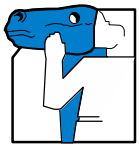
Aan Trees en Wannes



Faculteit Diergeneeskunde

Vakgroep Voortplanting, Verloskunde en Bedrijfsdiergeneeskunde

Epidemiology and control of classical swine fever: experimental assessment of virus transmission and potential benefits of emergency vaccination.

Proefschrift ter verkrijging van de graad van Doctor in de Diergeneeskundige Wetenschappen (PhD) aan de Faculteit Diergeneeskunde, Universiteit Gent, November 2002

door

Jeroen Dewulf

Promotor: **Prof. Dr. A. de Kruif**

Co-promotoren: **Dr. F. Koenen en Dr. H. Laevens**

Foto omslag: Een eeuw in beeld (1990) www.hbvl.be

ISBN: 90-5864-024-8

The results of science remain hypotheses that may have been well tested, but not established: not shown to be true. Of course, they may be true. But even if they fail to be true, they are splendid hypotheses, opening the way to still better ones.

Karl R. Popper, A World of Prospensities, 1990

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LIST OF ABBREVIATIONS

EU:	European union
DPLA:	densely populated livestock area
CSF:	classical swine fever
CSFV:	classical swine fever virus
FMD:	foot and mouth disease
IBR:	infectious bovine rhinotracheitis
BVD:	bovine viral diarrhoea
BD:	border disease
HRP1:	high risk period 1
HRP2:	high risk period 2
Post-HRP:	post high risk period
PCR:	polymerase chain reaction
RT-PCR:	reverse transcriptase polymerase chain reaction
VN:	virus neutralisation
VI:	virus isolation
DNA :	deoxyribonucleic acid
RNA :	ribonucleic acid
ELISA :	enzyme-linked immunosorbent assay
CTB-ELISA:	complex trapping blocking enzyme-linked immunosorbent assay
R_0 :	basic reproduction ratio
R:	reproduction ratio (in vaccinated population)
ANOVA:	analysis of variance
SPF:	specific pathogen free
MAS:	market authorisation strategy
DDS:	delayed destruction strategy
NSFL:	national swine fever laboratories
CI:	confidence interval
SD:	standard deviation

CHAPTER 1:

INTRODUCTION

Introduction

Classical swine fever (CSF) is an important disease of list A of the "Office International des Epizooties" (OIE). List A diseases are defined as: *transmissible diseases which have the potential for very serious and rapid spread, irrespective of national borders, which are of serious socio-economic or public health consequence, and are of major importance in the international trade of animals and animal products* (OIE, 2001).

Because of the large socio-economic impact of CSF outbreaks, huge efforts have been made to control this disease. During the 20th century several countries succeeded in eradicating the virus by implementing relatively simple control measures (e.g. Denmark, 1933; Australia, 1963; Canada, 1964; USA, 1977). Others, inside as well as outside the European Union (EU), have experienced major difficulties in their attempt to eradicate the classical swine fever virus (CSFV) (Dahle and Liess, 1992; Laevens, 1999; van Oirschot, 1999).

In the establishment of a common European market, with free movement of people, goods and services, it was decided, in 1980, that all member states ought to eradicate CSFV on their territory (Council directive 80/217/EEC) and implement a policy of eradication and non-vaccination (Council directive 2001/89/EC). It wasn't until 1990 that a generalised vaccination prohibition was implemented in all EU member states. As a consequence of this policy, the whole European pig population became fully susceptible to CSF. This evolution, in combination with the aggregation of pig production in certain regions within the EU, has resulted in a vulnerable pig production system. Despite the preventive measures taken to protect these susceptible populations, CSF virus introduction, in regions that were previously CSF-free, still occurs regularly (Table 1).

Table 1: CSF outbreaks in domestic pigs in the EU member states since the vaccination stop (1990).

Country	'90	'91	'92	'93	'94	'95	'96	'97	'98	'99	'00	'01	'02*
Austria	-	-	-	-	-	2	0	0	0	0	0	0	0
Belgium	113	0	0	7	48	0	0	8	0	0	0	0	0
Denmark	0	0	0	0	0	0	0	0	0	0	0	0	0
Finland	-	-	-	-	-	0	0	0	0	0	0	0	0
France	4	1	1	1	0	0	0	0	0	0	0	0	1
Germany	118	6	13	105	117	54	4	44	11	6	2	5	6
Greece	0	0	0	0	0	0	0	0	0	0	0	0	0
Ireland	0	0	0	0	0	0	0	0	0	0	0	0	0
Italy	15	15	20	12	25	42	46	44	18	9	3	5	0
Luxemburg	0	0	0	0	0	0	0	0	0	0	0	0	8
The Netherlands	2	0	5	0	0	0	0	424	5	0	0	0	0
Portugal	0	0	0	0	0	0	0	0	0	0	0	0	0
Spain	0	0	0	0	0	0	0	78	21	0	0	0	20
Sweden	-	-	-	-	-	0	0	0	0	0	16	0	0
United Kingdom	0	0	0	0	0	0	0	0	0	0	16	0	0
Total	252	22	39	125	190	98	50	598	55	15	21	10	35

* Updated until 19 August 2002

Laevens, 1999; OIE, 2002

In these cases, the rapid eradication of the CSFV is attempted by blocking all possible between-herd transmission routes, and by pre-emptive eradication of all herds at risk through contact with an infected herd, during its infectious period. The efficiency of this strategy depends on how well all possible between-herd virus transmission routes are understood, and,

based on this knowledge**,** the accuracy of predictions of potentially infected herds. Also, the duration of the interval between infection and detection of a herd (infectious period), and the speed and efficiency of the implementation of control measures, is of great importance for the success of eradication. However, due to the incomplete understanding of the epidemiology of CSF, many preventive measures are general in nature (e.g. pre-emptive eradication of the neighbourhood) and therefore often result in the eradication of uninfected herds. When CSF outbreaks are situated in regions with high pig densities, the effects are often devastating (Miry et al., 1991; Vanthemsche, 1995; Koenen et al., 1996; Elbers et al., 1999) (Table 2).

Table 2: Consequences of some CSF outbreaks in areas with high pig densities

Year	Country	Number of infected herds	Number of killed pigs	Direct costs (million €)
1990	Belgium	113	1.000.000	212
1993-94	Belgium	55	790.000	75
1997-98	The Netherlands	429	12.000.000	1.321

Laevens, 1999; Meuwissen et al., 1999

The figures in Table 2 only include the direct costs and need at least to be doubled if the indirect costs are taken into account.

Although the current strategy has proven to be effective in the sense that the authorities have been able to control each CSF outbreak, there is growing criticism due to the huge amount of pre-emptively killed animals (Terpstra, 1998). This massive killing and destruction of mostly non-infected animals is debatable on the grounds of economical, ethical and animal welfare arguments. From an economic viewpoint**,** controlling a CSF outbreak in a densely populated livestock area is extremely costly (Table 2). These financial consequences have an impact on the agricultural sector, as well as on the entire community. From an ethical point of view**,** given the food scarcity in large parts of the world, it is argued that the destruction of thousands of tons of qualitatively fine meat is unacceptable. The animal welfare point of view due to argues that the welfare of animals is often harmed during the control of CSF outbreaks, overcrowded pens and the on-farm destruction of animals.

Reference List

Anonymous, (1980) Council directive 80/217/EEC introducing Community measures for the control of classical swine fever. *Official Journal of the European Communities 80/217/EEC*, 11-23

Anonymous, (2001) Council Directive 2001/89/EC on community measures for the control of classical swine fever. *Official Journal of the European Communities 2001/89/EC*,

Dahle, J. and Liess, B. (1992) A review on classical swine fever infections in pigs: epizootiology, clinical disease and pathology. *Comp. Immunol. Microbiol. Infect. Dis. 15*, 203-211.

Elbers, A.R., Stegeman, A., Moser, H., Ekker, H. M., Smak, J. A., and Pluimers, F. H. (1999) The classical swine fever epidemic 1997-1998 in The Netherlands: descriptive epidemiology. *Prev. Vet. Med. 42*, 157-184.

Koenen, F., Van Caenegem, G., Vermeersch, J. P., Vandenheede, J., and Deluyker, H. (1996) Epidemiological characteristics of an outbreak of classical swine fever in an area of high pig density. *Vet. Rec. 139*, 367-371.

Laevens, H. (1999) Epizootiology of classical swine fever in the European union. In: Epizootiology of classical swine fever: experimental infections simulating field conditions, and risk factors for virus transmission in the neighbourhood of an infected herd. PhD thesis, Ghent, Ghent University. pp. 103-121

Meuwissen, M.P., Horst, S. H., Huirne, R. B., and Dijkhuizen, A. A. (1999) A model to estimate the financial consequences of classical swine fever outbreaks: principles and outcomes. *Prev. Vet. Med. 42*, 249-270.

Miry, C., Castryck, F., Koenen, F., Broers, A., and Segers, E. (1991) Quelques aspects de l'épizootie de peste porcine classique en Belgique en 1990. *Epidémiol. Santé Animal* 20, 23-32.

Terpstra, C. (1998) Preventive emptying: a compensation for a lack of training. *Tijdschr. Diergeneeskd.123*, 324-325.

Office International des Epizooties, (2001) The International Animal Health Code. Edition 2001, http://www.oie.int, Office International des Epizooties, Paris, France.

Office International des Epizooties, (2002) official Animla Health status of Member States, Handistatus II. http://www.oie.int, Office International des Epizooties, Paris, France.

van Oirschot, J.T. (1999) Classical Swine Fever. In Diseases of Swine, vol. 8, Editors: Straw, B. E., D'Allaire, S., Mengeling, W. L., and Taylor, D. J. Ames, Iowa, Iowa State University Press, pp. 159-172.

Vanthemsche, P. (1995). Classical swine fever 1993-1994 in Belgium. In the proceedings of the 8th Annual meeting of the Dutch society for veterinary epidemiology and economics. 13 December 1995, Lelystad, p 25.

CHAPTER 2:

AIMS OF THE STUDY

Given the disadvantages of the current control strategy, efforts should be made to improve or to change this strategy, so that the effects of a CSF outbreak become less devastating. In this thesis, two main possible changes are considered: refining the current measures, and reintroduction of (emergency) vaccination.

The current control measures are based upon the available knowledge of the epidemiology of CSF. To refine these measures, a better understanding of the routes of between-herd transmission is necessary. In order to evaluate their importance in the field, several routes of between-herd transmission of CSF are examined under experimental conditions in the first part of this thesis. Additionally, within-herd virus transmission in breeding herds is examined to be able to design new methods for early detection of a CSF infection.

More specifically, the following questions were answered:

1. Is airborne transmission of CSFV possible?
2. Can CSFV infect pets and rodents and result in an active infection?
3. Is transmission of CSFV possible via excretions of infectious pigs in the early stages of an infection?
4. How does horizontal as well as vertical transmission of CSFV proceed in gilts housed in a sow-box housing system?
5. How does the within-herd virus transmission and the clinical response after infection influence the diagnosis in breeding herds?

The second possibility is the reintroduction of vaccination. When vaccination is considered as a tool in the control of CSF, several scenarios are possible. Firstly, a distinction must be drawn between prophylactic and emergency vaccination. In prophylactic vaccination, all animals within a certain region or country are vaccinated in order to prevent an outbreak of the disease. In the framework of the current EU control policy (Council Directive 2001/89/EC) this prophylactic vaccination is not allowed and will most probably not be allowed in the future. Therefore, the option of reintroduction of prophylactic vaccination is not further elaborated in this thesis. The possibility of emergency vaccination is admitted under certain circumstances. When emergency vaccination is considered, a conventional live

vaccine, as well as a sub-unit marker vaccine, can be used. The question of whether vaccination against CSF, with a marker or a conventional vaccine, could be potentially more beneficial than the current eradication strategy, is elaborated in the second part of this thesis.

Therefore, the following questions were answered:

1. Is an E2 sub-unit marker vaccine capable of stopping or reducing horizontal as well as vertical virus transmission in fattening and breeder pigs exposed to a natural contact infection?
2. What is the effect of vaccination with an E2 sub-unit marker vaccine or a conventional C-strain vaccine on horizontal virus transmission when the infection occurs shortly after vaccination (1 or 2 weeks) or simultaneously with the vaccination?

CHAPTER 3:

EXPERIMENTAL ASSESSMENT OF CLASSICAL SWINE FEVER VIRUS TRANSMISSION.

CHAPTER 3.1:

TRANSMISSION ROUTES OF CLASSICAL SWINE FEVER VIRUS. A REVIEW OF PRESENT KNOWLEDGE.

ABSTRACT

Classical swine fever (CSF) is an important pig disease with huge economical and social implications. Since 1980, the control of CSF in the European Union has been based on a strategy of non-vaccination and eradication of infected herds (Council directive 80/217/EEC). As a result, the total domestic pig population in the EU has become fully susceptible to the CSF virus. On several occasions this has led to huge epidemics that could only be controlled by implementing drastic measures. These control measures are based on the knowledge of the transmission routes of CSF virus. Yet, the importance, and even the existence, of many routes of CSF virus transmission are still uncertain. This leads inevitably to discussions about which control measures should be used.

This chapter gives a review on the current knowledge of the different routes of direct and indirect transmission of CSF virus. In addition, the areas where current knowledge is insufficient or inconclusive are indicated.

INTRODUCTION

Classical swine fever (CSF) is an important infectious viral disease in domestic pigs and wild boar (Terpstra, 1988). The CSF virus is an enveloped RNA virus, belonging to the family of the *Flaviviridae*, genus *pestivirus,* and is closely related to bovine viral diarrhoea virus and border disease virus (Moennig, 1992; van Oirschot, 1999). The disease was recognised about 170 years ago, and efforts to control it started in the nineteenth century. Nevertheless, it remains a lingering problem in many parts of the world where it has both an economical impact on swine production and a constraining effect on international trade (Edwards et al., 2000a).

During the 1980s, CSF control in the European Union (EU) moved from a diversity of national control programs to a uniform approach, based on a strategy of non-vaccination and eradication of infected herds (Council directive 80/217/EEC). As a result of this non-vaccination strategy, the total domestic pig population in the EU has become fully susceptible to the CSF virus. This susceptible population, combined with the development of areas with dense pig populations in several parts of the EU, has created a dangerous situation, leading occasionally to epidemics with sometimes disastrous consequences (Lamsens, 1992; Vanthemsche, 1995; Meuwissen et al., 1999; Stegeman et al., 2000b; Mintiens et al., 2001).

In order to prevent CSF introduction, and to optimise control programmes, a thorough knowledge of the different virus transmission routes of CSF is a prerequisite.

In this chapter, a review is given on the current knowledge of the different routes of direct and indirect CSF virus transmission.

DIRECT VIRUS TRANSMISSION

Horizontal transmission

Direct animal contact is generally accepted as the most important route for within-herd as well as for between-herd virus transmission. CSF infected pigs excrete the virus through oronasal and lacrimal secretions as well as through urine and faeces (Liess, 1987; Terpstra, 1988; Depner et al., 1994; van Oirschot, 1999). Susceptible pigs can become infected through oral, nasal, aerogenic, conjunctival, genital and various parenteral routes (Terpstra, 1988).
The basic reproduction ratio (R_0), indicating the average number of secondary cases caused by one typical infectious animal, is a quantitative representation of disease transmission in a susceptible population. This can be calculated for within and between-herd spread. This R_0 is influenced by the infectiousness of an infectious pig (herd), the susceptibility of a susceptible pig (herd), the contact structure between pigs (herds), and will vary in different situations. The R_0 for within-herd spread via direct contact has been quantified in weaner pigs, $R_0 = 81.3$ (Laevens et al., 1998) and slaughter pigs, $R_0 = 13.7$ (Laevens et al., 1999a). These high figures clearly indicate that CSF virus spreads very easily through direct contact. Furthermore, an age dependency for the transmission rate, similar to the age dependent clinical course of the infection (van Oirschot 1988), can be observed. The transmission rate for between-herd spread can also be quantified. Obviously this transmission rate (R_h) will largely depend on the different control measures implemented during the epidemic**.** In the 1997-1998 CSF epidemic in the Netherlands, during the period before the first outbreak, the R_h was calculated at 6.8 (Stegeman et al., 1999). During the periods following the epidemic, a broad spectrum of control measures were implemented, reducing the R_h to less than 1, which eventually stopped the epidemic. Note that the R_h is not only the result of direct horizontal transmission, also indirect transmission routes have been considered to calculate this transmission rate.

Vertical transmission

As a result of an infection during the second trimester of their pregnancy, sows may give birth to stillborn, mummified, peri-natal dying and normal sero-converted piglets, and also to congenitally infected piglets that are apparently normal (Liess, 1984; Wensvoort and Terpstra, 1985; Westergaard, 1996; van Oirschot, 1999). These piglets are immune tolerant and persistently shed the virus until they die after several weeks or months (van Oirschot, 1979a; van Oirschot, 1979b; Liess, 1984; Terpstra, 1988). In the past it was found that this so called "carrier sow syndrome" may occur in up to 43% of pregnant sows in a herd (Wensvoort and Terpstra, 1985). This vertical virus transmission route can be of huge importance in the epidemiology of CSF, since asymptomatic and persistently viraemic piglets can maintain an infection over a long period of time, or can re-ignite an outbreak after a period of apparent absence (van Oirschot, 1999).

INDIRECT VIRUS TRANSMISSION

The different routes of direct virus transmission are well known and measures to prevent these types of transmissions are relatively straightforward. However, during outbreaks, it has been observed that halting all direct virus transmission routes is insufficient to stop a CSF epidemic. This illustrates the importance of the indirect spread in the epidemiology of CSF. Yet, the different routes of indirect virus transmission are not so well studied and understood. Consequently, the importance, and sometimes even the occurrence**,** of some transmission routes remain unclear.

Swill feeding

It is well established that CSF virus can readily be detected in pigs that die or are killed during acute swine fever infection, including the prodromal period (Edwards, 2000b). In pork and pork products, the virus can remain infectious for months. When meat is stored, cooled or frozen, the survival of the virus can even be prolonged for years (Dahle and Liess, 1992; van Oirschot, 1999). When waste food from the human food chain (swill), containing uncooked infected meat, is fed to pigs it can initiate a new focus of infection (Terpstra, 1988). Swill

feeding was allowed, if an official license was given to process the swill with heat treatment to inactivate infectious particles. However, feeding untreated garbage from restaurants and barracks to pigs has often been reported as a source of CSF (Dahle and Liess, 1992; Fritzemeier et al., 2000). According to a recent European directive swill feeding is now prohibited in the EU, even after heat treatment (Council Directive 2001/89).

Transport vehicles

Another indirect virus transmission route, identified in several epidemiological studies, are livestock trucks that have been in contact with infectious animals (Kramer et al., 1995; Stegeman et al., 1997; Teuffert et al. 1998; Benard et al., 1999; Stegeman et al., 2000a, 2000b; Elbers et al. 2001). It is believed that these trucks are contaminated through secretions and excretions of the infectious animals. However, there is little published experimental evidence for this type of indirect virus transmission. Hughes and Gustafson (1960) reported that only 2 out of 10 pigs exposed to secretions and excretions of clinically diseased pigs became infected. In preliminary experiments by Koenen (unpublished data), challenging susceptible pigs with faeces from infectious pigs did not result in infection.
Virus transmission via other vehicles that frequently visit farms is also mentioned sometimes. Yet, very little experimental or epidemiological evidence is reported to evaluate this virus transmission route.

Human

Visitors such as veterinarians, inseminators, pig handlers, screening teams, etc. have also been identified as potential virus carriers, causing between-herd as well as within-herd spread (Stegeman et al. 2000a). The actual virus transmission is believed to occur via contaminated instruments, equipment or drugs, or via contaminated clothing and footwear (Terpstra 1988). The importance of iatrogenic transmission was demonstrated in Germany in the seventies where 38 herds were infected by vaccination teams using the same vaccine and needles for different holdings (Dahle and Liess, 1992). However, in modern pig holdings, the habit of sharing equipment or using the same syringes and needles in different herds has become rare. Nevertheless, it remains important for within-herd transmission.

The role of contaminated clothing and footwear is uncertain. Some argue that they are of little significance because the amount of virus carried around will be marginal and most likely below the minimum infective dose (Terpstra, 1988). A recent epidemiological study found that visitors entering a pig herd, without wearing an overcoat and boots supplied by the farmer, were a significant risk factor for herd infection. (Elbers et al., 2001). Yet, there is little available experimental proof supporting the importance of contaminated clothing and footwear. In one experiment a pen, which had only airborne contact with an adjacent infected pen, was compared to a pen that had airborne contact plus contact via contaminated clothing and footwear. It was found that the additional contact of contaminated clothing did not significantly influence the transmission of the CSF virus (Laevens et al., 1998). However, more experiments are needed to clarify the importance of contaminated clothing and footwear in the absence of airborne contact.

Liquid manure

In general, virus excreted with urine and faeces will enter the liquid manure storage tank where it will disappear over varying periods of time (Westergaard, 1996). In liquid manure kept at 20°C, CSF virus can remain for up to 2 weeks (Edwards, 2000b). However, the amount of virus excreted with urine and faeces is low when compared to titres in blood (Ressang, 1973; Depner et al., 1994). Moreover, the quantities that stay airborne during the land spreading of liquid manure are negligible. Therefore, it is believed that land spreading of liquid manure is unlikely to be an important factor in the disease transmission (Terpstra, 1988).

Wild Boar

As mentioned before, CSF infections also occur in wild boar (Aubert et al., 1994; Depner et al., 1995). The population of wild boar in the EU has been roughly estimated between 800,000 and 1 million head, but its density is highly variable from area to area and from country to country (Laddomada, 2000). CSF virus has been detected in wild boar populations in several EU member states, e.g. in Austria, France, Italy, Germany and Luxemburg (Westergaard, 1996; Albina et al., 2000; Laddomada, 2000; Kaden and Lange, 2001; Koenen, personal communication). These endemic, infected wild boar populations represent permanent

virus reservoirs that pose a constant threat to the domestic pig populations (Moennig, 2000). Domestic pigs may contract the virus directly from wild boar (animal contact) or, more often, indirectly. Transmission via direct contact may occur in regions where domestic pigs are kept outdoors (Laddomada, 2000). Indirect virus transmission may occur when susceptible pigs are infected by the contaminated equipment or clothing of pig farmers who are also hunters, or when wild boar carcasses are illegally fed to susceptible pigs (Kramer et al., 1995; Fritzemeier et al., 2000; Moennig, 2000). Between 1993 and 1998, 92 primary CSF outbreaks in domestic pig herds were recorded in Germany, 59% of them due to direct or indirect contact with wild boar or wild boar meat (Fritzemeier et al., 2000).

Artificial insemination

The risk of virus spread via contaminated semen was recognised during the 1997-1998 CSF epidemic in the Netherlands (Elbers et al., 1999; Stegeman et al., 2000b), and later on confirmed experimentally (de Smit et al., 1999). Obviously, virus spread via artificial insemination centres is extremely dangerous, since a large number of herds distributed over a large area, can become infected. In the 1997 epidemic, 1680 pig herds received suspect semen. Of these, 36 herds became infected, presumably due to this suspect semen (Hennecken et al., 2000).

Arthropods

The role and importance of arthropods in the within as well as between-herd spread of CSF is unclear. Some past experiments indicate that transmission of the virus is possible via several arthropods, eg. house fly, stable fly, mosquitoes. It has even been reported that these arthropods could harbour the virus for up to 72 hours (Terpstra, 1988; Dahle and Liess, 1992; Westergaard, 1996). However all these experiments date from the seventies or earlier. In recent scientific literature no evidence of this mode of virus transmission has been reported. In recent years, the effort to isolate CSF virus from stable flies collected in stables housing CSF infected pigs was unsuccessful on several occasions (Koenen, unpublished data). Given the uncertainty of virus spread via arthropods, it must be taken into account that stamping out an infected herd will change the normal habitat for arthropods and stimulate a resident population to move and seek new residences (Westergaard, 1996).

Other animals

In general, pestiviruses share ruminants and pigs as common hosts. Based on experimental inoculations it is known that CSF virus can be transmitted to goats, sheep, calves and deer (Shimizu and Kumagai, 1989; Moennig, 1992a). These inoculations lead to sub-clinical infections and antibody response (Harkness and Roeder, 1988; Dahle and Liess, 1992; Moennig and Plagemann 1992b). In one case, after intravenous inoculation of goats, transplacental infection of the foetuses occurred. However in the kids born alive, only neutralizing antibodies, and no virus, could be found (Shimizu and Kumagai, 1989). Until now, there is no evidence that infection of ruminants with CSF virus occurs under natural conditions (Harkness and Roeder, 1988). Therefore the importance of ruminants in the epidemiology of CSF is believed to be minimal.

Pets, rats and birds are also sometimes mentioned as possible virus transmitters because they are frequently found at pig farms and live in close contact with pigs (Ellis et al., 1977; Miry et al., 1991; van Oirschot, 1999). Moreover, they are very mobile. In an experiment where rats, trained to feed in close contact with infected pigs, were moved to susceptible pigs, no virus transmission could be effectuated. Even rats that had eaten from a pig that died from CSF did not transmit the disease (Terpstra, 1988). There is also no experimental evidence that suggests that dogs or cats can transmit the virus (Westergaard, 1996). One experiment reported that sparrows were able to transmit the virus over a short distance (Hughes and Gustafson, 1960).

Local spread

The term "neighbourhood infection" refers to a situation where a known CSF infected herd is located in the neighbourhood (area with a radius of 1 km) of a new outbreak, and where none of the above mentioned routes could be identified as the source of virus transmission (Westergaard, 1996). The fact that no known infection route can be identified may be due to the fact that it did not occur, or that it was not reported during the epidemiological tracing. Non-reporting of contacts may be a result of the fact that people do not remember all contacts that occurred in the previous weeks, or to the occurrence of illegal contacts. However, not all neighbourhood infections can be explained by “not reported contacts”. Subsequently, there

are only a few possibilities remaining: unknown transmission routes and airborne transmission.

The spread of CSF virus via a currently unknown transmission mode can never be excluded. However, in view of the importance of CSF and the number of studies that have been carried out on the epidemiology of CSF, this possibility is unlikely.

The possibility of airborne transmission of CSF remains undecided**.** Recent CSF epidemics show that the risk of neighbourhood infections decreases with increasing distance to the primary infected herd (Roberts, 1995; Koenen et al., 1996; Staubach et al., 1997; Stegeman et al., 2000a). A recent epidemiological study, based on the data of the Belgian epizootic of 1993-1994, has indicated that the chance of a neighbouring herd becoming infected was dependent on i) the size of the neighbouring herd, ii) the distance of the neighbouring herd to the primary herd, and, iii) the neighbouring herd (based on field observations of prevailing wind conditions) being downwind from the primary infected herd (Laevens, 1999b; Mintiens et al., 2000). When disease transmission depends on risk factors like herd size, distance between neighbouring herds, the size of the nearest herd and animal density of the area, then airborne virus transmission between-herds might be involved as this factor determines the plume dispersal (Stärk, 1998a).
Experimentally it was already shown in 1960 that the CSF virus could spread airborne and infect susceptible pigs over short distances (Hughes and Gustafson, 1960). Afterwards, these findings were confirmed in experiments by Terpsta (1988), Laevens et al. (1998,1999), and Gonzales et al. (2001). On the other hand, isolation of CSF virus from air samples has so far been unsuccessful. (Stärk et al., 1998b).

CONCLUSIONS

As a conclusion it can be stated that the relevance and importance of many, especially indirect, virus transmission routes remains unclear. This lack of knowledge hampers the development of efficient and selective disease control measures. Therefore more epidemiological as well as experimental research should be performed on the possible types of CSF virus transmission.

REFERENCE LIST

Albina, E., Mesplede, A., Chenut, G., Le Potier, M. F., Bourbao, G., Le Gal, S., and Leforban, Y. (2000) A serological survey on classical swine fever (CSF), Aujeszky's disease (AD) and porcine reproductive and respiratory syndrome (PRRS) virus infections in French wild boars from 1991 to 1998. *Vet. Microbiol. 77*, 43-57.

Anonymous (1980) Council directive 80/217/EEC. introducing Community measures for the control of classical swine fever. *Official Journal of the European Communities* 80/217/EEC, 11-23.

Anonymous, (2001). Council Directive 2001/89/EC on community measures for the control of classical swine fever. *Official Journal of the European Communities 2001/89/EC*

Aubert, M., Picard, M., Fouquet, E., Conde, J., Cruciere, C., Ferry, R., Albina, E., Barrat, J., and Vedeau, F. (1994) La peste porcine classique du sanglier en Europe. *Ann. Med. Vet. 138*, 239-247.

Benard, H.J., Stark, K.D., Morris, R.S., Pfeiffer, D.U., and Moser, H. (1999) The 1997-1998 classical swine fever epidemic in The Netherlands-a survival analysis. *Prev. Vet. Med. 42*, 235-248.

Dahle, J. and Liess, B. (1992) A review on classical swine fever infections in pigs: epizootiology, clinical disease and pathology. *Comp. of Imm. and Microb. of Infect. Diseases 15*, 203-211.

de Smit, A.J., Bouma, A., Terpstra, C., and van Oirschot, J.T. (1999) Transmission of classical swine fever virus by artificial insemination. *Vet. Microbiol. 67*, 239-249.

Depner, K., Gruber, A., and Liess, B. (1994) Experimental infection of weaner pigs with a field isolate of Hog Cholera/Classical Swine Fever Virus derived from a recent outbreak in Lower Saxony. 1: Clinical, virological and serological findings. *Wien. Tierärztl. Mschr. 81*, 370-373.

Depner, K.R., Muller, A., Gruber, A., Rodriguez, A., Bickhardt, K., and Liess, B. (1995) Classical swine fever in wild boar (Sus scrofa), experimental infections and viral persistence. *Dtsch. Tierärztl. Wochenschr. 102*, 381-384.

Edwards, S., Fukusho, A., Lefevre, P., Lipowski, A., Pejsak, Z., Roehe, P., and Westergaard, J. (2000a) Classical swine fever: the global situation. *Vet. Microbiol. 73,* 103-119.

Edwards, S. (2000b) Survival and inactivation of classical swine fever virus. *Vet. Microbiol. 73*, 175-181.

Elbers, A., Stegeman, A., and de Jong, M. C. (2001) Factors associated with the introduction of Classical Swine Fever virus into pig herds in the central area of the 1997-1998 epidemic in The Netherlands. In the proceedings of the 9th conference of the Society for Veterinary Epidemiology and Preventive Medicine, 6-14, Noordwijkerhout, The Netherlands.

Elbers, A.R., Stegeman, A., Moser, H., Ekker, H. M., Smak, J.A., and Pluimers, F.H. (1999) The classical swine fever epidemic 1997-1998 in The Netherlands: descriptive epidemiology. *Prev. Vet. Med. 42*, 157-184.

Ellis, P.R., James, A.D., and Shaw, A.P. (1977) Studies on the epidemiology and economics of swine fever eradication in the EEC. *EUR 5738e*,

Fritzemeier, J., Teuffert, J., Greiser-Wilke, I., Staubach, C., Schluter, H., and Moennig, V. (2000) Epidemiology of classical swine fever in Germany in the 1990s. *Vet. Microbiol. 77*, 29-41.

Gonzalez, C., Pijoan, C., Ciprian, A., Correa, P., and Mendoza, S. (2001) The effect of vaccination with the PAV-250 strain classical swine fever (CSF) virus on the airborne transmission of CSF virus. *J. Vet. Med. Sci. 63*, 991-996.

Harkness, J.W. and Roeder, P.L. (1988) The comparative biology of classical swine fever. In Classical swine fever and related viral infections. Editors: Liess, B. Martinus Nijhoff Publishing, Dordrecht, The Netherlands, 234-288.

Hennecken, M., Stegeman, J.A., Elbers, A.R., Van Nes, A., Smak, J.A., and Verheijden, J.H. (2000) Transmission of classical swine fever virus by artificial insemination during the

1997-1998 epidemic in The Netherlands: a descriptive epidemiological study. *Vet. Q. 22*, 228-233.

Hughes, R.W. and Gustafson, D. P. (1960) Some factors that May Influence Hog Cholera transmission. *Am. J. Vet. Res. 21*, 464-471.

Kaden, V. and Lange, B. (2001) Oral immunisation against classical swine fever (CSF): onset and duration of immunity. *Vet. Microbiol. 82*, 301-310.

Koenen, F., Van Caenegem, G., Vermeersch, J.P., Vandenheede, J., and Deluyker, H. (1996) Epidemiological characteristics of an outbreak of classical swine fever in an area of high pig density. *Vet. Rec. 139*, 367-371.

Kramer, M., Ahl, R., Teuffert, J, Kroscheski, W., Schlüter, H., and Otte, J. (1995) Classical swine fever in Germany, some epidemiological aspects. In the Proceedings of meeting of the society for veterinary epidemiology and preventive medicine. 29-31 March 1995, Reading, UK, pp.110-118

Laddomada, A. (2000) Incidence and control of CSF in wild boar in Europe. *Vet. Microbiol. 73*, 121-130.

Laevens, H., Koenen, F., Deluyker, H., and Berkvens, D. (1998) An experimental infection with classical swine fever virus in weaner pigs. I. Transmission of the virus, course of the disease, and antibody response. *Vet. Q. 20*, 41-45.

Laevens, H., Koenen, F., Deluyker, H., and de Kruif, A. (1999a) Experimental infection of slaughter pigs with classical swine fever virus: transmission of the virus, course of the disease and antibody response. *Vet. Rec. 145*, 243-248.

Laevens, H. (1999b) Risk factors for the transmission of classical swine fever virus to herds in the close neighbourhood of an infected herd. In: Epizootiology of classical swine fever: Experimental Infections Simulating Field Conditions, and Risk Factors for Virus Transmission in the Neighbourhood of an Infected Herd. PhD thesis, Ghent, Ghent University. pp. 103 – 122.

Lamsens, G. (1992) Klassieke varkenspest Epizoötie 1990. Ministerie van Landbouw, Diergeneeskundige Inspectie, Brussels, Belgium.

Liess, B. (1984) Persistent infections of Hog Cholera: a review. *Prev. Vet. Med. 2*, 109-113.

Liess, B. (1987) Pathogenesis and epidemiology of Hog Cholera. *Ann. Rech. Vet. 18*, 139-145.

Meuwissen, M.P., Horst, S.H., Huirne, R.B., and Dijkhuizen, A.A. (1999) A model to estimate the financial consequences of classical swine fever outbreaks: principles and outcomes. *Prev. Vet. Med. 42,* 249-270.

Mintiens, K., Laevens, H., Deluyker, H., Dewulf, J., Koenen, F., and de Kruif, A. (2000) Estimation of the likelyhood for "Neighbourhood Infections" during classical swine fever epidemics based on spatial risk assesment of real outbreak data. In proceedings of 9th Symposium of the International Society for Veterinary Epidemiology and Economics. Breckenridge, Colorado, 6-11 August 2000, pp. 712-714.

Mintiens, K., Deluyker, H., Laevens, H., Koenen, F., Dewulf, J., and de Kruif, A. (2001) Descriptive epidemiology of a Classical Swine Fever outbreak in the Limburg province of Belgium in 1997. *J. Vet. Med. B. 48*, 143-149.

Miry, C., Castryck, F., Koenen, F., Broers, A., and Segers, E. (1991) Quelques aspects de l'épizootie de peste porcine classique en Belgique en 1990. *Epidémiol. Santé Animal 20*, 23-32.

Moennig, V. (1992) The hog cholera virus. *Comp Immunol. Microbiol. Infect. Dis. 15*, 189-201.

Moennig, V. (2000) Introduction to classical swine fever: virus, disease and control policy. *Vet. Microbiol. 73*, 93-102.

Moennig, V. and Plagemann, P.G. (1992) The pestiviruses. *Adv. Virus Res. 41*, 53-98.

Ressang, A.A. (1973) Studies on the Pathogenesis of Hog Cholera. *Zentralbl. Veterinärmed. B. 20*, 256-271.

Roberts, M. (1995) Evaluation of optimal size of restriction zones in disease control with particular references to classical swine fever. In the Proceedings of meeting of the society for veterinary epidemiology and preventive medicine. 29-31 March 1995, Reading, UK, p.119.

Shimizu, M. and Kumagai, T. (1989) Experimental infection of pregnant goats with swine fever virus. *Vet. Microbiol. 20*, 207-214.

Stärk, K.D.C. (1998) The role of infectious aerosols in disease transmission in pigs. In systems for the prevention and control of infectious disease in pigs, PhD thesis, Palmerston North, New-Zeeland, Massey University. pp. 65-89.

Stärk, K.D.C., Frey, J., Nicolet, J., Thür, B., and Morris, R. S. (1998) Assessment of aerosol transmission in the epidemiology of infectious disease in swine using air sampling and polymerase chain reaction assays. In the Proceedings of the The 15th International Pig Veterinary Society Congress, 5-9 June 1998, Birmingham, UK, p. 252

Staubach, C., Teuffert, J, and Thulke, H. H. (1997) Risk Analysis and local spread mechanisms of classical swine fever. In the Proceedings of the 8th conference of the international society for veterinary epidemiology and economics. Juli 1997, Paris, France, pp. 06.12.1-06.12.3.

Stegeman, A., Elbers, A.R., Smak, J., and de Jong, M.C. (1999) Quantification of the transmission of classical swine fever virus between herds during the 1997-1998 epidemic in The Netherlands. *Prev. Vet. Med. 42*, 219-234.

Stegeman, A., Elbers, A., and de Jong, M.C. (2000a) Rate of inter-herd transmission of classical swine fever virus by different types of contact. In proceedings of 9th Symposium of the International Society for Veterinary Epidemiology and Economics. Breckenridge, Colorado, 6-11 August 2000, pp. 1095-1096.

Stegeman, A., Elbers, A., de Smit, H., Moser, H., Smak, J., and Pluimers, F. (2000b) The 1997-1998 epidemic of classical swine fever in the Netherlands. *Vet. Microbiol. 73,* 183-196.

Stegeman, J.A., Elbers, A.R., de Smit, A.J., Moser, H., & de Jong, M.C. (1997) Between-herd transmission of classical swine fever virus during the 1997 epidemic in the Netherlands. In the proceedings of the 10th annual meeting of the Dutch society for veterinary epidemiology and economics. 20 November 1997, Boxtel, The Netherlands, p. 25

Terpstra, C. (1988) Epizootiology of Hog-Cholera. In Classical swine fever and related viral infections. Editors: Liess, B. Martinus Nijhoff Publishing, Dordrecht, The Netherlands, pp. 201-216.

Teuffert, J., Kramer, M., and Schlüter, H. (1998) The epidemiologie of classical swine fever (CSF) in Germany under special consideration of the tasks of the veterinary practitioners. *Prak. Tier. coll. Vet. XXVIII* 45-49.

van Oirschot, J.T. (1979a) Experimental production of congenital persistent swine fever infections. I. Clinical, pathological and virological observations. *Vet. Microbiol. 4*, 117-132.

van Oirschot, J.T. (1979b) Experimental production of congenital persistent swine fever infections. II. Effect on functions of the immune system. *Vet. Microbiol. 4*, 133-147.

van Oirschot, J.T. (1988) Description of the virus infection. In Classical swine fever and related viral infections. Editors: Liess, B. Martinus Nijhoff Publishing, Dordrecht, The Netherlands, pp.1-25.

van Oirschot, J.T. (1999) Classical swine fever. In Diseases of Swine, vol. 8,. Editors: Straw, B. E., D'Allaire, S., Mengeling, W. L., and Taylor, D. J. Ames, Iowa, Iowa State University Press, pp.159-172.

Vanthemsche, P. (1995) Classical swine fever 1993-1994 in Belgium. In: Proceedings of the 8th Annual Meeting of the Dutch Society for Veterinary Epidemiology and Economics 13 December 1995, Lelystad, p. 25

Wensvoort, G. and Terpstra, C. (1985) Varkenspest: een veranderend ziektebeeld. *Tijdsch. Diergeneesk. 110*, 263-269.

Westergaard, J. M., (1996) Epidemiology of classical swine fever, In proceedings of Workshop on diagnostic procedures and measures to control classical swine fever in domestic pigs and the European wild boar. Poland, National Veterinary Research Institute, Pulaway, Poland. pp. 119-130

CHAPTER 3.2:

AIRBORNE TRANSMISSION OF CLASSICAL SWINE FEVER UNDER EXPERIMENTAL CONDITIONS.

J. Dewulf , H. Laevens , F. Koenen , K. Mintiens,

and A. de Kruif

Veterinary Record (2000) 147, 735-738

Abstract

The aim of this study was to examine airborne virus transmission of classical swine fever (CSF) and to estimate the influence of compartmentalisation and air currents on airborne virus transmission.

Therefore 61 pigs were housed in an isolation unit with 3 compartments and 5 pens. Each compartment had its own ventilation system resulting in air currents from compartment A (pens 1-3) towards compartment B (pen 4), but not towards compartment C (pen 5). CSF virus was introduced by experimental inoculation of one pig in the middle pen (pen 2) of compartment A.

The possibility of airborne transport of CSF has been clearly demonstrated in this experiment, as the virus was able to spread towards compartment B, following the prevalent air currents. Compartmentalisation only had a retarding effect on the virus transmission. The absence of infection in pen 5, which was not different from pen 4 except for the ventilation system, indicates that the virus spread was clearly effected by the air current.

It remains difficult to extrapolate these results into the field and to assess the importance of airborne virus transmission between neighbouring herds. However, in a fully susceptible population and in densely populated regions even a transmission route with minor importance may have major economical consequences.

Introduction

Many possible risk factors contributing to the between-herd spread of classical swine fever (CSF) in unvaccinated pig populations have been described in literature. The most cited are movements of infected pigs, feeding of waste food containing material from infectious animals, transmission by improperly disinfected vehicles, and spread by humans (Terpstra, 1988; Edwards, 1989; Dahle and Liess, 1992; Stegeman et al., 1997; van Oirschot, 1999). During the 1997 outbreak in the Netherlands it became clear that CSF could also be spread by artificial insemination (Elbers et al., 1999).

During recent CSF epidemics in Belgium, Germany and the Netherlands a large number of secondary cases was located near primary outbreaks (Roberts, 1995; Koenen et al., 1996; Staubach et al., 1997). If such outbreaks are located within a distance of 1 km of a previously infected herd they are classified as neighbourhood infections (Vanthemsche, 1995; Pittler et al., 1996; Elbers et al., 1999). In many of those neighbourhood infections none of the "traditional" transmission routes could be identified as being responsible for virus introduction (Roberts, 1995; Laevens, 1999).

The transmission routes causing those neighbourhood infections remain unknown. Factors such as transmission by arthropods, birds, pets and rodents have been suggested (Terpstra, 1988), but their importance remains questionable. Even without a definitive knowledge on the real routes of transmission it was shown that the risk for neighbourhood infections decreased with increasing distance to the primary infected herd (Roberts, 1995; Koenen et al., 1996; Staubach et al., 1997). Recently an epidemiological study based on the data of the Belgian epizootic of 1993-1994 has indicated that the chance of a neighbouring herd becoming infected was dependent on the size of the neighbouring herd, the distance of the neighbouring herd to the primary herd and the number of observations of the neighbouring herd laying downwind from the primary infected herd (Laevens, 1999). When disease transmission depends on risk factors like size of infected herd, distance to nearest infected herds, size of the nearest herd and animal density of the area, airborne virus transmission between herds might be involved as these factors determine the plume dispersal (Stärk, 1998).

Already in 1960 it was found from experiments that the CSF virus could spread aerogenically and infect susceptible pigs over short distances (Hughes and Gustafson, 1960). More recently, these findings were experimentally confirmed by others (Terpstra, 1987; Laevens et al., 1998; Laevens et al., 1999).

This paper describes an experiment conducted to confirm the previous findings of airborne virus transmission and to evaluate the influence of compartmentalisation and air currents on airborne virus transmission.

Materials and methods

Animals

Conventional weaner pigs of 12-15 kg (n = 61) originating from an isolated pig herd and controlled for the absence of bovine viral diarrhoea virus (BVDV) and CSFV antigen and antibodies were used. On the day of experimental inoculation the average weight of the pigs was 50 kg.

Virus

The isolate used for the experimental infection was originally obtained from the first CSF-infected herd of the 1993-1994 Belgian epizootic. The isolate was verified to be free of African swine fever virus and BVDV. By using monoclonal antibodies, it was characterised to be similar to an isolate known as the 'souche Lorraine'(Koenen and Lefebvre, 1995). Virus infectivity was 10^3 median cell culture infective dose ($TCID_{50}$ / ml), passage level 45 in PK_{15} cell cultures.

Experimental design

Upon arrival, the weaner pigs were randomly allocated to one of 5 pens of an isolation unit (Figure 1). After an acclimatisation period one randomly selected pig of pen 2 (compartment A) was experimentally inoculated by deep intramuscular injection (2 ml) plus intranasal inoculation (2 ml).

In order to investigate the effect of compartmentalisation and air currents on virus spread, pens were situated in three different compartments with different ventilation systems. Three adjacent pens (1 to 3) (length x width x height = 610 x 245 x 130 cm) were located in compartment A. The partition of the pens in compartment A was made by solid walls (130 cm height) preventing direct contact between pigs of different pens. In each of these pens 15 weaner pigs were housed.

Pens 4 (9 pigs) and 5 (8 pigs) (length x width x height = 325 x 245 x 130 cm) were situated in compartment B and C, respectively. Full walls, which were not 100% airtight, especially at the entrances, were used for compartmentalisation. Each compartment had its own, independent, ventilation system (Figure 1).

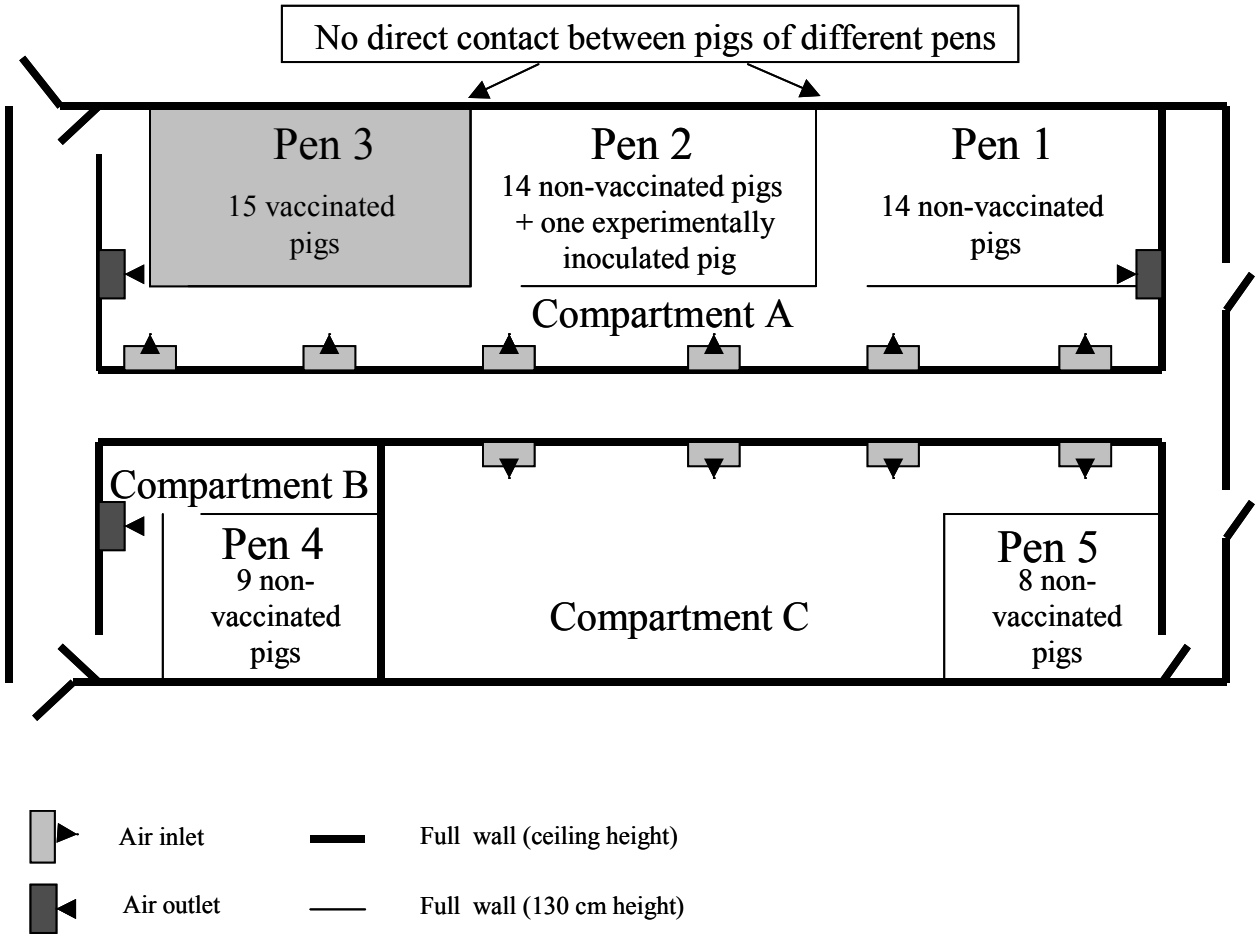


Figure 1: Ground plan of the isolation unit and the three compartments and five pens.

The air currents within and between the compartments were examined with fumigation experiments and are shown in Figure 2. The major air currents were visualised by making smoke on several places in the different pens and compartments. The minor and discrete air

currents were examined using small amounts of smoke on more than 100 different locations in the isolation unit. All fumigation experiments were repeated several times. Compartment A had several air inlets, through which heated air from outside the building was introduced, and two air outlets, through which air was evacuated towards filtering installations. Compartment B had no air inlets and one air outlet through which air was also evacuated towards the filtering installations, this resulted in a discrete negative air pressure in compartment B. Compartment C on the other hand, had several air inlets introducing air from outside the building, but no air outlets. This resulted in a discrete positive air pressure.

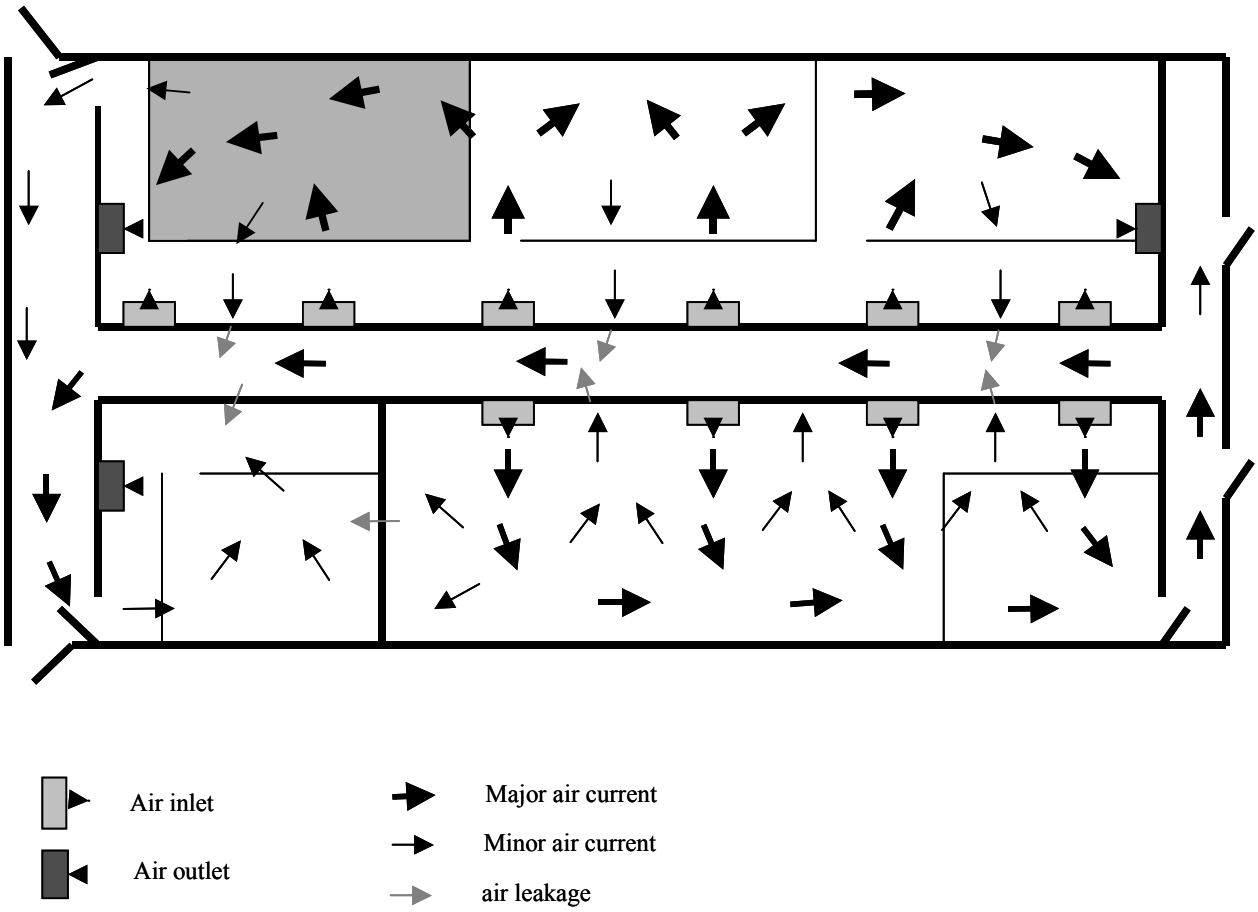


Figure 2: Schematic diagram of the air flows within and between the compartments.

Simultaneously with this experiment a vaccination experiment was conducted in compartment A. The results of this experiment have been described previously (Dewulf et al., 2000). Vaccinated pigs in pen 3 were considered not to be infectious during the experiment and therefore only the results of pens 1 and 2 (non-vaccinated pigs) in compartment A are presented here.

Sample collection and clinical examination

The first sample collection from all pigs took place upon arrival. During the post-inoculation period blood samples were taken from all pigs two days prior to inoculation and subsequently every other day until 60 days post-inoculation (dpi). Simultaneously with the sample collection all pigs were examined clinically. The following information was gathered: rectal temperature, liveliness (apathy), body condition (cachexia), coughing, conjunctivitis, diarrhoea, ataxia, and erythema. Mortality and feed and water intake per pen were recorded daily.

Pens were visited following a strict protocol, in order to ensure that the only way of virus spread between pens and compartments was airborne. The isolation unit was always entered with washed overalls and thoroughly cleaned and disinfected footwear. The pens were visited in the following order: pen 5 → pen 4 → pen 1 → pen 3 → pen 2. Between the visits of the successive pens, overall, gloves, footwear, and head covering were changed, except between pens 3 and 2. Additionally, footwear was disinfected before entering and after leaving each pen to avoid contamination of the corridors. All materials necessary for blood sampling, rectal temperature monitoring, cleansing of the pens, and feeding of the pigs were provided per pen. The pen floors were hosed every other day, care being taken to avoid water contact with the pigs in the other pens. Manure was drained through a grating leading to an underground manure drain. Although the manure drain was the same for the three pens of compartment A and for the two pens of compartments B and C, it was impossible for the pigs to make contact with the manure from other pens. From every pig that died or had to be euthanised, tissue samples (tonsil, muscles of shoulder and rump, mesenterial, ileocecale and maxillary lymph node, kidney, spleen, heart, lung, liver, brain, eye fluid, blood, faeces, urine) were collected.

Sample analyses

For virus isolation (VI) from blood, 100 µl blood was inoculated in duplicate onto a non-confluent monolayer of PK_{15} cells cultured in multiwell plates (24 wells / plate). After 48 hours, the cells were fixed with isopropanol and stained with a polyclonal fluorescein-conjugated anti-CSF immunoglobulin. For antibody detection in the serum the virus neutralisation (VN) test and the CTB-ELISA (Ceditest), (Wensvoort et al., 1988) were used.

Data analyses

Since blood samples were collected every other day, the viraemic period was judged to have started one day prior to the first positive VI and ended one day prior to the first of at least two subsequent negative VI's. When a pig died during the viraemic period, the day of death was taken to be the end of the viraemic period. Based on findings in the experimentally inoculated pig and on findings in a previous experiment (Laevens et al., 1998) it was assumed that all pigs got infected three days prior to the beginning of its viraemic period. Additionally, it was assumed that a pig was infectious during the viraemic period.

The possibility of airborne virus spread was examined by looking at proof of infection in the different pens of the different compartments.

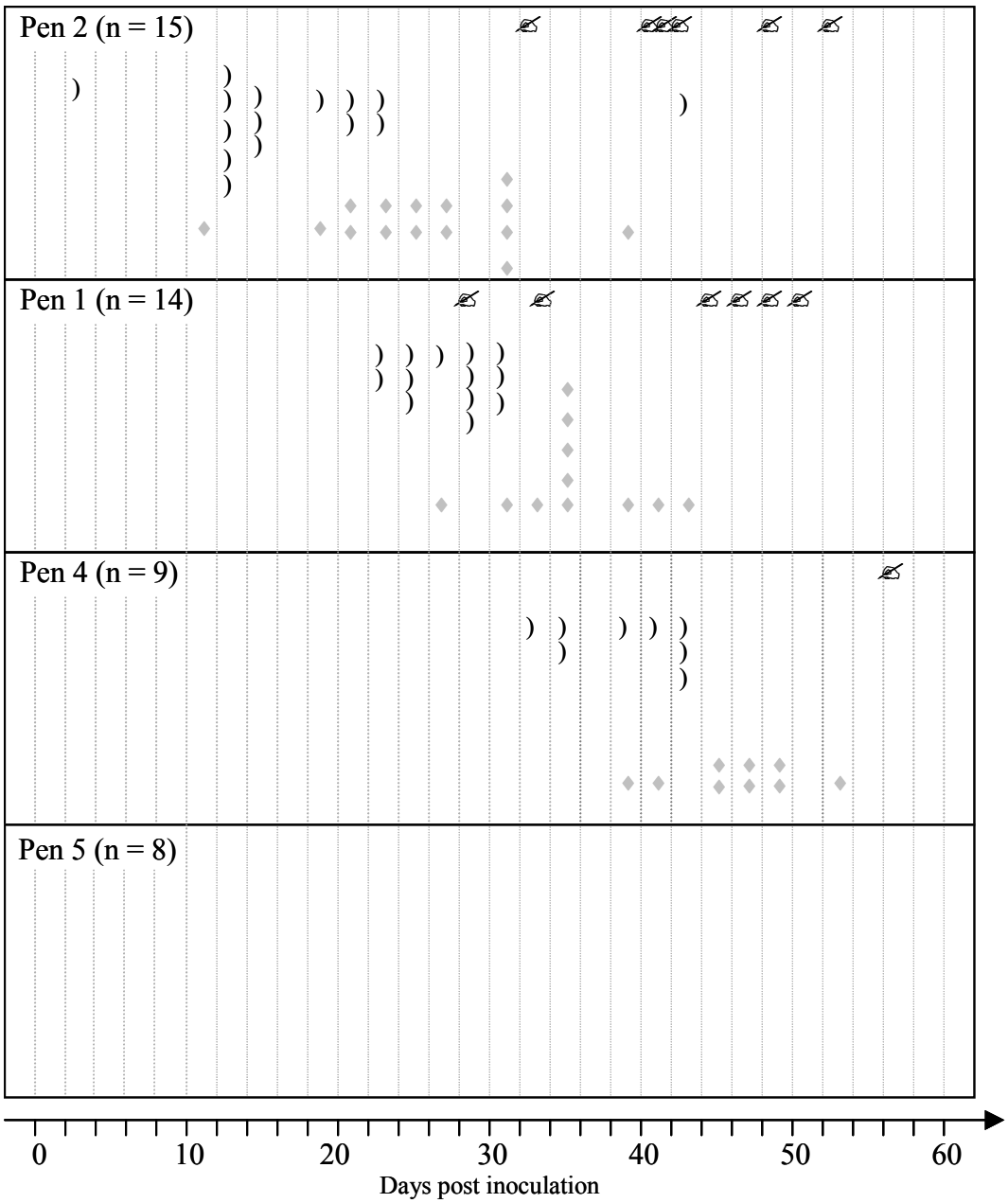
The effect of air currents on the airborne virus spread was examined by comparing the survival times of the pigs in pen 4 (air drawn from the surrounding compartments) with the survival times of the pigs in pen 5 (air blown into the surrounding compartments) (Kaplan-Meier survival analysis, SPSS). In the analysis the day of experimental infection of the pig in pen 2 was taken as start of the time at risk and the day of first viraemia of the pigs in pens 4 and 5 as event time.

The influence of compartmentalisation on virus spread was examined by comparing the survival times of the pigs in pen 1 (indirect contact, same compartment) with the survival times of the pigs in pen 4 (indirect contact, different compartment) (Kaplan-Meier survival analysis, SPSS). However, the real effect of compartmentalisation on airborne virus spread may be confounded by differences in the speed of virus transmission within pens 1 and 4. Therefore, the period in which all pigs in pens 1 and 4 became viraemic, from the moment that the first pig in the pen became viraemic (initial event), was compared (Kaplan-Meier survival analysis, SPSS).

Results

One pig in pen 1 died during the acclimatisation period due to an unknown reason. Therefore only data of 14 pigs in pen 1 were recorded.

The experimentally inoculated pig (pen 2) became viraemic 3 dpi (Figure 3). The pen mates became viraemic between 13 and 43 dpi. In pen 1, first viraemia was observed 23 dpi, 13 pigs became viraemic between 23 and 31 dpi, and one pig died before viraemia could be detected. In pen 4, eight out of the nine pigs became viraemic between 33 and 43 dpi. In one pig no viraemia was detected but seroconversion was observed before the end of the observation period. In pen 5 no proof of infection (viraemia or seroconversion) could be detected (Figure 3). Since it was assumed that every pig got infected three days prior to the beginning of its viraemic period, the days of first infection in the different pens could be determined. The first contact infection within pen 2 occurred 10 dpi. In pens 1 and 4, the first infection occurred on 20 and 30 dpi, respectively. On 10, 20, and 30, dpi, 1, 7 and 14 pigs, respectively, were viraemic. In pen 1, 3 pigs died before they seroconverted. Seroconversion occurred on average 7.8 days (s.d.= 4.8) after the onset of viraemia.



) : First day of viraemic period (each point representing a pig)

♦ : First seroconversion (each point representing a pig)

✍ : Death of pigs at a given point of time

Figure 3: First day of the viraemic period in individual pigs, the day on which each pig seroconverted and the days when pigs died.

The cumulative proportion of non-viraemic pigs in time in pens 1, 4 and 5 is shown in Figure 4. As a result of the air currents, no pigs in pen 5 got infected. The different infection statuses of pens 4 and 5 resulted in a significant difference ($p<0.001$) of the survivor functions between pens 4 and 5.

The time in which all pigs in pens 1 and 4 became viraemic was not different ($p=0.22$) between pens, indicating that once the first pig in a pen became viraemic, virus transmission within a pen is similar for all pens. Therefore the difference ($p<0.05$) between pens 1 and 4, that was found in the survivor functions giving the evolution in time of the cumulative proportion of non-viraemic pigs from the moment of experimental inoculation (initial event), was the result of the retarding effect of compartmentalisation on virus spread.

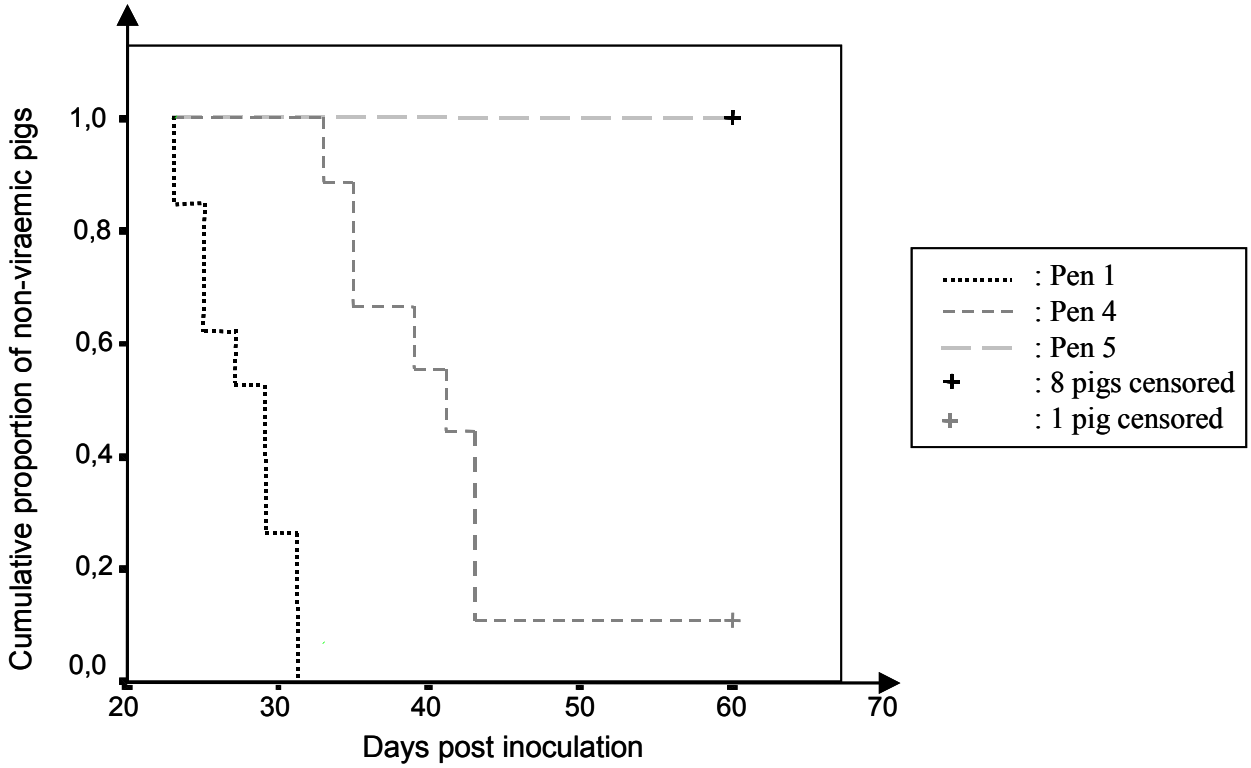


Figure 4: Cumulative proportions of non-viraemic pigs in pens 1, 4 and 5 at intervals after the experimental inoculation of one pig in pen 2.

Discussion

Airborne disease transmission includes three steps: 1) aerosol generation, 2) aerosol transport to susceptible animals, and 3) inhalation of infectious aerosols by susceptible animals (Stärk, 1998).

Aerosols are generated by animals through sneezing and coughing and in a lesser extent by normally exhaled breath. Furthermore faeces and urine splashes can generate aerosols. No exact data are available on the amount of virus excreted by a CSF infected pig. However, it has been described that a CSF infected pig excretes large amounts of virus with oral fluids and smaller quantities with urine, faeces, nasal and lachrymal fluids (Terpstra, 1988). The concentration of infectious agents in aerosols is directly proportional to the strength of the aerosol source. Indicators of the source strength are the number and the concentration of infectious animals (Stärk, 1998). This is consistent with the findings in this experiment where it was found that the source strength of one infectious pig was sufficient for infecting pigs within the same pen, whereas 7 infectious pigs were needed for virus transmission towards an adjacent pen. Virus transmission towards another compartment only occurred when the aerosol source strength was as large as 14 infectious pigs.

Attempts to isolate CSF virus from the air in rooms housing experimentally infected pigs have not been successful yet (Stärk et al., 1998). However the possibility of airborne transport of infectious aerosols has been clearly demonstrated in this experiment, as the virus was able to spread towards another compartment following the prevalent air currents. The absence of infection in pen 5, which was not different from pen 4 except for the ventilation system, indicates that the virus spread was clearly affected by the air currents.

Under experimental conditions pigs have been infected by oral, nasal, aerogenic, conjunctival, genital and various parenteral routes. Most of these routes are likely to occur in one way or another under natural conditions as well (Terpstra, 1988). The minimal intranasal infectious dose for fatal disease with the Alfort strain was reported to be less than 10 $TCID_{50}$ per pig (Liess, 1987; Dahle and Liess, 1995). This indicates that only a small amount of infectious aerosols is needed to initiate an infection.

Combining currently known experimental and epidemiological data it can be concluded that airborne spread of CSF virus is possible. However, it remains difficult to estimate the epidemiological importance of this transmission route under field conditions. Yet, in a fully susceptible population and in densely populated regions even a transmission route with minor importance may have major consequences.

Reference List

Dahle, J. and Liess, B. (1992) A review on classical swine fever infections in pigs: epizootiology, clinical disease and pathology. *Comp. of Imm. and Microb. of Infect. Diseases 15*, 203-211.

Dahle, J. and Liess, B. (1995) Comparative study with cloned classical swine fever virus strains ALFORT and GLENTORF: clinical, pathological, virological and serological findings in weaner pigs. *Wien. Tierärz. Moonatschr. 82*, 232-238.

Dewulf, J., Laevens, H., Koenen, F., Vanderhallen, H., Mintiens, K., Deluyker, H., de Kruif, A.. (2000) An experimental infection with classical swine fever in E2 sub-unit marker-vaccine vaccinated and in non-vaccinated pigs. *Vaccine. 19*, 475-482.

Edwards, S. (1989) Epidemiology and control of classical swine fever. In the Proceedings of the Society for veterinary epidemiology and preventive medicine. 12-14 April 1989, Exeter, UK, p.74.

Elbers, A.R., Stegeman, A., Moser, H., Ekker, H. M., Smak, J.A., and Pluimers, F.H. (1999) The classical swine fever epidemic 1997-1998 in The Netherlands: descriptive epidemiology. *Prev. Vet. Med. 42*, 157-184.

Hughes, R.W. and Gustafson, D. P. (1960) Some factors that May Influence Hog Cholera transmission. *Am. J. Vet. Res. 21*, 464-471.

Koenen, F. and Lefebvre, J. (1995) Kinetics of an experimental infection with a classical swine fever (CSF) field isolate. In the Proceedings of the 3rd Congress of European Society of Veterinary Virology. 4-7 September 1994, Interlaken, pp. 322-326.

Koenen, F., Van Caenegem, G., Vermeersch, J.P., Vandenheede, J., and Deluyker, H. (1996) Epidemiological characteristics of an outbreak of classical swine fever in an area of high pig density. *Vet. Rec. 139*, 367-371.

Laevens, H. (1999b) Risk factors for the transmission of classical swine fever virus to herds in the close neighbourhood of an infected herd. In: Epizootiology of classical swine

fever: Experimental Infections Simulating Field Conditions, and Risk Factors for Virus Transmission in the Neighbourhood of an Infected Herd. PhD thesis, Ghent, Ghent University. pp. 103 – 122.

Laevens, H., Koenen, F., Deluyker, H., and Berkvens, D. (1998) An experimental infection with classical swine fever virus in weaner pigs. I. Transmission of the virus, course of the disease, and antibody response. *Vet. Q. 20*, 41-45.

Laevens, H., Koenen, F., Deluyker, H., and de Kruif, A. (1999a) Experimental infection of slaughter pigs with classical swine fever virus: transmission of the virus, course of the disease and antibody response. *Vet. Rec. 145*, 243-248.

Liess, B. (1987) Pathogenesis and epidemiology of Hog Cholera. *Ann. Rech. Vet. 18*, 139-145.

Pittler, H., Fiedler, J., Polton, B. (1996) Swine fever epidemic in Germany 1993-1995, problems and consequences. In the Proceedings of the14th International pig Veterinary congress. 7-10 July 1996, Bologna, p. 402.

Roberts, M. (1995) Evaluation of optimal size of restriction zones in disease control with particular references to classical swine fever. In the Proceedings of meeting of the society for veterinary epidemiology and preventive medicine. 29-31 March 1995, Reading, UK, p.119.

Stärk, K.D.C. (1998) The role of infectious aerosols in disease transmission in pigs. In systems for the prevention and control of infectious disease in pigs, PhD thesis, Palmerston North, New-Zeeland, Massey University. pp. 65-89.

Stärk, K.D.C., Frey, J., Nicolet, J., Thür, B., and Morris, R. S. (1998) Assessment of aerosol transmission in the epidemiology of infectious disease in swine using air sampling and polymerase chain reaction assays. In the Proceedings of the The 15th International Pig Veterinary Society Congress, 5-9 June 1998, Birmingham, UK, p. 252

Staubach, C., Teuffert, J, and Thulke, H. H. (1997) Risk Analysis and local spread mechanisms of classical swine fever. In the Proceedings of the 8th conference of the international society for veterinary epidemiology and economics. Juli 1997, Paris, France, pp. 06.12.1-06.12.3.

Stegeman, J.A., Elbers, A.R., de Smit, A.J., Moser, H., & de Jong, M.C. (1997) Between-herd transmission of classical swine fever virus during the 1997 epidemic in the Netherlands. In the proceedings of the 10th annual meeting of the Dutch society for veterinary epidemiology and economics. 20 November 1997, Boxtel, The Netherlands, p. 25

Terpstra, C. (1987) Epizootiology of swine fever. *Vet. Quart. 9*, 50-60

Terpstra, C. (1988) Epizootiology of Hog-Cholera. In Classical swine fever and related viral infections. Editors: Liess, B. Martinus Nijhoff Publishing, Dordrecht, The Netherlands, pp. 201-216.

van Oirschot, J.T. (1999) Classical swine fever. In Diseases of Swine, vol. 8,. Editors: Straw, B. E., D'Allaire, S., Mengeling, W. L., and Taylor, D. J. Ames, Iowa, Iowa State University Press, pp.159-172.

Vanthemsche, P. (1995) Classical swine fever 1993-1994 in Belgium. In: Proceedings of the 8th Annual Meeting of the Dutch Society for Veterinary Epidemiology and Economics 13 December 1995, Lelystad, p. 25.

Wensvoort, G., Bloemraad, M., Terpstra, C. (1988) An enzyme immunoassay employing monoclonal antibodies and detecting specifically antibodies to classical swine fever virus. *Vet. Microbiol. 17*, 129-140.

CHAPTER 3.3:

EVALUATION OF THE POTENTIAL OF DOGS, CATS, AND RATS TO SPREAD CLASSICAL SWINE FEVER VIRUS.

J. Dewulf , H. Laevens , F. Koenen , K. Mintiens,

and A. de Kruif

Veterinary Record (2001) 149, 212-213

Abstract

The aim of this experiment was to examine whether classical swine fever (CSF) virus can infect pets or rodents and, as a result, lead to replication dependent virus dissemination in the neighbourhood of an infected herd. Therefore, 3 dogs, 3 cats and 4 rats were intra-nasally and orally challenged with high doses of CSF virus. After an observation period of 43 days all animals were euthanised.

At the end of the experiment all blood samples were negative for antibodies and CSF virus. Also all tissue samples were negative both on virus isolation as well as on PCR. During the whole observation period no clinical symptoms were observed.

The results of this experiment provide further evidence that dogs, cats or rats are unlikely to represent significant biological reservoirs. However mechanical spread of CSF virus by pets and rodents remains possible. The likelihood of mechanical spread is difficult to assess and was beyond the objectives of this experiment.

Introduction

During recent classical swine fever (CSF) epidemics in densely populated livestock areas in Europe, large numbers of secondary infections have been observed in the vicinity of primary infected herds (Vanthemsche, 1995; Koenen et al., 1996; Elbers et al., 1999). For many of these neighbourhood infections none of the "traditional" transmission routes for CSF, e.g. direct animal contact, swill feeding or transport contact, were found to be responsible for the virus spread (Terpstra, 1988; Koenen et al., 1996). In some of these cases pets and rodents have been suggested as possible virus transmitters (Hughes and Gustafson, 1960; Terpstra, 1988). However, there are only a limited number of experiments that examine the possibility of this type of virus transmission (Hughes and Gustafson, 1960; Terpstra, 1988). Therefore, it remains difficult for policy makers to decide whether pets and rodents present on an infected farm, should be killed together with the pigs on the day of eradication.

The aim of this experiment was to determine whether CSF virus can infect pets or rodents and, as a result lead to replication dependant virus dissemination (active infection) in the neighbourhood of an infected herd.

Materials and methods

Three cats, three dogs and four rats, purchased from specialised breeders (Iffa Credo, France) were used. They were housed in specifically designed, individual cages.
Before challenge, blood samples were taken from all animals to verify that the animals were free of antibodies against classical swine fever virus (CSFV) and bovine viral diarrhoea virus (BVDV).
After blood sampling all animals were intra-nasally and orally challenged using different volumes of inoculum per species. The intra-nasal inoculum was equally divided in the two nostrils.

- Dogs: 1.5 ml intranasal + 0.5 ml oral.
- Cats: 0.8 ml intranasal + 0.2 ml oral.
- Rats: 0.4 ml intranasal + 0.1 ml oral.

The isolate used for the experimental inoculation was originally obtained from the first CSF-infected herd of the 1993-1994 Belgian epizootic, and was characterised to be similar to an isolate known as the 'souche Lorraine' (Koenen and Lefebvre, 1995). Virus infectiousness was 10^3 median tissue culture infective dose ($TCID_{50}$ / ml).

The animals were sedated before challenge, using medethomidine (Domosedan®, Pfizer animal health, Belgium), in order to minimize discomfort and to assure that the total inoculum was fully and properly administered.

During the observation period all animals were inspected daily, and clinically examined by a veterinarian every three days.

At the end of the 43 days observation period all animals were euthanised and blood (whole blood and serum) and tissue samples (kidney, spleen, tonsils, heart and liver) were collected.

For virus isolation (VI) in blood, 100µl whole blood was inoculated in duplicate onto a non-confluent monolayer of PK15 cells cultured in multiwell plates (24 wells / plate). For VI in tissue samples one cm^3 of each organ was put into 9ml minimal essential medium (MEM) and ground with an ultra-Turrax (Janke and Kunkel). After centrifugation for 10 min at 4000g, 300µl of the supernatant was inoculated in duplicate onto a non-confluent monolayer of PK_{15} cells cultured in multiwell plates (24 wells / plate). After 48 hours, the cells were fixed with isopropanol and stained with a polyclonal fluorescein-conjugated anti-CSF immunoglobulin. Additionally, a single tube RT-nPCR test (McGoldrick et al., 1999), using a positive blood or organ sample from an experimentally infected pig as positive control, was used to detect virus in tissue samples. For antibody detection in serum, the virus neutralisation (VN) test and the CTB-ELISA (Ceditest) were used (Wensvoort et al., 1988).

Results

Before inoculation, all blood samples were negative both for the presence of antibodies against CSF and for CSF virus. After the observation period, all blood samples remained negative for antibodies and CSF virus.
All tissue samples were negative on VI as well as on PCR analysis.
During the whole post inoculation period no clinical symptoms were observed.

Discussion

Since dogs, cats or rats are not the natural hosts, two outcomes of an active infection with CSFV were possible: persistent infection without antibody development or acute infection followed by viral clearance and seroconversion. In this experiment, no CSF virus and no antibodies against CSF could be detected in any of the animals at the end of the observation period. Therefore, it may be assumed that no or very minimal viral replication has occurred. The amount of virus that the animals were exposed to is comparable to the average amount of virus excreted by an infected pig in the field and is 100 times higher than the minimal infectious dose for fatal disease in pigs, which was reported to be less than 10 TCID50 per pig (Liess, 1987; Dahle and Liess, 1995).

The findings of this experiment corroborate with the statement of Hughes and Gustafson (1960) that CSFV transmission by rats and dogs is unlikely. In an experiment by Terpstra (1988), it was found that rats that were fed in close contact with CSF infected pigs were not able to transmit the infection to susceptible animals.

In conclusion it can be stated that the results of this experiment provides further evidence that dogs, cats or rats are unlikely to represent significant biological reservoirs. However mechanical spread of CSF virus by pets and rodents remains possible. The likelihood of mechanical spread is difficult to assess and was beyond the objectives of this experiment.

In terms of safety measures during outbreaks it can be suggested that it is not necessary to kill pets and rodents as long as they are prevented from carrying potentially contaminated materials towards neighbouring herds.

Reference List

Dahle, J. and Liess, B. (1995) Comparative study with cloned classical swine fever virus strains ALFORT and GLENTORF: clinical, pathological, virological and serological findings in weaner pigs. *Wien. Tierärz. Moonatschr. 82*, 232-238.

Elbers, A.R., Stegeman, A., Moser, H., Ekker, H. M., Smak, J.A., and Pluimers, F.H. (1999) The classical swine fever epidemic 1997-1998 in The Netherlands: descriptive epidemiology. *Prev. Vet. Med. 42*, 157-184.

Hughes, R.W. and Gustafson, D. P. (1960) Some factors that May Influence Hog Cholera transmission. *Am. J. Vet. Res. 21*, 464-471.

Koenen, F. and Lefebvre, J. (1995) Kinetics of an experimental infection with a classical swine fever (CSF) field isolate. In the Proceedings of the 3rd Congress of European Society of Veterinary Virology. 4-7 September 1994, Interlaken, pp. 322-326.

Koenen, F., Van Caenegem, G., Vermeersch, J.P., Vandenheede, J., and Deluyker, H. (1996) Epidemiological characteristics of an outbreak of classical swine fever in an area of high pig density. *Vet. Rec. 139*, 367-371.

Liess, B. (1987) Pathogenesis and epidemiology of Hog Cholera. *Ann. Rech. Vet. 18*, 139-145.

McGoldrick, A., Bensaude, E., Ibata, G., Sharp, G., Paton, D.J., (1999). Closed one-tube reverse transcription nested polymerase chain reaction for the detection of pestiviral RNA with fluorescent probes. *J. Virol. Methods* 79, 85-95.

Terpstra, C. (1988) Epizootiology of Hog-Cholera. In Classical swine fever and related viral infections. Editors: Liess, B. Martinus Nijhoff Publishing, Dordrecht, The Netherlands, pp. 201-216.

Vanthemsche, P. (1995) Classical swine fever 1993-1994 in Belgium. In: Proceedings of the 8th Annual Meeting of the Dutch Society for Veterinary Epidemiology and Economics 13 December 1995, Lelystad, p. 25.

Wensvoort, G., Bloemraad, M., Terpstra, C. (1988) An enzyme immunoassay employing monoclonal antibodies and detecting specifically antibodies to classical swine fever virus. *Vet. Microbiol. 17*, 129-140.

CHAPTER 3.4:

AN EXPERIMENTAL INFECTION TO INVESTIGATE THE INDIRECT TRANSMISSION OF CLASSICAL SWINE FEVER VIRUS BY EXCRETIONS OF INFECTED PIGS.

J. Dewulf , H. Laevens , F. Koenen , K. Mintiens,

and A. de Kruif

Accepted for publication in Journal of Veterinary Medicine serie B

Abstract

In this experiment transmission of classical swine fever (CSF) virus via excretions of infected pigs was investigated under experimental conditions. Five pairs of pigs were experimentally infected with CSF virus. Eight days after experimental infection, when all pigs were viraemic for at least 3 days, the pens were depopulated and 20 hours later restocked with 5 pairs of susceptible pigs who stayed in these pens for 35 days. During the first 3 weeks of the experiment the pens were neither cleaned nor disinfected. During the observation period none of the susceptible pigs became infected. This result indicates that CSF virus spread via excretions is of minor importance in the early stages of infection. For extrapolation of these findings to the field situation and to increase the validity of the conclusions further research is needed to evaluate the effect of factors like virus strain, interval, …, that may influence the outcome of the experiment.

Introduction

Different routes for between-herd transmission of the classical swine fever (CSF) virus have been described. The most frequently cited are direct animal contact, mechanical vectors such as vehicles, equipment, and persons, artificial insemination with semen from infected boars and neighbourhood contacts. The majority of these virus transmission routes were identified based on epidemiological research and expert opinions (Terpstra, 1988; Horst et al., 1997; Teuffert et al., 1998; Laevens, 1999b; Mintiens et al., 2000; Stegeman et al., 2000; Elbers et al., 2001). Whereas, only a limited number were identified or confirmed under experimental conditions (Hughes and Gustafson, 1960; Terpstra, 1988; de Smit et al., 1999; Dewulf et al., 2000).

In case of transmission of CSF virus through mechanical vectors it is believed that these vectors are contaminated through excretions of infectious pigs and that the susceptible pigs are infected through contact with these excretions. Subsequently, the risk of transmission is influenced by the probability of virus spread via the excretions and by the probability of infection through contact with these excretions. In general, it is stated that CSF infected pigs are excreting the virus through oronasal and lacrimal excretions as well as through urine and faeces (Terpstra, 1988; van Oirschot, 1999) and can become infected through oral, nasal, aerogenic, conjunctival, genital and various parenteral routes (Terpstra, 1988). Furthermore it is described that pigs may shed virus before the onset of the disease and continue to do so during the entire disease period (van Oirschot, 1999). However, these general rules are based on very limited experimental information. In one experiment the CSF virus could be isolated from the faeces and urine 6 to 7 days post inoculation (Ressang and Bool, 1972), whereas in an other study virus could only be isolated from faeces from day 14 post inoculation onward. However, in none of these experiments an attempt was made to reinfect susceptible animals with these positive excretions. Information on the possibility of infection of susceptible pigs with excretions of infectious pigs is even sparser. Only one experiment is described in which 2 out of 10 pigs exposed to secretions and excretions of clinically diseased pigs became infected (Hughes and Gustafson, 1960).

Because of the importance that is addressed to the indirect virus transmission via mechanical vectors during CSF epidemics on the one hand, and the limited experimental information that is available on this route of virus transmission on the other hand, an experiment was set up to evaluate the importance of virus spread through excretions of infected pigs. More specific a frequently occurring field situation, where susceptible pigs are transported with a vehicle that previously transported infectious pigs, was mimicked in an experimental setting.

Material and methods

Animals

Twenty conventional weaner pigs of 12-15 kg, originating from an isolated pig herd and controlled for the absence of bovine viral diarrhoea virus (BVDV) and CSFV antibodies, were used.

Virus

The isolate used for the experimental inoculation was originally obtained from the first CSF-infected herd of the 1993-1994 Belgian epizootic. The isolate was verified to be free of African swine fever virus and BVD virus. By using monoclonal antibodies, it was characterised to be similar to an isolate known as the 'souche Lorraine' (Koenen and Lefebvre, 1994). Virus infectiousness was 10^3 median tissue culture infective dose ($TCID_{50}$ / ml). The inoculum was obtained from the original isolate after two passages on PK_{15} cells.

Experimental design

Upon arrival, 10 pigs were randomly allocated to 5 pens (2 pigs per pen) situated in one compartment. All pens were the same size (length x width x height = 150 x 200 x 150 cm) with a full concrete floor, and separated by a full wall. Therefore, no direct contact was possible between pigs or manure of the different pens. After an acclimatisation period of 6 days all pigs were individually inoculated with CSF virus by deep intramuscular injection (2 ml) plus intranasal inoculation (2 ml). During an eight days period (infection period), the

infection status of the inoculated pigs was checked by clinical examination, rectal temperature monitoring, and blood sampling. After this period the pens were depopulated and the pigs were euthanised.

Twenty hours after depopulation pens were restocked with 10 susceptible weaner pigs (random allocation, 2 pigs per pen). The susceptible pigs stayed in the pens during the next 35 days (observation period). After the observation period all pigs were euthanised. During the infection period, in the period between depopulation and restocking, and the first two weeks of the observation period, the pens were neither cleaned nor disinfected.

During the whole experiment the temperature in the pens was around 20°C.

Sample collection and analysis

First sample collection from all pigs took place upon arrival. During the infection period (inoculated pigs, 8 days) and the first two weeks of the observation period (susceptible pigs) clotted and heparinised blood samples for virus, antigen, and antibody detection were collected every two days. During the last three weeks of the observation period blood samples were taken weekly. The pens were always visited following the same route beginning with pen 1 and ending with pen 5. Between the visits of the pens footwear was disinfected. All material necessary for rectal temperature monitoring and blood sampling was provided per pen.

On the blood samples collected from the experimentally inoculated pigs (infection period) virus was detected using virus isolation (VI) in whole blood and serum, whereas during the observation period virus was detected using VI in whole blood, serum and leucocytes. Additionally, a single tube RT-nPCR test (McGoldrick et al., 1999), including a positive blood sample from an experimentally infected pig as positive control, was used to detect virus in blood samples during the observation period. In all blood samples antigen was detected using Ag ELISA (CHEKIT CSFV III, Dr. Bommeli AG, Liebefeld-Bern) and antibodies against CSF virus as well as BVD virus, were detected using virus-neutralisation (VN) test (Holm-Jensen 1981). From every pig that died or was euthanised, tissue (kidney, spleen, tonsils, heart and liver), blood and faeces (directly from colon) samples and nasal swabs were collected for VI. Additionally, both at the moment of depopulation and at the moment of restocking, 2 excretion samples were collected in each pen for VI.

For VI in blood samples, 100µl whole blood, serum or buffy coat was inoculated in duplicate onto a non-confluent monolayer of PK_{15} cells cultured in multiwell plates (24 wells / plate). For VI in tissue samples and faeces one cm^3 of each organ or faeces sample was put into 9ml minimal essential medium (MEM) and grounded with an ultra-Turrax (Janke and Kunkel). After centrifugation for 10 min at 4000g, 300µl of the supernatant was inoculated in duplicate onto a non-confluent monolayer of PK_{15} cells cultured in multiwell plates (24 wells / plate). After 48 hours, the cells were fixed with isopropanol and stained with a polyclonal fluorescein-conjugated anti-CSF immunoglobulin. Virus titration was done using ten fold dilutions. During the whole experiment rectal temperature was monitored daily.

Results

The experimental inoculation succeeded in all pigs. Since viraemia is assumed to start halfway between the last negative result and the first positive result, the average time between experimental inoculation and onset of viraemia, based on the VI in whole blood, was estimated to be 3.2 days (Table 1). On the day of depopulation blood samples of all pigs were positive in VI as well as Ag ELISA. Virus titres in whole blood varied between 10^{-3} and 10^{-4}. At the end of the infection period clinical symptoms such as erythema, conjunctivitis, diarrhoea and ataxia were present.

Table 1: Virus detection by means of VI in whole blood

	Incub. period				Observation period										
1.1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0
1.2	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0
2.1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0
2.2	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0
3.1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0
3.2	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0
4.1	0	1	1	1	0	0	0	0	0	0	0	0	nd	nd	nd
4.2	0	1	1	1	0	0	0	0	0	0	0	0	0	0	nd
5.1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0
5.2	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0
	0	2	4	6	8	9	11	13	15	17	19	21	23	30	37	44

Days post experimental inoculation

Also the rectal temperature of all pigs rose above 40°C (Figure 1). Due to the limited period (8 days) between inoculation and depopulation no antibodies were found in these pigs. At the

moment of depopulation virus could be isolated from most of the tissue samples of the experimentally inoculated pigs (Table 2). Moreover, in four pigs virus could be isolated from the nasal swabs with titres varying between 10^{-1} and 10^{-2}. However, in only one pig virus could be isolated in faeces collected directly from the colon (titre 10^{-1}) (Table 2). In the excretion samples collected in the pens at the moment of depopulation and at the moment of restocking no virus could be isolated.

At the moment of restocking the floor of each pen was almost fully covered with excretions. Consequently, an intense contact of the susceptible pigs with the excretions was possible. During the observation period all blood samples of all pigs remained negative for VI in whole blood (Table 1), leucocytes and serum, as well as for RT-nPCR and Ag-ELISA. Also no antibodies could be detected at any moment during the observation period. These negative results were confirmed by the negative results in all tissue samples. Furthermore, no clinical signs or fever could be detected during the observation period (Figure1).

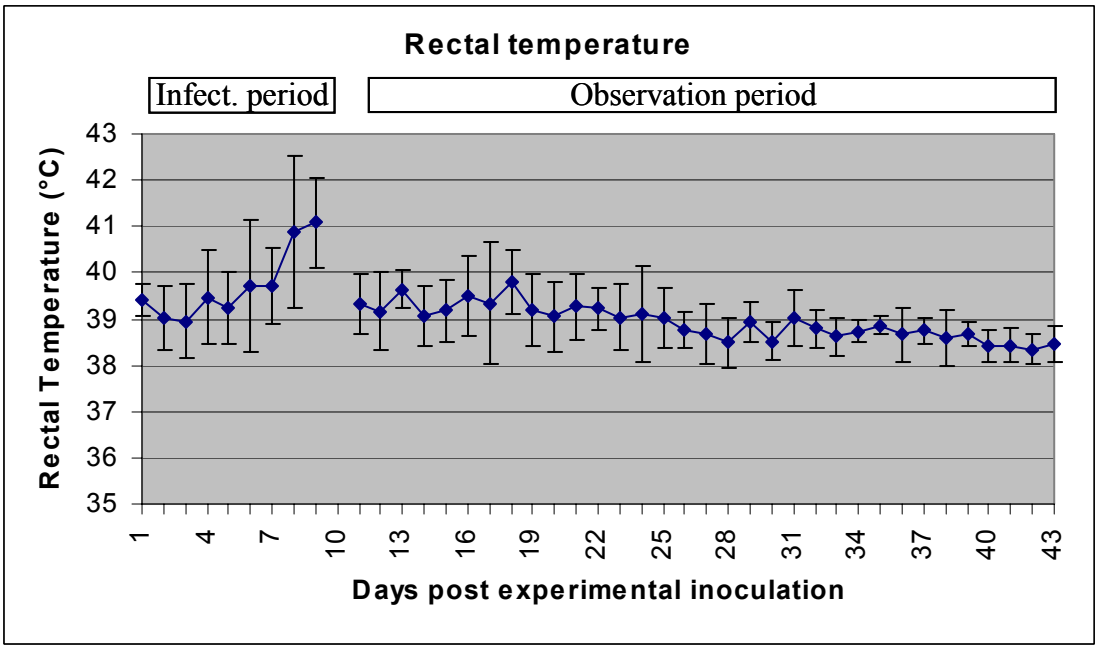


Figure 1: Average rectal temperature curve (95% confidence interval)

Two pigs (nrs 4.1 and 4.2) died 17 and 32 days after restocking, respectively. The death of pig nr 4.1 was due to stress during the blood sampling, whereas for pig nr 4.2 the cause of death remained unknown. However, since all blood as well as tissue samples of both pigs were

negative during the whole observation period and after death it was concluded that they did not die as a result of a CSF infection.

Table 2: Virus Isolation in different tissues and in blood, faeces, and nasal fluid samples after slaughter

Virus Isolation in experimentally inoculated pigs

	tonsil	kidney	spleen	hart	liver	faeces	nas. fl.*	blood
1.1	1	1	1	1	1	1	0	1
1.2	1	1	1	1	0	0	0	1
2.1	1	1	1	1	1	0	1	1
2.2	1	1	1	1	1	0	1	1
3.1	1	1	1	1	1	0	1	1
3.2	1	1	1	1	0	0	1	1
4.1	1	1	1	1	0	0	0	1
4.2	1	1	1	1	1	0	0	1
5.1	1	1	1	1	0	0	0	1
5.2	1	1	1	1	0	0	0	1

Virus Isolation in susceptible pigs

	tonsil	kidney	spleen	hart	liver	faeces	nas. fl.*	blood
1.1	0	0	0	0	0	0	nd	0
1.2	0	0	0	0	0	0	nd	0
2.1	0	0	0	0	0	0	nd	0
2.2	0	0	0	0	0	0	nd	0
3.1	0	0	0	0	0	0	nd	0
3.2	0	0	0	0	0	0	nd	0
4.1	0	0	0	0	0	0	nd	0
4.2	0	0	0	0	0	0	nd	0
5.1	0	0	0	0	0	0	nd	0
5.2	0	0	0	0	0	0	nd	0

1 Positive result

0 Negative result

nd not done

* Nasal fluid

Discussion

The experiment was designed to correspond as much as possible to a field situation where susceptible pigs are transported with a vehicle that previously transported infectious pigs. Therefore the incubation period was deliberately limited to 8 days to allow all pigs to become viraemic but to avoid the pigs to become undeniably clinically diseased since visibly diseased animals are unlikely to be transported during a CSF epidemic.
Although all experimentally inoculated pigs were euthanised before they became clearly diseased, it may be assumed that they all were developing a severe infection. This conclusion is based on the one hand on the observation that already 8 days post infection all pigs had fever, were developing the first clinical symptoms and had high virus titres in blood and on the other hand it is known from several previous experiments, using the same virus strain and infection dose, that the 'souche Lorraine' consistently causes severe clinical symptoms and mortality in piglets, fattening pigs as well as sows (Laevens et al., 1998; Laevens et al., 1999a; Dewulf et al., 2000; Dewulf et al., 2001).

The time interval between depopulation and restocking was set to be 20 hours mimicking a vehicle transporting infectious pigs on one day and susceptible pigs the next day. However this time interval is believed to be of minor importance since CSF virus can remain infective up to 2 weeks in liquid manure kept at 20°C (Westergaard, 1996; Edwards, 2000). The fact that the pens were neither cleaned nor disinfected between depopulation and restocking mimics a worst-case scenario where the mandatory hygienic procedures of cleaning and disinfection between subsequent animal transports were totally ignored.

Although most of the pigs were already viraemic 5 days prior to depopulation, and virus was isolated in the faeces of one pig and the nasal excretions of 4 pigs, the results of the experiment indicate that the amount of virus excreted was insufficient to contaminate the pens in such a way that the susceptible pigs became infected. The one pig in which virus could be isolated from the faeces was viraemic for 5 days and developed fever during the last two days before euthanasia. On the day of euthanasia there was also conjunctivitis detectable. However, these results do not differ from the other inoculated pigs in which no virus could be detected in the faeces. The isolation of virus in nasal excretions and faeces already 8 days post

infection does not fully correspond with the results of Depner et al. (1994) who found that virus could only be isolated in nose fluid and faeces as early as 10 and 14 days post inoculation, respectively. The differences may be caused by biological variation, test sensitivity or virus strain.

The extrapolation of results of this experiment to the field situation remains a difficult task. On the one hand it can be concluded that the virus transmission via excretions is of minor importance in the early stages of the infection. Referring to the results of Depner et al. (1994) it could even be assumed that virus spread via excretion becomes only important when the infected pigs are clinically diseased. On the other hand this conclusion should be drawn very carefully since many factors, such as virus strain, infection dose, length of the interval infection depopulation, etc., may have influenced the outcome of the experiment. Moreover the results of this experiment conflict with the importance that is appointed to the transmission of CSF virus via contaminated mechanical vectors in several epidemiological studies (Stegeman et al. 1997, Elbers et al. 2001). Therefore, this experiment should be repeated to evaluate the effect of the potentially influencing factors and to increase the power of the conclusions.

However, based on the results of this experiment, it can be concluded at least that transmission of CSF virus by contaminated mechanical vectors is not as self-evident as assumed before and merits further research. Finally it should be stressed that the results of this experiment may in no means be interpreted as that hygienic procedures should be neglected during the control of a CSF outbreak.

Reference List

de Smit, A.J., Bouma, A., Terpstra, C., and van Oirschot, J.T. (1999) Transmission of classical swine fever virus by artificial insemination. *Vet. Microbiol. 67,* 239-249.

Depner, K., Gruber, A., and Liess, B. (1994) Experimental infection of weaner pigs with a field isolate of Hog Cholera/Classical Swine Fever Virus derived from a recent outbreak in Lower Saxony. 1: Clinical, virological and serological findings. *Wien. Tierärztl. Mschr. 81*, 370-373.

Dewulf, J., Laevens, H., Koenen, F., Mintiens, K., de Kruif, A. (2000a) Airborne transmission of classical swine fever virus under experimental conditions. *Vet. Rec.147*, 735-738.

Dewulf, J., Laevens, H., Koenen, F., Vanderhallen, H., Mintiens, K., Deluyker, H., de Kruif, A. (2000b) An experimental infection with classical swine fever in E2 sub-unit marker-vaccine vaccinated and in non-vaccinated pigs. *Vaccine 19,* 475-482.

Dewulf, J., Laevens, H., Koenen, F., Mintiens, K., de Kruif, A. (2001) An experimental infection with classical swine fever virus in pregnant sows: transmission of the virus, course of the disease, antibody response and effect on gestation. *J. Vet. Med. B 48*, 583-591.

Edwards, S. (2000) Survival and inactivation of classical swine fever virus. *Vet. Microbiol. 73*, 175-181.

Elbers, A., Stegeman, A., and de Jong, M. C. (2001) Factors associated with the introduction of Classical Swine Fever virus into pig herds in the central area of the 1997-1998 epidemic in The Netherlands. In the proceedings of the 9th conference of the Society for Veterinary Epidemiology and Preventive Medicine, 6-14, Noordwijkerhout, The Netherlands.

Holm-Jensen, M. (1981) Detection of antibodies against hog holera virus and bovine viral diarrhoea in porcine serum. A comparative examination using CF, PLA, and NPLA assay. *Acta Vet. Scan. 22*, 85-98.

Horst, H. S., Huirne, R. B., Dijkhuizen, A. A. (1997) Risks and economic consequences of introducing classical swine fever into The Netherlands by feeding swill to swine. *Rev. Sci. Tech. 16,* 207-214.

Hughes, R.W. and Gustafson, D. P. (1960) Some factors that May Influence Hog Cholera transmission. *Am. J. Vet. Res. 21*, 464-471.

Koenen, F. and Lefebvre, J. (1995) Kinetics of an experimental infection with a classical swine fever (CSF) field isolate. In the Proceedings of the 3rd Congress of European Society of Veterinary Virology. 4-7 September 1994, Interlaken, pp. 322-326.

Laevens, H. (1999b) Risk factors for the transmission of classical swine fever virus to herds in the close neighbourhood of an infected herd. In: Epizootiology of classical swine fever: Experimental Infections Simulating Field Conditions, and Risk Factors for Virus Transmission in the Neighbourhood of an Infected Herd. PhD thesis, Ghent, Ghent University. pp. 103 – 122.

Laevens, H., Koenen, F., Deluyker, H., and Berkvens, D. (1998) An experimental infection with classical swine fever virus in weaner pigs. I. Transmission of the virus, course of the disease, and antibody response. *Vet. Q. 20*, 41-45.

Laevens, H., Koenen, F., Deluyker, H., and de Kruif, A. (1999a) Experimental infection of slaughter pigs with classical swine fever virus: transmission of the virus, course of the disease and antibody response. *Vet. Rec. 145*, 243-248.

McGoldrick, A., Bensaude, E., Ibata, G., Sharp, G., Paton, D.J., (1999). Closed one-tube reverse transcription nested polymerase chain reaction for the detection of pestiviral RNA with fluorescent probes. *J. Virol. Methods* 79, 85-95.

Mintiens, K., Laevens, H., Deluyker, H., Dewulf, J., Koenen, F., and de Kruif, A. (2000) Estimation of the likelyhood for "Neighbourhood Infections" during classical swine fever epidemics based on spatial risk assesment of real outbreak data. In proceedings of 9th Symposium of the International Society for Veterinary Epidemiology and Economics. Breckenridge, Colorado, 6-11 August 2000, pp. 712-714.

Ressang, A. A. and Bool, P. H. (1972) Virus excretion in vaccinated pigs subject to contact infection with virulent hog cholera strains. *Zentralbl.Veterinarmed. 19,* 739-752.

Stegeman, A., Elbers, A., and de Jong, M.C. (2000) Rate of inter-herd transmission of classical swine fever virus by different types of contact. In proceedings of 9th Symposium of the International Society for Veterinary Epidemiology and Economics. Breckenridge, Colorado, 6-11 August 2000, pp. 1095-1096.

Stegeman, J.A., Elbers, A.R., de Smit, A.J., Moser, H., & de Jong, M.C. (1997) Between-herd transmission of classical swine fever virus during the 1997 epidemic in the Netherlands. In the proceedings of the 10th annual meeting of the Dutch society for veterinary epidemiology and economics. 20 November 1997, Boxtel, The Netherlands, p. 25

Terpstra, C. (1988) Epizootiology of Hog-Cholera. In Classical swine fever and related viral infections. Editors: Liess, B. Martinus Nijhoff Publishing, Dordrecht, The Netherlands, pp. 201-216.

Teuffert, J., Kramer, M., and Schlüter, H. (1998) The epidemiologie of classical swine fever (CSF) in Germany under special consideration of the tasks of the veterinary practitioners. *Prak. Tier. coll. Vet. XXVIII* 45-49.

van Oirschot, J.T. (1999) Classical swine fever. In Diseases of Swine, vol. 8,. Editors: Straw, B. E., D'Allaire, S., Mengeling, W. L., and Taylor, D. J. Ames, Iowa, Iowa State University Press, pp.159-172.

Westergaard, J. M., (1996) Epidemiology of classical swine fever, In proceedings of Workshop on diagnostic procedures and measures to control classical swine fever in domestic pigs and the European wild boar. Poland, National Veterinary Research Institute, Pulaway, Poland. pp. 119-130

CHAPTER 3.5:

AN EXPERIMENTAL INFECTION WITH CLASSICAL SWINE FEVER VIRUS IN PREGNANT SOWS: TRANSMISSION OF THE VIRUS, COURSE OF THE DISEASE, ANTIBODY RESPONSE, AND EFFECT ON GESTATION.

J. Dewulf , H. Laevens , F. Koenen , K. Mintiens,

and A. de Kruif

Journal of Veterinary Medicine serie B (2001) 48, 583-591

Abstract

An experimental infection with classical swine fever (CSF) virus in twelve conventional gilts, housed in a sow-box housing system, was conducted in order to evaluate horizontal transmission, clinical, virological and serological response, and the effect on gestation. Two out of the twelve gilts, of which 10 were pregnant, were experimentally inoculated. They became viraemic for the first time 6 days post inoculation (dpi). Contact gilts became viraemic between 18 and 21 days post inoculation. On the basis of virological findings and the martingale estimate of R_0 (13.0) it was concluded that the two experimentally inoculated gilts infected all contact gilts, although random contacts between gilts were not possible. The presence of a CSF infection could be diagnosed earlier and during a longer period when leukocyte count or polymerase chain reaction were used in comparison with virus isolation in whole blood ($p<0.05$). The observed clinical symptoms were atypical and highly variable between the gilts, which hampered clinical diagnosis. The pregnant gilts got infected between day 43 and 67 of gestation. In all cases vertical virus transmission occurred and this resulted partially in abortion and/or mummification.

Introduction

Classical swine fever (CSF) is known as a highly contagious pig disease causing considerable economic losses. In 1980 the European Union (EU) adopted an eradication strategy for CSF (Council Directive 80/217, EU). Since the control of CSF in the EU is based on a policy of non-vaccination and stamping-out. This policy has resulted in an eradication of the disease in most of the member states of the EU.

However, recent outbreaks have shown that CSF epidemics in densely populated pig areas are difficult to control and can have dramatic consequences (Elbers et al., 1999). In the 1997-1998 CSF epidemic in the Netherlands, it once again has been proven that the early detection of the primary CSF infected herd is crucial to minimize the size of an outbreak. The longer CSF remains undetected, the larger the opportunities are for the virus to become widespread (Horst et al., 1998; Elbers et al., 1999). The most important hindrance to detect a present CSF infection in an early stage is the appearance of atypical clinical symptoms and the relatively large chance to miss an infection if only a limited number of blood samples are taken (Koenen et al., 1996).

In order to design a surveillance system that maximises the possibility of detecting a present infection, it is essential to have detailed information on the clinical picture and on the dynamics of the infection. Moreover, information of the within-herd virus spread is of great importance to assess the risk of between-herd virus spread.
The spread of CSF in weaner and slaughter pigs has already been investigated (Laevens et al., 1998; Laevens et al., 1999). Similar experiments in sows housed in a sow-box housing system have not yet been conducted.

In the present study the transmission of CSF virus among gilts housed in a sow-box housing system was examined. Furthermore, the virological and serological response, the clinical symptoms, and the effect on gestation, following a CSF infection are described.

Materials and methods

Animals

Twelve conventional gilts, 8 months of age, originating from a selection herd and controlled for the absence of bovine viral diarrhoea (BVD) and CSF antigen and antibodies were used.

Virus

The isolate used for the experimental inoculation was originally obtained from the first CSF-infected herd of the 1993-1994 Belgian epizootic. The isolate was verified to be free of African swine fever virus and BVD virus. By using monoclonal antibodies, it was characterised to be similar to an isolate known as the 'souche Lorraine' (Koenen and Lefebvre, 1995). Virus infectiousness was 10^3 median tissue culture infective dose ($CTID_{50}$ / ml).

Experimental design

Upon arrival, gilts were housed in individual sow boxes where oestrus detection was carried out on a daily basis. Within a range of 24 days oestrus was observed in all gilts. During oestrus, gilts were inseminated twice. Twenty-five days after the last insemination gilts were chequed on pregnancy using ultrasound. After pregnancy diagnosis, gilts were transferred to an isolation unit where they were again housed in individual sow-boxes. The two gilts that were inseminated first (longest period of gestation) were housed in boxes 3 and 10, respectively. The two gilts that were not pregnant were housed in the middle boxes (6 and 7). The remaining 8 gilts were randomly allocated to the remaining boxes. Direct nose-to-nose contact was only possible between neighbouring pigs.

Following a ten days acclimatisation period after arrival at the isolation unit, two gilts, housed in box 3 and box 10, were experimentally inoculated with CSF virus through deep intramuscular injection (2 ml) plus intranasal inoculation (2 ml). After experimental inoculation, sows were not released from the boxes until the end of the experiment or at the

moment of death. All gilts that survived the infection were slaughtered one week before the end of gestation.

In a 75-days post-inoculation period, the boxes were visited following a strict route starting as far as possible from the experimentally inoculated gilts and moving towards the sources of infection. By applying this visiting procedure, it was ensured that the virus was not transferred from infected to uninfected gilts during sample collection. Additionally, all materials necessary for blood sampling and rectal temperature monitoring were provided for each box separately.

Sample collection and clinical examination

Clotted and heparinised blood samples were collected from all gilts upon arrival. Again, blood samples were taken upon arrival at the isolation unit and two days prior to inoculation. During the post-inoculation period, blood samples were collected from all gilts every 3 days until 54 days post inoculation (dpi), and every 6 days between 54 and 75 dpi. Additionally, swabs of nasal secretion and faeces were collected from the experimentally inoculated gilts every 3 days during the first 30 dpi. Simultaneously with sample collection, all gilts were examined clinically. The following symptoms were recorded: liveliness (apathy), body condition (cachexia), coughing, conjunctivitis, diarrhoea, ataxia, and erythema. Rectal temperature, feed intake and mortality were recorded daily.

From every pig that died or had to be euthanised, tissue samples (tonsil, muscles of shoulder and rump, mesenterial, ileocecal and maxillary lymph node, kidney, spleen, heart, lung, liver, brain, eye fluid, blood, faeces, urine) were collected. After dead or after abortion, blood and tissue samples (tonsil, kidney, spleen, heart, and lung) were collected from the foetuses.

Sample analyses

For virus isolation (VI) in blood, 100 µl whole blood was inoculated in duplicate onto a non-confluent monolayer of PK_{15} cells cultured in multiwell plates (24 wells / plate). After 48 hours, the cells were fixed with isopropanol and stained with a polyclonal fluorescein-conjugated anti-CSF immunoglobulin. Additionally, a single tube reverse transcriptase (RT)-nested polymerase chain reaction (nPCR) test (McGoldrick et al., 1999) was used to detect

viraemia in serum. The same single tube RT-nPCR test was used to detect CSF virus in nasal secretion and faeces of the experimentally inoculated pigs.
For antibody detection in serum, the virus neutralisation (VN) test and the CTB-ELISA (Ceditest) (Wensvoort et al., 1988) were used. Leukocyte count was carried out using the Coulter-Counter ZM (Analis).

Data analyses

The basic reproduction ratio (R_0), a measure of transmission of infection, and defined as the mean number of new infections arising from one typical infectious case introduced in a totally susceptible population, was calculated using the martingale (de Jong and Kimman, 1994) and the maximum likelihood (Bouma et al., 1996) estimator.
The martingale estimator is defined as:

$$R_{0mrt} = \frac{N}{C - Z} \sum_{i=S_t+1}^{S_0} \frac{1}{i}$$

To calculate Z (the sum of fractions of infectious periods that were spent at the time when no susceptibles remained), the day of infection was estimated for all gilts and it was assumed that the gilts were infectious during their entire viraemic period. The "SIR" (Susceptible-Infective-Removed) model was used to describe the final size distribution in terms of R_{0mrt} (de Jong and Kimman, 1994). Statistical tests for R_{0mrt} were performed as described by Kroese and de Jong (in preparation) (H_0: $R_0 \leq 1$).

The maximum likelihood estimator is calculated numerically from:

$$R_{0mle} = \max \prod_{i=1}^{n} F\left\langle X_i, R_0 \,\middle|\, N, S_0, I_0 \right\rangle$$

Fever was defined as a rectal temperature > 39.0°C. This is the one-sided upper 95% confidence limit (CL) calculated on the average rectal temperature of each gilt during the last three days before experimental inoculation. Leukopenia was defined in a similar way and the one-sided lower 95% CL limit was equal to 11,500 cells/ml.

Periods during which a given clinical symptom occurred started with the first of at least two subsequent observations of a given clinical symptom and ended with the first of at least two subsequent observations for which the given clinical symptom was absent. Periods of positive VI, PCR and Leukopenia were defined in a similar way.

The time to first leukopenia, positive PCR and positive serology was compared with the time to first positive VI in blood using a paired sample T-Test (SPSS, Chicago, USA). Also the period during which Leukopenia was present and during which PCR was positive, was compared with the period during which VI in blood was positive using a paired sample T-Test (SPSS, Chicago, USA).

Results

Both experimentally inoculated gilts were first detected positive for CSF on VI at 6 dpi. At the same time virus was also detected (PCR) for the first time in the nasal secretion and faeces of these gilts. The number of gilts with first positive VI, PCR and VN test at each time point is shown in Figure 1. In gilt 7 no viraemia was detected using VI, yet PCR and VN were positive.

Based on the results of VI in the experimentally inoculated gilts, the moment of infection of the contact infected gilts was estimated to be two observations (6 days) before the first positive VI. Since there was no positive VI in gilt 7 the moment of infection of gilt 7 was estimated based on the results of the PCR in serum. The first positive PCR in serum occurred on average 1.64 days before the first positive VI (Figure 2). Therefore the day of infection was estimated to be 4.36 days (6 – 1.64) before the first positive PCR. However, since there were only observations every three days, the estimated day of infection of gilt 7 was equal to one observation (3 days) before the first positive PCR.

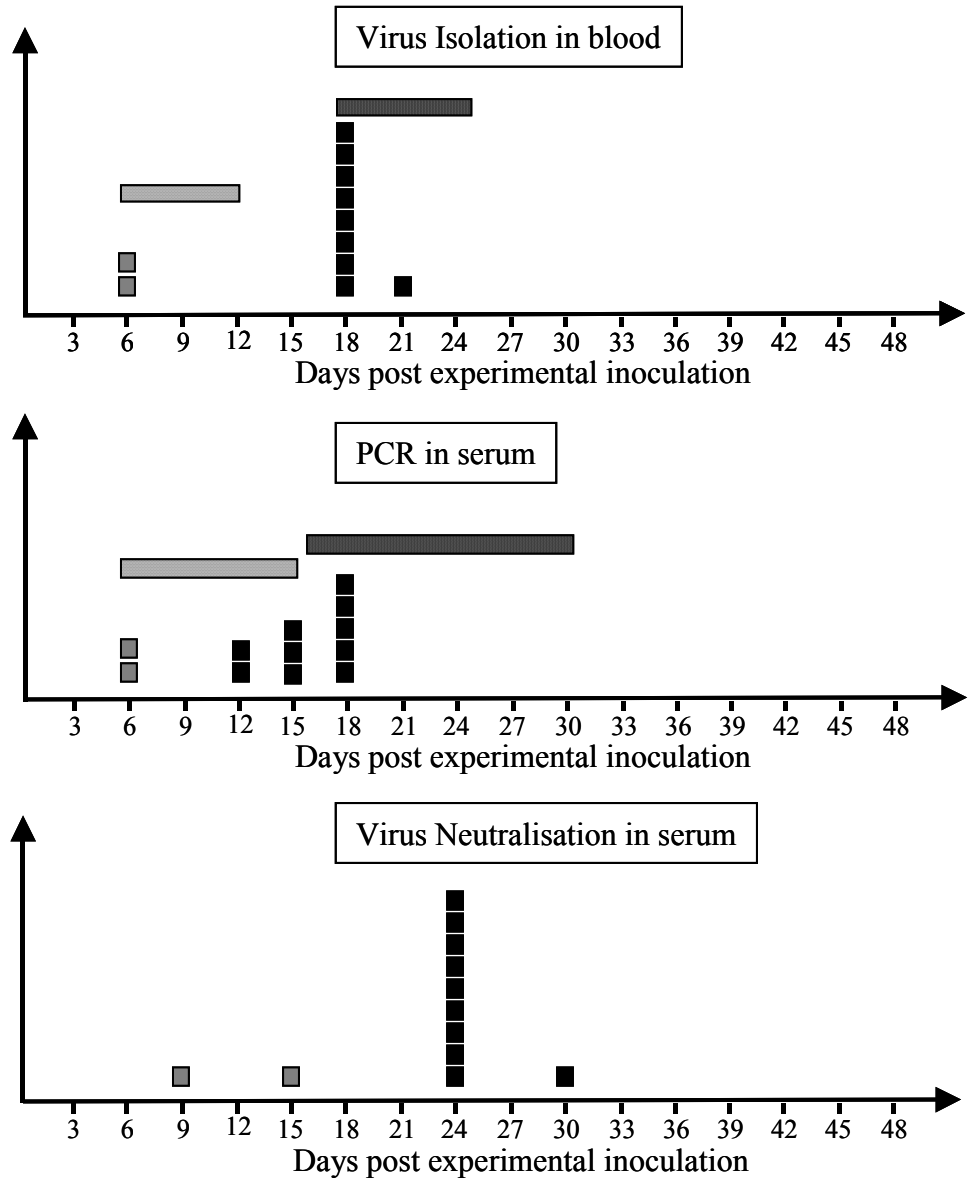
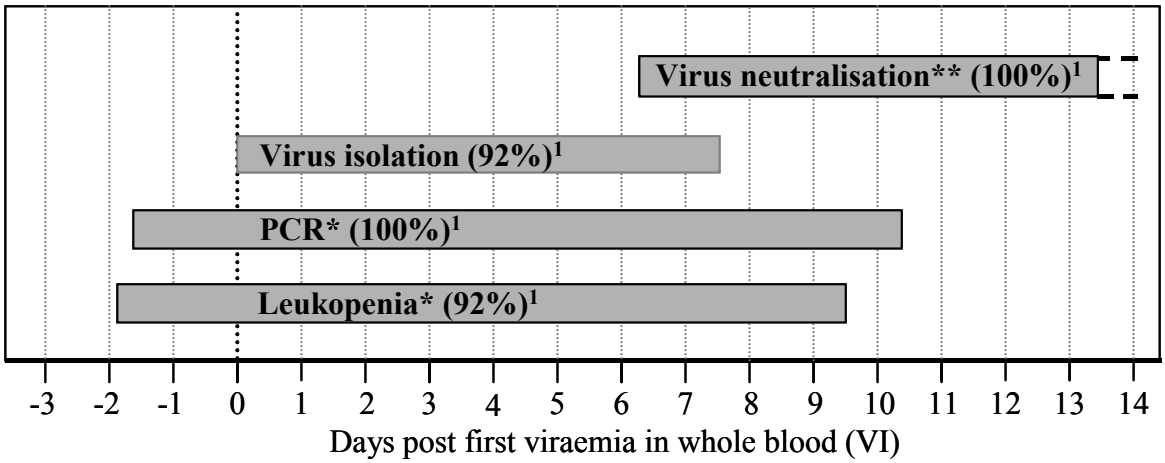


Figure 1: Virological and serological response after infection

The martingale estimate of R_0 was calculated to be 13.0 (H_0: $R_0 \leq 1$; $p<0.01$). Since no susceptible gilts remained at the end of the experiment, the maximum likelihood estimate of R_0 was $+\infty$. The lower boundary of the 95% CI of the R_{0mle} was 1.24.

In Figure 2 the diagnostic techniques are compared, with VI in whole blood as reference. Both leukopenia (1.8 days) and positive PCR in serum (1.6 days) occurred significantly ($p<0.05$)

earlier than positive VI. Antibodies (VN test) were detected on average 6.3 days after the first positive VI ($p<0.01$). The average period during which leukopenia was present (10.5 days) and PCR was positive (12 days) was also significantly longer ($p = 0.015$ and $p = 0.049$, respectively) in comparison with the period during which VI in whole blood (7 days) could be observed.



* significantly earlier and longer than virus isolation ($p < 0.05$)
** significantly later than virus isolation ($p < 0.01$)
1 percentage of gilts with positive test

Figure 2: Different diagnostic methods, using virus isolation (VI) as reference.

The clinical symptoms are summarised in Figures 3 and 4. Eight out of the twelve gilts showed fever (>39.0 °C). Fever appeared on average 5 days after infection, varying from 1 to10 days. The duration of fever varied between 2 and 31 days. The occurrence and the duration of the other clinical symptoms were also highly variable. For example gilt 6 remained without any clinical symptom during the whole observation period, although leukopenia and fever were observed, whereas gilts 8, 9, and 10 showed conjunctivitis and erythema without having fever. Gilts 2 and 3 died 15 and 20 days after infection, respectively (Figure 5). They both showed severe clinical illness before dying.

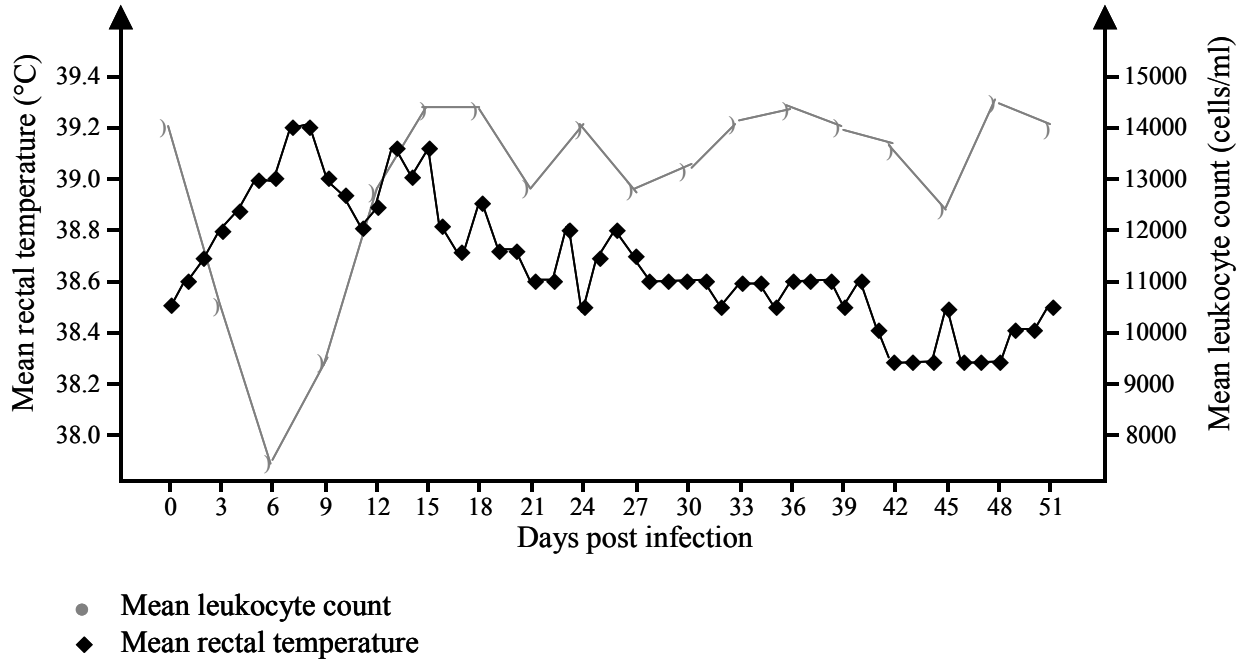
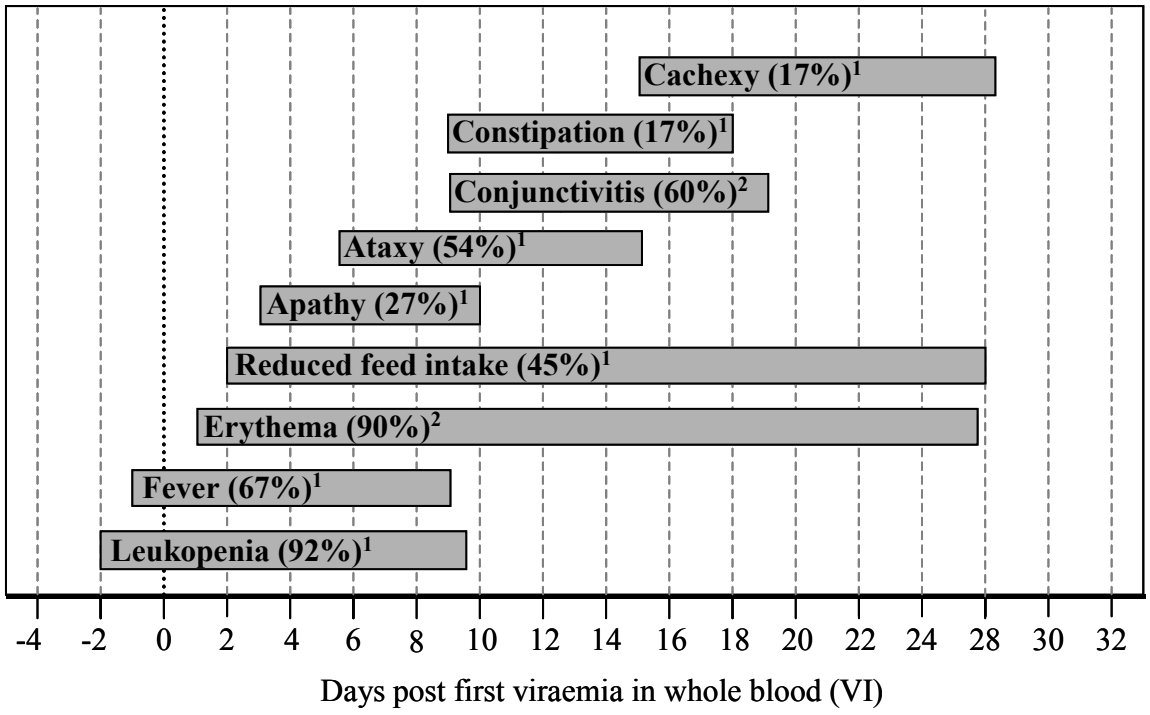


Figure 3: Evolution in temperature and leukocyte count.



[1] percentage of gilts with positive test, based on observations of 11 gilts, since no viraemia (VI) was detected in gilt 7

[2] percentage of gilts with positive test, based on observations of only 10 gilts, since gilt 12 showed already erythema and conjunctivitis before the day of experimental inoculation

Figure 4: Different clinical symptoms, using the beginning of viraemia as reference.

On the day of experimental inoculation the inoculated gilts were both on day 55 of gestation. The other gilts were between day 31 and 55 of gestation. All gilts got infected between day 43 and 67 of gestation (Figure 5). Four out of the 10 pregnant gilts aborted. The abortions occurred between 13 and 49 days after infection. In all pregnant gilts (aborted + euthanised) the offspring was at least partially infected (table 1). Also mummification of a part of the offspring occurred (table 1). None of the infected offspring had seroconverted against CSF.

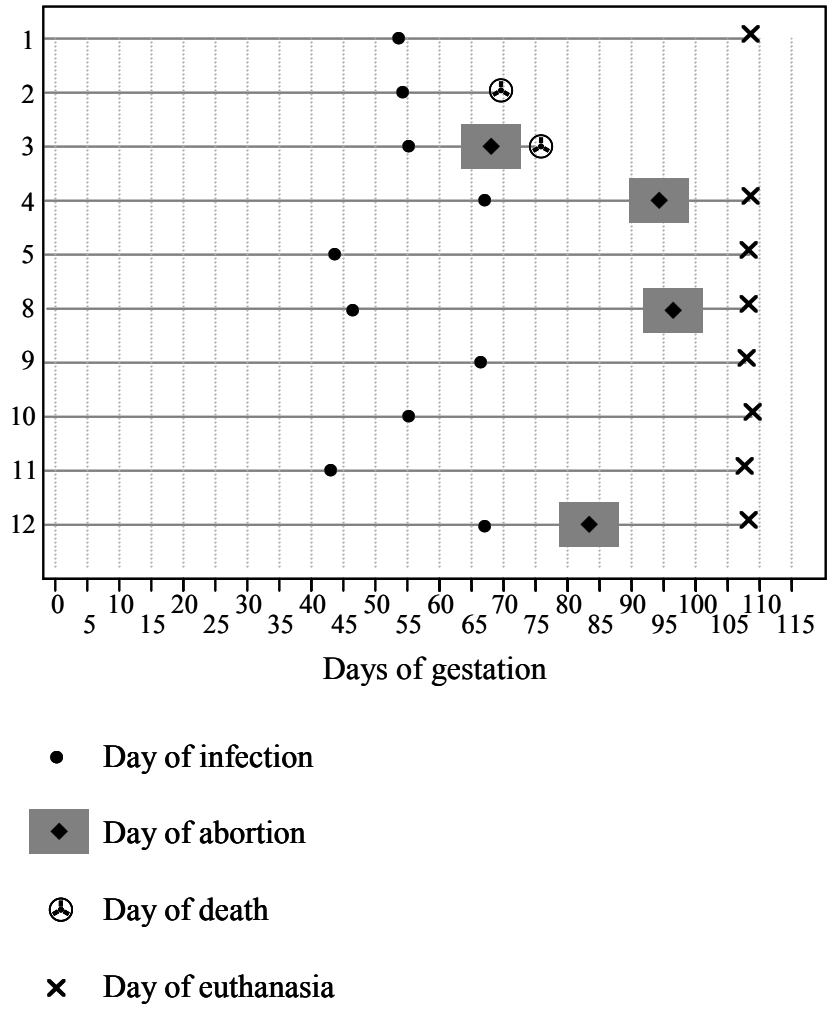


Figure 5: Moment of infection in relation to the day of gestation.

Table 1: Virological result of the offspring

Sow number	Piglets (n)	Mummified piglets (n)	Virus Isolation*		
			Spleen	Kidney	Tonsils
1	10	2	8	7	8
2	12	0	12	12	not done
3	10	0	9	8	not done
4	14	0	6	8	5
5	11	8	2	3	3
8	12	5	6	4	7
9	7	0	7	7	6
10	14	6	6	7	5
11	14	7	7	7	5
12	9	0	6	6	not done

n = Number

* Number of piglets with positive virus isolation results. No virus isolation was done in mummified piglets.

Discussion

During the 1997 epidemic in The Netherlands 322 out of 429 outbreaks were detected based on the presence of clinical signs (Elbers et al., 1999). This illustrates the importance of regular clinical examinations during an outbreak. However, detecting a present CSF infection by clinical examination seems to be more difficult in breeding herds than in fattening herds. In fact, during the 1993-1994 epidemic in Belgium it was found that the time between the first occurrence of clinical signs and the reporting of CSF suspicion was longer when the disease was introduced in sows, boars or suckling piglets as compared with fattening pigs (Koenen et al., 1996).

The extended time between the detection of the first clinical symptoms and the suspicion of a CSF infection in breeding herds compared to fattening herds may be the result of a combination of factors.
First, the clinical symptoms in sows, following a CSF infection, are atypical and discrete and do not incline immediately CSF suspicion, unless the fact that the farmers spent more time in a sow unit which makes the inspection of the sows more intense (Elbers et al., 1999). Secondly, in a sow-box housing system, virus spread may proceed much slower, since it is generally assumed that direct contact between infected and susceptible pigs is the principal way of virus transmission (Edwards, 2000).

The atypical and discrete clinical symptoms and the low mortality rate following a CSF infection in sows are probably the most important factors causing a delayed diagnosis. In this experiment the first clinical symptoms that could be observed were fever and leukopenia. Other clinical symptoms (apathy, ataxia, conjunctivitis, constipation, cachexia) occurred later on and in a variable number of gilts. The symptoms are comparable with observations in the field during outbreaks (Koenen et al., 1996; Elbers et al., 1999). In comparison with experimental infections with the same strain in weaner and slaughter pigs (Laevens en al., 1998; Laevens et al., 1999) clinical symptoms were less severe in gilts. This is in agreement with previous studies where it was found that the clinical course of the infection is influenced by the age of the infected animal (Depner et al., 1994; Koenen and Lefebvre, 1995; van

Oirschot, 1999). It should however be emphasised, that a large individual variability in the occurrence of the clinical symptoms was observed.

The “carrier-sow” syndrome remains important in the epidemiology of CSF, especially at the beginning and the end of an outbreak when the control measures are less strict. In this experiment, part of the sows aborted after infection and part of the sows produced litters with mummified and living piglets. These findings are in comparison to what has been described in literature (Plateau et al., 1980; Meyer et al., 1981; Terpstra, 1988; Dahle and Liess, 1992). It is difficult to asses weather the living piglets were all viable since the sows were euthanised some days before the end of the gestation period. The observation that in several gilts only part of the litter was infected has also been described in literature (van Oirschot, 1979; Meyer et al., 1981).

The second possible explanation for the delayed diagnosis of a CSF infection in sows is the slower virus transmission in sows, especially in sow-box housing systems. The dynamics of a CSF infection in sows may differ from an infection in weaner or slaughter pigs because of the difference in age and housing system. The relation between age and the severity of the clinical symptoms has been discussed previously. However, the effect of age on the virus transmission has not been fully explained yet.

In this experiment it was found that both experimental inoculated gilts became viraemic between 3 (last negative response on VI) and 6 (first positive response on VI) dpi. These results are consistent with previous experimental inoculations in weaner and slaughter pigs (Depner et al., 1994; Laevens et al., 1998; Laevens et al., 1999; Dewulf et al., 2000) and indicate that age has no major effect on the time between infection and viraemia.

The calculated R_{0mrt} (13.0), which is comparable to what has been found in previous experiments for slaughter pigs (13.7) (Laevens et al., 1999), and the observation that the two experimentally inoculated gilts infected all contact gilts, indicates that the virus spread in gilts proceeds relatively fast. These results also demonstrate clearly that CSF virus spread is indifferent to direct nose-to-nose contact. Therefore, airborne virus transmission may be more important in a sow-box housing system than previously accepted.

In view of the atypical and variable clinical symptoms, confirmation of a suspected infection should be done by diagnostic tests. It has been shown that leukocyte count and PCR are the two techniques that respond first, on average 2 days before the VI. Leukocyte count is a fast and easy technique that is sensitive however not at all specific. PCR on the other hand is sensitive and specific but it is labour intensive and expensive. To limit the workload, a first selection of the samples based on leukocyte count followed by a PCR on the samples with leukopenia may be preferred. An additional advantage of leukocyte count and PCR is that viraemia can be detected during a longer period compared to VI. The serology is of little use for an early detection, it is of great importance for screening purposes, due to the large number of samples that can be processed and due to the long detectable period.

In conclusion it can be stated that there is no major difference in the dynamics of a CSF infection between breeding and fattening pigs. Therefore, the late clinical detection of a present CSF infection is mainly due to the atypical and discrete clinical symptoms. As a preventive measure it may be recommended that in the presence of an unknown disease in sows, with atypical clinical symptoms as described, blood samples should be taken for CSF diagnosis. Leukocyte count with PCR as confirmation test is very suitable for an early diagnosis.

References

Anonymous (1980) Council directive 80/217/EEC. introducing Community measures for the control of classical swine fever. *Official Journal of the European Communities 80/217/EEC,* 11-23.

Bouma, A., de Jong, M.C., Kimman, T.G. (1996) Transmission of two pseudorabies virus strains that differ in virulence and virus excretion in groups of vaccinated pigs. *A. J. of Vet. Res. 57*. 43-47.

Dahle, J. and Liess, B. (1992) A review on classical swine fever infections in pigs: epizootiology, clinical disease and pathology. *Comp. of Imm. and Microb. of Infect. Diseases 15*, 203-211.

de Jong, M.C. and Kimman, T. G. (1994) Experimental quantification of vaccine-induced reduction in virus transmission. *Vaccine 12,* 761-766.

Depner, K., Gruber, A., and Liess, B. (1994) Experimental infection of weaner pigs with a field isolate of Hog Cholera/Classical Swine Fever Virus derived from a recent outbreak in Lower Saxony. 1: Clinical, virological and serological findings. *Wien. Tierärztl. Mschr. 81*, 370-373.

Dewulf, J., Laevens, H., Koenen, F., Vanderhallen, H., Mintiens, K., Deluyker, H., de Kruif, A., (2000) An experimental infection with classical swine fever in E2 sub-unit marker-vaccine vaccinated and in non-vaccinated pigs. *Vaccine 19*, 475-482

Edwards, S. (2000) Survival and inactivation of classical swine fever virus. *Vet. Microbiol. 73*, 175-181.

Elbers, A.R., Stegeman, A., Moser, H., Ekker, H. M., Smak, J.A., and Pluimers, F.H. (1999) The classical swine fever epidemic 1997-1998 in The Netherlands: descriptive epidemiology. *Prev. Vet. Med. 42*, 157-184.

Horst H.S., Dijkhuizen, A.A., Huirne, R.B. and de Leeuw, P.W. (1998). Introduction of contagious animal diseases into the Netherlands: elicitation of expert opinions. *Livest. Prod. Science 53*, 253-264.

Koenen, F., Lefebvre, J. (1994) Kinetics of an experimental infection with a classical swine fever (CSF) field isolate. In the Proceedings of the 3rd Congress of European Society of Veterinary Virology. 4-7 September 1994, Interlaken, pp. 322-326.

Koenen, F., Van Caenegem, G., Vermeersch, J.P., Vandenheede, J., and Deluyker, H. (1996) Epidemiological characteristics of an outbreak of classical swine fever in an area of high pig density. *Vet. Rec. 139*, 367-371.

Kroese, A. H., de Jong, M. C. (2002) Design and analysis of transmission experiments, In preparation

Laevens, H., Koenen, F., Deluyker, H., and Berkvens, D. (1998) An experimental infection with classical swine fever virus in weaner pigs. I. Transmission of the virus, course of the disease, and antibody response. *Vet. Q. 20*, 41-45.

Laevens, H., Koenen, F., Deluyker, H., and de Kruif, A. (1999) Experimental infection of slaughter pigs with classical swine fever virus: transmission of the virus, course of the disease and antibody response. *Vet. Rec. 145*, 243-248.

McGoldrick A., Bensaude E., Ibata G., Sharp G., Paton D.J., (1999). Closed one-tube reverse transcription nested polymerase chain reaction for the detection of pestiviral RNA with fluorescent probes. *J. Virol. Methods 79*, 85-95.

Meyer, H., Liess, B., Frey, H.R., Hermanns, W., Trautwein, G. (1981). Experimental transplacental transmission of Hog Cholera Virus in pigs. IV. Virological and serological studies in newborn piglets. *Zentralbl. Veterinarmed. [B] 28*, 659-668.

Plateau, E., Vannier, P., Tillon, J.P. (1980). Atypical Hog Cholera infection: Viral isolation and clinical study of in utero transmission. *A. J. of Vet. Res. 41*, 2012-2015.

Terpstra, C. (1988) Epizootiology of Hog-Cholera. In Classical swine fever and related viral infections. Editors: Liess, B. Martinus Nijhoff Publishing, Dordrecht, The Netherlands, pp. 201-216.

van Oirschot, J.T. (1999) Classical swine fever. In Diseases of Swine, vol. 8,. Editors: Straw, B. E., D'Allaire, S., Mengeling, W. L., and Taylor, D. J. Ames, Iowa, Iowa State University Press, pp.159-172.

Wensvoort, G., Bloemraad, M., Terpstra, C. (1988). An enzyme immunoassay employing monoclonal antibodies and detecting specifically antibodies to classical swine fever virus. *Vet. Microbiol. 17*, 129-140.

CHAPTER 3.6:

GENERAL DISCUSSION.

Introduction

Due to the non-vaccination policy, the domestic pig population in the European Union (EU) has become fully susceptible to the classical swine fever virus (CSFV). As a consequence of the highly contagious nature of the disease, and the geographically clustered structure of the pig industry, the introduction of the virus in a domestic pig population is always a serious threat and sometimes develops into a major epidemic (Miry et al., 1991; Vanthemsche, 1995; Koenen et al., 1996; Elbers et al., 1999).

In order to try to avoid these major epidemics, and to minimise the damage caused by a CSF outbreak, a series of control measures are implemented once a CSF infected herd is detected (Council Directive 2001/89/EC). In general, these measures can be divided into four categories: (i) stamping out and disinfection of the infected herd; (ii) implementation of protection (minimum 3 km) and surveillance (minimum 10 km) zones which include prohibited transport of all animals, strict sanitary measures for persons, vehicles and equipment, and clinical and serological surveillance of herds located in these zones; (iii) epidemiological forward and backward tracing to identify high risk contact herds; (iv) stamping out and disinfection of high risk contact herds identified by epidemiological tracing and geographical location.

The objective of these measures is to minimise the size of an outbreak by eliminating known and potential sources of infection, by blocking all possible routes of between-herd virus transmissions, and by clinical and serological surveillance for early detection of new infections. However, based upon current, incomplete understanding of the epidemiology of CSF, many of these preventive measures are very general in nature (e.g. eradication of all herds in the neighbourhood of an infected herd) and therefore often result in the pre-emptive culling of uninfected herds.

A thorough knowledge of the epidemiology of CSF is a prerequisite for optimising the measures which limit the economic, ethical, and animal welfare consequences of the control strategy. In general, the risk of between-herd virus transmission is influenced by: (i) the

amount of virus excreted by an infected herd and (ii) the possible routes of between-herd transmission. In this thesis, some epidemiological characteristics influencing the amount of virus excreted by an infected herd (chapter 3.5), as well as some epidemiological characteristics influencing the between-herd transmission of the virus (chapters 3.2, 3.3, 3.4), are studied.

Virus excretion by infected herds

The amount of virus excreted by an infected herd depends on the number of infectious animals present in the herd, and the duration of the infectious period of the herd. These parameters are influenced by: (i) the within-herd virus transmission, and (ii) the speed and accuracy of detection of an infected herd.

A. Within-herd virus transmission

The most appropriate parameter to quantify the within-herd virus transmission is the basic reproduction ratio (R_0). This R_0 gives the average number of secondary cases caused by one typically infectious animal in a fully susceptible population (de Jong and Diekmann, 1992). The R_0 for CSF has already been quantified in weaning piglets (81.3) (Laevens et al., 1998), and slaughter pigs (13.7) (Laevens et al., 1999a). Using a newly developed calculation method, these numbers were adjusted to 100 and 15.5, respectively (Klinkenberg et al., 2002a). Based on these transmission rates, it can be assumed that there is a certain age dependency for the transmission rate. This observation has recently been confirmed in other CSF transmission experiments (Klinkenberg et al., 2002b). In chapter 3.5, for the first time, the R_0 for a CSF infection in gilts was estimated. In contrast to what was expected, the R_0 (13.0) was relatively large and almost equal to the R_0 observed in slaughter pigs. Moreover, it was observed that the two experimentally inoculated gilts infected all contact gilts. These results indicate that CSF virus transmission is independent of direct nose-to-nose contact, and that the age dependent reduction in disease transmission does not seem to proceed after a certain age. Therefore, in the field, it may be expected that virus transmission in breeding farms proceeds as efficiently as in fattening farms and, by consequence, the number of infectious animals is expected to increase at the same pace. However, due to the relatively

small number of animals used to estimate the transmission rate, and the biological heterogeneity present in a population, ideally several repetitions of the transmission experiments are needed to confirm these results. Also the effect of compartmentalisation on the transmission rate should be evaluated.

B. Detection of an infected herd

During the 1997-1998 CSF epidemic in the Netherlands, 322 out of 429 outbreaks were detected through the presence of clinical signs (Elbers et al., 1999). This illustrates that clinical inspection is a very important diagnostic tool for the early detection of CSF. Despite the awareness of CSF-caused clinical symptoms during a CSF outbreak, the atypical and discrete symptoms, and the low mortality rate following a CSF infection in sows, result too often in a long interval between infection and detection in breeding herds. In our experiment, described in chapter 3.5, the first clinical symptoms that could be observed were fever and erythema. Other clinical symptoms such as apathy, ataxia, conjunctivitis, constipation, cachexia, and abortion occurred later on and in a variable number of gilts. These findings are comparable with field observations during outbreaks (Koenen et al., 1996; Elbers et al., 1999). As a consequence, the diagnosis of CSF in breeding pigs is often made only after several other possibilities have been ruled out, resulting in a longer average time interval between the first clinical signs in one animal and the reporting of suspected CSF (Koenen et al., 1996).

Given the difficulty of clinical diagnosis, confirmation of a suspected infection should be made by laboratory tests. In chapter 3.5 it was found that leukocyte count and RT-PCR are the two techniques that respond first, and in which the viraemia is detectable for the longest period. Moreover, it was found that the viraemic period largely corresponds to the period of fever (> 39.0°C). A diagnostic procedure which guarantees a high sensitivity, and simultaneously limits the laboratory workload, is to select and sample pigs with an increased body temperature, perform a leukocyte count (high sensitivity, low specificity) on all samples, and then perform a RT-PCR (high sensitivity, high specificity) on the samples with a decreased leukocyte count (< 12.000).

The combination of the efficient within-herd transmission and the difficulty of clinical diagnosis in breeding herds makes these herds highly epidemiologically important. Moreover, it is known that breeding herds are also dangerous because of the possibility of vertical virus transmission (Chapter 3.5) (Wensvoort and Terpstra, 1985; Westergaard, 1996; van Oirschot, 1999). Based on these findings, it is advisable that well-trained veterinarians carry out frequent epidemiological surveillance in breeding herds during CSF outbreaks. Recording of the rectal temperature of a representative number of sows, and blood sampling of all sows with fever (> 39.0°C) or other clinical symptoms, should be a compulsory part of this surveillance. In the laboratory, all blood samples ought to be analysed using leukocyte count, and, on the samples with leukopenia, a RT-PCR virus detection should be performed.

Between-herd transmission of the CSF virus

When between-herd contacts are considered, a differentiation must be made between direct (animal) contacts and indirect contacts. The indirect contacts may be subdivided into distance dependent (distance from source of infection to the contact herd is a limiting factor) and distance independent contacts (distance is not a limiting factor). The frequency of occurrence of these different contacts is influenced by the type and organisation of the pig industry (Nielen et al., 1996) and the control measures (e.g. transport standstill) implemented during the successive periods of an outbreak (Jalvingh et al., 1999).

A. Direct animal contact

The transmission via direct animal contact is undoubtedly the most efficient way of virus spread. The introduction of an infectious piglet or breeding pig into a susceptible herd will almost always lead to a major outbreak of CSF in that herd. The probability of a minor outbreak, given a direct contact, can be calculated based on the estimations of the transmission rate and is equal to the inverse of the transmission rate (R_0^{-1}) (de Jong, personal communication). For breeding pigs this is 1/13 = 7.7% (chaper 3.5) whereas for piglets this is only 1/81 = 1.2% (Laevens et al., 1998).

Between-herd transmission via direct animal contact is primarily important during the high-risk period 1 (HRP1). The HRP1 starts with the introduction of the virus in an area that was

previously CSF virus free, and ends with the notification of the first case. The HRP2 begins with the notification of the first case and ends when all control measures are considered effective. The post-HRP starts with the end of the HRP2 and ends when all control measures are lifted (Horst et al., 1998). During the HRP1, no restrictive measures are in place and potentially infected animals can be transported freely between herds. In the 1997 epidemic in The Netherlands, 17% of the 39 herds infected during the HRP1 were most likely infected via direct animal contact. During the HRP2 and the post-HRP (390 infected herds) the relative importance of direct animal contact dropped to 2% (Elbers et al., 1999). These numbers illustrate that control measures such as movement standstill are able to prevent horizontal virus transmission via direct animal contact rather efficiently.

Given the risk of between-herd virus transmission associated with direct animal contact, the pre-emptive culling of herds that have had direct animal contact with the primary infected herd during the infectious period is believed to be a necessary measure. Moreover, this measure has never been criticised because of the obvious risk associated with direct animal contacts.

B. Indirect contact

Although the standard control measures, such as eradication of the infected herds and movement standstill, are able to prevent between-herd virus transmission through direct animal contact rather efficiently, it has repeatedly been observed that these measures are insufficient to fully stop the spread of the virus (Koenen et al., 1996; Elbers et al., 1999; Stegeman et al., 1999). Yet, the prevention of indirect virus transmission is much more difficult because of the incomplete understanding of routes causing this indirect transmission.

When discussing the indirect virus transmission route, a distinction needs to be made between the distance independent and the distance dependent routes.

Distance independent indirect virus transmission

Although indirect virus transmission through mechanical vectors (vehicles, persons, equipment,…) is mentioned in all reviews on the epidemiology of CSF (Hughes and

Gustafson, 1960; Terpstra, 1988; Dahle and Liess, 1992; Westergaard, 1996; van Oirschot, 1999; chapter 3.1) and is often identified in epidemiological studies (Kramer et al., 1995; Terpstra and de Smit, 2000; Elbers et al., 2001; Stegeman et al., 2002), there is very limited experimental evidence to support the importance assigned to this type of transmission. Despite this lack of information, the detection of these indirect contacts often results in very drastic control measures. As an example, several herds in the province of West-Flanders were pre-emptively culled during the 1997 CSF outbreak in Belgium because of a contact with an empty pig transportation lorry which had had contact with an infected area in the province of Limburg (personal communication, Lamsens).

In the experiment described in chapter 3.4 an attempt was made to evaluate, under experimental conditions, the probability of virus transmission through excretions of infectious pigs. The experiment was designed in such a way that it simulated a field situation where infectious pigs were transported and the same vehicle was subsequently used to transport susceptible pigs. This scenario has repeatedly been described as a suspected route of indirect virus transmission (Elbers et al., 1999). The results of the experiment showed that the probability of virus transmission through excretions and secretions of infectious pigs is very small in the early stages of the infection.

The extrapolation of these rather unexpected results of the field situation remains a difficult task, since many factors, such as virus strain, infection dose, length of the interval between infection and depopulation, temperature of the neighbourhood, etc., may have influenced the outcome of the experiment. Moreover, the results of this experiment do not concur with the importance that is attributed to the transmission of CSF virus via contaminated mechanical vectors in several epidemiological studies (Elbers et al., 2001; Stegeman et al., 2002). It should be stressed that in our experiment only the probability of transmission was estimated, whereas the risk attributed to a specific transmission route is composed of the probability of transmission during one occurrence, the frequency of occurrence, and the consequences involved. A transmission route with a small probability but a high frequency of occurrence and severe consequences, may still be recognised as a high risk. Therefore, our experiment should be repeated to evaluate the effect of the potentially influencing factors, and to increase the strength of the conclusion. If further research confirms the initial findings, the pre-emptive culling of herds because of indirect mechanical contacts should be revised.

Finally, it should be stressed that the results of this experiment may in no way be interpreted to mean that hygienic procedures could be neglected during the control of a CSF outbreak.

Distance dependent indirect virus transmission

Based on epidemiological studies of CSF outbreaks in recent history, it was concluded by several authors that the risk of secondary infections decreased with increasing distance to the primary infected herd (Roberts, 1995; Koenen et al., 1996; Staubach et al., 1997; Laevens, 1999b; Mintiens et al., 2001; Stegeman et al., 2002). This phenomenon is most prominent within a short distance (1 km) of the primary infected herd. Therefore, all secondary infections that occur within a 1 km radius around a primary infected herd, and where none of the known virus transmission routes could be identified via epidemiological tracing, are considered "neighbourhood infections" (Westergaard, 1996). During the HRP1 of the 1997-98 epidemic in the Netherlands, 22% of the secondary cases were categorised as neighbourhood infections, whereas in the HRP II and the post-HRP this increased to 39% (Elbers et al., 1999).

Although the underlying mechanisms causing these neighbourhood infections are still not completely understood**,** field observations have been used as a pragmatic justification for the pre-emptive culling of all herds in the surroundings of an infected herd (1 km radius). The usefulness of this measure was illustrated during the 1997-98 epidemic in the Netherlands, where control of the outbreak only became possible after pre-emptive culling in the neighbourhood of infected herds (Stegeman et al., 1999).

Despite the fact that this measure is believed to be cost-efficient, since it reduces the size of an outbreak, (Nielen et al., 1999), it still has far-reaching financial consequences. Moreover, the massive killing and destruction of mostly non-infected animals is perceived by the public as wasteful and ethically unacceptable (Terpstra, 1998). This is worsened by the fact that there is no agreement on the mechanism of virus transmission causing these neighbourhood infections. It is difficult to defend the necessity of such a drastic control measure, either to the sector or to the public, if the underlying reasons are not fully understood. Therefore, trying to understand the transmission routes causing these neighbourhood infections is of huge importance. As described in chapter 3.1, there are only a few possible explanations: (i) illegal

or unreported contacts; (ii) an unknown transmission route; (iii) pets and rodents; and (iv) airborne contact.

(i) The occurrence of unreported and perhaps illegal contacts during past epidemics is probable. However, for obvious reasons, it is difficult to quantify the relative importance of this transmission route. In the future, these illegal contacts should be avoided through clear risk communication towards all people concerned, combined with intensive surveillance in infected regions.

(ii) The possibility of virus spread via a presently unknown transmission mode is unlikely. In a recent epidemiological study that was undertaken to identify factors associated with the introduction of the CSF virus into a herd, it was concluded that there was no evidence for any new important route of infection (Elbers et al., 2001).

(iii) Pets and rodents are sometimes suspected as possible virus transmitters between herds (Ellis et al., 1977; Miry et al., 1991; van Oirschot, 1999). This suspicion is based on several arguments: (a) pets and/or rodents are present in almost all farms; (b) they are very mobile and therefore can easily move from one herd to another; and (c) it is thought that at the moment of depopulation of an infected herd, the rodent population migrates to adjacent herds. In an experiment carried out to evaluate the probability of mechanical virus transmission by rats, it was found that they were not able to transmit the virus (Terpstra, 1988). The probability of pets or rats acting as intermediate hosts was evaluated in the experiment described in chapter 3.3. In this experiment neither CSF virus nor antibodies against CSF could be detected in any of the animals at the end of the observation period. Therefore, it may be stated that dogs, cats or rats are unlikely to represent a biological reservoir. This conclusion is in accordance with the findings of a recent epidemiological study that found no association between the presence of cats, rats or mice around the premises, and the risk of infection during a CSF outbreak (Elbers et al., 2001). Consequently, it seems unnecessary to kill pets and rodents on infected farms to control CSF.

(iv) Given the fact that neighbourhood transmission through unknown transmission routes and pets or rodents is unlikely, and the fact that the unreported or illegal contacts are most probably not responsible for the explanation of all neighbourhood contacts, the only

remaining route is that of airborne transmission. As described in chapter 3.1, airborne transmission was already demonstrated experimentally in 1960 by Hughes and Gustafson and later on confirmed by others (Terpstra, 1988; Laevens et al., 1999a). In the experiment described in chapter 3.2, the possibility of airborne transmission was demonstrated once again. Moreover, this experiment demonstrated that the virus was able to spread, following the prevalent air currents, towards another compartment. Based on these experimental data, it can be concluded that airborne transmission within and between pens and compartments is possible. Given the fact that large-scale experiments to evaluate the between-herd transmission via airborne spread are impossible, data gathered during outbreaks need to be used. In an epidemiological study based on the data of the 1993-1994 Belgian epidemic, it was found that the chance of a neighbouring herd becoming infected was dependent on the size of the neighbouring herd, the distance of the neighbouring herd to the primary herd and the number of observations of the neighbouring herd laying downwind from the primary infected herd (Laevens, 1999b; Mintiens et al., 2001). Analysis of the data of the Dutch epidemic of 1997-98 indicated that aerosols, produced during the cleaning of the material used to depopulate infected herds, were an important risk factor for the spread of the virus towards neighbouring herds within a radius of 250 meters (Elbers et al., 2001).

Based on the above mentioned experimental and epidemiological findings, it can be concluded that airborne transmission of CSF is possible within as well as between herds. However, in some studies, airborne transmission is found only in a radius of 250 m, whereas in others it is found in the 1 km zone. Consequently, the maximum distance that the virus can spread airborne remains unclear, and the radius of the neighbourhood which should be pre-emptively slaughtered is uncertain. Moreover, it is to be expected that airborne transmission is largely influenced by climatological and geographical parameters, but factors such as virus strain may also influence the transmission. Therefore, more experimental data should be gathered, and the development of software, which is able to predict airborne transmission when given all necessary geographical and climatological information, should be encouraged. A combination of software and the experimental data might lead to possible identification of those regions that are at risk from airborne infection. This knowledge would refine the control measures to a large extent, and could limit the pre-emptive culling to those herds lying in the high-risk zones.

Conclusions

In a CSF outbreak it will always remain a difficult task for decision makers to find a balance between control measures. Drastic control measures prevent further spread of the virus, but have the drawback that many herds are unnecessarily culled (control strategy with a high sensitivity and a low specificity). More specific control measures limit the number of pre-emptively culled herds, but involve a greater risk of missing an infected herd (control strategy with a somewhat lower sensitivity but a higher specificity). The scientific information presented in this thesis may help in designing better control measures with high sensitivity as well as high specificity.

Reference List

Anonymous (2001) Council Directive 2001/89/EC on community measures for the control of classical swine fever. *Official Journal of the European Communities* 2001/89/EC,

Dahle, J. and Liess, B. (1992) A review on classical swine fever infections in pigs: epizootiology, clinical disease and pathology. *Comp. Immunol. Microbiol. Infect. Dis. 15*, 203-211.

de Jong, M.C. and Diekmann, O. (1992) A method to calculate - for computer-silumated infections - the threshold value R_0 that predicts whether or not the infection will spread. *Prev. Vet. Med.12*, 269-285.

Elbers, A.R., Stegeman, A., Moser, H., Ekker, H. M., Smak, J. A., and Pluimers, F. H. (1999) The classical swine fever epidemic 1997-1998 in The Netherlands: descriptive epidemiology. *Prev. Vet. Med. 42*, 157-184.

Elbers, A., Stegeman, A., and de Jong, M. C. (2001) Factors associated with the introduction of Classical Swine Fever virus into pig herds in the central area of the 1997-1998 epidemic in The Netherlands. In the proceedings of the 9th conference of the Society for Veterinary Epidemiology and Preventive Medicine, Noordwijkerhout, The Netherlands, pp 6-14.

Ellis, P.R., James, A. D., and Shaw, A. P. (1977) Studies on the epidemiology and economics of swine fever eradication in the EEC. *EUR 5738e*.

Horst H.S., Dijkhuizen, A.A., Huirne, R.B. and de Leeuw, P.W. (1998). Introduction of contagious animal diseases into the Netherlands: elicitation of expert opinions. *Livest. Prod. Science 53*, 253-264.

Hughes, R.W. and Gustafson, D. P. (1960) Some factors that May Influence Hog Cholera transmission. *Am. J. Vet. Res. 21*, 464-471.

Jalvingh, A.W., Nielen, M., Maurice, H., Stegeman, A.J., Elbers, A.R., Dijkhuizen, A.A. (1999) Spatial and stochastic simulation to evaluate the impact of events and control

measures on the 1997-1998 classical swine fever epidemic in The Netherlands. I. Description of simulation model. *Prev. Vet. Med. 42*, 271-295.

Koenen, F., Van Caenegem, G., Vermeersch, J. P., Vandenheede, J., and Deluyker, H. (1996) Epidemiological characteristics of an outbreak of classical swine fever in an area of high pig density. *Vet. Rec. 139*, 367-371.

Kramer, M., Ahl, R., Teuffert, J., Kroscheski, W., Schlüter, H., and Otte, J. (1995) Classical swine fever in Germany, some epidemiological aspects. In the Proceedings of meeting of the society for veterinary epidemiology and preventive medicine. 29-31 March 1995, Reading, UK, pp. 110-118

Klinkenberg, D., De Bree, J., Laevens, H. and de Jong, M.C. (2002a) Within- and between-pen transmission of Classical Swine Fever Virus: a new method to estimate the basic reproduction ratio from transmission experiments. *Epidemiol. Infect. 128,* 293-299.

Klinkenberg, D., Moormann, R.J.M., de Smit, D., Bouma, A., de Jong, M.C. (2002b) Influence of maternal antibodies on efficacy of a subunit vaccine: transmission of classical swine fever between pigs vaccinated at 2 weeks of age. *Vaccine 20,* 3005-3013.

Laevens, H., Koenen, F., Deluyker, H., and Berkvens, D. (1998) An experimental infection with classical swine fever virus in weaner pigs. I. Transmission of the virus, course of the disease, and antibody response. *Vet.Q. 20*, 41-45.

Laevens, H. (1999) Risk factors for the transmission of classical swine fever virus to herds in the close neighbourhood of an infected herd. In: Epizootiology of classical swine fever: experimental infections simulating field conditions, and risk factors for virus transmission in the neighbourhood of an infected herd. PhD thesis, Ghent, Ghent University. pp. 103-121

Laevens, H., Koenen, F., Deluyker, H., and de Kruif, A. (1999) Experimental infection of slaughter pigs with classical swine fever virus: transmission of the virus, course of the disease and antibody response. *Vet. Rec. 145*, 243-248.

Mintiens, K., Laevens, H., Dewulf, J., Boelaert, F., Van Vlaenderen, I. and Koenen, F. (2001) Prediction of the likelihood of spread of classical swine fever virus through

"neighbourhood infection" for different regions in Belgium. In the proceedings of the conference of the Society for Veterinary Epidemiology and Preventive Medicine, 28-30 march 2001, Noordwijkerhout, pp 1-5.

Miry, C., Castryck, F., Koenen, F., Broers, A., and Segers, E. (1991) Quelques aspects de l'épizootie de peste porcine classique en Belgique en 1990. *Epidémiol. Santé Animal 20*, 23-32.

Nielen, M., Jalvingh, A.W. and Horst, H.S. (1996) Quantification of contacts between Dutch farms to assess the potential risk of foot-and-mouth disease spread. *Prev. Vet. Med. 28,* 143-158.

Nielen, M., Jalvingh, A.W., Meuwissen, M.P., Horst, S.H. and Dijkhuizen, A.A. (1999) Spatial and stochastic simulation to evaluate the impact of events and control measures on the 1997-1998 classical swine fever epidemic in The Netherlands. II. Comparison of control strategies. *Prev. Vet. Med. 42*, 297-317.

Roberts, M. (1995) Evaluation of optimal size of restriction zones in disease control with particular references to classical swine fever. In the Proceedings of meeting of the society for veterinary epidemiology and preventive medicine. 29-31 March 1995, Reading, UK, p. 119

Staubach, C., Teuffert, J., and Thulke, H. H. (1997) Risk Analysis and local spread mechanisms of classical swine fever. In the Proceedings of the 8th conference of the international society for veterinary epidemiology and economics. Juli 1997, Paris, France, pp. 06.12.1-06.12.3.

Stegeman, A., Elbers, A. R., Smak, J., and de Jong, M. C. (1999) Quantification of the transmission of classical swine fever virus between herds during the 1997-1998 epidemic in The Netherlands. *Prev.Vet.Med.* 42, 219-234.

Stegeman, J.A., Elbers, A.R., Boum, A. and de Jong. M.C. (2002) Rate of inter-herd transmission of classical swine fever virus by different types of contact during the 1997-8 epidemic in The Netherlands. *Epidemiol. Infect. 128,* 285-291.

Terpstra, C. (1988) Epizootiology of Hog-Cholera. In Classical swine fever and related viral infections. Editors: Liess, B. Martinus Nijhoff Publishing, Dordrecht, The Netherlands, pp. 201-216.

Terpstra, C. (1998) Preventive emptying: a compensation for a lack of training. *Tijdschr. Diergeneeskd.123,* 324-325.

Terpstra, C. and de Smit, A.J. (2000) The 1997/1998 epizootic of swine fever in the netherlands: control strategies under a non-vaccination regimen. *Vet. Microbiol. 77*, 3-15.

van Oirschot, J.T. (1999) Classical Swine Fever. In Diseases of Swine, vol. 8, Editors: Straw, B. E., D'Allaire, S., Mengeling, W. L., and Taylor, D. J. Ames, Iowa, Iowa State University Press, pp. 159-172.

Vanthemsche, P. (1995). Classical swine fever 1993-1994 in Belgium. In the proceedings of the 8th Annual meeting of the Dutch society for veterinary epidemiology and economics. 13 December 1995, Lelystad, p 25.

Wensvoort, G. and Terpstra, C. (1985) Varkenspest: een veranderend ziektebeeld. *Tijdschr. Diergeneeskd. 110*, 263-269.

Westergaard, J. M., (1996) Epidemiology of classical swine fever, In proceedings of Workshop on diagnostic procedures and measures to control classical swine fever in domestic pigs and the European wild boar. Poland, National Veterinary Research Institute, Pulaway, Poland, pp. 119-130

CHAPTER 4:

THE POTENTIAL BENEFITS OF VACCINATION IN THE CONTROL OF CLASSICAL SWINE FEVER.

CHAPTER 4.1:

THE USE OF VACCINATION IN THE CONTROL OF CLASSICAL SWINE FEVER, A HISTORICAL REVIEW AND RECENT DEVELOPMENTS.

J. Dewulf , H. Laevens , F. Koenen , K. Mintiens,

and A. de Kruif

Vlaams Diergeneeskundig Tijdschrift (2001), 70, 453-458

(updated and extended manuscript)

Introduction

Classical swine fever (CSF) was described for the first time in 1833 in Ohio, USA (Edwards et al., 2000). Later on in the 19th century, it became clear that a European pig disease known as "pig fever" was identical to the disease described in the USA (Terpstra and Robijns, 1977; Edwards, 1989). By the end of the 19th century, CSF was scattered all over the world. The CSF virus belongs to the genus *Pestivirus*, which is part of the Flaviviridae family (Murphy et al., 1995).

With the growing importance of pig farming in the global food supply, it soon became obvious that CSF was an important economic threat. Therefore, long before the aetiology and epidemiology of CSF were understood (Bendixen, 1998), measurements were taken to prevent the introduction and/or spread of CSF in pig populations. With the increasing knowledge of the aetiology and epidemiology of CSF, many countries succeeded in eradicating the virus by taking relative simple measures such as import bans of live animals and prohibition of swill feeding. Examples are Denmark (1933), Ireland (1956), Australia (1963), Canada (1964), Switzerland (1974) and the USA (1977) (Dahle en Liess, 1992; Laevens, 1999; van Oirschot, 1999). Other countries, inside as well as outside the European Union (EU), experienced many more problems in their attempts to eradicate CSF. Over the years, several techniques, including vaccination, were used with variable success (Terpstra, 1991).

In the last decade, huge investments were made in the development of marker vaccines against CSF. Marker vaccination has already proven its efficiency in different countries in the control of animal diseases such as Aujeszky's disease (van Oirschot et al., 1990) and infectious bovine rhinotracheitis (van Oirschot et al., 1996). Therefore, there were great expectations for marker vaccines to serve as an ethical and economically attractive alternative in the control of future CSF outbreaks.

In this chapter, a review is given on the use of conventional vaccines in the past control of CSF. The development of marker vaccines against CSF is discussed, with attention to the possibilities and limitations of these vaccines and the accompanying diagnostic tests.

Development and efficacy of conventional CSF vaccines

The first method to protect pigs from CSF was described by Niles in 1910, and consisted of simultaneously inoculating pigs with wild virus and antiserum. This technique was impractical and the results uncertain. Mcbryde and Cole (1936) produced the first real vaccine. It was composed of suspensions of tissues and blood derived from infectious pigs, inactivated with formalin or crystal violet. In the following years the production of these types of vaccines was further refined, and until the 70^{ies} they were commonly used (Aynaud, 1988). The inactivated vaccines were safe, although the protective properties were mediocre (Van Bekkum, 1966; Pilchard, 1967). Moreover, the vaccines were incapable of preventing transplacental infection of foetuses (Stewart et al., 1972). This vertical virus transmission path is of huge importance in the epidemiology of CSF, since symptomless persistent viraemic piglets can be produced this way. Such persistent infected piglets can maintain an infection over a long time, or they can re-ignite an outbreak after a period of apparent absence (van Oirschot, 1999).

Because of the mediocre protective properties of the inactivated vaccines, further research was done in order to improve the vaccines. From the 70^{ies} onwards, live vaccines were produced by serial passages in rabbits (C-strain) or in cell cultures (GPE strain, Thiverval strain). These vaccines provide excellent clinical protection as soon as 3 days after administration and last for at least one and a half years (Aynaud, 1988; van Oirschot, 1994; Pensaert and Van Reeth, 1997). Another important advantage of live vaccines is that they also prevent the vertical (transplacental) virus spread (Stewart et al. 1972). Yet, there is no information available on the effect of vaccination on the reduction of replication and on shedding of the virulent challenge virus. Therefore, it is not exactly known from which day onwards these vaccines reduce or prevent horizontal virus transmission (van Oirschot, 1997).

The use of conventional vaccines in the field

Prior to 1980, every country within the EU decided independently whether or not to use vaccination against CSF. The vaccination strategies varied from generalised systematic vaccination to localised emergency vaccinations, in combination with sanitary measures (Ellis et al., 1977; Terpstra and Robijns, 1977; Bendixen, 1988; Lamsens, 1992).

With the introduction of the rapidly active live vaccines, the strategy of emergency vaccination in regions suffering from an outbreak became more common. The infected region was delimited and, within this region, all pigs older than 2 weeks were vaccinated. This mass vaccination was conducted over a period of no more than 2 to 3 weeks and, for at least the following year, all piglets were revaccinated at the age of 6 to 7 weeks. Emergency vaccination was always combined with eradication of infected herds. Using this strategy, it was possible to eradicate CSF from an infected region within a period of one year (Terpstra and Robijns, 1977).

Change to a policy of non-vaccination

Due to a combination of the excellent protective properties of the live vaccines and the growing knowledge on the epidemiology of CSF, most of the member states of the EU managed to decrease the yearly number of outbreaks during the seventies (Ellis et al., 1977; Bironf and Leunen, 1988; Laevens 1999). This relatively favourable situation did not prevent large differences in CSF incidence between member states of the EU. Moreover, it was impossible to distinguish serologically between infected and vaccinated animals using the conventional vaccines. All this resulted in several trading restrictions.

In the establishment of an internal market comprising of free movement of goods, persons, services and capital, it was decided in 1980 that all EU member states should totally eradicate CSF virus from their territory (Council directive 80/217/EEC) and subsequently implement a policy of non-vaccination. This decision was largely trade driven, since trading partners

increasingly required "official CSF-free status". It was also calculated that, in the long-term, a non-vaccination strategy would be more profitable than the vaccination strategy as it was executed until then (Ellis et al., 1977). It was not until the first of January 1990 that a general and definitive vaccination prohibition was installed in all EU member states. Ever since, the control of CSF within the EU has been based on prevention of introduction and rapid eradication of the virus.

Criticism on the non-vaccination policy

Several huge outbreaks of CSF during the nineties have shown that present knowledge on the epidemiology and diagnosis of CSF is insufficient to quickly control a CSF outbreak, while still complying with the European non-vaccination and eradication policy, especially when the outbreak occurs in densely populated livestock areas (DPLA). (Lamsens, 1992; Vanthemsche, 1995; Meuwissen et al., 1999; Stegeman et al., 2000). Moreover, these recent epidemics have exemplified that, in absence of vaccination, CSF outbreaks in DPLAs can only be fought successfully by extensive pre-emptive culling in the neighbourhood of infected herds (Stegeman et al., 1999). Since it reduces the size of an outbreak, this method is believed to be cost-effective, despite the fact that it has far-reaching financial consequences (Nielen et al., 1999). Moreover, the massive killing and destruction of mostly non-infected animals is perceived as wasteful and ethically unacceptable (Terpstra, 1998).

Emergency vaccination with the conventional vaccine

Although a policy of non-vaccination has been definitively implemented in the EU since 1990, there is still a case for emergency vaccination when several requirements are met. Emergency vaccinations can only be executed in restricted areas, when an outbreak seems to run out of control, and with the permission of the European Commission (Anonymous, 1994; Dahle and Liess, 1995).

These emergency vaccinations consist of a single vaccination, with a live vaccine, of all pigs older than 2 weeks and within a period of 2 weeks. This is followed by revaccination of all piglets between the age of 6 and 7 weeks, for a period of one year (Anonymous, 1994). An

important drawback of these emergency vaccinations is that the country concerned loses its "official CSF-free" status and receives a total export ban on all pigs and derived products. This export ban is maintained until the "official CSF-free" status is regained, which can be, at the earliest, one and a half years after the beginning of vaccination (Anonymous, 1997). In case of an outbreak without vaccination the "official CSF-free" status is only temporally suspended (until 45 days after the last infected herd is diagnosed). Because of the severe economical consequences, no emergency vaccinations have been performed since 1990 (Terpstra, 1991; Anonymous, 1997; Moennig, 1998).

Development of marker vaccines

The huge economic and ethical consequences inherent in a policy of non-vaccination and eradication, combined with the economic impossibility of implementing emergency vaccinations with conventional vaccines, has led to the development of marker vaccines that may be used as an alternative for the current eradication and non-vaccination policy.

The essential goal of marker vaccination is to make the vaccinated animals develop a pattern of antibodies that protects against a natural infection, but is distinguishable from that of an animal which has recovered from a field virus infection. Pigs infected with a natural CSF virus develop antibodies against 2 envelope glycoproteins E^{rns} and E2, and one non-structural glycoproteine NS3 (Wensvoort et al., 1988; Paton et al., 1991).

First generation marker vaccines

During the development of a marker vaccine it became apparent that the E2 glycoproteine, in purified form, was capable of inducing a protective immunity (Rumenapf et al., 1991; Van Zijl et al., 1991; Hulst et al., 1993; König et al., 1995; Van Rijn et al., 1996; Peeters et al., 1997). This led to the development of a sub-unit vaccine that exists only of these E2 glycoproteins. The sub-unit vaccine is produced in cultures of insect cells infected with a baculo virus vector. This vector stimulates the insect cells to produce E2 glycoproteins (Hulst et al., 1993). Pigs vaccinated with such an E2 sub-unit marker vaccine develop only antibodies against the E2 glycoproteine, whereas pigs that are naturally infected develop

antibodies against 3 different glycoproteins. Consequently, it becomes possible to distinguish between an infected and a vaccinated pig by means of an ELISA test that only detects E^{rns} glycoproteins (Moormann et al., 2000). Currently there are two E2 sub-unit marker vaccines commercially available: *Porcilis Pesti®, Intervet* and *Bayovac CSF marker®, Bayer.* Also, two similar discriminatory diagnostic ELISA tests are available (*Chekit CSF-Marker®, Bommeli AG; Ceditest CSFV-E^{rns}®, ID-Lelystad*). Both tests are competitive ELISAs, detecting antibodies against the E^{rns} glycoprotein of CSF virus.

Second generation marker vaccines

Besides the development of sub-unit marker vaccines, efforts were made in the development of live marker vaccines. Two types of live vaccines are under investigation: recombinant vaccines, and DNA vaccines.

One possibility for the transmissible recombinant vaccines is to use the conventional vaccine strain (C-strain), and replace a part of the viral RNA with the homologous RNA of the bovine viral diarrhoea (BVD) virus (de Smit et al., 2000b; de Smit et al., 2001). Another, is to use a vector virus (porcine adeno virus), in which a part of the RNA of the CSF virus is inserted (Hammond et al., 2000; Hammond et al., 2001a, Hammond et al., 2001b). Recently, progress has been made in the development of non-transmissible recombinant marker vaccines (Widjojoatmodjo et al., 2000; van Gennip et al., 2002).
The DNA vaccines consist of purified DNA that codes for the production of E2 glycoproteins in vaccinated animals. These E2 glycoproteins subsequently induce the development of antibodies (Andrew et al., 2000; Hammond et al., 2001a; Markowska-Daniel et al., 2001, Yu et al., 2001).

Efficacy of the marker vaccines and diagnostic tests under experimental circumstances

For the E2 sub-unit marker vaccine, a sufficient number of experiments were carried out to be able to evaluate its protective properties. The recombinant vaccines, as well as the DNA vaccines, are still in the experimental stage of development.

During the development of the E2 sub-unit vaccines, several experiments were carried out in which it was demonstrated that SPF piglets were protected against the clinical course of the disease two weeks after double vaccination, or 6 weeks after single vaccination (Hulst et al., 1993; König et al., 1995; Van Rijn et al., 1996). More recently it was shown that 32µg E2 in a water-in-oil adjuvans provokes a protective immunity only 3 weeks 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 to reduce horizontal and vertical transmission is more relevant than the clinical protection (van Oirschot, 1997).

Horizontal virus transmission

The horizontal virus transmission can be quantified using the reproduction ratio (R_0). This is a measure of transmission of infection, and is defined as the mean number of new infections arising from one typical infectious case (de Jong and Kimman, 1994). If the R_0 becomes smaller than 1, one infectious animal infects less than one new animal. In this situation the epidemic will run dead.

The effect of vaccination on the horizontal virus spread can be evaluated in several different ways. One possibility is to vaccinate an entire group of pigs and subsequently infect a number of animals from the group. In this way, the combined effect of decreased virus shedding and increased protection against infection in vaccinated animals is examined. Such an experiment with CSF infections in vaccinated SPF piglets found that horizontal virus transmission within the vaccinated group was already prevented 10 days after a single vaccination (Bouma et al., 2000). In comparable experiments with conventional piglets, executed in several reference laboratories, it was shown that even 21 days after vaccination a limited virus transmission was still possible (Uttenthal et al., 2001). This difference in vaccine efficacy in SPF and conventional pigs has also been described in transmission experiments with Aujeszky's disease (Van Nes et al., 2001).

If the aim of the experiment is to evaluate the effect of vaccination on virus shedding, it is sufficient to infect previously vaccinated pigs and put them in contact with susceptible pigs. A similar experiment found that SPF piglets, infected 3 weeks post vaccination and brought into contact with susceptible piglets, did not shed the virus in 7 out of 8 groups. In one group the vaccinated piglets became infectious and the contact pigs were infected (Bouma et al., 1999). Experiments to evaluate the protective properties of the vaccine, so that it prevents infection in vaccinated animals brought in contact with non-vaccinated infected animals, are described in chapter 4.2 and 4.3.

Vertical virus transmission

The vertical virus transmission was also examined in several experiments. De Smit et al. (2000a) found that double vaccination of pregnant sows prevented the transplacental infections of their piglets. They also reported that single vaccination prevented transplacental infection in 8 out of 9 sows. Ahrens et al. (2000) found that double vaccination was capable of preventing vertical virus spread in 9 out of 10 sows. On the other hand, experiments conducted by different reference laboratories found that in pregnant sows, infected 2 weeks post vaccination, the transplacental infection of the offspring occurred in 100% of the cases. In sows infected after double vaccination, the transplacental infection occurred in 5 out of the 12 sows (Depner et al., 2001).

Discriminatory diagnostic tests

The detection of a CSF infection after vaccination with a marker vaccine depends entirely on the discriminatory ELISAs. No confirmatory test, able to differentiate between BVD/BD and CSF antibodies once the animals have been vaccinated with a marker vaccine, is available for a “CSF positive” ELISA result.

The only source of information on the reliability of the discriminatory tests is the result of a number of experiments conducted by reference laboratories (Floegel-Niesmann, 2001). The sensitivity of both discriminatory ELISAs (Chekit CSF-Marker®: 94.1%; Ceditest CSFV-E^{rns}®: 73.5%) is lower than the sensitivity of the conventional CSF antibody ELISAs. If the

discriminatory ELISAs were to be used on a herd basis, a CSF infected herd might still be detected if the number of random samples is increased accordingly. Yet, the specificity of the test is the limiting factor in the case of large sample sizes. The specificity of both tests was evaluated on reference sera and field sera respectively (Chekit CSF-Marker®: 70.6% and 98%; Ceditest CSFV-E^{rns}®: 91.8% and 100%). Based on the results of these experiments, it was concluded by the author that the limitations of the discriminatory ELISAs were the major factor that would prevent the use of the marker vaccine under field conditions (Floegel-Niesmann, 2001).

Conclusions

Given the growing criticism on the non-vaccination and eradication strategy, the current control policy of CSF needs to be revised. Therefore reintroduction of emergency vaccination may be a valid alternative. However there are still many unanswered questions concerning the applicability and efficacy of both marker vaccines and conventional vaccines. These questions hamper the development of feasible and reliable vaccination strategies that are able to control CSF in more efficient epidemiological, ethical and economical ways than the current non-vaccination strategy.

Reference List

Ahrens, U., Kaden, V., Drexler, C., and Visser, N. (2000) Efficacy of the classical swine fever (CSF) markervaccine Porcilis® pesti in pregnant sows. *Vet. Microbiol. 77*, 83-97.

Andrew, M.E., Morrissy, C. J., Lenghaus, C., Oke, P. G., Sproat, K. W., Hodgson, A. L., Johnson, M. A., and Coupar, B. E. (2000) Protection of pigs against classical swine fever with DNA-delivered gp55. *Vaccine 18,* 1932-1938.

Anonymous (1980) Council directive 80/217/EEC. introducing Community measures for the control of classical swine fever. *Official Journal of the European Communities* 80/217/EEC, 11-23.

Anonymous (1994) Report from the scientific veterinary committee on guidelines for a Classical Swine Fever emergency vaccination programme. VI/7389/94-EN.

Anonymous (1997) The use of marker vaccines in the control of infectious diseases in particular classical swine fever, Report of the Scientific Veterinary Committee. Scientific Veterinary Committee of the European Commission

Aynaud, J.M. (1988) Principles of vaccination. In Classical swine fever and related viral infections. Editors: Liess, B. Martinus Nijhoff Publishing, Dordrecht, The Netherlands, pp. 165-180.

Bendixen, H.J. (1988) Control of Classical Swine Fever. In Classical swine fever and related viral infections. Editors: Liess, B. Martinus Nijhoff Publishing, Dordrecht, The Netherlands, pp. 217-232.

Bironf, P. and Leunen, J. (1988) Vaccines. In Classical swine fever and related viral infections. Editors: Liess, B. Martinus Nijhoff Publishing, Dordrecht, The Netherlands, pp. 181-200.

Bouma, A., de Smit, A. J., de Kluijver, E. P., Terpstra, C., and Moormann, R. J. (1999) Efficacy and stability of a subunit vaccine based on glycoprotein E2 of classical swine fever virus. *Vet. Microbiol. 66,* 101-114.

Bouma, A., de Smit, A. J., de Jong, M. C., de Kluijver, E. P., and Moormann, R. J. (2000) Determination of the onset of the herd-immunity induced by the E2 sub-unit vaccine against classical swine fever virus. *Vaccine 18*, 1374-1381.

Dahle, J. and Liess, B. (1992) A review on classical swine fever infections in pigs: epizootiology, clinical disease and pathology. *Comp. of Imm. and Microb. of Infect. Diseases 15*, 203-211.

Dahle, J. and Liess, B. (1995) Assessment of safety and protective value of a cell culture modified strain "C" vaccine of hog cholera/classical swine fever virus. *Berl. und Münchr Tierärz. Wochenschrif. 108,* 20-25.

de Jong, M.C. and Kimman, T. G. (1994) Experimental quantification of vaccine-induced reduction in virus transmission. *Vaccine 12,* 761-766.

de Smit, A.J., Bouma, A., de Kluijver, E. P., Terpstra, C., and Moormann, R. J. (2000a) Prevention of transplacental transmission of moderate-virulent classical swine fever virus after single or double vaccination with an E2 subunit vaccine. *Vet. Q. 22,* 150-153.

de Smit, A.J. (2000b) Laboratory diagnosis, epizootiology, and efficacy of marker vaccines in classical swine fever: a review. *Vet.Q. 22*, 182-188.

de Smit, A.J., Bouma, A., van Gennip, H. G., de Kluijver, E. P., and Moormann, R. J. (2001) Chimeric (marker) C-strain viruses induce clinical protection against virulent classical swine fever virus (CSFV) and reduce transmission of CSFV between vaccinated pigs. *Vaccine 19,* 1467-1476.

Depner, K.R., Bouma, A., Koenen, F., Klinkenberg, D., Lange, E., de Smit, H., and Vanderhallen, H. (2001) Classical swine fever (CSF) marker vaccine. Trial II. Challenge study in pregnant sows. *Vet.Microbiol. 83,* 107-120.

Edwards, S. (1989) Epidemiology and control of classical swine fever. In: Proceedings of the Society for Veterinary Epidemiology and Preventive Medicine, 12-14 April 1989, Exeter, p. 74

Edwards, S., Fukusho, A., Lefevre, P., Lipowski, A., Pejsak, Z., Roehe, P., and Westergaard, J. (2000) Classical swine fever: the global situation. *Vet. Microbiol 73,* 103-119.

Ellis, P.R., James, A. D., and Shaw, A. P. (1977) Studies on the epidemiology and economics of swine fever eradication in the EEC. *EUR 5738e*,

Floegel-Niesmann, G. (2001) Classical swine fever (CSF) marker vaccine. Trial III. Evaluation of discriminatory ELISAs. *Vet.Microbiol. 83,* 121-136.

Hammond, J.M., McCoy, R. J., Jansen, E. S., Morrissy, C. J., Hodgson, A. L., and Johnson, M. A. (2000) Vaccination with a single dose of a recombinant porcine adenovirus expressing the classical swine fever virus gp55 (E2) gene protects pigs against classical swine fever. *Vaccine 18,* 1040-1050.

Hammond, J.M., Jansen, E. S., Morrissy, C. J., Goff, W. V., Meehan, G. C., Williamson, M. M., Lenghaus, C., Sproat, K. W., Andrew, M. E., Coupar, B. E., and Johnson, M. A. (2001a) A prime-boost vaccination strategy using naked DNA followed by recombinant porcine adenovirus protects pigs from classical swine fever. *Vet. Microbiol. 80,* 101-119.

Hammond, J.M., Jansen, E. S., Morrissy, C. J., Williamson, M. M., Hodgson, A. L., and Johnson, M. A. (2001b) Oral and sub-cutaneous vaccination of commercial pigs with a recombinant porcine adenovirus expressing the classical swine fever virus gp55 gene. *Arch.Virol. 146,* 1787-1793.

Hulst, M.M., Westra, D. F., Wensvoort, G., and Moormann, R. J. (1993) Glycoprotein E1 of hog cholera virus expressed in insect cells protects swine from hog cholera. *J. of Virol. 67,* 5435-5442.

König, M., Lengsfeld, T., Pauly, T., Stark, R., and Thiel, H. J. (1995) Classical swine fever virus: independent induction of protective immunity by two structural glycoproteins. *J. of Virol. 69*, 6479-6486

Laevens, H. (1999) Risk factors for the transmission of classical swine fever virus to herds in the close neighbourhood of an infected herd. In: Epizootiology of classical swine fever: Experimental Infections Simulating Field Conditions, and Risk Factors for

Virus Transmission in the Neighbourhood of an Infected Herd. PhD thesis, Ghent, Ghent University. pp. 103 – 122.

Lamsens, G. (1992) Klassieke varkenspest Epizoötie 1990. Ministerie van Landbouw, Diergeneeskundige Inspectie, Brussels, Belgium.

Markowska-Daniel, I., Collins, R. A., and Pejsak, Z. (2001) Evaluation of genetic vaccine against classical swine fever. *Vaccine 19*, 2480-2484.

Mcbryde, C. and Cole, C. G. (1936) Crystal-violet vaccine for the prevention of Hog Cholera. *J. A. Vet. Med. Assoc. 89*, 652-663.

Meuwissen, M.P., Horst, S. H., Huirne, R. B., and Dijkhuizen, A. A. (1999) A model to estimate the financial consequences of classical swine fever outbreaks: principles and outcomes. *Prev. Vet. Med. 42,* 249-270.

Moennig, V. (1998) Concluding remarks on the OIE symposium on classical swine fever. OIE symposium on classical swine fever, Birmingham, 9-10 July 1998.

Moormann, R.J., Bouma, A., Kramps, J. A., Terpstra, C., and De Smit, H. J. (2000) Development of a classical swine fever subunit marker vaccine and companion diagnostic test. *Vet. Microbiol. 73,* 209-219.

Murphy, F.A., Fauquet, C. M., Bischop, D. H. L., Ghabrial, S. S., Jarvis, A. W., Martelli, G. P., Mayo, M. A., and Summers, M. D. (1995) Virus Taxonomy, Sixth Report of the International Committee on Taxonomy of Viruses. *Arch.of Virol. Suppl. 10,* 415-427.

Nielen, M., Jalvingh, A.W., Meuwissen, M.P., Horst, S.H. and Dijkhuizen, A.A. (1999) Spatial and stochastic simulation to evaluate the impact of events and control measures on the 1997-1998 classical swine fever epidemic in The Netherlands. II. Comparison of control strategies. *Prev. Vet. Med. 42*, 297-317.

Paton, D.J., Ibata, G., Edwards, S., and Wensvoort, G. (1991) An ELISA detecting antibody to conserved pestivirus epitopes. *J. of Virol. Methods 31*, 315-324.

Peeters, B., Hulst, M., Gielkens, A., and Kimman, T. (1997) Biologically safe, non-transmissible pseudorabies virus vector vaccine protects pigs against both Aujeszky's disease and classical swine fever. *J. of General Virol. 78,* 3311-3315.

Pensaert, M. and Van Reeth, K., Vaccines for swine. In: Veterinary Vaccinology, editors: Pastoret, P., Blancou, J., Vannier, P., Verschueren, C., Amsterdam, Elsevier; 1997. pp. 374-375.

Pilchard, I. (1967) Experimental inactivated virus hog cholera vaccines: induction period of immunity. *American J. of Vet. Res. 28,* 915-923.

Rumenapf, T., Stark, R., Meyers, G., and Thiel, H. J. (1991) Structural proteins of hog cholera virus expressed by vaccinia virus: further characterization and induction of protective immunity. *J. of Virol. 65,* 589-597.

Stegeman, A., Elbers, A. R., Smak, J., and de Jong, M. C. (1999) Quantification of the transmission of classical swine fever virus between herds during the 1997-1998 epidemic in The Netherlands. *Prev.Vet.Med.* 42, 219-234.

Stegeman, A., Elbers, A., de Smit, H., Moser, H., Smak, J., and Pluimers, F. (2000) The 1997-1998 epidemic of classical swine fever in the Netherlands. *Vet. Microbiol. 73,* 183-196.

Stewart, W.C., Carbrey, E. A., and Kresse, J. I. (1972) Transplacental hog cholera infection in immune sows. *A. J. of Vet. Res. 33*, 791-798.

Terpstra, C. and Robijns, K. G. (1977) Experience with regional vaccination against swine fever in enzootic areas for limited periods using C-strain virus. *Tijds. Diergeneesk. 102,* 106-112.

Terpstra, C. (1991) Hog cholera: an update of present knowledge. *Br.Vet. J.* 147, 397-406.

Terpstra, C. (1998) Preventive emptying: a compensation for a lack of training. *Tijdschr. Diergeneeskd.123,* 324-325.

Uttenthal, A., Le Potier, M., Romero, L., De Mia, G. M., and Floegel-Niesmann, G. (2001) Classical swine fever (CSF) marker vaccine. Trial I. Challenge studies in weaner pigs. *Vet.Microbiol. 83,* 85-106.

Van Bekkum, J.G. (1966) Serological aspects of the vaccination against Hog Cholera with crystal violet vaccine. *Tijdschr. Diergeneeskd. 91,* 149-158.

van Gennip, H.G., Bouma, A., van Rijn, P. A., Widjojoatmodjo, M. N., and Moormann, R. J. (2002) Experimental non-transmissible marker vaccines for classical swine fever (CSF) by trans-complementation of E(rns) or E2 of CSFV. *Vaccine 20,* 1544-1556.

van Oirschot, J.T., Gielkens, A. L., Moormann, R. J., and Berns, A. J. (1990) Markervaccines, virus protein-specific antibody assays and the control of Aujeszky's disease. *Vet. Microbiol. 23,* 85-101.

Van Nes, A., De Jong, M.C., Schoevers, E.J., Van Oirschot, J.T., Verheijden, J.H. (2001) Pseudorabies virus is transmitted among vaccinated conventional pigs, but not among vaccinated SPF pigs. *Vet. Microbiol. 80*, 303-312

van Oirschot, J.T. (1994) Vaccination in food animal populations. *Vaccine 12,* 415-418.

van Oirschot, J.T., Kaashoek, M. J., Rijsewijk, F. A., and Stegeman, J. A. (1996) The use of markervaccines in eradication of herpesviruses. *J. of Biotech.. 44*, 75-81.

van Oirschot, J.T. (1997) Rapid diagnosis and rapid vaccines in animal disease outbreaks. In the proceedings of the 10th annual meeting of the Dutch society of veterinary epidemiology and economy, Boxtel, The Netherlands, pp. 65-74.

van Oirschot, J.T. (1999) Classical swine fever. In Diseases of Swine, vol. 8,. Editors: Straw, B. E., D'Allaire, S., Mengeling, W. L., and Taylor, D. J. Ames, Iowa, Iowa State University Press, pp.159-172.

van Rijn, P.A., Bossers, A., Wensvoort, G., and Moormann, R. J. (1996) Classical swine fever virus (CSFV) envelope glycoprotein E2 containing one structural antigenic unit protects pigs from lethal CSFV challenge. *J. of General Virol. 77,* 2737-2745.

van Zijl, M., Wensvoort, G., de Kluyver, E., Hulst, M., van der, Gulden H., Gielkens, A., Berns, A., and Moormann, R. (1991) Live attenuated pseudorabies virus expressing envelope glycoprotein E1 of hog cholera virus protects swine against both pseudorabies and hog cholera. *J. of Virol. 65,* 2761-2765.

Vanthemsche, P. (1995) Classical swine fever 1993-1994 in Belgium. In: Proceedings of the 8th Annual Meeting of the Dutch Society for Veterinary Epidemiology and Economics 13 December 1995, Lelystad, p. 25

Wensvoort, G., Bloemraad, M., and Terpstra, C. (1988) An enzyme immunoassay employing monoclonal antibodies and detecting specifically antibodies to classical swine fever virus. *Vet. Microbiol. 17,* 129-140.

Widjojoatmodjo, M.N., van Gennip, H. G., Bouma, A., van Rijn, P. A., and Moormann, R. J. (2000) Classical swine fever virus E(rns) deletion mutants: trans- complementation and potential use as non-transmissible, modified, live- attenuated markervaccines. *J. of Virol. 74,* 2973-2980.

Yu, X., Tu, C., Li, H., Hu, R., Chen, C., Li, Z., Zhang, M., and Yin, Z. (2001) DNA-mediated protection against classical swine fever virus. *Vaccine 19*, 1520-1525.

CHAPTER 4.2:

AN EXPERIMENTAL INFECTION WITH CLASSICAL SWINE FEVER IN E2 SUB-UNIT MARKER VACCINE VACCINATED AND IN NON-VACCINATED PIGS.

J. Dewulf , H. Laevens , F. Koenen , H. Vanderhallen, K. Mintiens, H. Deluyker, and A. de Kruif

Vaccine (2001), 19, 475-482

Abstract

The clinical and virological protection induced by an E2 sub-unit marker vaccine against classical swine fever (CSF) was examined during an experimental infection in vaccinated and non-vaccinated pigs. Forty-five pigs were equally distributed over 3 adjacent pens of an isolation unit, there was only indirect (airborne) contact between pigs in the different pens. In pen 3 all pigs were vaccinated twice with a four weeks interval. Pigs in pens 1 and 2 were not vaccinated. Two weeks after booster vaccination, one randomly selected pig in the middle pen was experimentally inoculated with CSF virus. After the initial virus spread in the infected pen, all pigs in the non-vaccinated adjacent pen were infected. In the vaccinated pen, seven out of fourteen pigs became infected during the experiment. Survival analysis showed that virus transmission by direct and indirect contact was significantly ($p<0.001$) delayed in vaccinated pigs as compared to non-vaccinated pigs. In the non-vaccinated pens over 40% of the pigs died and typical clinical signs were noticed. In the vaccinated pen no mortality and no clinical symptoms were observed.

Although double vaccination with an E2 sub-unit marker vaccine was able to prevent the clinical course of the disease it was unable to prevent infection through indirect contact. This finding combined with the slow serological response after vaccination will complicate the possible use of the vaccine in emergency vaccination programs

Introduction

Since 1990, the control of classical swine fever (CSF) in the European Union is based on a policy of non-vaccination and stamping-out. This policy has been imposed partially because of lack of a vaccine that is both efficacious and enables differentiation between vaccinated and infected pigs (Pensaert and Van Reeth, 1997). Recent experience shows that, without application of emergency vaccination, the control of outbreaks in non-vaccinated population through eradication may be very expensive, in particular for areas with high pig density (Miry et al., 1991; Vanthemsche, 1995; Elbers et al., 1999)

In recent years, progress has been made in the development of marker vaccines and accompanying discriminatory diagnostic tests. It has been shown that the E2 sub-unit marker vaccines were able to protect pigs against the clinical course of a CSF infection (König et al., 1995; Van Rijn et al., 1996). It was suggested that these new marker vaccines would be potentially suitable in emergency vaccination programs during an outbreak of CSF (Bouma et al., 1999; Bouma et al., 2000).

A report of the Scientific Veterinary Committee of the European Community on the use of marker vaccines in the control of CSF states that emergency marker vaccines should only be applied in well-defined regions of restricted size with a high pig density (Anonymous, 1997). In order to assess the applicability of an E2 sub-unit marker vaccine in emergency vaccination strategies, information about the vaccine induced clinical and virological protection is a prerequisite.

This paper describes the effects of a double vaccination with an E2 sub-unit marker vaccine against CSF on the clinical course of the disease and on the virus transmission. Therefore vaccinated and non-vaccinated pigs were compared during an experimental CSF infection. In order to be able to test the effects of the vaccine under ideal circumstances, pigs were vaccinated twice, according to the producer's recommendations.

Materials and methods

Virus

The isolate used for the experimental infection was originally obtained from the first CSF-infected herd of the 1993-1994 Belgian epizootic. The isolate was verified to be free of African swine fever virus and bovine viral diarrhoea virus (BVDV). By using monoclonal antibodies, it was characterised to be similar to an isolate known as the 'souche Lorraine' (Koenen and Lefebvre, 1995). Virus infectiousness was 10^3 median cell culture infective dose ($CCID_{50}$) / ml.

Vaccine

The vaccine used was a sub-unit marker vaccine (Porcilis® Pesti, Intervet, The Netherlands) consisting of the E2 glycoprotein of the CSFV, strain Alfort/Tübingen. The glycoprotein was produced by means of a Baculo virus expressing the protein in insect cells. One dose (2 ml) of the vaccine contains at least 40 EU, inducing at least an average serum virus neutralisation (VN) titre of 5 $\log_2$ in pigs in the potency test (Intervet, 1997).

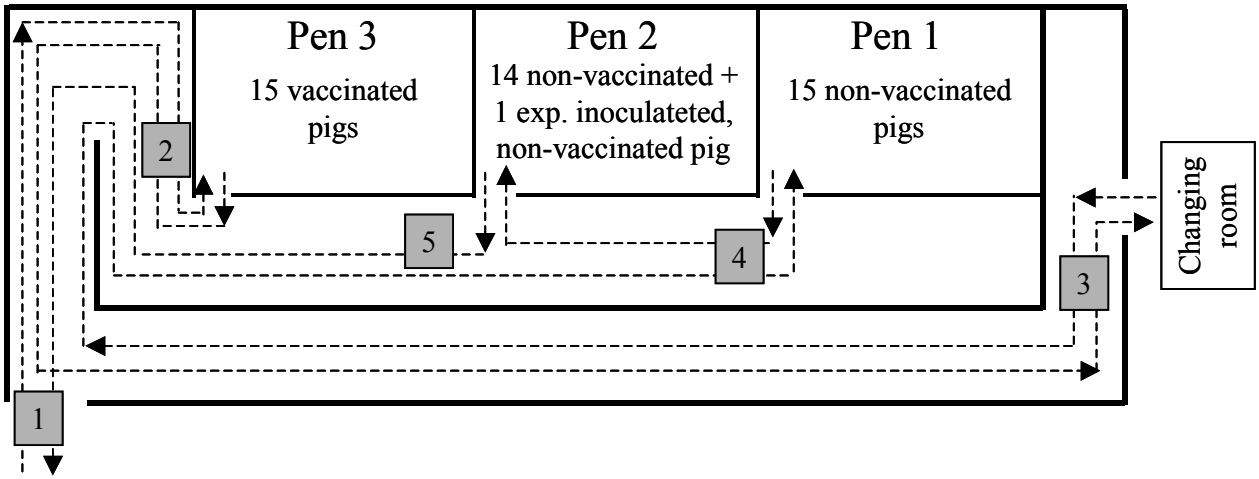
Animals

Forty-five conventional weaner pigs of 12-15 kg originating from an isolated pig herd and controlled on the absence of BVDV and CSFV antigen and antibodies were used for the experiment. On the day of experimental inoculation the average weight of the pigs was 50 kg.

Experimental design

Upon arrival, the weaner pigs were randomly allocated to one of 3 adjacent pens in an isolation unit. In each pen, 15 weaner pigs were housed (Figure 1). Pens were partitioned by a

solid wall, preventing direct contact between pigs of different pens. The dimensions of the pens were 610 x 245 x 130 cm (length x width x height).



1 Entering the unit with washed overall and thoroughly cleaned and disinfected footwear

2 Disinfection of footwear before entering and after leaving pen 3

3 Changing clothes, gloves, footwear and head covering between pens 3 and 1

4 Disinfection of footwear before entering and after leaving pen 1

5 Disinfection of footwear after leaving pen 2

Figure 1: Ground plan of the isolation unit and visiting procedure during post-inoculation period.

The experiment was divided in 3 subsequent periods: the acclimatisation-, the vaccination- and the post-inoculation period.

During the acclimatisation period (17 days) all pigs were examined clinically three times a week. The vaccination period started when the pigs of pen 3 were primo vaccinated. This was only done after confirmation that all pigs in pen 3 were in good health. All pigs of pen 3 were vaccinated twice, with a four weeks interval, by deep intramuscular injection of 2 ml vaccine, according to the producer's recommendations.

The post-inoculation period started with the inoculation, by deep intramuscular injection (2 ml) plus intranasal inoculation (2 ml), of a randomly selected pig in pen 2. This was 14 days after booster vaccination of the pigs in pen 3. During the 60-days post-inoculation (dpi)

period, pens were visited every other day, according to a strict route, in order to make sure that the only way of virus spread between pens was airborne (Figure 1). Additionally, to prevent direct or indirect contact between pigs in different pens, all equipment used for blood sampling, rectal temperature monitoring, cleansing of the pens, and feeding of the pigs was provided per pen.

Clinical examination

All pigs were clinically examined three times a week during the acclimatisation period and weekly during the vaccination period. The vaccinated pigs were examined for five consecutive days after vaccination. During the post-inoculation period all pigs were examined every other day. The following information was monitored during clinical examination: rectal temperature, liveliness (apathy), body condition (cachexy), coughing, conjunctivitis, diarrhoea, ataxia, and erythema. Mortality and feed and water intake were recorded daily.

Sample collection

Blood samples were taken from all pigs upon arrival, two days prior to inoculation, and subsequently every other day until 60 dpi. Moreover, from the vaccinated pigs blood samples were taken on 0, 8, 15, 18, 21, 24, 28, 32, 35 and 39 days post primo-vaccination (dppv) in order to follow the antibody response after vaccination.
From every pig that died or was euthanased, samples (tonsils, muscles of shoulder and rump, mesenterial, ileocecale and maxillary lymph nodes, kidney, spleen, heart, lung, liver, brain, eye fluid, blood, faeces, urine) were collected.

Sample analyses

For virus isolation (VI) from blood, 100 µl blood was inoculated in duplicate onto a non-confluent monolayer of PK_{15} cells cultured in multiwell plates (24 wells / plate). After 48 hours, the cells were fixed with isopropanol and stained with a polyclonal fluorescein-conjugated anti-CSF immunoglobulin. Additionally, for pigs in pens 1 and 3 a single tube RT-nPCR test (McGoldrick et al., 1999) was used to detect viraemia. For antibody detection in

the serum, the VN test and the CTB-ELISA (Ceditest) were used. Additionally, for pigs in pens 1 and 3 the discriminating diagnostic kit (Chekit CSF-Marker®, Bommeli AG.) identifying antibodies against the E^{rns} glycoprotein of the CSF virus, was used.

Data analyses

Since blood samples were collected every other day, the viraemic period, detected with VI, started one day prior to first positive VI and ended one day prior to the first of at least two subsequent negative VI. When a pig died during the viraemic period, the day of death was the end of the viraemic period. The viraemic period, detected with PCR, was defined in a similar way.

In order to be able to quantify virus transmission, some assumptions were made. Firstly, it was assumed that non-vaccinated pigs became infected 4 days prior to first VI. This was based on observations of the experimentally inoculated pig and on similar observations in previous experiments (Laevens et al., 1998). Secondly, and based on the first assumption, it was assumed that a non-vaccinated pig became infected by direct contact if the first positive VI of the pig was observed 4 days or more after the first pig in the same pen became positive on VI. Finally, it was assumed that non-vaccinated as well as vaccinated pigs were infectious during their entire viraemic period (detected with VI). Pigs in which viraemia was only detectable with PCR, were considered not infectious.

Virus transmission from pen 2 towards (indirect contact) and within (direct contact) adjacent pens was analysed with Kaplan-Meier survival analysis (SPSS). Therefore, survivor functions of pens 1 and 3 were compared, with the day of experimental inoculation of the pig in pen 2 as initial event and the day of first viraemia (PCR) of the pigs in pens 1 and 3 as final event.

To compare virus transmission within pens 1 and 3, again Kaplan-Meier survival analysis (SPSS) was used. Therefore, the time in which the pigs per pen became viraemic (PCR) (final event), from the moment the first pig in the same pen became viraemic (PCR) (initial event), were compared.

Finally, virus transmission by indirect contact from pigs in pen 2 to vaccinated or non-vaccinated pigs in adjacent pens was quantified with Cox proportional hazard analysis (SPSS). Therefore the day of experimental inoculation in pen 2 was the initial event and the day of first viraemia (PCR) of indirect infected pigs in pens 1 and 3 was the final event.

Differences in duration of the viraemic period between pens were estimated with one-way ANOVA (SPSS).
To estimate whether the protective capacity of the vaccine could be related to the antibody titres induced by vaccination, a paired comparison was carried out. Therefore, the vaccine induced antibody titre on the day of first viraemia (PCR) in an infected, vaccinated pig was compared with the antibody titre of a randomly selected vaccinated pig that had not become viraemic yet at that time. Differences in antibody titres were analysed with the Wilcoxon Signed Ranks Test (SPSS).

Fever was defined as a rectal temperature > 40.1°C. This was the one-sided upper 95% confidence interval calculated on the observations of seven, four and zero days before experimental inoculation. For each pig the febrile period was defined and started one day prior to the first of at least two subsequent observations of fever and ended one day prior to the first of at least two subsequent observations of normal rectal temperature (<40.1°C). Periods during which a given clinical symptom occurred were defined in a similar way.
Differences in number of pigs per pen affected at least once by a given clinical symptom were analysed with Chi-square test (SPSS). Differences in numbers of observations of a given clinical symptom between pens were analysed with one-way ANOVA (SPSS).

Results

One pig in pen 1 died during the acclimatisation period. Thus only data of 14 pigs in pen 1 are reported. One pig in pen 3 died 30 dpi, 9 days before the first pig in pen 3 became viraemic. On autopsy there were no pathological findings referring to a CSF infection, and CSFV could not be isolated from samples collected during autopsy. Additionally, blood was negative for antibodies against CSF. Therefore, it was assumed that this pig did not die as a result of a CSF infection, and in consequence data of this pig are not reported.

The experimentally inoculated pig (pen 2) became viraemic (VI) 3 dpi. The pen mates became first viraemic between 13 and 43 dpi, the mean viraemic period was 13.6 days (range 2-29 days) (Figure 2).

In pen 1, all pigs became viraemic (VI) between 23 and 31 dpi, with a mean viraemic period of 15.6 days (range 5-36 days). The mean viraemic period (VI) was similar ($p=0.801$) in pens 1 and 2. In pen 3 there were no positive cases detected with VI.

Using PCR, viraemia was detected in seven pigs of pen 3. These pigs became viraemic between 39 and 57 dpi. The mean duration of the viraemia was 6.3 days (range 4-10 days). This period was significantly shorter ($p<0.01$) as compared with the mean duration of viraemia in pen 1 (PCR) which was 19.4 days (range 3-38 days). Pigs in pen 1 became viraemic (PCR) between 19 and 31 dpi. Using PCR, viraemia could be detected on average two days prior to detection of viraemia with VI.

The period during which pigs became viraemic (PCR), as a result of indirect or direct contact infections, was significantly ($p<0.001$) shorter in pen 1 as compared to pen 3. Once the first pig in a pen became viraemic, virus transmission within the pen (the period during which the following pigs were infected) was completed significantly earlier ($p<0.001$) in pen 1 as compared to pen 3.

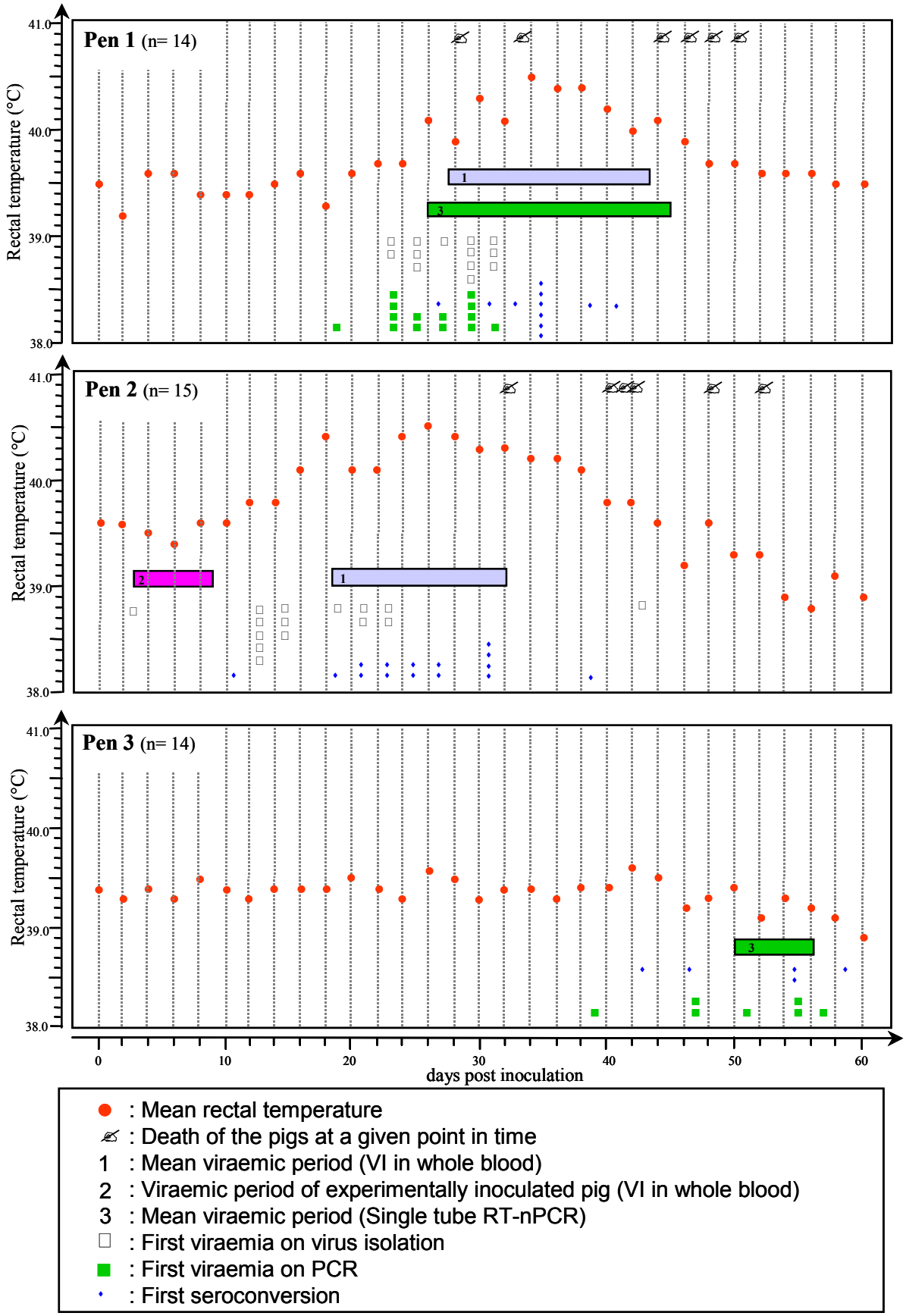


Figure 2: Virological and clinical findings of a CSF infection after inoculation of 1 pig in pen 2.

Based on the assumptions mentioned previously, only the first viraemic pig in pen 1 was considered infected by indirect contact. The pen mates, which became viraemic 4 days or more after the first pig became viraemic, were considered infected by direct contact. In pen 3 all viraemic pigs were considered infected by indirect contact, since viraemia in pen 3, which was only detectable with PCR, was considered not infectious. Cox proportional hazard analysis indicated that a non-vaccinated pig was 65.3 times more likely to become viraemic by indirect contact as compared with a vaccinated pig. However, this rate ratio did not differ significantly from 1 ($p = 0.61$).

The mean time between primo-vaccination and first vaccine-induced seroconversion (VN test) was 27 days (range 23 - 33 days). On the day of booster vaccination 11 out of 15 pigs had detectable antibody titres. The vaccine induced antibody titres on the day of experimental inoculation varied between 1/60 and 1/2560, with an average of 1/420. There was no significant difference ($p=0.69$) in vaccine induced antibody titres between vaccinated pigs on the day of first viraemia (PCR) and randomly selected, vaccinated pigs that had not become viraemic yet at that time.

The experimentally inoculated pig seroconverted 11 dpi, i.e.8 days after the onset of viraemia (Figure 2). The mean time between first positive VI and first positive VN in non-vaccinated pigs was 8 days (S.D. = 5.2) (Figure 2). Three pigs of pen 1 died before antibody response could be detected.

During the post-inoculation period, 5 out of the 15 vaccinated pigs seroconverted against the wild virus (Figure 2). Four out of these five pigs had been viraemic (PCR). The first positive virological diagnosis was, on average, 7 days (S.D. = 2.0) prior to the first positive differential ELISA.

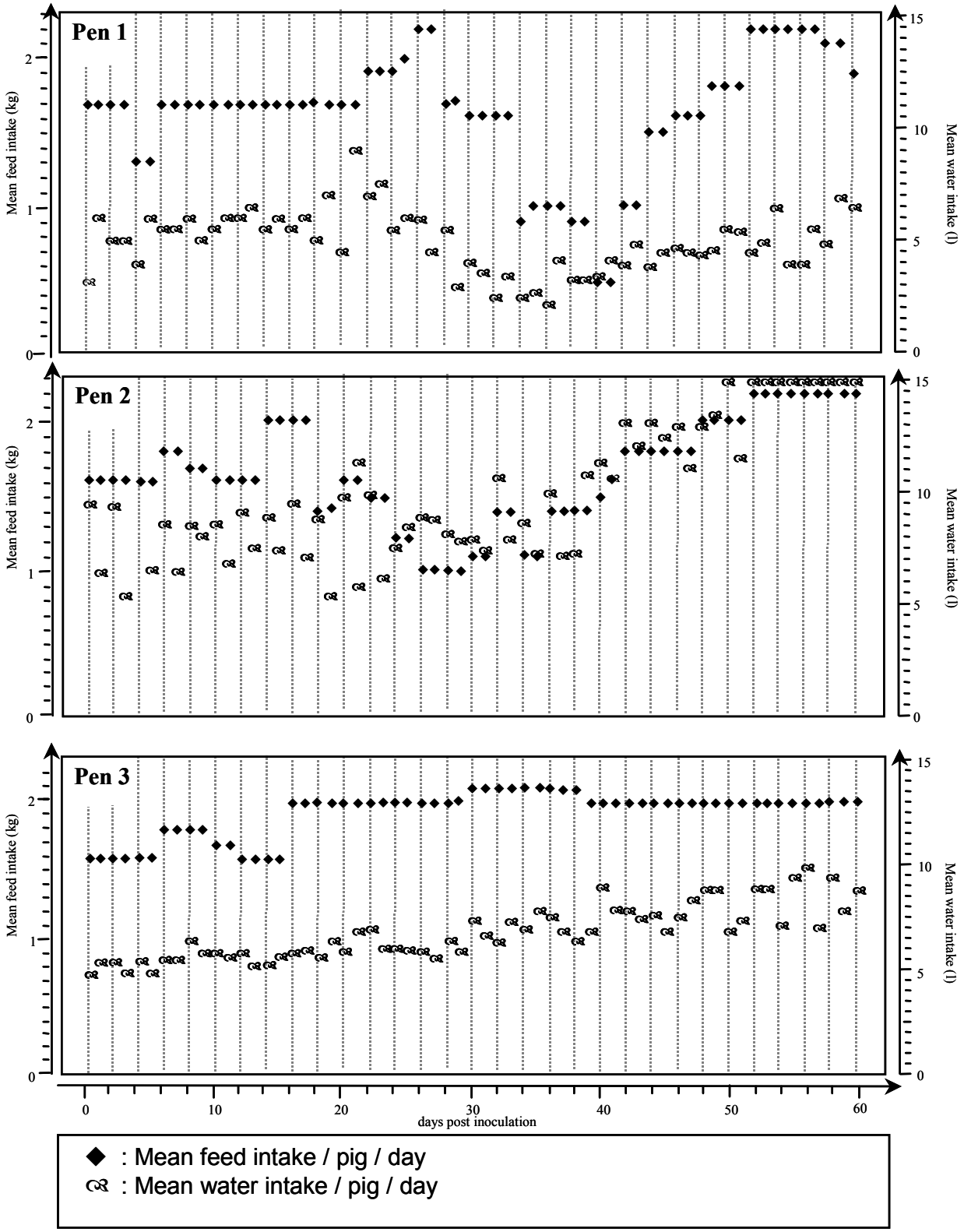


Figure 3: Course of feed and water intake in pens 1, 2, and 3.

In each of the non-vaccinated pens, the clinical course was similar as in previous experiments by Laevens et al. (1998, 1999). The initial clinical symptoms observed after virus was introduced in the pens were an increase of the mean rectal temperature and a decrease of the feed intake (Figures 2 and 3). Although water intake was more variable than feed intake, the shape of the curve was similar to the one of the feed intake (Figure 3).

In vaccinated pigs, mean rectal temperature and feed and water intake remained stable during the post inoculation period (Figures 2 and 3).

The results of mortality and clinical symptoms monitored during post-inoculation are summarised in table 1. It is obvious that in the vaccinated pen almost no clinical symptoms and no mortality were observed.

Clinical sign	**Pen 1** (n=14)		**Pen 2** (n=15)		**Pen 3** (n=14)	
	n[1]	Observations[2]	n[1]	Observations[2]	n[1]	Observations[2]
Fever*	10	9.3 (3.9)	12	9.7 (3.9)	1	5.0
Apathy*	9	7.0 (3.5)	9	5.8 (3.5)	0	-
Ataxy*	8	6.3 (3.5)	9	5.7 (5.9)	0	-
Cachexy	4	5.5 (2.9)	5	4.8 (4.2)	0	-
Diarrhoea	1	2.0	2	5.0 (1.4)	0	-
Conjunctivitis*	11	6.0 (3.0)	11	9.7 (5.8)	0	-
Coughing	0	-	4	2.0 (0.8)	0	-
Erythema*	13	10.4 (4.1)	15	11.5 (4.1)	1	5.0

[1]number of animals per pen with two or more subsequent observations of a given clinical symptom

[2]mean number of observations and its standard deviation

*Significant difference between the pens in the overall chi-square test ($p < 0.01$). Pairwise comparisons showed a significant difference ($p < 0.01$) between pens 1 and 3 and between pens 2 and 3, but not between pens 1 and 2 ($p > 0.05$).

Table 1: Clinical response during the post-inoculation period.

Discussion

Recent epizootics have shown that controlling CSF outbreaks may be very difficult with the current control strategy, in particular in areas with high pig density (Miry et al., 1991; Vanthemsche, 1995; Elbers et al., 1999). Therefore vaccination with marker vaccines could be of help in reducing the size of an outbreak.

In the report of the Scientific Veterinary Committee of the European Community on the use of marker vaccines in the control of CSF, minimum demands concerning the effect of vaccination were stated. Two of these demands were that vaccination should provide protection against any natural contact infection and that the protective effect of vaccination should be achieved within the shortest possible period of time (Anonymous, 1997).

As protection is concerned, it was shown in this experiment that vaccination was able to protect pigs from a clinical course of disease. This is consistent with previous findings (Rumenapf et al., 1991; van Zijl et al., 1991; Hulst et al., 1993; König et al., 1995; van Rijn et al., 1996; Peeters et al., 1997; Bouma et al., 2000). However, within the framework of emergency vaccination, clinical protection is not the primary aim. It might even be contra-indicated since it can mask a present infection (Table 1, Figures 1 & 2). Consequently, clinical examination of the pigs at weekly interval, which has been used as a screening test during recent epidemics, will be useless in vaccinated populations. Therefore the presence of a CSF infection in vaccinated populations has to be detected with more labour intensive virological or serological screening tests.

Essentially vaccination against CSF is used to reduce the virus spread and make the epidemic fade out. Therefore, the more important question is whether the vaccine is able to prevent virus multiplication following a natural contact infection. In this experiment it was demonstrated that, although the time to infection was significantly delayed in vaccinated pigs as compared to non-vaccinated pigs, vaccination was unable to fully protect pigs against indirect contact infections. Although, viraemia in vaccinated pigs was only detected by means of PCR techniques, which makes it impossible to determine whether infectious antigens or harmless antigen-antibody complexes were detected, it contains the possibility of virus spread by infected vaccinated pigs. Recently it has been found that virus transmission by direct

contact from E2 sub-unit marker vaccine vaccinated pigs, inoculated with CSFV, towards sentinel pigs was prevented in nearly all groups (Bouma et al., 1999). This also indicates that, although virus spread by vaccinated pigs was largely reduced, it was not totally prevented. Consequently, it will be essential within an emergency vaccination campaign that all infected pigs can be discriminated from vaccinated pigs. Whether uninfected vaccinated pigs should be killed depends on the discriminatory test and on the possibilities of slaughtering and/or exporting uninfected but vaccinated pigs.

In this set-up, the effectiveness of the vaccine may be somewhat under-estimated since vaccinated pigs where exposed to a severe infection load. In an emergency situation it is likely that all pigs in a region will be vaccinated which will, most probably, reduce the infection load. However, it should be stressed that the protective properties of the vaccine were examined under ideal circumstances, meaning that the experimental infection only took place two weeks after the booster vaccination. It is likely that the circumstances in which the vaccine will be used in emergency vaccination programs will be less favourable.

The second demand, mentioned in the rapport of the Scientific Veterinary Committee, was that the effect of vaccination should be achieved within the shortest possible period of time. In this experiment, serological response following vaccination was found on average 27 days after the primo vaccination. Although the relationship between serum antibody titres and protection is not fully clarified, humoral antibodies can be assumed to play a major role in the protection to CSF infection (Terpstra and Wensvoort, 1987). Therefore this slow serological response gave an indication about the slow immunological response after vaccination. This slow response is not surprising since the E2 sub-unit marker vaccine is a dead vaccine only consisting of one glycoprotein. On the other hand, recent transmission experiments have demonstrated with 95% certainty that the E2 sub-unit marker vaccine could prevent virus transmission 2 weeks after vaccination (Bouma et al., 2000). Yet, in the epizootic of 1990 in Belgium it was found that 48 % of the outbreaks occurred in less than two weeks after the nearest neighbouring outbreak (Roberts, 1995). This illustrates that even a 2 weeks interval between vaccination and protection will be an important hindrance for the use of the vaccine in emergency vaccinations programs. Furthermore, vaccination in the neighbourhood of an outbreak includes the risk of vaccination in inapparently infected herds. In the past it has been established that vaccination of inapparently infected pigs may lead to spread of the virus by needle, and initiate an outbreak in the herd (Terpstra and Robijns, 1977). However, even if a

first generation of contact infections cannot be prevented, it is likely that the occurrence of a second generation, which might occur after 4 weeks, can be prevented.

Based on the results of this experiment it can be concluded that an indirect infection of well-vaccinated pigs can compromise a rapid CSF eradication. In addition the potential use of the E2 sub-unit marker vaccine in emergency vaccination programs will be complicated by the slow induction of protection after vaccination.

Reference List

Anonymous (1997) The use of marker vaccines in the control of infectious diseases in particular classical swine fever, *Report of the Scientific Veterinary Committee*. Scientific Veterinary Committee of the European Commission

Bouma, A., de Smit, A. J., de Kluijver, E. P., Terpstra, C., and Moormann, R. J. (1999) Efficacy and stability of a subunit vaccine based on glycoprotein E2 of classical swine fever virus. *Vet. Microbiol. 66*, 101-114.

Bouma, A., de Smit, A. J., de Jong, M. C., de Kluijver, E. P., and Moormann, R. J. (2000) Determination of the onset of the herd-immunity induced by the E2 sub-unit vaccine against classical swine fever virus. *Vaccine 18*, 1374-1381.

Elbers, A.R., Stegeman, A., Moser, H., Ekker, H. M., Smak, J. A., and Pluimers, F. H. (1999) The classical swine fever epidemic 1997-1998 in The Netherlands: descriptive epidemiology. *Prev.Vet.Med. 42,* 157-184.

Hulst, M.M., Westra, D. F., Wensvoort, G., and Moormann, R. J. (1993) Glycoprotein E1 of hog cholera virus expressed in insect cells protects swine from hog cholera. *J. of Virol. 67,* 5435-5442.

Intervet (1997) Porcilis Pesti, Overview of information currently available. pp. 56-71.

Koenen, F., Lefebvre, J. (1994) Kinetics of an experimental infection with a classical swine fever (CSF) field isolate. In the Proceedings of the 3rd Congress of European Society of Veterinary Virology. 4-7 September 1994, Interlaken, pp. 322-326

König, M., Lengsfeld, T., Pauly, T., Stark, R., and Thiel, H. J. (1995) Classical swine fever virus: independent induction of protective immunity by two structural glycoproteins. *J. of Virol. 69*, 6479-6486.

Laevens, H., Koenen, F., Deluyker, H., and Berkvens, D. (1998) An experimental infection with classical swine fever virus in weaner pigs. I. Transmission of the virus, course of the disease, and antibody response. *Vet.Q. 20*, 41-45.

Laevens, H., Koenen, F., Deluyker, H., and de Kruif, A. (1999) Experimental infection of slaughter pigs with classical swine fever virus: transmission of the virus, course of the disease and antibody response. *Vet. Rec. 145*, 243-248.

McGoldrick A., Bensaude E., Ibata G., Sharp G., Paton D.J., (1999). Closed one-tube reverse transcription nested polymerase chain reaction for the detection of pestiviral RNA with fluorescent probes. *J. Virol. Methods 79*, 85-95.

Miry, C., Castryck, F., Koenen, F., Broers, A., and Segers, E. (1991) Quelques aspects de l'épizootie de peste porcine classique en Belgique en 1990. *Epidémiol.Santé Animal 20*, 23-32.

Peeters, B., Hulst, M., Gielkens, A., and Kimman, T. (1997) Biologically safe, non-transmissible pseudorabies virus vector vaccine protects pigs against both Aujeszky's disease and classical swine fever. *J. of General Virol. 78,* 3311-3315.

Pensaert, M. and Van Reeth, K., Vaccines for swine. In: Veterinary Vaccinology, editors: Pastoret, P., Blancou, J., Vannier, P., Verschueren, C., Amsterdam, Elsevier; 1997. pp. 374-375.

Roberts, M. (1995) Evaluation of optimal size of restriction zones in disease control with particular references to classical swine fever. In the Proceedings of meeting of the society for veterinary epidemiology and preventive medicine. 29-31 March 1995, Reading, UK, p. 119

Rumenapf, T., Stark, R., Meyers, G., and Thiel, H. J. (1991) Structural proteins of hog cholera virus expressed by vaccinia virus: further characterization and induction of protective immunity. *J. of Virol. 65,* 589-597.

Terpstra, C. and Robijns, K. G. (1977) Experience with regional vaccination against swine fever in enzootic areas for limited periods using C-strain virus. *Tijdschr. Diergeneesk. 102,* 106-112.

Terpstra C. and Wensvoort G., (1987) Influence of the vaccination regime on the herd immune response for swine fever. *Vet. Microbiol. 13*, 143-151.

van Rijn, P.A., Bossers, A., Wensvoort, G., and Moormann, R. J. (1996) Classical swine fever virus (CSFV) envelope glycoprotein E2 containing one structural antigenic unit protects pigs from lethal CSFV challenge. *J. of General Virol. 77,* 2737-2745.

van Zijl, M., Wensvoort, G., de Kluyver, E., Hulst, M., van der, Gulden H., Gielkens, A., Berns, A., and Moormann, R. (1991) Live attenuated pseudorabies virus expressing envelope glycoprotein E1 of hog cholera virus protects swine against both pseudorabies and hog cholera. *J. of Virol. 65,* 2761-2765.

Vanthemsche, P. (1995) Classical swine fever 1993-1994 in Belgium. In: Proceedings of the 8th Annual Meeting of the Dutch Society for Veterinary Epidemiology and Economics 13 December 1995, Lelystad, p. 25

CHAPTER 4.3:

AN E2 SUB-UNIT MARKER VACCINE DOES NOT PREVENT HORIZONTAL OR VERTICAL TRANSMISSION OF CLASSICAL SWINE FEVER VIRUS.

J. Dewulf , H. Laevens , F. Koenen , K. Mintiens,

and A. de Kruif

Vaccine (2002), 20, 86-91

Abstract

An experimental infection with classical swine fever (CSF) virus in E2 sub-unit marker vaccine vaccinated gilts was conducted in order to evaluate the effect of vaccination on virus transmission and course of the disease. Therefore, clinical signs as well as horizontal and vertical virus transmission were monitored in two inoculated, non-vaccinated and ten vaccinated conventional gilts, housed in individual sow boxes. Within 10 days post inoculation, all vaccinated gilts became infected. Depending on the definition of the infectious period 2 different estimates of R_0 were calculated (R_0 = 14.8 and 3.3), both significantly larger than 1 ($p<0.01$). In 3 out of the 8 vaccinated pregnant gilts vertical virus transmission occurred, resulting in infected offspring. Based on the results of this experiment, it can be concluded that double vaccination with an E2 sub-unit marker vaccine only protects pregnant gilts from the clinical course of the disease but does not prevent horizontal nor vertical spread of the CSF virus.

Introduction

Classical swine fever (CSF) is known as a highly contagious pig disease causing considerable economic losses. In 1980 the European Union (EU) adopted an eradication strategy for CSF (Council Directive 80/217/EEC). Since, the control of CSF in the EU is based on a policy of non-vaccination and stamping-out. This policy has been imposed, partially because of the lack of a vaccine that both was efficacious and enabled differentiation between vaccinated and infected pigs (Pensaert and Van Reeth, 1997). Recent epidemics have shown that, without application of emergency vaccination, the control of outbreaks in non-vaccinated populations through eradication may be very expensive, particularly in areas with high pig densities (Miry et al., 1991; Vanthemsche, 1995; Elbers et al., 1999).

In the last 10 years, progress has been made in the development of marker-vaccines and accompanying discriminatory diagnostic tests (Moormann et al. 2000). For the evaluation of the efficacy of a CSF vaccine it is important to know to what extent vaccination will reduce horizontal as well as vertical virus transmission. Transplacental infection of the offspring can induce persistently infected piglets (Terpstra, 1988; van Oirschot, 1999). These piglets are of great epidemiological importance since they can maintain a CSF infection in the pig population over a long period.

This paper describes the effect of double vaccination with an E2 sub-unit marker vaccine against CSF on the virus transmission among gilts housed in a sow-box housing system. Furthermore, the virological and serological response, the clinical symptoms, and the effect on gestation, following a CSF infection in vaccinated pregnant gilts are described.

Materials and methods

Animals

Twelve conventional gilts, 8 months of age, originating from a selection herd and checked on the absence of bovine viral diarrhoea (BVD) and CSF antigen and antibodies were used.

Virus

The isolate used for inoculation was originally obtained from the first infected herd of the 1993-1994 Belgian CSF epizootic. The isolate was verified to be free of African swine fever virus and BVD virus. By using a panel of monoclonal antibodies, it was characterised by Prof Ahl (Tübingen) to be antigenically similar to an isolate known as the 'souche Lorraine' (Koenen and Lefebvre, 1994) which has been described as a moderate virulent strain (Laevens, 1999). The virus was cultivated on PK_{15} cells and 2 passages were carried out. The titre was 10^3 median tissue culture infective dose ($TCID_{50}$ / ml).

Vaccine

The vaccine used was a sub-unit marker vaccine (Porcilis® Pesti, Intervet International BV) consisting of the E2 glycoprotein of the CSF virus, strain Alfort/Tübingen. The glycoprotein was produced by means of a baculovirus expressing the protein in insect cells. One dose (2 ml) of the vaccine contains at least 40 Elisa Units, inducing at least an average virus neutralization (VN) titre in serum of 5 $\log_2$ in pigs in the potency test (Intervet, 1997).

Experimental design

Upon arrival, 12 gilts were housed in individual sow boxes. After an acclimatisation period of 10 days, 10 randomly chosen gilts were vaccinated. Four weeks later a second vaccination was given. Between primo and booster vaccination the reproductive cycle of the gilts was

synchronised using altrenogest (Regumate®, Hoechst Roussel Vet). The synchronization period ended on the day of booster vaccination. In the 6 subsequent days oestrus detection was carried out on a daily basis. During oestrus, all gilts were inseminated twice. Twenty-five days after the last insemination, the gilts were checked on pregnancy using ultrasound. Nine were pregnant of which 8 were vaccinated and one was not vaccinated. After examining the pregnancy all gilts, including the 3 non-pregnant gilts, were transferred to an isolation unit and again housed in individual sow-boxes. The two non-vaccinated gilts were randomly assigned to boxes 3 and 10, respectively. The remaining 10 gilts were randomly allocated to the remaining boxes. Direct nose-to-nose contact was only possible between neighbouring gilts.

Following a 10 days acclimatisation period after arrival at the isolation unit (46 days after booster vaccination), the two non-vaccinated gilts, housed in box 3 and box 10, were inoculated with CSF virus through deep intra-muscular injection (2 ml) plus intranasal inoculation (2 ml). After inoculation, the gilts were not released from the boxes until the end of the experiment or the moment of death. All gilts that survived the infection were slaughtered approximately one week before the end of gestation.

In the 70-days post-inoculation period, the boxes were visited following a strict route starting as far as possible from the inoculated gilts and moving towards the sources of infection. By applying this visiting procedure, it was ensured that the virus was not transferred from infected to uninfected gilts during sample collection. Additionally, all materials necessary for blood sampling and rectal temperature monitoring were provided for each box separately.

Sample collection and clinical examination

Clotted and heparinised blood samples were collected from all gilts upon arrival. Again, blood samples were taken upon arrival at the isolation unit and 2 days prior to inoculation. During the post-inoculation period, blood samples were collected from all gilts every 3 days until 42 days post inoculation (dpi), and subsequently every 7 days until 70 dpi. Additionally, swabs of nasal secretion and faeces were collected every 3 days during the first 18 dpi. Simultaneously with sample collection, all gilts were examined clinically. The following symptoms were recorded: liveliness (apathy), body condition (cachexia), coughing,

conjunctivitis, diarrhoea, ataxia, and erythema. Rectal temperature, feed intake and mortality were recorded daily.

From every gilt that died or was euthanatised, tissue samples (tonsil, muscles of shoulder and rump, mesenterial, ileocecal and maxillary lymph nodes, kidney, spleen, heart, lung, liver, brain, eye fluid, blood, faeces, urine) were collected. After death or after abortion, blood and tissue samples (tonsil, kidney, spleen, heart, and lung) were collected from the foetuses.

Sample analyses

For virus isolation (VI) in blood or leucocytes, 100µl whole blood or 100 µl buffy coat on heparinised blood was inoculated in duplicate onto a non-confluent monolayer of PK_{15} cells cultured in multiwell plates (24 wells / plate). For VI in tissue samples one cm^3 of each organ was homogenised into 9ml minimal essential medium (MEM) and grinded with an ultra-Turrax. After centrifugation for 10 min at 4000g, 300µl of the supernatant was inoculated in duplicate onto a non-confluent monolayer of PK_{15} cells cultured in multiwell plates (24 wells / plate). After 48 hours, the cells were fixed with isopropanol and stained with a polyclonal fluorescein-conjugated anti-CSF immunoglobulin.

Additionally, a single tube RT-nPCR test (McGoldrick et al., 1999) was used to detect viraemia in serum. The same single tube RT-nPCR test was used to detect CSF virus in nasal secretion and faeces. We did not use virus isolation for nasal secretions and faeces.

For antibody detection in serum, the virus neutralization (VN) as described by Holm-Jensen (1981) using the $Alfort_{187}$ strain, and the Herd Check CSFV ELISA (IDEXX, Scandinavia, Osterbybruk, Sweden) were used. The discriminating diagnostic kit (Chekit® CSF-Marker, Dr. Bommeli AG, Switzerland), identifying antibodies against the E^{rns} glycoprotein of the CSF virus, was used to distinguish between antibody response from vaccinated and infected gilts.

Leukocyte count was carried out using the Coulter-Counter ZM (Analis).

Data analyses

The basic reproduction ratio (R_0), a measure of transmission of infection, and defined as the mean number of new infections arising from one typical infectious case introduced in a susceptible population, was calculated using the martingale and the maximum likelihood estimator.

The martingale estimator is defined as:

$$R_{0mrt} = \frac{N}{C - Z} \sum_{i=S_t+1}^{S_0} \frac{1}{i}$$

where N is the total number of animals at the beginning of the outbreak, C is the total number of cases that occurred during the observation period, Z is the sum of fractions of infectious periods that were spent at the time when no susceptibles remained, S_0 is the number of susceptibles at the beginning of the observation period, and S_t is the number of susceptibles at the end of the observation period (de Jong and Kimman, 1994). To calculate Z, the day of infection and the duration of the infectious period were estimated for all contact infected gilts. The duration of the infectious period was defined in two different ways: 1) gilts were assumed infectious during their entire viraemic period diagnosed with PCR and, 2) gilts were assumed infectious during their entire viraemic period diagnosed with VI, resulting in two different values for R_0. The "SIR" (Susceptible-Infective-Removed) model was used to describe the final size distribution in terms of R_{0mrt} (Kroese and de Jong, in preparation). Hypothesis testing (H_0: $R_0 = 1$) of R_{0mrt} was performed as described by Kroese and de Jong.

The maximum likelihood estimator is calculated numerically from:

$$R_{0mle} = \max \prod_{i=1}^{n} F\left\langle X_i, R_0 \,\middle|\, N, S_0, I_0 \right\rangle$$

where $F(X_i,R_0 \mid N,S_0,I_0)$ is the likelihood function for the observed value X_i. X_i is the total number of pigs that become infected, N, S_0, and I_0 are the total number of animals, the number of susceptible animals and the number of infectious animals at the beginning of the outbreak, respectively (Bouma et al., 1996).

Fever was defined as a rectal temperature > 38.9°C. This is the one-sided upper limit of the 95% confidence interval (CI) calculated on the average rectal temperature of all gilts during the last three days before inoculation. Leukopenia was defined in a similar way. The one-sided lower limit of the 95% CI was equal to 12,000 cells/ml.
Periods during which a given clinical symptom occurred started with the first of at least two subsequent observations of a given clinical symptom and ended with the first of at least two

subsequent observations for which the given clinical symptom was absent. Periods of positive VI, PCR and leukopenia were defined in a similar way.

The mean time between infection and the obvious rise of antibody titres in the VN test and the mean time between infection and the first positive differentiating ELISA test were compared using a paired sample T-Test (SPSS, Chicago, USA).

Results

In the vaccinated gilts almost no clinical symptoms were observed. Only one gilt showed some discrete cachexia and erythema during 2 subsequent observations. However, these symptoms occurred at the very beginning of the observation period and prior to the estimated day of infection of this gilt, therefore it is highly probable that these symptoms were not related with the CSF virus infection. Three out of the 10 vaccinated gilts showed fever (>38.9°C) during an average period of 4 days (S.D. 1.64 days). The periods of fever were synchronous with the viraemic periods of these gilts. No leukopenia was detected in the contact-infected gilts. In both inoculated non-vaccinated gilts typical clinical symptoms such as erythema, conjunctivitis and ataxia were observed. In gilt nr 10 also severe leucopenia was found. Eventually, both gilts died between 19 and 21 dpi, after having had high fever (average 40.5°C) for more than 12 days.

Both inoculated, non-vaccinated gilts were first detected positive for CSF on VI in whole blood and leucocytes 6 dpi. With PCR, these gilts were first positive 6 and 3 dpi. Using VI in whole blood and leucocytes, no viraemia could be detected in the vaccinated gilts. However, using PCR, viraemia was detectable in all but one of the contact infected gilts for the first time between 9 and 12dpi (Figure 1). The average duration of viraemia in vaccinated gilts was 3.7 days. In 6 out of the 10 vaccinated gilts viral RNA was also detected in faeces between 9 and 12 dpi. In nasal secretions viral RNA was detected in 4 out of the 10 vaccinated gilts between 15 and 18 dpi. The detection of viral RNA in faeces was synchronously with the viraemic period detected in serum. Except for one case in which viral RNA could be detected in nasal secretion after the end of viraemia detected in serum, viral RNA was isolated in nasal secretions synchronously with the viraemic period detected in serum.

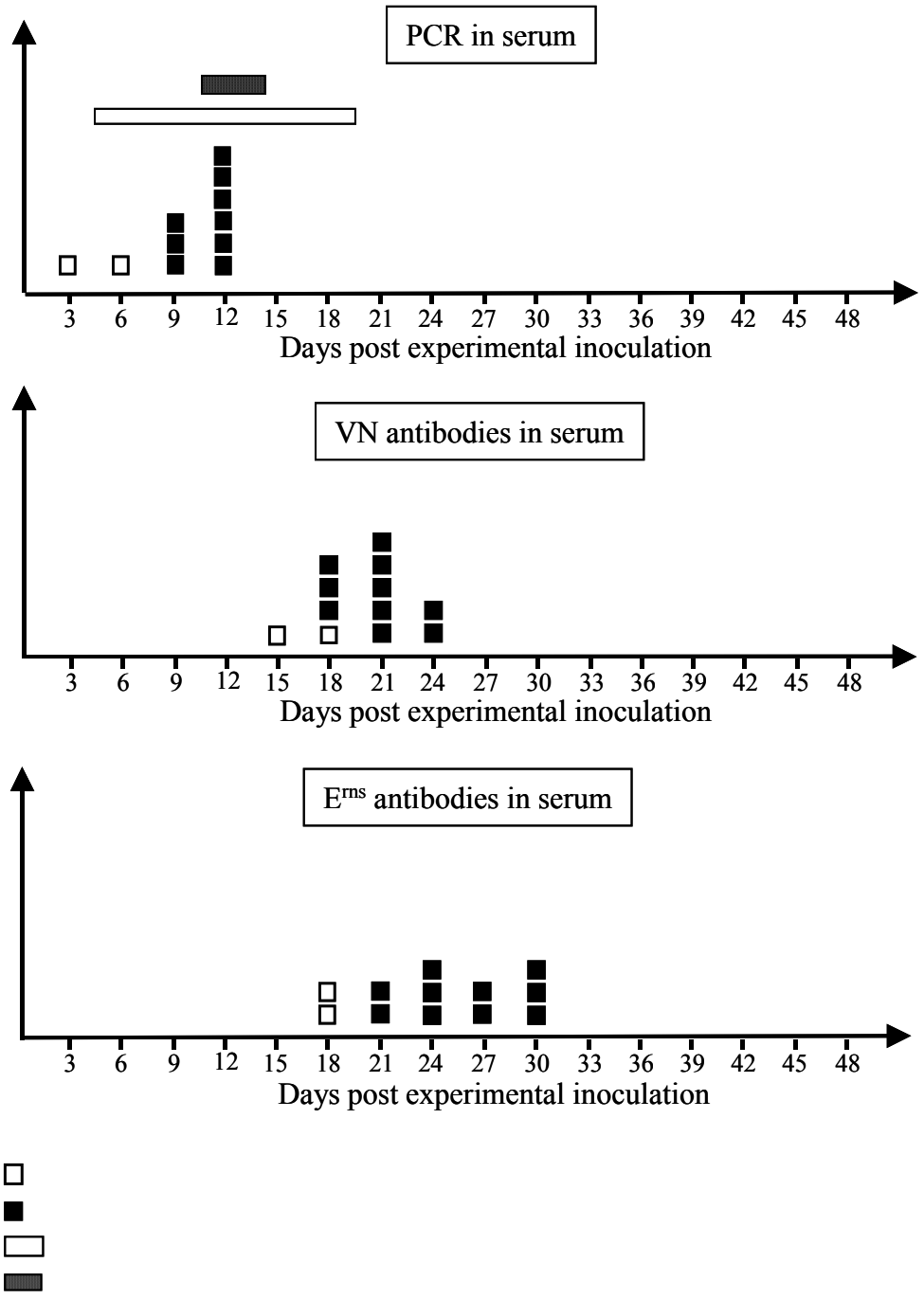


Figure 1: Virus and antibody detection after infection.

A sampling interval of 3 days includes that an animal became viraemic in between the last negative and the first positive sample. Therefore, the beginning of the viraemic period was set to be 1.5 days before the first positive sample. Based on the results of the inoculated gilts (beginning of viraemia 1.5 and 4.5 dpi, respectively) the beginning of the viraemic period detected by PCR was set to be on average 3 days post infection. Subsequently, the moment of

infection of the contact infected gilts was estimated to be 3 days before the beginning of the viraemic period detected by PCR, which is equal to 4.5 days before the first positive PCR.

The mean time between infection and obvious rise of antibody titres in the VN test was 12.8 days (S.D. 2.1 days) (Figure 2). The mean time between infection and first positive differentiating ELISA test was 17.2 days (S.D. 3.0 days) (Figure 1). The differentiating ELISA test responded significantly ($p<0.01$) later than the VN test. In gilt nr 8 no viraemia was detected using PCR. Yet the gilt reacted positive in the discriminating ELISA test and therefore was assumed infected too. Based on the serological results, the day of infection was estimated to be 9.5 dpi.

Two different martingale estimates of R_0 were calculated depending on the definition of the infectious period. When assuming that a gilt was infectious during the entire viraemic period detectable with PCR, the R_{0mrt} was estimated at 14.8. When assuming that a gilt was only infectious during the viraemic period detectable with VI the R_{0mrt} was estimated at 3.3. In both cases the R_{0mrt} is significantly larger than 1 ($p<0.01$). Since no susceptible gilts remained at the end of the experiment, the maximum likelihood estimate of R_0 was $+\infty$. The lower limit of the 95% CI of the R_{0mle} was 1.24. This also indicates that the R_0 is significantly larger than 1.

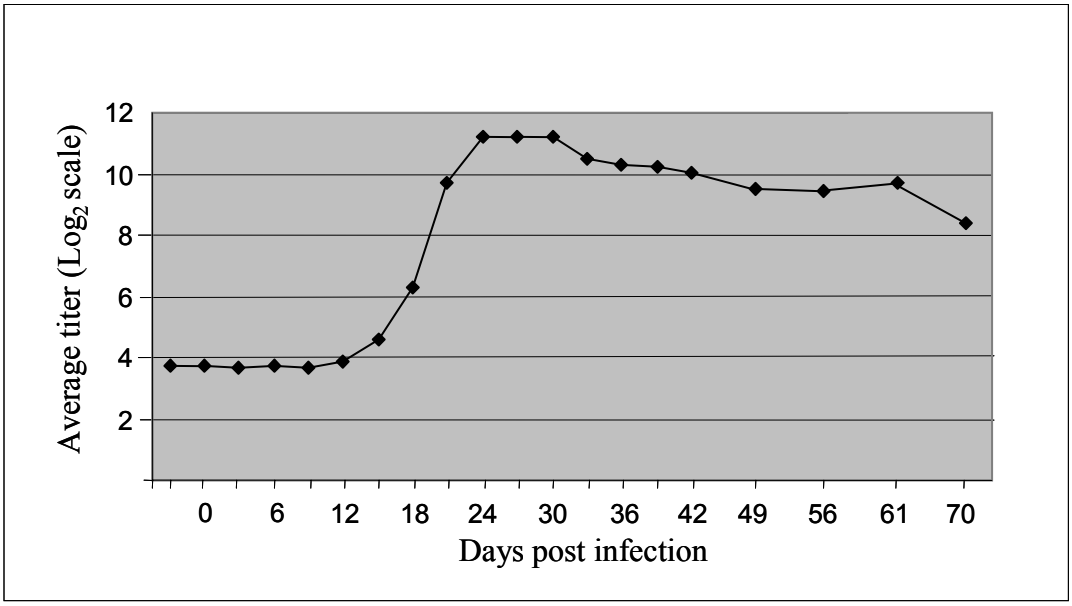


Figure 2: Average VN antibody titre in serum in vaccinated gilts

On the day of inoculation, the vaccinated gilts were on day 42-43 of gestation. On the estimated day of infection, the gilts were between day 46 and 52 of gestation (Table 1). In 3 out of the 8 vaccinated pregnant gilts, the offspring was at least partially infected with CSF virus (Table 1). Additionally, gilt nr 5 gave birth 4 days before the end of the normal pregnancy period (on day 111 of the gestation). None of the infected offspring had seroconverted against CSF.

Table 1: Virological findings in the offspring of the vaccinated pregnant gilts

Gilt number	Days of Gestation*	Piglets (n)	Mummified piglets (n)	Virus Isolation**		
				Spleen	Kidney	Tonsils
1	47	17	0	0	0	0
5	46	17	5	7	8	7
6	51	11	0	0	0	0
7	51	11	0	0	0	0
8	52	6	0	0	0	0
9	50	17	0	0	0	0
11	50	13	3	10	10	10
12	49	3	0	1	1	1

n = Number
* Days of gestation on the estimated day of infection
** Number of piglets from which virus was isolated. No virus isolation was done from mummified piglets.

Discussion

In the report of the Scientific Veterinary Committee of the European Community on the use of marker vaccines in the control of CSF, several minimum demands concerning the effect of vaccination were stated. Two of these demands were that vaccination should provide protection against any natural contact infection and prevent transplacental infection (Anonymous, 1997).
As clinical protection is concerned, a comparison of the symptoms in the vaccinated and the inoculated (non-vaccinated) gilts and a comparison with a similar experiment in non-vaccinated sows (Dewulf et al., 2001) shows that vaccination was able to protect gilts from the clinical course of disease. This is consistent with previous findings in sows (Ahrens et al., 2000; de Smit et al., 2000; Moormann et al., 2000) and in grower and finishing pigs (König et al., 1995; van Rijn et al., 1996; Dewulf et al., 2000). However, within the framework of emergency vaccination, clinical protection is not the primary aim. It might even be contra-indicated since it can mask a present infection. Therefore the more important question is whether vaccination is able to protect against infection and to prevent further virus spread.

In this experiment it was clearly demonstrated that double vaccination was unable to protect gilts from infection. The fact that viraemia in vaccinated gilts was only detectable by means of PCR is consistent with previous findings in vaccinated grower pigs (Dewulf et al., 2000) and may be explained by a limited viraemia which remains under the detection limit of VI in whole blood as well as in leucocytes. Since virus was also found in the offspring it could be concluded that, although viraemia was not detectable by means of VI, it really consisted of infectious virus. The finding of viral RNA in faeces and nasal secretions of vaccinated gilts may suggest that also horizontal spread occurred. Yet, since virus detection in faeces and serum was done using PCR it is impossible to demonstrate infectious virus.
The infection of all vaccinated gilts within a short period of time resulted in an R_0 that is significantly larger than 1 and comparable with the R_{0mrt} calculated from a similar experiment in non-vaccinated gilts (R_{0mrt} = 13.0) (Dewulf et al., 2001). As stated before, it is difficult to determine, based on the results of this experiment, whether infected vaccinated gilts can spread the virus horizontally. However, even if horizontal spread by vaccinated gilts occurred it is likely that the amount of virus excreted was much lower than the amount of virus spread

by the inoculated non-vaccinated gilts. Therefore the R_0 calculated in this experiment, where vaccinated gilts were in contact with both vaccinated and non-vaccinated infected gilts, quantifies the reduction of the susceptibility after vaccination and not the reduction of infectivity after vaccination. In a population where all pigs are vaccinated the R_0 may be smaller. On the other hand, the challenge in this experiment only took place several weeks after double vaccination, whereas it is likely that in emergency vaccination campaigns the interval between vaccination and infection is expected to be much shorter, resulting in a sub-optimal protective effect of the vaccine.

The second demand, mentioned in the report of the Scientific Veterinary Committee, was that vaccination of pregnant sows should prevent transplacental infections. In this experiment, transplacental infection of the offspring was observed in 3 out of the 8 vaccinated pregnant gilts. This result is only partially consistent with previous findings in literature where in some experiments transplacental infection in double vaccinated sows was totally prevented (de Smit et al., 2000; Moormann et al., 2000) or was observed in a smaller number of sows (Ahrens et al., 2000). These different results may be due to a difference in vaccine efficacy, the use of another challenge virus strain, difference in inoculation procedure and dose, and several other host factors such as age, breed and nutritional condition (van Oirschot et al., 1988).

Since it was shown in this experiment that the E2 sub-unit vaccine was unable to prevent vaccinated gilts from infection, powerful diagnostic techniques to identify the infected vaccinated animals will be essential in an emergency vaccination scenario using this marker vaccine. Especially since, the use of routine diagnostic techniques such as clinical examination, VI, antibody ELISA, to detect a CSF infection will be of no use in a vaccinated population. In this experiment, detection of infected vaccinated gilts was only possible via PCR or the discriminating ELISA test. Yet, PCR is a very labour intensive technique and therefore not suitable for mass diagnosis. Moreover the very short duration of the viraemic period makes PCR of limited use. The discriminating ELISA test, which is less labour intensive, has the important drawback that antibodies are on average detected only 17.2 days post infection.

In conclusion it can be stated, based on the results of this experiment, that the tested vaccine does not fulfil the minimum demands of the Scientific Veterinary Committee since it does not prevent horizontal nor vertical spread of CSF virus.

Reference List

Ahrens, U., Kaden, V., Drexler, C., and Visser, N. (2000) Efficacy of the classical swine fever (CSF) markervaccine Porcilis® pesti in pregnant sows. Vet. Microbiol. 77, 83-97.

Anonymous (1980) Council directive 80/217/EEC. introducing Community measures for the control of classical swine fever. Official Journal of the European Communities 80/217/EEC, 11-23.

Anonymous (1997) The use of marker vaccines in the control of infectious diseases in particular classical swine fever, Report of the Scientific Veterinary Committee. Scientific Veterinary Committee of the European Commission.

Bouma, A., de Jong, M.C., Kimman, T.G. (1996) Transmission of two pseudorabies virus strains that differ in virulence and virus excretion in groups of vaccinated pigs. *A. J. of Vet. Res. 57*. 43-47.

de Jong, M.C. and Kimman, T. G. (1994) Experimental quantification of vaccine-induced reduction in virus transmission. *Vaccine 12,* 761-766.

de Smit, A.J., Bouma, A., de Kluijver, E. P., Terpstra, C., and Moormann, R. J. (2000) Prevention of transplacental transmission of moderate-virulent classical swine fever virus after single or double vaccination with an E2 subunit vaccine. *Vet. Q. 22,* 150-153.

Dewulf, J., Laevens, H., Koenen, F., Vanderhallen, H., Mintiens, K., Deluyker, H., de Kruif, A., (2000) An experimental infection with classical swine fever in E2 sub-unit marker-vaccine vaccinated and in non-vaccinated pigs. *Vaccine 19*, 475-482.

Dewulf, J., Laevens, H., Koenen, F., Mintiens, K., de Kruif, A. (2001) An experimental infection with classical swine fever virus in pregnant sows: transmission of the virus, course of the disease, antibody response and effect on gestation. *J. Vet. Med.. B, 48*, 583-591.

Elbers, A.R., Stegeman, A., Moser, H., Ekker, H. M., Smak, J. A., and Pluimers, F. H. (1999) The classical swine fever epidemic 1997-1998 in The Netherlands: descriptive epidemiology. *Prev.Vet.Med. 42,* 157-184.

Holm-Jensen, M. (1981) Detection of antibodies against hog holera virus and bovine viral diarrhoea in porcine serum. A comparative examination using CF, PLA, and NPLA assay. *A. Vet. Scan. 22,* 85-98.

Intervet (1997) Porcilis Pesti, Overview of information currently available. pp. 56-71.

Koenen, F., Lefebvre, J. (1994) Kinetics of an experimental infection with a classical swine fever (CSF) field isolate. In the Proceedings of the 3rd Congress of European Society of Veterinary Virology. 4-7 September 1994, Interlaken, pp. 322-326.

König, M., Lengsfeld, T., Pauly, T., Stark, R., and Thiel, H. J. (1995) Classical swine fever virus: independent induction of protective immunity by two structural glycoproteins. *J. of Virol. 69*, 6479-6486.

Kroese, A. H., de Jong, M. C. (2002) Design and analysis of transmission experiments, In preparation.

Laevens, H. (1999) Risk factors for the transmission of classical swine fever virus to herds in the close neighbourhood of an infected herd. In: Epizootiology of classical swine fever: Experimental Infections Simulating Field Conditions, and Risk Factors for Virus Transmission in the Neighbourhood of an Infected Herd. PhD thesis, Ghent, Ghent University. pp. 103 – 122.

McGoldrick A., Bensaude E., Ibata G., Sharp G., Paton D.J., (1999). Closed one-tube reverse transcription nested polymerase chain reaction for the detection of pestiviral RNA with fluorescent probes. *J. Virol. Methods 79*, 85-95.

Miry, C., Castryck, F., Koenen, F., Broers, A., and Segers, E. (1991) Quelques aspects de l'épizootie de peste porcine classique en Belgique en 1990. *Epidémiol.Santé Animal 20*, 23-32.

Moormann, R.J., Bouma, A., Kramps, J. A., Terpstra, C., and De Smit, H. J. (2000) Development of a classical swine fever subunit marker vaccine and companion diagnostic test. *Vet. Microbiol. 73,* 209-219.

Pensaert, M. and Van Reeth, K., Vaccines for swine. In: Veterinary Vaccinology, editors: Pastoret, P., Blancou, J., Vannier, P., Verschueren, C., Amsterdam, Elsevier; 1997. pp. 374-375.

Terpstra, C. (1988) Epizootiology of Hog-Cholera. In Classical swine fever and related viral infections. Editors: Liess, B. Martinus Nijhoff Publishing, Dordrecht, The Netherlands, pp. 201-216.

van Oirschot, J.T. (1988) Description of the virus infection. In Classical swine fever and related viral infections. Editors: Liess, B. Martinus Nijhoff Publishing, Dordrecht, The Netherlands, pp. 1-25.

van Oirschot, J.T. (1999) Classical swine fever. In Diseases of Swine, vol. 8,. Editors: Straw, B. E., D'Allaire, S., Mengeling, W. L., and Taylor, D. J. Ames, Iowa, Iowa State University Press, pp.159-172.

van Rijn, P.A., Bossers, A., Wensvoort, G., and Moormann, R. J. (1996) Classical swine fever virus (CSFV) envelope glycoprotein E2 containing one structural antigenic unit protects pigs from lethal CSFV challenge. *J. of General Virol. 77,* 2737-2745.

Vanthemsche, P. (1995) Classical swine fever 1993-1994 in Belgium. In: Proceedings of the 8th Annual Meeting of the Dutch Society for Veterinary Epidemiology and Economics 13 December 1995, Lelystad, p. 25

CHAPTER 4.4:

A COMPARATIVE STUDY FOR EMERGENCY VACCINATION AGAINST CLASSICAL SWINE FEVER WITH AN E2 SUB-UNIT MARKER VACCINE AND A C-STRAIN VACCINE.

Abstract

Two types of vaccines against CSF virus are commercially available at present: E2 sub-unit marker vaccines and the conventional live C-strain vaccines. To evaluate the potential use of both vaccines in the framework of an emergency vaccination scenario, 3 comparable experiments were carried out in which groups of weaner pigs, vaccinated with a marker vaccine or a C-strain vaccine, were challenged with CSF virus at 0, 7, and 14 days post vaccination (dpv).

Using the marker vaccine, the virus transmission was totally prevented when the challenge occurred 14 days post vaccination, resulting in a transmission ratio (R) of 0. When the challenge occurred 0 or 7 days post vaccination the R's were $+\infty$ and 3.5, respectively. The interval of 14 days between vaccination and prevention of virus transmission reduces the usefulness of the marker vaccine, unless a large neighbourhood is vaccinated.

Using the conventional vaccine, the virus transmission was already totally prevented when the challenge occurred on the same day as vaccination (R = 0). Therefore, this vaccine may be an additional tool for a rapid and efficient prevention of neighbourhood infections during CSF outbreaks.

Introduction

The control of classical swine fever (CSF) in the European Union (EU) has been based on a policy of non-vaccination and stamping-out since 1980. However, recent outbreaks have shown that the control of CSF in non-vaccinated populations through stamping out may be very expensive, particularly in areas with high pig densities (Koenen et al., 1996; Meuwissen et al., 1999). This is partially due to the large number of animals that are pre-emptively slaughtered when trying to cope with the virus spread in the neighbourhood of infected herds. Ethically, this strategy has become more and more debatable (Terpstra, 1998).

Although it is still not fully understood which routes of transmission are responsible for neighbourhood infections, it is clear that pre-emptive eradication of the neighbourhood of an infected herd is an effective and even a possibly indispensable measure in the control of a CSF epidemic in areas with high pig densities (Koenen et al., 1996; Staubach et al., 1997; Elbers et al., 1999). The purpose of this measure is to prevent major within-herd outbreaks in order to reduce the virus infection load in a neighbourhood. This reduced infection load subsequently results in a reduction of the between-herd virus transmission.

Theoretically, the same goal could be achieved by vaccination, instead of eradication of neighbouring herds. This should result in a decreased infectivity of infected vaccinated animals and a decreased susceptibility of not yet infected vaccinated animals (de Jong and Kimman, 1994). To be equally as efficient as the eradication strategy, it is essential that the interval between vaccination and onset of immunity (reduction of infectivity and susceptibility) is as short as possible.

There are two types of vaccines against CSF virus commercially available at present: E2 sub-unit marker vaccines and the conventional live C-strain vaccines.

To evaluate the potential use of both vaccines in an emergency vaccination scenario, 3 comparable experiments were carried out in which groups of weaner pigs, vaccinated with a marker vaccine or a C-strain vaccine, were challenged with CSF virus 0, 7, and 14 days post

vaccination (dpv). The clinical protection and the reduction of virus transmission, induced by both vaccines, were compared.

Materials and methods

Animals

In each of the 3 experiments, 32 clinically healthy, conventional weaner pigs originating from the same herd were used. These animals were checked for the absence of bovine viral diarrhoea (BVD) and CSF antigen and antibodies.

Virus

The isolate used for challenge exposure was originally obtained from the first infected herd of the 1993-1994 Belgian CSF epizootic. The isolate was verified to be free of African swine fever virus and BVD virus. By using a panel of monoclonal antibodies, Prof Ahl (Tübingen) characterised the isolate as antigenically similar to an isolate known as the 'souche Lorraine' (Koenen and Lefebvre, 1994) which has been described as a moderately virulent strain (Laevens, 1999). The virus was cultivated on PK_{15} cells and 2 passages were carried out. The titre was 10^3 median tissue culture infective doses ($TCID_{50}$ / ml).

Vaccine

In each experiment 2 vaccines were used:

1. Conventional C-Strain vaccine

The conventional vaccine used (Pestiffa®, Merial France) is the so-called Chinese strain or C-strain, which is a modified live vaccine, produced by serial passages in rabbits (Aynaud, 1988).

2. E2 sub-unit marker vaccine

The marker vaccine used (Porcilis® Pesti, Intervet International BV) consists of the E2 glycoprotein of the CSF virus strain Alfort/Tübingen. The glycoprotein was produced by means of a baculovirus stimulating insect cells to express the glycoprotein (Hulst et al., 1993).

Experimental design

All three experiments were set up in a similar way.
Upon arrival, 32 conventional weaner pigs of 12-15 kg were randomly allocated to 4 pens (8 pigs per pen) in two separated compartments (two pens per compartment). After an acclimatisation period of 7 to 14 days, all pigs of pens 1 and 2 (compartment A) were vaccinated with the marker vaccine, whereas all pigs of pens 3 and 4 (compartment B) were vaccinated with the C-strain vaccine. At zero (experiment A), seven (experiment B) and fourteen (experiment C) dpv, 2 randomly selected pigs per pen were challenged with virulent CSF virus by deep intramuscular injection (2 ml). Before the challenge exposure, the pigs were moved to a separate pen where they remained until six hours after challenge exposure. Before the reintroduction into their respective pens, all challenged pigs were washed with clean water. During the observation period (period between challenge exposure and end of the experiment), the health and infection status of the challenged and contact pigs was monitored. After the observation period all remaining pigs were euthanised.

Pens were always visited in the following order: compartment B (pen 4 $\rightarrow$ pen 3), compartment A (pen 2 $\rightarrow$ pen 1). Between visits of pens within the same compartment, gloves were changed and footwear was disinfected. Between the visits of compartments B and A, overalls, gloves and footwear were changed. All materials necessary for blood sampling, rectal temperature monitoring, cleansing of the pens, and feeding of the pigs were provided per pen.

Sample collection and clinical examination

During the acclimatisation period, clotted and heparinised blood samples were collected from all pigs upon arrival, and one week later. In the interval between vaccination and infection,

blood samples were collected 3 times a week, and during the observation period blood samples were collected every other day. Simultaneously with sample collection, all pigs were examined clinically. The following symptoms were recorded: liveliness (apathy), body condition (cachexia), coughing, conjunctivitis, diarrhoea, ataxia, and haemorrhages. Rectal temperature was recorded daily.
Tissue samples (tonsil, kidney, spleen, heart, liver) were collected from every pig that died or was euthanised.

Sample analyses

For virus isolation (VI) in blood or leukocytes, 100µl whole blood or 100 µl buffy coat was challenged in duplicate onto a non-confluent monolayer of PK_{15} cells cultured in multiwell plates (24 wells / plate). For VI in tissue samples, one cm^3 of each organ was homogenised into 9 ml minimal essential medium (MEM) and ground with an ultra-Turrax. After centrifugation for 10 min at 4000g, 300µl of the supernatant was challenged in duplicate onto a non-confluent monolayer of PK_{15} cells cultured in multiwell plates (24 wells / plate). After 48 hours, the cells were fixed with isopropanol and stained with a polyclonal fluorescein-conjugated anti-CSF immunoglobulin.

For antibody detection in serum, the virus neutralization (VN) test (Holm-Jensen, 1981) using the $Alfort_{187}$ strain and the Herd Check CSFV ELISA (Ab-ELISA) test (IDEXX, Scandinavia, Osterbybruk, Sweden) were used. The discriminating ELISA (D-ELISA) test (Chekit® CSF-Marker, Dr. Bommeli AG, Switzerland), identifying antibodies against the E^{rns} glycoprotein of the CSF virus, was used in the marker-vaccinated pigs to distinguish between antibody response of vaccinated and infected pigs.

Leukocyte count was carried out using the Coulter-Counter ZM (Analis).

Data analyses

The reproduction ratio (R), a measure of transmission of infection, and defined as the average number of new infections arising from one typical infectious case, was calculated numerically using the maximum likelihood estimator.

$$R = \max \prod_{i=1}^{n} F\left\langle X_i, R \middle| N, S_0, I_0 \right\rangle$$

$F(X_i,R \mid N,S_0,I_0)$ is the likelihood function for the observed value X_i. Where X_i is the total number of pigs that become infected, N, S_0, and I_0 are the total number of animals, the number of susceptible animals and the number of infectious animals at the beginning of the outbreak, respectively (Bouma et al., 1996). 95 % confidence intervals (CI) were constructed symmetrically around the estimated value of R (Bouma et al., 2000).
Fever was defined as a rectal temperature > 40,0°C. This is the one-sided upper limit (rounded off) of the 95% CI calculated on the average rectal temperature of all weaner pigs during all observations before the challenge exposure. Leukopenia was defined in a similar way. The one-sided lower limit (rounded off) of the 95% CI was equal to 12 000 cells/ml.

Periods during which a given clinical symptom occurred started with the first of at least two subsequent observations of a given clinical symptom, and ended with the first of at least two subsequent observations for which the given clinical symptom was absent. Periods of fever and leukopenia were defined in a similar way.

The duration of the interval between challenge exposure and a first positive blood sample, and the duration of the viraemia, detected with different diagnostic tests, were compared using a paired sample T-test (SPSS 10.0, Chicago, USA).

Results

Clinical symptoms and rectal temperature

✓ Experiment A (challenge = 0 dpv)

All challenged pigs developed severe clinical symptoms in the marker as well as in the C-strain-vaccinated pens (Table 1). Seven out of the eight challenged pigs died due to the CSF infection (Table 1). In the marker-vaccinated contact pigs, the clinical symptoms were less severe and were only present in a limited number of the pigs (Table 1). In the C-strain-vaccinated contact pigs, only one pig developed a number of clinical symptoms and eventually died.

Table 1: Summary of clinical symptoms in challenged and contact pigs in experiment A

	Marker vaccine		C-strain vaccine	
	Challenged	Contact	Challenged	Contact
Apathy	1/4 (8 days)*	1/12 (6 days)	1/4 (4 days)	0/12
Cachexia	2/4 (4 days)	1/12 (14 days)	2/4 (9 days)	1/12 (4 days)
Conjunctivitis	3/4 (12 days)	3/12 (13 days)	4/4 (16 days)	1/12 (6 days)
Diarrhoea	1/4 (6 days)	0/12	0/12	1/12 (4 days)
Ataxia	3/4 (8 days)	1/12 (4 days)	2/4 (11 days)	0/12
Mortality	4/4	3/12	3/4	1/12

* Average duration of the clinical symptom.

The average rectal temperature of the challenged and contact pigs in both the marker and C-strain-vaccinated groups is presented in Figure 1.

✓ Experiment B (challenge = 7 dpv)

In the marker-vaccinated pens, all challenged pigs became clinically ill. Symptoms that were most frequently observed were: fever, conjunctivitis, cachexia, apathy, and diarrhoea.

Eventually 3 out of the 4 challenged pigs died. In the contact pigs, the clinical symptoms were restricted to transient fever (n = 6) and conjunctivitis (n = 3) and no mortality occurred.
In the C-strain-vaccinated pens, none of the challenged or contact pigs became clinically ill or died. The average rectal temperature of the challenged and contact pigs in both the marker- and C-strain-vaccinated groups is presented in Figure 1.

✓ Experiment C (challenge = 14 dpv)

In pen 3 (C-strain vaccine) one pig was euthanised during the observation period because of a broken leg. Therefore there were only 5 contact pigs in this pen.

In the marker-vaccinated pens, 3 out of the 4 challenged pigs developed fever which lasted,on average, 4 days. None of the contact pigs developed fever.
In the C-strain-vaccinated pigs no fever was detectable in the challenged or contact pigs (Figure 1).
No other clinical symptoms or mortality were observed in either the marker or the C-strain-vaccinated pens.

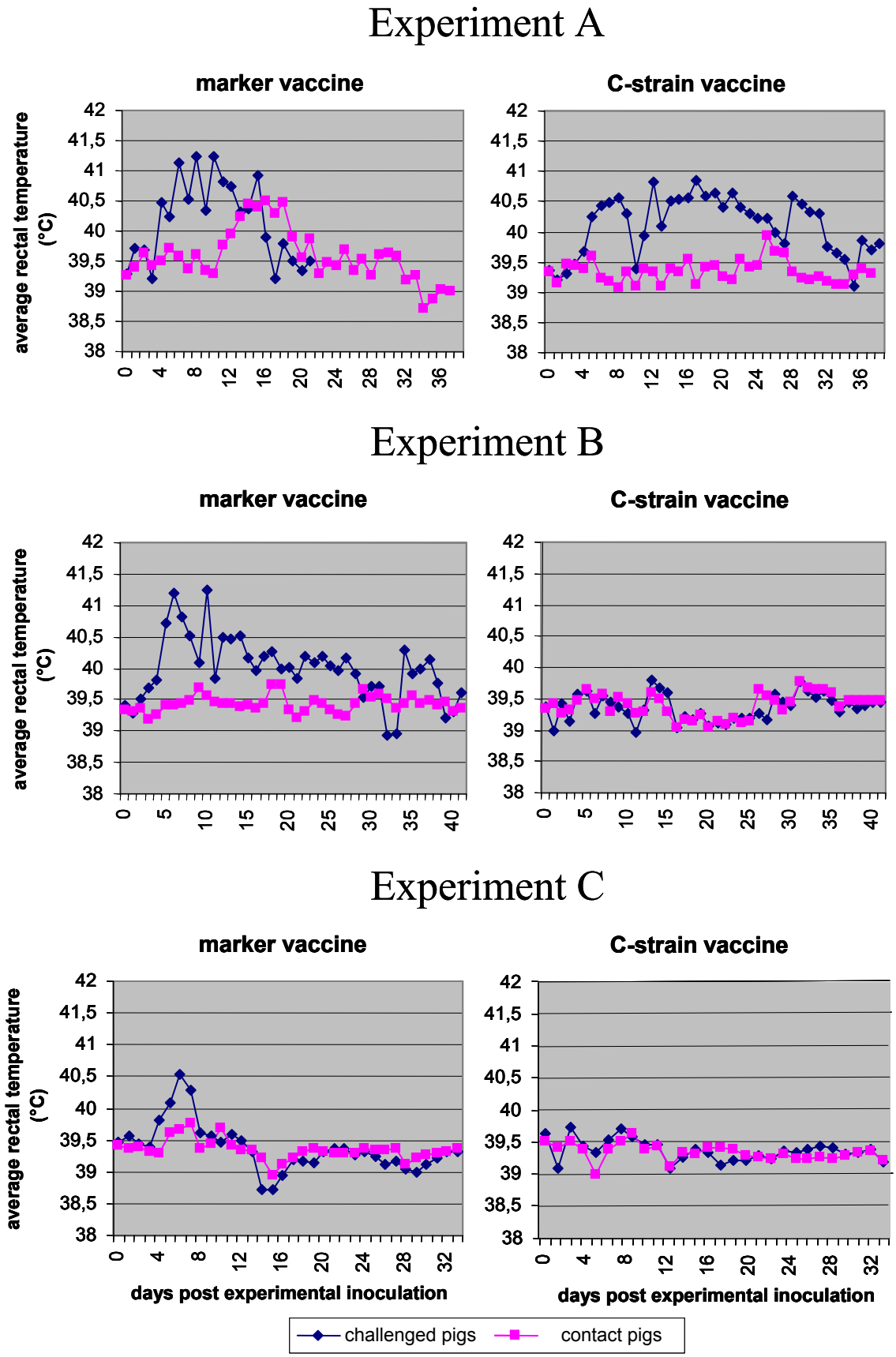


Figure 1: Average rectal temperature of challenged and contact pigs.

Virus isolation and leukocyte count

The results of VI in whole blood were highly comparable to the results of VI in leukocytes in all experiments. The average duration of the interval between challenge exposure and first positive sample was 0.26 days shorter, and the average length of the viraemic period was 0.58 days longer when using the VI in leukocytes in comparison to VI in whole blood. Both differences were not significantly different from 0 (n = 31, P = 0.16 and P = 0.09 respectively). Therefore, only the results of the VI in leukocytes are presented.

✓ Experiment A (challenge = 0 dpv)

In the marker-vaccinated pens, all challenged pigs and 10 out of the 12 contact pigs became viraemic (Table 2). In the C-strain-vaccinated pens, all challenged but none of the contact pigs became viraemic (Table 2).

✓ Experiment B (challenge = 7 dpv)

In the marker-vaccinated pens, all challenged pigs and 5 out of the 12 contact pigs became viraemic (Table 2). In the C-strain-vaccinated pens, none of the challenged or contact pigs became viraemic (Table 2).

✓ Experiment C (challenge = 14 dpv)

In the marker-vaccinated pens, all challenged pigs and none of the contact pigs became viraemic (Table 2). In the C-strain-vaccinated pens, none of the challenged or contact pigs became viraemic (Table 2).

Table 2: Results of VI and leukocyte count for the different experiments.

			VI in leukocytes			Leukocyte count		
			# positive pigs	First positive sample (SD)*	Average duration (SD)	# positive pigs	First positive sample (SD)	Average duration (SD)
Exp. A (challenge= 0 dpv)	Marker vaccine	Challenged (n = 4)	4	4 (0)	15 (2.6)	3	4.7 (1.2)	11.3 (7.0)
		Contact (n = 12)	10	13 (1.1)	11.4 (5.2)	9	13.8 (1.2)	10.7 (5.8)
	C-strain vaccine	Challenged (n = 4)	4	4 (0)	27.5 (7.9)	4	4 (0)	25 (5.8)
		Contact (n = 12)	0	/	/	0	/	/
Exp. B (challenge= 7 dpv)	Marker vaccine	Challenged (n = 4)	4	4 (0)	17 (12.9)	4	4.5 (1)	12.5 (6.6)
		Contact (n = 12)	5	17.6 (2.6)	2 (0)	6	14.5 (1.9)	5.5 (1)
	C-strain vaccine	Challenged (n = 4)	0	/	/	1	22 /	4 /
		Contact (n = 12)	0	/	/	2	29 (1.4)	6 (2.8)
Exp. C (challenge= 14 dpv)	Marker vaccine	Challenged (n = 4)	4	5 (1.2)	3.5 (1.9)	2	4 (0)	6 (0)
		Contact (n = 12)	0	/	/	0	/	/
	C-strain vaccine	Challenged (n = 4)	0	/	/	0	/	/
		Contact (n = 11)	0	/	/	0	/	/

* Average duration of the interval between challenge and first positive sample in days (SD = standard deviation)

Serology

The results of the VN and the Ab-ELISA were comparable. Positive results were, on average, 0.63 days (not significantly different from 0, n = 90, $P = 0.41$) earlier using the VN test in comparison to the Ab-ELISA. Therefore, only the results of the VN test are presented.

✓ Experiment A (challenge 0 dpv)

In the marker- as well as the C-strain-vaccinated pens, some of the challenged pigs did not react positively in the different serological test (Table 3). This is probably because they died shortly after the infection and before the serological response became detectable.
In the marker-vaccinated pens, all contact pigs reacted positively in the VN and the D-ELISA (Table 3).

✓ Experiment B (challenge 7 dpv)

All challenged and contact pigs reacted positively in the VN, in both the marker- and C-strain vaccinated pens (Table 3).

✓ Experiment C (challenge 14 dpv)

All challenged and contact pigs reacted positively in the VN, in both the marker- and C-strain vaccinated pens (Table 3).
In the D-ELISA, in the marker-vaccinated pens, only the challenged pigs became positive whereas the contact pigs remained negative.

Table 3: Serological results for the different experiments.

			Virus Neutralisation		Discriminating ELISA	
			# positive pigs	First positive sample (SD)*	# positive pigs	First positive sample (SD)
Exp. A (challenge = 0 dpv)	Marker vaccine	Challenged (n = 4)	2	17 days (1.4)	3	14 days (0)
		Contact (n = 12)	12	15 days (2.3)	12	22.7 days (2.6)
	C-strain vaccine	Challenged (n = 4)	3	13.3 days (2.3)	nd	nd
		Contact (n = 12)	12	13.5 days (2.7)	nd	nd
Exp. B (challenge = 7 dpv)	Marker vaccine	Challenged (n = 4)	4	10.5 days (5.3)	3	18 days (8.7)
		Contact (n = 12)	12	12 days (4.7)	11	27 days (6.3)
	C-strain vaccine	Challenged (n = 4)	4	3 days (1.2)	nd	nd
		Contact (n = 12)	12	4.8 days (2.8)	nd	nd
Exp. C (challenge = 14 dpv)	Marker vaccine	Challenged (n = 4)	4	12 days (6.9)	4	12.5 days (1.9)
		Contact (n = 12)	12	11.8 days (5.9)	0	/
	C-strain vaccine	Challenged (n = 4)	4	6 days (2.8)	nd	nd
		Contact (n = 11)	11	3.63 days (2.4)	nd	nd

* Average duration of the interval between challenge and first positive sample (SD = standard deviation)

nd: not done

Reproduction ratio

✓ Experiment A (challenge = 0 dpv)

Based on the results of the VI, it was concluded that 10 and 0 contact pigs became infected in the marker- and C-strain-vaccinated pens, respectively, resulting in an R of 2.88 (1.47-10.77) for the marker-vaccinated pens and an R of 0 (0-1.48) for the C-strain vaccinated pens (Table 4).
When the results of the D-ELISA were used to determine the number of infected contact pigs, it was found that in the marker-vaccinated pens all contact pigs became infected. This results in an R of +∞ (plus infinite) (1.87-+∞) (Table 4).

✓ Experiment B (challenge = 7 dpv)

Based on the results of the VI, it was concluded that 5 contact pigs became infected in the marker-vaccinated pens, resulting in an R of 1.03 (0.30-2.99).
In the C-strain-vaccinated pens, neither the challenged nor the contact pigs became infected. Therefore no reproduction ratio could be calculated (Table 4).
Although viraemia was only detectable in 5 marker-vaccinated contact pigs, 11 of them reacted positively in the D-ELISA. When these results are used to calculate the reproduction ratio, the overall R for the marker-vaccinated pens becomes 3.53 (1.62-10.94) (Table 4).

✓ Experiment C (challenge = 14 dpv)

Based on the results of the VI, it was found that all challenged pigs, but none of the contact pigs, became infected in the marker-vaccinated pens. This results in a reproduction ratio of 0 (0 – 1.49) (Table 4). The same result is obtained when the results of the D-ELISA are used to determine the number of infected contact pigs. In the C-strain-vaccinated pens, no viraemia was detectable in the challenged or the contact pigs and no R could be calculated (Table 4).

Table 4: Different values of the reproduction ratio's in the different experiments.

			# of contact infected pigs	R per pen (CI)**	Overall R (CI)
Exp. A (0 days)	Marker vaccine (VI)*	Pen 1	4	1.6 (0.5-6.9)	2.9 (1.5-10.8)
		Pen 2	6	+∞ (1.1-+∞)	
	Marker vaccine (D-ELISA)*	Pen 1	6	+∞ (1.1-+∞)	+∞ (1.9-+∞)
		Pen 2	6	+∞ (1.1-+∞)	
	C-strain vaccine (VI)*	Pen 3	0	0 (0-4.6)	0 (0-1.5)
		Pen 4	0	0 (0-4.6)	
Exp. B (7 days)	Marker vaccine (VI)	Pen 1	2	0.8 (0.2-5.8)	1.0 (0.3-3.0)
		Pen 2	3	1.2 (0.3-9.4)	
	Marker vaccine (D-ELISA)	Pen 1	6	+∞ (1.1-+∞)	3.5 (1.6-10.9)
		Pen 2	5	2.4 (0.7-7.5)	
	C-strain vaccine (VI)	Pen 3	/	/	/
		Pen 4	/	/	/
Exp. C (14 days)	Marker vaccine (VI)	Pen 1	0	0 (0-4.6)	0 (0-1.5)
		Pen 2	0	0 (0-4.6)	
	Marker vaccine (D-ELISA)	Pen 1	0	0 (0-4.6)	0 (0-1.5)
		Pen 2	0	0 (0-4.6)	
	C-strain vaccine (VI)	Pen 3	/	/	/
		Pen 4	/	/	/

* test used to determine the number of contact infections

** CI = 95 % confidence interval

Discussion

A crucial feature of an emergency vaccination program is that the vaccine used is able to stop the transmission of the virus from infected to contact pigs as soon as possible after vaccination. The purpose of this study was to quantify transmission of CSF virus among pigs vaccinated with an E2 sub-unit marker vaccine and pigs vaccinated with a conventional C-strain vaccine at different time intervals after vaccination.

The fact that no non-vaccinated control groups were included in the experiments does not influence the interpretation of the results, since the challenge model used was successful in all experiments. This can be derived from the fact that in each experiment at least one group of pigs became viraemic.

The horizontal virus transmission was fully prevented (R = 0) by the marker vaccine when the infection occurred 14 dpv. The reproduction ratio, calculated on the results of the VI test (R = 1.0), suggests that virus transmission was already largely reduced 7 dpv. However, these results are somewhat misleading since the results of the D-ELISA indicate that 11 instead of 5 contact pigs became infected, resulting in an R of 3.5. This leads to the conclusion that CSF virus transmission is not yet sufficiently reduced by the marker vaccine when the challenge exposure occurs 7 dpv. The difference between the VI and the D-ELISA may be due to the fact that a viraemia with a short duration may be missed when samples are only take every two days, or that the viraemia remains under the detection limit of the VI technique. In previous experiments it was also found that marker-vaccinated pigs did react positively in the D-ELISA without having had a detectable viraemia in VI (Dewulf et al., 2000; Dewulf et al., 2001). At 0 dpv, no reduction of the virus transmission was observed.
The results of these transmission experiments are highly comparable to the results of similar experiments described by Bouma et al. (2000).
The clinical protection in pigs vaccinated with the marker vaccine is present 7 to 10 days before virus transmission is prevented, since it has been observed that the contact pigs were already partially clinically protected in experiment A and fully protected it in experiment B.

In the conventional vaccinated pens, virus transmission was already fully prevented when the vaccination occurred on the same day of the challenge exposure, despite the fact that the challenged pigs developed severe viraemia and became clinically diseased. This remarkable finding may be explained by the fact that it takes, on average, 4 days for a challenged pig to become infectious (Terpstra, 1988). During these 4 days the contact pigs have already developed a sufficient immunity to prevent the infection. On the other hand, it needs to be stressed that the point estimate of R (=0), estimated based upon the results of these experiments is not significantly smaller than 1, indicating that the experiments should be repeated to increase the validity of the conclusion. Besides, it is impossible to confirm the virological results by a serological test, since there is no discriminating test available to make a serological differentiation between conventional-vaccinated and infected pigs. Therefore, it is possible that the contact pigs did become infected and consequently the R we calculated could be an underestimation of the reality. Nevertheless, if there was still a transmission of the virus, this did not lead to a detectable viraemia, nor any kind of clinical symptoms, indicating that it is unlikely that these contact pigs are themselves a source of further infectivity. When the challenge exposure occurred 7 or 14 dpv, even the challenged pigs did not develop viraemia and, by consequence, there was also no detectable transmission of the virus towards the contact pigs. All pigs vaccinated with the C-strain vaccine in the different experiments were also clinically protected, except the challenged pigs and one contact pig in experiment A. It remains indistinct whether the one clinically diseased contact pig in experiment A was a result of a CSF infection, since there was no detectable viraemia.

Our findings have important implications for the use of the different vaccines in an emergency vaccination scenario.

It is clear that the interval of 14 days between vaccination and full prevention of virus transmission is an important restriction when considering the use of a marker vaccine. Within-herd virus transmission still proceeds, and herds can still become infectious towards neighbouring herds during this interval. Therefore, a neighbourhood with a large radius would have to be vaccinated to adequately stop between-herd transmission. Further research is necessary to determine the minimal size of this neighbourhood. This large vaccination region is not necessarily a drawback because, provided that the marker-vaccinated animals can be undoubtedly distinguished from the infected animals, the vaccinated animals do not lose their market value when a marker vaccine is used.

Based upon the results of our experiments we can conclude that virus transmission was already prevented by the conventional C-strain vaccine from day 0 onwards. If these results are confirmed in further experiments, it would mean that in all situations where depopulation of an infected neighbourhood in one day is practically or logistically unfeasible, vaccination of the neighbourhood with the conventional vaccine, in anticipation of depopulation, is more beneficial than the current strategy. This measure would also reduce the time pressure and logistical restraints during an epidemic, since vaccinated neighbourhoods are no longer a danger for further virus transmission. The risk of virus spread by vaccination teams is an argument that is often mentioned against this strategy. However, even if a vaccination team introduced the virus in a herd, this would not lead to a major outbreak because it was found that the virus does not spread if the infection and the vaccination occur on the same day (experiment A).

Nevertheless, there are also some potential disadvantages to the strategy of emergency vaccination. In the past, as a result of the spread of the virus through infected needles, vaccination on already infected but still undetected farms led to major outbreaks (Terpstra and Robijns, 1977). Therefore, in an emergency vaccination strategy (marker vaccine or conventional vaccine), it is essential to impose hygienic measures such as the frequent changing of needles.

Conclusions

Our resulst indicate that the conventional C-strain vaccine prevents the virus transmission from day 0 post vaccination onwards. If these results are confirmed in further experiments, this vaccine may become an additional tool for the current eradication strategy. Unless a large neighbourhood is vaccinated, the 14 days interval between vaccination and prevention of virus transmission through a marker vaccine will limit its preventive effect on neighbourhood infections.

References

Aynaud, J.M. (1988) Principles of vaccination. In Classical swine fever and related viral infections. Editors: Liess, B. Martinus Nijhoff Publishing, Dordrecht, The Netherlands, pp. 165-180.

Bouma, A., de Jong, M.C., Kimman, T.G. (1996) Transmission of two pseudorabies virus strains that differ in virulence and virus excretion in groups of vaccinated pigs. A. J. of Vet. Res. 57. 43-47.

Bouma, A., de Smit, A. J., de Jong, M. C., de Kluijver, E. P., and Moormann, R. J. (2000) Determination of the onset of the herd-immunity induced by the E2 sub-unit vaccine against classical swine fever virus. *Vaccine 18*, 1374-1381.

de Jong, M.C. and Kimman, T. G. (1994) Experimental quantification of vaccine-induced reduction in virus transmission. *Vaccine 12,* 761-766.

Dewulf, J., Laevens, H., Koenen, F., Mintiens, K., & de Kruif, A. (2001) An E2 sub-unit marker vaccine does not prevent horizontal or vertical transmission of classical swine fever virus. *Vaccine 20,* 86-91

Dewulf, J., Laevens, H., Koenen, F., Vanderhallen, H., Mintiens, K., Deluyker, H., de Kruif, A., (2000) An experimental infection with classical swine fever in E2 sub-unit marker-vaccine vaccinated and in non-vaccinated pigs. *Vaccine 19*, 475-482

Elbers, A.R., Stegeman, A., Moser, H., Ekker, H. M., Smak, J. A., and Pluimers, F. H. (1999) The classical swine fever epidemic 1997-1998 in The Netherlands: descriptive epidemiology. *Prev.Vet.Med. 42,* 157-184.

Hulst, M.M., Westra, D. F., Wensvoort, G., and Moormann, R. J. (1993) Glycoprotein E1 of hog cholera virus expressed in insect cells protects swine from hog cholera. *J. of Virol. 67,* 5435-5442.

Koenen, F., Lefebvre, J. (1994) Kinetics of an experimental infection with a classical swine fever (CSF) field isolate. In the Proceedings of the 3rd Congress of European Society of Veterinary Virology. 4-7 September 1994, Interlaken, pp. 322-326

Koenen, F., Van Caenegem, G., Vermeersch, J.P., Vandenheede, J., and Deluyker, H. (1996) Epidemiological characteristics of an outbreak of classical swine fever in an area of high pig density. *Vet. Rec. 139*, 367-371.

Laevens, H. (1999) Risk factors for the transmission of classical swine fever virus to herds in the close neighbourhood of an infected herd. In: Epizootiology of classical swine fever: Experimental Infections Simulating Field Conditions, and Risk Factors for Virus Transmission in the Neighbourhood of an Infected Herd. PhD thesis, Ghent, Ghent University. pp. 103 – 122.

Meuwissen, M.P., Horst, S. H., Huirne, R. B., and Dijkhuizen, A. A. (1999) A model to estimate the financial consequences of classical swine fever outbreaks: principles and outcomes. *Prev. Vet. Med. 42,* 249-270.

Staubach, C., Teuffert, J, and Thulke, H. H. (1997) Risk Analysis and local spread mechanisms of classical swine fever. In the Proceedings of the 8th conference of the international society for veterinary epidemiology and economics. Juli 1997, Paris, France, pp. 06.12.1-06.12.3.

Terpstra, C. and Robijns, K. G. (1977) Experience with regional vaccination against swine fever in enzootic areas for limited periods using C-strain virus. *Tijds. Diergeneesk. 102,* 106-112.

Terpstra, C. (1988) Epizootiology of Hog-Cholera. In Classical swine fever and related viral infections. Editors: Liess, B. Martinus Nijhoff Publishing, Dordrecht, The Netherlands, pp. 201-216.

Terpstra, C. (1998) Preventive emptying: a compensation for a lack of training. *Tijds. Diergeneeskd.123,* 324-325.

CHAPTER 4.5:

GENERAL DISCUSSION

Introduction

When vaccination is considered as a control strategy for CSF, a first, major distinction needs to be made between generalised prophylactic vaccination and restricted emergency vaccination. Within the EU, there is an agreement that the ultimate goal in all member states remains to obtain official CSF-free status. This status can only be achieved when the following requirements are met: (i) no CSF has been detected for at least the preceding 12 months, (ii) an absence of CSF vaccinated pigs, (iii) vaccination against CSF has not been authorised for at least the preceding 12 months (Council Directive 80/1095/EEC). The reintroduction of generalised vaccination would make it impossible to achieve this status and is seen as a step backwards. Therefore, it is not considered for the moment and will most probably not be considered in the near future. This possibility will not further be elaborated in this discussion.

Emergency vaccination, in principle, is not in disagreement with the EU non-vaccination policy (Council Directive 2001/89/EC) when certain criteria are met: only in well-defined, restricted areas, only when an outbreak seems to run out of control, and with the agreement of the European Commission (Anonymous, 1994; Dahle and Liess, 1995).

The consequences of these emergency vaccinations for CSF status and for the export position of the region or country involved, largely depend on the type of vaccine used (conventional vaccine versus marker vaccine) and the destination of the vaccinated animals (destruction versus market). Regardless of which strategy is used, emergency vaccination will always be complementary to other control strategies such as eradication of infected herds, movement restrictions and hygienic measures. Therefore, the implementation of any vaccination strategy will never replace these basic control measures.

A number of possible vaccination scenarios will be discussed in the light of the results obtained in our experiments and the knowledge currently available in literature. The results of each scenario will be analysed and compared to the current control strategy in terms of epidemiological, economic, ethical and animal welfare consequences.

Market authorisation strategy

In the market authorisation strategy (MAS), as in all other emergency vaccination strategies, vaccination is applied to reduce the number of secondary outbreaks in the neighbourhood of an infected herd, through immunising this neighbourhood. The specific element of the MAS is that pig meat originating from vaccinated pigs is admitted to the (local or intra-community) market. This strategy can be applied using a conventional vaccine as well as a marker vaccine.

Conventional vaccine

The use of a conventional vaccine in such a strategy is regulated by the guidelines for CSF emergency vaccination (Anonymous, 1994) and the Council Directive 2001/89/EC. Following these guidelines, vaccination is divided in two phases. In the first phase, which may not take longer than 2 weeks, all pigs older than 2 weeks are vaccinated. In the second phase, all animals introduced in the vaccination area, as well as all new-born piglets, are vaccinated. This second phase lasts at least 12 months after the last outbreak in the region. The directive also stipulates that the vaccinated pigs can only be slaughtered for the local market and the country involved loses its official CSF free status during the entire vaccination period. This is a result of the fact that no serological distinction can be made between conventionally vaccinated and infected pigs. The official CSF free status can be regained, at the earliest, 6 months after the end of the vaccination campaign.

This strategy proved its merits during the 70^{ies} and 80^{ies} where it was used on several occasions with good results (Terpstra and Robijns, 1977; Bendixen, 1988). From epidemiological and ethical points of view, this strategy has many advantages since it is possible to eradicate the virus from a certain region in a period of one year, without pre-emptive slaughtering of neighbouring or contact herds. However, the movement restrictions and export ban of at least one and a half years have huge economic consequences, especially for exporting countries, so this strategy hasn't been used since the introduction of the vaccination stop in the EU (Terpstra, 1991; Anonymous, 1997; Moennig, 1998).

Marker vaccine

Theoretically speaking, serological discrimination between vaccinated and infected pigs is possible when a marker vaccine is used. Serological discrimination can verify whether or not vaccinated pigs or derived products have been in contact with the CSF virus, and makes intra-community trade possible. However, there is no current European legislation to regulate the use of marker vaccines in the control of CSF or to stipulate the possible trade restrictions involved. Therefore, it will be essential to convince all individual trade partners, inside as well as outside the EU, of the quality of the applied strategy and the discriminating diagnostic tests.

In a MAS, several parameters should be considered when evaluating the quality of a marker vaccine and its accompanying discriminating test: (i) prevention of infection after natural contact infections, (ii) reduction of horizontal as well as vertical virus transmission, (iii) duration of the interval between vaccination and onset of immunity, and (iv) characteristics of the diagnostic tests.

(i) In the two experiments described in chapters 4.2 and 4.3, vaccinated pigs were brought in contact with non-vaccinated, experimentally inoculated pigs. This set-up was deliberately chosen to evaluate the protective properties of the vaccine against natural contact infections. The results of both experiments clearly demonstrate that even after double vaccination with an E2 sub-unit marker vaccine, the vaccinated pigs are clinically, but not virologically, protected against natural contact infections. This means marker vaccination will not prevent infection of the vaccinated animals as long as sources of infection remain present. An example of such a persistent virus source may be the infected wild boar populations of several European countries (Laddomada, 2000).

(ii) In the experiment described in chapter 4.3, it was found that transplacental virus transmission still could occur in double vaccinated sows after a natural contact infection. This finding was recently confirmed by the results of a large-scale trial in several national swine

fever laboratories (NSFL) of the EU member states (Depner et al., 2001). This transplacental transmission may result in the birth of viraemic piglets, which can again infect other contact animals. Based on this mechanism, a smouldering epidemic may persist undetected in the population. Once the emergency vaccination is stopped, a re-ignition of the epidemic may occur. It is remarkable that the results obtained in the experiment described in chapter 4.3 and in the NSFL trial differ largely from the results obtained in other experiments, where it was found that vertical transmission was fully prevented after double vaccination with a marker vaccine (de Smit et al., 2000a). Even after a single vaccination, the transplacental transmission was largely reduced (de Smit et al., 2000a). The most obvious explanation is that the results are strongly influenced by the different virus strain used for challenge in the different experiments.

(iii) The length of the interval between vaccination and onset of immunity will determine the time before a reduction of the virus transmission is achieved. When the within-herd virus transmission is reduced or even prevented, the infectiousness of an infected herd will decrease. If the interval between vaccination and onset of immunity is short, herds can't become infectious anymore shortly after they have been vaccinated, and the first generation of secondary cases may already be prevented. In this case, only a small neighbourhood needs to be vaccinated to be able to stop the between-herd transmission through local spread. If the interval is longer, a herd that is infected shortly before or after vaccination may still become infectious, and only the second or third generation of secondary cases will be prevented. In such a situation, a larger vaccination region is necessary. Therefore, the interval between vaccination and onset of immunity determines the radius of the vaccination area. Further research is needed to translate this interval into the exact radius of the vaccination area. The minimal vaccination-challenge interval necessary to prevent horizontal transmission, when an E2 sub-unit marker vaccine is used (after single vaccination), is approximately 14 days (chapter 4.4). These results were also confirmed in several other experiments (Moormann et al., 2000; Uttenthal et al., 2001). As a result, within-herd virus transmission may proceed until 2 weeks after vaccination, and these herds may become infectious themselves. Based upon the data gathered during the 1990 CSF outbreak in Belgium and the 1993-94 outbreak in Germany, it was found that in 48% and 64% of the neighbourhood infections (1 km radius), the time elapsed between confirmation of an outbreak and confirmation of the nearest outbreak was less than 2 weeks (Roberts, 1995). In these cases, the immunity induced by the

marker vaccine is not able to prevent these herds becoming infectious. Therefore, a larger vaccination area (more than 1 km) needs to be imposed in order to prevent at least the second generation of neighbourhood infections.

(iv) The larger the vaccination area becomes, the more pigs that need to be vaccinated. Assuming that there are excellent diagnostic tools available to differentiate between vaccinated and infected pigs, thus identifying remaining sources of infection, this is not necessarily a problem since vaccinated animals do not lose their market value in the MAS. These diagnostic tools will be of utmost importance, since it has been described above that the marker-vaccination does not prevent infection and vertical virus transmission after a natural contact infection.

During outbreaks of CSF in unvaccinated populations, clinical diagnosis is the most important diagnostic tool for early detection of infections (de Smit et al., 1999; Elbers et al., 2002). In chapter 4.4 it was shown that the marker vaccine is capable of protecting the vaccinated pigs against the clinical course of the disease as soon as 7 days post vaccination, despite the fact that these pigs developed serious viraemia. Consequently, the clinical diagnosis becomes useless in a vaccinated population. Our experiments also found that viraemia in double vaccinated pigs was not detectable using the virus isolation (VI) technique, even though these pigs did seroconvert against the wild virus (chapter 4.2, 4.3, 4.4). Moreover, in the vaccinated gilts, transplacental infection of the offspring occurred. This is an undeniable proof that infectious virus did circulate in these sows. As a result, the only remaining tool for an early detection of infection in a marker-vaccinated population is the RT-PCR (chapter 4.2, 4.3). The use of this technique is laborious due to the complexity of the sample preparation and less suitable for large sampling procedures.

For the serological diagnosis, two different discriminating antibody ELISAs are available (*Chekit CSF-Marker®, Bommeli AG; Ceditest CSFV-E^{rns}®, ID-Lelystad)*. These tests are the only available laboratory tests that can be used on a large scale during a CSF outbreak. The experiments described in chapter 4.3 and 4.4 showed that the interval between infection and

the first positive result in the discriminatory ELISA was, on average, 17.2 and 14.1 days respectively. Thus, an infection will already be present for a relative long period before it becomes detectable. When considering the test characteristics of the discriminating ELISAs, the sensitivity as well as the specificity of the tests needs to be evaluated. In our experiment in vaccinated weaner piglets (chapter 4.2) it was found that 4 out of the 7 RT-PCR positive piglets were also positively detected by the discriminating ELISA, but one piglet was found positive in the discriminating ELISA without a positive RT-PCR diagnosis in the preceding weeks. Since the infection occurred late in the experiment it might be that the 3 remaining pigs would still have seroconverted if they had been followed for a longer period. In the experiments described in chapter 4.3 and 4.4 all animals that were infected also reacted positively in the discriminating ELISA test. In an extensive trial set-up to evaluate the test characteristics, and carried out by several NSFL, it was found that both commercially available discriminatory ELISAs were less sensitive (Chekit CSF-Marker®: 94.1%; Ceditest CSFV-E^{rns}®: 73.5%) than the conventional CSF antibody ELISAs (Floegel-Niesmann, 2001). If the discriminatory ELISAs were to be used on a herd basis, a CSF infected herd may still be detected if the number of blood samples taken is increased according to the limited sensitivity and the expected reduced prevalence (de Smit, 2000b). If the number of samples is increased, the number of false positive results will increase proportionally. Given the limited specificity of both tests (Chekit CSF-Marker®: 70.6% and 98%; Ceditest CSFV-E^{rns}®: 91.8% and 100% in reference and field sera respectively), a large number of false positive results will occur (Floegel-Niesmann, 2001; Moormann et al., 2000). These false positive results will hamper the serological screening tests since there is no confirmatory test available (Meuwissen et al., 1999). In some cases a diagnosis on herd level will be insufficient and the CSF status of the individual animals will need to be evaluated, e.g. pregnant sows or pigs for export. Given the limited sensitivity, diagnosis on the individual pig is impossible using the current available discriminatory ELISAs.

Summarising, it may be concluded that, using an E2 sub-unit marker vaccine, the horizontal transmission of the CSF virus can probably be prevented if a large enough area is vaccinated. This would result in a reduction of the number of pigs that need to be pre-emptively slaughtered, which is an improvement from an ethical point of view. However, the incomplete prevention of the vertical virus transmission involves the risk of an invisible slumbering of the

epidemic, which might result in an escape of the virus outside the vaccination region, or in a flare up of the epidemic when the vaccination is stopped. This would, of course, result in a further spread of the disease and undo the perceived advantages of the vaccination. Also the poor performances of the discriminating ELISAs will largely hamper the use of a marker vaccine. Therefore, it is questionable whether the MAS with a marker vaccine will be an epidemiological or ethical improvement on the current non-vaccination and eradication strategy.

Delayed destruction strategy

The delayed destruction strategy (DDS) is largely comparable to the current control strategy in the sense that all animals in the infected herds, the neighbouring herds and all dangerous contact herds are killed. The major difference is that in the DDS, all herds in the neighbourhood of an infected herd (1 km radius) are vaccinated first, but several weeks after this vaccination the herds are depopulated, and all meat coming from the vaccinated pigs is destroyed. A well-known example of this DDS is the control of the recent foot and mouth disease epidemic in the Netherlands (Bouma et al., 2001).

In the DDS, the prevention of the horizontal virus transmission and the interval between vaccination and onset of immunity are the two most important elements. They determine the radius of the area that needs to be vaccinated (see above) and, indirectly, the number of herds that are to be eradicated afterwards. The prevention of vertical transmission and the performances of the discriminating diagnostic tests are of lesser importance, since all vaccinated pigs are killed eventually. The DDS can be executed using a conventional or a marker vaccine.

Conventional vaccine

The results of the experiments described in chapter 4.4 show that, using a conventional C-strain vaccine, the virus transmission is already fully prevented when the challenge occurrs on the same day of vaccination. This indicates that the first generation of secondary cases has already been prevented. If these results are confirmed in new experiments, it can be expected

that vaccination of the neighbourhood (1 km radius) with a conventional vaccine will be at least as efficient as depopulation of the neighbourhood in the prevention of local virus transmission. The vaccinated pigs may remain alive for several weeks without being a risk for further virus spread. This will result in a significant gain of time, which will largely overcome the logistic restraints of killing large numbers of animals within a very short period of time. The time gain may also result in a reduction of the psychological pressure of all parties involved, leading to a more animal-friendly way of working during the depopulation of the herds. From the ethical point of view there is no direct improvement, since all herds in the neighbourhood of an infected herd will be eradicated. However, due to the efficient prevention of between herd transmission, and the reduced risk of virus spread during the depopulation of potentially infectious neighbouring herds, it may be expected that the overall size of the epidemic will be reduced using this strategy. A reduction of the total size of the epidemic will, of course, be an ethical as well as a financial improvement. A simulation model, which has been developed to evaluate the financial implications of different marker vaccination strategies (Mangen et al. 2001), could be used to more exactly forecast the epidemiological and economic effects of this strategy.

Because all vaccinated pigs are killed and destroyed in the DDS, the implementation of this strategy will not adversely affect the time of suspension of the "officially CSF free" status. An end screening could be applied to declare the region free again of CSF after the last vaccinated herd is destroyed.

There are also some disadvantages and possible hazards to the DSS with a conventional vaccine. The general disadvantages of each vaccination strategy are: a false sense of security which may result in a relaxation of other control and bio-security measures, a diversion of efforts from other control measures, risk of spread of the virus by vaccination teams and risk of a major outbreak in a vaccinated herd that was infected shortly before vaccination (Anonymous, 1997). The latter can occur when, by coincidence, one of the first vaccinated pigs is viraemic and, subsequently, the virus is carried around by the infected needle (Terpstra and Robijns 1977). There are also some disadvantages that are specifically related to the use of a conventional vaccine. All vaccinated herds will have to be totally quarantined to avoid

mixing of vaccinated and non-vaccinated animals, because vaccinated and infected pigs cannot be discriminated from each other. When using conventional vaccines, it is impossible to evaluate the number of secondary outbreaks, which occur in the vaccinated regions. Although this will not influence the course of the epidemic, this information is essential when, at the end of an outbreak, the efficacy of the control strategy needs to be evaluated.

Marker vaccine

While the marker vaccine starts to prevent the transmission 14 days post vaccination (chapter 4.4), only the second or third transmission generation will be prevented. This results in a large neighbourhood that needs to be vaccinated before the local virus transmission is stopped (see above). In a DDS this large neighbourhood is very harmful since it will result in a large number of herds that are to be eradicated. The most important advantage of the marker vaccine (distinction between vaccinated and infected animals) is not crucial in this strategy since all vaccinated animals are killed and destroyed eventually.

Therefore, it is believed that from the epidemiological point of view, the use of an E2 sub-unit marker vaccine in a DDS will not be more beneficial than the current eradication strategy.

General conclusions

It may be stated that the properties of the current available E2 sub-unit marker vaccine and the accompanying tests are inadequate to be a valid alternative for the current eradication strategy. Further research concerning the development of better (live) marker vaccines and diagnostics (chapter 4.1) is continuing. However, these new vaccines haven't been tested sufficiently to be able to evaluate their usefulness for CSF control strategies. The DDS using a conventional C-strain vaccine may be a useful improvement of the current eradication policy. The expected benefits of this strategy are a rapid prevention of neighbourhood virus transmission, which may result in a reduced total size of the outbreak and a reduction of logistic constraints (rendering capacity) and psychological pressure.

Reference List

Anonymous (1980) Council directive 80/1095/EEC. Laying down conditions designed to render and keep the territory of the Community free from classical swine fever. Official Journal of the European Communities 80/217/EEC.

Anonymous (1994) Report from the scientific veterinary committee on guidelines for a Classical Swine Fever emergency vaccination programme. VI/7389/94-EN.

Anonymous (1997) The use of marker vaccines in the control of infectious diseases in particular classical swine fever, Report of the Scientific Veterinary Committee. Scientific Veterinary Committee of the European Commission

Anonymous (2001) Council Directive 2001/89/EC on community measures for the control of classical swine fever. *Official Journal of the European Communities* 2001/89/EC.

Bendixen, H.J. (1988) Control of Classical Swine Fever. In Classical swine fever and related viral infections. Editors: Liess, B. Martinus Nijhoff Publishing, Dordrecht, The Netherlands, pp. 217-232.

Bouma, A., Elbers, A., Bartels, A.R.W., De Koeijer, A., Velleman, P., Moll, L., Dekker, A., Van der Wal, P., Pluimers, F., de Jong, M.C., (2001). The epidemic of Foot-and Mouth disease in the Netherlands in 2001. In the proceedings of the 14th congress of the Dutch society of veterinary epidemiology and economics. Wageningen, 12 December, pp. 9-16.

Dahle, J. and Liess, B. (1995) Assessment of safety and protective value of a cell culture modified strain "C" vaccine of hog cholera/classical swine fever virus. *Berl. und Münchr Tierärz. Wochenschrif. 108,* 20-25.

de Smit, A.J., Eble, P.L., de Kluijver, E.P., Bloemraad, M., Bouma, A., (1999). Laboratory decision-making during the classical swine fever epidemic of 1997-1998 in The Netherlands. *Prev. Vet. Med. 42*, 185-199.

de Smit, A.J., Bouma, A., de Kluijver, E. P., Terpstra, C., and Moormann, R. J. (2000a) Prevention of transplacental transmission of moderate-virulent classical swine fever virus after single or double vaccination with an E2 subunit vaccine. *Vet. Q. 22,* 150-153.

de Smit, A.J. (2000b) Laboratory diagnosis, epizootiology, and efficacy of marker vaccines in classical swine fever: a review. *Vet.Q. 22*, 182-188.

Depner, K.R., Bouma, A., Koenen, F., Klinkenberg, D., Lange, E., de Smit, H., and Vanderhallen, H. (2001) Classical swine fever (CSF) marker vaccine. Trial II. Challenge study in pregnant sows. *Vet.Microbiol. 83,* 107-120.

Elbers, A.R., Bouma, A., Stegeman, J.A., (2002). Quantitative assessment of clinical signs for the detection of classical swine fever outbreaks during an epidemic. *Vet. Microbiol. 85*, 323-332.

Floegel-Niesmann, G. (2001) Classical swine fever (CSF) marker vaccine. Trial III. Evaluation of discriminatory ELISAs. *Vet.Microbiol. 83,* 121-136.

Laddomada, A. (2000) Incidence and control of CSF in wild boar in Europe. *Vet. Microbiol. 73*, 121-130.

Mangen, M.J., Jalvingh, A.W., Nielen, M., Mourits, M.C., Klinkenberg, D., Dijkhuizen, A.A., (2001). Spatial and stochastic simulation to compare two emergency-vaccination strategies with a marker vaccine in the 1997/1998 Dutch Classical Swine Fever epidemic. *Prev. Vet. Med. 48*, 177-200.

Meuwissen, M.P., Horst, S. H., Huirne, R. B., and Dijkhuizen, A. A. (1999) A model to estimate the financial consequences of classical swine fever outbreaks: principles and outcomes. *Prev. Vet. Med. 42,* 249-270.

Moennig, V. (1998) Concluding remarks on the OIE symposium on classical swine fever. OIE symposium on classical swine fever, Birmingham, 9-10 July 1998.

Moormann, R.J., Bouma, A., Kramps, J. A., Terpstra, C., and De Smit, H. J. (2000) Development of a classical swine fever subunit marker vaccine and companion diagnostic test. *Vet. Microbiol. 73,* 209-219.

Roberts, M. (1995) Evaluation of optimal size of restriction zones in disease control with particular references to classical swine fever. In the Proceedings of meeting of the society for veterinary epidemiology and preventive medicine. 29-31 March 1995, Reading, UK, p.119.

Terpstra, C. (1991) Hog cholera: an update of present knowledge. *Br.Vet. J.* 147, 397-406.

Terpstra, C. and Robijns, K. G. (1977) Experience with regional vaccination against swine fever in enzootic areas for limited periods using C-strain virus. *Tijds. Diergeneesk. 102,* 106-112.

Uttenthal, A., Le Potier, M., Romero, L., De Mia, G. M., and Floegel-Niesmann, G. (2001) Classical swine fever (CSF) marker vaccine. Trial I. Challenge studies in weaner pigs. *Vet.Microbiol. 83,* 85-106.

CHAPTER 5:

SUMMARY

This thesis is based on the results of a number of experimental infections with classical swine fever (CSF) virus in both non-vaccinated and vaccinated pigs. With this data we hope to provide answers to the obstructions which interfere with the control of CSF. These obstructions occur in two different areas:

1. Empirically, during several CSF epidemics in densely populated livestock areas (e.g. Flanders), it has been found that outbreaks can only be controlled when all herds, situated either in the neighbourhood of an infected herd, or that have been in contact with an infected herd or region, are pre-emptively eradicated. This results in the slaughter of a large number of uninfected herds. Based on these findings, several questions arise: what are the exact between-herd transmission routes causing this virus spread, is it really necessary to pre-emptively eradicate all these herds, and is it possible to diagnose infected herds earlier?

2. Vaccination has been abandoned as a tool in the control of CSF since 1990. Due to growing criticism of the current control strategy and the development of new marker vaccines against CSF, interest in the control of CSF outbreaks through vaccination has been growing. These new marker vaccines, and the conventional vaccines, can be used in several different vaccination scenarios. The question is whether one of these scenarios may be more beneficial than the current eradication strategy.

Therefore, the specific aims of the study were (chapter 2):

1. To asses the possibility and importance of CSF virus transmission through different transmission routes such as airborne transmission, transmission through pets and rodents, and transmission through excretions.
2. To quantify the transmission of CSF virus in breeding herds and to evaluate how this influences the diagnosis.
3. To quantify the effect of vaccination with a marker vaccine on both horizontal and vertical virus transmission in growing and breeder pigs that have been exposed to a natural contact infection.

4. To assess the duration of the interval between vaccination and onset of immunity using an E2 sub-unit marker vaccine or a conventional C-strain vaccine.

The thesis is divided in two major parts. In the first part (Chapter 3), several aspects of the within- and between-herd transmission of CSF virus are studied and discussed.

The different routes of CSF virus transmission are reviewed in Chapter 3.1. The areas of incomplete or inconclusive knowledge are indicated.

In Chapter 3.2, the possibility of airborne transmission of CSF virus was studied, and the effect of compartmentalisation and air currents on this airborne virus transmission was evaluated. Therefore, 61 pigs were housed in an isolation unit with 3 compartments and 5 pens. Each compartment had its own ventilation system resulting in air currents from compartment A (pens 1-3) towards compartment B (pen 4), but not towards compartment C (pen 5). CSF virus was introduced by a challenge exposure of 1 pig in the middle pen (pen 2) of compartment A.
As it was found that the virus was able to spread from compartment A towards compartment B following the prevailing air currents, it was concluded that the possibility of airborne transmission of CSF was clearly demonstrated in this experiment. The absence of infection in pen 5, which was no different from pen 4 except for the ventilation system, indicates that the air currents influenced the virus spread. The compartmentalisation had a retarding effect on the virus transmission but did not prevent it.
It is difficult to extrapolate these experimental results into the field, and to assess the importance of airborne virus transmission between neighbouring herds. However, the combination of experimental proof and epidemiological indications of the occurrence of airborne transmission, indicate that it is very likely that airborne transmission of CSF virus plays a role in between-herd transmission. This is especially so in densely populated regions where pig herds are located close to each other.

The aim of the experiment, described in Chapter 3.3, was to examine whether CSF virus can infect pets or rodents and, as a result, lead to replication dependant virus dissemination in the neighbourhood of an infected herd. Therefore, 3 dogs, 3 cats and 4 rats were intra-nasally and orally challenged with high doses of CSF virus. After an observation period of 43 days all

animals were euthanised and blood and tissue samples were examined for the presence of CSF virus or antibodies against CSF virus. At the end of the experiment, all blood and tissue samples were negative both on virus isolation and on RT-PCR. Also no antibodies against CSF virus were found. During the whole observation period, no clinical symptoms were observed. The results of this experiment provide further evidence that dogs, cats or rats are unlikely to represent significant biological reservoirs.

In Chapter 3.4, the possibility of transmission of CSF virus through excretions of infected pigs was investigated under experimental conditions. Five pairs of pigs were individually challenged with CSF virus. Eight days after challenge, when all pigs had been viraemic for at least 3 days, the pens were depopulated. Twenty hours later, the same pens were restocked with 5 pairs of susceptible pigs that stayed in these pens for 35 days. During the first 3 weeks of the experiment the pens were neither cleaned nor disinfected.
None of the susceptible pigs, brought in contact with the excretions and secretions of the infectious pigs, became infected. This result indicates that CSF virus spread through excretions is of minor importance in the early stages of infection. Before extrapolating these findings to the field, further research is needed to increase the power of the conclusions, and to evaluate the effect of factors (eg virus strain, interval, temperature, ….) which may influence the outcome of the experiment.

The experiment described in Chapter 3.5 was set-up to quantify horizontal and vertical transmission of CSF virus among pregnant gilts. Therefore, 12 conventional gilts, housed in a sow-box housing system, were used. Two out of the twelve gilts, ten of which were pregnant, were challenged with the CSF virus. They became viraemic for the first time 6 days post inoculation (dpi). All contact gilts became viraemic between 18 and 21 days post inoculation. The basic reproduction ratio (R_0), a measure of virus transmission, was estimated according to the martingale estimator, to be 13.0. Moreover, it was found that the 2 experimentally inoculated gilts infected all contact gilts, despite the fact that no random contacts between gilts were possible. The pregnant gilts were infected between day 43 and 67 of gestation. In all cases vertical virus transmission occurred and this resulted in cases of abortion and/or mummification. The presence of a CSF infection was able to be diagnosed earlier and for a longer period when a leukocyte count or a RT-PCR was used, when compared to virus

isolation in whole blood ($p<0.05$). The observed clinical symptoms were atypical and highly variable between the gilts, which hampered clinical diagnosis.

The implications of the results found in the previously described experiments, for the control of CSF epidemics, are discussed in Chapter 3.6.

In the second part of this thesis (Chapter 4), the potential effects of vaccination on the epidemiology and control of CSF are studied and discussed.

First (Chapter 4.1), a review is given on the past use of vaccination against CSF, the reasons why vaccination was abandoned, and the development of new marker vaccines against CSF. Subsequently, the results of several experiments, conducted to evaluate different properties of the E2 sub-unit marker vaccine, are described and discussed.

In the first experiment (Chapter 4.2), the clinical and virological protection induced by an E2 sub-unit marker vaccine against CSF was examined in fattening pigs. For this purpose, 45 pigs were equally distributed over 3 adjacent pens of an isolation unit. There was only indirect (airborne) contact between pigs in the different pens. In pen 3, all pigs were vaccinated twice, the revaccination occurring after a 4 weeks interval. Pigs in pens 1 and 2 were not vaccinated. Then, 2 weeks after booster vaccination, 1 randomly selected pig in the middle pen was challenged with CSF virus. After the initial virus spread in the infected pen, all pigs in the non-vaccinated adjacent pen were infected. In the vaccinated pen, 7 out of 14 pigs became infected during the experiment. Survival analysis showed that virus transmission by direct and indirect contact was significantly ($p<0.001$) delayed in vaccinated pigs when compared to non-vaccinated pigs. In the non-vaccinated pens, over 40% of the pigs died and typical clinical signs were noticed. In the vaccinated pen, no mortality and no clinical symptoms were observed. It was concluded that, although double vaccination with an E2 sub-unit marker vaccine was able to prevent the clinical course of the disease, it was unable to prevent infection through indirect contact. It is believed that this will complicate the possible use of the vaccine in emergency vaccination programs.

The results of an experimental infection with CSF virus in marker-vaccinated gilts are described in Chapter 4.3. This experiment was conducted to examine the effect of vaccination

on horizontal and vertical virus transmission in pregnant gilts. Therefore, 12 gilts, housed in individual sow boxes, were used. Of the 12 gilts, 10 were double vaccinated with an E2 sub-unit marker vaccine. The 2 non-vaccinated gilts were challenged with CSF virus 2 weeks after the booster vaccination of the vaccinated gilts.

Within 10 days post inoculation, all vaccinated gilts became infected. Depending on the definition of the infectious period, 2 different estimates of R_0 were calculated (R_0 = 14.8 and 3.3), both significantly larger than 1 ($p<0.01$). In 3 out of the 8 vaccinated pregnant gilts, vertical virus transmission occurred, resulting in infected offspring.

Based on the results of this experiment, it was concluded that double vaccination with an E2 sub-unit marker vaccine only protects pregnant gilts from the clinical course of the disease but does not prevent horizontal or vertical spread of the CSF virus. This will largely complicate the use of the E2 sub-unit marker vaccine in breeding herds.

In Chapter 4.4, the two commercially available vaccines against CSF (E2 sub-unit marker vaccines and conventional live C-strain vaccine) were compared to evaluate their potential use in an emergency vaccination scenario. For this purpose, 3 comparable experiments were carried out in which groups of weaner pigs, singularly vaccinated with a marker vaccine or a C-strain vaccine, were challenged with CSF virus 0, 7, and 14 days post vaccination (dpv). The challenge occurred through inoculation of 2 randomly chosen pigs per pen with a wild virus strain. Using the marker vaccine, the virus transmission was totally prevented when the challenge occurred 14 days post vaccination, resulting in a transmission ratio (R_0) of 0. When the challenge occurred 0 or 7 days post vaccination the R_0's were $+\infty$ and 3.5, respectively. The interval of 14 days between vaccination and prevention of virus transmission will seriously reduce the usefulness of the marker vaccine, unless a large neighbourhood is vaccinated.

Using the conventional vaccine, the virus transmission was already totally prevented when the challenge occurred at the same day of vaccination (R_0 = 0). Therefore, this vaccine may be an additional tool for a rapid and efficient prevention of neighbourhood infections during CSF outbreaks.

The combined results of all the vaccination experiments, and the implications for potential vaccination scenarios, are discussed in Chapter 4.5.

CHAPTER 5:

SAMENVATTING

De basis van dit proefschrift wordt gevormd door een aantal experimentele infecties met het klassieke varkenspest (KVP) virus bij gevaccineerde en niet gevaccineerde varkens. Met behulp van de gegevens verzameld gedurende deze experimentele infecties, is gepoogd een antwoord te vinden op een reeks vragen en / of problemen die de bestrijding van KVP bemoeilijken. Deze vragen hebben betrekking op twee specifieke domeinen:

1. Gedurende verschillende grote uitbraken van KVP in varkens-dense gebieden in Europa (o.a. Oost- en West-Vlaanderen), werd empirisch aangetoond dat deze uitbraken enkel te bestrijden vielen indien de varkens van alle bedrijven die gelokaliseerd waren in de nabijheid van een geïnfecteerd bedrijf of die op één of andere manier in contact waren geweest met een geïnfecteerd bedrijf, preventief werden opgeruimd. Dit resulteerde in het afslachten van een groot aantal varkensstapels waarvan een grote meerderheid eigenlijk niet geïnfecteerd was. Uiteraard roept deze manier van handelen vele vragen op zoals: (1) welke zijn de concrete virustransmissieroutes die deze "tussenbedrijfstransmissie" veroorzaken, (2) is het wel noodzakelijk om al deze risicobedrijven preventief op te ruimen, en (3) bestaan er technieken om de geïnfecteerde bedrijven sneller en accurater te detecteren?
2. Sedert 1990 wordt geen gebruik meer gemaakt van vaccinatie als hulpmiddel bij de bestrijding van KVP. Als gevolg van de groeiende kritiek op de huidige bestrijding en de ontwikkeling van nieuwe markervaccins, bestaat er tegenwoordig hernieuwde interesse in de mogelijkheden van vaccinatie bij de bestrijding van KVP. Deze nieuwe markervaccins, maar ook de conventionele vaccins, kunnen in verschillende scenario's worden ingezet. De vraag is echter welke scenario's in aanmerking komen, en in hoeverre één of meerdere van deze scenario's efficiënter zou zijn dan de huidige bestrijdingsstrategie.

Meer specifiek werd in de twee delen van dit proefschrift gepoogd de volgende vragen te beantwoorden (Hoofdstuk 2):

1. Kan het KVP virus verspreid worden via aërogene transmissie, via honden, katten en ratten en via excreties van infectieuze dieren? En zo ja, wat is het belang van deze verschillende transmissieroutes?
2. Hoe snel verspreidt het KVP virus zich bij zeugen die gehuisvest zijn in individuele boxen? Wat is de invloed van virustransmissie onder deze omstandigheden op de moeilijke diagnostiek van KVP op zeugenbedrijven?

3. Wat is het effect van vaccinatie met een markervaccin op de horizontale en verticale virustransmissie bij zowel vleesvarkens als zeugen die werden blootgesteld aan een natuurlijke contact infectie?
4. Wat is de minimale lengte van het interval tussen vaccinatie en verhindering van virustransmissie, na enkelvoudige vaccinatie met een conventioneel - of een markervaccin?

Het eerste deel van dit proefschrift (Hoofdstuk 3) handelt over KVP virustransmissie.

In Hoofdstuk 3.1 wordt een overzicht gegeven van de verschillende mogelijke manieren van KVP virustransmissie. Hierbij wordt ook aangeduid over welke virustransmissieroutes er onvoldoende gegevens beschikbaar zijn om de mogelijkheid van voorkomen en het belang ervan correct te kunnen inschatten.

De aërogene virustransmissie werd onderzocht in het experiment beschreven in Hoofdstuk 3.2. Daartoe werd gebruik gemaakt van 61 biggen die werden ondergebracht in een isolatiestal met 5 hokken en 3 compartimenten. Ieder compartiment beschikte over een eigen ventilatiesysteem waardoor het mogelijk was om specifieke luchtstromen te doen ontstaan. Op die manier werd ervoor gezorgd dat er een luchtstroom was van compartiment A (hokken 1 tot en met 3) naar compartiment B (hok 4), maar niet naar compartiment C (hok 5). Het virus werd geïntroduceerd door middel van een experimentele inoculatie van één dier in het middelste hok (hok 2) van compartiment A. In de loop van het experiment raakten eerst alle dieren in compartiment A geïnfecteerd en vervolgens ook die in compartiment B. De dieren in compartiment C werden niet geïnfecteerd. Hieruit kan worden besloten dat aërogene transmissie van het KVP virus wel degelijk mogelijk is. Bovendien werd aangetoond dat de aërogene transmissie verliep volgens de dominante luchtstromen en dat compartimentering een vertragend maar geen verhinderend effect had. Uiteraard is het moeilijk om op basis van experimentele resultaten uitspraken te doen over het belang van de aërogene verspreiding in het veld, maar de combinatie van experimenteel bewijs en epidemiologische indicaties uit het veld, duiden hoe langer hoe meer aan dat het zeer waarschijnlijk is dat aërogene transmissie een rol speelt is de verspreiding van het KVP virus tussen nabijgelegen bedrijven.

Het doel van het experiment beschreven in Hoofdstuk 3.3, was na te gaan in hoeverre honden, katten en ratten kunnen geïnfecteerd geraken door het KVP virus en vervolgens het virus kunnen uitscheiden en verspreiden. Hiertoe werden 3 honden, 3 katten en 4 ratten intra-nasaal en oraal geïnfecteerd met een hoge dosis KVP virus. Na een observatie periode van 43 dagen werden alle dieren geëuthanaseerd en werden bloed- en weefselstalen verzameld en onderzocht op de aanwezigheid van het KVP virus en/of antistoffen tegenover het KVP virus. Op het einde van het experiment bleek dat er zowel met behulp van de virusisolatie als de RT-PCR geen virus kon worden gedetecteerd. Er werden geen antistoffen tegenr het KVP virus teruggevonden. De resultaten van dit experiment geven bijkomende informatie die erop wijst dat het onwaarschijnlijk is dat honden, katten en ratten een biologisch reservoir vormen voor het KVP virus.

In Hoofdstuk 3.4 wordt de mogelijkheid van transmissie van het KVP virus via excreties van infectieuze varkens onderzocht onder experimentele omstandigheden. Hiertoe werden 5 paar varkens gehuisvest in aparte hokjes. Alle varkens werden individueel geïnoculeerd met het wild KVP virus. Acht dagen na inoculatie, op het moment dat alle varkens reeds minstens gedurende 3 dagen viraemisch waren, werden de hokken ontruimd. Twintig uur na deze ontruiming werden dezelfde hokken opnieuw bevolkt met 5 paar gevoelige varkens die gedurende 35 dagen in deze hokken aanwezig bleven. Gedurende de eerste 3 weken van het experiment, en dus ook tussen de ontruiming en de herbevolking, werden de hokken noch gereinigd noch ontsmet. Uit de resultaten van dit experiment bleek dat geen enkel van de gevoelige contact dieren geïnfecteerd werd. Hieruit kan worden geconcludeerd dat de verspreiding van het KVP virus via de excreties en secreties van infectieuze dieren van beperkt belang is in het beginstadium van de infectie. Vooraleer deze verrassende resultaten worden geëxtrapoleerd naar het veld en er mogelijke conclusies worden aan verbonden, is meer onderzoek noodzakelijk om de betrouwbaarheid van deze bevindingen te verhogen en om de invloed van verschillende factoren (o.a. virus stam, interval infectie - depopulatie, omgevingstemperatuur,….) op het resultaat, te onderzoeken.

Het experiment dat wordt beschreven in Hoofdstuk 3.5, werd opgezet om de horizontale en verticale virustransmissie bij drachtige gelten te onderzoeken en te kwantificeren. Daartoe werden 12 conventionele gelten, waarvan er 10 drachtig waren, gehuisvest in zeugenboxen. Twee van de 12 dieren werden experimenteel geïnoculeerd met het wilde KVP virus. Deze

twee gelten werden viraemisch vanaf dag 6 na inoculatie. Alle 10 contact dieren werden op hun beurt voor het eerst viraemisch tussen 18 en 21 dagen na inoculatie. De reproductie ratio (R_0), een kwantitatieve maat voor de virustransmissie, werd geschat op 13 (martingale schatter). Bovendien kon uit de resultaten worden afgeleid dat de twee geïnoculeerde varkens alle andere dieren hadden geïnfecteerd, niettegenstaande het feit dat er geen random contact mogelijk was tussen de gelten. De drachtige gelten werden geïnfecteerd op een moment dat ze tussen de 43 en 67 dagen drachtig waren. Bij alle gelten werd er verticale virusoverdracht waargenomen. Dit resulteerde in gevallen van abortus en/of mummificatie. De aanwezigheid van het KVP virus kon eerder en gedurende een langere tijd waargenomen worden wanneer gebruik werd gemaakt van leukocytentelling of de RT-PCR, in plaats van de virusisolatie techniek ($p<0.05$). De klinische symptomen die werden waargenomen waren atypisch en zeer variabel, wat de klinische diagnostiek sterk bemoeilijkte.

De implicaties van deze resultaten voor de bestrijding van KVP onder veld omstandigheden worden bediscussieerd in Hoofdstuk 3.6.

In het tweede deel van dit proefschrift (Hoofdstuk 4), wordt ingegaan op de mogelijke effecten van vaccinatie op de epidemiologie en bestrijding van KVP.

In Hoofdstuk 4.1 wordt een overzicht gegeven van de ontwikkeling en het gebruik van verschillende vaccins tegen KVP. Verder, wordt ingegaan op de redenen waarom vaccinatie werd afgeschaft in de EU en wordt de ontwikkeling van nieuwe (marker) vaccins tegen KVP besproken. Ook worden de resultaten van verschillende experimentele infecties met het E2 sub-unit markervaccin beschreven en toegelicht.

In Hoofdstuk 4.2 wordt een experiment beschreven dat werd uitgevoerd om de klinische en virologische bescherming, die wordt geïnduceerd door een dubbele vaccinatie met een E2 sub-unit markervaccin bij vleesvarkens, te evalueren. Hiertoe werden 45 vleesvarkens verdeeld over 3 naast elkaar gelegen hokken. Tussen de dieren uit de verschillende hokken was er enkel indirect (aërogeen) contact mogelijk. In hok 3 werden alle varkens dubbel gevaccineerd (boostervaccinatie 4 weken na primo vaccinatie) met het E2 sub-unit markervaccin. De varkens in de hokken 1 en 2 werden niet gevaccineerd. Twee weken na de boostervaccinatie werd één random geselecteerd varken uit het middelste hok (hok 2)

experimenteel geïnoculeerd met het wilde virus. Na de initiële virusverspreiding in het geïnfecteerde hok, werden ook alle varkens in het niet gevaccineerde naburige hok geïnfecteerd. In het gevaccineerde hok werden 7 van de 14 dieren geïnfecteerd in de loop van het experiment. Met behulp van survival analyse kon worden aangetoond dat virustransmissie via direct en indirect contact significant ($p<0.001$) werd vertraagd in het gevaccineerde hok in vergelijking met het niet gevaccineerde hok. In de niet gevaccineerde hokken stierven meer dan 40% van de dieren ten gevolge van de KVP infectie, terwijl in het gevaccineerde hok geen mortaliteit noch klinische symptomen werden opgemerkt. Op basis van deze resultaten werd besloten dat een dubbele vaccinatie met een E2 sub-unit markervaccin in staat was het klinisch verloop van een KVP infectie te verhinderen, maar niet in staat was te verhinderen dat de dieren geïnfecteerd raken door indirect contact. Deze bevindingen kunnen een belemmering zijn voor het gebruik van het E2 sub-unit markervaccin.

In het experiment beschreven in Hoofdstuk 4.3, werd het effect van een dubbele vaccinatie, met een E2 sub-unit markervaccin, op de horizontale en verticale virustransmissie onderzocht, bij drachtige gelten. Daartoe werden 12 gelten gehuisvest in individuele boxen. Tien van de 12 dieren werden dubbel gevaccineerd met een E2 sub-unit markervaccin. De twee niet gevaccineerde gelten werden experimenteel geïnoculeerd met het wilde virus twee weken na de booster vaccinatie. Binnen de tien dagen na inoculatie raakten alle gevaccineerde gelten geïnfecteerd met het wilde virus. Afhankelijk van de definitie van de infectieuze periode, werden 2 verschillende reproductie ratio's (R) berekend: $R_0 = 14.8$ en $R_0 = 3.3$. Beide zijn significant ($p<0.01$) groter dan 1 wat aanduidt dat de infectie verder zal spreiden. In drie van de acht drachtige gevaccineerde gelten werd ook verticale virustransmissie waargenomen met geïnfecteerde foeti als gevolg. Gedurende het ganse verloop van het experiment werden geen klinische symptomen waargenomen bij de gevaccineerde gelten. Gebaseerd op deze resultaten kan besloten worden dat vaccinatie met een E2 sub-unit markervaccin in staat is om het klinisch verloop van de infectie te verhinderen maar niet in staat is om de horizontale of verticale virustransmissie tegen te gaan. Dit is uiteraard een belangrijke beperking voor het gebruik van het vaccin onder veldomstandigheden.

In Hoofdstuk 4.4, wordt een experiment beschreven waarbij de twee commercieel beschikbare vaccins tegen KVP (E2 sub-unit markervaccin en het conventionele levende C-stam vaccin), worden vergeleken in functie van hun mogelijke inzetbaarheid voor noodvaccinatie

scenario's. Hiertoe werden 3 gelijkaardige experimenten opgezet waarbij telkens twee maal twee groepen van acht gespeende biggen enkelvoudig werden gevaccineerd met het conventionele vaccin of het markervaccin. Telkens werden 2 random gekozen varkens per groep experimenteel geïnoculeerd met het wilde virus op respectievelijk 0, 7 en 14 dagen na vaccinatie. Vervolgens werd de verspreiding van het virus en het klinisch verloop van de infectie nagegaan. Met het markervaccin, werd de horizontale virustransmissie volledig verhinderd vanaf 14 dagen na vaccinatie, wat resulteerde in een reproductie ratio (R) van 0. Als de infectie plaatsvond op 0 en 7 dagen na vaccinatie kon er wel nog virustransmissie plaats vinden, resulterend in R's van respectievelijk $+\infty$ en 3.5. Het minimale interval van 14 dagen tussen vaccinatie en het verhinderen van virustransmissie is natuurlijk een belangrijke beperking voor het gebruik van het markervaccin, tenzij een grote regio wordt gevaccineerd. Met behulp van het conventionele vaccin, wordt de virustransmissie reeds verhinderd wanneer de vaccinatie en de infectie op dezelfde dag plaatsvinden (R = 0). Daardoor kan het conventionele vaccin een bijkomend middel zijn voor een snelle en efficiënte verhindering van buurtinfecties. Uiteraard kan bij gebruik van het conventionele vaccin geen onderscheid gemaakt worden tussen gevaccineerde en geïnfecteerde dieren waardoor alle gevaccineerde dieren uiteindelijk zullen moeten opgeruimd worden.

In Hoofdstuk 4.5 worden de resultaten bekomen in de verschillende experimenten van Hoofdstuk 4 bediscussieerd en wordt aangeduid wat de implicaties zijn van deze bevindingen voor het mogelijke gebruik van vaccinatie bij de bestrijding van KVP.

Dankwoord

Zoals zo velen vatte ik de studies diergeneeskunde aan met de vaste overtuiging om, Dr. Vlimmen achterna, de praktijk in te gaan. Nu tien jaar later ben ik allesbehalve een Dr. Vlimmen geworden en spendeer ik het merendeel van mijn tijd in de boeken of achter mijn computer. Maar wat bijzonder is, is dat ik het nog leuk vind bovenop. Het uitspitten van een onderwerp tot op de bodem en het steeds kritisch in vraag stellen van resultaten van anderen maar vooral van jezelf blijkt uitermate boeiend te zijn. Het maken van dit proefschrift vond ik dan ook een zeer verrijkende ervaring.

Het is passen om aan het eind van dit proefschrift iedereen te danken die op één of andere manier heeft meegewerkt aan het welslagen ervan.

Prof. Dr. A. de Kruif, als promotor van dit werk wil ik u bedanken voor het vertrouwen dat u schonk in mij en voor de positieve ingesteldheid waarmee u mij begeleid hebt. U vier jaar bezig te zien is niet enkel een professionele maar ook een menselijke verrijking.

Bijzondere woorden van dank gaan natuurlijk uit naar de twee co-promotors van dit werk Dr. F. Koenen en Dr. H. Laevens die mij dagdagelijks met raad en daad hebben bij gestaan bij de uitvoering van dit onderzoek.

Frank, van u heb ik geleerd hoe varkenspest in mekaar zit en wat kan en wat niet kan met een virus. Uw nuchtere kijk op de zaken en uw grote ervaring met varkenspest, in het labo maar ook in het veld, heeft vaak de noodzakelijke nuances aangebracht die ik in mijn enthousiasme soms over het hoofd zag. Ook dank ik u voor de vele uren die je hebt gespendeerd aan het uitvoeren van analyses en het aflezen van platen, goed wetende dat uw tijd vaak schaars is.

Hans, ik heb het onschatbare geluk gehad om te mogen werken op een project waar jij voorheen reeds 3 jaar mee bezig was geweest en dus gebruik heb kunnen maken van alle know-how die jij reeds had opgebouwd. Naast uw kennis van varkenspest heb je ook uw passie voor epidemiologie op mij overgedragen. Ik heb het gevoel dat ik in de laatste vier jaar naast een goede leermeester ook een goede vriend heb leren kennen en hoop dan ook van harte dat we in de toekomst nog veel zullen kunnen samenwerken. Zonder overdrijven mag

gerust gesteld worden dat een zeer groot deel van de verdienste van dit proefschrift eigenlijk u toekomt, waarvoor dank.

Ook Dr. K. Mintiens wil ik danken voor de vele hulp bij het opzetten en uitvoeren van dit project en het kritisch nalezen van al mijn schrijfsels.

Prof. Dr. H. Deluyker wil ik danken omdat hij mee gestaan heeft aan de wieg van dit project en mij in de juiste richting heeft gelanceerd.

Uiteraard kan een dergelijk onderzoek niet slagen zonder de technische hulp van velen. Daarom ben ik zeer veel dank verschuldigd aan de medewerkers van het CODA, zowel in het proefstation in Machelen als in het laboratorium van Dr. Koenen, voor de hulp bij de ontelbare bloednames en labo analyses, vaak in weekends en gedurende vakantie periodes.

Een bijzonder woordje van dank ook aan Prof. Dr. M. Pensaert. U bent van bij de aanvang betrokken geweest bij dit project als extern adviseur. En alhoewel uw kritiek vaak scherp was, heb ik snel moeten ondervinden dat ze ook steeds terecht was en dat heeft zeker bijgedragen tot de kwaliteit van dit werk. Weten dat een kritische geest uw werk zal beoordelen houdt een mens scherp.

Ook Dr. M. Nielen zou ik willen danken voor de kritische en waardevolle adviezen die hebben bijgedragen bij het finaliseren van dit werk.

Uiteraard wil ook alle overige leden van de examencommissie danken: Prof. Dr. M. Verdonck, Prof. Dr. P. Deprez, Prof. Dr. R. Ducatelle, Prof. Dr. P. Vanthemsche en Prof. Dr. L. Duchateau.

Dr. M. Corijn, Sharon Evans en mijn vader verdienen veel dank voor het zorgvuldig nalezen en verbeteren van al mijn teksten.

Dit onderzoek werd financieel gesteund door het bestuur voor onderzoek en ontwikkeling van het voormalige Ministerie van landbouw en het huidige Ministerie van Volksgezondheid en door de varkenssector zelf via het begrotingsfonds voor de kwaliteit van dieren en dierlijke

producten, waarvoor dank. Het financieren van wetenschappelijk onderzoek, waarvan de goede afloop niet steeds op voorhand kan voorspeld worden, getuigd mijn inziens van een goed en toekomstgericht beleid van zowel de overheid als de sector. Ik hoop dan ook van harte dat dit in de toekomst met onverminderde inspanningen en enthousiasme wordt verder gezet. Ook Dr. X. Van Huffel wil ik danken voor het vertrouwen in ons onderzoek en voor de begeleiding van het project.

Prof. Dr. M. de Jong, Prof. Dr. A. Stegeman, Dr. M. Nielen, Dr. K. Frankena, Dr. C. Kruitwagen en alle andere lesgevers en medestudenten van de Masters opleiding in Veterinaire epidemiologie en economie wil ik danken voor de vele epidemiologische en statistische kennis die ze mij bijbrachten zodat het de moeite waard was om gedurende bijna twee jaar te pendelen tussen Merelbeke en Utrecht.

Tevens dank ik alle collega's en medewerkers van de vakgroep voor de gezellige werksfeer en voor de directe of indirecte bijdrage die elk op zijn manier heeft geleverd voor het tot stand komen van dit proefschrift. Bedankt voor het geven van goede raad, het verbeteren van teksten, het overnemen van diensten, de bemoedigende woorden, de interesse,…… . Alle jonge onderzoekers die aan hun doctoraat aan het werken zijn wens ik zeer veel inspiratie en doorzettingsvermogen toe.

Mijn oprechte dank gaat ook uit naar mijn ouders omdat deze mij steeds enthousiast gesteund hebben, zowel tijdens mijn studies als tijdens mijn onderzoek. Jullie interesse voor mijn werk was steeds een grote motivatie.

Ook mijn schoonvader wil ik in het bijzonder vernoemen omdat hij mij steeds kon bevoorraden met de allerlaatste informatie over wat er om gaat in de landbouwsector. Ook voor een onderzoeker is het niet slecht om op de hoogte te zijn van wat er zich in het veld afspeelt en te weten wat de prijs van de varkens is.

Trees, jouw wil ik bedanken om er steeds te zijn als het nodig was. Maar bovenal wil ik je danken voor onze fantastische zoon en voor de goede zorgen waarmee je hem steeds omringt. Ik zie je graag.

Tot slot nog eens merci aan alle vrienden en familie die er voor zorgen dat het leven goed en gezellig is en diegene die aan dit dankwoord ontsnapt zouden zijn.

Jeroen Dewulf.

Personalia

Jeroen Dewulf werd op 1 maart 1974 geboren te Gent. Na het behalen van het diploma hoger secundair onderwijs aan het Don Bosco college te Zwijnaarde (Wetenschappelijke B), begon hij in 1992 met de studie Diergeneeskunde aan de Universiteit Gent. Hij behaalde in 1998 het diploma van Dierenarts met onderscheiding.

Onmiddellijk daarna trad hij in dienst als wetenschappelijk medewerker bij de vakgroep Voortplanting, Verloskunde en Bedrijfsdiergeneeskunde. Sedert 1 januari 1999 werkt hij binnen deze vakgroep aan een onderzoeksproject getiteld "epidemiologie, diagnose en controle van klassieke varkenspest en mogelijke toepassingen van een marker vaccin". Dit onderzoek loopt in samenwerking met het CODA en wordt gefinancierd door het Ministerie van middenstand en landbouw en het Begrotingsfonds voor de gezondheid en de kwaliteit van de dieren en de dierlijke producten. Tussen 2000 en 2002 volgde hij de opleiding "Master of Science in Veterinary Epidemiology and Economics" aan de Faculteit Diergeneeskunde van de Universiteit Utrecht, waar hij als meest verdienstelijke student het diploma van Master of Science "cum laude" behaalde. In 2002 behaalde hij tevens het getuigschrift van de doctoraatsopleiding in de diergeneeskundige wetenschappen.

Jeroen Dewulf is auteur of mede-auteur van 17 publicaties in internationale en nationale tijdschriften en nam actief deel aan verschillende nationale en 5 internationale congressen.

Publicaties

Publicaties in internationale wetenschappelijke tijdschriften

Dewulf, J., Laevens, H., Koenen, F., Vanderhallen, H., Mintiens, K., Deluyker, H., & de Kruif, A. 2000. An Experimental Infection with Classical Swine Fever in E2 Sub-Unit Marker-Vaccine Vaccinated and in Non-Vaccinated Pigs. Vaccine, Vol. 19, Issue 4-5 pp. 475-482

Dewulf, J., Koenen, F., Laevens, H., Mintiens, K., & de Kruif, A. 2000 Airborne transmission of classical swine fever virus under experimental conditions. Veterinary Record, Vol. 147, pp. 735-738.

Mintiens, K., Deluyker, H., Laevens, H., Koenen, F., **Dewulf, J**., & de Kruif, A. 2001, Descriptive epidemiology of a classical swine fever outbreak in the Limburg province of Belgium in 1997. Journal of Veterinary Medicine series B, Vol. 48, pp. 143-149

Dewulf, J., Laevens, H., Koenen, F., Mintiens, K., & de Kruif, A. 2001, An Experimental Infection with Classical Swine Fever in Pregnant sows: Transmission of the virus, course of the disease, antibody response and effect on gestation. Journal of Veterinary Medicine series B, Vol 48, Issue 8, pp. 583-592

Dewulf, J., Laevens, H., Koenen, F., Mintiens, K., & de Kruif, A. 2001, An Experimental Infection to evaluate the potential spread of the Classical Swine Fever virus by pets and rodents. Veterinary Record, Vol 149, pp. 212-213

Dewulf, J., Laevens, H., Koenen, F., Mintiens, K., & de Kruif, A. 2002, An E2 sub-unit marker vaccine does not prevent horizontal or vertical transmission of classical swine fever virus, Vaccine, Vol. 20, Issue 1-2, pp. 86-91

Van Soom, A., Yuan, QY., Peelman, L., De Matos, DG., **Dewulf, J**., Laevens, H., de Kruif, A., 2002, Prevalence of apoptosis and inner cell allocation in bovine embryos cultured under different oxygen tensions with or without cysteine addition, Theriogenology, 57 (5), 1453-1465

Pasmans, F., De Herdt, P., **Dewulf, J.,** Haesebrouck, F. 2002, Pathogenesis of infections with Salmonella enterica subsp. enterica serovar Muenchen in the turtle Trachemys scripta scripta. Veterinary Microbiology, 87, 315-325

Verberckmoes, S., Van Soom, A., De Pauw, I., **Dewulf, J**., & de Kruif, A. 2002. Migration of bovine spermatozoa in a synthetic medium and its relation to in vivo bull fertility. Theriogenology, 58, 1027-1037

Dewulf, J., Laevens, H., Koenen, F., Mintiens, K., & de Kruif, A. 2002, An experimental infection to investigate the indirect transmission of classical Swine Fever virus by excretions of infected pigs. Accepted for publication in Journal of veterinary series B,

Mintiens, K., Laevens, H., **Dewulf, J.,** Boelaert, F. 2002. Risk, analysis of the spread of classical swine fever virus through 'neighbourhood infections' for different regions in Belgium, accepted for publication in Preventive Veterinary Medicine.

Nollet, H., Van Ham, L., Gasthuys, F., **Dewulf, J.,** Vanderstraeten, G., Deprez, P. 2002, Influence of detomidine and buprenorphine on the motor evoked potentials in horses, accepted for publication in Veterinary Record.

Publicaties in nationale wetenschappelijke tijdschriften

Boel, K., Deprez, P., **Dewulf, J**., Vlaminck, L., Steenhaut, M. 2001, Evaluatie van risicofactoren voor postoperatieve ileus bij paarden, Vlaams Diergeneeskundig Tijdschrift, 70, 298-303

Opsomer, G., **Dewulf, J**., De Vliegher, S. de Kruif, A. 2001, Hoe staat de moderne rundveehouder tegenover diergeneeskundige begeleiding? Uitslag van een enquête. Vlaams Diergeneeskundig Tijdschrift, 70, 307-313

Hoflack, G., **Dewulf, J**., De Vliegher, S. de Kruif, A. 2002, Geslachtsdeterminatie van de foetus bij koeien met behulp van het ovatec/trac toestel, Vlaams Diergeneeskundig Tijdschrift, 71,74-80

Laevens, H., **Dewulf, J**., Koenen, F., Mintiens, K. 2001, Epidemiologie van klassieke varkenspest in de Europese Unie, Vlaams Diergeneeskundig Tijdschrift, 70, 446-452.

Dewulf, J., Laevens, H., Koenen, F., Mintiens, K., & de Kruif, A. 2001, Het gebruik van vaccinatie bij de bestrijding van klassieke varkenspest, een historisch overzicht en mogelijkheden voor de toekomst, Vlaams Diergeneeskundig Tijdschrift, 70, 453-458.

The difficulty lies not so much in developing new ideas as in escaping from old ones."

John Maynard Keynes (1883-1946)