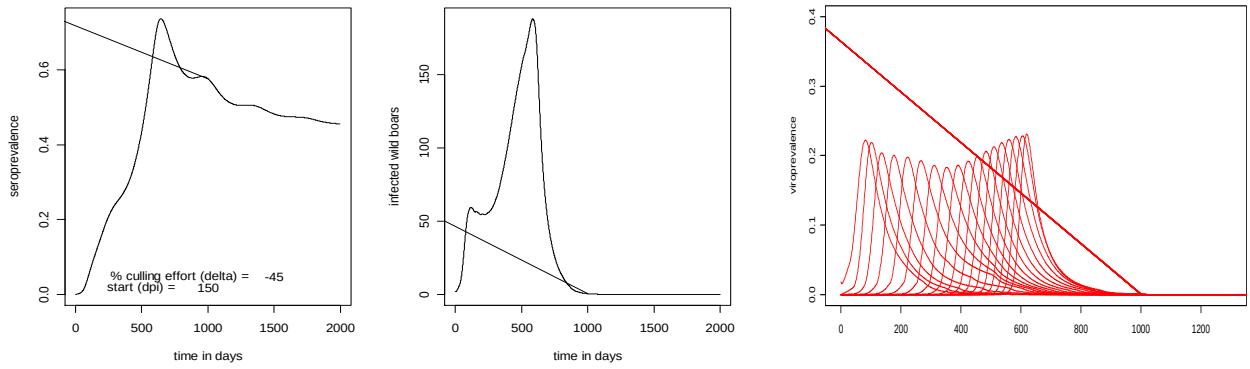


Figure 7. Infection dynamic: without hunting

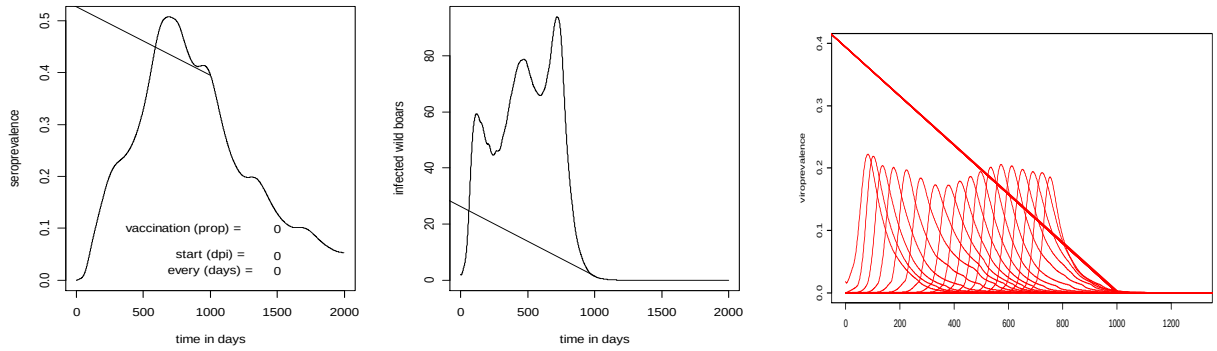


Figure 8. Effect of hunting on the infection dynamic: hunting 45% (default value)

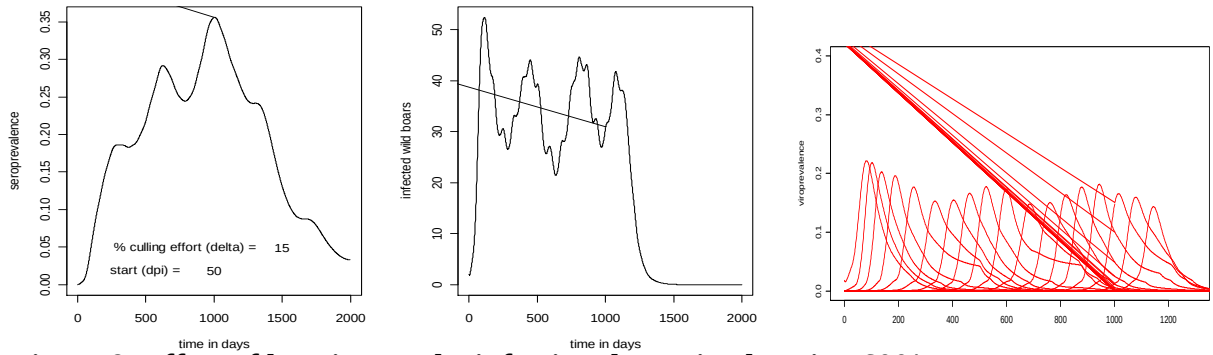


Figure 9. Effect of hunting on the infection dynamic: hunting 60%

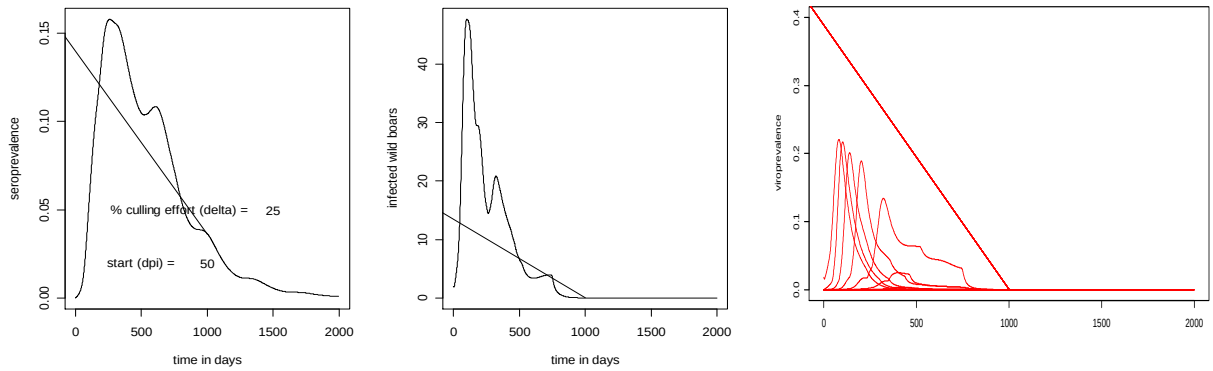


Figure 10. Effect of hunting on the infection dynamic: hunting rate > 70% year^{-1}

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)

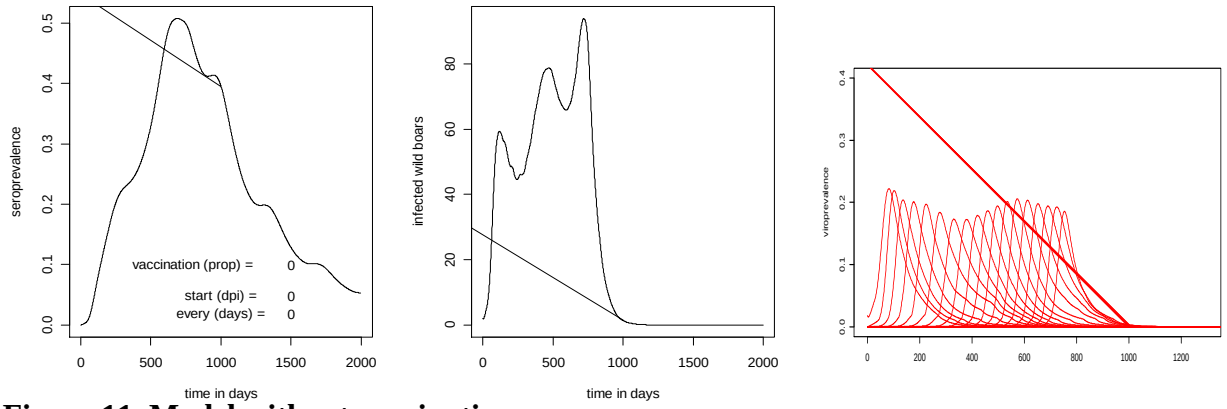


Figure 11. Model without vaccination

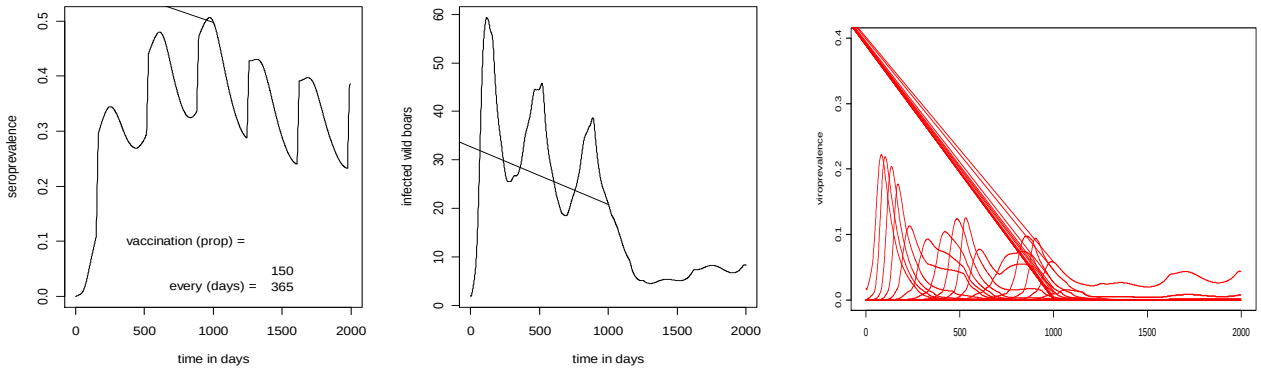


Figure 12.Effect of vaccination when 20% of susceptible individuals resulted vaccinated

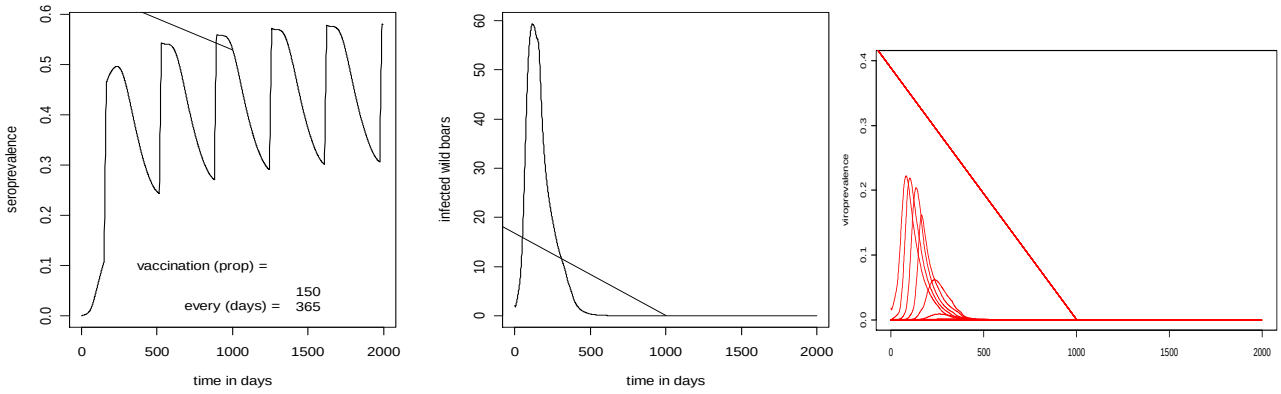


Figure 13. Effect of vaccination when 40% of susceptible individuals resulted vaccinated

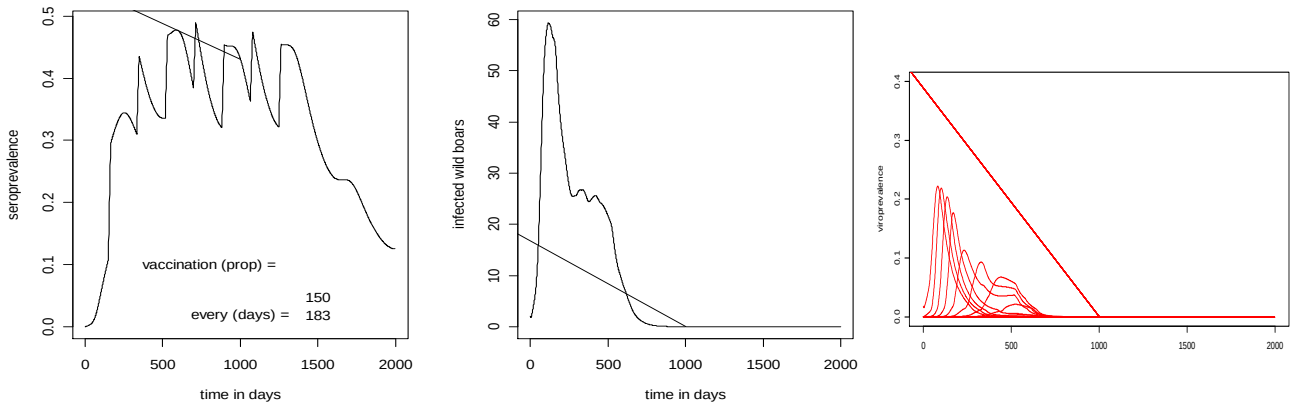


Figure 14. Effect of vaccination when 2 repeated vaccination (6 months time delayed) each of one reach 20% of susceptible individuals

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.

6.4.1. 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 (Annex B, table 2) 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 shedder 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 shedder and immunotollerant were included in the model (Figure 9).

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).

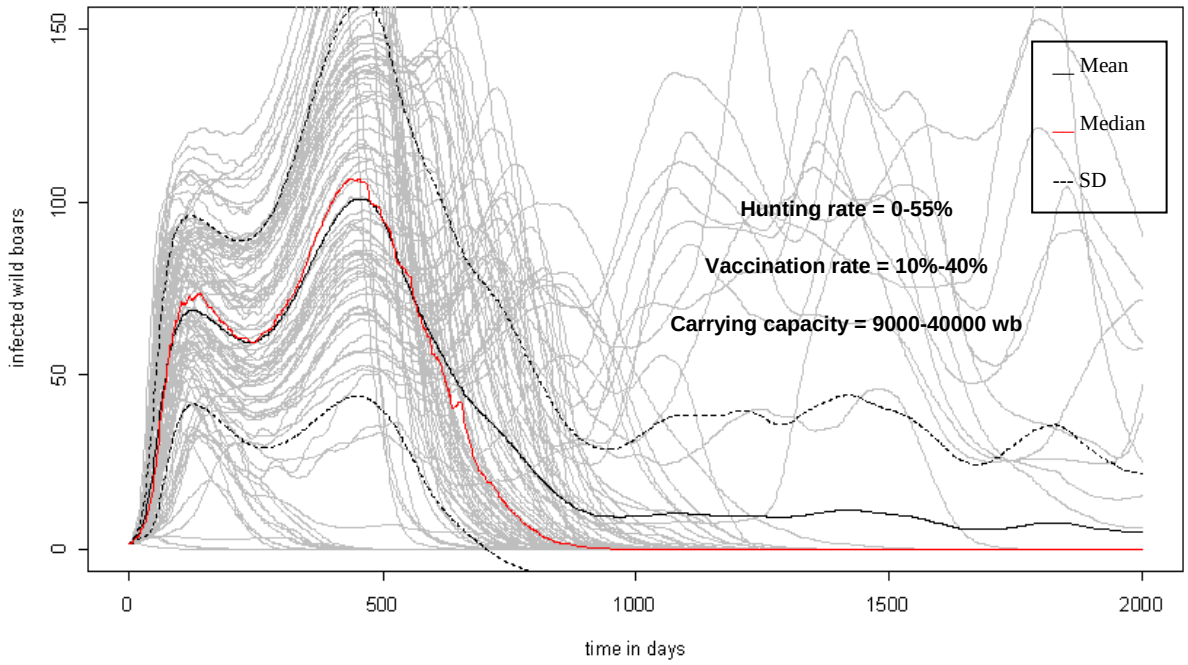


Figure 15. Probability to have endemic persistence (10%) after 5.5 years in large populations

In summary, vaccination can be considered as one of efficacious tool in managing the infection. Nevertheless, the sole scientific field proved vaccination action is to maintain a high level of herd immunity. Although field evidence (Table 9) by frequency arguments bolsters the view of oral vaccination as an efficacious measure for eradication too, there is need for further understanding how in fact the measure interacts on the population level with disease induced immunity when elimination of the virus from a population is observed, and when it is not observed. The more definitive evaluation of efficacy of vaccination to eradicate the infection will be possible with upcoming empirical and theoretical experiments, but beyond when marker vaccines will become available for oral application in field.

7. SURVEILLANCE AND MONITORING OF CSF IN WILD BOARS

7.1. Aims and Principles

Chapter 3 elaborates on the principles and purpose of surveillance and monitoring systems for domestic pig populations. Most of these issues are applied for wild boar surveillance systems. 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), Appendix 3.8.1.). 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 was not possible in wild boar vaccination programs. Adding tetracycline to CSF vaccine baits was 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 non-infected 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 wild life to domestic pigs.

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

7.2.1. Samples

The main sources for the detection of virus or antibodies are sera, tonsils and spleen. Non - invasivesamples 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:

a. the main source of sampling is hunting activity;

b. hunted animals are very often stressed particularly when the dog drive system is used; in this case haemolysis is a common finding;

c. 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.

d. 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.

7.2.2. 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 (Table 13)

Table 10. Relation between supposed wild boar density and hunting rate (Source: EFSA Questionnaire)

Area	wild boar /sqkm	% Hunted wild boars	Hunt. Bag/sqkm
1	4,34	70,53	3,05
2	0,72	34	0,24
4	0,82	55	0,45
5	9,72	43	4,2
6	11,42	45,9	5,24
7	1,52	38,8	0,6
8	0,89	75,3	0,67
9	#	#	#
10	0,94	96	0,9
11	0,49	74,5	0,36
12	0,000246	#	#

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

Area	Hunt. Bag/sqkm	Viro sample sqkm	% Hunt. bag sampled	Sero sample sqkm	% Hunt. bag sero sampled
1	3,05	0,245	8,03	0,1	3,28
2	0,24	0,018	7,50	0,0036	1,50
4	0,45	#	#	0,0041	0,91
5	4,2	3,23	76,90	2,79	66,43
6	5,24	5,18	98,85	4,62	88,17
7	0,6	0,144	24,00	#	#
8	0,67	0,0024	0,36	0,0008	0,12
9	#	0,078	#	0,044	#
10	0,9	0,83	92,22	0,77	85,56
11	0,36	0,172	47,78	0,148	41,11
12	#	#	#	#	#

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 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).

7.2.3. Investigating the presence of infection

7.2.3.1. 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.

7.2.3.2. 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.

7.2.3.3. 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 (Table 10,

Table 11) 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-epidemilogical characters of the disease but it is more intensiye and costly than the first option.

7.2.4. 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 term of sero prevalence in the whole vaccinated population often irrespective of gender and age classes. Very often statistical test are used in order to

demonstrate vaccination efficacy. In such circumstancies 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 when an adequate sample size is reached, will reduce dramatically the efficacy of the survey.

It is worth mention 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.

7.2.5. 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 step 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 over estimate or underestimate the real prevalence of the infection and a possible failure in exactly identify other infected areas.

Another critical issue is the time 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 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.

7.2.6. Demonstration of freedom of CSF

Details concerning freedom of disease are presented in Chapter 3. 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

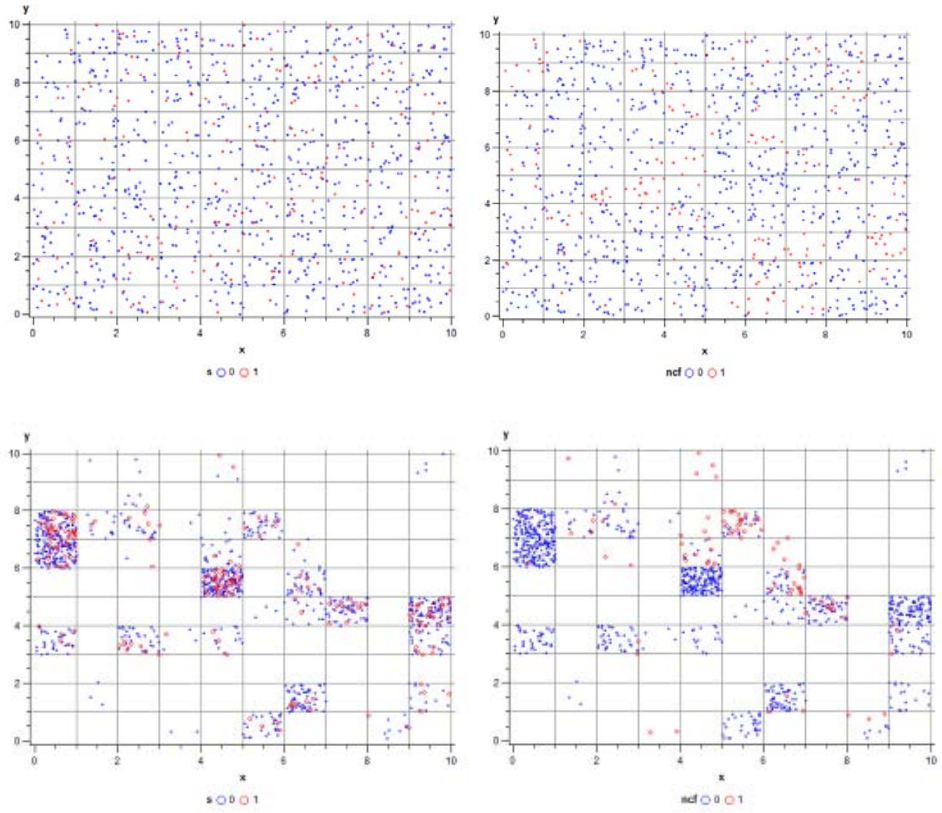
modelling approaches that may be used to answer the above question. The aim of the model is to show how the hunting system and the wild boar distribution will affect the capacity of the surveillance system.

7.2.7. 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 (Chapter 7) and difficult to quantify given sparse empirical studies (Chapter 4 + 6). Nevertheless hunting based surveys are required to monitor CSF when the mortality event of the epidemic has passed (Chapter 5) 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 (Chapter 4). The survey design often targets the overall mean disease prevalence; however, CSF prevalence is known to differ spatially as any contagious infection does (Chapter 5). The sampling for diagnostic testing is based on hunted animals, however, hunting is known to be not random (Chapter 6) 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.



Red: sampled animals, blue: Non sampled animals

Left-top: Random distribution of wild-boars + equal sampling fraction (S1);
Right-top: Random distribution of wild-boars + Unequal sampling fraction (S2);
Left-bottom: Clustered distribution of wild-boars + Equal sampling fraction (S3);
Clustered distribution of wild-boars + Unequal sampling fraction (S4):

Figure 16. Different scenarios of spatial population and sample distribution

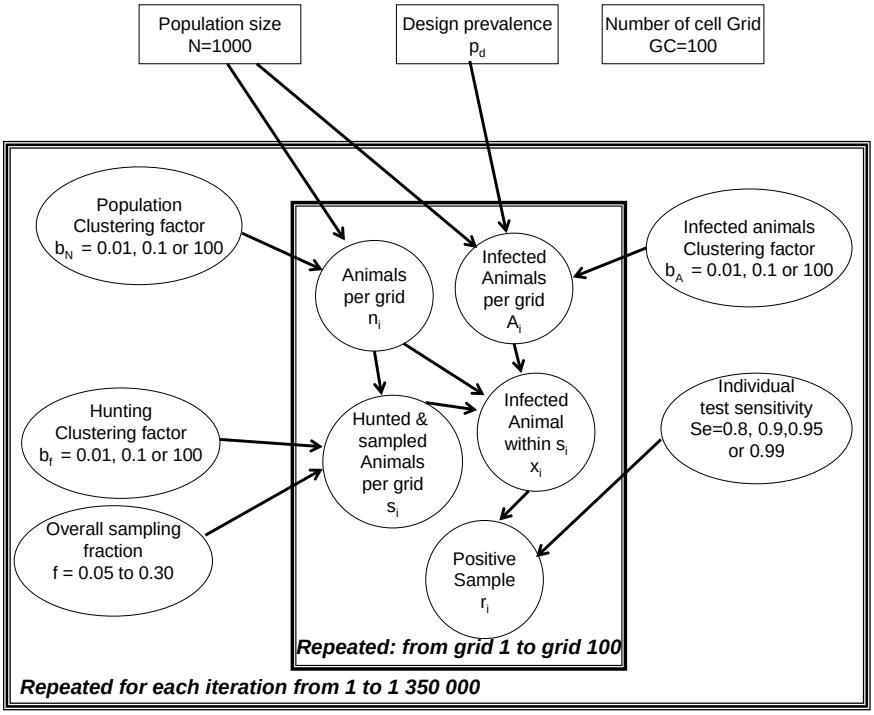
7.2.7.1. Model

The simulation model is built on spatial units (hunting districts, geographic coordinates, or ecologically determined sub-areas). In the following these spatial units are represented by cells of a regular grid (Figure 16). Between spatial units, differences are allowed regarding their surface area, their edge configuration, their wild boar number, or regarding to the hunting scheme applied within. However, within each cell wild boar hunting is performed consistently and the size is small enough to prevent marked difference in disease exposure (see "epidemiological sampling units" in Chapter 7.2.3.2).

Sensitivity estimates are derived from the model by Monte Carlo simulations, while assuming presence of the disease albeit with low prevalence. Simulations generate random spatial configurations of animals, infection and hunting. Finally, diagnostic testing of animals sampled out of the spatial units is simulated considering respective individual test sensitivity (e.g. 90%). Prior to each simulation run wild boar animals (e.g. 1000) are randomly distributed over the grid cells (e.g. 100); the infected animals are randomly assigned to these wild boars (e.g. 10 of 1000); and finally the hunting intensity is specified by a fraction of the total wild boars that has to be shot for sampling (e.g. 25%), a variable part of this sample is taken from each grid cell (e.g. 0% to 100% wild boars per single grid cell). Random assignment of wild boars number, local hunting intensity and individual infections to the grid cells is performed by drawing from probability distributions. This probability distribution generates either uniform assignment (e.g.

all grid cells have same number of wild boars), or clustered assignments (e.g. some grid cells contain nearly all wild boars) depending of a clustering parameter (Figure 16). After stochastic simulation of the sampling and testing procedure the simulated survey ends up with at least one positive result or not (Figure 17). Counting this binomial outcome for thousands of repeated surveys will provide an estimate of the sensitivity of the sampling system (SeSS) to demonstrate the disease i.e. the probability to detect at least one positive animal if the disease is prevalent with the assumed design level (e.g. 1%, or 10 of 1000). Formal details and complete result tables are provided as Annex B, section 2.

For the simulations the diagnostic test used does not affect the outcome (apart from the related individual test sensitivity, e.g. 90%). Logically, when antibody tests are used "infected" animals in the population are those which are recovered, when rRT-PCR is in mind then "infected" refers to virus positive animals.



See text for description and refer to the Annex B (section 2) for pseudo-code describing the model algorithm to simulate sampling surveys.

Before the simulation starts the inputs N, GC, A and Se are fixed. Further on, before each iteration the parameters bN, bf, bA are specified.

Figure 17. Schematic representation of the simulation model.

7.2.8. Sensitivity of CSF surveillance system – simulation of field conditions

Table 12 provides re-assessed sensitivity of the surveillance system when natural complexities are considered (Figure 16). Compared to the example figure of 92% calculated for the particular sampling fraction of 25% in the "ideal" situation of complete uniformity, one identifies huge impact of the introduced complexities from Table 12. The worst sensitivities are obtained when wild boars, hunting intensity, and disease distribution are all strongly clustered (right bottom of Table, SeSS = 11%). With the same surveillance sample (i.e. 25% of animals, in an area with 1000 wild boars, 1% prevalence, and 90% individual test sensitivity) the resulting SeSS can range between 11% and 77% depending on the (mostly unknown) natural heterogeneities, compared to a predicted SeSS of 92% in the theoretical situation of complete uniformity.

Table 12. Model result: Sensitivity of the simulated sampling system assuming different levels of clustering in wild boar, sampling, or disease distribution

Scenario (see Figure 16)	Disease is randomly distributed: bA=100	Disease is moderately clustered: bA=0.1	Disease is highly clustered (emergence) bA=0.01
S1 Random distribution of wild-boars + equal sampling fraction: bN=100; bf=100	77%	76%	73%
S3 Clustered distribution of wild-boars + Equal sampling fraction: bN=0.01; bf=100	47%	36%	27%
S2 Random distribution of wild-boars + unequal sampling fraction: bN=100; bf=0.01	33%	28%	17%
S4 Clustered distribution of wild-boars + unequal sampling fraction: bN=0.01; bf=0.01	17%	13%	11%

N = 1000, number of grid cells (GC) = 100, prevalence=1%, individual test sensitivity = 90%, sampling fraction = 25%.Often it will be unknown to which part of the table an area belongs. Therefore the huge range of resulting sensitivity indicates the uncertainty left after a completely negative survey. The selected point values provide an illustrative example.

The characteristics of the simulated surveillance system that describe clustering of wild boars and clustering of infected animals (parameters bN and bA) can not be controlled in the field.

However, bf could be modified to some extend by providing a less variable hunting pressure compared between spatial units. The simple increase in the sample which is continuously characterised by highly variable hunting fraction in the spatial units, or by clustered sampling, will not improve the sensitivity of the surveillance system.

7.2.8.1. Interpretation and discussion

The aim of this model simulation was to show the importance of non-uniformity or clustering in wild boar, sampling intensity, or disease distribution. How such clustering can be assessed in the field is another research question.

The model refers to spatial units, or sampling units that form epidemiologically defined sampling units (see Chapter 7.2.3.2). Such units are assumed to vary by e.g. the density, the size, and the spatial distribution of the wild boar sub-populations. The sub-populations may additionally have different micro-epidemiological characteristics but are defined small enough to represent an epidemiologically independent sub-population regarding the CSF spread. Hence, for such sub-populations a joined surveillance sample is reasonable. The grid cells of the model represent these units.

For the model following assumptions and limitations are valid:

1. The model assumes that the wild boar distribution is constant during the considered period of time.

2. The proportion of hunted animals is set independently of the number of animals in the grid cell (i.e. defined as fraction), but the total number hunted per grid cell varies with the animal number in the cell.

3. Hunting is considered independent of infection status (infected or uninfected), hence targeted sampling is excluded from the evaluation (i.e. conservative assumption).

4. The number of hunted animals per time period cumulated over all grid cells provides the random sample taken for "diagnostic investigation". Hunting which does not contribute to the surveillance sample is not simulated. The overall sampling fraction (f) is obtained by dividing the sample size (S) per the total population size (N).

5. The model assumes that the number of infected animals is constant during the considered period of time.

6. The total number of infected animals in the simulation area is corresponding to design prevalence (e.g. 1%).

7. The inputs of the model are:

a. Total number of animals in the simulation area (N).

b. The number of spatial sub-populations, i.e. grid cells (GC).

c. The number of infected animals (A; fixes prevalence via N).

d. The total sample size (S) given by sampling fraction (f; S=f*N or f=S/N).

e. The individual test sensitivity (Se).

f. The three cluster parameters bN, bf, bA for the spatial distribution of wild boars, sample, and infection.

8. The output measures are:

a. a. The successful detection of the present disease, or failure.

b. b The probability of (a.) provides the estimated Sensitivity of the applied surveillance sample/system (SeSS)

The indicative assessment revealed the strong effect of clustering in wild boar distribution on the SeSS (see Table 12). As priority, this effect has to be singled out from the other two confounders: clustering in sampling, or of the disease.

In difference to the field situation, the simulation of surveillance in a model allowed for the consideration of potential confounding factors (wild boar habitat, hunting pattern, disease occurrence) and an assessment of their effect on the surveillance system sensitivity.

- The sample size (here quantified as sampling fraction) is not the only factor that dictates the overall sensitivity of a surveillance system.
- The uncontrolled and mostly unknown variability of the sensitivity of a surveillance system due to the natural heterogeneities in wild boar, hunted sample, and disease distribution (e.g. 11%-77% for constant sampling fraction of 25%) might exceed the variability in SeSS introduced by different sampling intensity (e.g. 37%-99% when sample size was varied by collecting a fraction between 5% and 40% of the population).
- Area specific data about wild boar structure, spatial and temporal hunting regime, or disease history may enhance the efficacy of the surveillance system by a better estimate of its sensitivity.

It seems beneficial to further standardise modes of data collection or to develop statistics that allow estimates about wild boar habitat and hunting pattern. Additionally, the results argue to guide variably sampling with reference to available knowledge about the infected area.

The simulation-based assessment of the sensitivity of surveillance systems (SeSS) with regard to effects of varying wild boar habitat, hunting patterns, and disease distribution revealed:

- The sample size is not the only factor that dictates the overall SeSS but also wild boar habitat, hunting patterns, and disease distribution.
- The uncontrolled and mostly unknown variability of the SeSS attributable to heterogeneous distribution of wild boars, samples, and disease might exceed the change in SeSS introduced by increasing sampling intensity.
- Area specific data about wild boar structure, spatial and temporal hunting regime, or disease history may enhance the efficacy of the surveillance system by a better estimate of its sensitivity.

8. THE EFFICACY OF SURVEILLANCE AND MEASURES TO CONTROL AND ERADICATE CSF IN WILD BOAR: DISCUSSION AND SYNTHESIS OF THE ABOVE INFORMATION

8.1. 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:

- 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 spatial-temporal spread; efficacious to demonstrate termination of an outbreak);
- 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.

8.2. Control measures

Two control measures are considered in this report: hunting and oral vaccination. Limited experience on the field application of other possible control measures such as barrier reinforcement or fertility reduction were available, hence the following focuses on hunting and oral vaccination (with C-strain if not stated otherwise).

8.2.1. Hunting

Intensified, but non-discriminatory hunting, has never been shown to be efficient neither in controlling nor in eradicating CSF, unless in very small and geographically isolated populations.

The main drawback comes from the complex population dynamics and the interference between practical hunting schemes and the age dependence of CSF epidemiology in the wild boar populations. Thus hunting alone is not sufficient to cut the virus transmission chain, instead it may even result in enhanced virus perpetuation. The attempt to focus hunting on high risk classes i.e. particular age (juvenile) or sex (breeding female) has not proven feasible (von Rüden et al., 2008). Additionally, targeting the hunting to the immune or less susceptible sub-population by the removal of adult wild boars (especially if combined with vaccination measures) did not accomplish the aim of the fully eradicating disease.

According to SEIR modelling procedure, the complexity of wild boar population dynamics, CSF transmission and population management by hunting leads to the following conclusions:

- Absence of hunting does not result in significant changes in disease spread;
- Low hunting rates increase the number of infected boars;
- Medium hunting rates can promote CSF persistence and spread;
- Only such high hunting rates that are found impossible to achieve in a field situation could contribute to CSF control.

Data from the questionnaire suggest that reducing wild boar numbers to a level that excludes CSF spread would require shooting at least half of the considered population (47.5-72%) (Table 13).

Table 13. Wild boar to be culled instantaneously to reach threshold density of virus extinction based on a density dependent process

Dataset	Time of observation	Surface (sq km)	Wild boar density (wild boar per sq km)	Median Nt (I.C. 95%)	% of individuals (and total n. of individuals) to be culled instantaneously to reach threshold density of virus extinction based on a density dependent process (Nt)
Mecklenburg Western Pomerania	1993-1996	5196	6.1	1.71 (1.5-2)	72% (22820)
Luxemburg	2002-2003	2182	3.2	1.68 (1.3-2.2)	47.5% (3316)
Varese Province	1997-2002	370	3.2	1.5 (1.4-1.6)	53.2% (630)
Rhineland Palatinate (sub-area/pre vaccination)	1999-2002	247	5.1	1.7 (1.6-1.9)	66,7% (840)

Additionally it is also confirmed that insufficient hunting, as usual in disease control, will thwart eradication, here e.g. by favouring transmission (Laddomada, 2000) or by generating an increased turnover of the population (Rossi et al., 2005b).

Although hunting was not found efficacious as control measure it will provide a substantial part of any MOSS for CSF in wild boar.

8.2.2. Vaccination

Theoretically, vaccination through several campaigns over several years (see chapter 6), is one measure to control or eradicate the disease by reducing the number of susceptible animals. The oral vaccination procedure is efficacious in maintaining high levels of immune animals even when, naturally, reduced virus incidence would lead to a decreased immunity level in the population. The vaccination-made maintenance of high level of immunity then enhances elimination of the virus. Therefore, vaccination can be considered as one of the tools to control and eradicate the infection.

Oral vaccination using the C-strain, has been demonstrated to be fully protective at the individual level (Chenut et al., 1999; Kaden et al., 2000a). Furthermore, the elimination of CSF from large areas repeatedly happened currently with the intensive application of oral vaccination of wild boars. Some field studies in line with oral vaccination demonstrate 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 several vaccination campaigns. Thus there is strong empirical evidence to support the efficacy of oral vaccination as measure to control and also to eradicate the disease (e.g. von Rüden, 2008). The clear demonstration of vaccination as efficacious in eradicating CSF from wild boar populations, however, is still lacking. This is due to the fact that the antibodies to the vaccine are indistinguishable from those to the field infection. Additionally, low incidence and limited sensitivity of the surveillance system might prevent the observation of control success.

Vaccination procedures have been adjusted several times also in the same areas according to a trial and error approach. The approach most recently used in successful programs consisted of

two/three repeated vaccination campaigns per year using at least 30-50 baits per sq km of forest. For the moment there is no agreed procedure to terminate vaccination.

The low bait intake of piglets is speculated as potential limitation, or the heterogeneity of transmission and vaccination in the population (Rossi et al., in preparation), or the variability in individual infectious courses (Kramer-Schadt et al., 2007). Also 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 (von Rüden et al.,2008; Kaden et al., 2006a). The effect of insufficient vaccination is suggested to be as dramatic for CSF in wild boar as for other diseases of the wild (Schenzle, 1995).

Thus due to limited understanding of host and virus related mechanisms, the experience from the field might limit efficacy of vaccination: making it one tool to control and eradicate the disease rather than the exclusive solution or solely preferred approach.

Pinpointing the lacking causal prove and understanding of the role of vaccination in the eradication context inconsistencies exist between collected data and epidemiological estimation. For example SEIR metapopulation modelling suggested that a rather high proportion of protected animals are needed to guarantee the final eradication of CSF in wild boars (herd immunity). Indeed, data collected in the field rarely (if ever) demonstrate that vaccination was able to reduce the number of susceptible animals below the critical density, nevertheless eradication in alignment with ongoing oral vaccination programs was observed in the field (see chapter 6).

Thus, oral vaccination alone may not eradicate CSF from wild boar in all regional or epidemic situations, in contrast to, for example, the standardised oral vaccination of foxes against rabies.

In summary: By frequency arguments field evidence bolsters the view of oral vaccination as an efficacious tool for control that also might end-up in eradication. Also the maintenance of high immunity in vaccinated populations is not questioned. However, there is need for further understanding how vaccination interacts on the population level with disease induced immunity.

9. SAFETY" OF FRESH MEAT FROM CSF FIELD VIRUS DERIVED FROM EMERGENCY VACCINATED DOMESTIC PIGS

9.1. General part

The ToR require an assessment 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 in the ToR. 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 (9.3.2).

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 (Chapter 3.3.2). 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.

9.2. Schemes applied to detect field virus in fresh meat

9.2.1. 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.

9.2.2. 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 E^{RNS} -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. E^{RNS} -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)

Table 14. CSFV status of animals or carcasses based on combined interpretation of different diagnostic tests

PCR	VI	E2-ELISA	E^{RNS}-ELISA	Interpretation	Conclusion*
Neg.	Neg	Neg	Neg	CSFV-free or sampling during incubation	Free
Pos.	Pos.	Neg.	Neg.	CSFV-infected, early time point after infection, infectious virus present	Positive
Neg.	Neg.	Pos.	Pos.	CSFV-infected, re-convalescent or vaccinated with MLV, no infectious virus present	Positive
Neg.	Neg.	Pos.	Neg.	Vaccinated with E2subV	Negative
Pos.	Neg.	Pos.	Pos.	CSFV-infected, re-convalescent or vaccinated with MLV; No infectious virus detectable CSFV-genomes detectable	Positive

* Positive according to the current CSF directive (2001/89/EC)

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 (see sub-chapter 2.4) have to be classified as "zero risk" animals for fresh meat.

9.2.3. Vaccination

As already described in 2.4, 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 (see Chapter 2). 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 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.

9.2.4. 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 RT-PCR 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 (see also sub-chapter 3.4.5).

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.

9.3. Model-based risk assessment of the risk for fresh meat originating from pigs after emergency vaccination

9.3.1. Background

In the following section conceptualisation, assessment and interpretations are based on the application of a simulation model of CSF-spread.

The ToR refers to the risk of fresh meat originating from pigs after emergency vaccination. There can only be a risk for fresh meat from vaccinated pigs if emergency vaccination is applied as "vaccinate-to-live", i.e. all vaccinated pigs will be slaughtered for market. The objective of this section is to assess the risk for fresh meat as a consequence of emergency vaccination, compared to the risk of the conventional strategy of pre-emptive culling.

According to legislation animals from the protection zone of a CSF outbreak can be traded after restrictions have been lifted. It is generally believed that the conventional non-vaccination strategy bears a negligible risk of having field virus in fresh meat. However, there are no scientifically sound figures to back up this assumption. Therefore, in a first step this scenario is assessed in the model.

This study does not address the consequences of trade and distribution of meat from vaccinated animals. No public health concerns have to be considered.

9.3.2. Concepts and basic termini

Infected herd: The concept of "infected" refers to any herd that contracted an infection and is not yet detected. In the following "infected herd" 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 (see Table 17). Particularly vaccinated herds may be "infected" without harbouring virus any more.

Regarding the risk for fresh meat "infected herds" play different roles:

- If a herd that contains infectious animals reaches the slaughterhouse there is a clear hazard.
- If a herd contains an animal that has antibodies against wild type virus there is no immediate risk for CSFV-contamination of fresh meat.

Infected before protection (ibp): At the herd level, the term characterises units that are vaccinated closely after introduction of the infection or units 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.

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.

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 (see Bergevoet et al., 2007). 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.

9.3.3. Control scenarios

Because it is not meaningful to calculate an absolute risk in terms of the ToR the relative risk was quantified by comparing different strategies applied to control the identical outbreaks. The compared strategies are:

"Cull": Stamping-out of CSF detected herds, standstill in 10km, and *pre-emptive culling* of premises within 1km radius around each detected case.

"Vac4": Stamping-out of CSF detected herds, standstill in 10km, and *emergency vaccination* of premises within 3km radius around the detected case, assuming *protection within 4 days* post vaccination ("blocking immunity").

"Vac14": Stamping-out of CSF detected herds, standstill in 10km, and *emergency vaccination* of premises within 3km radius around the detected case, assuming *protection within 14 days* post vaccination ("blocking immunity") and *DIVA property*.

The scenarios follow up the outbreak with all its control measures until final screening gave for the first time a completely negative diagnostic result meaning that all restrictions would have been lifted as the next step.

9.3.4. Risk assessment

9.3.4.1. Approach

The risk assessment is based on a spatially-explicit simulation model developed to simulate CSF outbreaks in geographic landscapes with pig holdings (Thulke et al., 2007). Due to the complexity of the processes that interact before a risk animal can reach the slaughterhouse simulation modelling is an optimal approach to the problem and well established in epidemiology (e.g. Bates, et al., 2003; Karsten et al., 2005; Fernandez et al., 2006; Bergevoet et al., 2007; Harvey et al., 2007).

The rationale of the modelling is to build a model from detailed expert rules corresponding to scientific literature or agreed by the WG. The consistency of the resulting model is tested with available data patterns on all levels of information (see Annex B, section 3 for the examples and Grimm et al., 2005, for the methodology). The structure of the model is based on pig herds and comparable to the generic NAADSM approach (North American Animal Disease Spread Model; Harvey et al., 2007).

An important advantage of a model is that conditions that are hidden in real life, e.g. undetected clinical disease, undetected infected animals/herds due to false negative test results become

visible in the model. Likewise the relative risk due to change of control strategies, e.g. without and with vaccination, can be measured before final screening.

Details on the model, parameterisation and simulation experiments are given in the Annex B, section 3.

9.3.4.2. Risk quantification

The risk of contamination of fresh meat is assumed to be proportional to the average number of infected herds either remaining before final screening or still present after lift-up of the restrictions.

9.3.4.3. General assumptions and limitations

Modelling uses assumptions that are close to realistic facts. The output of the model should be considered also with respect to assumptions made and compared to available field data.

Herds detected as CSF-positive are stamped out in all scenarios.

Outbreak simulations of alternative emergency control strategies are evaluated exclusively with respect to the risk for fresh meat. The outcome of a low risk for fresh meat associated to a particular control concept does not mean that this strategy is also superior with respect to other aspects e.g. Eradication success, final size and duration of an outbreak, or associated costs and losses.

The model does not create false positive laboratory results because these do not influence the risk for fresh meat, except for a potential prolongation of restriction thus somewhat decreasing the risk for fresh meat.

Table 15. Diagnostic tests considered in the RA model depending on the control strategy applied and on the herd status

Herd status before lift-up	Pre-emptive cull	Vaccination conventional	Vaccination with marker
Herd not vaccinated	rRT-PCR	rRT-PCR	rRT-PCR
Herd vaccinated with MLV	n.a.	rRT-PCR	n.a.
Herd vaccinated with E2subV	n.a.	n.a.	AB-ELISA-ERNS

Table 15 provides an overview about strategy-test combinations considered in the current risk assessment. The RA is performed with regard to the risk for fresh meat. The standard model therefore applies rRT-PCR whenever not testing in E2SubV-vaccinated herds; exceptions are mentioned. The reason why ELISA-E2 is not used in standard simulation is that whenever %AB+ < %rRT-PCR+ the reduced sensitivity of the ELISA-E2 and delayed diagnostic results of the ELISA-E2 is disadvantageous. See for a simulation example last row (Table 16).

Susceptibility, infectiousness and immunity are modelled on the herd level and represented in an all or nothing fashion (for sensitivity investigation also model runs with age-dependent and number dependent infectiousness were performed but main findings did not change). Particularly for vaccinated herds that contracted a timely infection, the maximum time the last infectious animal remains beyond the date when all herd mates got fully protected is set to the mean infectious period (Bergevoet et al., 2007).

In simulations the final screening is performed in adherence to the legislation (Directive 2001/89/EC) and scheduled 30 days after the last infected herd was detected. During the risk

assessment the value of lift-up time was not amended albeit for demonstration purpose one example simulation applied 36 days together with the "Vac14" scenario.

The risk assessment reflects the core ideal of emergency vaccination. The success rate of vaccination is assumed 100% for animals in vaccinated herds thus imperfect compliance as well as vaccine failures are excluded.

The simulation does not differentiate between fattening and breeding herds. Vaccinated sows may no longer be used for breeding; they are treated as fattening pigs.

9.3.4.4. Simulations

Simulations followed a full outbreak with potentially increasing area of intervention measures after any new case detection (e.g. 1km pre-emptive culling; 3km emergency vaccination). Simulations were performed until the final screening of the whole cumulated intervention area was negative.

Simulation results are shown for the low density scenario. Higher density (data not shown) increases the individual values but does not change the qualitative differences shown by the diagram.

9.3.5. Results

9.3.5.1. The complete outbreak

The aim of the complete outbreak simulation was to enumerate infected herds that contained incubating or virus-positive animals that have passed the final screening without detection. As these herds eventually are being sent to slaughter they comprise serious exposure for fresh meat.

Table 16. Risk herds from simulated outbreaks for different scenarios

Scenario	Outbreak without risk herds [1]	Outbreak with risk herds [2]	Percentage of risk herds equal to 1 [3]	Mean number of risk herds per outbreak (risk herds cumulated over 1000 simulated outbreaks) [4]
Cull	98.2%	1.8%	68%	0.025 [25]
Vac4	99.0%	1.0%	78%	0.014 [14]
Vac14	97.7%	2.3%	90%	0.025 [25]
E2Vac14	97.8%	2.2%	68%	0.033 [33]

1 - Percentage of full outbreak simulations without risk herds out of the final zone of intervention;
2 - Percent outbreak simulations that left at least one herd with incubating or virus-positive animals (100% minus the value in the previous column);
3 - Percentage of total number of outbreaks with risk herds with only one risk herd;
4 - The average number of risk herds per outbreak respective the total number of herds risky for fresh meat cumulated over 1000 outbreaks.

As shown in Table 16, the risk for fresh meat associated with the pre-emptive culling strategy was found to be minimal. However, in general none of the simulated standard vaccination scenarios ("Vac4" and "Vac14") was inferior to the preemptive culling (differences not significant). The scenario in which E2-ELISA serology was used instead of rRT-PCR for diagnosis in non-vaccinated herds (row "E2Vac14") did not improve the performance of the intervention against the outbreak with regard to fresh meat. However, the number of hazard events is marginally increased when exclusively serology is used.

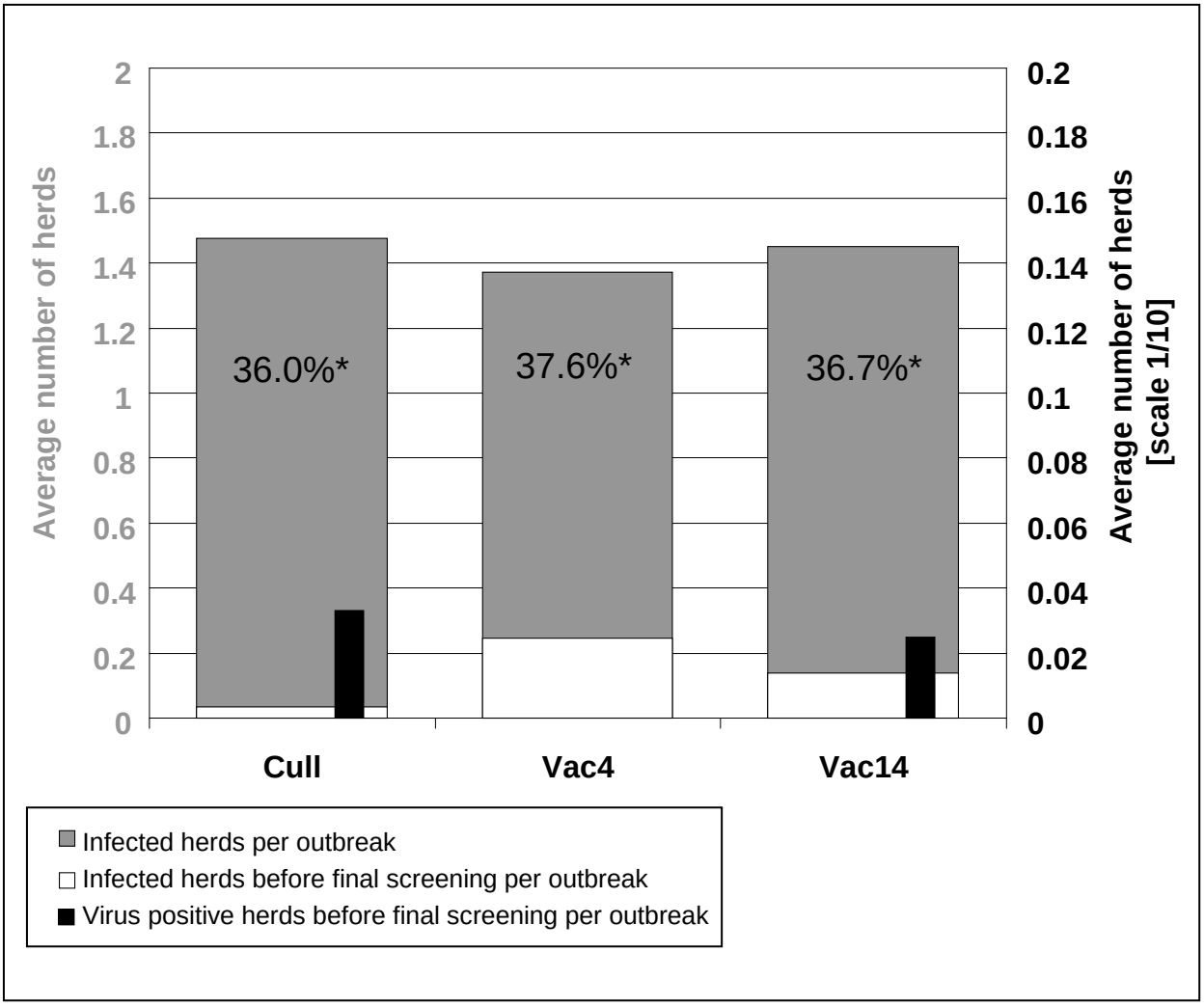
The finding that vaccination strategies do not increase the amount of risky herds after lift-up compared to pre-emptive culling is in contrast to common expectation. Therefore the status of the total number of infected herds before final screening and after lift-up has to be considered in the next sections.

9.3.5.2. Focal analysis

The next part of the assessment was to understand how areas actually vaccinated contribute to the relative risk for fresh meat. To do so it was necessary to investigate the relative performance of the proposed control strategies independent of the randomly realised size or duration of the full outbreak simulation. Therefore the data gathered from the 3km zone around the first case detected in simulation were analysed more detailed. Data in Figure 18 (focal 3km zone, risk due to control strategy) and Figure 19 (focal 3km zone, risk due to final diagnostic screening) summarize the situation in this zone.

Thick grey bars in Figure 18 show the average number of herds infected within the 3km zone around the first notified outbreak, and thick white bars indicate how many of those remain undetected until the first final screening. The thin black bars show how many of the latter still harbour virus positive animals. Although all three strategies result in "infected" herds remaining undetected until final screening (white), not all strategies produce virus positive herds (black bars). Furthermore, in all strategies more than one third of all notified outbreaks remain without follow-up outbreaks in the 3km zone (i.e. 36%; 38%; 37% respectively for each strategy).

The scenario "Vac4" caused slightly fewer infected herds (grey thick bars). This is due to faster protection of the whole 3 km control zone ("Vac4", 4 days) compared to the marker vaccine with a slower onset of protection ("Vac14", 14 days). With the strategy "Cull" the ring between 1km and 3km is only subjected to standstill as long as no new detection occurs therein or in close neighbourhood.



On the left scale, the grey thick bars show the number of infected herds within the 3km zone (the originating detection in the centre is not counted). White thick bars represent the average number out of these herds that remained undetected until final screening. On the right scale, thin black bars show a further subset i.e. the herds that contain incubating or infectious animals. Simulation results are shown for the low density scenario (1 herd and on average 1000 pigs per km²).

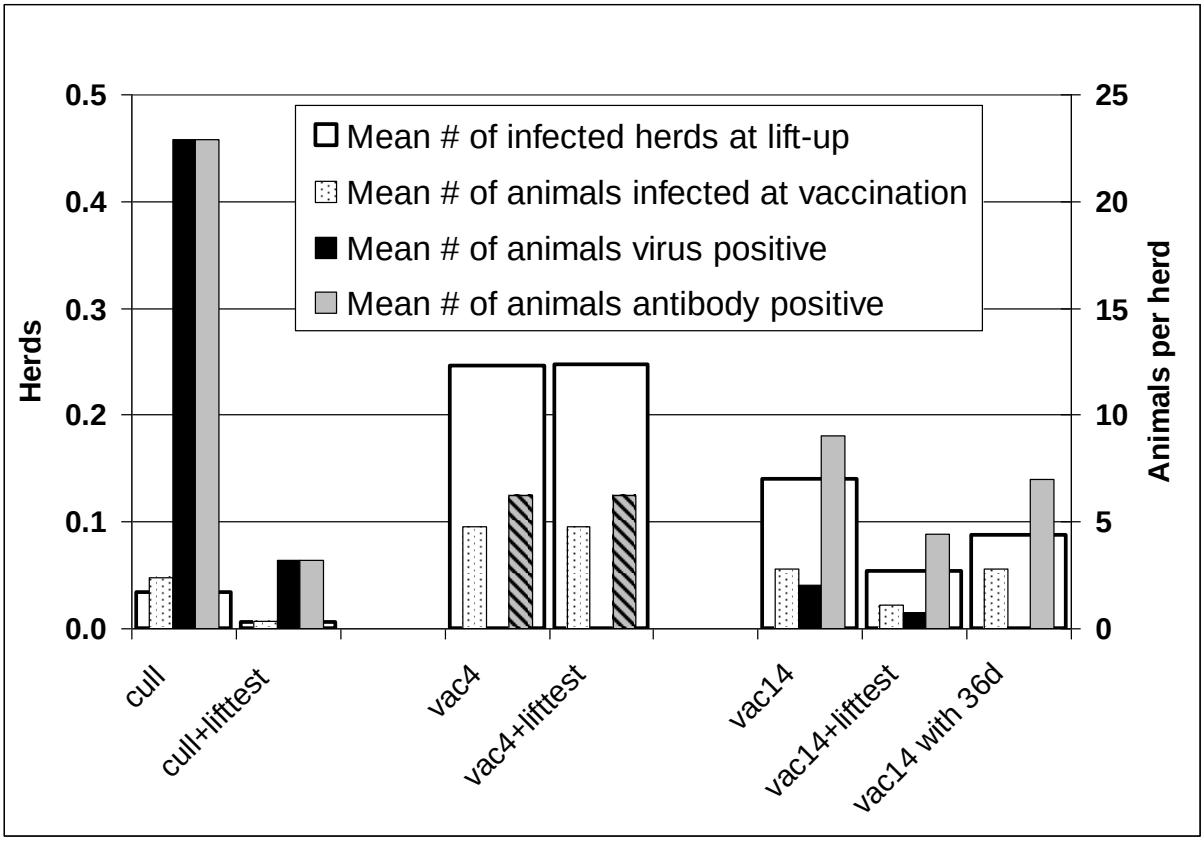
*Proportion of outbreaks in which no second infected herd occurred inside the 3km zone after emergency cull of the detected herd in the centre.

Figure 18. Simulation results for the 3km zone around the first notified outbreak herd before final screening.

The scenario "Cull" left the lowest number of infected herds undetected until final screening (white thick bars). This is due to the number of continued clinic detections in the 1km to 3km ring as disease prevalence and clinical visibility in infected undetected herds is continuously rising. In vaccination scenarios more infected herds remained undetected because of the small numbers of animals affected by outbreaks in vaccinated herds.

Scenario "Vac4" (fast protection) was found to be completely safe already before final screening (no thin black bar). The scenarios "Cull" and "Vac14" left virus positive herds for final screening. As the "Vac14" scenario assumes later onset of protection in the model an equally later lift-up might solve for the difference between both vaccination scenarios. Indeed, after experimentally postponing the lift-up in the "Vac14" scenario no virus positive animals remained (see Figure 19: data point "vac14 with 36d" where the lift-up experimentally was performed 36 days after the last case detection). The appropriate number of days for the lift up

time depends on the dynamics of infection and immunity (Bergevoet et al., 2007), and the identification of the adapted value for either of the vaccines was not in the scope of this assessment. The lessons learnt, however address the time of lift-up: for vaccinated herds the time period before protection, i.e. zero susceptibility of all vaccinated animals in a herd, directly prolongs the time required for a safe lift-up.



Scenario description: in addition to the abbreviations used previously, "strategy name+lifttest" same strategy as before but continued until lift-up i.e. after completely negative final screening result. Scenario "Vac14 with 36d" same as "Vac14" but the lift-up condition was experimental prolonged to 36 days.

Data are grouped for each scenario: in the background thick white bars represent the average number of infected herds that remained undetected, thin bars represent the average number of infected animals over all these herds. The animals of the black bar series would test virus positive or are incubating. The animals represented by the gray bar series would test positive in either ELISA (after 14-21 days p.i. with E2, or after 21-35 days p.i. with E^{RNS}) as they have antibodies against the field virus. Note: grey striped bars (cluster "vac4") represent animals from herds vaccinated with MLV so that detection by serology is not possible. Dotted bars represent the virus-positive animals present at time of administration of the vaccine in the herd. For the three strategies the information is read twice from the model once before final screening starts (cull, vac4, vac14) and after lift-up (cull+lifttest, vac4+lifttest, vac14+lifttest). The thick bars of the former copy the white thick series from the figure before.

Figure 19. Simulation results for the 3km zone around the first notified outbreak herd before final screening including a prolonged lift-up time of 36 days.

Figure 19 details the effect of the final screening procedure in improving the safety of the lift-up. The two thick white bars for each strategy quantify the difference in the number of infected herds before and after final screening. Therefore the situation in the 3km zone is evaluated when the last outbreak was 30 days ago (left, exactly the same as in Figure 18), and second time after lift-up (i.e. when final screening was negative, right thick white bars).

Comparison of the number of infected herds that remained undetected between both situations reveals: During final screening procedures in the "Cull" scenario about 80% of infected herds

were removed, in this case per 1000 herds 27 out of 33 infected herds were detected. For the scenario "Vac14" still about 60% of the infected herds were detected during final screening, in this case per 1000 herds 86 out of 140 infected herds were detected. In the "Vac4" scenario this number was zero, in this case per 1000 herds 0 out of 246 infected herds were detected.

The reason for the apparent "underperformance" of the fast protective vaccine ("Vac4") will become clear when the absence or presence of virus positive and field-virus antibody positive animals is taken into account. This information is displayed by the thin bar series of Figure 19. For "Vac4" scenario there are no animals that are virus positive or incubating (black bars) and in contrast all animals are antibody-positive for field virus (grey bars series). The latter are detectable in vaccinated herds in the "Vac4" model scenario; but indistinguishable from vaccine induced antibodies under field conditions (gray-black striped bars). For this reason the final screening cannot reduce infected herds in the "Vac4" scenario due to the missing DIVA property of their antibodies. Assuming ideal compliance no virus-positive animals are left in these vaccinated herds at the time of lift-up because they have either recovered or died.

The conventional culling strategy potentially leaves virus-positive animals at time of final screening and after lift-up (black thin bars). However, the numerous "diagnostic targets" in this non-vaccination scenario enhance the probability of detection of some but not necessarily all of the infected herds during final screening (see Figure 19, "Cull" vs. "Cull+lifttest"). Those false negative virus positive infected herds remaining after final screening will result in virus positive animals being presented at the slaughterhouse. A similar scenario is likely to arise in the "Vac14" but can be avoided when time of lift-up is adjusted to the slow onset of protection of the DIVA vaccine (see the three data for "Vac14", "Vac14+lifttest", and "Vac14+36d").

Considering the risk for fresh meat alone and assuming ideal compliance, emergency vaccination concept is clearly superior to conventional culling: Although the number of undetected infected herds after lift-up was more than 7 (3) times greater for the "Vac4" ("Vac14+36") scenario compared to the "Cull" scenario, all infected herds of "Vac4" ("Vac14+36d") were safe for fresh meat, because no virus positive animals came to slaughter. The main purpose of the risk assessment was to identify the link between control scenario, necessity of final screening, and risk for fresh meat. For a less ideal level of compliance see discussion

The main difference between the "Vac4" and "Vac14" (Figure 19) is the significance of grey bars representing field virus antibody-positive animals. In Vac14 those are detectable using DIVA ELISA, while in the Vac4 scenario they cannot be distinguished from field virus antibodies, and therefore will remain after lift-up. Although the latter animals may be of concern with respect to trade legislation, they will not be risky for fresh meat.

9.3.5.3. Evaluation and discussion

The RA gave clear indications that a properly adjusted vaccination strategy is likely to be safer for fresh meat than the conventional strategy including pre-emptive culling. It was clearly shown that the base-line risk for remaining virus-positive pigs using the conventional culling strategy is not zero.

The greater safety after emergency vaccination is caused by the limited size of an outbreak in vaccinated herds that contracted an infection, or were infected close before vaccination. If no long-term carriers are supposed, such "micro-epidemics" must quickly reach the end at which the animals in the herd are vaccine-protected, immune by recovery or removed by dead (Figure 19). Theoretically, the pre-emptive slaughter of the 3km vaccination area will be equally safe as no single animal is retained. But this is not a considered option, both for associated cost ratio (>

1:30 per animal) and for ethical reasons. Hence, conventional culling clears the 1km zone and therefore cannot achieve a comparable safety.

In order to achieve safety of vaccination strategies it is crucial to know the time an infected vaccinated herd might harbour infectious animals even after full protection of the uninfected herd mates. The infection, immunity and lowered susceptibility after vaccination naturally are dynamic processes within the individual pig and potentially follow probability distributions over time (Bergevoet et al., 2007). Because there is limited knowledge about the very precise form of these distributions the risk assessment was performed with a herd-based modelling approach. For example infectiousness of herds is represented in an all or nothing fashion (for sensitivity investigation also model runs with age-dependent and number dependent infectiousness were performed but main findings did not change). In case of infected herds that are protected after vaccination, temporal development of infectiousness was represented by adding a further mean infectious period (15 days; Bergevoet et al., 2007) after day of latest protection observed with the vaccine (Chapter 2).

With the model rules at hand an adapted lift-up time can be calculated: After detection of a new outbreak herd, approximately 7 days will elapse before an emergency vaccination could start. Thereafter it takes time until the herd is fully protected ("blocking immunity", 4 days in scenario "Vac4", and 14 days in "Vac14"). Finally, the protected herd is assumed infectious for the above mentioned 15 days (equal in both vaccination scenarios). Hence, according to model rules, the total time from the detection of the outbreak herd until safety of emergency vaccinated herds add up to 26 days in scenario "Vac4" respective 36 days in "Vac14". Consequently, 26 days after the last outbreak detection (less than the 30 days lift-up time supposed by legislation) in the model no virus positive animals can occur in infected vaccinated herds of scenario Vac4. This safe situation will occur after 36 days in the "Vac14" scenario (Figure 19, see "vac14 with 36d"). The identification of the exact value of lift-up time was not the scope of this assessment, but more general lessons were learnt: Both, the time period between herd vaccination and herd protection, and the supposed maximum time that regular infectious animals sustain after protection of vaccinated herd mates must be considered to calculate an adapted time for safe lift-up adapted to the applied vaccine.

Although the identification of the most appropriate value of lift-up time for either of the vaccines (and a reasonable maximal infectiousness) was not the scope of this assessment, more general lessons were learnt: Both, the time period between herd vaccination and herd protection, and the supposed maximum time that regular infectious animals remain after protection in vaccinated herds can be used to calculate a time for safe lift-up adapted to the applied vaccine.

Chronically infected animals (being infectious for >30 days) stay infective for a much longer period than assumed in the RA model. Chronic infection is usually caused in naïve animals but never observed in already vaccinated animals (see chapter 2.4). In the model the chronically infected animal would have been at least three weeks infected at time of final screening. For animals infected for such a long time, it can safely be assumed that they show obvious clinical signs. Taken the high awareness during final screening it will be highly likely that such animals do not escape the diagnostic investigation and hence will lead to the detection of the herd before lift-up. In marker vaccinated sows there is the possibility that carrier sows will emerge after field virus infection and transplacental infection of piglets. To minimise the risk from these animals, offspring from marker vaccinated sows may be tested using rRT-PCR.

None of the considered strategies can reduce the risk in the intervention area to absolute zero. Interestingly this result is identical to the independent findings by Bergevoet et al. (2007). For the strategies analysed about 98%-99% all outbreaks will not affect the safety for fresh meat.

These results are in accord with findings of Bergevoet et al. (2007), who evaluated the application E2subV and different sampling strategies for final screening. Comparing the equivalent final screening scheme (ES3 in Bergevoet et al., 2007) their study determines a probability to stay free after declaring freedom of infection (Table 1.6. in Bergevoet et al., 2007) of 95% for the pre-emptive culling and 92%-94% for the 2km resp. 5km vaccination with E2subV. The slightly less optimistic values are related to the lower sensitivity of the serologic test applied in final screening (see Table 16 in this report) the smaller radius assumed for the protection zone (10km vs. 3km in Bergevoet et al., 2007), and the occurrence of chronic courses (individual infectious periods are drawn from a gamma probability density function, Bergevoet et al., 2007, pg. 40). In addition the inability of the marker vaccine to induce complete protection was also taken into account (pg. 42 in Bergevoet et al., 2007). In essence both models lead to the conclusion that both conventional culling and emergency vaccination harbours a minimal risk for remaining infectious animals after lifting restrictions. Emergency vaccination does not change the risk markedly when compared to culling (Bergevoet et al., 2007, pg. 66).

The model-based RA did not consider the occurrence of reduced compliance in control application for all scenarios e.g. less prudent human administering the vaccine or failing people that perform screening diagnosis in conventional culling strategies. Such kind of simplification is very common when reasoning disease management strategies (Depner et al., 2005), but also in rather quantitative risk assessments (e.g. Bergevoet et al., 2007). The appeal of the simplification comes from the need to understand the strict logic of a proposed approach to control a disease. In that sense, the ToR was answered by an appropriate design and analysis of the model but assuming that a highly efficacious vaccine (e.g. the MLV) results in protected animals whenever they are designated for vaccination and not yet infected. Additionally, for CSFV outbreak control as well as for many other diseases sparse or even lacking data are available concerning the relevant level of compliance in emergency practise that could be considered in a quantitative risk assessment.

The other side of the coin is that the problem raised in the ToR implicitly might touch also the effect of e.g. non-compliance during vaccine administration although this is not explicitly stated and no field studies are available on the topic.

The expert judgement would carry forward the knowledge that usually a less ideal compliance of a control scheme will result in less perfect outcome. To explore the qualitative statement tentative analyses were performed with the model to demonstrate how the assumption of an extreme of 10% or a more relevant 2% level of non-compliance will change the safety for fresh meat by assuming respective proportions of vaccinated herds as completely unprotected. Indeed in conjunction with expectation then a minimal number of herds vaccinated with an effective vaccine still harbour virus positive animals before final screening (see Annex B, section 3). But to the opposite, a rigorous prolongation of the lift-up time – in the model - can balance for the drawback because for example 2% herd mates that remain unprotected will allow for a negligible outbreak only and finally all die or recover. Hence, again after a limited time to wait no animals risky for fresh meat will remain. Then the situation falls back to the results described above.

It is not possible with the existing knowledge, the data unavailable and the exiting models to calculate in rigorous manner which control scenario (“Cull”, “Vac4”, “Vac14”) will lead for a given lift-up time and level of compliance to the most or the fewest number of undetected infections. Nevertheless, indicative model analysis reconfirmed that with reasonable high level of compliance the total amount of risk for fresh meat was kept minimal and the longer the lift-up time can be scheduled the lower the risk will be particularly for the emergency vaccination

scenarios. Although reduced compliance will reduce the safety of intervention, prolongation of lift-up time seems to raise a promising candidate to balance the negative consequences for emergency vaccination. In that sense the presented results should be expected robust against reasonably small lack of compliance

9.3.5.4. General conclusions

The model simulations indicate that conventional CSF control strategies (e.g. pre-emptive culling) pose certain risk for fresh meat to contain CSFV. That risk is expected to be lower properlly designed emergency vaccination strategy together with the targeted search of chronically infected animals in vaccinated herds during final screening. However, if the quality of the administration procedure of the vaccine (level of compliance) reduces the resulting efficacy, this will relatively reduce safety for fresh meat. Hence, the practical quality level of vaccine administration and the role of micro-epidemics in partly-protected herds were identified as urgent research need. Time of protection after vaccination is directly correlated to the time when meat can be considered safe from virus contamination.

This report has not assessed wether the use of a vaccine which provides faster protection is superior in controlling the outbreak.

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ANNEX B - TECHNICAL DESCRIPTION OF THE MODELS

SCIENTIFIC REPORT

Control and eradication of Classic Swine Fever in wild boar[1] and

Animal health safety of fresh meat derived from pigs vaccinated against Classic Swine Fever[2]

Scientific opinions of the Panel on Animal Health and Welfare

(Question No EFSA-Q-2007-200)

(Question No EFSA-Q-2008-427)

Adopted on 12 December 2008

This annex to the opinion, published on 3 July 2009, replaces the earlier version published on 30 January 2009[3].

[1] For citation purposes: Scientific Opinion of the Panel on AHAW on a request from Commission on "Control and eradication of Classic Swine Fever in wild boar". *The EFSA Journal* (2009) 932, 1-18

[2] For citation purposes: Scientific Opinion of the Panel on AHAW on a request from Commission on "Animal health safety of fresh meat derived from pigs vaccinated against Classic Swine Fever". *The EFSA Journal* (2009) 933, 1-16

[3] The year of the Question No EFSA-Q-2008-427 has been corrected from 2007 to 2008. No further changes have been introduced in the opinion or its annexes. To avoid confusion, the original version of the opinion has been removed from the website, but is available on request as is a version showing all the changes made

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ANNEX B. MODELS

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

1.1. Model Parameters

Table 1. Model parameters

Parameter	Description	Default value	Bibliographic source
t	Time step	1 day	-
Sj	Susceptible < 4 months	10	-
Ej	Latent < 4 months	0	-
Ij	Infectious < 4 months	0	-
It	Immunotollerant	0	-
Rp	Passively immunized < 4 months	0	-
Rj	Recovered < 4 months	0	-
S	Susceptible > 4 months	100	-
E	Latent > 4 months	0	-
I	Infectious > 4 months	1	-
R	Recovered > 4 months	0	-
C	Chronic > 4 months	0	-
N(t)	Population	Sj+Ej+Ij+It+Rp+Rj+S+E+I+R+C	-
K	Carrying capacity	240	-
β	Coefficient of transmission	0.25 day-1	P5-Individual Progress Report 2005
aj	Lethality 0-4 months	70% in 15 days	Depner et al., 1994 e 2007 ; Dewulf et al., 2001; Mittelholzer et al., 2000; van Oirschot, 1990; Kaden et al., 2004
a	Lethality > 4 months	5% in 15 days	
g	Recovered rate	13 days	Uttenthal et al., 2003; Depner et al., 1995; van Oirschot, 1990
pl	Incubation rate	6 days	Uttenthal et al., 2003; Depner et al., 2007 ; Dewulf et al., 2001 e 2002; Depner et al., 1994 ; Ribbens et al. 2004 ; Kaden et al., 2004; Leavens et al., 1999
c	Hunting rate	45% year-1	EU 6th FPP-SSP/8.1 c.n.501559; Lemel, 1999
cj	Hunting rate (<4 months)	20% year-1	EU 6th FPP-SSP/8.1 c.n.501559
im	Loss of passive immunity	Half-life = 14 days	Coggins, 1964
pj	Transiting rate from <4 months to >4 months	120 days	-
na	Potential natality (low population density)	2,98 newborn per wild boar year-1 (effective annual rate of 1.5 animal per individual)	Fenati e Armaroli, 2004
uj	Max mortality rate (<4 months) (high population density)	(90%-c) year -1	Lebedeva, 1956
μ	Natural Mortality (>4 months)	3% year -1	Lemel, 1999
Pci	Proportion of immunotolerants	0.3	-
mci	Proportion of Chronics	0.25	-
di	Immunotollerants survival	Mean ~ 60 gg	Moennig et al , 2003 ; Meyer et al., 1981 ; van Oirschot e Terpstra, 1977
it	Chronic survival	Mean ~ 60 gg	Dahle and Liess, 1992; Moennig et al. , 2003; van Oirschot, 1990
mi	Max migration rate (high population density)	13 animals year-1 (6 animals year-1 as the proportion of 12-15 months in the population)	Anrzejewski e Jezierski, 1978

Table 2. Stochastic simulation parameters

Parameter	Parameter distribution values	Initial value	Literature
Recovered rate (g)	Uniform min: 10 days ; max: 20 days	13 days	Uttenthal et al., 2003 ; Depner et al., 1995; van Oirschot, 1990
Latency (pl)	Uniform min: 3 days; max: 10 days	6 days	Uttenthal et al., 2003 ; Depner et al., 2007 ; Dewulf et al., 2001 and 2002 ; Depner et al., 1994 ; Ribbens et al., 2004 ; Kaden et al., 2004; Leavens et al., 1999
Lethality (a)	Uniform min: 7 days; max: 20 days	15 days	Depner et al., 1994 e 2007 ; Dewulf et al., 2001; Mittelholzer et al., 2000; van Oirschot, 1990; Kaden et al., 2004
Beta (β)	Uniform min: 0.2 day-1; max: 0.3 day-1	0.25	P5-Individual Progress Report 2005
Proportion of Immunotollerants	Uniform min: 0.1 days; max: 0.5 days	0.3	-
Proportion of chronics	Uniform min: 0 days; max: 0.05 days	0.25	-
Immunotollerants survival	Weibull Shape (α): 1.5; scale (β): 60	Mean ~ 60 gg	Moennig et al , 2004 ; Meyer et al., 1981 ; van Oirschot e Terpstra, 1977
Chronics survival	Weibull Shape (α): 2.5; scale (β): 60	Mean ~ 60 gg	Dahle and Liess, 1993; Moennig et al. , 2003; van Oirschot, 1990

Chronic infection is characterized by three phase: a) clinic disease, b) remission and c) clinical exacerbation, where in b) the viraemia is reduced or can completely disappear. Chronic infectivity has been reduced of 1/3.

1.2. Model validation

Table 3. Goodenss of fit test for both the model: basic (no long virus shedder) and modified (with long virus shedder).

Model	WRMSE	Optimised Value	Worst case
Basic	0.07575846	0.05467576	0.0733529
With long virus shedder	0.06651072		

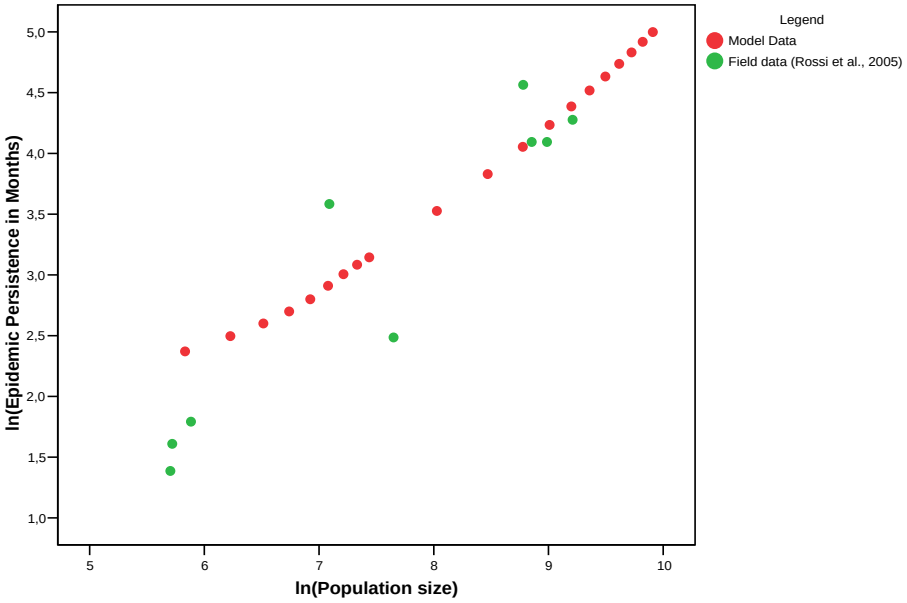


Figure 1. Comparison between model and field data (Rossi et al., 2005) about virus persistence at different population size.

1.3. Sensitivity analysis

Sensitivity analysis of all model parameter was tested for the virus persistence (Figure 2, Table 4). The coefficient of transmission (β) resulted as the most sensitive parameter, then the latency period (incubation), adult mortality and recovery rate. Small positive changes (increase) of these parameters produce large variation of virus persistence that decrease except for carrying capacity and adult mortality.

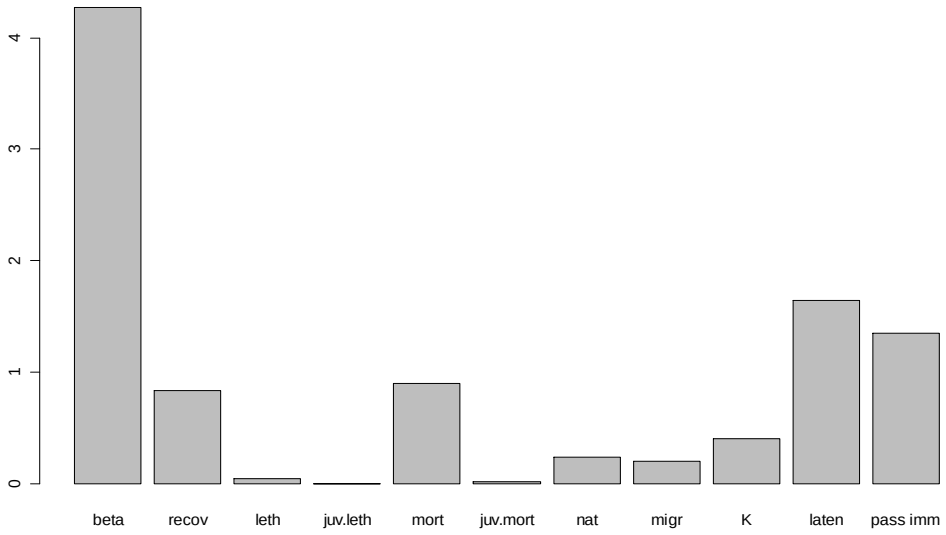


Figure 2. Sensitivity analsysis of the model parameters for virus persistence.

Table 4. Parameter sensitivity values in respect to virus persistence.

Parameters	Sensitivity
Coefficient of transmission	-4.26926181
Recovery rate	-0.84018249
Lethality	-0.04375738
Juvenile lethality	0.00000000
Mortality	0.90126906
Juvenile mortality	0.01990321
Natality	-0.23531179
Migration rate	-0.20483550
Carrying capacity	0.40817536
Latency (incubation)	-1.64484380
Passive Immunity	-1.35447377

1.4. Metapopulation Equations

1.4.1. Basic model (acute or sub-acute infection)

$$\frac{dSj_i}{dt} = S_i \cdot stn(a,t,nn_i,K_i) \cdot Sj_i \cdot (tc(j,t) + uj \cdot nn_i / K_i + pj$$
$$- (trasm(b,I_i,nn_i,el) + trasm(b,Ij_i,nn_i,el) \cdot Rp_i \cdot im)$$

$$\frac{dEj_i}{dt} = -Ej_i \cdot (tc(j,t) + uj \cdot nn_i / K_i + pl)$$
$$+ Sj_i \cdot (trasm(b,I_i,nn_i,el) + trasm(b,Ij,nn,el))$$

$$\frac{dIj_i}{dt} = -Ej_i \cdot pl - Ij_i \cdot (tc(j,t) + uj \cdot nn_i / K_i + g + aj)$$
$$\frac{dRj_i}{dt} = Ij_i \cdot g - Rj_i \cdot (tc(j,t) + uj \cdot nn_i / K_i + pj)$$
$$\frac{dRp_i}{dt} = R_i \cdot stn(a,t,nn_i,K_i) \cdot Rp_i \cdot (tc(j,t) + uj \cdot nn_i / K_i + im)$$
$$\frac{dS_i}{dt} = -S_i \cdot (tc(,t) + u) + \sum_{u=1}^{h} miS_{iu} \cdot S_u \cdot nn_u / K_u - \sum_{u=1}^{h} miS_{ui} \cdot S_i \cdot nn_i / K_i + Sj_i \cdot pj$$
$$- S_i \cdot (trasm(b,I_i,nn_i,el) + trasm(,Ij_i,nn_i,el)) \cdot Rp \cdot im$$
$$\frac{dE_i}{dt} = -E_i \cdot (pl + stc(,t) + u) + \sum_{u=1}^{h} miE_{iu} \cdot E_u \cdot nn_u / K_u - \sum_{u=1}^{h} miE_{ui} \cdot E_i \cdot nn_i / K_i$$
$$+ S_i \cdot (trasm(b,I_i,nn_i,el) + trasm(,Ij_i,nn_i,el))$$
$$\frac{dI_i}{dt} = E_i \cdot pl - I_i \cdot (pci + g + a + stc(,t) + u) + \sum_{u=1}^{h} miI_{iu} \cdot I_u \cdot nn_u / K_u$$
$$- \sum_{u=1}^{h} miI_{ui} \cdot I_i \cdot nn_i / K_i$$
$$\frac{dR_i}{dt} = I_i \cdot g - Rj_i \cdot pj - R_i \cdot (tc(,t) + u) + \sum_{u=1}^{h} miR_{iu} \cdot R_u \cdot nn_u / K_u$$
$$- \sum_{u=1}^{h} miR_{ui} \cdot R_i \cdot nn_i / K_i$$

1.4.2. Modified model that includes long shedder chronics and immunotollerant animals

$$\frac{dSj_i}{dt} = S_i \cdot stn(a,t,nn_i,K_i) \cdot Sj_i \cdot (tc(j,t) + uj \cdot nn_i / K_i + pj - (trasm(b,I_i,nn_i,el)$$
$$+ trasm(,Ij_i,nn_i,el) + trasm(,It_i,nn_i,el) + trasm(,C_i \cdot tc,nn_i,el) \cdot Rp_i \cdot im)$$
$$\frac{dEj_i}{dt} = -Ej_i \cdot (tc(j,t) + uj \cdot nn_i / K_i + pl) + Sj_i \cdot (trasm(b,I_i,nn_i,el)$$
$$+ trasm(,Ij_i,nn_i,el) + trasm(,It_i,nn_i,el) + trasm(,C_i \cdot tc,nn_i,el))$$
$$\frac{dIj_i}{dt} = -Ej_i \cdot pl - Ij_i \cdot (tc(j,t) + uj \cdot nn_i / K_i + g + aj)$$
$$\frac{dIt_i}{dt} = I_i \cdot it \cdot stn(a,t,nn_i,K_i) \cdot It_i \cdot (tc(j,t) + uj \cdot nn_i / K_i + pj + di)$$
$$\frac{dRj_i}{dt} = Ij_i \cdot g - Rj_i \cdot (tc(j,t) + uj \cdot nn_i / K_i + pj)$$

$$\frac{dRp_i}{dt} = R_i \cdot stn(a,t,nn_i,K_i) - Rp_i \cdot (tc(j,t) \cdot uj \cdot nn_i / K_i + im)$$

$$\frac{dS_i}{dt} = -S_i \cdot (tc(i,t) \cdot u) + \sum_{u=1}^{h} miS_{iu} \cdot S_u \cdot nn_u / K_u - \sum_{u=1}^{h} miS_{ui} \cdot S_i \cdot nn_i / K_i + Sj_i \cdot pj$$

$$- S_i \cdot \begin{pmatrix} trasm(b, I_i, nn_i, el) + trasm(b, Ij_i, nn_i, el) + trasm(b, It_i, nn_i, el) \\ + trasm(b, C_i \cdot tc, nn_i, el) \end{pmatrix}$$

$$\frac{dE_i}{dt} = -E_i \cdot (pl + stc(i,t) \cdot u) + \sum_{u=1}^{h} miE_{iu} \cdot E_u \cdot nn_u / K_u - \sum_{u=1}^{h} miE_{ui} \cdot E_i \cdot nn_i / K_i$$

$$+ S_i \cdot \begin{pmatrix} trasm(b, I_i, nn_i, el) + trasm(b, Ij_i, nn_i, el) + trasm(b, It_i, nn_i, el) \\ + trasm(b, C_i \cdot tc, nn_i, el) \end{pmatrix}$$

$$\frac{dI_i}{dt} = E_i \cdot pl - I_i \cdot (pci + g + a + stc(i,t) \cdot u) + \sum_{u=1}^{h} miI_{iu} \cdot I_u \cdot nn_u / K_u$$

$$- \sum_{u=1}^{h} miI_{ui} \cdot I_i \cdot nn_i / K_i$$

$$\frac{dR_i}{dt} = I_i \cdot g - Rj_i \cdot pj - R_i \cdot (tc(i,t) \cdot u) + \sum_{u=1}^{h} miR_{iu} \cdot R_u \cdot nn_u / K_u$$

$$- \sum_{u=1}^{h} miR_{ui} \cdot R_i \cdot nn_i / K_i$$

$$\frac{dC_i}{dt} = I_i \cdot pci - C_i \cdot (tc(i,t) \cdot mci) + \sum_{u=1}^{h} miC_{iu} \cdot C_u \cdot nn_u / K_u$$

$$- \sum_{u=1}^{h} miC_{ui} \cdot C_i \cdot nn_i / K$$

1.4.3. Model with vaccination

$$\frac{dSj_i}{dt} = S_i \cdot stn(a,t,nn_i,K_i) - Sj_i \cdot (vac(j_i,t,i_i) + stc(j,t) \cdot uj \cdot nn_i / K_i + pj$$

$$- (trasm(b, I_i, nn_i, el) + trasm(b, Ij_i, nn_i, el) + trasm(b, It_i, nn_i, el) + trasm(b, C_i, nn_i, el) - Rp_i \cdot im)$$

$$\frac{dEj_i}{dt} = -Ej_i \cdot (tc(j,t) \cdot uj \cdot nn_i / K_i + pl)$$

$$+ Sj_i \cdot (trasm(b, I_i, nn_i, el) + trasm(b, Ij_i, nn_i, el) + trasm(b, It_i, nn_i, el) + trasm(b, C_i, nn_i, el))$$

$$\frac{dIj_i}{dt} = -Ej_i \cdot pl - Ij_i \cdot (stc(j,t) \cdot uj \cdot nn_i / K_i + g + aj)$$

$$\frac{dIt_i}{dt} = I_i \cdot it \cdot stn(a,t,nn_i,K_i) - It_i \cdot (tc(j,t) \cdot uj \cdot nn_i / K_i + pj + di)$$

$$\frac{dRj_i}{dt} = Ij_i \cdot g - Rj_i \cdot (tc(j,t) \cdot uj \cdot nn_i / K_i + pj) + Sj_i \cdot vac(j_i,t,i_i)$$

$$\frac{dRp_i}{dt} = R_i \cdot stn(a,t,nn_i,K_i) - Rp_i \cdot (tc(j,t) \cdot uj \cdot nn_i / K_i + im)$$

$$\frac{dS_i}{dt} = -S_i \cdot (vac(i,t,i_i) + stc(i,t) \cdot u) + \sum_{u=1}^{h} miS_{iu} \cdot S_u \cdot nn_u / K_u - \sum_{u=1}^{h} miS_{ui} \cdot S_i \cdot nn_i / K_i$$

$$+ Sj_i \cdot pj - S_i \cdot \begin{pmatrix} trasm(b, I_i, nn_i, el) + trasm(b, Ij_i, nn_i, el) + trasm(b, It_i, nn_i, el) \\ + trasm(b, C_i, nn_i, el) \end{pmatrix} - Rp \cdot im$$

$$\frac{dE_i}{dt} = -E_i \cdot (pl + stc(t,u)) + \sum_{u=1}^{h} miE_{iu} \cdot E_u \cdot nn_u / K_u - \sum_{u=1}^{h} miE_{ui} \cdot E_i \cdot nn_i / K_i$$
$$+ S_i \cdot \begin{pmatrix} trasm(\phi, I_i, nn_i, el) + trasm(\phi, Ij_i, nn_i, el) + trasm(\phi, It_i, nn_i, el) \\ + trasm(\phi, C_i, nn_i, el) \end{pmatrix}$$

$$\frac{dI_i}{dt} = E_i \cdot pl - I_i \cdot (pci + g + a + stc(t,u)) + \sum_{u=1}^{h} miI_{iu} \cdot I_u \cdot nn_u / K_u - \sum_{u=1}^{h} miI_{ui} \cdot I_i \cdot nn_i / K_i$$

$$\frac{dR_i}{dt} = I_i \cdot g - Rj_i \cdot pj - R_i \cdot (tc(t,u)) + \sum_{u=1}^{h} miR_{iu} \cdot R_u \cdot nn_u / K_u - \sum_{u=1}^{h} miR_{ui} \cdot R_i \cdot nn_i / K_i$$
$$+ Si \cdot vac(t, i_i)$$

$$\frac{dC_i}{dt} = I_i \cdot pci - C_i \cdot (tc(t,u) + mci)$$

2. Uncertain sensitivity of MOSS in Wild Boar

Pseudo-code describing the model algorithm to simulate sampling surveys

Details of the simulation algorithm that calculates the sensitivity of a Monte-Carlo sampling process aiming at the detection of a low prevalent disease in the population

2.1. Step1: Initialise the simulation.

Input the total number of animals in the simulation area (N; e.g. 1000).

Input the number of grid cells (GC; e.g. 100).

Input the number of infected animals (A; e.g. 10; or prevalence i.e. 1%).

Input the total sample size S or sampling fraction f (S is the product of the sampling fraction f and the total number of animals in the simulation area: S = f * N or equivalently f = S/N).

2.2. Step2: Generation of the spatial wild boar distribution.

Input the degree of clustering in cells of wild boars (i.e. parameter bN).

The closer the value of the parameter bN is the more the wild boars are clumped in few grid cells. For increasing parameter values (e.g. bN > 100) the wild boar distribution will tend to be more uniformly random throughout the cells.

Assign the animals randomly to grid cells using a binomial beta random function:

For each cell i (i in 1 to GC) the random number of animals ni is drawn from:

ni= Binomial (Beta (bN,bN*(GC-i)), Ni)

where $Ni=N-\sum_{j=1}^{i-1} n_j$

n0= 0

2.3. Step3: Generation of the spatial distribution of infected wild boar

Input the degree of clustering in cells of infected wild boars (i.e. parameter bA).

The closer bA is to zero the more the infected animals cluster in few cells. For increasing parameter values the infected wild boars will be more uniformly distributed.

Assign the infected animals randomly to grid cells using a binomial beta random function:

For each cell i (i in 1 to GC) the random number of infected animals ai is drawn from:

ai= Binomial(Beta(bA,bA*(GC-i)), Ai)

where $Ai=A-\sum_{j=1}^{i-1} a_j$

2.4. Step4: Select the sample

Input the degree of clustering in cells of samples taken (i.e. parameter bf).

The closer bf is to zero the more animals of the total sample are from few cells only. For increasing parameter values the sampled wild boars will be taken more uniformly from all grid cells.

Assign the sampled animals randomly to grid cells using a binomial beta random function:

For each cell i (i in 1 to GC) the random number of sampled animals si is drawn from:

si= Binomial(beta (bf,bf*(GC-i)),Si)

si= min(si,ni)

where $Si=S-\sum_{j=1}^{i-1} s_j$ and S=f x N

2.5. Step5: Simulation of infected animals within the sample from the grid cells

The number of infected animals (xi) that reach the sample of a grid cell (si) is randomly drawn from:

xi= Hypergeometric(ni,si,ai)

2.6. Step6: Simulation of the diagnostic test

The number of infected animals in the sample (xi) that test positive in an individual diagnostic test (ri) with individual test sensitivity Se, is randomly drawn from:

ri= Binomial(xi,Se)

2.7. Step7: Report survey result

The disease is detected if for one grid cell j the rj is greater than zero.

3. Model-Based Risk Assessment of the risk for fresh meat originating from pigs after emergency vaccination

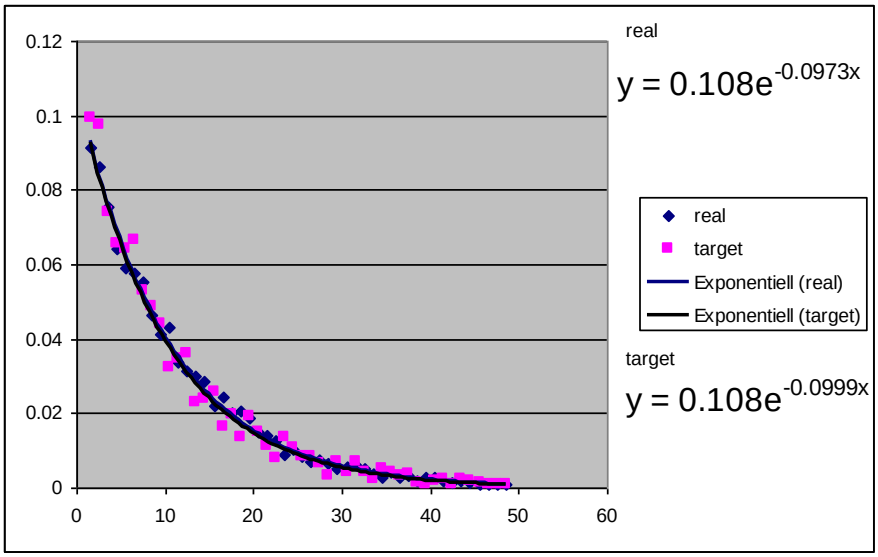
3.1. Model parameters

Table 5. Risk assessment (RA) model - Parameters relevant for the RA-Meat

Simulation Parameters	Parameter values	Source	Explanation
HerdDensity	1 & 3	Scenario	Average per sq km
PigsPerHerd	1000 & 3000	Scenario	Average per sq km
DaysNeededTillSell	120	WG (Work Group)	Time till finishing/slaughter of finisher pigs above 60 days old
Disease & Transmission			
HerdIncubationDays	4	WG	Herd capable to transmit (days)
D500	0.003813242	Stegeman, 2002	Local infection probability per herd less than 500m apart of an infectious herd
D1000	0.001110579	Stegeman, 2002	As before but between 500m & 1000m
RegionalInfectProb	0.073	Fitted	Probability per day per infectious farm to cause an infection (Fit: observed secondary infections per HRP - Dutch data; Fig.A-RAmodel 1+2)
RegionalMaxInfectDist	50	Scenario	Maximum distance of regional transmission
RegionalMeanInfectDist	10	Fitted	Mean of distance distribution (neg.exp.) (Fit: observed transmission distances - Dutch data; Fig.A-RAmodel 1+2)
Simulation Parameters	**Parameter values**	**Source**	**Explanation**
EpidemicGrowthRate	0.0822	Fitted	Probability per infectious capita per day to infect a herd mate (Fit: observed time profiles of sero-positive animals – Dutch data; Fig. A-RAmodel 3)
Detection			
MinFarmerDetectTime	21	Literature	Lower limit of detection (days; farmer's suspicion)
MaxFarmerDetectTime	55	Literature	Upper limit of detection (days; farmer's suspicion)
FirstDetectDelay	14	Literature	Delay during high risk period (days)
TestSystem	1 or 0	Scenario	Test system 1=virus / 0=antibodies (1 = rRT-PCR / 0 = E2-ELISA and Erns-ELISA)
TestDaysTillDetect	1: 3 days 0: 16 or 28	WG – Report (mean of interval)	Testability after infection; earlier testing will be false negative (days)
TestSensitivity	1: 100% 0: 90%	WG-Report	Sensitivity of the applied test after TestDaysTillDetect days post infection
TestSample	0 or 1	WG-Report	0 = census test, all animals, pooled; 1 = sample test: minimum of (60 animals; 10% herd size), and one per pen assuming clustered disease occurrence
TracingEfficiency	0.8	Scenario	Probability to establish an infection sourcing from & targeting at the herd
LiftupCondition	30	WG-Report	Time period without any new detection before final screening can start (WaitTimeTillFree)
Standstill			
StandstillRadius	10	Scenario	Radius of zone affected by standstill (km)
StandstillDelay	1	WG	Time after detection before standstill is established (days)
StandstillEfficency	0.8	Scenario	Probability to suppress regional infections
Surveillance			
SurveillanceRadius	10	Scenario	radius of zone affected by surveillance (km)
SurveillanceDetect Reduction	7	Literature	Shortens time till detection at regular visit of an expert (days; 1 visit per week)

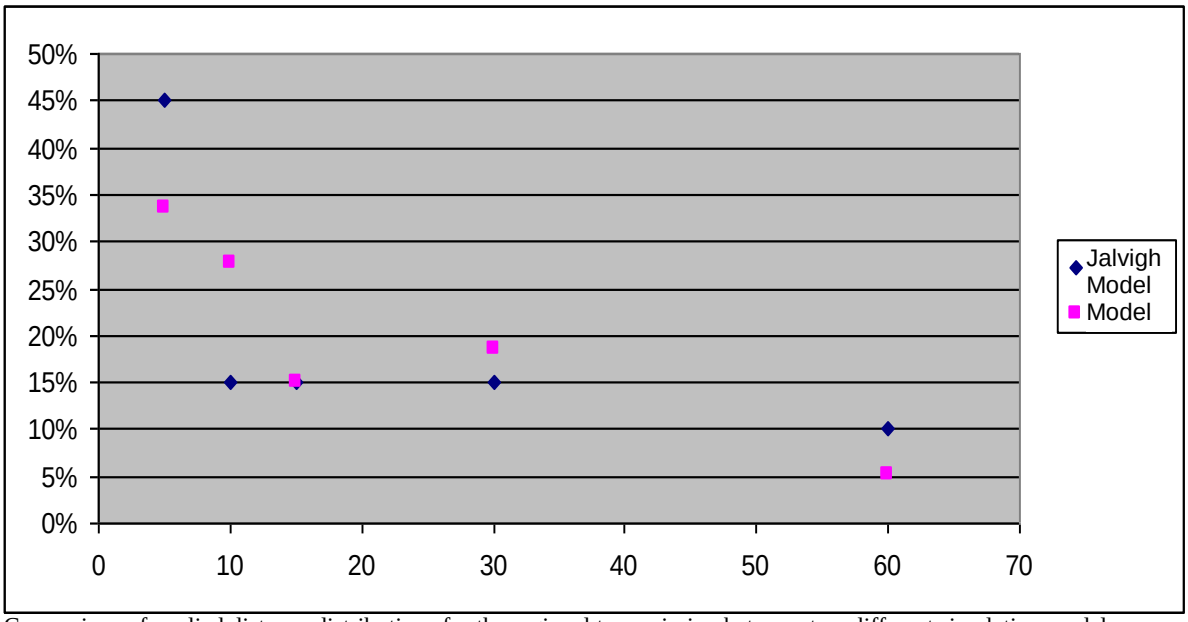
Pre-emptive Culling			
CullRadius	1	Directive	Radius of culling zone (km)
CullDelay	3	WG	Time after detection (days) until first animal is pre-emptively culled; may be delayed by limited capacities (see CullCapacityPerDay)
CullCapacityPerDay	7150 or ∞	WG	Animals per day (all culls: emergency + pre-emptive + welfare)
Vaccination			
VacRadius	3	Directive	Radius of vaccination zone (km)
VaccDelay	7	WG	Time after detection (days) until first animal is treated by vaccination teams; may be delayed by limited capacities (see VacCapacityPerDay)
VacCapacityPerDay	14300 or ∞	WG	Animals per day
VacTimeTillImmune	4 & 14	WG-Report	Days until a vaccinated farm turns protected. If earlier than an infected vaccinated farm turns infectious, the infected farm turns immune after last infected animal recovered (see VacInfectiousDays)
VacInfectiousDays	15	Scenario	Days a vaccinated and infected herd remains infectious after "the rest of the vaccinated non-infected animals" became protected; Equals mean infectious period after Bergevoet et al. (2007)

3.2. Patterns and Data used to fit model parameters:



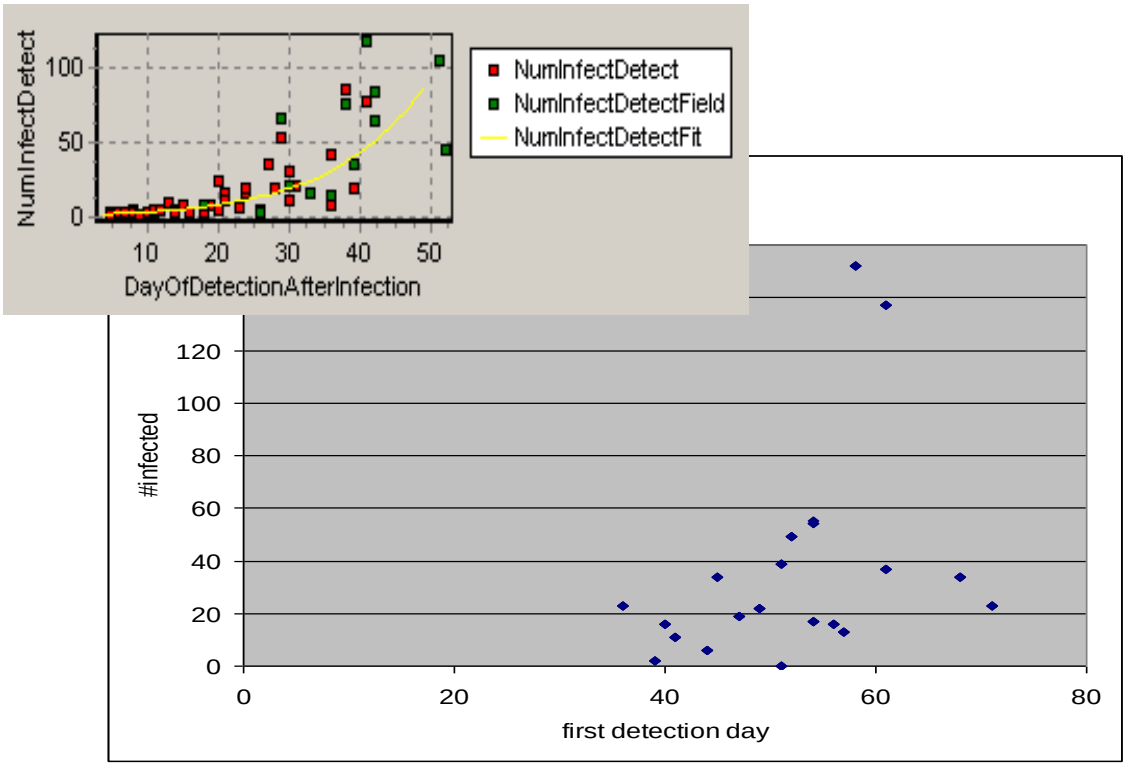
Observed distance distribution of regional transmission events (real - blue).
The observed data follow a negative exponential distribution which is used to parameterize the model.
Simulated data are reread from the model algorithm determining the regional transmission (target - pink).
Source: Dutch data.

Figure 3. A - RA model 1



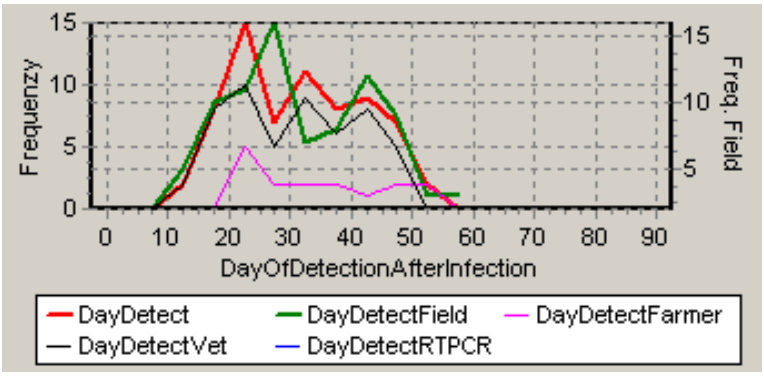
Comparison of applied distance distributions for the regional transmission between two different simulation models.
Pink square: The data as reread from the algorithm determining the regional transmission events.
Blue rhombus: The distance distribution applied to simulate regional CSF transmission
Based on the model by Jalvigh et al. (1999; see reference list report)

Figure 4. A - RA model 2



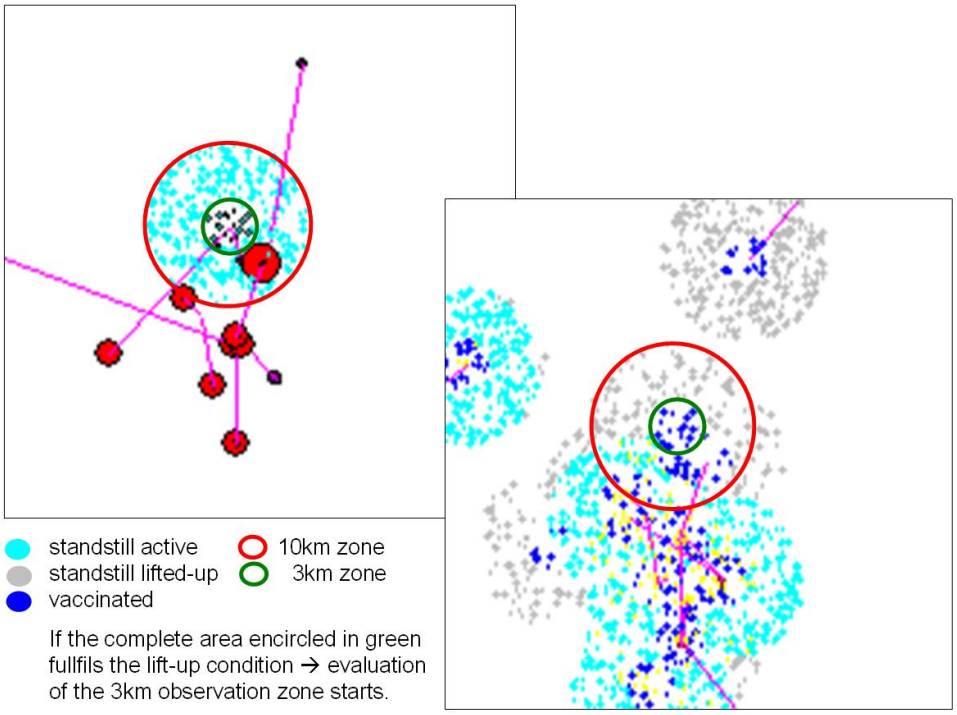
Time-line of sero-positive animals detected in infected herds as function of the age of infection (backwards tracing was used to determine the day of infection). The grey insert shows the same data (green squares) compared to the data reread from the model algorithm that realises epidemic growth (red squares)
Source: Dutch data.

Figure 5. A - RA model 3



Distribution of time between infection and detection for traced herds with known date of infection (green line). The same data are reread from the model simulation that perfectly counted the time between infection and detection for all infected herds (red line). Other colours represent the proportion contributed by the detection routes.
(The simulation example does not show the contribution of PCR testing as the field data comprise the HRP only.)
Source: Dutch data.

Figure 6. A - RA model 4



Snapshot of the spatially explicit simulation model that depicts the logic of the evaluation of the 3km observation zone in the RA Meat.
The left box shows the initial detection (centre of the green circle line) already stamped out and the activated standstill zone of 10km radius (red circle line). Red dots mark additional outbreaks still not detected (red filled circles growing according to the number of infectious animals). Pink lines show the geography of regional transmission events.
The right box shows the same location but with the situation at the point in time when the 3km observation zone had lacked new detections inside or new overlap with other established zones for at least 30 days. At this point the data are read for evaluation before the full outbreak simulation continues. In the simulation the physically final lift-up only was performed if the full area ever affected fulfilled the lift-up condition simultaneously. Susceptible and not involved farms around the intervention zones are not shown. Blue farms symbolize vaccinated farms. Grey farms symbolize farms with no detection since at least 30 days in their 10km vicinity. Yellow herds needed to be welfare slaughtered (aged up to +30days above finishing).

Figure 7. A – RA model 5

3.3. Flowchart

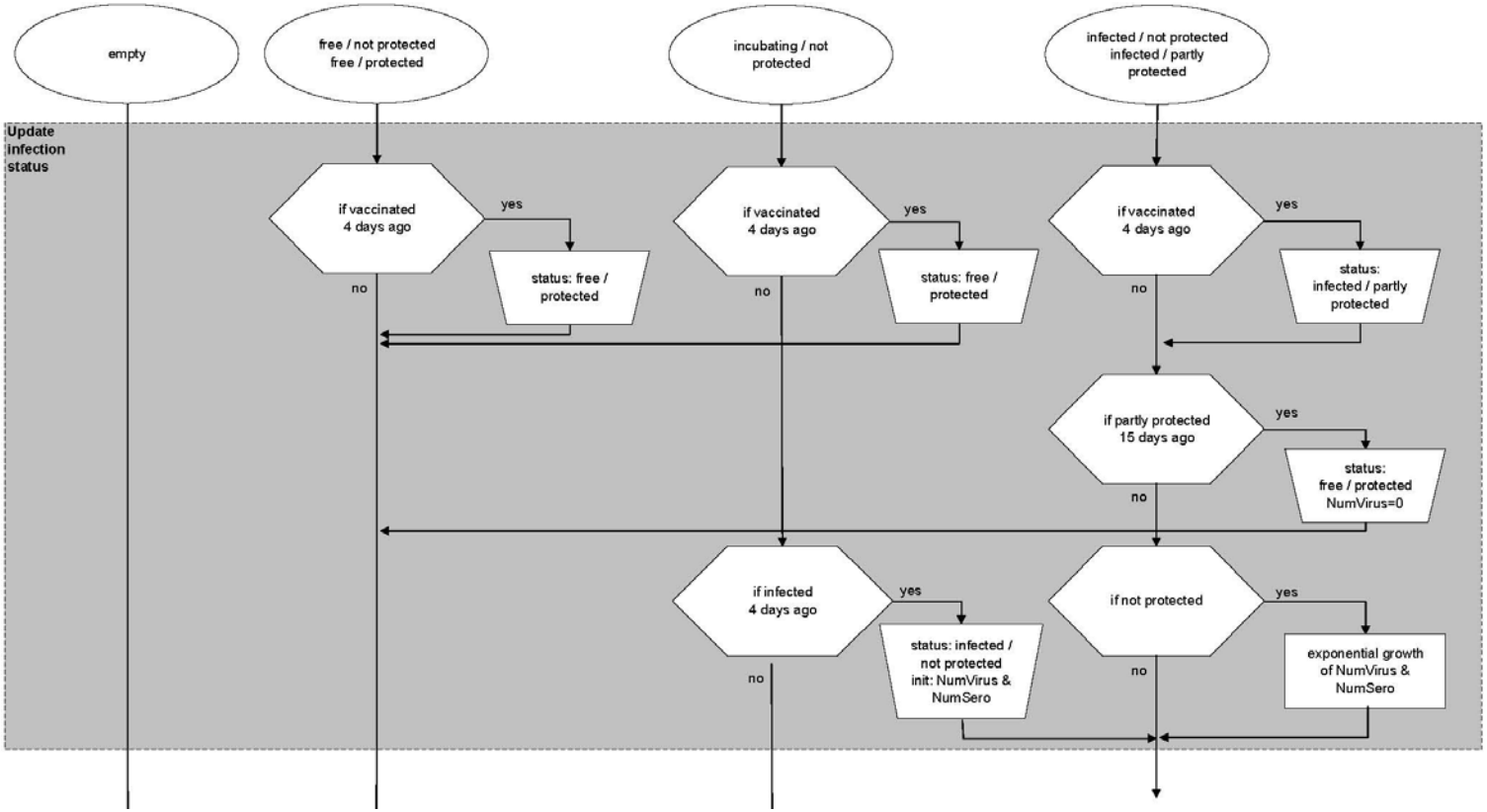


Figure 8. Model-based RA of the risk for fresh meat originating from pigs after emergency vaccination - Flowchart (a)

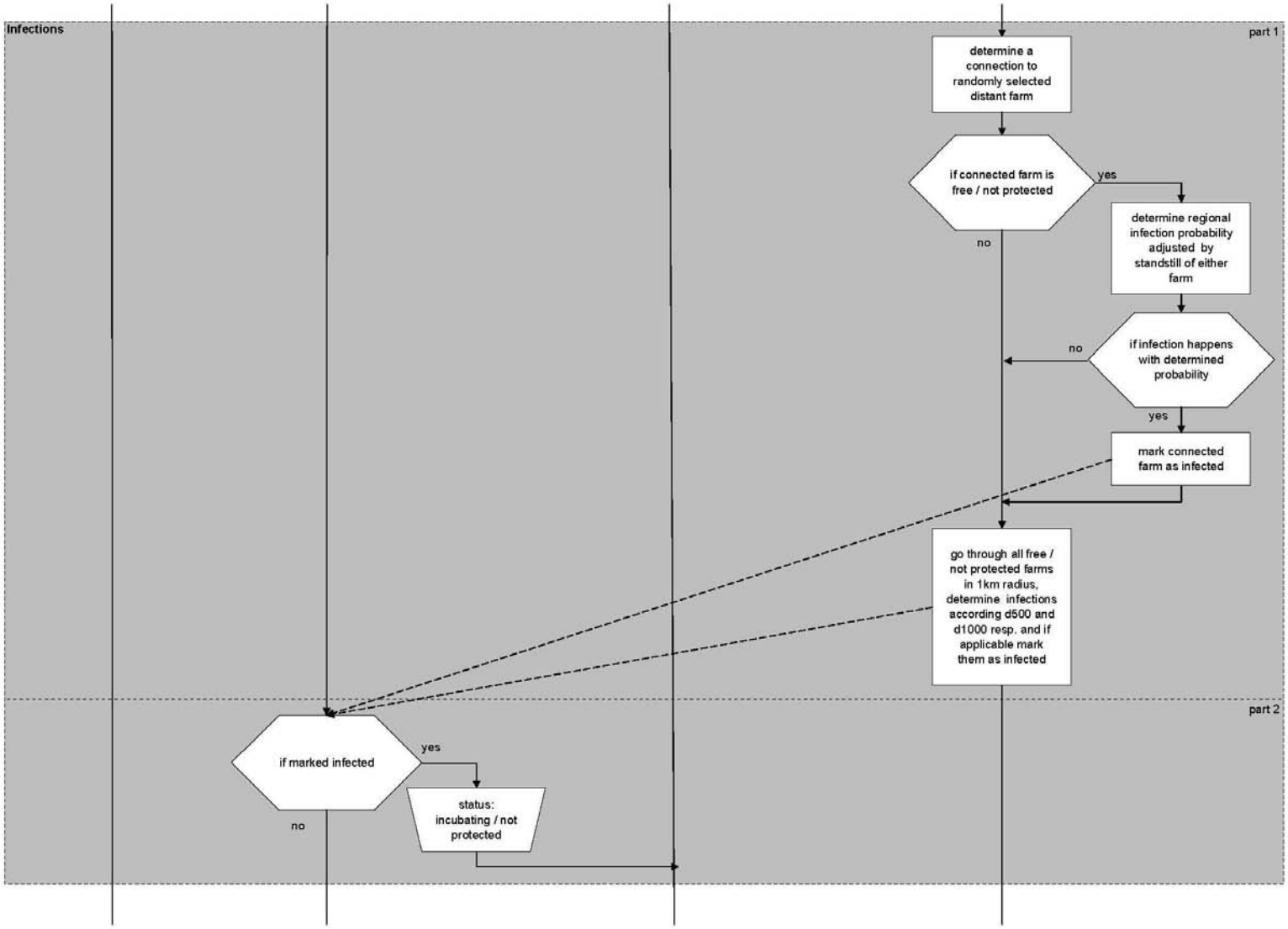


Figure 9. Model-based RA of the risk for fresh meat originating from pigs after emergency vaccination - Flowchart (b)

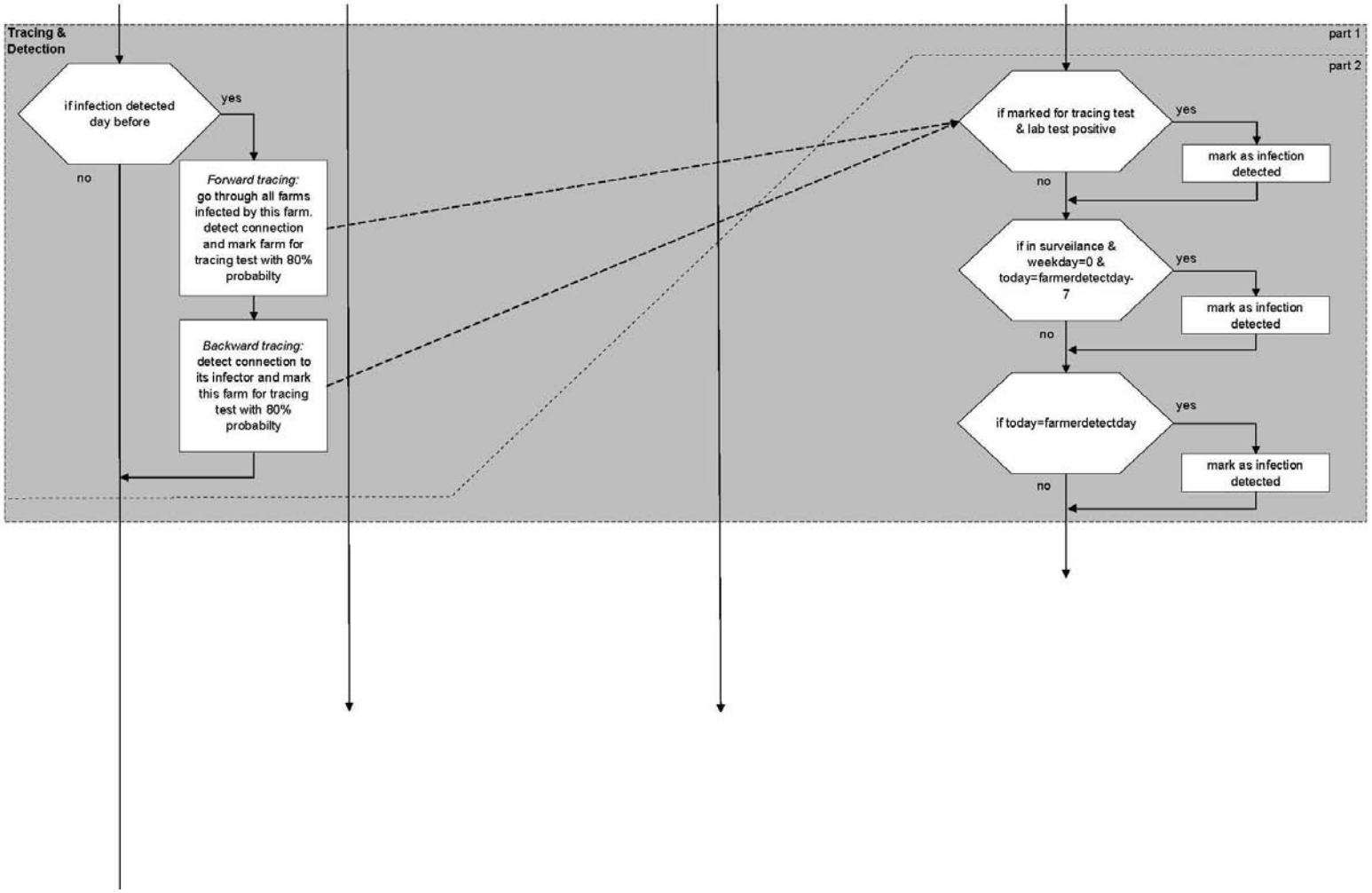


Figure 10. Model-based RA of the risk for fresh meat originating from pigs after emergency vaccination - Flowchart (c)

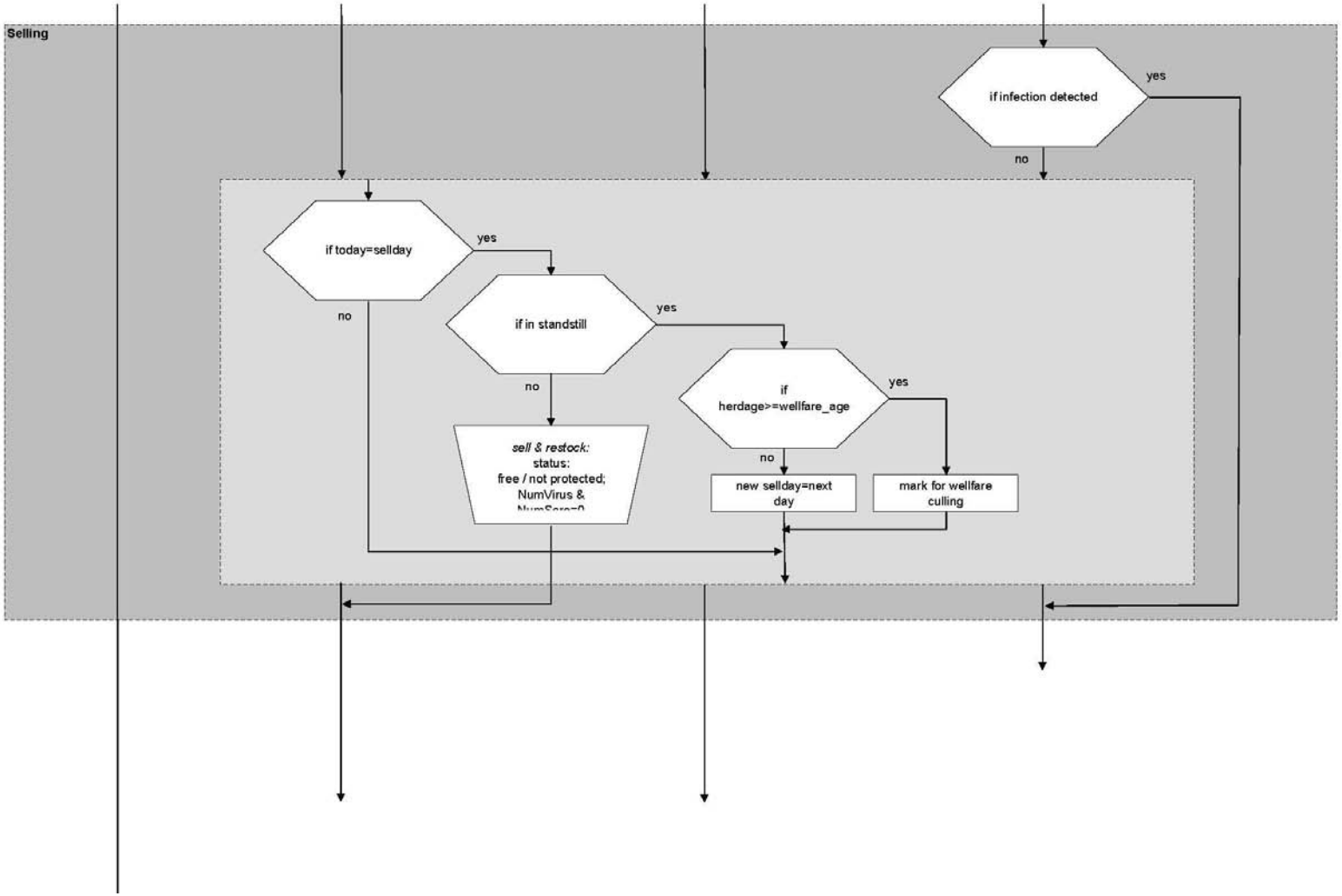


Figure 11. Model-based RA of the risk for fresh meat originating from pigs after emergency vaccination - Flowchart (d)

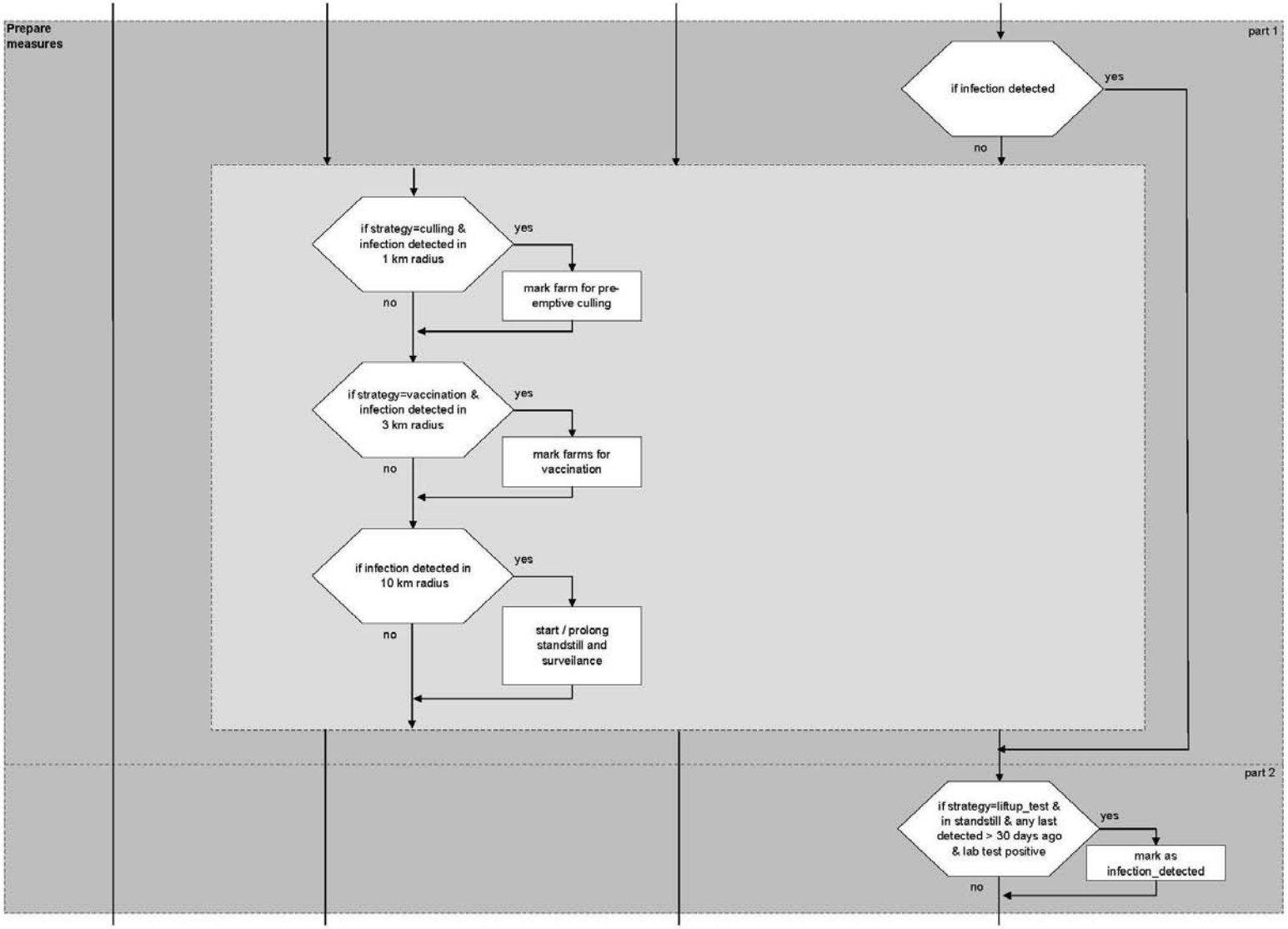


Figure 12. Model-based RA of the risk for fresh meat originating from pigs after EV - Flowchart (e)

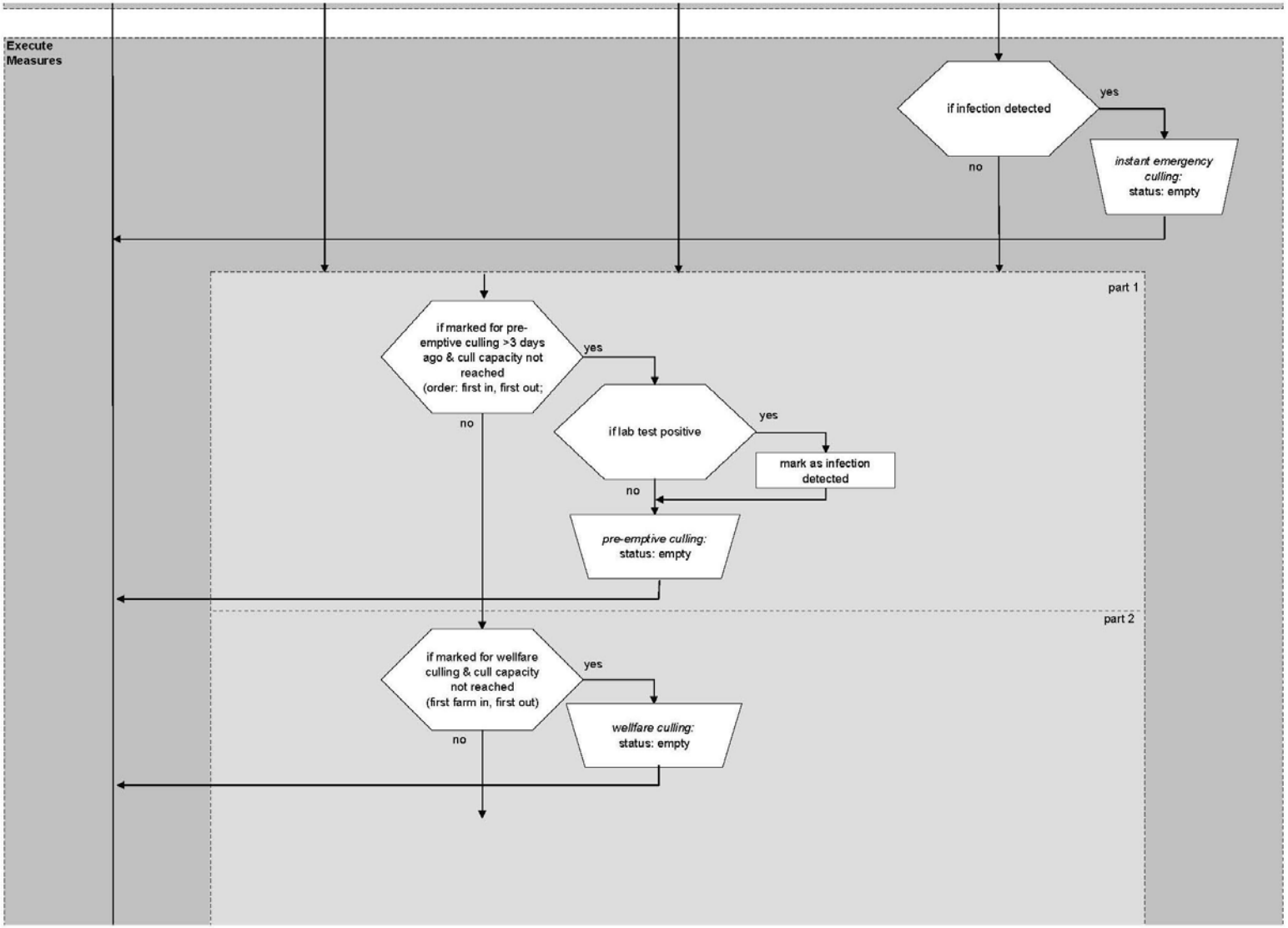


Figure 13. Model-based RA of the risk for fresh meat originating from pigs after EV - Flowchart (f)

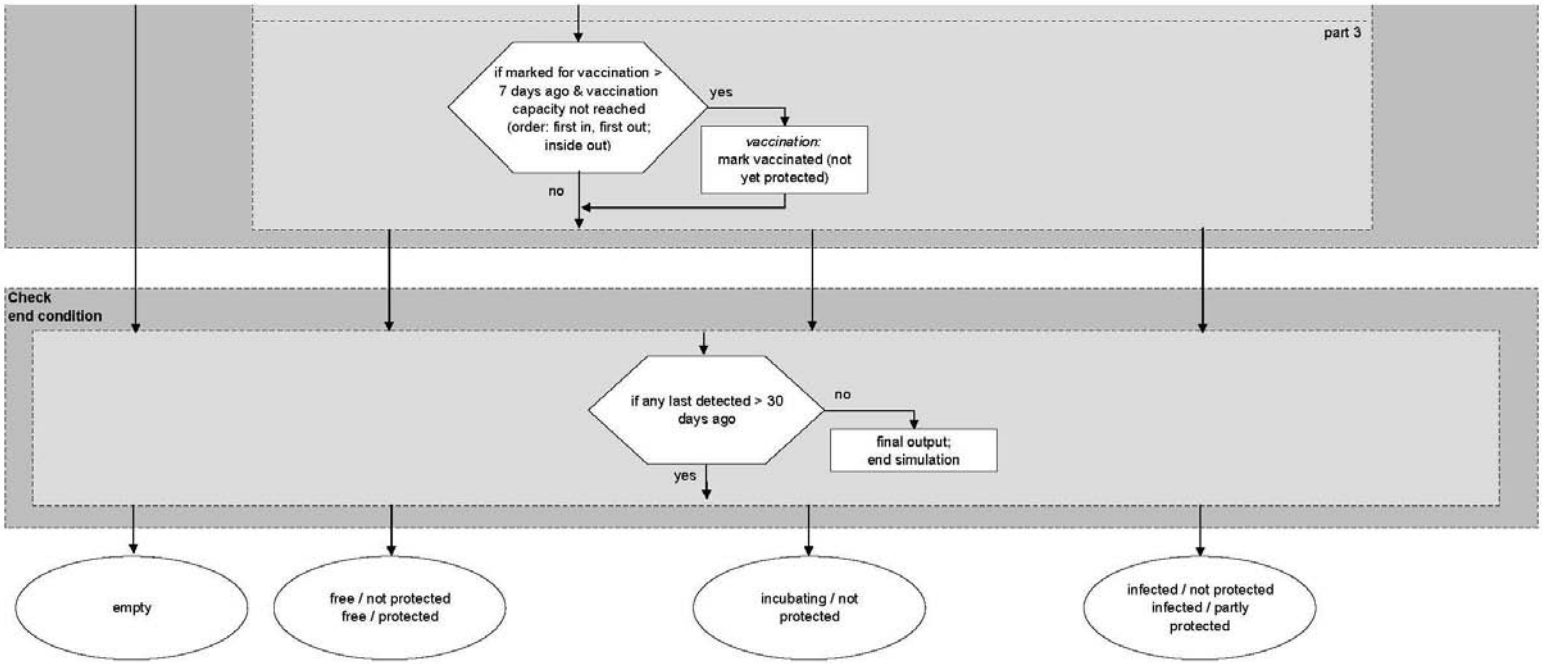


Figure 14. Model-based RA of the risk for fresh meat originating from pigs after EV - Flowchart (g)