Mathematical epidemiology and the control of classical swine fever virus

Mathematische epidemiologie en de bestrijding van klassieke-varkenspestvirus

(met een samenvatting in het Nederlands)

Proefschrift
ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de Rector Magnificus, prof. dr. W.H. Gispen, ingevolge het besluit van het College voor Promoties in het openbaar te verdedigen op donderdag 24 april 2003 des middags te 4.15 uur

door

Dieudonné Klinkenberg

Geboren op 28 februari 1975, te Apeldoorn

Promotoren: Prof. dr. ir. M.C.M. de Jong
Quantitative Veterinary Epidemiology
Department of Animal Sciences, Wageningen University
Faculty of Veterinary Medicine, Utrecht University
Institute of Animal Science and Health, Lelystad

Prof. dr. J.H.M. Verheijden
Farm Animal Health
Faculty of Veterinary Medicine, Utrecht University

Co-promotor: Dr. R.J.M. Moormann
Research Director of Infection Biology
Division of Infectious Diseases and Chain Quality
Institute of Animal Science and Health, Lelystad

The research described in this thesis was carried out at the Institute for Animal Science and Health (ID-Lelystad) in Lelystad, The Netherlands. The research was part of the project WBI.4139, financed by STW (Technology Foundation), Utrecht, The Netherlands.

Financial support in printing expenses was kindly provided by:
AUV Dierenartsencoöperatie, Graduate School of Animal Health, ID-Lelystad, STW Technology Foundation

Aan mijn ouders

ISBN 90-393-3317-3

Omslagtekening: Don Klinkenberg
Druk: Ponsen & Looijen BV, Wageningen

Contents

Chapter 1

General introduction

1.1. Introduction

Classical swine fever (CSF) is feared for the major impact that CSF epidemics can have on the economy of the pig production sector, on the economy and welfare of pig farmers, and on the welfare of pigs. After the large CSF epidemic in the Netherlands in 1997 and 1998, which led to the killing and destruction of approximately 12 million pigs, discussions were raised on the effectiveness of the used control strategy and on the ethics of destroying for the most part healthy pigs.

The damage caused by CSF epidemics has to be reduced by preventing entry of CSF virus (CSFV) into the domestic pig population, and by efficient control of CSFV during CSF epidemics. Generally, prevention has to take account of the risk of importing CSFV infected pigs, which made the virus enter the Netherlands in 1997 (Elbers et al., 1999; Fritzemeier et al., 2000; Sharpe et al., 2001; Taylor, 1995), and of the two routes by which CSFV can enter a pig herd from outside the domestic pig population. The first is swill feeding, which caused CSFV entry in many recent CSF epidemics (Elbers et al., 1999; Fritzemeier et al., 2000; Sharpe et al., 2001; Taylor, 1995), although it is not allowed by EU legislation (Anonymous, 2001). The second is contact with infected wild boar, which has caused many CSF cases in Germany and Italy (Sardinia) (Fritzemeier et al., 2000; Laddomada, 2000). It is tried to control CSF in wild boar by hunting and by oral vaccination programs by means of baits (Chenut et al., 1999; Kaden and Lange, 2001; Kaden et al., 2000; Laddomada, 2000). Although prevention of virus entry is an important aspect of CSF control, this thesis merely focuses on control of CSF epidemics after virus entry.

In 1998, an STW(Technology Foundation)-funded project was started to carry out research to improve the control of CSF epidemics in the Netherlands. The project has focused on management and organisation of CSF epidemic control (Crauwels et al., 2001; Crauwels et al., 2000), on the economics of CSF epidemics (Mangen, 2002), and on the epidemiology of CSF. In this thesis, the epidemiological research of the project is described. The goal of the epidemiological part was to describe the transmission of CSFV within and between pig herds by means of mathematical models, and to use these models to answer relevant questions regarding CSF control.

Chapter 1 serves to describe the historical context of CSF control, which is the basis of the current EU legislation on the control of CSF epidemics. In addition, it points out the shortcomings of the current CSF control strategy and comes up with two pillars of optimal control of CSF epidemics. First, preparation before epidemics

to ensure quick and effective action upon CSFV detection, and second, information during epidemics to enable steering of the control strategy for each epidemic specifically. Both pillars need attention to minimise the damage of CSF epidemics. Finally, the role of epidemiological research to contribute to improvement of control of CSF epidemics is explained, and an overview of the remaining chapters in this thesis is given.

1.2. Early history of CSF eradication

Many authors have described aspects of the history of classical swine fever eradication (Beynon, 1969; Campbell, 1969; Dahle and Liess, 1992; Dunne, 1966; Robijns, 1971; Saulmon, 1973; Taylor, 1995). Classical swine fever, also known as hog cholera, was first reported in the 1830's in Ohio, in the United States (Saulmon, 1973). The first European reports date from the 1860's (Beynon, 1969; Saulmon, 1973) and the first case in the Netherlands was described in 1899 (Robijns, 1971). In the beginning of the 20th century, the causative agent was shown to be a virus (Campbell, 1969; Saulmon, 1973), which was quickly followed by the development of the first vaccines, consisting of virus and serum (Saulmon, 1973). In 1936, the crystal-violet (CV) vaccine was developed, which consisted of blood from infected pigs in which the virus was inactivated by crystal-violet (Campbell, 1969; Saulmon, 1973). In the 1950's, the first attenuated strains of CSF virus came into use as modified-live vaccines (Dunne, 1966; Saulmon, 1973).

CSF caused great losses in affected farms, and therefore eradication of the virus was desired. Great Britain started eradication already in 1879, by making CSF a notifiable disease (Beynon, 1969; Dahle and Liess, 1992). Various control strategies were used in the years after, like slaughter of infected pigs and their contacts, the use of virus/serum vaccines, and isolation of infected herds (Beynon, 1969). In 1953, the British implemented an officially-registered vaccination scheme with the CV vaccine (Beynon, 1969; Campbell, 1969), but in 1963, it was realised that the best eradication strategy would be by slaughter and disinfection of infected farms, and by imposing an import ban for pigs and pig products from CSFV infected countries (Beynon, 1969). Additional measures were tracing and killing of virus-exposed pigs, and movement restrictions in infected areas. In 1964, vaccination was prohibited, because it was believed that vaccinated herds could still become infectious and because it made diagnosis of CSFV infections very complicated: vaccines interfered with diagnostic tests and reduced clinical symptoms (Beynon, 1969; Campbell, 1969). In 1966, the last outbreak was detected before Great Britain was CSF free for

the first time, although occasional small epidemics have occurred since (Beynon, 1969; Sharpe et al., 2001; Taylor, 1995).

The United States also had a negative experience with the use of vaccines, but for other reasons than Great Britain. The US eradication program started in 1961, but the use of modified-live vaccines hampered control seriously, because the vaccines themselves caused about 30% of the outbreaks (Saulmon, 1973). Therefore, as of 1968 more and more states prohibited the use of vaccines until production of such vaccines in the US was completely stopped in 1971 (Saulmon, 1973). Another major problem of CSF eradication in the US appeared to be the movement of infected swine, which repeatedly caused new infected areas (Saulmon, 1973). This led to federal quarantines on infected states and intensified control, which in the end resulted in eradication of CSF in 1973 (Dahle and Liess, 1992).

In the Netherlands, CSF eradication was started in 1936 by making it a notifiable disease. Isolation of infected farms was the only action upon CSF diagnosis until 1961, when slaughter of infected animals was added as a control measure (Robijns, 1971). The uninfected animals in infected farms were vaccinated with an attenuated live vaccine in combination with a highly immune serum. In the subsequent years, the control program was more and more intensified until in 1968, infected farms were stamped out and disinfected, and vaccination was discouraged (Robijns, 1971). Incidence decreased in those years, although CSF remained endemic in the Ede/Barneveld region and virus introductions from abroad led to occasional epidemics (Robijns, 1971). Vaccination was reintroduced as a control measure in 1973 (Terpstra, 1978; Terpstra and Robijns, 1977; Terpstra and Wensvoort, 1987), because of the development of the C-strain attenuated vaccine and the notion that eradication was impossible without European co-operation (Robijns, 1971). It appeared possible to clear CSFV from an infected area by a massive vaccination campaign, but new virus entries prevented keeping the areas CSFV free (Pensaert, 1978; Terpstra, 1978; Terpstra, 1988; Terpstra and Robijns, 1977).

1.3. CSF eradication in the EU

Because of the harmonisation of the free market within the European Community, the joining of the CSF free countries Great Britain, Ireland and Denmark to the EC in 1973 had to lead to a community CSF policy (Dahle and Liess, 1992; Edwards et al., 2000; Vandeputte and Chappuis, 1999). In 1980, EC legislation was implemented to reach the CSF-free status for all EC countries (Anonymous, 1980).

The European non-vaccination policy is based on the distinction of health statuses (Van Oirschot, 1994). It is directly derived from the International Animal

Health Code (IAHC) of the OIE, which serves to regulate international trade (OIE, 2002; OIE is the International Office of Epizootics). The IAHC gives the highest health status to regions with an uninfected and unvaccinated pig population. Those regions are economically the most profitable, because they are allowed to refuse animal imports from regions with a lower health status. In accordance with the IAHC, the EC legislation states that regions are declared CSF free for the first time if no vaccinated animals are present, and no disease has been detected and no vaccination has been applied in the past 12 months (Vandeputte and Chappuis, 1999). If CSF outbreaks are diagnosed in these CSF free areas, they have to be followed by thorough surveillance before an area can be declared CSF free again, and the 12-month delay is not needed.

Immediately after implementation of the EC legislation, all countries with CSF started eradication programs, in which increased surveillance and mass vaccination of heavily infected regions with the C-strain vaccine were the most important measures (Pittler, 1986; Vandeputte and Chappuis, 1999). Vaccination was stopped if no disease was detected anymore. By this eradication program, the Netherlands became CSF free in 1986 (Terpstra, 1988; Terpstra et al., 2000; Vandeputte and Chappuis, 1999).

Although vaccination in the CSF eradication campaigns in Great Britain and the USA seemed to hamper eradication, it appeared a successful strategy in the EC in the 1980's. This can be attributed to the fact that vaccination campaigns should reduce virus transmission to achieve eradication, whereas vaccines were often judged by their capability to reduce clinical disease. In the British and American campaigns, the vaccination programs probably did not reduce virus transmission sufficiently, as the inactivated crystal-violet vaccine (used in Britain) could not prevent outbreaks and the modified-live vaccines (used in the USA) even caused outbreaks themselves (Campbell, 1969; Dunne, 1966). Then, obviously, vaccination even frustrates eradication because it hampers detection (Beynon, 1969; Campbell, 1969; Dunne, 1966). In the European campaigns in the 1980's, the used C-strain vaccine was able to inhibit virus multiplication in tonsils (Biront et al., 1987; Taylor, 1995) and to stop virus spread, thereby causing CSFV eradication with full vaccination coverage in affected areas (Terpstra, 1988; Terpstra and Wensvoort, 1987; Vandeputte and Chappuis, 1999). To put it in epidemiological terminology, the European vaccination campaigns succeeded in reducing the reproduction ratio between herds R_h (the average number of herds infected by one typical infectious herds) to below the threshold value 1, which ascertains extinction of the disease.

1.4. Current CSF control in the EU (2002)

Maintenance of an uninfected pig population in the European Union has not been easy up to now (Vagsholm, 1996). Epidemics have, for instance, occurred in the UK in 1971, 1986 and 2001 (Sharpe et al., 2001; Taylor, 1995; Williams and Matthews, 1988), in Belgium in 1993/1994 (Koenen et al., 1996), in Germany in 1994 (Staubach et al., 1997), and in the Netherlands in 1997/1998 (Moennig, 2000; Stegeman et al., 2000; Vandeputte and Chappuis, 1999). The Dutch epidemic had originated in Germany and led to some cases in Belgium, Italy, and Spain (Edwards et al., 2000; Elbers et al., 1999; Mintiens et al., 2001; Moennig, 2000; Terpstra et al., 2000; Vandeputte and Chappuis, 1999). Furthermore, in Germany and Italy (Sardinia) many cases occurred in the 1990's due to contact of domestic pigs with infected wild boar (Fritzemeier et al., 2000; Laddomada, 2000).

CSF epidemics in the EU are controlled by use of EU-prescribed control measures (Anonymous, 2001; Edwards et al., 2000; Stegeman et al., 2000; Vandeputte and Chappuis, 1999). According to these control measures, infected herds are stamped out and protection and surveillance zones are installed in areas of 3 and 10 km around the infected herds, respectively. In these zones a transport ban is imposed, and in the protection zone each herd has to be inspected clinically within 7 days. Furthermore, the infectious contacts of the detected herds are traced, so as to determine the possible sources of infection and to find out which herds might have been infected by the detected herds. Before declaring the surveillance zone CSF free, each herd has to be inspected and serologically tested to exclude CSFV infection.

In addition to the EU-prescribed control measures, preventive slaughter was applied during some CSF epidemics, namely, the Belgian 1993/1994 epidemic, the German 1994 epidemic, and – though not immediately – during the Dutch 1997/1998 epidemic (Koenen et al., 1996; Staubach et al., 1997; Stegeman et al., 2000; Vandeputte and Chappuis, 1999). Subject to preventive slaughter were not only herds with traced contacts with infected herds, but also herds located within 1 km of detected herds, because it appeared that neighbouring herds had an increased probability of being infected (Koenen et al., 1996; Staubach et al., 1997; Stegeman et al., 2000; Terpstra et al., 2000; Vandeputte and Chappuis, 1999).

The experience of the CSF epidemics in the 1990's, especially the Dutch epidemic in 1997/1998, has revealed that CSF control is still a problem (Edwards et al., 2000). The direct costs of the control program can become very high if the epidemic takes long, and the indirect costs due to trade restrictions can even be much higher (Mangen et al., 2002; Meuwissen et al., 1999; Saatkamp et al., 2000). Also, during an epidemic, many animals are killed and destroyed, animals from

infected herds, animals from preventively slaughtered herds (if preventive slaughter is applied), but also animals from uninfected herds because of welfare slaughter (Stegeman et al., 2000; Terpstra et al., 2000). Aside from the economic loss (destruction of capital), destruction of animals from probably uninfected farms raises ethical concerns and is questioned by the public opinion (Saatkamp et al., 2000; Terpstra et al., 2000; Van Oirschot, 1994). Finally, farmers suffer the uncertainty and stress caused by an epidemic (Terpstra et al., 2000).

1.5. CSF control in the future

Because CSF is still endemic in wild boar in regions in the EU, and because the last decade has shown that swill feeding still causes CSF outbreaks every now and then, it is important to be as well-prepared as possible to new CSF epidemics. The harmful consequences of CSF epidemics are minimal when the number of slaughtered farms is kept as small as possible and when the duration of the epidemic is reduced. Optimal control of CSF epidemics rests on two pillars:

1. *Preparation.* As soon as CSF is diagnosed, it is essential to act quickly and adequately. Therefore, the control organisation has to be clear on all management levels, from government to surveillance teams. It must also be clear which control measures are compulsory, and which are optional. Knowledge on the likely effect of specific control strategies, epidemiological as well as economic, is of great importance for quick and adequate action. In this light, a high level of agreement on the possible control strategies by all parties involved, e.g. the farmers, the veterinarians, and the general public, will be the ultimate goal of the preparation.
2. *Information.* Each CSF epidemic is unique in factors like virus strain, location of infected herds, and random effects. In spite of all preparation, during each ongoing epidemic information is needed for steering the control. For instance, contact tracing must reveal information about possibly infected farms, to enable a quicker detection. On the strategic level, where the decisions are made on the control strategy, the overall effectiveness of the current control strategy needs to be evaluated to decide upon the change of the strategy. Essential for good execution of the control program is clear communication to all parties involved, in order to explain why certain control measures are taken.

Both pillars have been addressed in the STW-funded project, started to improve the control of CSF epidemics after CSFV enters the domestic pig population. Also in

the present thesis, which describes the epidemiological part of the project, contributions are made to both preparation to epidemics and information during epidemics:

1. *Preparation.*

During the Dutch CSF epidemic in 1997/1998, the basic set of EU-prescribed control measures appeared insufficient, which made the use of preventive slaughter as an additional measure necessary (Stegeman et al., 2000; Stegeman et al., 1999b). Vaccination was not an option: because the European legislation considered vaccinated pigs as CSFV-infected, it would have taken much longer before the affected areas were officially virus-free, unless the vaccinated animals were slaughtered at the end (Vagsholm, 1996). In the last decade, two E2 subunit marker vaccines have been developed, vaccines with an accompanying diagnostic test to establish CSFV infection through serology (Hulst et al., 1993; Moormann et al., 2000; Moormann et al., 1997; Van Oirschot, 1994; Van Oirschot, 1999). In the most recent EU CSF legislation (Anonymous, 2001), the use of marker vaccines is taken into account, thereby making the option to use emergency vaccination and market the meat of vaccinated pigs under strict conditions more realistic.

Four chapters of this thesis deal with research carried out to quantify the effectiveness of the E2 subunit marker vaccines during CSF epidemics (Hulst et al., 1993; Moormann et al., 2000; Moormann et al., 1997). Chapters two, three, and four discuss transmission experiments, in which CSFV transmission was studied. In transmission experiments, some virus-infected animals are housed together with uninfected contact animals (Bouma et al., 1996; Bouma et al., 2000; De Jong and Kimman, 1994; Kroese and De Jong, 2001). Subsequently, CSFV transmission is followed in time by several diagnostic tests, like virus isolation on blood samples, or serological tests to examine the virus-induced antibody response.

A mathematical model of pathogen transmission, the so-called SIR (susceptible-infectious-removed) model (cf. Anderson and May, 1991; Diekmann and Heesterbeek, 2000; Kermack and McKendrick, 1991), is used for statistical inference, for example, to test the effectiveness of vaccination or to estimate the reproduction ratio between individual animals R_i. R_i is an important epidemiological parameter and is defined – comparable to R_h on herd level – as the average number of animals that is infected by one typical infectious animal in an infinite, susceptible population (cf. Anderson and May, 1991; Diekmann and Heesterbeek, 2000). R_i has an important threshold property: only if it is larger than 1, epidemics can occur, otherwise the disease will certainly die out. By statistical inference on R_i, the

effectiveness of vaccination in experiments can be extrapolated to the effectiveness in larger populations, like groups of pigs in pig farms.

- The second chapter describes a method to estimate R_i from transmission experiments with CSFV. The method is illustrated by using previously published CSFV transmission experiments. The method is also used in chapters 3 (though slightly altered) and 4, which describe the analysis of CSFV transmission experiments to test the E2 vaccine.
- In the third chapter, a series of experiments with two different E2 vaccines is analysed. The experiments were carried out to determine how long the vaccines take to achieve an R_i smaller than 1 and to establish whether the vaccines are equally effective.
- The fourth chapter describes a transmission experiment, in which the effect of maternal antibodies on the effectiveness of vaccination is investigated, when piglets are vaccinated at the age of two weeks. This is an important issue, since maternal antibodies are known to inhibit the active immune response upon vaccination with the C-strain vaccine (Bironi et al., 1987; Precausta et al., 1978; Precausta et al., 1983; Terpstra and Tielen, 1976; Terpstra and Wensvoort, 1987).

Whereas chapters 2 through 4 deal with virus transmission within groups of pigs, chapter five extrapolates the results to CSFV transmission between pig herds:

- In chapter 5, various vaccination strategies are compared in a mathematical model of CSFV transmission between pig herds. The model describes the virus transmission in the context of the basic EU control measures during the 1997/1998 Dutch epidemic. Control strategies are compared with respect to the average duration of epidemics and the average number of infected herds. Tested control measures include vaccination of finishing herds and of piglets in multiplier herds. Furthermore, the epidemiological consequences of a limited transport permission to continue production and do away with welfare problems are considered.

2. *Information.*

Aside from the general research into transmission dynamics of CSFV and the effectiveness of control measures and control scenarios, mathematical epidemiological models can also be used to interpret epidemiological data in ongoing epidemics:

- In chapter 6, a previously published method to analyse simple epidemiological data (Meester et al., 2002) is tested with simulated epidemics. The method is based on a branching process model to describe CSFV transmission between

pig herds, and the only data that is needed is the number of detected herds per week. It is tested whether the model parameters can be accurately estimated, and whether the method is able to give an unbiased estimate for the number of undetected infected herds during an epidemic.

3. *Concluding chapter*

- The thesis concludes with a chapter (chapter 7) on the use of the E2 subunit vaccines in an emergency vaccination campaign. The relevant publications are discussed and two potential problems are recognised, relating to vertical transmission of CSFV (from sow to the unborn foetus) and to the serological endscreening after the epidemic. The chapter gives possible solutions to both problems and concludes that emergency vaccination with the E2 vaccines can be a successful control measure.

Chapter 2

Within- and between-pen transmission of classical swine fever virus: a new method to estimate the basic reproduction ratio from transmission experiments

D. Klinkenberg, J. de Bree, H. Laevens, M.C.M. de Jong

Reprinted from *Epidemiology & Infection*, Vol 128, Pages 293-299, Copyright (2002), with permission from Cambridge University Press

Abstract

We present a method to estimate basic reproduction ratio R_0 from transmission experiments. By using previously published data of experiments with Classical Swine Fever Virus more extensively, we obtained smaller confidence intervals than the martingale method used in the original papers. Moreover, our method allows simultaneous estimation of a reproduction ratio within pens R_{0w} and a modified reproduction ratio between pens R'_{0b}. Resulting estimates of R_{0w} and R'_{0b} for weaner pigs were 100 (95% CI 54.4-186) and 7.77 (4.68-12.9), respectively. For slaughter pigs they were 15.5 (6.20-38.7) and 3.39 (1.54-7.45), respectively. We believe, because of the smaller confidence intervals we were able to obtain, that the method presented here is better suited for use in future experiments.

2.1. Introduction

Classical Swine Fever (CSF) or hog cholera is a highly contagious pig disease (Dahle and Liess, 1992; Taylor, 1995; Terpstra, 1987), an epidemic of which can cause huge problems like reduction in animal welfare, and high economic losses as a result of export limitations and mass destruction (Meuwissen et al., 1999). The disease is caused by the Classical Swine Fever Virus (CSFV) (Dahle and Liess, 1992; Taylor, 1995; Terpstra, 1987). Transmission of the virus between pigs can be quantified by estimating parameters from transmission experiments, in which a number of pigs within a pen are inoculated with the virus and the transmission process is followed (De Jong and Kimman, 1994). An important parameter of virus transmission is the basic reproduction ratio R_0, defined as the number of secondary infected individuals caused by one typical infectious individual in an infinite susceptible population. If R_0 is smaller than 1, then on average every infectious animal infects less than one other animal causing the outbreak to wane. If on the other hand R_0 is greater than 1, major outbreaks can occur (Anderson and May, 1979).

In 1998 and 1999 Laevens et al. did two transmission experiments with CSFV; one with weaner pigs and the other with slaughter pigs (Laevens et al., 1998; Laevens et al., 1999). In both experiments there were 3 adjacent pens with either 15 weaner pigs or 6 slaughter pigs in each pen. In the middle pen one pig was inoculated with CSFV and every 2 days blood samples of all the pigs were taken to

measure viraemia. From these measurements the infectious period of every pig was reconstructed by assuming that a pig is infectious when it is viraemic. Subsequently R_0 was estimated using the martingale estimation method, based on the stochastic *SIR* model (Becker, 1989; De Jong and Kimman, 1994). This model describes transmission of a virus in a group of animals by describing the change in the numbers of susceptible (*S*) and infectious (*I*) animals in terms of these numbers and the total number of animals (*N*). In the model, infection of susceptible animals and recovery of infectious animals are assumed to be generated by a Poisson process with rates $\beta SI/N$ and αI, where β and α are the transmission and recovery parameter, respectively. The R_0 is estimated from the number of animals ultimately infected during the experiment, when no susceptible or no infectious animals remain. The sum of the fractions of infectious periods remaining when the last susceptible animal is infected is used if relevant. Laevens et al. (1998; 1999) used only the data of the middle pen to estimate R_0 because in the other pens transmission was not solely caused by infectious animals in the same pen. The estimates obtained were 81.3 (s.e. 109, i.e., 95% CI: –132-295) and 13.7 (s.e. 13.7, i.e., 95% CI: –13.2-40.6) for weaner and slaughter pigs, respectively. This meant that despite the fact that the infection process took place very quickly and all animals were infected, the estimated R_0 s were not significantly greater than 1. Since some aspects of the data were not used for the estimation (infection times and infectious periods of all animals known for all three pens), searching for an alternative estimation method would be worthwhile, using as much information from the data as possible. Hopefully this will lead to a smaller confidence interval.

In an attempt to obtain an R_0 estimate with a smaller confidence interval, we did separate estimations of β, the infectivity parameter, and α, the recovery parameter, which are used to calculate R_0 ($R_0 = \beta/\alpha$). For β estimation, the infection process was partitioned into intervals with known numbers of infection cases (*C*) and susceptible (*S*) and infectious (*I*) animals. These sets of (*S*, *I*, *C*) were used to construct a likelihood function, which we maximised to get a maximum likelihood estimator for β. For α estimation, the lengths of the infectious periods were used to fit a generalised linear model.

2.2. Materials and methods

We used the data obtained in the transmission experiments of Laevens et al (for more detail see Laevens et al. (1998; 1999)). In both experiments there were three

adjacent pens with equal numbers of pigs: 15 weaner pigs in one experiment and 6 slaughter pigs in the other. One of the pigs in the middle pen was inoculated with CSFV and every two days blood samples were taken from all animals, which were tested for viraemia. From this data the infectious period of each pig was reconstructed, assuming that the animal is infectious when it is viraemic.

By assuming a latent period of 6 days (infected but not yet infectious) (Taylor, 1995), we were able to reconstruct the entire virus transmission process in the three pens. These reconstructions enabled us to estimate the parameters, by using the following stochastic *SIR* model (Anderson and May, 1979), incorporating both within- and between-pen transmission:

$$rate(S \to S-1) = (\beta_w I_w / N_w + \beta_b I_b / N_b)S \tag{2.1}$$

$$rate(I \to I-1) = \alpha I . \tag{2.2}$$

In this model, β_w is the within-pen transmission parameter defined as the expected number of new infections in the same pen per day per typical infectious animal in a fully susceptible population. Likewise, β_b is the between-pen transmission parameter defined as the expected number of new infections in other pens per day per typical infectious animal in a fully susceptible population. The parameter α represents the recovery rate per infectious animal. Because there are two transmission parameters β_w and β_b, we also make a distinction between a within-pen reproduction ratio R_{0w} and a between-pen reproduction ratio R_{0b}. R_{0w} is defined as the expected number of secondary infected animals caused by one typical infectious animal in the same pen. R_{0b} is defined as the expected number of secondary infected pens caused by one typical infectious pen, considering a pen as infected when at least one pig is infected. Estimates for R_{0w} and R_{0b} can be calculated as follows:

$$R_{0w} = \frac{\beta_w}{\alpha} \tag{2.3}$$

$$R_{0b} = R'_{0b} \cdot E(I_{tot}) = \frac{\beta_b}{\alpha} \cdot E(I_{tot}) . \tag{2.4}$$

In this equation, $E(I_{tot})$ is the expected number of animals ultimately infected within one pen. $E(I_{tot})$ can under our model assumptions easily be determined if R_{0w} is known (Diekmann and Heesterbeek, 2000), but will not be further discussed

in this paper. R'_{0b} is the expected number of secondary infected pens caused by one typical infectious *animal*. R'_{0b}, being independent of $E(I_{tot})$, is the parameter that will be estimated in this paper. For notational convenience, we have introduced the vectors $\vec{\beta} = (\beta_w, \beta_b)$, $\log\vec{\beta} = (\log\beta_w, \log\beta_b)$, $\vec{R}_0 = (R_{0w}, R'_{0b})$, and $\log\vec{R}_0 = (\log R_{0w}, \log R'_{0b})$. Because infection and recovery are independent processes, $\vec{R}_0$ was calculated from separate estimations of $\vec{\beta}$ and α.

In order to estimate transmission parameters $\vec{\beta}$, the infection process has been divided into time intervals of two days, the intervals between two subsequent samplings. For each interval, the number of susceptible pigs at the start of the interval (*S*), the number of infectious pigs (*I*) and the number of new cases (*C*) was determined (Table 2.1). In each time interval *k*, the probability of a susceptible animal escaping infection from the constant rate $(\beta_w I_{wk}/N_{wk} + \beta_b I_{bk}/N_{bk})$ is, according to the Poisson distribution, $e^{-(\beta_w I_{wk}/N_{wk} + \beta_b I_{bk}/N_{bk})}$. Therefore, the probability of getting C_k cases, with S_k susceptibles and i_k as the fraction of infectious pigs (I_k/N_k) in the same pen and j_k as the fraction of infectious pigs in the other pens is, according to the binomial distribution:

$$prob(C_k | i_k, j_k, S_k) = \binom{S_k}{C_k} \left(1 - e^{-\beta_w i_k - \beta_b j_k}\right)^{C_k} \left(e^{-\beta_w i_k - \beta_b j_k}\right)^{S_k - C_k}. \qquad (2.5)$$

The probabilities for all time intervals have been used to make up the log-likelihood function, which may be written as:

$$\log L(\beta_w, \beta_b) = \sum_k \left[C_k \log\left(e^{\beta_w i_k + \beta_b j_k} - 1\right) - S_k(\beta_w i_k + \beta_b j_k)\right], \qquad (2.6)$$

where $\log\binom{S_k}{C_k}$ has been omitted because it plays no role. Maximising this function results in maximum likelihood estimators for β_w and β_b.

Three methods were used to derive confidence intervals for β_w. After comparing several features (e.g. mathematical background, practical value), a decision was made as to which method should be used for interval estimation of β_b, R_{0w} and R'_{0b}. The first method, which we shall refer to as the construction method, is based on the likelihood ratio and on the equivalence of testing and construction of

Table 2.1. Course of transmission experiments

Time (days):		4-6	6-8	8-10	10-12	12-14	14-16	16-18	18-20	20-22	22-24
Weaner pigs											
pen 1	S	15	15	15	15	13	7	4	2		
	I	0	0	0	0	0	0	1	5		
	C	0	0	0	2	6	3	2	2		
	N	15	15	15	15	15	15	15	15		
pen 2	S	14	5	0	0	0	0	0	0		
	I	1	1	1	5.5	12	11.5	10	10		
	C	9	5	0	0	0	0	0	0		
	N	15	15	15	15	14.5	11.5	10	10		
pen 3	S	13	13	13	13	6	3	1	0		
	I	0	0	0	0	0	0	3.5	8.5		
	C	0	0	0	7	3	2	1	0		
	N	14	14	14	14	13	13	13	13		
Slaughter pigs											
pen 1	S	5	5	5	5	5	4	4	3	1	1
	I	0	0	0	0	0	0	0	0.5	1	1.5
	C	0	0	0	0	1	0	1	2	0	1
	N	6	6	6	6	6	6	6	6	5	5
pen 2	S	5	4	3	1	0	0	0	0	0	0
	I	0.5	1	1	1	1.5	3	4.5	5	4.5	4
	C	1	1	2	1	0	0	0	0	0	0
	N	6	6	6	6	6	6	6	6	6	6
pen 3	S	6	6	6	6	6	6	6	2	0	0
	I	0	0	0	0	0	0	0	0	0	2
	C	0	0	0	0	0	0	4	2	0	0
	N	6	6	6	6	6	6	6	6	6	6

Division of the virus transmission process in two-day time periods, stratified by pen. Time starts at day of inoculation. *S* is the number of susceptible animals at the start of the interval; *I* is the number of infectious animals; *C* is the number of new cases and *N* is the total number of animals, where 0.5 is an animal present for only one of two days in a certain category.

a confidence interval. The test used here is derived from the observation that the likelihood ratio for testing one value of β_w (H_0: $\beta_w = \beta_0$) against another value of β_w (H_A: $\beta_w = \beta' < \beta_0$) is a monotonic and decreasing function of each *C*. It allowed us to construct a critical region for the *C* by using the probability function of *C* itself, without invoking any approximate probability distribution of the likelihood ratio. For details, see the appendix. With this method confidence intervals can be constructed for one of the two βs (β_w or β_b) treating the other as a constant at its estimate. Unfortunately, the computation is almost prohibitively time-

consuming, and just how to construct a confidence area for the parameter *vector* $\vec{\beta}$ or how to determine confidence intervals for R_0 is not clear. The second method is the likelihood ratio (λ) test as described by Neyman & Pearson (reference in Birkes, 1998), which relies on the asymptotic chi-square distribution of $-2\log\lambda$ with, in our case, 1 degree of freedom. This method calculates 95% confidence limits by solving the equation $-2\log\lambda = 3.84$ for one of the two βs (β_w or β_b) treating the other as a constant at its estimate. This is a much faster method than the first one; nonetheless it suffers from the same construction difficulties with regard to simultaneous confidence intervals. The third method is based on the asymptotic (multivariate) normal distribution of a maximum likelihood estimator (Beaumont, 1980). The assumption is made that the estimator of $\log\vec{\beta}$ (instead of $\vec{\beta}$), being also a ML-estimator, is asymptotically normally distributed because then non-realistic (negative) values of β_w and β_b cannot occur. This results in the following covariance matrix **M**:

$$\mathrm{M} = -\begin{pmatrix} \partial^2 \log L / \partial(\log\beta_w)^2 & \partial^2 \log L / \partial(\log\beta_w)(\log\beta_b) \\ \partial^2 \log L / \partial(\log\beta_w)(\log\beta_b) & \partial^2 \log L / \partial(\log\beta_b)^2 \end{pmatrix}^{-1} . \qquad (2.7)$$

This method is computationally fast and, since it provides an estimate of the covariance matrix, it obviously enables construction of confidence areas for $\log\vec{\beta}$ and $\log\vec{R}_0$ [1].

The recovery/death rate parameter α has been estimated using a generalised linear model for survival analysis with censoring, as described by Aitken et al. (1989). In this model for each animal two explanatory variables T_k and y_k can be observed. The first one, T_k, is the observed length of the infectious period. The second one, y_k, is a censoring variable: y_k is 1 if T_k is the true survival time,

[1] Note that, if only one transmission parameter is estimated, this likelihood variance method is in fact the same as a generalised linear model with response variate *C*, binomially distributed with index *S*, and a complementary log-log LINK function, and $\log(I/N)$ as offset. Because in this case we want to estimate two transmission parameters simultaneously, it is not possible to use this GLM.

whereas y_k is 0 if the true survival time is greater than T_k. The likelihood function reads as follows:

$$L(\alpha) = \prod_{k=1}^{n} \left(\alpha e^{-\alpha T_k}\right)^{y_k} \left(e^{-\alpha T_k}\right)^{1-y_k} = \prod_{k=1}^{n} \alpha^{y_k} e^{-\alpha T_k} = \prod_{k=1}^{n} (\alpha T_k)^{y_k} e^{-\alpha T_k} \Big/ \prod_{k=1}^{n} T_k{}^{y_k} \quad . \tag{2.8}$$

The kernel of this likelihood is the same as it would be with a set of *n* observations y_k each having an independent Poisson distribution with mean αT_k (see Aitkin et al., 1989). The analysis was performed in Genstat (1998), using the RSURVIVAL procedure, where y_k denotes the response variate, $\log T_k$ the offset, and the model is fitted with a log LINK function and a Poisson distribution. The output is an estimate of $\log \alpha$ and its estimated variance.

The estimator of $\log \vec{R}_0$ is given by:

$$\log \vec{R}_0 = \log \vec{\beta} \; - \log \alpha \; . \tag{2.9}$$

Derivation of a confidence area for $\log \vec{R}_0$ is done by adding the covariance matrices for $\log \vec{\beta}$ and $\log \alpha$:

$$\operatorname{var}\left(\log \vec{R}_0\right) = \operatorname{var}\left(\log \vec{\beta}\right) + \operatorname{var}\left(\log \alpha\right) \begin{pmatrix} 1 & 1 \\ 1 & 1 \end{pmatrix} . \tag{2.10}$$

The estimated $\log \vec{R}_0$s and $\operatorname{var}\left(\log \vec{R}_0\right)$s for weaner and slaughter pigs were used to construct a confidence area for the difference of the two $\log \vec{R}_0$s, and to assess whether R_{0w} or R'_{0b} differ significantly between weaner and slaughter pigs.

2.3. Results

The maximum likelihood estimation method used to estimate transmission parameters β_w and β_b produced point estimates of 8.52 and 0.656 for weaner pigs and 1.85 and 0.402 for slaughter pigs, respectively. Three methods were used to determine 95% confidence intervals for β_w. With the construction method based on the likelihood ratio λ, the intervals for β_w obtained were (4.77-14.1) and

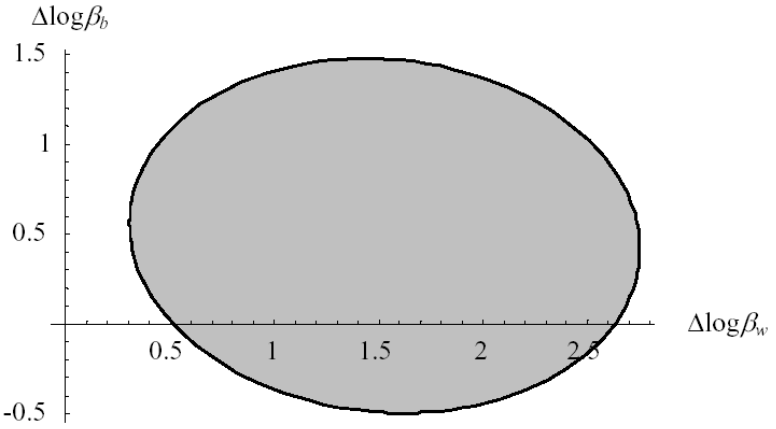


Figure 2.1. Shaded area is the 95% confidence area for $\Delta \log \vec{\beta}$

(0.704-3.63) for weaner pigs and slaughter pigs, respectively. With the $\log \lambda$ method the intervals were (4.78-14.1) and (0.709-3.79), respectively, and the likelihood variance method produced intervals of (4.98-14.6) and (0.817-4.18). Because the construction method does not assume specific distributions based on asymptotic features, we believe that the estimated confidence intervals from this method would be the most precise. The $\log \lambda$ method, which is much faster than the numerical method, performed quite well, while the likelihood variance method resulted in slightly upwards shifted intervals. However, we decided to use this last-mentioned method for further calculations, because the obtained covariance matrices for $\vec{\beta}$ together with the variances for α can be used to estimate covariance matrices for $\vec{R}_0$.

The covariance matrices **M** of $\log \vec{\beta}$ thus calculated were:

$$\mathbf{M}_{\text{weaner}} = \begin{pmatrix} 0.0752 & -0.00128 \\ -0.00128 & 0.0438 \end{pmatrix} \tag{2.11}$$

$$\mathbf{M}_{\text{slaughter}} = \begin{pmatrix} 0.175 & -0.0132 \\ -0.0132 & 0.118 \end{pmatrix} \tag{2.12}$$

To compare the estimated $\log \vec{\beta}$ s of weaner and slaughter pigs, the difference of the two was calculated ($\Delta \log \vec{\beta}$), together with the accompanying covariance matrix,

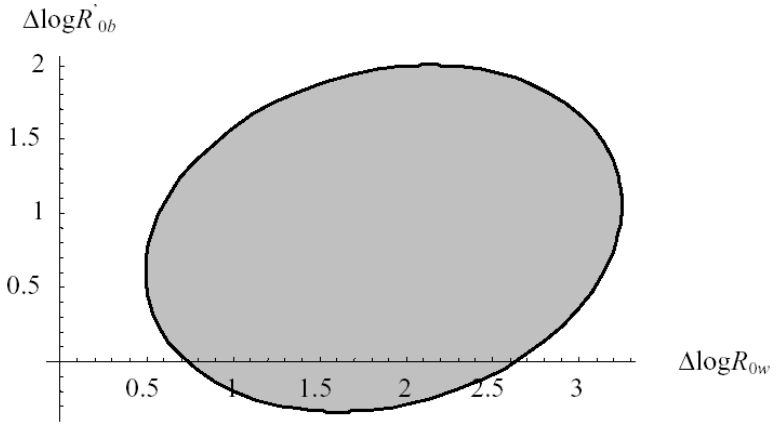


Figure 2.2: Shaded area is the 95% confidence area for $\Delta \log \vec{R}_0$

$\mathbf{M}_{\mathbf{weaner}}$ + $\mathbf{M}_{\mathbf{slaughter}}$. The 95% confidence area of this difference in figure 2.1 shows that this area does not cross the line $\Delta \log \beta_w = 0$ and therefore the $\log \beta_w$s of weaner and slaughter pigs differ significantly. This is not the case for the $\log \beta_b$ s. Estimation of recovery parameter α resulted in a $\log \alpha$ for weaner pigs of –2.47 with variance 0.0231 and for slaughter pigs of –2.13 with variance 0.0433.

Estimation of $\log \vec{R}_0$ resulted in these vectors and covariance matrices:

$$\log \vec{R}_{0weaner} = \begin{pmatrix} 4.61 \\ 2.05 \end{pmatrix} \text{ and covariance matrix } \begin{pmatrix} 0.0983 & 0.0218 \\ 0.0218 & 0.0669 \end{pmatrix} \tag{2.13}$$

$$\log \vec{R}_{0slaughter} = \begin{pmatrix} 2.74 \\ 1.22 \end{pmatrix} \text{ and covariance matrix } \begin{pmatrix} 0.218 & 0.0300 \\ 0.0300 & 0.162 \end{pmatrix} \tag{2.14}$$

This means that the estimated R_{0w} and R'_{0b} for weaner pigs were 100 (CI 54.4-186) and 7.77 (CI 4.68-12.9), and for slaughter pigs 15.5 (CI 6.20-38.7) and 3.39 (CI 1.54-7.45), respectively. Testing whether $\log \vec{R}_{0weaner}$ differs from $\log \vec{R}_{0slaughter}$ has been done by calculating the difference and accompanying covariance matrix, and subsequently plotting the 95% confidence area (Figure 2.2). It illustrates that the confidence area does not cross the line $\Delta \log R_{0w} = 0$, but does cross the line $\Delta \log R'_{0b} = 0$. Therefore, the conclusion is that R_{0w} differs between weaner and slaughter pigs, but R'_{0b} does not.

2.4. Discussion

The maximum likelihood method presented in this paper resulted in a much smaller confidence interval of R_{0w} than the martingale method (Laevens et al., 1998; Laevens et al., 1999). This was probably due to the more extensive use of the data, by dividing the virus transmission process into intervals with known numbers of new cases and susceptible and infectious pigs. Also, the maximum likelihood method uses data from all the pens, in contrast with the martingale method, which only uses the data of the middle (the primarily infected) pen. We are convinced that the method presented here is more suitable to be used in data analysis of future experiments.

Three different methods were used to calculate confidence intervals for β_w: a construction method based on the likelihood ratio λ, the $\log\lambda$ method, and the likelihood variance method. The construction method is not based on asymptotic features (i.e. many data points), and is in this sense a reliable method. However, its disadvantages were that the calculation time was long, it was impossible to construct a confidence area for two parameters (β_w and β_b) simultaneously, and it was not possible to use the results to construct intervals for R_0. The other two methods are based on asymptotic features of the $-2\log\lambda$ and of the likelihood function itself. Both of these methods are fast. The advantage of the $\log\lambda$ method is that it uses the likelihood ratio, just like the construction method, and that the results are very similar. The advantage of the likelihood variance method, however, is that it allows derivation of confidence areas for β_w and β_b simultaneously and that the estimated variances can be used to obtain variances of the $\log\bar{R}_0$ estimates. That is why this likelihood variance method is used to estimate variances for β_b and $\log\bar{R}_0$ as well.

With the maximum likelihood method presented in this paper, the R_{0w} and R'_{0b} appeared to be significantly greater than 1 for both weaner and slaughter pigs. This conclusion could not be made from the martingale estimation, but was expected because of the large outbreak in both experiments (all animals infected) and the ability of the virus to cause CSF epidemics.

A more surprising result was the significant difference between the two age groups: the R_{0w} of weaner pigs is larger than the R_{0w} of slaughter pigs. This can be due to several causes, which should be judged by the fact that the R'_{0b}s do not differ. First, the resistance to infection in younger pigs could be lower (higher susceptibility). Second, the smaller volume of younger pigs could be responsible for a higher virus concentration in the animals and consequently a higher virus excretion

(higher infectiousness). Third, weaner pigs might have more intensive contacts with each other, which is the most probable cause, because the first two mentioned would also result in higher R'_{0b} s. However, it is also possible that the R'_{0b} s do differ, but that this was not observed in these experiments.

From an epidemiological point of view, the difference between the groups can be important because virus transmission in units with younger pigs (weaner pigs in a sow herd) will be quicker than in units with older pigs (in a finishing herd). Therefore it is important to know whether this difference exists with other CSF strains as well. If the difference is mainly due to more intensive animal contacts, this is to be expected.

Acknowledgement

The authors thank STW (Technology Foundation), Utrecht, The Netherlands, for financial support.

Appendix 2.

Here a numerical method is derived to construct confidence intervals (CI) for the transmission parameters $\vec{\beta}$. To keep the derivation more orderly, it is shown here for only one transmission parameter β, as if there were only within-pen transmission. When the other parameter is kept constant, as in the examples in the text, the derivation is similar. The log-likelihood equation with one parameter β is, analogous to (2.6):

$$\log L(\beta) = \sum_k \left[C_k \log\left(e^{\beta i_k} - 1\right) - S_k(\beta i_k) \right] \tag{2.15}$$

Now, with the equivalence of testing and CI construction in mind, a test is suggested of H_0: $\beta = \beta_0$, against H_A: $\beta = \beta' < \beta_0$. Then, letting β' tend to β_0, a test will be obtained to test β_0 against any $\beta' < \beta_0$. This test can be used to construct an upper limit of a confidence interval. A similar procedure is followed for the lower limit.

The test, Φ, is based on the likelihood ratio (λ) (Lehmann, 1959):

$$\Phi = \begin{cases} 1 \text{ if } \log\lambda \geq d \\ 0 \text{ if } \log\lambda < d \end{cases}, \tag{2.16}$$

where d is determined by $E_{\beta_0}(\Phi) = 0.05$ (for a 95% CI). H_0 is rejected when $\Phi = 1$ and H_0 is not rejected when $\Phi = 0$. In (2.16), $\log\lambda$ is:

$$\log\lambda = \log\left[\frac{\prod_{j=1}^{m}\prod_{k=1}^{n_j}\binom{S_j - \sum_{l=0}^{k-1} C_{jl}}{C_{jk}}\left(1-e^{-\beta' i_{jk}}\right)^{C_{jk}}\left(e^{-\beta' i_{jk}}\right)^{S_j - \sum_{l=1}^{k} C_{jl}}}{\prod_{j=1}^{m}\prod_{k=1}^{n_j}\binom{S_j - \sum_{l=0}^{k-1} C_{jl}}{C_{jk}}\left(1-e^{-\beta_0 i_{jk}}\right)^{C_{jk}}\left(e^{-\beta_0 i_{jk}}\right)^{S_j - \sum_{l=1}^{k} C_{jl}}}\right] =$$

$$\sum_{j=1}^{m}\sum_{k=1}^{n_j}\left[C_{jk}\left(\log\frac{1-e^{-\beta' i_{jk}}}{1-e^{-\beta_0 i_{jk}}}\right) + \left(\sum_{l=1}^{k} C_{jl} - S_j\right)\left(i_{jk}\left(\beta' - \beta_0\right)\right)\right] =$$

$$\sum_{j=1}^{m}\sum_{k=1}^{n_j}\left[C_{jk}\left(a_{jk} + \sum_{l=k}^{n_j} b_{jl}\right) - S_j b_{jk}\right], \qquad (2.17)$$

where $a_{jk} = \log\frac{1-e^{-\beta' i_{jk}}}{1-e^{-\beta_0 i_{jk}}}$ and $b_{jk} = i_{jk}\left(\beta' - \beta_0\right)$.

Observe that $\log\lambda$ is monotonically decreasing in every C_{jk}:

$$\frac{\partial\log\lambda}{\partial C_{jk}} = a_{jk} + \sum_{l=k}^{n_j} b_{jl} \le 0 \Leftrightarrow$$

$$\beta'\sum_{l=k}^{n_j} i_{jl} + \log\left[1-e^{-\beta' i_{jk}}\right] \le \beta_0\sum_{l=k}^{n_j} i_{jl} + \log\left[1-e^{-\beta_0 i_{jk}}\right],$$

which is true since $g_{jk}(\beta) = \beta\sum_{l=k}^{n_j} i_{jl} + \log\left[1-e^{-\beta i_{jk}}\right]$ is a monotonic and increasing function of β.

Hence, $\log\lambda$ can be used to construct a test for $\beta' < \beta_0$.

The test is constructed for any $\beta' < \beta_0$ (upper limit) by letting β' tend to β_0 ($\beta' \uparrow \beta_0$), which results in:

$$a_{jk} + \sum_{l=k}^{n_j} b_{jl} = \left[Log\left[1 - e^{-\beta' i_{jk}}\right] + \sum_{l=k}^{n_j} i_{jl}\beta' \right] - \left[Log\left[1 - e^{-\beta_0 i_{jk}}\right] + \sum_{l=k}^{n_j} i_{jl}\beta_0 \right] \approx$$

(via Taylor expansion)

$$(\beta' - \beta_0)\left(\frac{i_{jk}}{e^{\beta_0 i_{jk}} - 1} + \sum_{l=k}^{n_j} i_{jl} \right) = (\beta' - \beta_0) r_{jk}, \tag{2.18}$$

where $r_{jk} = \left(\frac{i_{jk}}{e^{\beta_0 i_{jk}} - 1} + \sum_{l=k}^{n_j} i_{jl} \right)$. Hence, $\log\lambda$ becomes:

$$\log\lambda = \sum_{j=1}^{m}\sum_{k=1}^{n_j}\left[C_{jk}\left((\beta' - \beta_0) r_{jk}\right) - S_j i_{jk}(\beta' - \beta_0)\right], \tag{2.19}$$

which determines the form of the test for the upper limit (since all other factors are independent of β_0):

$$\Psi = \begin{cases} 1 \text{ if } \sum_{j=1}^{m}\sum_{k=1}^{n_j} C_{jk} r_{jk} \le d \\ 0 \text{ if } \sum_{j=1}^{m}\sum_{k=1}^{n_j} C_{jk} r_{jk} > d \end{cases} \tag{2.20}$$

For the case $\beta' > \beta_0$ (lower limit), the derivation is the same, except for the inequality signs in formula (2.19), which are switched.

The test is used for an iterative search of that β_0 for which holds:

$E(\Psi) = 0.025$, and

$\sum_{j=1}^{m}\sum_{k=1}^{n_j} C_{jk} r_{jk} = d$

Chapter 3

E2 subunit marker vaccines reduce transmission of classical swine fever virus sufficiently to halt epidemics

D. Klinkenberg, A. Bouma, G. Floegel-Niesmann, M.C.M. de Jong

Submitted to *Preventive Veterinary Medicine*

Abstract

This paper presents a more comprehensive analysis of a previously published Classical Swine Fever Virus (CSFV) transmission experiment. In the experiment, two E2 subunit marker vaccines, one from Bayer, Germany (A) and one from Intervet, the Netherlands (B), had been tested to establish the time interval between vaccination and protection against horizontal virus transmission. The original analysis led to the conclusion that the vaccines mutually differed and that they reduced transmission as from 14 days after vaccination with either vaccine. In the analysis, however, virus transmission was not quantified. Therefore, it did not allow for extrapolation of the results to larger populations. Because the expected vaccine effectiveness on population level will be important for the decision to use emergency vaccination during a CSFV epidemic or not, a more comprehensive analysis was deemed necessary. The new analysis tested the vaccines in their ability to decrease the basic reproduction ratio R_0, which is defined as the average number of susceptible animals that will be infected by one infectious animal in a susceptible population. An important property of R_0 is its threshold value 1: if $R_0 < 1$, no epidemics can occur. Two methods for analysis were used. The first method, the final size method, determined for each tested vaccination-challenge interval whether R_0 was significantly reduced. It appeared that R_0 was significantly reduced after 14 days with vaccine A and after 21 days with vaccine B. The second method, the entire course method, was used to determine the time the vaccines needed to bring R_0 below 1, and to determine R_0 in an unvaccinated and in a vaccinated population. With both vaccines R_0 was significantly smaller than 1 after three weeks. In an unvaccinated population, R_0 was estimated at 9.9. Vaccine A let R_0 decrease to 0.047, whereas vaccine B reduced R_0 to 0.41. Although R_0 with vaccine A significantly differed from R_0 with vaccine B, stochastic simulations showed that either vaccine will limit most CSFV outbreaks in a pig unit of 100 pigs to only one infected pig (95% CI with vaccine A: 1-2; with vaccine B: 1-6).

3.1. Introduction

Classical swine fever (CSF, syn. hog cholera) is an infectious disease of swine, caused by the CSF virus (CSFV) (for more general information, cf. Taylor, 1995; Van Oirschot, 1992). Introduction of CSFV into a susceptible pig population can cause large epidemics which can affect many pig farms and become very costly, as seen in the Dutch CSFV epidemic of 1997/1998 (Elbers et al., 1999; Meuwissen et

al., 1999). Vaccination might reduce size and costs of CSFV epidemics, but the legislation of the European Union (EU) does not allow prophylactic vaccination against CSF since 1980 (Anonymous, 1980; Anonymous, 2001). Although use of the C-strain vaccine has been shown to accomplish CSFV eradication in the 1980s (Terpstra and Wensvoort, 1987) and the EU does allow emergency vaccinations during epidemics (Anonymous, 2001), they have never been applied because of the resulting export restrictions (Moennig, 2000).

The reason for export restrictions is that vaccinated animals cannot be distinguished from infected animals, and therefore trade with vaccinated animals is a risk. Because of this, marker vaccines have been developed, based on the E2-glycoprotein of the virus (Hulst et al., 1993; König et al., 1995). Vaccination with an E2 marker vaccine induces antibodies against only the E2 protein of the virus. An ELISA was designed to detect antibodies against the E^{rns} protein of the virus and can be used as a discriminating test (Moormann et al., 2000; van Rijn et al., 1999).

Applying emergency vaccination with an E2 vaccine will only be useful if the vaccine is sufficiently capable of reducing the size and costs of an epidemic. Therefore, the EU financed three marker vaccine experiments to test the efficacy of two E2 marker vaccines, BAYOVAC CSF marker (Bayer, Germany) and PORCILIS PESTI (Intervet, The Netherlands) (Depner et al., 2001; Floegel-Niesmann, 2001; Uttenthal et al., 2001). One of the experiments was to establish the effect on horizontal virus transmission (Uttenthal et al., 2001). The main conclusions of this experiment were that there is a difference in virus transmission between the vaccines, and that vaccination reduces virus shedding and spreading as from 14 days after vaccination.

A problem with the analysis of Uttenthal et al. (2001) is that it does not answer the question whether the vaccines *sufficiently* reduce CSFV transmission to prevent major outbreaks on pig farms. Since the conclusions were based on the Fisher's exact test and on qualitative data analysis, it is impossible to predict the effect of vaccination on transmission, if the virus would enter a vaccinated pig herd. Better predictions can be made if transmission of an infectious agent is quantified by the basic reproduction ratio, R_0: the average number of individuals that is infected by one infectious individual in an entirely susceptible population (cf. Anderson and May, 1991; Diekmann and Heesterbeek, 2000). A nice property of R_0 is its threshold value: if R_0 is smaller than 1, only minor outbreaks can occur and only if R_0 is larger than 1, the virus can spread and major outbreaks can occur. Because of this threshold property, it is very useful to analyse transmission experiments by use of R_0, either by testing the null-hypothesis that R_0 is equal in two groups, or by estimating R_0 in the different groups and testing whether it is smaller (or larger) than 1.

This paper presents an analysis of the EU horizontal-transmission trials with respect to R_0. The objective of the analysis was to determine whether the vaccines are able to reduce R_0 to a value smaller than 1, and to determine the time from vaccination until R_0 becomes smaller than 1. Since the trials were conducted in five different laboratories, a meta-analysis of all trials was conducted. A meta-analysis was considered appropriate, since many important aspects of the trials were the same in all laboratories: the animals' age and health status (no other infections), the vaccine batches, the CSFV challenge strain, and the diagnostic tests used to assess CSFV infection.

In our analysis, we used two methods, both based on a mathematical model that describes the transmission of infectious agents, the SIR model (Bailey, 1975). With the first method, the final size method (Kroese and De Jong, 2001), it was tested whether the R_0 of the vaccine groups was significantly reduced compared to the control group. With the second method, the entire course method (Chapter 2), two epidemiological parameters were estimated with time after vaccination, viz. the transmission parameter β and the recovery parameter α. The estimates for β and α were used to determine the time lag between vaccination and the situation where $R_0 < 1$, and to estimate R_0 with and without vaccination.

3.2. Materials and Methods

3.2.1. Experimental procedures

The experimental procedures of the transmission trials are described in detail by Uttenthal et al. (2001). Here we give a summary, where we focus on the aspects that are important for interpretation of the results with respect to reduction of virus transmission.

3.2.1.1. Experimental design

A total of 190 conventional weaner pigs of 5-6 weeks of age had been used in 19 transmission trials with ten pigs per trial. The 19 trials had been divided into nine treatment groups. Four treatment groups consisting of two trials had been vaccinated with vaccine A (BAYOVAC® CSF marker E2/98/B001, Bayer, Germany). Another four treatment groups of two trials had been vaccinated with vaccine B (PORCILIS® PESTI January 1999, Intervet, The Netherlands). The ninth treatment group of three trials was used as the unvaccinated control group.

All trials had started by challenging five out of ten animals with a CSFV field strain isolated in Germany (Paderborn). Four different time intervals between vaccination and challenge were studied in the four treatment groups per vaccine. The intervals were 7 days (groups A7 and B7), 10 days (groups A10 and B10), 14 days (groups A14 and B14), and 21 days (groups A21 and B21). The transmission trials of the unvaccinated control group (group C) had started at day 0. After challenge, every two to eight days the animals had been sampled for determination of viraemia and antibodies against the E^{rns}-epitope of the CSFV. Body temperature had been measured daily. At the end of the trials, tissue samples had been taken for virus detection.

3.2.1.2. Organisation of the experiment

Four national swine fever laboratories in Italy, Spain, France, and Denmark and the EU reference laboratory for CSF in Germany had been involved in carrying out the transmission trials. Besides the unavoidable differences between the laboratories with regard to management and handling of the animals, more specific differences are listed in Table 3.1. Because of the differences, Uttenthal et al. (2001) had decided only to compare groups within laboratories. Because of the major similarities between the trials in the different laboratories, we considered a meta-analysis of all trials appropriate. The meta-analysis made a more comprehensive analysis possible.

3.2.2. Statistical analysis

3.2.2.1. Transmission trials and R_0

Transmission trials start with only five infectious pigs and five susceptible[1] contact pigs, whereas the basic reproduction ratio R_0 is defined in an infinite population (cf. Anderson and May, 1991; Diekmann and Heesterbeek, 2000). The stochastic SIR model (Bailey, 1975) can be used to translate the data of transmission trials to R_0. 'SIR' refers to the assumption that each animal is a member of one of the classes S (susceptible), I (infectious), or R (removed, i.e. dead or immune). The animals can proceed from the S class to the I class by infection, due to the presence of infectious animals. Each infectious animal infects susceptible animals with rate $\beta s/N$, where s

[1] In this paper, 'susceptible' means 'not yet infected', so vaccinated animals can also be susceptible.

Table 3.1. Comparison of the experimental procedures in the participating laboratories

Country	Groups	Challenge		Irregularities
		route	dose ($TCID_{50}$)	
France	A14, A21, B14, B21, C	Intranasal	$10^{2.54}$	some pigs were BVD^{+} [a]
Denmark	A14, A21, B14, B21, C	Oral	$10^{4.4}$	All trials in one isolation unit
Spain	A7, A10, B7, B10	Intranasal	10^{5}	
Italy	A7, A10, B7, B10	Intranasal	10^{5}	
Germany	C	Oronasal	10^{3}	

[a] At time of challenge, 20% of the French pigs was still BVD^{+}. Before vaccination, BVD^{+} pigs had been equally distributed among the five trials.

is the actual number of susceptible pigs, N the total number of present pigs, and β is the transmission parameter. Animals in the I class can move to the R class by recovery or death, which happens at rate α, the recovery parameter. The average length of the infectious period is $1/\alpha$.

From the SIR model, R_0 can be determined by regarding an infinite susceptible population, in which $s \approx N$. This results in an infection rate of β and a basic reproduction ratio, which is the average number of new infections per day times the average length of the infectious period, of $R_0 = \beta/\alpha$ (Diekmann and Heesterbeek, 2000).

3.2.2.2. The final size method

The final size method (De Jong and Kimman, 1994; Kroese and De Jong, 2001) was used to test whether the R_0 in the eight different vaccine groups was significantly lower than the R_0 in the control group C. The null-hypothesis to test a vaccine group V (e.g. group A7) against the control group C was H_0: $R_{0,V} = R_{0,C}$. The method was used, because it was perfectly fit for the design of the trials and because it was used in previous transmission experiments (Bouma et al., 2000; Chapter 4). Interpretation of the results is however not straightforward, because the method assumes a constant R_0 in each trial, which was probably unrealistic for the vaccination-challenge intervals of 7 and 10 days, and maybe for 14 and 21 days as well. In the discussion section, the interpretation will be addressed.

The data needed for the final size method were the initial state and the final size of each transmission trial. The initial state consists of the number of challenged (infectious) animals i_0 and the number of contact (susceptible) animals s_0 at the start of the transmission trial. The final size is the number of contact animals that was still susceptible by the end of the trial, s_t. Therefore, for each contact animal it had to be determined whether it had been infected during the trial. As in Uttenthal et al. (2001), a contact animal was defined infected if it had been positive at least once in the E^{rns} ELISA, virus isolation from blood samples, or virus detection from tissue samples.

The test statistic for the final size method was the difference in the number of contact infections between the vaccine and control group. The number of contact infections was $s_t - s_0$ for each trial. The calculated *P*-value is the probability that the difference (or larger) is observed given H_0. A *P*-value < 0.05 was regarded statistically significant.

3.2.2.3. The entire course method

The entire course method was used to estimate the parameters β and α of the SIR model in the second, third, and fourth week after vaccination, and from the fifth week onwards. Although it is a complicated method, which needs more data and assumptions than the final size method, it can cope with the fact that the parameters change with time. Moreover, the estimates of β and α can be used to estimate R_0. Because a changing R_0 due to a changing β or α is hard to interpret, R_0 was only determined for situations with constant β and α, which was in the unvaccinated group C and three weeks after vaccination in groups A and B.

The data needed for the entire course method are exact reconstructions of all transmission trials, that is, for each animal the time of infection, the period of infectiousness, and the time of death were needed. Exact reconstructions could not be made directly from the data, because they were not precise enough. Therefore, 100 possible exact reconstructions were drawn using distributions of the infection times, and of the start and end of the infectious periods for each animal. The distributions were obtained from the viraemia and serology data.

In short, the entire course method consisted of three successive steps. The first was the interpretation of the viraemia and serology data to obtain distributions for the time of infection and the start and end of the infectious period of each animal. The second was the drawing of 100 exact reconstructions with these distributions. The third was the estimation of β and α from these exact reconstructions.

3.2.2.4. Entire course method step 1: data interpretation

To determine the start and end of the infectious period, the assumption was made that infectiousness had coincided with viraemia, as had been assumed in previous analyses (Chapter 2; Chapter 4; Laevens et al., 1998; Laevens et al., 1999). In all trials, blood samples had been taken in time intervals of two to eight days for virus isolation. It was assumed that the start of the infectious period of each animal was uniformly distributed within the time interval before the first positive sample. The end of the infectious period was either uniformly distributed within the time interval after the last positive sample or it was determined by death of the animal when it had still been virus-positive by then.

The time of infection could be reconstructed in one of four possibilities. First, if the animal had been challenged, the time of infection was equal to the time of challenge. Second, if the animal had not shown viraemia and had not been seropositive in the E^{rns} ELISA (the discriminatory ELISA), it had not been infected at all, so there was no infection time. Third, if the animal had shown viraemia, it was assumed that the infection time had been one latent period before the start of the infectious period. The latent period is the interval between becoming infected and infectious. It was determined from the challenged animals in the same laboratory as the mode of their latent periods. Fourth, if the animal had not shown viraemia, but had been seropositive, the interval of seroconversion was determined and the moment of seroconversion was assumed to be uniformly distributed within the interval. Subsequently, a distribution for the time between infection and first seropositivity was needed. It was assumed that this was a lognormal distribution of which the parameters — μ_A and σ_A^2 for the test with vaccine A, or μ_B and σ_B^2 for the test with vaccine B — were determined from the data of the challenged animals (see Appendix 3A).

For some situations, the interpretation of the viraemia and serology data as described could not be followed. Then, exceptions were necessary and the data had to be interpreted case by case. The exceptions are given in the Tables with the Results section (Tables 3.2 – 3.5).

3.2.2.5. Entire course method step 2: exact reconstructions

Each transmission trial was reconstructed 100 times in five steps:

1. If the animal had been infectious, the start of the infectious period was drawn from the interval of first viraemia.
2. If the animal had been infectious, the end of the infectious period was drawn from the interval of last viraemia or it was set at the day of death.

3. If the animal had been infected, the time of infection was determined. For challenged pigs, it was the challenge day. For contact pigs, it was the length of the latent period subtracted from the start of the infectious period, or it was drawn from the appropriate distribution by using the interval of first seropositivity.
4. The day of death of the animal. This was used to define the population size in the trial at each time.
5. Finally, it was checked whether each drawn infection time coincided with at least one animal that was infectious. If that was not the case, the process was restarted at step 1 until a realistic reconstruction was obtained.

3.2.2.6. Entire course method step 3: parameter estimation

The reconstructions were divided into three sets, all vaccine A trials, all vaccine B trials and all group C trials. The group C trials were used to estimate β, α, and R_0 in an unvaccinated population. Each set of vaccine trials was used to estimate β and α in the second, third, and fourth week after vaccination, and from the fifth week onwards. Then it was tested whether β and α were significantly different from week to week. If β (or α) did not differ significantly in successive weeks, the weeks were pooled and β (or α) was re-estimated for the longer period of two or more weeks. The β and α from the periods the longest after vaccination, which include the fifth week onwards, were used to estimate R_0 in a vaccinated population.

Before estimation was possible, assumptions were necessary on how β and α depend on time after vaccination. In the most complex situation, the recovery and infection rates of an infectious animal at time t_{now} depend on (1) the time that the animal was infected t_{inf} and (2) t_{now}. Since the recovery rate reflects the ability of the animal's immune system to clear the virus, it highly depends on the state of the immune system at the time of infection. Hence, α was assumed to depend only on t_{inf}. The infection rate of an animal reflects the animal's immune response too, but it also depends on the susceptibility of the yet uninfected animals. Therefore, β would depend on both t_{inf} as t_{now}, which would create a model with too many different β's in relation to the available data. Because the immune response is already covered by α, it was assumed that β only depends on t_{now}. In short, the α of a certain week is the rate by which animals that are infected in that week will recover and the β of a certain week is, multiplied by s/N, the rate by which infectious animals cause new infections within that very week.

Both β and α were estimated with a generalised linear model (GLM). The method to estimate α had been used before in Chapter 2, although it had to be

adjusted because of the use of 100 reconstructions. A similar method was used to estimate β (see Appendix 3B). The results consisted of one estimate for $\log\beta$ with variance and one estimate for $\log\alpha$ with variance, for the week or weeks of interest. These were used to test whether the parameters were different in the successive weeks and for construction of confidence intervals. The estimates and variances were also used to estimate $\log R_0$ and its variance:

$$\log R_0 = \log\beta - \log\alpha$$
$$\mathrm{var}(\log R_0) = \mathrm{var}(\log\beta) + \mathrm{var}(\log\alpha),$$

from which an estimate and a 95% confidence interval for R_0 were derived. Note that the $\mathrm{cov}(\log\beta, \log\alpha)$ is assumed to be 0. Because $\mathrm{cov}(\log\beta, \log\alpha)$ will not be negative, – a larger α will lead to shorter infectious periods, which has to be compensated by a larger β to obtain the same number of contact infections – $\mathrm{var}(\log R_0)$ will be overestimated. Overestimation results in too wide confidence intervals for R_0.

3.2.3. Vaccination and outbreak size

As mentioned before, it is important to know if the vaccines are able to sufficiently reduce the outbreak size. Therefore, simulations were conducted in which one pig in a group of 100 pigs was infected by CSFV and virus transmission occurred according to the SIR model with the estimated parameters β and α. The parameters were allowed to change if the estimated values changed per week after vaccination.

A comparison was made between an unvaccinated group and groups completely protected by either vaccine A or B. In addition it was examined to what extent the outbreak sizes will be reduced if virus would enter a herd only 7 days after vaccination, when the vaccines are not yet completely protective.

The simulations were carried out in Mathematica® (Wolfram, 1999). For each of the five different situations, 1000 simulations were performed. The situations were compared with the number of pigs that were ultimately infected during the simulation, which is the final size of the outbreak.

3.3. Results

3.3.1. The final size method

Table 3.2. The final size method: data and results.

Trial	s_0 [a]	i_0 [b]	s_t [c]	# c.i. [d]	P [e]
C - Germany	5	5	0		
C - Denmark [f]	7	3	0	18	-
C - France [f]	6	4	0		
A7 - Spain [g]	4	6	0		
A7 - Italy	5	5	5	4	<0.05
A10 - Spain [h]	4	5	2		
A10 - Italy	5	5	0	7	0.11
A14 - Denmark	5	5	3		
A14 - France	5	5	3	4	<0.05
A21 - Denmark	5	5	4		
A21 - France	5	5	5	1	<0.05
B7 - Spain	5	5	0		
B7 - Italy [h]	4	5	2	7	0.11
B10 - Spain	5	5	0		
B10 - Italy	5	5	1	9	0.23
B14 - Denmark	5	5	0		
B14 - France	5	5	0	10	1
B21 - Denmark	5	5	1		
B21 - France	5	5	5	4	<0.05

[a] The number of susceptible animals at the start of the trial

[b] The number of infected animals at the start of the trial

[c] The number of susceptible animals at the end of the trial

[d] The total number of contact infection in the treatment group

[e] The *P*-value of the final size test for testing against group C

[f] Because in these trials one and two pigs were only viraemic at the time of the other contact pigs, it was assumed that the inoculation had been unsuccessful and the pigs were regarded as contact pigs

[g] In this trial, one animal was viraemic at day 11, just like the challenged animals, and has therefore been treated as a challenged animal

[h] In these trials, one pig had died before the challenge day

The data of all transmission trials are listed in Table 3.2 and were used for the test with H_0: $R_{0,V} = R_{0,C}$. The resulting *P*-values are shown in Table 3.2 as well. CSFV transmission was significantly reduced in groups A7, A14, and A21, however not in group A10. Transmission was also significantly reduced in group B21.

3.3.2. The entire course method

First, the parameters μ and σ^2 of the distribution of the challenge-seropositivity interval were determined for both E^{rns} ELISAs. The challenged animals were found to seroconvert by ELISA A in the following time intervals after challenge:

8 – 10 (3×), 9 – 11 (8×), 10 – 12 (7×), 12 – 14 (2×), 12 – 15, 14 – 16, 14 – 21, 15 – 18, 21 – 25, 21 – 28, 28 – 35 (4×), 35 – 42 (5×),

from which μ_A was estimated at 2.73 and σ_A^2 at 0.279. With the vaccine B ELISA, the time intervals of seroconversion were found to be

6 – 8, 8 – 10 (9×), 9 – 12, 10 – 12 (12×), 12 – 14 (7×), 12 – 15 (3×), 12 – 17 (4×), 14 – 21 (2×), 17 – 23 (3×), 18 – 22, 21 – 28,

from which μ_B was estimated at 2.49 and σ_B^2 at 0.0635.

The data for the entire course method are listed in Table 3.3 for all groups A, in Table 3.4 for all groups B, and in Table 3.5 for group C. Table 3.6 lists the estimates of β and α from the vaccine-A trials and from the group-C trials and Table 3.7 lists the estimates from the vaccine-B trials and the group-C trials.

The β estimated from group C was 0.65 (95% CI: 0.40 – 1.1), whereas the α was estimated at 0.065 (95% CI: 0.045 – 0.096). The resulting R_0 in unvaccinated pigs was 9.9 (95% CI: 5.3 – 18).

For vaccine A (Table 3.6), the transmission parameter β did not differ significantly between time intervals. Consequently, all data were pooled and β was estimated for the whole period after vaccination, which led to an estimate of 0.29 (95% CI: 0.17 – 0.48), which was significantly lower than the β of group C. Recovery parameter α was estimated in the same time intervals as β. The α appeared to increase weekly until at least the fourth week after vaccination. From the fifth week onwards, only one data point was available, with $T_k = 0$. Therefore, α was re-estimated for the whole period from 21 days after vaccination at 6.1 (95% CI: 2.4 – 16), which was significantly higher than the α of group C. As explained in section 2.2.3, the basic reproduction ratio R_0 was only estimated with constant β and α, which was 21 days after vaccination: R_0 was 0.047 (95% CI: 0.016 – 0.14). Thus, vaccine A significantly reduced R_0, which reached a value significantly smaller than 1.

Table 3.3. Reconstruction of the transmission trials of group A.

Trial	Time of infection[b] (days[a])	Start of infectious period[c] (days[a])	End of infectious period[d] (days[a])	Trial	Time of infection[b] (days[a])	Start of infectious period[c] (days[a])	End of infectious period[d] (days[a])
Spain – A7	7	9 – 11	24	Denmark – A14	14	18 – 20	20 – 22
	7	9 – 11	15 – 17		14	-	-
	7	9 – 11	17 – 19		14	-	-
	7	9 – 11	34		14	18 – 20	20 – 22
	7	9 – 11	31		14	- [h]	- [h]
	(-3)	17 – 19	21 – 28		-	-	-
	(-3)	17 – 19	28 – 35		-	-	-
	(-3)	17 – 19	21 – 28		-	-	-
	7 [e]	9 – 11	21 – 28		49 – 56	- [h]	- [h]
	(-3)	17 – 19	19 – 21		42 – 49	-	-
Italy – A7	7	14 – 16	21 – 25	France – A14	14	-	-
	7	14 – 16	22		14	-	-
	7	16 – 18	25 – 28		14	-	-
	7	14 – 16	25 – 28		14	18 – 20 [i]	18 – 20 [i]
	7	14 – 16	18 – 21		14	-	-
	-	-	-		-	-	-
	-	-	-		-	-	-
	-	-	-		42 – 49	-	-
	-	-	-		-	-	-
	-	-	-		42 – 49	-	-
Spain – A10	10	12 – 14	20 – 22	Denmark – A21	21	27 – 29 [h]	29 – 31 [h]
	10	12 – 14	22 – 24		21	- [h]	- [h]
	10	12 – 14	22 – 24		21	-	-
	10	10 – 12	14 – 16		21	- [h]	[h]-
	10	12 – 14	18 – 20		21	-	-
	(-3)	16 – 18	18 – 20		-	-	-
	(-3)	18 – 20	22 – 24		-	- [h]	- [h]
	-	-	-		-	-	-
	-	-	-		-	- [h]	- [h]
	- [f]	- [f]	- [f]		56 – 63	-	-
Italy – A10	10	17 – 19	28 – 31	France – A21	21	-	-
	10	19 – 21	28 – 31		21	-	-
	10	17 – 19	26 – 28 [g]		21	-	-
	10	19 – 21	31 – 35		21	-	-
	10	19 – 21	28 – 31		21	-	-
	28 – 31	-	-		-	-	-
	38 – 46	-	-		-	-	-
	(-8)	26 – 28	32		-	-	-
	26 – 28	-	-		-	-	-
	31 – 35	-	-		-	-	-

Caption to Table 3.3.
[a] Days since vaccination
[b] Single positive numbers are challenge times; negative numbers between brackets are the latent periods, to be subtracted from the start of the infectious period to obtain the infection times; double numbers are the intervals in which the seropositivity started; dashes are for uninfected animals
[c] Double numbers are the intervals in which the infectious periods started; dashes are for animals that were not infectious
[d] Single numbers are the times of death and hence the ends of the infectiousness; double numbers are the intervals in which the infectious period ended
[e] This animal was viraemic at day 11, just like the challenged animals, and has therefore been treated as a challenged animal
[f] This animal was dead before the start of the experiment
[g] This animal died at day 37
[h] These animals had for at least one day only one virus-positive well of six replicates, which was considered a false-positive
[i] This infectious period was based on fever (T > 40 °C) and was necessary to explain the two contact infections

For vaccine B (Table 3.7), β did not change from week to week after vaccination either, so β was ultimately estimated at 0.34 (95% CI: 0.23 – 0.49). This differed significantly from the non-vaccination β and was almost equal to the β with vaccine A. The course of α looked similar for both vaccines as well. Unlike the trials with vaccine A, the trials with vaccine B did contain enough data for the time after the fourth week, from which a further increase of α was not observed. After pooling the last two time intervals, the α after 21 days was estimated at 0.82 (95% CI: 0.51 – 1.3), which was significantly different from the non-vaccination α, and also from the α with vaccine A. The resulting R_0 was 0.41 (95% CI: 0.22 – 0.75), which was significantly different from the unvaccinated group C, and also from vaccine A. Although the two vaccines differed with respect to α and R_0, they both let R_0 significantly decrease to a value below 1.

3.3.3. Vaccination and outbreak size

The outbreak simulations in a non-vaccinated population resulted in either small or large outbreaks: 90 of the 1000 simulations resulted in a final size of 1 or 2 infected animals, whereas in 910 simulations the final size was 99 or 100. For the vaccinated groups there was no distinction between small and large outbreaks possible: the median and 95% confidence intervals are shown in Table 3.8.

Table 3.4. Reconstruction of the transmission trials of group B.

Trial	Time of infection[b] (days[a])	Start of infectious period[c] (days[a])	End of infectious period[d] (days[a])	Trial	Time of infection[b] (days[a])	Start of infectious period[c] (days[a])	End of infectious period[d] (days[a])
Spain – B7	7	9 – 11	20	Denmark – B14	14	18 – 20	22 – 24
	7	9 – 11	19 – 21		14	22 – 24 [g]	24 – 26 [g]
	7	9 – 11	28		14	18 – 20	24 – 26
	7	9 – 11	21 – 28		14	20 – 22 [g]	24 – 26 [g]
	7	9 – 11	14		14	18 – 20	20 – 22
	(-3)	11 – 13	28 – 35		(-5)	26 – 28 [g*]	28 – 35 [g*]
	(-3)	17 – 19	28 – 35		(-5)	35 – 42	42 – 49
	(-3)	17 – 19	21		35 – 42	-	-
	(-3)	17 – 19	21 – 28		42 – 49	-	-
	(-3)	15 – 17	28 – 35		28 – 35	-	-
Italy – B7	7	11 – 14	24 – 30	France – B14	14	18 – 20	20 – 22
	7	7 – 11	31		14	-	-
	7	7 – 11	41		14	16 – 18	20 – 22
	7	11 – 14	34		14	-	
	7	11 – 16	22		14	-	-
	38 – 44	-	-		35 – 42	-	-
	30 – 38	-	-		35 – 42	-	-
	-	-	-		35 – 42	-	-
	-	-	-		35 – 42	-	-
	-[e]	-[e]	-[e]		28 – 31	-	-
Spain – B10	10	12 – 14	28	Denmark – B21	21	-	-
	10	12 – 14	24 – 31		21	25 – 27	29 – 31
	10	14 – 16	22 – 24		21	-	-
	10	14 – 16	24 – 31		21	25 – 27	31 – 33
	10	14 – 16	16 – 18		21	25 – 27	27 – 29
	(-3)	16 – 18 [f]	31 – 38 [f]		49 – 56	-	-
	(-3)	16 – 18 [f]	31 – 38 [f]		42 – 49	- [g]	- [g]
	(-3)	14 – 16	28		42 – 49	-	-
	(-3)	22 – 24	31 – 38		33 – 35	- [g]	- [g]
	(-3)	18 – 20	24 – 31		-	- [g]	- [g]
Italy – B10	10	14 – 17	22 – 27	France – B21	21	-	-
	10	10 – 14	17 – 19		21	-	-
	10	17 – 19	27 – 41		21	-	-
	10	17 – 19	27 – 33		21	-	-
	10	10 – 14	45		21	-	-
	27 – 33	-	-		-	-	-
	27 – 33	-	-		-	-	-
	33 – 41	-	-		-	-	-
	-	-	-		-	-	-
	41 – 47	-	-		-	-	-

Caption to Table 3.4.
[a] Days since vaccination
[b] Single positive numbers are challenge times; negative numbers between brackets are the latent periods, to be subtracted from the start of the infectious period to obtain the infection times; double numbers are the intervals in which the seropositivity started; dashes are for uninfected animals
[c] Double numbers are the intervals in which the infectious periods started; dashes are for animals that were not infectious
[d] Single numbers are the times of death and hence the ends of the infectiousness; double numbers are the intervals in which the infectious periods ended
[e] This animal was dead before the start of the experiment
[f] Within a series of positive samples, these animals had at least one negative sample, which was considered false-negative
[g] These animals had for at least one day only one virus-positive well of six replicates, which was considered a false-positive
[g*] For this animal, the one positive of six replicates was considered positive to explain the subsequent contact infection

When CSFV enters a pig unit 21 days after vaccination, the number of infected pigs will remain very small with either vaccine. In most cases, only the initially infected pig will be infected and sometimes, especially with vaccine B, a few more will. Also when CSFV enters a pig unit only a week after vaccination, when α is still increasing for another two weeks, the size of the outbreak will be considerably reduced. With vaccine A, the upper 95% limit of the 1000 simulations reveals that only 21 out of 100 pigs will be infected, so there is only a 2.5% chance that this will be more. With vaccine B, the upper limit is 41 infected animals.

3.4. Discussion

This paper described an analysis of CSFV transmission trials that were carried out in the EU to test two E2 subunit marker vaccines. With the final size method, the reduction of CSFV transmission was tested. Virus transmission was significantly reduced when virus was introduced 7, 14 or 21 days after vaccination with vaccine A (produced by Bayer, Germany). Transmission was also reduced 21 days after vaccination with vaccine B (produced by Intervet, The Netherlands). The entire course method was used to estimate the parameters β, α, and R_0 of the SIR model. Vaccination with both vaccines reduced the transmission parameter β by about 50%. More importantly, the vaccines increased the recovery parameter α, vaccine A even more than vaccine B. Both vaccines reduced R_0 to a value significantly below 1.

Table 3.5. Reconstruction the transmission trials of group C

Trial	Time of infection[b] (days[a])	Start of infectious period[c] (days[a])	End of infectious period[d] (days[a])
Germany – C	0	2 – 4	16
	0	4 – 6	22
	0	4 – 6	8
	0	4 – 6	25
	0	4 – 6	16
	(-5)	12 – 15	18
	(-5)	12 – 15	57
	(-5)	12 – 15	30
	(-5)	12 – 15	45
	(-5)	12 – 15	32
Denmark – C	(-5) [e]	14 –21	29
	0	4 – 6 [f]	12 – 14 [f]
	(-5) [e]	14 – 21	30
	0	4 – 6 [g]	31 [g]
	0	4 – 6	43 [h]
	(-5)	14 – 21	31
	(-5)	14 – 21	28 – 35
	(-5)	10 – 12	31
	(-5)	12 – 14	21 – 28
	(-5)	10 – 12 [f]	28 – 35 [f]
France – C	0	6 – 8	29
	(-5) [e]	12 – 14	14 – 16
	0	4 – 6	8 – 10
	0	4 – 6	26
	0	2 – 4 [g]	14 – 16 [g]
	(-5)	8 – 10 [g]	17 – 21 [g]
	(-5)	12 – 14	14 – 16
	(-5)	12 – 14	14 – 16
	(-5)	12 – 14	23
	(-5)	12 – 14	17 – 21

[a] Days since start of the transmission trial

[b] Single positive numbers are challenge times; negative numbers between brackets are the latent periods, to be subtracted from the start of the infectious period to obtain the infection times

[c] The intervals in which the infectious periods started

[d] Single numbers are the times of death and hence the ends of the infectiousness; double numbers are the intervals in which the infectious periods ended

[e] Because this pig was only viraemic at the time of the other contact pigs, it was assumed that the inoculation had been unsuccessful and the pig was regarded as a contact pig

[f] These animals had for at least one day only one virus-positive well of six replicates, which was considered a false-positive

[g] Within a series of positive samples, these animals had at least one negative sample, which was considered false-negative

[h] The pig was still infectious, but the experiment stopped at this time.

The estimated β and α were used in simulations of outbreaks in a pig unit of 100 pigs. It appeared that the size of an outbreak will be greatly reduced if the pigs are vaccinated. Already when virus enters the unit only 7 days after vaccination, the number of animals that become infected is considerably lower than in the unvaccinated group.

With the final size method, the implicit assumption is made that R_0, and thus β and α, are constant over time. This was probably not the case in at least some of the

Table 3.6. Estimates and 95% confidence intervals of β, α, and R_0 without vaccination and with time after vaccination with vaccine A.

		Control	Day 7 – 14	Day 14 – 21	Day 21 – 28	Day 28 – ∞
β	per interval [a]	0.65 0.40 – 1.1	0.081 $2.5\ 10^{-10}$ – $2.7\ 10^{7}$	0.42 0.22 – 0.79	0.16 $4.7\ 10^{-6}$ – $5.5\ 10^{3}$	1.2 $3.3\ 10^{-7}$ – $3.8\ 10^{6}$
	after joining [b]	0.65 0.40 – 1.1	0.29 0.17 – 0.48			
α	per interval [a]	0.065 0.045 – 0.094	0.098 0.063 – 0.15	0.50 0.31 – 0.79	5.5 2.1 – 14	∞ [d]
	after joining [b]	0.065 0.045 – 0.094	0.098 0.063 – 0.15	0.50 0.31 – 0.79	6.1 2.4 – 16	
R_0	after joining [c]	9.9 5.3 – 18	ND [e]	ND [e]	0.047 0.016 – 0.14	

[a] Estimates without vaccination and per week after vaccination

[b] Estimates without vaccination and per period after vaccination within which the estimates for the subsequent weeks did not differ significantly

[c] Estimates without vaccination and with maximum protection by the vaccine

[d] Only one animal of the Denmark-A21 trial was, in some of the 100 exact reconstructions, part of the interval and had an infectious period of 0 days.

[e] Not determined, for the vaccine had not reached maximum protection yet.

Table 3.7. Estimates and 95% confidence intervals of β, α, and R_0 without vaccination and with time after vaccination with vaccine B.

		Control	Day 7 – 14	Day 14 – 21	Day 21 – 28	Day 28 – ∞
β	per interval [a]	0.65 0.40 – 1.1	0.25 0.077 – 0.83	0.35 0.18 – 0.69	0.26 0.088 – 0.79	0.50 0.20 – 1.2
	after joining [b]	0.65 0.40 – 1.1	0.34 0.23 – 0.49			
α	per interval [a]	0.065 0.045 – 0.094	0.069 0.046 – 0.10	0.28 0.16 – 0.46	0.86 0.48 – 1.5	0.74 0.25 – 2.2
	after joining [b]	0.065 0.045 – 0.094	0.069 0.046 – 0.10	0.28 0.16 – 0.46	0.82 0.51 – 1.3	
R_0	after joining [c]	9.9 5.3 – 18	ND [d]	ND [d]	0.41 0.22 – 0.75	

[a] Estimates without vaccination and per week after vaccination

[b] Estimates without vaccination and per period after vaccination within which the estimates for the subsequent weeks did not differ significantly

[c] Estimates without vaccination and with maximum protection by the vaccine

[d] Not determined, for the vaccine had not reached maximum protection yet.

EU transmission trials, which makes it important to discuss the meaning of the obtained *P*-values. A *P*-value larger than 0.05 (groups A10, B7, B10, and B14) would usually mean that virus transmission was not significantly reduced in the regarded groups. Because it is known from groups A21 and B21 that transmission is significantly reduced after 21 days, most of the transmission in groups A10, B7, B10, and B14 must have taken place in the initial stage of the trials. Thus, if $P > 0.05$, the vaccine is not shown to be effective after the regarded vaccination-challenge interval. On the other hand, if the *P*-value is smaller than 0.05 (groups A7, A14, A21, and B21), it is not certain that the vaccine reduced transmission immediately after the regarded interval. It can only be concluded that the vaccine was effective soon enough to prevent much virus transmission. In group A7, protection was probably insufficient in the first days, because otherwise group A10 should have shown a significant reduction as well. In groups A14, A21, and B21, immediate reduction of transmission is very likely if the results of the entire course method are considered: R_0 was probably smaller than 1 during these trials.

Table 3.8. Results of the simulations of CSFV outbreaks in a pig unit with 100 pigs. The outbreaks started with 1 infectious pig.

Vaccine	Interval between vaccination and virus entry	Final size	95% CI
No vaccine	--	[a]	[a]
Vaccine A	7 days	5	1 – 21
Vaccine A	21 days	1	1 – 2
Vaccine B	7 days	11	1 – 41
Vaccine B	21 days	1	1 – 6

[a] Without vaccination, either small outbreaks of 1 or 2 (90 of 1000 simulations) or large outbreaks of 99 or 100 infected animals (910 of 1000 simulations) occurred.

A second implicit assumption was that the specificity and the sensitivity of the E^{rns}-ELISAs were 100%, while they were 92% (sp.) and 74% (se.) for E^{rns} ELISA A, and 71% (sp.) and 94% (se.) for E^{rns} ELISA B (De Smit et al., 2000b; Floegel Niesmann, 2001). Due to the low specificity, observed contact infections in the vaccine groups might have been false-positive. Then, the effectiveness of the vaccines was underestimated in the final size analysis. By opposite reasons, the low sensitivity might have caused an overestimation of the vaccines' effects, but this is not likely because of the multiple tests on each animal. The effects of imperfect tests on the results of the entire course method are more complicated. If a too lowly sensitive test misses a contact infection, β is obviously underestimated, but so is α, since the unobserved infected animal was not observed by viraemia either and consequently had a 0 days infectious period. Since β is estimated by less data than α, the estimate of β is probably more sensitive to an incorrect test result. Hence, a

low sensitivity may underestimate R_0. However, there is no need to question the vaccines' effectiveness, since R_0 will still be smaller than 1 if β *is* underestimated (and unchanged by vaccination) and α is not.

The next step is to see whether the difference in sensitivity can explain the difference between the two vaccines, as suggested by (Uttenthal et al., 2001). Since increased sensitivity would lead to a higher β and a higher α at the same time, β and α should have higher estimates with vaccine B, if both vaccines are equally effective. However, β is the same for both vaccines and α is higher with vaccine A instead of B, so the difference in sensitivity cannot explain the difference in R_0 between the two vaccines. Hence, the difference in R_0 between the two vaccines is probably a biological difference, although its nature remains unclear. Speculations have been made on the adjuvants and the quality or quantity of antigen per vaccination dose (Depner et al., 2001).

The reason not to analyse all trials simultaneously is that they were carried out in different laboratories. Namely, the risk of such a meta-analysis is that observed 'treatment' effects are not solely due to the treatment, but also to other differences. Therefore, the consequences of the laboratory differences should be considered for interpretation of the results. In the EU transmission experiment, three interpretation problems could arise:

1. Comparison of groups A7, A10, B7, and B10 to group C. The conclusions from trials A7, A10, B7, and B10 were that the vaccines were not yet sufficiently effective before 14 (final size method) or 21 (entire course method) days after vaccination. Hence, there is no risk of an observed 'treatment' effect not caused by the treatment.
2. Comparison of groups A7, A10, B7, and B10 to groups A14, A21, B14, and B21. The groups have not been directly compared, but used simultaneously to estimate β and α. However, an indirect comparison was made because the 7 and 10 days groups mainly provided data for weeks 2 and 3 after vaccination, and the 14 and 21 days groups for weeks 4 and 5. It might have led to a wrong conclusion about the course of β over time, but will not have affected the conclusions that R_0 was smaller than 1 after 21 days or the actual estimates of R_0.
3. The use of the German group-C trial. Since it was the only trial carried out in Germany, Uttenthal et al. (2001) did not use this trial in their analysis at all. The reason to include the trial in our analysis after all was that the results were very similar to the other two trials of group C, and to all control trials of other CSFV transmission experiments as well (Chapter 4; Bouma et al., 2000; Laevens et al., 1998; Laevens et al., 1999).

The final assumption that needs attention is that viraemia is the indicator for infectiousness, which had been assumed in the analysis of other transmission experiments as well (Chapter 4; Laevens et al., 1998; Laevens et al., 1999). A problem with the assumption is that virus excretion might also occur while viraemia is not observed, though possibly at lower levels. However, that will be a problem with any assumption and is related to the assumption of constant infectiousness in the SIR model. Here, viraemia is used to indicate infectiousness, because viraemia is a sign of virus replication and it is likely that virus excretion can occur when virus is replicating within the animal.

Uttenthal et al. (2001) analysed the same experiment and used the Fischer's exact test to show a difference between the two vaccines in the number of contact infections, especially after 14 days. Qualitative data inspection led to the conclusion that the vaccines reduced virus shedding from 14 days after vaccination, but that no complete protection was achieved. No conclusion could be made on whether the protection would be sufficient. Both analysis methods in our paper, the final size method as well as the entire course method, made use of the SIR model of virus transmission between animals. The effect of the vaccines was investigated by obtaining inference on the parameters of the SIR model, i.e. β, α, and R_0, either by testing whether treatment groups differ or by directly estimating the parameters. The advantage of using the parameters of the SIR model is that it is easy to extrapolate the results to larger populations, which was shown by the simulations.

Vaccine A was also tested by Bouma et al. (2000), who showed that in SPF pigs, transmission was already significantly reduced 10 days after vaccination. After 14 days, R_0 was significantly smaller than 1. The later onset of protection in the EU trials might be explained by the use of conventional pigs, i.e. non-SPF pigs. Also, the heterogeneity due to the different laboratories might have reduced the power of the statistical tests and hence delayed finding a significant reduction in transmission. Vaccine B was also tested by Dewulf et al., who studied horizontal transmission between weaner pigs (Dewulf et al., 2000) and sows (Dewulf et al., 2001), but only vaccinated the contact pigs and not the challenged pigs. Their conclusion was that the vaccine did not prevent contact infection, which is in accordance with our findings that the largest effect of the vaccines lies in the increase of α, and not in the decrease of β.

The decision to use vaccination as an emergency measure during a CSFV epidemic will depend on many factors. Policy makers will have to weigh epidemiological, economic, and ethical arguments, brought up by the European Union legislation, the pig industry, and the public opinion. Important aspect of the discussion will always be the effectiveness of a vaccination campaign. The ultimate effect of emergency vaccination should be that a CSFV epidemic will come quickly

to an end, with less herds affected. However, it cannot be predicted directly from the results of the analyses how fast an epidemic may stop and how many herds may be infected. Extrapolation of the between-animal transmission to between-herd transmission, e.g. by means of mathematical modelling, is needed. What can be concluded, however, is that virus transmission between animals will be sufficiently reduced three weeks after vaccination with either marker vaccine. Already when virus enters a herd only one week after vaccination, the size of the outbreak will be much smaller than without vaccination. Hence, from the epidemiological point of view, emergency vaccination looks very promising.

Acknowledgements

The first author gratefully acknowledges financial support from STW (Technology Foundation), Utrecht, The Netherlands. All authors thank the EU Commission for financing the project; Bayer AG, Leverkusen, Germany and Intervet BV, Boxmeer, The Netherlands for supplying the vaccines; and ID-Lelystad, The Netherlands and Dr Bommeli AG, Bern, Switzerland for supplying the Ceditest and Chekit ELISA tests.

Appendix 3A

The distribution parameters μ_A and σ_A^2 for the E^{rns}-ELISA A, and μ_B and σ_B^2 for ELISA B were estimated from the serological data of the challenged animals. Because the animals in group C had been tested with both ELISAs, they were used for estimation of both μ_A and σ_A^2, and μ_B and σ_B^2. Animals that had not become seropositive during the trials were omitted, because it concerns a distribution given that the animal becomes seropositive within the duration of the trials.

If the animal is tested with E^{rns}-ELISA A, the probability that the random variable $T_{seropos.}$ falls within interval (t_1, t_2) is

$$P\left(T_{seropos.} \in (t_1, t_2)\right) = \int_{t_1}^{t_2} PDF_{\mu_A, \sigma_A^2}(t)dt \ ,$$

in which $PDF_{\mu_A, \sigma_A^2}(t)$ is the probability density function of the lognormal distribution with mean μ_A and variance σ_A^2, and t is the time since challenge. Hence,

the data of all challenged and tested animals k were used to construct the log-likelihood function of μ_A and σ_A^2:

$$L\left(\mu_A, \sigma_A^2\right) = \sum_k \log\left[\int_{t_{1,k}}^{t_{2,k}} PDF_{\mu_A,\sigma_A^2}(t)dt\right] \tag{3.1}$$

Maximising Eq. (3.1) for the dataset of E^{rms}-test A revealed the estimates for μ_A and σ_A^2. Estimates for μ_B and σ_B^2 were obtained analogously.

Appendix 3B

3B.1. Estimation of β

The method for estimation of β could be used for any desired time interval of the transmission trials. Within the interval under consideration, it was assumed that β was a constant parameter.

For estimation of β, a survival analysis was used. Survival analysis makes use of a hazard function $h(t,\beta)$:

$$h(t,\beta) = \beta \cdot i(t) = \beta \frac{\#\,\text{infectious animals}(t)}{\text{total}\,\#\,\text{animals}(t)},$$

which represents the pressure which the susceptible animals are subject to until they are infected. Each transmission trial had a different $h(t,\beta)$, where $i(t)$ is the infectiousness function of the trial.

To estimate β in a specific time interval, each susceptible animal that had been subject to one of the $h(t,\beta)$ within that interval made up one record. For example, if we consider the week from day 14 until day 21 with vaccine A, the dataset consisted of all animals from the trials of treatment groups A7, A10, and A14 that had still been susceptible at day 14 after vaccination. Each record k consisted of two data, y_k and t_k. The y_k denoted whether the t_k was truncated: y_k was equal to 1 if animal k had been infected within the considered time interval, otherwise it was 0. The t_k was either equal to the time of infection of the animal (if $y_k = 1$) or to the end of the time interval (if $y_k = 0$).

If the time interval started at time t_{start}, the accumulated infectiousness I_k for each animal k at time t_k was calculated as

$$I_k = \int_{t_{start}}^{t_k} i_k(t)dt ,$$

in which $i_k(t)$ was the infectiousness function of the trial which animal k had been part of. Now the probability of observing (y_k, t_k) was

$$\begin{aligned} P(Y_k = y_k \cap T_k = t_k) &= [(\beta \cdot i_k(t_k))\exp(-\beta \cdot I_k)]^{y_k} [\exp(-\beta \cdot I_k)]^{1-y_k} . \\ &= (\beta \cdot i_k(t_k))^{y_k} \exp(-\beta \cdot I_k) \end{aligned}$$

From this probability, the likelihood function for β could be constructed as follows:

$$\begin{aligned} L(\beta) &= \prod_{k=1}^{n} \left[(\beta \cdot i_k(t_k)\exp(-\beta \cdot I_k))^{y_k} (\exp(-\beta \cdot I_k))^{1-y_k}\right] \\ &= \prod_{k=1}^{n} \left[(\beta \cdot i_k(t_k))^{y_k} \exp(-\beta \cdot I_k)\right] \\ &= \prod_{k=1}^{n} \left[(\beta \cdot I_k)^{y_k} \exp(-\beta \cdot I_k)\right] \Big/ \prod_{k=1}^{n} \left[(I_k / i_k(t_k))^{y_k}\right] \end{aligned} .$$

The kernel of this likelihood is the same as it would be with a set of n observations y_k, each having an independent Poisson distribution with mean βI_k (see Aitkin et al., 1989). Therefore, the analysis was performed with a generalised linear model (GLM), where y_k denoted the response variate, $\log I_k$ the offset, and the model was fitted with a log LINK function and a Poisson distribution. The output was an estimate of $\log\beta$ and its estimated variance. The GLM was programmed in Mathematica® (Wolfram, 1999).

The 100 exact reconstructions of each transmission trial resulted in 100 estimates for β for each considered time interval. The average of the 100 estimates was considered as the final maximum likelihood estimate. The variance of the final maximum likelihood estimator consisted of two parts, viz. the average of the 100 estimated variances and the variance of the 100 estimates (Rao, 1973):

$$\mathrm{var}_{\vartheta}(\hat{\beta}(\vartheta)) = \mathrm{var}_{\vartheta}[E(\hat{\beta}(\vartheta)|\vartheta)] + E_{\vartheta}[\mathrm{var}(\hat{\beta}(\vartheta)|\vartheta)],$$

in which $\mathcal{9}$ represents the vector of all data (the infection times, the starting and ending times of the infectious periods, and the times of death of the animals) and $\hat{\beta}(\mathcal{9})$ is the estimator of β as a function of the data $\mathcal{9}$.

3B.2. Estimation of α

As with the estimation of β, the method for estimating α can be used for any desired time interval. The α of the considered time interval is the recovery rate of each animal infected within the time interval. The estimation method for α was a survival analysis as well. The hazard function $h(t,\alpha)$ of recovery was

$$h(t,\alpha) = \alpha$$

and was therefore independent of time and transmission trial.

Each animal that became infected (not infectious) within the time interval under consideration, made up one record. Each record k consisted of two data, y_k and T_k. The y_k was 1 if the animal had recovered before the end of the experiment, and was 0 otherwise. The T_k was the length of the infectious period. A likelihood function could be constructed similarly to the likelihood function for β, and α could be estimated by use of a GLM with a log LINK function and a Poisson distribution, and with y_k as the response variate and $\log T_k$ as the offset. An estimate for $\log\alpha$ was obtained, with the variance of its estimator. In Chapter 2, the same method had been used for estimation of α.

A final maximum likelihood estimate for $\log\alpha$ was obtained by averaging the 100 estimates from all exactly reconstructed transmission trials. A variance of the estimator of $\log\alpha$ was determined similarly to that of $\log\beta$, by adding the average variance of the 100 estimates to the variance of the 100 estimates.

Chapter 4

Influence of maternal antibodies on efficacy of a subunit vaccine: transmission of classical swine fever virus between pigs vaccinated at two weeks of age

D. Klinkenberg, R.J.M. Moormann, A.J. de Smit, A. Bouma, M.C.M. de Jong

Reprinted from *Vaccine*, Vol 20, Pages 3005-3013, Copyright (2002), with permission from Elsevier

Abstract

This study shows the effectiveness of vaccination with an E2 subunit vaccine against classical swine fever in two-week old piglets. Half of the piglets were carrying maternally derived antibodies at the time of vaccination. Three and six months later, antibody levels were compared between the two treatments. Moreover, reduction of virus transmission was investigated at three and six months by doing transmission experiments. The vaccine was found to be capable of reducing virus transmission significantly at both time intervals. Maternal immunity reduced vaccination-induced antibody levels after three and six months and possibly led to a less effective protection against virus transmission after six months.

4.1. Introduction

Classical swine fever (CSF) is a contagious pig disease caused by classical swine fever virus (CSFV) (Dahle and Liess, 1992; Taylor, 1995; Terpstra, 1987). Occasional epidemics of CSFV in domestic pigs can cause huge problems, as exemplified by the Dutch epidemic in 1997/1998. The epidemic caused the killing of over 12 million pigs and a loss of US$ 2.3 billion (Meuwissen et al., 1999; Stegeman et al., 2000). New epidemics might occur in the future as re-introduction of the virus in the domestic pig population is a constant threat (Edwards et al., 2000; Moennig, 2000). Although preventive slaughter of pig herds that had been at risk for infection was a major control measure in the Dutch epidemic (Stegeman et al., 1999b), in future epidemics, vaccination might be a better option for ethical reasons (Terpstra et al., 2000). Emergency vaccinations during CSF epidemics are allowed by the EU (Anonymous, 1980), but have not been applied yet. This is, because with the hitherto used vaccines and diagnostic tests infection of vaccinated pigs cannot be detected, so that emergency vaccinations would result in prolonged trade bans by other countries and are therefore economically not profitable (Terpstra et al., 2000).

This problem could be overcome by a marker vaccine against CSFV with an accompanying diagnostic test to detect infection in vaccinated animals, provided that such a vaccine would be internationally accepted. One such marker vaccine is based on the glycoprotein E2 of the virus, produced in an insect cell-baculovirus expression system (Hulst et al., 1993; Moormann et al., 2000). Several experiments have shown that this vaccine can prevent clinical signs in and virus transmission between animals (Bouma et al., 2000; Bouma et al., 1999; Hulst et al., 1993). In SPF (specified pathogen free) pigs, transmission appeared to be significantly lower if

infection occurred at 10 days after vaccination (Bouma et al., 2000). In conventional pigs, transmission appeared to be significantly lower only after 14 days. Estimations of the basic reproduction ratio R_0, i.e., the average number of secondary infections caused by one infectious individual in a fully susceptible population, appeared to decrease from 6.8 without vaccination to 0.36 three weeks after vaccination (Klinkenberg, unpublished results). Stochastic simulations of a CSF epidemic with vaccination based on the 1997-1998 epidemic in the Netherlands showed that vaccination might lead to a reduction of the magnitude and the costs of the epidemic (Mangen et al., 2001).

If, in case of application of the vaccine in emergency situations, young pigs are immunised in the first weeks after birth, two complications could arise. First, if the sow has been vaccinated, its piglets receive maternally derived antibodies (MDA) via the colostrum, which may interfere with the immune response to the vaccine. MDA have been shown to affect antibody responses to non-live vaccines (Siegrist, 2001) and to the C-strain vaccine against CSF, a modified live vaccine (Precausta et al., 1978). Also, the potential of MDA-related reduction of vaccine efficacy in affecting transmission has been observed in experiments with vaccination against pseudorabies virus (Bouma et al., 1997). Second, the efficacy of the vaccine will possibly decrease as the piglets get older. Decrease of antibody titre over time occurs in some pigs vaccinated with the E2 vaccine (De Smit et al., 2001a), which might result in a finishing pig population with insufficiently protected pigs, allowing virus transmission within a herd and to other herds. The aim of the research described in this paper was to determine whether vaccination of piglets at two weeks of age, with or without presence of MDA, reduced transmission of CSFV three and six months later.

4.2. Materials and Methods

4.2.1. Experimental design

The whole experiment consisted of six different transmission trials, in which transmission of CSFV was investigated. Each of these transmission trials was conducted with a group of ten animals with the same vaccination history and age. Groups C and D were born of twice vaccinated sows and were vaccinated themselves at the age of two weeks. Groups E and F were born of naive sows and were vaccinated at the age of two weeks. Finally, groups G and H were born of naive sows and were not vaccinated. The transmission trials with groups C, E, and G

started when the animals were at the age of about 3.5 months, whereas the trials with groups D, F, and H started three months later, with animals of about 6.5 months old.

4.2.2. Animals and vaccination

Six pregnant sows from the conventional pig herd of the Veterinary Faculty in Utrecht were transported to ID-Lelystad, where the whole experiment was carried out. The sows were free of antibodies against pestiviruses. Three of the sows received a double dose (4 ml.) of E2 marker vaccine (16 μg/ml inactivated E2 antigen in a Double Oil Emulsion adjuvant), intramuscularly (i.m.) behind the ear, at day 70 of pregnancy. The same sows were vaccinated a second time, four weeks later, with a single dose (2 ml) i.m. The other three sows were not vaccinated, but instead they were given PBS twice, 4 and 2 ml i.m.

After farrowing, twenty piglets from the sows in group A were randomly (by random stratification by litter, sex, and weight) allotted to groups C and D. Twenty piglets from the sows in group B were randomly allotted to E and F. Twenty piglets born of non-vaccinated sows were obtained from the pig herd of the Veterinary Faculty and were allotted to groups G and H. Groups C, D, E, and F were vaccinated once with 2 ml i.m. in the neck at two weeks of age. Groups G and H remained untreated.

About three months post-vaccination, when the animals were 3 to 3.5 months of age (depending on the litter they came from), groups C, E, and G were transported to the high containment unit of ID-Lelystad. About six months post-vaccination, groups D, F, and H were transported to the high containment unit. All groups were housed separately and the pigs were allowed to acclimatise for one week, before the transmission trials started. During the trials, they were fed on complete foodpellets (Hope Farms) twice a day and were free to drink water from a nipple ad libitum.

4.2.3. Transmission trials

The transmission trials (for general information, see Kroese and De Jong, 2001) lasted six weeks. In each trial, five of the ten pigs were separated from the others and inoculated intranasally with 10^5 $TCID_{50}$ of CSF strain CSF277, a field isolate from Germany (Paderborn) (Greiser Wilke et al., 1998) and used at passage level 5. After 24 hours, the remaining five pigs per trial were placed with the inoculated animals as contact animals. Six weeks later, all pigs that had not died during the trial were killed for post-mortem examination.

4.2.4. Clinical observations

During the transmission trials, the pigs were observed clinically every day. In addition, rectal body temperatures were assessed daily in the first three weeks, which was prolonged in group G (no vaccination, 3 months) because some animals still had fever after three weeks. Fever was defined as a body temperature of at least 40.5ºC.

4.2.5. Sampling

EDTA-blood samples from all the pigs were collected on days 0, 2, 4, 7, 9, 11, 14, 16, 18, 21, 28, and 35 after inoculation. From these blood samples peripheral blood leukocytes were extracted as described in De Smit et al. (2001b), with one difference: the leukocytes were resuspended in a K1000 medium with 2% antibiotic stock before storage at –70°C. Oral swabs were taken on days 0, 2, 4, 7, 9, 11, 14, 16, and 18 after inoculation to monitor viral excretion. The samples were stored at –70ºC until they were tested collectively.

Serum blood samples of all the pigs were taken just before vaccination, just before inoculation and during the transmission experiments at weekly intervals until death or euthanasia. Serum samples of the vaccinated dams were taken four weeks after farrowing. Samples were stored at –20°C until testing at the end of the experiment.

At post-mortem examination, tissue samples were collected from tonsil, spleen, kidney, and ileum, for a direct immunofluorescence test for the presence of viral antigen.

4.2.6. Testing

The EDTA-blood samples were used to monitor the leukocytes and thrombocytes in the blood. Cell counts were performed with a Medonic® CA cell counter: a decrease of the numbers of leukocytes (leukopenia) or thrombocytes (thrombocytopenia) is a typical sign of CSF (Dahle and Liess, 1992; Taylor, 1995). Leukopenia and thrombocytopenia were defined as cell and platelet counts that were considerably lower (1 day < half of the maximum value of the first week's counts [days 0, 2, and 4 after challenge]).

Presence of virus in the leukocyte samples and oral swabs was tested by virus isolation (De Smit et al., 2001b; Wensvoort et al., 1986). Antigen detection in the post-mortem tissue samples was checked in an immunofluorescence test (IFT, Ressang and Den Boer, 1967).

The serum samples were tested for antibodies by two ELISAs and by a neutralising peroxidase-linked assay (NPLA). The ELISAs were the Ceditest® E2-ELISA (Colijn et al., 1997), which detects antibodies against the E2-vaccine and the corresponding epitope on the CSFV; and the Ceditest® E^{rns}-ELISA (De Smit et al., 2000b), which detects antibodies against the E^{rns} epitope on the CSFV only, and is therefore the test that distinguishes between vaccinated and infected animals. The NPLA (Terpstra et al., 1984) determines the antibody titre against the whole virus. The antibody titre was expressed as the reciprocal of the highest dilution that neutralised all virus present.

4.2.7. Data analysis

The vaccine-induced antibody (VIA) titres were examined in three steps to investigate the effect of maternal immunity on vaccination effectiveness. In the first step, it was tested whether the MDA titres at two weeks of age differed between the piglets (groups C and D) of the three mother sows. For this, a Kruskal-Wallis test (Sokal and Rohlf, 1981) was used. In the second step, it was tested whether the MDA titres at two weeks of age were related with the VIA titres at three or six months of age, before the transmission experiments started (groups C and D). This was done with a two-sided Spearman's rank correlation test (Sokal and Rohlf, 1981). In the third step, it was tested whether the VIA titres in the MDA^+-vaccinated animals differed from the titres of the MDA^--vaccinated animals. A two-sided Mann-Whitney U test (Sokal and Rohlf, 1981) was used to test group C versus E (three months post vaccination) and group D versus F (6 months post vaccination).

The effect of vaccination on the transmission of CSFV was analysed with the statistical test of Kroese and De Jong (2001). This method, which is based on the stochastic SIR model that describes the transmission dynamics of infectious diseases between individuals, compares treatments pairwise with respect to their effect on virus transmission. The test statistic that is needed for this test, is the difference in the numbers of contact infections between the two treatment groups, where a contact infection is a contact animal that got infected during the trial. The test calculates the probability (P) of the observed difference in the numbers of contact infections, under the null-hypothesis: $R_{0,control} = R_{0,vaccine}$. If P is smaller than 0.05, the groups differ significantly with respect to virus transmission.

In this experiment, group C was tested against G, group E against G, group D against H, and group F against H, in each case a vaccinated group against a control group. A significant difference would lead to the conclusion that the vaccine protects against CSFV transmission. For each group, the number of contact infections had to be determined. A contact animal was considered a contact infection

if it had been positive at least once in the IFT, the virus isolation from leukocytes, or the E^{rns} ELISA.

Basic reproduction ratios (R_0s) were estimated in each group using two methods. The first is a maximum likelihood method based on the number of initially infectious animals (I_0) and the number of ultimately infected animals (I_t) (final size method (Kroese and De Jong, 2001)). The second is a maximum likelihood method with Generalised Linear Models (GLM), based on the exact course of the local epidemic within the group (GLM method (Chapter 2)). For the GLM method, the periods during which the animals were infectious towards other animals had to be determined. This was done by assuming that animals are infectious when they are viraemic, which made it possible to reconstruct the course of each experiment. Both estimation methods were used, because on the one hand the final size method needs no assumptions on the course of the infectiousness of the individual animals and can therefore be used with little data, but on the other hand the GLM method, which needs more data, gives narrower confidence intervals. If the confidence intervals obtained did not include the value 1 but only values smaller (larger) than 1, it could be concluded that R_0 was significantly smaller (larger) than 1.

4.3. Results

4.3.1. MDA and VIA titres

The MDA titres at the time of vaccination of groups C and D are shown in Table 4.1, as well as the VIA titres at the starting time of the transmission trials of groups C, D, E, and F. It appeared that there was a significant difference between the three sows in their piglets' MDA titres ($P < 0.01$): a lower antibody titre in the sow seems to lead to a lower titre in its piglets. The MDA titre is not related to the VIA titre in the same pig, neither at three months ($P = 0.21$) nor at six months ($P = 0.67$). However, MDA^+-vaccinated pigs do differ from MDA^--vaccinated pigs with respect to their antibody titres, after three months ($P < 0.01$) as well as after six months ($P < 0.01$).

4.3.2. Description of the trials

Figure 4.1 shows the data of oral swab and leukocyte virus isolation, IFT, and E^{rns}-ELISA. Each trial is described separately and the numbers of the animals refer to Figure 4.1.

Table 4.1. Maternal antibody titres at the day of vaccination and vaccine-induced antibody titres at the day of the start of the transmission trials. The titres are expressed as the ^{2}log(titre/10).

	Animal no[a]	Animal age				Animal no	Animal age	
		2 weeks[b]	3 months[c]	6 months[c]			3 months[d]	6 months[d]
Group C	1 (I)	8	6.5	Not determined	Group E	11	8.5	Not determined
	2 (I)	8.5	<0[e]			12	7	
	3 (I)	7.5	7			13	8	
	4 (I)	7.5	6			14	11	
	5 (I)	7.5	7			15	10.5	
	6 (I)	7.5	7			16	4	
	7 (II)	6	7			17	7.5	
	8 (II)	7	7			18	9	
	9 (III)	4.5	6			19	7.5	
	10 (III)	5	7.5			20	9.5	
Group D	31 (I)	9	Not determined	8	Group F	41	Not determined	9
	32 (I)	6.5		5.5		42		7.5
	33 (I)	7.5		6.5		43		8
	34 (I)	8.5		6.5		44		7
	35 (I)	9.5		7.5		45		8
	36 (I)	8.5		4		46		7
	37 (II)	6.5		5.5		47		8.5
	38 (III)	5		6		48		7.5
	39 (III)	5		7		49		9
	40 (III)	5.5		8		50		9

[a] Between brackets are the numbers of the dams, which had the following antibody titres 4 weeks post-farrowing: (I) 6.5, (II) 5.5, (III) 2.5.

[b] The maternal antibody titres. There was a significant difference in antibody titres between the piglets of the three sows ($P < 0.01$).

[c] The vaccine-induced antibody titres in the MDA^+-vaccinated animals at the start of the transmission experiments. There was no significant relation between the maternal titres at two weeks and the vaccine-induced titres at three months ($P = 0.21$) or at six months ($P = 0.67$).

[d] The vaccine-induced antibody titres in the MDA^--vaccinated animals at the start of the transmission trials. There was a significant effect of maternal immunity on the vaccine-induced titre at three months ($P < 0.01$) and at six months ($P < 0.01$).

[e] During the transmission trial, the titre of this animal was comparable to the titres of the animals with the same treatment (nos. 1, 3, 4, 5).

	Animal		Days since inoculation													E^{rns}	IFT
	no	c/i	Vacc	0	2	4	7	9	11	14	16	18	21	28	35		
Group C	1	c	–	–	–	–	–	–	–	–	–	–				–	– – – –
	2	c	–	–	–	–	–	–	–	–	–	–				–	– – – –
	3	c	–	–	–	–	–	–	–	–	–	–				–	– – – –
MDA+	4	c	–	–	–	–	–	–	–	–	–	–				–	– – – –
3 months	5	c	–	–	–	–	–	–	–	–	–	–				–	– – – –
	6	i	–	–	–	–	–	–	–	–	–	–				+	– – – –
	7	i	–	–	–	–	–	–	–	–	–	–				–	– – – –
	8	i	–	–	–	–	–	–	–	–	–	–				+	– – – –
	9	i	–	–	–	–	–	–	–	–	–	–				+	– – – –
	10	i	–	–	–	–	–	–	–	–	–	–				+	– – – –
Group E	11	c	–	–	–	–	–	–	–	–	–	–				–	– – – –
	12	c	–	–	–	–	–	–	–	–	–	–				–	– – – –
	13	c	–	–	–	–	–	–	–	–	–	–				–	– – – –
MDA–	14	c	–	–	–	–	–	–	–	–	–	–				–	– – – –
3 months	15	c	–	–	–	–	–	–	–	–	–	–				–	– – – –
	16	i	–	–	–	–	–	–	–	–	–	–				+	– – – –
	17	i	–	–	–	–	–	–	–	–	–	–				+	– – – –
	18	i	–	–	–	–	–	–	–	–	–	–				+	– – – –
	19	i	–	–	–	–	–	–	–	–	–	–				+	– – – –
	20	i	–	–	–	–	–	–	–	–	–	–				+	– – – –
Group G	21	c	–	–	–	–	–	–	–	–	–	–				+	– – – –
	22	c	–	–	–	–	–	–	–	+	+	–			x	+	– – – –
	23	c	–	–	–	–	–	–	–	–	+	–				+	– – – –
control	24	c	–	–	–	–	–	–	–	+	+	+				+	+ + + +
3 months	25	c	–	–	–	–	–	–	–	–	+	+				+	+ + + +
	26	i	–	–	–	–	–	+	+	+	+	+			x	+	+ + + +
	27	i	–	–	–	–	–	–	–	–	–	–				+	– – – –
	28	i	–	–	–	–	–	–	+	+	–	+		x		+	+ + + +
	29	i	–	–	–	–	–	–	+	–	–	–				+	– – – –
	30	i	–	–	–	+	+	–	–	–	–	–				+	– – – –
Group D	31	c	–	–	–	–	–	–	–	–	–	–				–	– – – –
	32	c	–	–	–	–	–	–	–	–	–	–				–	– – – –
	33	c	–	–	–	–	–	–	–	–	–	–				–	– – – –
MDA+	34	c	–	–	–	–	–	–	–	–	–	–				+	– – – –
6 months	35	c	–	–	–	–	–	–	–	–	–	–				–	– – – –
	36	i	–	–	–	–	–	–	–	–	–	–				+	– – – –
	37	i	–	–	–	–	–	–	–	–	–	–				+	– – – –
	38	i	–	–	–	–	–	–	–	–	–	–				+	– – – –
	39	i	–	–	–	–	–	–	–	–	–	–				+	– – – –
	40	i	–	–	–	–	–	–	–	–	–	–				+	– – – –
Group F	41	c	–	–	–	–	–	–	–	–	–	–				–	– – – –
	42	c	–	–	–	–	–	–	–	–	–	–				–	– – – –
	43	c	–	–	–	–	–	–	–	–	–	–				–	– – – –
MDA–	44	c	–	–	–	–	–	–	–	–	–	–				–	– – – –
6 months	45	c	–	–	–	–	–	–	–	–	–	–				–	– – – –
	46	i	–	–	–	–	–	–	–	–	–	–				+	– – – –
	47	i	–	–	–	–	–	–	–	–	–	–				–	– – – –
	48	i	–	–	–	–	–	–	–	–	–	–				+	– – – –
	49	i	–	–	–	–	–	–	–	–	–	–				+	– – – –
	50	i	–	–	–	–	–	–	–	–	–	–				+	– – – –
Group H	51	c	–	–	–	–	–	–	–	–	–	–				+	– – – –
	52	c	–	–	–	–	–	–	–	–	–	–				+	– – – –
	53	c	–	–	–	–	–	–	–	–	–	–				+	– – – –
control	54	c	–	–	–	–	–	–	–	–	–	–				+	– – – –
6 months	55	c	–	–	–	–	–	–	–	–	–	–				+	– – – –
	56	i	–	–	–	–	–	+	+	–	–	–		x		+	+ – + +
	57	i	–	–	–	–	–	+	–	–	–	–				+	– – – –
	58	i	–	–	–	–	–	+	–	–	–	–				+	– – – –
	59	i	–	–	–	–	–	+	–	–	–	–				+	– – – –
	60	i	–	–	–	–	–	–	–	–	–	–				+	– – – –

← Figure 1. Course of the experiment in all trials. Each row represents one animal, identified by a number and either an 'i', if it was an inoculated animal, or a 'c', if it was a contact animal. Day 'Vacc' is the day of vaccination, or, in the case of groups G and H, 14 days before day 0. Indicated are for each day the data of the virus isolation from the oral swabs (+/– at individual days; empty fields on days without sampling), and the data of the virus isolation from the EDTA blood samples (shaded areas denote viraemia, no shading means negative test result unless the animal had died). The last two columns show the data of the E^{rns}-ELISA (+ if the animal had been positive at least once), and the data of the IFT (the four +/ –-signs denote the IFT results from tonsil, kidney, spleen, and ileum, respectively). 'x', day as from which the animal was dead (might have died at a day not indicated in the figure).

4.3.2.1. Group C: vaccination when MDA^+, transmission experiment after 3 months

No clinical signs developed in group C, and only inoculated animal 10 had two days of fever (41.2ºC and 41.3ºC after 7 and 8 days, respectively). Leukopenia developed in inoculated pigs 6 and 9, and thrombocytopenia in contact pig 4. All organs were virus negative, as were the oral swabs. Four inoculated animals showed one day of viraemia and these animals were also E^{rns}-positive at some time. NPLA showed an increase in antibody titre of all inoculated animals except no. 7. All the tests on this animal were negative so we assumed that the inoculation had not been successful. Therefore, the animal was considered susceptible in the statistical analysis. The number of contact infections was 0.

4.3.2.2. Group E: vaccination when MDA^-, transmission experiment after 3 months

None of the pigs in group E showed clinical signs. Leukopenia was detected in four inoculated pigs (nos. 16, 17, 19, 20). Two inoculated pigs exhibited one day of viraemia, while IFT and oral swabs were negative for all animals during the entire experiment. All inoculated animals were E^{rns}-positive. The number of contact infections was 0.

4.3.2.3. Group G: control (MDA^-, no vaccination), transmission experiment after 3 months

All pigs of group G developed clinical signs ranging from dullness to diarrhoea and crippling. All animals got fever, the maximum temperature ranging between 40.7°C and 42.0°C. Two inoculated and two contact animals (nos. 22, 24, 26, 28) were killed during the experiment when moribund. All tested organs of three of these animals and of one other contact animal were IFT-positive. All animals developed

leukopenia, and all pigs but one (no. 27) got thrombocytopenia as well. The oral swabs of four inoculated and four contact animals were virus-positive at least one day. All the pigs had developed viraemia and were positive in the E^{rns}-ELISA. The number of contact infections was 5.

4.3.2.4. Group D: vaccination when MDA^{+}, transmission experiment after 6 months

No clinical signs were observed in group D. Thrombocytopenia appeared to have developed in three contact animals (nos. 31-33), and leukopenia was observed in the five inoculated pigs. All oral swabs were negative, no viraemia was seen and all organs were negative in the IFT. All inoculated animals appeared to have E^{rns}-antibodies, and one contact animal was E^{rns}-ELISA positive (no. 34). Antibody titres increased after inoculation in all inoculated pigs. The number of contact infections was 1.

4.3.2.5. Group F: vaccination when MDA^{-}, transmission experiment after 6 months

The pigs in group F had no clinical signs. The two inoculated pigs 47 and 48 developed thrombocytopenia; no. 48 also showed leukopenia. Oral swabs and leukocyte virus isolation were always negative and only four of the inoculated pigs were E^{rns}-positive. Because the fifth, E^{rns}-negative, pig (no. 47) did show a large increase in antibody titre, as observed in the NPLA test, inoculation was assumed to have been successful in this animal. The number of contact infections was 0.

4.3.2.6. Group H: control (MDA^{-}, no vaccination), transmission experiment after 6 months

Four inoculated and four contact pigs (nos. 52-56, 58-60) developed clinical signs, mostly dullness and anorexia. Four inoculated pigs (nos. 56, 58-60) also got fever, the maximum temperature ranging between 40.5°C and 41.3°C. One inoculated moribund animal was killed during the experiment. This was the only animal that tested IFT-positive in all organs but the kidney. Two inoculated pigs and one contact pig (nos. 51, 56, 59) developed thrombocytopenia; three inoculated and all contact pigs (nos. 51-57, 59) showed leukopenia. Only four inoculated animals had positive oral swabs, but all ten pigs were viraemic for one or more days. All pigs were E^{rns}-positive. The number of contact infections was 5.

Table 4.2. Final size results and statistical analysis of the transmission trials.

Group	Treatment[a]			Final size[b]		*P*-value[c]	final size method[d]		GLM method[e]	
	MDA	vacc	time lag	I_0	I_t		R_0	95% CI	R_0	95% CI
C	+	+	3	4	4	0.017	0.0	(0.0 - 2.5)	ND[g]	ND[g]
D	+	+	6	5	6	0.053	0.38	(0.020 - 3.2)	ND[g]	ND[g]
E	–	+	3	5	5	0.013	0.0	(0.0 - 2.2)	ND[g]	ND[g]
F	–	+	6	5	5	0.013	0.0	(0.0 - 2.2)	ND[g]	ND[g]
G	–	–	3	5	10	ND[f]	∞	(0.68 - ∞)	16	(5.0 - 51)
H	–	–	6	5	10	ND[f]	∞	(0.68 - ∞)	4.0	(1.4 - 12)

[a] Summary of the treatment of the groups: MDA, presence of MDA at the time of vaccination; vacc, vaccination at two weeks of age; time lag, time lag between vaccination and start of the transmission trial.

[b] The final size results of the transmission trials: I_0, number of successfully virus-inoculated animals; I_t, number of animals ultimately infected.

[c] *P*-value of the final size-based test with H_0: $R_{0,\text{vaccine}} = R_{0,\text{control}}$ (Kroese and De Jong, 2001). Groups C and E are tested against G, groups D and F are tested against H.

[d] R_0 estimations and 95% CI with the final size method as described in Kroese and De Jong (2001).

[e] R_0 estimations and 95% CI with the GLM method as described in Chapter 2.

[f] Not determined, because these are the control groups.

[g] Not determined, because of lack of data.

4.3.3. Analysis of the transmission experiments

Table 2 presents the results of the statistical evaluation of the transmission experiments. First, the difference in the number of contact infections between the vaccinated groups (C, D, E, F) and the control groups (G, H) was used to test whether vaccination reduced CSFV transmission. The transmission was reduced in the groups C, E, and F ($P < 0.05$), but not in group D. Therefore, it is concluded that transmission was not significantly reduced 6 months after vaccination in MDA^+ pigs.

Second, the reproduction ratio R_0 in the different groups was estimated. The final size method estimated R_0 in all four vaccinated groups (C, D, E, F) to be below 1, and in both control groups (G, H) above 1. However, none of these R_0s significantly differed from 1. The GLM method could only be properly used in the control groups G and H, since in the vaccine groups not enough infectiousness — viraemia was assumed to denote infectiousness — was available. R_0 appeared to be significantly above 1 in both control groups ($P < 0.01$). The GLM method estimated R_0 after six

months (group H) smaller than R_0 after three months (group G), but this difference was not statistically significant.

4.4. Discussion

The objective of this study was to determine whether vaccination of piglets at two weeks of age, reduced transmission of CSFV three and six months later. Because maternal immunity might affect the vaccine effectiveness in young pigs, vaccination of MDA^+ pigs has been compared with vaccination of MDA^- pigs.

First, the antibody titres of the pigs were examined in order to test the effect of MDA on the VIA titres. It was shown that the presence of MDA at the time of vaccination led to lower VIA titres (Table 1). The titres of pigs from vaccinated sows (averages of the ^{2}log(titre/10): 6.1 after three months; 6.5 after six months) were significantly lower than the titre of pigs from unvaccinated sows (averages: 8.3 after three months; 8.1 after six months). This effect has also been observed with the C-strain vaccine, a modified live CSF vaccine (Precausta et al., 1983; Terpstra and Tielen, 1976) and is probably caused by neutralisation of the vaccine by the MDA. Neutralisation of the vaccine by MDA would also be expected to lead to different vaccine responses between MDA^+ pigs, i.e., a negative correlation between the MDA titre and the VIA titre in the same pig. Such a negative correlation could lead to a very heterogeneously protected pig population, with subgroups of poorly protected pigs within which CSFV can circulate ($R_0 > 1$ within subgroups). A negative correlation could, however, not be shown in this experiment (Table 1), although it has been described with the C-strain vaccine (Precausta et al., 1978; Terpstra and Tielen, 1976). The difference can possibly be explained by the different natures of the E2 vaccine and the C-strain vaccine, the latter of which is suggested to have a relatively high sensitivity of the vaccination response to the presence of maternal antibodies (Precausta et al., 1978). More likely, however, is that there is an effect of the MDA titre on the VIA titre, but that this could not be shown with only ten animals per time interval. In any case, since there is no very clear-cut effect, vaccination of MDA^+ pigs is not expected to lead to subgroups of poorly protected pigs.

A question may be raised concerning the level of the antibodies at three or six months, whether this may still consist of MDA, which would mean that the VIA titres are in fact even more reduced in MDA^+-vaccinated pigs than already shown. This is, however, rather unlikely, since the MDA titres reach a minimum level from about 10 weeks of age onwards (Precausta et al. (1978, 1983); Soos et al. (2001); Terpstra and Tielen (1976) for the C-strain vaccine; Lipowski et al. (2000) for the

E2-vaccine). Moreover, MDA of piglets from sows with the same treatment as in this paper had titres below the detection limit at an age of 84 days (Moormann, unpublished results).

Second, the effectiveness of vaccination with respect to virus transmission was investigated. This was done three and six months post-vaccination, in MDA^+-vaccinated as well as MDA^--vaccinated animals. The experiments indicate that, after vaccination at a very young age, the reduction of transmission of CSFV will still be significant at slaughter age, at least if the piglets were MDA^-. In the case of the vaccination of MDA^+ piglets, a statistically significant reduction of transmission after six months could not be proved (group D). Because of the apparently reduced protection and the lower antibody titres in MDA^+-vaccinated pigs after six months, there seems to be a relation between antibody titre and virus transmission. Although the relation cannot be excluded, it is not certain either: the antibody titres in the MDA^+ group C were lower than in the MDA^- group E three months after vaccination, but virus transmission was significantly reduced in both groups C and E. Moreover, the conclusion that maternal immunity at the time of vaccination hampers the reduction of CSFV transmission six months after vaccination, would be too definite for two reasons. The first is that the power of the statistical test was only 33%, with one vaccine group (if $R_0 = 0.38$ as estimated) and one control group (if $R_0 = 4.0$ as estimated). The second reason is that the method of counting contact infections is rather conservative. The statistical method used to test the reduction of transmission in the vaccinated groups assumes equal infectivity of all infected pigs, whether inoculated or contact infected. Whether the contact infection in group D is a true contact infection in terms of this assumption, is questionable, since it was only once positive in the E^{rns} ELISA and never in any other test, including cell counts and temperature measurements. An interesting observation was that, in the control groups, R_0 after six months was estimated to be smaller than R_0 after three months. Although this was not a significant result, it is in accordance with estimations from other experiments with CSFV, where R_0 did differ significantly between weaner pigs (6 weeks) and slaughter pigs (6 months) (Chapter 2).

The results of the presented experiment, together with the results of earlier transmission experiments in E2 vaccinated animals (Bouma et al., 2000), give confidence in the effectiveness of emergency vaccination as a control measure during CSF epidemics. CSFV transmission appears to be significantly reduced during the entire economic life of finishing pigs, when they are vaccinated once at a young age. R_0 appears to be smaller than 1, so that no major outbreaks can occur. A point of concern is the possible negative effect of maternal immunity on the vaccine effectiveness. For the development of control strategies, it might be considered to avoid the risk of having vaccinated pigs that become susceptible again. This can be

done, e.g., by vaccinating at a later age, or by not vaccinating sows at all. In the case of the C-strain vaccine, age at vaccination appeared to be of importance for the size of the MDA-caused inhibitory effect on VIA development (Precausta et al., 1983). Not vaccinating sows may sound unlogical at first, but if all other pigs within the herd are vaccinated, herd immunity may remain secured. Another advantage is that, if persistently infected piglets would be born (piglets which shed CSFV without getting diseased, (Van Oirschot and Terpstra, 1977)), the very young unprotected piglets and the sows can have a 'signalling' function by showing clinical signs after infection by the persistently infected piglets. The vaccine, namely, does not fully prevent birth of these piglets (Depner et al., 2001; Dewulf et al., 2001).

Further investigations into effectiveness of different control scenarios can be done by means of mathematical modelling virus spread between herds. The specific design of transmission trials, with homogeneous groups of animals instead of vaccinated infectious animals and unvaccinated sentinels, make it easier to extrapolate the data to the field situation with mathematical models. That is, because the actual effectiveness of vaccination of pig herds is reflected by the vaccine's effectiveness on susceptibility and infectivity together. Moreover, the vaccine can be judged in terms of the reproduction ratio R_0, which facilitates the interpretation of its effect on transmission within pig herds.

In this particular experiment we used conventional pigs instead of SPF pigs. The advantage of SPF animals would be that the animals are probably more similar leading to less variance in the results and a higher power of the experiment. Therefore, using SPF animals to evaluate vaccine efficacy can be very effective. However, a major disadvantage is that conclusions drawn from experiments with SPF animals do not necessarily apply to conventional animals, as is suggested by Van Nes et al. (2001) in the case of PRV transmission between pigs. More significant results in different settings augment the robustness of the conclusion that the vaccine is indeed capable of reducing transmission.

An important factor of a marker vaccine is the quality of its accompanying diagnostic test. The accompanying test of the E2 vaccine is the E^{rns} ELISA and in this experiment two animals (in groups C and F) were not found positive in the E^{rns} ELISA after inoculation. One of these pigs (in group C) had no increase in CSFV antibody titre and did not show any other sign of CSFV infection, so we assumed that inoculation had not caused infection. The animal was considered susceptible in the statistical analysis. Considering it infectious would, however, not have altered the conclusion that virus transmission was significantly reduced in group C. The other inoculated E^{rns}-negative animal did show an antibody (booster) response, which means that the E^{rns} ELISA result was false-negative. Occurrence of false-negativity is in accordance with experiments for determination of the E^{rns} ELISA test

characteristics, in which the sensitivity was estimated at about 74% (De Smit et al., 2000b; Floegel Niesmann, 2001). False-negative in this case means that the E^{rns} ELISA was unable to detect antibodies in an animal that had been in contact with the virus. In principle, every test will suffer from this inability to some degree if the amount of virus that the animal had been in contact with is small enough. However, it is undesirable for a test to score an animal false-negative, when it is a risk in terms of infectiousness. It is unlikely that false-negatives for the E^{rns} ELISA are very infectious since even E^{rns}-positive (vaccinated) animals were hardly able to transmit virus to contact animals, which has been seen in the experiments presented in this paper and in the experiments of Bouma et al. (2000).

Acknowledgements

We owe much gratitude to H. Rutgers, A. Timmerman and co-workers for the care of the animals and to W. Hoogers, L. Mastenbroek, M. Koopmans, and E. de Kluijver for their technical assistance. We thank three anonymous referees for helpful comments on the manuscript. This research project was supported by Bayer Animal Health Division, Germany, and STW (Technology Foundation), Utrecht, The Netherlands.

Chapter 5

Quantification of the effect of control strategies on classical swine fever epidemics

D. Klinkenberg , A. Everts-van der Wind, E.A.M. Graat, M.C.M. de Jong

Submitted to *Mathematical Biosciences*

Abstract

Emergency vaccination during an epidemic of Classical Swine Fever Virus (CSFV) has become a serious option because of the ethical problems of strategies with massive culling and the availability of a marker vaccine that reduces virus transmission. Here we present a model of between-herd CSFV transmission, which quantifies the effect of control strategies with and without vaccination. We estimate the model parameters from data of the Dutch CSFV epidemic of 1997/1998. With the model, a set of control strategies is compared, consisting of five control measures in several combinations. Consequently, the following general requirements of successful strategies can be formulated. First, to achieve extinction of a CSFV epidemic, transmission through transport should be prevented and the indirect virus transmission, i.e. all transmission not through animal contacts, should at least be halved, either by vaccination or by culling of the susceptible pig population. Second, to minimise the size and duration of an epidemic, the extinction requirements should be met quickly and indirect virus transmission should be reduced by far more than a half. Although the origin of the model parameters let the requirements in fact be only applicable for the southeastern part of the Netherlands, it is argued that epidemics in other areas will not need stricter control strategies.

5.1. Introduction

Classical swine fever (CSF) is a viral disease of swine (Taylor, 1995). The entry of classical swine fever virus (CSFV) into populations of non-vaccinated domestic pigs can cause large epidemics. Nonetheless, the domestic pig population of the European Union (EU) is not preventively vaccinated against CSFV, because importing countries do not accept vaccinated pigs (Anonymous, 1980). They consider vaccinated pigs as infected, which is due to the fact that most antibody tests react positively in vaccinated animals. This problem was to be solved with the E^{rns}-antibody ELISA, designed to be used with the E2 subunit marker vaccine which only evokes antibodies against the E2 subunit and not against E^{rns}. However, the E^{rns} ELISA has a sensitivity of only 75% (Depner et al., 2001).

In 1997, a CSFV entry into the Netherlands led to a large epidemic (hereafter called the Dutch CSFV epidemic), which lasted 1.5 years and in which 429 herds were infected (Elbers et al., 1999). In part the epidemic was so large and long-lasting because the initial set of control measures, as prescribed by the EU, was insufficient to bring the basic reproduction ratio between herds, R_h, to a value below

1. Additional control measures appeared necessary, among which preventive slaughter of herds with traced contacts with infected herds or in close vicinity of infected herds (Stegeman et al., 1999b). Preventive slaughter, however, has major disadvantages, because the killing of healthy pigs, if not for consumption, is economically and ethically undesirable (Terpstra et al., 2000).

Future introductions of CSFV should therefore be followed by an alternative control strategy, without preventive killing but with the effect of reducing R_h sufficiently to end the epidemic quickly. A future strategy might include emergency vaccination, e.g. with an E2 subunit vaccine, which reduces the basic reproduction ratio between individual pigs to below 1 (Bouma et al., 2000; Chapter 3). Vaccination of the entire pig population will therefore certainly lead to extinction of a CSF epidemic. However, the economic consequences might be far-reaching since importing countries will not resume import of live pigs and pig products as long as the entire population is not CSFV-free. If importing countries do not accept a sensitivity of 75% to prove virus-freedom, all vaccinated animals will have to be removed before resuming export. If sufficiently effective, it would be preferable to use strategies with only partial vaccination, e.g. only vaccination of fattening pigs. These could already be replaced by unvaccinated piglets before the end of the epidemic.

Considerations like these make it desirable to know the requirements of a good control strategy. To determine these requirements, the effects of various control strategies should be quantified. Nielen et al. (1999) and Mangen et al. (2001) studied the epidemiological and economic effects of a number of control strategies, in part defined by the reaction of trading partners — whether they will or will not import vaccinated pigs. In both these studies, simulation models with a very detailed structure were used. The models, for example, were spatially explicit and simulated every single contact between farms. The approach enabled the authors to make detailed economic analyses, but leads to questions regarding the reliability of the exact outcomes since many parameter values had to be chosen without data. Moreover, a general insight into the requirements of a good control strategy cannot be obtained. This points out the need for a quantitative analysis of control strategies with reliable parameter estimates from epidemic data.

In this paper, we present a mathematical model of CSFV transmission between pig herds. We use the model to link data on CSFV transmission and assumptions on the effect of control measures. In section 2, the model structure is presented. In section 3, the model is used to construct likelihood functions, which are used to estimate the model parameters from data of the 1997/1998 CSFV epidemic in the Netherlands. Section 4 describes how five control measures are incorporated into the model. In section 5, the estimated parameters are used to quantify the effects of the

five control measures, which are applied in all possible combinations. From the analysis, general requirements for good control strategies are deduced. Finally, in section 6 the results are interpreted and discussed in relation to previous publications, model assumptions, and the future of CSFV control.

5.2. Model structure

The model describes the transmission of CSFV between pig herds in the Netherlands. In the basic model, the set of EU control measures is applied, as was applied in the first ten weeks of the Dutch CSFV epidemic (Stegeman et al., 1999b). The most important control measures are the culling of infected herds, a transport prohibition, the tracing and testing of infectious contacts, and the implementation of hygiene measures and surveillance in the affected area. In the basic model, however, the complete transport prohibition is relaxed and animal transport from multiplier to finishing herds and from finishing herds to the slaughterhouse is permitted. This relaxation causes heterogeneity in the contact pattern, and therefore two herd types are distinguished in the model, multiplier herds and finishing herds. Multiplier herds contain sows and produce 23 piglets per sow per year (Siva software, 2001), which are transported to the finishing herds at an age of ten weeks. This leads to a piglet to sow ratio of (23·10·7):365 = 1610:365 within multiplier herds. The pigs remain 100 days on the finishing herds, until slaughter.

The virus transmission between pig herds is modelled as a branching process of infected herds, which means that the number of available susceptible herds to be infected is not limiting. Immediately after infection, virus spreads within the herd. The herd gives rise to new infected herds by a Poisson process with a variable rate proportional to the number of infectious animals within the herd. The infection process stops when infection of the herd is detected, as detection is immediately followed by culling of the herd. Although virus entry into a herd can result in a minor outbreak, only major outbreaks within herds are modelled since these are the most important for further virus transmission between herds. For an overview of all model parameters, variables, and functions, see Appendix 5A.

5.2.1. Within-herd transmission

Virus transmission within a herd starts with one infectious pig, immediately after infection of the herd. We assume that this is followed by a linear birth-death process (Cox and Miller, 1965) of infected pigs, independent of herd type. In

epidemiological terms, birth would be equivalent to infection and death to recovery. The linear birth-death process is described by

$$\frac{dp_0(t)}{dt} = \mu p_1(t) \tag{5.1.1}$$

$$\frac{dp_1(t)}{dt} = -(\lambda + \mu)p_1(t) + 2\mu p_2(t) \tag{5.1.2}$$

$$\frac{dp_i(t)}{dt} = -i(\lambda + \mu)p_i(t) + (i-1)\lambda p_{i-1}(t) + (i+1)\mu p_{i+1}(t), \quad \text{if } i \geq 2 \tag{5.1.3}$$

in which $p_i(t)$ is the probability of having i infectious pigs at time t, λ is the per capita infection rate and μ is the recovery rate (Cox and Miller, 1965).

The solution to this set of differential equations with initial conditions $p_1(0) = 1$ and $p_i(0) = 0 \forall i \neq 1$ is given by (after Cox and Miller (1965), p. 166)

$$p_0(t) = \frac{\exp(rt) - 1}{R \cdot \exp(rt) - 1} \tag{5.2.1}$$

$$p_i(t) = (1 - p_0(t))(1 - Rp_0(t))(Rp_0(t))^{i-1}, \tag{5.2.2}$$

in which $r = \lambda - \mu$, the mean exponential growth rate of the number of infectious pigs, and $R = \lambda/\mu$, the basic reproduction ratio between animals. In the model, R is assumed to exceed 1 (and hence, $r > 0$), otherwise no major outbreaks can occur within herds.

We now define the stochastic variable $I(t)$[1] as the number of infected animals at time t since infection of the herd and conditioned on non-extinction, as the model only takes major outbreaks in herds into account:

$$P(I(t) = i) = \frac{p_i(t)}{1 - p_0(t)} = (1 - Rp_0(t))(Rp_0(t))^{i-1}. \tag{5.3}$$

[1] Throughout the paper, upper case letters denote stochastic variables, whereas lower case letters denote parameters or ordinary variables. Note that r and R are two different model parameters.

By letting $t \to \infty$, a continuous approximation to the discrete distribution for $I(t)$ can be made (see Appendix 5B):

$$I(t) = H \exp(rt), \text{ in which} \tag{5.4.1}$$

$$H \cong pdf(h) = \frac{R-1}{R} \exp\left(-\frac{R-1}{R} h\right). \tag{5.4.2}$$

The approximation (5.4) is used instead of the real solution (5.2) to keep the model more manageable. In short, the within-herd transmission of CSFV is described by a deterministic exponential curve of which the height is random and has an exponential distribution.

5.2.2. Herd detection

Infected herds can be detected at any time after they are infected. Detection of infected herds takes place by a Poisson process with an increasing rate $\alpha I(t) = \alpha H \exp(rt)$. The first realisation is the detection time. Parameter α is the detection rate per infectious pig. After detection of a herd, it is immediately culled and cannot give rise to new infected herds anymore.

5.2.3. Between-herd transmission: indirect contacts

Each infectious herd can transmit the virus to susceptible herds via indirect contacts, i.e. all potentially infectious contacts except transport of infectious pigs. Transmission takes place by a Poisson process, thereby giving rise to new infectious herds with increasing transmission rates $\beta_f I(t) = \beta_f H \exp(rt)$ for infecting finishing herds and $\beta_m I(t) = \beta_m H \exp(rt)$ for infecting multiplier herds, where t is the time since infection of the infectious (source) herd. The type of source herd is assumed irrelevant. Parameters β_f and β_m are the transmission rates per infectious pig per indirect contact per day. The sum $\beta_{ind} = \beta_f + \beta_m$ is the total rate by which an infectious animal in an infectious herd gives rise to new infectious herds through indirect contacts.

5.2.4. Between-herd transmission: transport contacts

Infectious herds can also transmit virus through transport of live pigs. In the model, this mode of transmission is restricted to multiplier herds infecting finishing herds. For simplicity, the assumption is made that this occurs analogously to the indirect-contact transmission, viz. by a Poisson process with rate $\beta_{tr} I(t) = \beta_{tr} H \exp(rt)$, in which β_{tr} is the transmission rate per infectious pig for transport contacts.

5.3. Parameter estimations

The described model contains six parameters: (1) r, the exponential growth parameter for the number of infectious pigs on a farm; (2) R, the basic reproduction ratio between animals on a farm, which describes the height of the exponential infectious curve within farms. The pair (r, R) is a reparametrisation of the parameter pair (λ, μ) of the within-herd transmission model: $r = \lambda - \mu$ and $R = \lambda/\mu$; (3) α, the detection parameter, which denotes the rate of herd detection per infectious pig on a farm; (4) β_{ind}, the between-herd transmission parameter that denotes the rate at which one infectious pig infects other herds by indirect herd contacts. The indirect transmission is split into two types, for the transmission to finishing herds and to multiplier herds, which are denoted by the parameters β_f and β_m, respectively. The values of β_f and β_m can be derived from their sum β_{ind} and from their ratio: (5) the β_f:β_m ratio, which determines the division of β_{ind} into β_f and β_m; (6) β_{tr}, the between-herd transmission parameter for transport contacts.

5.3.1. Methods

5.3.1.1. Estimation of r, R, α, and β_{ind}

The first four parameters r, R, α, and β_{ind} have been estimated simultaneously by using data from the 1997/1998 epidemic in the Netherlands under the assumption that the data had arisen according to the described mathematical model. From the data, three stochastic processes could be distinguished and the log-likelihood functions $L_1(r, R)$, $L_2(r, R, \alpha)$, and $L_3(r, R, \alpha, \beta_{ind})$ for these processes were formulated. The sum of these log-likelihood functions $L(r, R, \alpha, \beta_{ind}) = L_1(r, R) + L_2(r, R, \alpha) + L_3(r, R, \alpha, \beta_{ind})$ has been maximised numerically in Mathematica® (Wolfram, 1999) for the four parameters simultaneously to obtain the maximum likelihood estimates.

The combined likelihood function has also been used to derive a distribution of the estimators. Since α and β_{ind} are positive by definition and r must be positive to enable within-herd transmission, it was assumed that the estimators for r, α, and β_{ind} were lognormally distributed. Since $R > 1$ to enable within herd transmission[2], it was assumed that the estimator for R-1 was lognormally distributed. A covariance matrix for log$r$, log(R-1), logα, and logβ_{ind} was obtained numerically in Mathematica® (Wolfram, 1999) by calculating

$$\text{var} = \begin{pmatrix} \dfrac{\partial^2 L}{(\partial \log r)^2} & \cdots & \cdots & \dfrac{\partial^2 L}{(\partial \log r)(\partial \log \beta_{ind})} \\ \dfrac{\partial^2 L}{(\partial \log r)(\partial \log(R-1))} & \ddots & & \vdots \\ \dfrac{\partial^2 L}{(\partial \log r)(\partial \log \alpha)} & & \ddots & \vdots \\ \dfrac{\partial^2 L}{(\partial \log r)(\partial \log \beta_{ind})} & \cdots & \cdots & \dfrac{\partial^2 L}{(\partial \log \beta_{ind})^2} \end{pmatrix}^{-1} \tag{5.5}$$

The goodness-of-fit of the model to the parameters has been tested for each likelihood equation separately, by calculation of the Pearson χ^2 statistic

$$\chi^2 = \sum_{i=1}^{n} \left[\frac{\left(y_i - E(Y_i)_i\right)}{\left(\text{var}(E(Y_i))\right)^2} \right],$$

in which n is the number of records, y_i the ith observation, $E(Y_i)$ the expected value of the ith observation, and var($E(Y_i)$) the estimated variance of the ith observation. The statistic is χ^2-distributed with $n - p$ degrees of freedom, where p is the number of parameters estimated with the regarded likelihood equation.

5.3.1.2. Log-likelihood function $L_1(r, R)$

The first log-likelihood function described the detection of infected animals in a random sample of animals as a function of time since infection of the herd. The function provided information on the within-herd transmission parameters r and R.

[2] Note that a basic reproduction ratio larger than 1 implies a net exponential increase: $R > 1 \Leftrightarrow r > 0$ (Diekmann and Heesterbeek, 2000).

Table 5.1. The dataset for $L_1(r,R)$. Each row represents the record of one herd.

t_{det} (days) [a]	n_{tot} [b]	n_{test} [c]	n_{pos} [d]	$E(N_{pos})$ [e]
12	3905	394	0	0.06
13	578	60	0	0.08
14	789	143	0	0.16
14	739	145	0	0.17
14	633	134	0	0.19
16	1598	226	0	0.18
18	2436	186	0	0.14
18	247	27	1	0.20
20	1888	216	0	0.16
20	1430	102	0	0.18
20	551	63	0	0.28
20	282	18	0	0.28
22	594	117	0	0.67
23	447	111	0	0.98
25	2400	137	0	0.30
25	1949	128	0	0.35
26	506	506	3	6.13
29	2624	361	9	1.24
29	1045	138	0	1.29
29	350	49	0	1.32
30	2510	188	0	0.79
30	2236	164	0	0.81
30	88	12	3	1.47
33	1885	238	2	2.07
36	585	83	2	3.51
38	1497	238	12	5.10
39	422	36	3	3.08
41	711	103	17	6.47
42	3657	132	3	1.76
42	1582	147	6	4.53
51	2998	229	8	4.13
52	321	42	6	6.70

[a] The time interval between infection and detection

[b] The number of animals in the herd

[c] The number of tested samples

[d] The number of positive samples

[e] The expected number of positive samples according to the model and the parameter estimates.

Of a set of 82 herds of the 1997/1998 epidemic of CSF in the Netherlands, the exact day of virus introduction is known from tracing (Stegeman et al., 1999b). Of this set of herds, those 32 finishing herds were selected, in which blood samples from animals throughout the herd had been taken to test for seroconversion. Of each of these herds, the known data were: the time between infection and detection t_{det}, the total number of animals n_{tot}, the number of sampled and tested animals n_{test}, and the number of animals tested seropositive n_{pos} (Table 5.1).

We assumed a perfect serological test with a sensitivity and a specificity of 1. Then, for each record, the number of seropositive animals n_{pos} was a realisation of a random draw of n_{test} animals out of a population of n_{tot} animals with N_{ser} true seropositives; the stochastic variable N_{pos} was therefore hypergeometrically distributed:

$$P\left(N_{pos} = n_{pos}\right) = \sum_{n_{ser}=n_{pos}}^{n_{tot}+n_{pos}-n_{test}} \left[\left(\binom{n_{ser}}{n_{pos}} \binom{n_{tot}-n_{ser}}{n_{test}-n_{pos}} \Big/ \binom{n_{tot}}{n_{test}} \right) P\left(N_{ser} = n_{ser}\right) \right] \tag{5.6}$$

The number of true seropositives N_{ser}, of which the distribution had to be determined to use equation (5.6), was the accumulated number of animals infected 18.45 days before t_{det}, 18.45 days being the average time until seropositivity of an individual animal (Stegeman et al., 1999b). The number of true seropositives N_{ser} consisted of the number of animals that had been infected at rate $\lambda I(t)$ by within-herd transmission plus the number of initially infected animals, which was the height H of the infectious curve at the time of infection of the herd:

$$N_{ser} = H + \int_0^{t_{det}} \lambda I\left(\tau - 18.45\right) d\tau \approx \frac{R}{R-1} H \exp\left(r\left(t_{det} - 18.45\right)\right). \tag{5.7}$$

Eq. (5.7) was used to derive the probability $P(N_{ser} = n_{ser})$, by approximating the continuous exponential distribution for N_{ser} by a discrete geometrical distribution:

$$\begin{aligned} &P\left(N_{ser} = n_{ser}\right) \\ &= P\left(N_{ser} \in \left[n_{ser}, n_{ser}+1\right)\right) \\ &= P\left(H \in \left[n_{ser} \frac{R-1}{R} \exp\left(-r\left(t_{det}-18.45\right)\right), \left(n_{ser}+1\right) \frac{R-1}{R} \exp\left(-r\left(t_{det}-18.45\right)\right) \right) \right) \end{aligned}$$

$$= \int_{n_{ser}\frac{R-1}{R}\exp(-r(t_{det}-18.45))}^{(n_{ser}+1)\frac{R-1}{R}\exp(-r(t_{det}-18.45))} \frac{R-1}{R}\exp\left(-\frac{R-1}{R}h\right)dh = \pi_0(1-\pi_0)^{n_{ser}}, \quad (5.8)$$

in which $\frac{R-1}{R}\exp\left(-\frac{R-1}{R}h\right)$ is the probability density function (*pdf*) for the height of the infectious curve H, and $\pi_0 = 1-\left(\exp\left(-\frac{R-1}{R}\right)\right)^2 \exp\left(-r(t_{det}-18.45)\right)$. By taking the logarithm of Eq. (5.6) and summing over all 32 observations, the function $L_1(r, R)$ was obtained.

5.3.1.3. Log-likelihood function $L_2(r, R, \alpha)$

The second log-likelihood function described the detection of infected herds. It mainly provided information on the detection parameter α. However, as detection depends on the within-herd dynamics, also the within-herd transmission parameters r and R were involved.

For 82 of the 429 herds of the Dutch CSF epidemic, the day of virus introduction is known. We used the interval in days between infection and detection as the detection times t_{det} of these 82 herds (Stegeman et al., 1999b):

10(2×), 12, 13, 14(3×), 16(3×), 18(3×), 20(4×), 21(2×), 22(3×), 23, 24(3×), 25(2×), 26(2×), 27(2×), 28, 29(6×), 30(4×), 33(3×), 34(2×), 35, 36, 37(2×), 38(4×), 39, 41(2×), 42(6×), 43, 44, 45, 47(2×), 48(3×), 49(2×), 50, 51, 52, 55, 56, 57(2×)

The detection times were considered as random draws from a probability distribution of the stochastic variable T_{det}, which could be expressed in terms of the parameters r, R, and α and became, integrated over all possible values of H:

$$pdf(t_{det}) = \int_0^\infty \alpha h \exp\left(rt_{det} - \frac{\alpha h}{r}\left(\exp(rt_{det})-1\right)\right)\frac{R-1}{R}\exp\left(-\frac{R-1}{R}h\right)dh$$

$$= \frac{\alpha\frac{R-1}{R}\exp(rt_{det})}{\left(\frac{\alpha}{r}\exp(rt_{det})-\frac{\alpha}{r}+\frac{R-1}{R}\right)^2} \quad (5.9)$$

In Eq. (5.9), $\alpha h\exp(rt_{det})$ is the detection rate at time t_{det}, $\exp(-\alpha h(\exp(rt_{det})-1)/r)$ is the probability that the herd has not been detected until t_{det}, and $\frac{R-1}{R}\exp\left(-\frac{R-1}{R}h\right)$ is the *pdf* for H. By summing the logarithm of Eq. (5.9) over the 82 observations, the function $L_2(r, R, \alpha)$ was acquired.

Table 5.2. The dataset for $L_3(r,R,\alpha,\beta_{ind})$. Each row represents the record of one week.

j [a]	c [b]	$E(C)$ [c]
22.9	9	8.4
27.2	16	10.0
30.3	10	11.2
35.9	12	13.2
41.2	12	15.2
50.1	17	18.5
55.6	20	20.5
59.0	20	21.8
67.5	27	24.9
68.6	26	25.3

[a] The average number of infectious herds

[b] The number of new infections

[c] The expected number of new infections according to the model and the parameter estimates.

5.3.1.4. Log-likelihood function $L_3(r, R, \alpha, \beta_{ind})$

The third log-likelihood function described the CSFV transmission between herds. Although it mainly provided information on transmission parameter β_{ind}, also the parameters r, R, and α were included, since these parameters together determine the average number of infectious animals within an infectious herd.

After the first detection of the Dutch CSFV epidemic, the compulsory set of EU measures came into force for 10 weeks. Of each week, the number of infectious herds j and the number of new infections c had been reconstructed by Stegeman et al. (1999a) (Table 5.2). According to the model, the numbers of new infections per week C were regarded as random draws from Poisson distributions of which the parameters depended on the number of infectious herds j and on r, R, α, and β_{ind}:

$$E(C) = \beta_{ind}\nu(r, R, \alpha)j \ . \tag{5.10}$$

Since β_{ind} is the average number of herds infected per infectious *animal* per *day*, the function $\nu(r,R,\alpha)$ had to convert one infectious herd into a number of infectious 'animal days'. Therefore, $\nu(r,R,\alpha)$ is the expected number of infectious 'animal days' per herd divided by the expected number of weeks from infection to detection of a herd:

$$\nu(r,R,\alpha)=\frac{E(\#\text{infectious animal days})(r,R,\alpha)}{E(\#\text{infectious weeks})(r,R,\alpha)}. \tag{5.11}$$

In Eq. (5.11), the numerator is equal to

$$\begin{aligned}&E(\#\text{infectious animal days})\\&\quad=\int_0^\infty\int_0^\infty h\exp\left(rt-\frac{\alpha h}{r}(\exp(rt)-1)\right)dt\,\frac{R-1}{R}\exp\left(-\frac{R-1}{R}h\right)dh,\\&\quad=\frac{1}{\alpha}\end{aligned}$$

where $h\exp(rt)$ is the infectiousness at time t, $\exp\left(-\frac{\alpha h}{r}(\exp(rt)-1)\right)$ the probability that the herd has not been detected at time t, and $\frac{R-1}{R}\exp\left(-\frac{R-1}{R}h\right)$ the *pdf* for H.

In Eq. (5.11), the denominator, i.e. the expected number of weeks that a herd is infectious, is

$$\begin{aligned}&E(\#\text{infectious weeks})\\&\quad=\frac{1}{7}E(\text{length of infectious period in days})\\&\quad=\frac{1}{7}\int_0^\infty\int_0^\infty \alpha h\exp\left(rt-\frac{\alpha h}{r}(\exp(rt)-1)\right)tdt\,\frac{R-1}{R}\exp\left(-\frac{R-1}{R}h\right)dh\\&\quad=\frac{1}{7}\int_0^\infty\frac{\alpha t\frac{R-1}{R}\exp(rt)}{\left(\frac{\alpha}{r}(\exp(rt)-1)+\frac{R-1}{R}\right)^2}dt,\end{aligned} \tag{5.12}$$

in which $\alpha h\exp\left(rt-\frac{\alpha h}{r}(\exp(rt)-1)\right)$ is the *pdf* for the detection time and $\frac{R-1}{R}\exp\left(-\frac{R-1}{R}h\right)$ the *pdf* for H. The division by 7 is to convert the expected number of days to the expected number of weeks.

This results in the probability of observing c new cases in a week being

$$P(C=c)=\frac{(\beta_{ind}\nu(r,R,\alpha)j)^c}{c!}\exp(-\beta_{ind}\nu(r,R,\alpha)j). \quad (5.13)$$

Summing the logarithms of Eq. (5.13) over the ten weekly intervals resulted in the function $L_3(r, R, \alpha, \beta_{ind})$.

5.3.1.5. The ratio $\beta_f:\beta_m$

The fifth model parameter is the ratio $\beta_f:\beta_m$, which is the ratio by which infectious herds of both types infect finishing and multiplier herds, respectively. For estimation of the ratio, the 429 infected herds of the Dutch CSFV epidemic were subdivided into three groups, according to the ratio of finishing pigs and sows in the herds. In a perfectly closed herd that does not sell or buy piglets, the ratio between the number of finishing pigs (each sow produces 23 piglets a year, which are all living as a finishing pig for 100 days) and the number of sows (each living 365 days a year) would be $23 \cdot 100/365 \approx 6.3$. Therefore, the groups were subdivided as follows: net piglet producers (finishing pig to sow ratio < 5.0), net piglet receivers (finishing pig to sow ratio > 7.5), and a third group (finishing pig to sow ratio between 5.0 and 7.5, breeding herds which supply gilts to herds with sows, and herds with unknown animal numbers). The third group is likely to have very few transport contacts, since only transport of piglets to finishing herds is permitted. Because infected third-group herds do not infect other herds by transport, just like the finishing herds, the third group has been included in the receiver group to determine the $\beta_f:\beta_m$ ratio.

5.3.1.6. The parameter β_{tr}

The parameter β_{tr} is the parameter for transmission through transport contacts. It is derived by first calculating the mean number of finishing herds that are infected by one infectious multiplier herd through transport, σ_{tr}. Subsequently, the parameter β_{tr} is chosen such that the mean number of infections through transport per herd according to the model will also be σ_{tr}.

The mean transport frequency of piglets from multiplier herds to finishing herds is approximately $32/365 \approx 1/11.4$ (one transport every 11.4 days) (Mangen, 2002). By assuming an average transmission probability of ±0.8, the frequency of transmission through transport becomes 1/14, thus once in two weeks. The expected number of contact infections due to transport of infectious pigs, σ_{tr}, then becomes:

$$\sigma_{tr} = \frac{1}{14} E\left(\text{length of herd's infectious period in days}\right)$$
$$= \frac{1}{14} \int_0^\infty \frac{\alpha t \frac{R-1}{R} \exp(rt)}{\left(\frac{\alpha}{r}\left(\exp(rt)-1\right) + \frac{R-1}{R} \right)^2} dt \,, \tag{5.14}$$

which was already derived in Eq. (5.12), apart from the division by 14.

In the model, the rate of transmission through transport is assumed to be proportional to the number of infectious animals at the infectious multiplier herd, i.e. equal to $\beta_{tr} I(t) = \beta_{tr} H \exp(rt)$. Therefore, with the expected total number of contact infections through transport being σ_{tr}, β_{tr} is equal to

$$\beta_{tr} = \alpha \sigma_{tr} \,. \tag{5.15}$$

5.3.2. Results

The estimates for log$r$, log(R–1), logα , and logβ_{ind} were –2.0, 0.60, –6.7, and –6.2 respectively. The covariance matrix of the estimators of these parameters was:

$$\text{var} = \begin{pmatrix} 0.0078 & 0.021 & -0.019 & -0.019 \\ 0.021 & 0.17 & -0.0094 & -0.010 \\ -0.019 & -0.0094 & 0.097 & 0.089 \\ -0.019 & -0.010 & 0.089 & 0.089 \end{pmatrix} \tag{5.16}$$

Transformed to the original model parameters, the point estimates with 95% confidence intervals are listed in Table 5.3. Table 5.3 also shows the means and 95% confidence intervals of β_{tr}. The 95% confidence limits of β_{tr} are the 250th and 9751st value of the ordered range of 10,000 determined β_{tr}s with parameters randomly drawn from the above distribution of log$r$, log(R-1), logα, and logβ_{ind}. To get an idea of the level of between-herd transmission with the estimated parameters, we have calculated the basic reproduction ratio between herds R_h as the largest eigenvalue of the next-generation matrix (Diekmann and Heesterbeek, 2000):

Table 5.3. The estimates and 95% confidence intervals of the model parameters and the basic reproduction ratios.

Parameter	Estimate	95% CI
r	0.13	0.11 - 0.16
R	2.8	1.8 – 5.1
α	0.0013	0.00068 - 0.0023
β_{ind}	0.0021	0.0012 - 0.0038
β_{tr}	0.0029	0.0016 - 0.0050 [a]
R_h without transport	1.7	1.4 - 2.0
R_h with transport	2.5	2.1 - 2.8 [a]

[a] These 95% CIs have been approximated by drawing 10,000 times from the parameter estimator distribution and determining β_{tr} and R_h for each parameter set. The 250th and 9751st values of the ordered ranges were the limits of the confidence intervals.

$$\begin{array}{c} \text{from:} \\ \text{to:} \end{array} \quad \begin{array}{cc} \text{fin. herd} & \text{mult. herd} \\ \text{fin. herd} \\ \text{mult. herd} \end{array} \quad \begin{pmatrix} \frac{\beta_f}{\alpha} & \frac{\beta_f + \beta_{tr}\varphi_{tr}}{\alpha} \\ \frac{\beta_m}{\alpha} & \frac{\beta_m}{\alpha} \end{pmatrix} \tag{5.17}$$

In matrix (5.17), $\varphi_{tr} = 1$ if transport is permitted and $\varphi_{tr} = 0$ if not. The estimated R_hs with and without transport are given in Table 5.3. The R_h without transport, i.e. the largest eigenvalue of (5.17), is equal to β_{ind}/α. Therefore, the variance of $\log R_h$ can be derived from the covariance matrix (5.16): $\text{var}(\log R_h) = \text{var}(\log\beta_{ind}) + \text{var}(\log\alpha) - 2\text{covar}(\log\beta_{ind},\log\alpha)$. The 95% CI for R_h with transport has been determined as for β_{tr}.

Subsequently, a χ^2 goodness-of-fit test was carried out for the three likelihood functions. All $E(N_{pos,i})$ for $L_1(r, R)$ and $E(C_i)$ for $L_3(r, R, \alpha, \beta_{ind})$ are given in Tables 5.1 and 5.2, respectively. The $E(T_{det,i})$ for $L_2(r, R, \alpha)$ were equal for all i: 32.0. The one-sided test results are $P = 0.26$ ($\chi^2 = 34.5$; d.f. = 30) for L_1, $P = 0.55$ ($\chi^2 = 76.6$; d.f. = 79) for L_2, and $P = 0.55$ ($\chi^2 = 4.97$; d.f. = 6) for L_3. Thus, the model cannot be rejected.

Finally, the division of the 429 infected herds of the Dutch CSF epidemic into groups resulted in 231 net piglet producers, 105 net piglet receivers, and 93 rest herds. By inclusion of the rest herds in the net piglet receiver group, a β_f:β_m ratio of approximately 1:1 is retrieved. The ratio 1:1 was used for the model calculations.

5.4. Control scenarios

The effects of five different control measures have been investigated with the described model, as well as all relevant combinations of these measures:

(A) Total transport prohibition
(B) Killing of young piglets (in combination with a breeding ban)
(C) Vaccination of all piglets (not sows) at multiplier herds, followed by recurrent vaccination of newborn piglets
(D) Single vaccination of all pigs at finishing herds
(E) Vaccination of piglets on arrival at finishing herds

Tested control scenarios consist of combinations of these measures and are indicated by codes referring to the above list, e.g. scenario AD holds a transport prohibition and a single vaccination of finishing herds. All tested scenarios are listed in Table 5.4. Missing letter combinations code for scenarios that are impossible, for example, all scenarios with control measures (A) and (E) together. To distinguish single control measures from control scenarios, the measures are always put between brackets, so (A) refers to a measure and A to a scenario.

The effect of control scenarios is modelled by multiplication of the transmission rate parameters β_m, β_f, and β_{tr} by the functions $\varphi_m(t)$, $\varphi_f(t)$, and $\varphi_{tr}(t)$, respectively. For each control scenario, the functions $\varphi(t)$ are different. The control scenarios start at $t = 0$. Assumptions for the functions $\varphi(t)$ are

(1) Animals vaccinated at $t = 0$ are instantaneously protected, i.e. not susceptible. Although in reality the vaccine significantly reduces transmission only after 2 weeks (Bouma et al., 2000), that is already sufficient to reduce the size of within-herd outbreaks even if the herd is infected just after vaccination. Protected animals cannot be infected, so the transmission rate for indirect infectious contacts is multiplied by the fraction of unvaccinated animals on the farm, which denotes the probability that the first animal to be infected is unvaccinated.
(2) On a multiplier herd, the piglet to sow ratio is 1610:365, as noted before. This means that, if there are no susceptible piglets present, $\varphi_m(t) = 365/(1610+365) = 365/1975$. When control measure (C) is applied, the vaccine is not assumed to protect at once, because vaccination is an ongoing process, which results in the continuous presence of yet insufficiently protected pigs. Hence, it is assumed that piglets are protected by vaccination from the age of four weeks onwards, namely, vaccination at two weeks of age plus two weeks for the vaccine to start its effect. Then, $\varphi_m(t) = (365+1610 \cdot 4/10)/1975 = 1009/1975$.

Table 5.4. The functions $\varphi(t)$ for each of the tested scenarios; time t is measured in days and $t = 0$ is defined as the initiation of the control scenarios.

	Scenario	Time interval	$\varphi_f(t)$	$\varphi_m(t)$	$\varphi_{tr}(t)$
constant $\varphi(t)$	ABCD	$t > 0$	0	365/1975	0
	ABC	$t > 0$	1	365/1975	0
	ABD	$t > 0$	0	1	0
	AB	$t > 0$	1	1	0
	ACD	$t > 0$	0	1009/1975	0
	AC	$t > 0$	1	1009/1975	0
	AD	$t > 0$	0	1	0
	A	$t > 0$	1	1	0
	BCD	$t > 0$	0	365/1975	0
	CD	$t > 0$	0	1009/1975	0
	DE	$t > 0$	0	1	1
	none	$t > 0$	1	1	1
changing $\varphi(t)$	BC	$0 < t \leq 100$	$1 - t/100$	365/1975	0
		$t > 100$	0	365/1975	0
	BDE	$0 < t \leq 70$	0	$1 - 23t/1975$	1
		$t > 70$	0	365/1975	0
	BD	$0 < t \leq 70$	$t/100$	$1 - 23t/1975$	1
		$70 < t \leq 100$	0.7	365/1975	0
		$100 < t \leq 170$	$1.7 - t/100$	365/1975	0
		$t > 170$	0	365/1975	0
	BE	$0 < t \leq 70$	$1 - t/100$	$1 - 23t/1975$	1
		$70 < t \leq 100$	$1 - t/100$	365/1975	0
		$t > 100$	0	365/1975	0
	B	$0 < t \leq 70$	1	$1 - 23t/1975$	1
		$70 < t \leq 170$	$1.7 - t/100$	365/1975	0
		$t > 170$	0	365/1975	0
	C	$0 < t \leq 100$	$1 - t/100$	1009/1975	0
		$t > 100$	0	1009/1975	0
	D	$0 < t \leq 100$	$t/100$	1	1
		$t > 100$	1	1	1
	E	$0 < t \leq 100$	$1 - t/100$	1	1
		$t > 100$	0	1	1

(3) Transmission through transport contacts is only prevented if there is no transport at all or if the transported piglets have been vaccinated at the multiplier herd. In all other cases transport of pigs from infected multiplier herds leads to major outbreaks in finishing herds with normal within-herd transmission rates.

(4) Vaccination does not affect virus transmission within herds, because in most cases the vaccinated and unvaccinated animals on a farm are separated (they are housed within weight classes, which are approximate age classes, which in most cases denote the vaccination status).

The functions $\varphi(t)$ for all tested control scenarios are listed in Table 5.4. As can be seen from Table 5.4, for some control scenarios, all $\varphi(t)$ remain constant from t = 0. For other scenarios, however, one or more functions had to be subdivided into time intervals, to account for the changing situation due to transport. For example, scenario B starts with a completely susceptible population, but in 70 days all piglets are removed from the multiplier herds and in another 100 days the finishing herds are emptied as well. Therefore, $\varphi_m(t)$ decreases linearly from 1 to 365/1975 within 70 days; $\varphi_{tr}(t) = 1$ until $t = 70$ and then becomes 0; and $\varphi_f(t)$ first remains 1 for 70 days and then decreases linearly from 1 to 0 within 100 days.

5.5. Model analysis

5.5.1. Methods

5.5.1.1. Probability of extinction

A common measure to characterise transmission of an infectious agent in a population of herds would be the basic reproduction ratio between herds R_h, the expected number of herds infected by one infectious herd in a population of susceptible herds. An $R_h < 1$ ascertains extinction of the infection, whereas an $R_h > 1$ denotes the possibility of major epidemics (Diekmann and Heesterbeek, 2000). For some of the scenarios, however, R_h is not a constant value in the initial stage since one of the functions $\varphi(t)$ changes with time. Therefore, we chose to determine the probability of extinction of an epidemic. The probability of extinction is directly related to the threshold property of R_h, since extinction will occur with probability 1 if in a control scenario R_h eventually reaches a constant value < 1.

The probability of extinction heavily depends on the epidemic situation at the time a control scenario is implemented, viz. on the number of undetected infected herds present in the population, the types of the infected herds (multiplier or finishing herds), and how much the infection has already spread in the herds. Regarding the progression of infection in infectious herds, we always assume that the herds have been infected at $t = 0$, implying that the entire infectious period still has to be completed. As for the initial numbers and types of infectious herds, the

probability of extinction has to be calculated only with one finishing herd or one multiplier herd as a starting condition. Subsequently, the probabilities of extinction for all other starting conditions can be calculated, because the model is a branching process: if the probability of extinction starting with one multiplier herd is z_m and the probability starting with one finishing herd is z_f, then the probability of extinction starting with x multiplier herds and y finishing herds will be $z_m^x z_f^y$.

For determination of the probabilities of extinction z_f and z_m, two types of control scenario could be distinguished. The first type comprised all scenarios with constant functions $\varphi(t)$, which were the scenarios ABCD, ABC, ABD, AB, ACD, AC, AD, A, BCD, CD, and DE (Table 5.4). For these control scenarios, R_h could be calculated as the largest eigenvalue of the next-generation matrix (Diekmann and Heesterbeek, 2000):

$$\begin{pmatrix} \dfrac{\beta_f \varphi_f(\cdot)}{\alpha} & \dfrac{\beta_f \varphi_f(\cdot) + \beta_{tr} \varphi_{tr}(\cdot)}{\alpha} \\ \dfrac{\beta_m \varphi_m(\cdot)}{\alpha} & \dfrac{\beta_m \varphi_m(\cdot)}{\alpha} \end{pmatrix} \tag{5.18}$$

Matrix (5.18) is a more general form of matrix (5.17), in which $\varphi_f(\cdot) = \varphi_m(\cdot) = 1$. If $R_h < 1$, then both z_f and z_m were equal to 1. If, on the other hand, $R_h > 1$, then it was possible to determine the probability of extinction with a method based on the properties of branching processes. In Appendix 5C, the following set of recursive equations is derived, from which z_f and z_m could be solved numerically:

$$z_f = \frac{1}{1 - \dfrac{\beta'_f}{\alpha}(z_f - 1) - \dfrac{\beta'_m}{\alpha}(z_m - 1)} \tag{5.19.1}$$

$$z_m = \frac{1}{1 - \dfrac{\beta'_f + \beta'_{tr}}{\alpha}(z_f - 1) - \dfrac{\beta'_m}{\alpha}(z_m - 1)} \tag{5.19.2}$$

In Eq. (5.19), $\beta'_f = \varphi_f(t)\ \beta_f$, $\beta'_m = \varphi_m(t)\ \beta_m$, and $\beta'_{tr} = \varphi_{tr}(t)\ \beta_{tr}$. For the point estimates of z_f and z_m, the point estimates of β_{ind} and α were used. Confidence intervals for z_f and z_m were determined by drawing 1000 times from the distribution of parameter estimators and determining z_f and z_m for each set of parameters. The 25th and 976th value of the ordered range denote the limits of the 95% confidence interval.

The second type of control scenario had at least one of the functions $\varphi(t)$ not constant. However, all functions $\varphi(t)$ in the long run did reach a constant value, so all control scenarios ultimately reached a constant R_h. According to the ultimate constant R_h, the second type could be divided into two subtypes. The first subtype consisted of all control scenarios where R_h becomes smaller than 1. Control scenarios BC, BDE, BD, BE, B, and C were of this subtype, for which $z_f = z_m = 1$. The second subtype comprised the scenarios where R_h ultimately exceeded 1 — scenarios D and E. For these scenarios, z_f and z_m had to be determined by 1000 repeated continuous time stochastic simulations (see Appendix 5D) with the model parameters set at their estimate. Starting with one finishing or multiplier herd, each simulation continued until either extinction or until a generation with at least 21 infectious herds was reached, the latter outcome indicating a major outbreak. Further, to get some insight into the variation of these z_f and z_m values due to uncertainty about the model parameters, for each of 1000 random draws from the distribution of the parameter estimators, 25 model simulations were executed from which z_f or z_m was estimated. The variance of these estimates was calculated from the simulation results and consists of two parts (Rao, 1973) [for notational convenience, we define $\boldsymbol{\vartheta} = (r, R, \alpha, \beta_{ind})^T$]:

$$\mathrm{var}_{\boldsymbol{\vartheta}}\left(Z_f(\boldsymbol{\vartheta})\right) = \mathrm{var}_{\boldsymbol{\vartheta}}\left\{E\left(Z_f(\boldsymbol{\vartheta})\middle|\boldsymbol{\vartheta}\right)\right\} + E_{\boldsymbol{\vartheta}}\left\{\mathrm{var}\left(Z_f(\boldsymbol{\vartheta})\middle|\boldsymbol{\vartheta}\right)\right\}, \tag{5.20}$$

in which $Z_f(\boldsymbol{\vartheta})$ is the estimator of z_f as a function of $\boldsymbol{\vartheta}$. The expected conditional variance in the second right-hand side (RHS) term reflects the variation due to stochastic effects and was equal to $z_f(\boldsymbol{\vartheta})(1\text{-}z_f(\boldsymbol{\vartheta}))/25$. It could be approximated by $\overline{z_f}\left(1-\overline{z_f}\right)\Big/25$, in which $\overline{z_f}$ is the average $\hat{z}_f(\boldsymbol{\vartheta})$. The variance of the conditional expectation in the first RHS term reflects the uncertainty about the model parameter estimates and was estimated by subtracting the expected variance from the observed variance by rewriting (5.20) as:

$$\hat{\mathrm{var}}_{\boldsymbol{\vartheta}}\left\{E\left(Z_f(\boldsymbol{\vartheta})\middle|\boldsymbol{\vartheta}\right)\right\} \approx \sum_i \frac{\left(\overline{z_f} - \hat{z}_f(\boldsymbol{\vartheta}_i)\right)^2}{1000} - \overline{z_f}\left(1-\overline{z_f}\right)/25 \tag{5.21}$$

By assuming a normal distribution of the conditional expected value, an approximate confidence interval for z_f was obtained, which may serve to indicate the effect of the uncertainty about the model parameter estimates. A confidence interval for z_m was determined analogously.

5.5.1.2. Duration and size of the epidemic

Although the probability of extinction is very useful in deciding which control strategies are insufficient, it does not distinguish between the scenarios in which extinction will be reached. Therefore, all scenarios with $z_f = z_m = 1$ were compared with respect to the duration and the size of the epidemic, i.e. the number of herds ultimately infected. The comparison was done by continuous time stochastic simulations of epidemics. Both duration and size of the epidemic largely depend on the situation at the time the control measures are implemented, as did the probability of extinction. The duration starting with one infectious herd, however, cannot so easily be extrapolated to situations with more infectious herds. Hence, we simulated with three starting conditions: one infectious finishing herd, one infectious multiplier herd, and five of both types of herds. The expected size of the epidemic is easier to extrapolate to other starting conditions, since this will always be a multiplication factor with respect to the initial number of infectious herds. Thus, the size of the epidemic has only been determined for the starting condition with five infectious herds of both types.

Simulations were performed as described in Appendix 5D. For each scenario and starting condition, 1000 simulations were done in which each simulation used another set of model parameters, randomly drawn from the distribution of the estimators. Of the 1000 simulations, the median time to extinction and the median size of the epidemic were determined together with the 95% interval by taking the 25th and 976th value of the ordered ranges of times and sizes.

5.5.1.3. Further model investigations

Finally, we made some plots to explore the sensitivity of the model outcomes for the four model parameters r, R, α, and β_{ind}, and for the calculated R_h (largest eigenvalue of matrix (5.18)); to explore the correlation between calculated outcomes z_f and z_m; and to explore the correlation between the size and duration of the simulated epidemics.

The variation in extinction times and epidemic sizes, as obtained with the simulations, is a combination of variation due to random effects of the stochastic simulation and variation due to uncertainty about the parameter values. The sensitivity plots were used to distinguish between these two sources of variation. If the variation is mainly due to random effects, no relation between the parameters and the outcomes can be observed. In that case, more precise estimates of the parameters will not help to better predict the effectiveness of the control measures.

Table 5.5. The probabilities of extinction for each of the tested scenarios if starting with one infected finishing herd (z_f) or one multiplier herd (z_m); $z_f = z_m = 1$ denotes an $R_h \leq 1$, whereas $z_f \leq 1.00$ and $z_m \leq 1.00$ denote an $R_h > 1$.

Scenario type [a]	Scenario	z_f	95% CI	z_m	95% CI
constant $\varphi(t)$	ABCD; ABD; ACD; AD; BCD; CD	1	1 - 1	1	1 - 1
	ABC	1.00	0.85 - 1	1.00	0.85 - 1
	AB	0.59	0.50 - 0.71	0.59	0.50 - 0.71
	AC	0.79	0.64 - 0.93	0.79	0.64 - 0.93
	A	0.59	0.50 - 0.71	0.59	0.50 - 0.71
	DE	0.69	0.62 - 0.75	0.46	0.40 - 0.54
	none	0.50	0.43 - 0.57	0.32	0.27 - 0.37
changing $\varphi(t)$ $R_h < 1$	BC; BDE; BD; BE; B; C	1	1 - 1	1	1 - 1
changing $\varphi(t)$ $R_h > 1$	D	0.61	0.57 - 0.64 [b]	0.38	0.30 - 0.46 [b]
	E	0.57	0.51 - 0.65 [b]	0.39	0.33 - 0.45 [b]

[a] Based on the functions $\varphi(t)$, two scenario types are distinguished: with constant $\varphi(t)$ and with changing $\varphi(t)$, the latter of which is subdivided into two subtypes: with R_h ultimately smaller than 1 and with R_h larger than 1.

[b] Approximate confidence intervals as calculated with Eq. (5.21) from 1000 estimates; each estimate was for a different parameter set, randomly drawn from the parameter distribution, and each estimate was based on 25 model simulations.

If, on the other hand, the model outcomes are related to the parameter values, better estimates can improve the predictions.

5.5.2. Results

Table 5.5 shows the estimated probabilities of extinction and the 95% confidence intervals for all tested scenarios. By comparison of the effectiveness of the strategies, we can come up with two conditions that have to be met to ensure extinction. First, virus transmission through transport should be prevented. This can be achieved by a transport prohibition (control measure (A)), by a breeding prohibition (control measure (B)), or by recurrent vaccination of multiplier herds (control measure (C)). Second, at least half of the indirect transmission should be prevented. This can be observed from comparing the effective scenario AD, in which the indirect transmission is reduced by 50%, and the ineffective scenario ABC, in which the indirect transmission is still present for 59%. The second

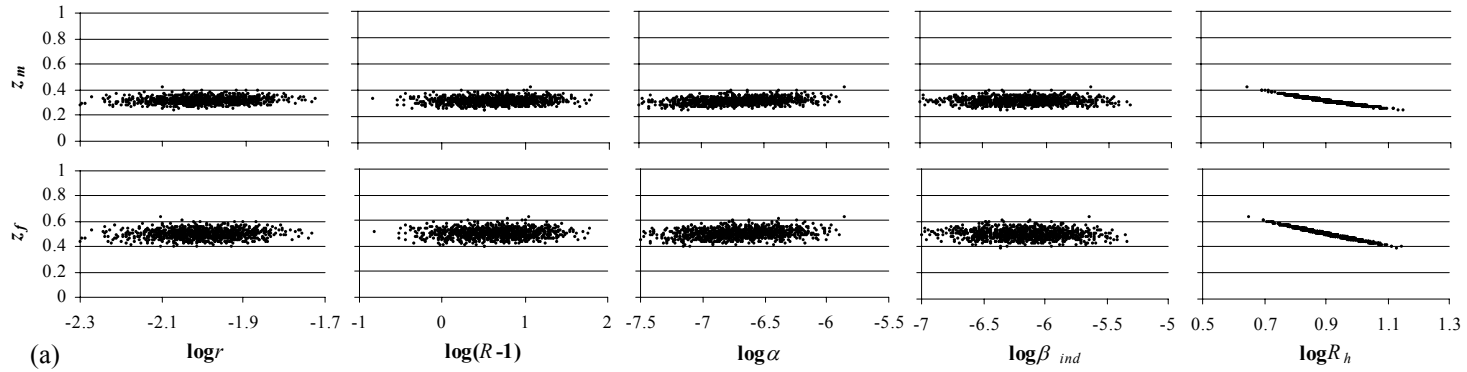
condition can also be explained from the estimate of R_h without transport, of which the upper limit of the 95% confidence interval is 2.0. Reducing the indirect transmission by a half results in an upper confidence limit of 1.0, which leads to extinction. Prevention of transmission can be accomplished by a breeding prohibition (control measure (B)), or by vaccination (control measures (C), (D), and (E)).

There are three possibilities of satisfying both conditions at the same time, namely, both control measures (A) and (D), only control measure (B), or only control measure (C). It is important to note that the second condition is met by control measures (B) and (C) because of the permit to transport pigs: the susceptible finishing pigs are either not replaced at all (in case of (B)), or replaced only by vaccinated pigs (in case of (C)). Consequently, if a transport prohibition is added as a control measure, the scenarios become less effective (compare scenarios ABC vs. BC, AB vs. B, and AC vs. C). This can be resolved by vaccinating the finishing herds.

For scenario 'none', Figure 5.1a shows the sensitivity plots of z_f and z_m against parameters r, R, β_{ind}, α, and R_h using the 1000 simulations for determining the confidence intervals for z_f and z_m. For all other scenarios with z_f and z_m smaller than 1, the sensitivity plots look similarly and are therefore not shown. It appears that R_h is the major determinant for both z_f and z_m. This is not very surprising, since the terms of the next-generation matrix (5.18), which determines R_h, also appear in the equations for z_f and z_m (5.19). Figure 5.1b shows a high correlation between the calculated z_f and z_m.

Table 5.6 shows the results of the simulations of the scenarios with certain extinction: the durations and sizes of the epidemics. In some of the simulations with scenario AD, the random draw of the model parameters led to an R_h above 1. If during such a simulation the number of infected herds grew so large that the probability of a major epidemic exceeded 95%, the simulation was stopped and duration and size of the epidemic were set to infinity. This happened in two of the 1000 simulations starting with one infectious herd and in seven of the 1000 simulations starting with ten infectious herds.

Close examination of the results in Table 5.6 reveals that there are two ways in which the effectiveness of a scenario can be improved. The first way is by a more severe reduction of indirect transmission between herds. This can best be seen by comparing the scenarios ABCD, ACD, and ABD. These all have constant functions $\varphi(t)$, but differ in the value of $\varphi_m(t)$, so they differ in the level of indirect transmission. R_h for these three scenarios is 0.16 for ABCD, 0.43 for ACD, and 0.85 for ABD. By comparison of the results in Table 5.6, it appears that a lower R_h



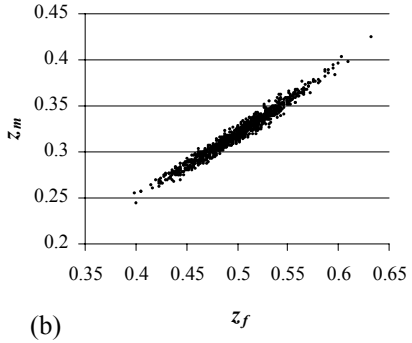


Figure 5.1. Investigation of z_f and z_m with respect to the distribution of the model parameters. Each dot represents one of 1000 draws from the parameter distribution.
(a) The sensitivity plots of z_m (top row) and z_f (bottom row) against the four transformed parameters log$r$, log(R-1), logα, and logβ_{ind}, and against the derived parameter logR_h.
(b) The plot of the correlation between z_m and z_f.

Table 5.6. The expected durations and sizes and 95% confidence intervals of the control scenarios with $z_f = z_m = 1$.

Scenario	duration (days)						size (herds)	
	1 f. [a]	95% CI	1 m. [b]	95% CI	5 + 5 [c]	95% CI	5 + 5 [c]	95% CI
ABCD	32	8 - 92	32	8 - 92	68	38 - 136	11	10 - 16
ABD	38	6 - 442	38	6 - 442	226	63 - 1070	42	13 - 452
ACD	35	7 - 151	35	7 - 151	103	47 - 239	16	10 - 35
AD	38	6 - 442	38	6 - 442	226	63 - 1070	42	13 - 452
BCD	32	8 - 92	32	8 - 92	68	38 - 136	11	10 - 16
BC	39	9 - 129	39	9 - 129	112	62 - 176	21	12 - 38
BDE	38	7 - 158	61	7 - 161	135	89 - 203	35	15 - 69
BD	43	6 - 215	72	7 - 213	194	108 - 264	67	20 - 157
BE	41	7 - 181	86	7 - 169	145	97 - 220	53	22 - 110
B	63	8 - 230	114	8 - 232	206	152 - 270	108.5	37 - 238
CD	35	7 - 151	35	7 - 151	103	47 - 239	16	10 - 35
C	46	7 - 197	46	7 - 197	157	76 - 293	33	15 - 73

[a] The initial situation of only one infected finishing herd

[b] The initial situation of only one infected multiplier herd

[c] The initial situation of five infected finishing herds and five infected multiplier herds

strongly reduces both duration and size. The second way to improve a scenario is by reaching the maximal reduction in indirect and direct transmission earlier. This is illustrated by comparing the scenarios ABCD, BDE, BE, and BD, which reach the same $R_h = 0.16$ after 0, 70, 100, and 170 days respectively. The expected duration and size increase in the same order, as seen in Table 5.6.

For scenario AD, the sensitivity of the duration and size for the model parameters r, R, β_{ind}, α, and R_h is shown in Figure 5.2a. The sensitivity plots are representative for all other scenarios. None of the parameters seem to determine the duration and size of the epidemic, although R_h is of importance as noted after comparison of the scenarios ABCD, ACD, and ABD above. Apparently, within the estimated range of R_h, stochasticity is the main source of variation in both duration and size. Figure 5.2b shows a clear correlation between size and duration of the simulated epidemics with scenario AD.

5.6. Discussion

We presented a mathematical model for the transmission of CSFV between pig herds. We showed that the model structure was in accordance with available data of

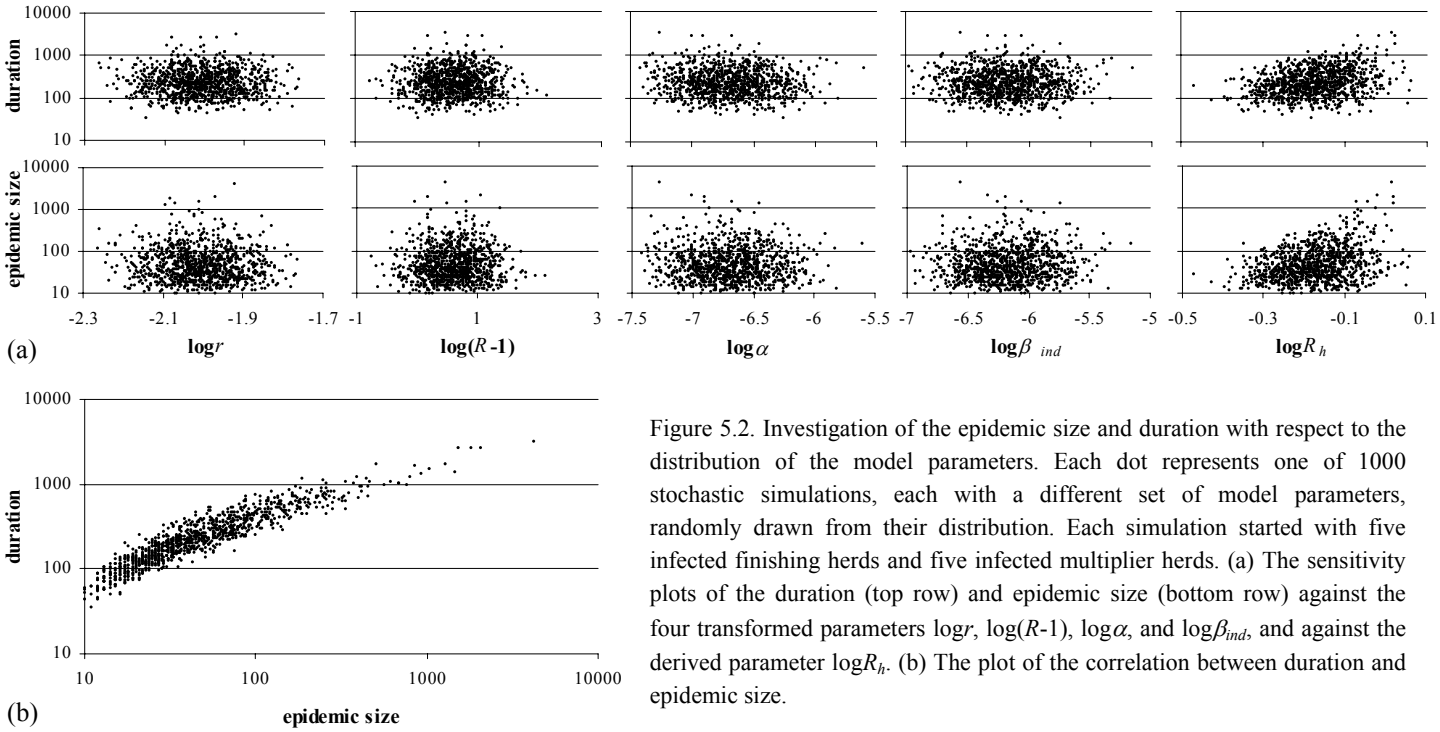


Figure 5.2. Investigation of the epidemic size and duration with respect to the distribution of the model parameters. Each dot represents one of 1000 stochastic simulations, each with a different set of model parameters, randomly drawn from their distribution. Each simulation started with five infected finishing herds and five infected multiplier herds. (a) The sensitivity plots of the duration (top row) and epidemic size (bottom row) against the four transformed parameters log$r$, log(R-1), logα, and logβ_{ind}, and against the derived parameter logR_h. (b) The plot of the correlation between duration and epidemic size.

the Dutch CSFV epidemic and we used the data to estimate the model parameters. With the model we were able to predict the effects of several scenarios for the control of CSFV epidemics. Two general conditions for extinction of CSFV epidemics could be derived and two criteria for improving the effectiveness of a scenario were discerned. Finally, the sensitivity analyses showed that the only parameter of real importance is R_h, which is mainly determined by the quotient of β_{ind} and α. A better estimate of R_h, however, will hardly improve the model predictions, as most of the uncertainty in outcome lies in the stochasticity of the epidemic process.

The conditions for extinction of an epidemic are (1) prevention of transmission through pig transports and (2) reduction of indirect virus transmission by at least 50%. A striking result was that a transport prohibition can have a negative effect on the effectiveness of a scenario compared to the same scenario without transport prohibition (scenarios ABC vs. BC, AB vs. B, and AC vs. C). That is because the second condition for extinction is met by scenarios BC, B, and C through removal of the susceptible pigs in the finishing herds, which is prevented by a transport prohibition. Permitting transport can be beneficial in another way as well, since it will 'wash out' the tracks of small outbreaks on the farms. These small outbreaks, which are not incorporated into the model but which will certainly arise during an epidemic, can lead to problems at the end of the epidemic, when an area has to be declared free of CSFV by a large-scale serological screening. Detection of minor outbreaks in a screening will lengthen the duration of trading and export limitations and increase the costs of the epidemic.

Two criteria for improving the effectiveness of CSFV control were distinguished. The first was a stricter reduction of indirect transmission, which leads to a decrease in R_h. If R_h is only just below 1, as with scenario ABD, the number of infected herds in the next generations will decrease slowly and it will take many generations before extinction is established. A more profound reduction in R_h will decrease the number of generations and, as a result, the epidemic will take less time and affect fewer herds. The second criterion was a quicker reduction of transmission, which leads to a low R_h earlier after start of the control strategy. If effectiveness is somehow delayed, as with scenario BDE, where transmission via transport is blocked only from 70 days, the first one or two generations of infected herds will still appear under a regime with $R_h > 1$. A quicker reduction of R_h will lead to considerably less infected herds, and hence an earlier extinction of the epidemic. The effect of quick action was also observed in the simulation study of Mangen et al. (2001), where it appeared that a delay in implementing control measures of 20 days could almost double the number of detected herds.

The sensitivity analysis showed that the only parameter of real importance is R_h. This parameter largely determines the probability of extinction as can be seen in Figure 5.1. As for the duration and size of the epidemic, R_h is of minor effect within its estimated range, although an increase in both duration and size is expected with higher R_h. Here, the stochastic effects determine the major part of the variation. The fact that r and R seem to be of no importance for the model outcomes does not mean that they have been entirely useless. Incorporating within-herd dynamics made it possible to do more reliable parameter estimations — otherwise the data would not fit to the model — and that also increased the reliability of the parameter values that do matter, namely, β_{ind} and α.

Since the parameter estimations are all based on the data of the Dutch CSFV epidemic in the southeastern part of the Netherlands, the model results in fact only account for that specific region. It is a very pig-dense area with relatively many multiplier herds. A lower pig density will probably reduce the value of β_{ind}, although the extent of this reduction will depend on the density-dependence of the contacts implicitly incorporated in β_{ind}. A lower multiplier herd fraction will lead to relatively fewer infected multiplier herds, which are most infectious if transport is allowed. In conclusion, if a control scenario comes out positive in this model, it will be effective in most other areas as well.

The within-herd exponential growth parameter r was estimated to be 0.13 (95% CI 0.11-0.16). Stegeman et al. (1999a) estimated r from 7 breeding herds of the same set of 82 herds we used and came up with an estimate of 0.11 (0.06-0.16), which is in reasonable agreement. When the parameter μ of the within herd transmission model (Eq. (5.1)) is calculated from the estimates of r and R, it is possible to estimate the mean generation time of the within herd transmission as $0.5\mu^{-1}$ = 6.8 days. The generation time can be compared to generation times estimated from transmission experiments with CSFV (Chapters 2, 3, 4), which are equal to the estimated latent period — the period an animal is infected but not yet infectious — plus half of the average infectious period. This results in generation times of 10.9 and 9.2 (weaner and adult pig groups of Chapter 2, respectively), and of 13.9 and 8.2 (3 months and 6 months old groups of Chapter 4, respectively; values of μ not presented in the paper). These are slightly higher than the estimate in this paper, but still reasonably close, considering the rough simplification of the within-herd transmission in our model.

The basic reproduction ratio between herds R_h was estimated to be 1.7 without transport contacts (95% CI 1.4-2.0). This is in fact an estimate for R_h in the first ten weeks of the Dutch CSFV epidemic. Stegeman et al. (1999b) estimated R_h in the same period at 1.3. The difference can be completely attributed for by the different

estimate of the mean duration of the infectious period, which is proportional to R_h. If calculated by Eq. (5.9), the infectious period in our model is 32 days, which is equal to the mean duration of the 82 herds in the dataset of the second log-likelihood function. Stegeman et al. (1999b) estimated the average infectious period at 25 days, from a dataset that for a major part contains infectious period lengths estimated from serological data.

An important aspect of our model compared to most existing models for CSF epidemics is its relatively simple structure (cf. Jalvingh et al., 1999; Mangen et al., 2001; Nielen et al., 1999). Only six parameters are included, of which five were directly estimated with data of the Dutch CSF epidemic and one (β_{tr}) was related to the average transport frequency. The simplicity enabled us to generalise the model outcomes and come up with requirements for good control strategies. Besides, it provided the opportunity to point out parameters for which more precise estimates are needed. Because the parameters could be estimated from data of a previous epidemic, we can be confident that the quantitative results are reliable. Nevertheless, two assumptions need further attention.

First, a branching process model as presented in this paper does not take the susceptible herds into account. This means that there is an implicit assumption of unlimited availability of susceptible herds. Hence, effects like local depletion or other spatial heterogeneities are not included. Because of the lack of spatial structure, some control measures cannot be incorporated into the model easily; for example, preventive slaughter or vaccination within short distance of infected herds. These control measures can only be incorporated by modelling their effect on the indirect between-herd transmission through adjustment of the functions $\varphi(t)$. The effects should then be estimated from data of previous epidemics. Lack of spatial structure also leads to the implicit assumption that all control measures are implemented in a large enough area. Escape of virus from the area would be the start of a new epidemic in terms of our model.

The second major assumption is the reduction of the population structure into two herd types, multiplier herds and finishing herds. This is by far not as detailed as the true diversity in herd types, ignoring the existence of mixed herds (with sows and finishers), breeding farms (supplying gilts for the multiplier herds), and artificial insemination stations. The reason to include herd diversity in a model would be the different epidemiological impact of different herd types. Regarding within-herd virus transmission, experiments have shown different transmission rates between weaner and slaughter pigs (Chapter 2). The sensitivity analyses in this paper, however, show that both parameters involved in within-herd transmission, r and R, appear to be of hardly any importance in determining the model outcomes (Fig. 5.1 and 5.2). It is, therefore, not expected that breaking down the population into herd

types with different within-herd transmission parameters would change the model results. As far as between-herd transmission is concerned, different herd types would be included to account for heterogeneity in the contact pattern. In the model, we included two herd types since we wanted to account for the possibility to relax the transport prohibition and allow transport of piglets to finishing herds and of finishing pigs to the slaughterhouse. As a result of the division into two herd types, a β_f:β_m ratio had to be determined. This was complicated by the diversity in herd types in the data set, which consisted for about a quarter of mixed and breeding herds. Because the herd type is only important for the rate at which the herd itself causes new infections, and not for the type of herd it is infected by, the rest group could simply be included in the finishing herds group. This led to a β_f:β_m ratio of 1:1.

The political decision to use a specific control strategy will not only depend on its epidemiological effectiveness. Other major roles will be played by the economic effectiveness and the public opinion. Regarding the economic effectiveness, costs of an epidemic are of course related to its duration and to the number of infected herds, but in addition other costs can result from implementing specific control measures. Especially if these control measures are related to export, the costs can become high (Mangen et al., 2001). An important aspect of export-related costs is the acceptance of vaccinated pigs by importing countries. The acceptance will depend on the quality of the diagnostic tests in vaccinated and unvaccinated pigs, but should also depend on the possible consequences of importing a false-negatively tested pig and the expected number of infected, yet undetected pigs. A risk analysis might be used to weigh these factors. Regarding the public opinion, we have seen that preventive slaughter has raised a major discussion on the ethics of killing and destroying healthy animals (Terpstra et al., 2000), which can influence the eventual decision on which control scenario to use. However, it will be helpful if the epidemiological aspects of the decision making are already quantified and available before a new CSFV entry occurs. We think the model results can prove useful for optimally preparing for CSFV epidemics.

Acknowledgements

The authors gratefully acknowledge financial support from STW (Technology Foundation), Utrecht, The Netherlands.

Appendix 5A

5A.1 Parameters

Input parameters

λ	within-herd transmission parameter
μ	animal recovery rate
R (:= λ/μ)	within-herd basic reproduction ratio
r (:= $\lambda - \mu$)	within-herd exponential growth rate
β_f	between-herd transmission parameter for infection of finishing herds through indirect contacts
β_m	between-herd transmission parameter for infection of multiplier herds through indirect contacts
β_{ind} (:= $\beta_f + \beta_m$)	total between-herd transmission parameter for indirect contacts
β_{tr}	between-herd transmission parameter for infection of finishing herds by multiplier herds through transport contacts
α	herd detection parameter

Derived parameters

$\beta'_f := \beta_f \cdot \varphi_f(\cdot)$	adjusted finishing herd transmission parameter for indirect contacts
$\beta'_m := \beta_m \cdot \varphi_m(\cdot)$	adjusted multiplier herd transmission parameter for indirect contacts
$\beta'_{tr} := \beta_{tr} \cdot \varphi_{tr}(\cdot)$	adjusted transmission parameter for transport contacts
R_h	between-herd basic reproduction ratio
z_f	probability of extinction if only one finishing herd is infected
z_m	probability of extinction if only one multiplier herd is infected

5A.2. Variables

General model

t	time since start of the control scenario
H	height of the within-herd exponential infectious curve

Likelihood function $L_1(r, R)$

t_{det}	time between infection and detection
n_{tot}	total number of animals in the herd
n_{test}	number of animals serologically tested
N_{ser}	total number of serologically positive animals in the herd
N_{pos}	number of serologically positive animals in the group of tested animals

Likelihood function $L_2(r, R, \alpha)$

T_{det}	time between infection and detection

Likelihood function $L_3(r, R, \alpha, \beta_{ind})$

j	average number of infected herds within the week
C	number of newly infected herds (cases) within the week

5A.3 Functions

General model

$I(t)$	number of infectious animals within the herd as a function of time since infection of the herd
$\varphi_f(t)$	reduction factor of the transmission to finishing herds through indirect contacts as a function of time since start of the control scenario
$\varphi_m(t)$	reduction factor of the transmission to multiplier herds through indirect contacts as a function of time since start of the control scenario
$\varphi_{tr}(t)$	reduction factor of the transmission from multiplier herds to finishing herds through transport contacts as a function of time since start of the control scenario

Likelihood function $L_3(r, R, \alpha, \beta_{ind})$

$\nu(r, R, \alpha)$	conversion factor for converting the number of infectious herds within a week to the number of infectious 'animal days' within a week

Appendix 5B

Here we derive the distribution of the continuous variable $I'(t)$, which has been used as an approximation to the distribution of the discrete variable $I(t)$, defined as

$$P(I(t)=i)=(1-Rp_0(t))(Rp_0(t))^{i-1}=\frac{1-Rp_0(t)}{Rp_0(t)}(Rp_0(t))^i. \tag{5.3}$$

By using Eq. (5.2.1), $P(I(t)=i)$ can be rewritten as

$$\begin{aligned}P(I(t)=i)&=\left(\frac{R-1}{R}\frac{1}{\exp(rt)-1}\right)\left(1-\frac{R-1}{R}\frac{1}{\exp(rt)-1/R}\right)^i\\&=\left(\frac{R-1}{R}\frac{\exp(-rt)}{1-\exp(-rt)}\right)\left(1-\frac{R-1}{R}\frac{\exp(-rt)}{1-\exp(-rt)/R}\right)^i\end{aligned}$$

For large t, the following approximations can be made:

$$\begin{aligned}&\frac{\exp(-rt)}{1-\exp(-rt)}\approx\exp(-rt)\\&\frac{\exp(-rt)}{1-\exp(-rt)/R}\approx\exp(-rt)\\&1-\frac{R-1}{R}\exp(-rt)\approx\exp\left(-\frac{R-1}{R}\exp(-rt)\right)\end{aligned}.$$

Hence, $P(I(t)=i)$ can be approximated by

$$P(I(t)=i)\approx\frac{R-1}{R}\exp(-rt)\exp\left(-\frac{R-1}{R}\exp(-rt)i\right),$$

which has the form of the *pdf* of an exponential distribution. Therefore, the continuous variable $I'(t)$ is distributed as

$$I'(t)\cong ExponentialDistribution\left[\frac{R-1}{R}\exp(-rt)\right]$$

By defining $H = I'(t)\exp(-rt)$ and by dropping the accent for notational convenience, $I(t)$ becomes

$$I(t) = H\exp(rt), \text{ with} \tag{5.4.1}$$

$$H \cong ExponentialDistribution\left[\frac{R-1}{R}\right] \tag{5.4.2}$$

Appendix 5C

Here we derive equations (5.19) for determining the probabilities of extinction z_f and z_m starting with one finishing herd or multiplier herd, respectively. Diekmann and Heesterbeek (Diekmann and Heesterbeek, 2000) derive a similar equation from a model with one type of infectious individual. Since both equations (5.19) are derived in a similar way, only the derivation for the first of the two equations is shown.

The probability z_f, that a chain of infected herds starting with one finishing herd will eventually go extinct, is

$$z_f = \sum_{k=0}^{\infty}\sum_{l=0}^{\infty} q_{k,l} {z_f}^k {z_m}^l, \tag{5C.1}$$

in which $q_{k,l}$ is the probability that a finishing herd infects k finishing herds and l multiplier herds:

$$\begin{aligned} q_{k,l} = \int_0^{\infty}\int_0^{\infty} &\frac{\left(\frac{\beta'_f h}{r}\left(\exp(rt)-1\right)\right)^k}{k!}\exp\left(-\frac{\beta'_f h}{r}\left(\exp(rt)-1\right)\right)... \\ &...\frac{\left(\frac{\beta'_m h}{r}\left(\exp(rt)-1\right)\right)^l}{l!}\exp\left(-\frac{\beta'_m h}{r}\left(\exp(rt)-1\right)\right)... \\ &...\alpha h\exp\left(rt-\frac{\alpha h}{r}\left(\exp(rt)-1\right)\right)\frac{R-1}{R}\exp\left(-\frac{R-1}{R}h\right)dtdh \end{aligned} \tag{5C.2}$$

The first two lines of Eq. (5C.2) are the probabilities of infecting k finishing herds and l multiplier herds according to a Poisson distribution with a mean depending on

the height h of the infectious curve and detection time t of the infectious finishing herd. The third line consists of the distributions for h and t over which the Poisson probabilities are integrated.

If $q_{k,l}$ is put into the formula for z_f, the generating function for the Poisson distribution can be used to obtain

$$z_f = \int_0^\infty \int_0^\infty \alpha h \exp\left[rt - \frac{h}{r}(\exp(rt)-1)\left(\alpha - \beta'_f\left(z_f - 1\right) - \beta'_m\left(z_m - 1\right)\right)\right] \ldots \\ \ldots \frac{R-1}{R} \exp\left(-\frac{R-1}{R} h \right) dtdh \tag{5C.3}$$

Evaluation of Eq. (5C.3) leads to the final result:

$$z_f = \frac{1}{1 - \frac{\beta'_f}{\alpha}\left(z_f - 1\right) - \frac{\beta'_m}{\alpha}\left(z_m - 1\right)} \tag{5.19.1}$$

Appendix 5D

Continuous time stochastic simulations were performed in Mathematica® (Wolfram, 1999). Epidemics were simulated in generations of infected herds. The initially infected herds were the 0th generation and were by definition infected at $t = 0$, the moment the control strategy was implemented. For each herd of the 0th generation, the following values were drawn from the appropriate distributions in the designated order:

1. The height of the infectious curve
2. The detection time
3. The numbers of finishing herds and multiplier herds in the 1st generation, infected by this herd
4. The infection times of these herds of the 1st generation

When for each herd of the 0th generation all the values had been drawn, the same 4 values were drawn for each herd of the 1st generation, etc.

The distributions for the 4 steps in the simulation for each herd in generation i were:

1. The height H of the infectious curve of the herd in generation i was exponentially distributed with parameter (R-1)/R
2. The detection time t_{det} of the herd in generation i was equal to its infection time (determined in step 4 of its source herd in generation i-1, or equal to 0 if i = 0) plus a random number from the distribution of the length of the infectious period δ, with probability density function:

$$pdf(\delta) = \alpha h \exp\left(r\delta - \frac{\alpha h}{r}\left(\exp(r\delta) - 1\right)\right), \tag{5D.1}$$

in which h was the height of the infectious curve, drawn in step 1.

3. The numbers of finishing and multiplier herds infected in generation i+1 by the considered herd in generation i were Poisson distributed with parameters depending on the herd type of the herd in generation i. If the herd is a finishing herd, the parameters of the Poisson distributions for the numbers of finishing herds σ_{ff} and multiplier herds σ_{mf} in generation i+1 were:

$$\sigma_{ff} = \beta_f \int_{t_{\inf}}^{t_{\det}} \varphi_f(\tau) \exp\left(r\left(\tau + t_{inf}\right)\right) d\tau \tag{5D.2.1}$$

$$\sigma_{mf} = \beta_m \int_{t_{\inf}}^{t_{\det}} \varphi_m(\tau) \exp\left(r\left(\tau + t_{inf}\right)\right) d\tau \tag{5D.2.2}$$

If the herd is a multiplier herd, the parameters of the Poisson distributions for the numbers of finishing herds σ_{fm} and multiplier herds σ_{mm} in generation i+1 were:

$$\sigma_{fm} = \beta_f \int_{t_{\inf}}^{t_{\det}} \varphi_f(\tau) \exp\left(r\left(\tau + t_{inf}\right)\right) d\tau + \beta_{tr} \int_{t_{\inf}}^{t_{\det}} \varphi_{tr}(\tau) \exp\left(r\left(\tau + t_{inf}\right)\right) d\tau \tag{5D.2.3}$$

$$\sigma_{mm} = \beta_m \int_{t_{\inf}}^{t_{\det}} \varphi_m(\tau) \exp\left(r\left(\tau + t_{inf}\right)\right) d\tau \tag{5D.2.4}$$

4. The infection times of the infected herds of generation i+1 were distributed according to the normalised infectious curve of the source herd in generation i. For example, the probability density function for infection times of finishing herds in generation i+1, $t_{inf,i+1}$, infected by a finishing herd in generation i with infection time $t_{inf,i}$ and detection time $t_{det,i}$, is

$$pdf\left(t_{inf,i+1}\right)=\begin{cases}0 & \text{, for} \quad t_{inf,i+1} < t_{inf,i} \\ \beta_f \varphi_f\left(t_{inf,i+1}\right)\exp\left(r\left(t_{inf,i+1}-t_{inf,i}\right)\right)/\sigma_{ff} & \text{, for } t_{inf,i} \le t_{inf,i+1} < t_{det,i} \\ 0 & \text{, for} \quad t_{inf,i+1} \ge t_{det,i}\end{cases} \quad \text{(5D.3)}$$

The *pdf*s for infection times of other herd types were derived analogously.

Chapter 6

Performance of real-time parameter estimation of classical swine fever epidemics

D. Klinkenberg, R.W.J. Meester, M.C.M. de Jong

Abstract

We test the performance of a real-time prediction model for Classical Swine Fever epidemics, introduced by Meester et al. (2002). The model is a two-type branching process, with two parameters representing farm infection and farm detection. A maximum likelihood (ML) method uses the number of detected cases per week to estimate the parameters of the ongoing epidemic. Subsequently, the estimates are used to obtain a distribution of the (unknown) current number of infected farms. We test the model with simulated epidemics and conclude that the proposed ML method has serious problems, which need further study. If the detection parameter would be estimated otherwise, the model can produce a reasonable prediction of the number of infected herds, which improves if the dataset contains more weeks. We suggest to study simpler models to understand the qualitative behaviour of the ML estimator. For the time being, for practical application, the use of additional data is suggested to force the ML method into a restricted region in parameter space.

6.1. Introduction

Epidemics of contagious animal diseases, like foot-and-mouth disease and classical swine fever, can have a major impact on the economy and public acceptance of animal production, as was confirmed during the 1997-1998 Dutch classical swine fever epidemic (Meuwissen et al., 1999; Terpstra et al., 2000). Epidemic control, therefore, should aim at minimal losses, both financial and in terms of the number of slaughtered animals. There is, however, no such thing as one optimal control strategy, since every epidemic will be unique in factors like virus strain, location, and random effects. Methods to analyse ongoing epidemics are therefore necessary to optimise the disease control.

Meester et al. (2002) proposed a branching process model to analyse the transmission of classical swine fever virus (CSFV) between pig farms in real time. They described a maximum likelihood (ML) method to estimate the parameters of the model in ongoing epidemics. Furthermore, they showed how to use the estimates to obtain a distribution of the current number of unobserved infected farms, and how to use this distribution for a prediction of the nearby future. The methods were applied to the data of the large Dutch CSFV epidemic in 1997-1998, and they concluded that the epidemic could have been analysed reasonably well. Its use during real CSFV epidemics in the future was suggested.

To use the model in a real ongoing epidemic, it is necessary to know the reliability of the results for different datasets. Important questions are whether CSFV epidemics can be described by the branching process model sufficiently well, and if so, whether the proposed estimation and prediction methods produce results that contribute to a better epidemic control. In this paper we address the second question and test the performance of the model with a set of simulated epidemics.

6.2. The model

The model is a discrete-time, two-type branching process with a one week time unit. The two types of herds in the model are *infectious* herds of type *i* and *depopulated* herds of type *d*. Furthermore, it is assumed that each epidemic starts with one herd of type *i* and no herds of type *d*. The numbers of infectious and depopulated herds in week k are random quantities and denoted by X_k and Z_k, respectively. We define the number of newly infected herds in week k by Y_k. The triplet $(X_{k+1}, Y_{k+1}, Z_{k+1})$ is related to X_k by:

$$Z_{k+1} \cong \text{BinomialDistribution}(X_k, \mu)$$
$$Y_{k+1} \cong \text{PoissonDistribution}(\lambda\,(X_k - Z_{k+1}/2))$$
$$X_{k+1} = X_k + Y_{k+1} - Z_{k+1}$$

In words, each herd of type *i* in week k has a probability μ to be detected and depopulated in week k+1, so to become of type *d*. Moreover, each herd of type *i* in week k infects a random number of susceptible herds in week k+1. This number is Poisson distributed with either a mean of λ, if the herd is still of type *i* in week k+1, or a mean of $\lambda/2$, if the herd is of type *d* in week k+1. Each herd of type *d* in week k does not reappear in week k+1. In Meester et al. (Meester et al., 2002), the Poisson distributions of the number of new infections per infectious herd per week (with parameters λ and $\lambda/2$) were right-truncated to save computation time. In reprogramming the model in Mathematica® (Wolfram, 1999) we did not use the truncation.

Two important epidemic quantities can be derived from λ and μ. The first is the basic reproduction ratio between herds R_h, defined as the average number of herds that is infected by one typical herd until it is detected. R_h is equal to:

$$R_h = \lambda(2-\mu)/(2\mu),$$

as already shown by Meester et al. (2002). The second is the average epidemic growth rate ρ, defined by the relation $E(X_{k+1}|X_k = x_k) = \rho x_k$, which is equal to:

$$\rho = (1-\mu)(1+\lambda)+\mu\lambda/2.$$

Both R_h and ρ have threshold value 1, below which an epidemic will die out with probability 1. Because of the transparent interpretation of ρ and because it made the estimation procedure faster (as will be explained below), we reparametrised the model from (λ, μ) to (ρ, μ).

After detection and depopulation of the first infected herd, it is very likely that a control strategy will be implemented that will affect the parameters ρ and μ. Also in a later stage of the epidemic, the parameters might change when the control measures are changed. It is possible to incorporate these changes into the model by defining different ρ's and μ's for different stages of the epidemic.

During an epidemic, only realisations of Z_k are observed, which are called d_k for each week k, where the week in which the first depopulation takes place is defined as week 1. The event $\{Z_k = d_k, Z_{k+1} = d_{k+1},\ldots, Z_m = d_m\}$, in which m is the current week, is called D_k^+. The event $D_1^+ = \{Z_1 = d_1,\ldots, Z_m = d_m\}$, with $d_1 > 0$, is simply denoted by D.

6.3. The simulations

We simulated epidemics with the described branching process model, by assuming two stages in the epidemic with different (ρ, μ) sets. The first stage ran until the first herd was detected and depopulated, which was followed by the second stage.

In the first stage, $(\rho_1, \mu_1) = (2, 0.2)$ in all simulations, which is considered realistic, since the accompanying R_h of 6.0 and average time between infection and detection of 5 weeks are close to what Stegeman et al. (1999b) reported for the Dutch 1997-1998 epidemic, 6.8 and 6.2 respectively. In the second stage, the four combinations with ρ_2 = 1.1 or 1.3, and μ_2 = 0.15 or 0.5 were used. We did not simulate with ρ_2 values smaller than 1, as these values are not expected to be a problem in real life. The μ_2 values are considered extreme, though not unrealistic, and were chosen so different as to make a proper distinction by the ML method more likely.

We let the simulations run until the 10th time step (week) after the first detection. The reason not to produce longer datasets was the very time-consuming estimation procedure. We ran 100 simulations per (ρ_2, μ_2) parameter set and

distinguished three types of simulated epidemics. The first were epidemics which were over within the first 4 weeks of the second stage, i.e. no infected herds were left. These epidemics were considered irrelevant for this model, since they need not to be analysed in reality. The second were epidemics with less than 5 detected herds in the first 4 weeks of the second stage. These epidemics were left out, because the dataset was considered too small, and hence too unspecific for the parameter set used. The third were the remaining epidemics with 5 or more detected herds in the first 4 weeks of the second stage. We used only the first 20 of these epidemics for the parameter estimations, initially to save time, and later because no more estimations were needed for our conclusions. The division of the 100 simulations per (ρ_2, μ_2) set over the three types of simulated epidemics is given in Table 6.1.

Table 6.1. Distribution of the simulations over three types

		$\rho_2 = 1.1$	$\rho_2 = 1.3$
$\mu_2 = 0.15$	extinct[a]	25	31
	too small[b]	31	8
	rest	44	61
$\mu_2 = 0.5$	extinct[a]	32	16
	too small[b]	8	4
	rest	60	80

[a] Epidemics that were extinct before week 5

[b] Epidemics with less than 5 detections in weeks 2-5

6.4. Estimation of the model parameters

We tested the ML method for estimation of the model parameters, as proposed by Meester et al. (2002). The ML method selects the parameters that maximise the probability $P(D)$, on a parameter grid.

6.4.1 The estimation procedure

The probability to observe D is computed by

$$P(D) = \sum_i P\left(D \middle| X_0 = i\right) P\left(X_0 = i\right).$$

In Meester et al. (2002), it is shown how to compute $P(X_0 = i)$. As far as $P(D|X_0 = i)$ is concerned, after calculating $P(Z_m = d_m|X_{m-1} = i)$ for all i, one can recursively compute $P(D_k^+|X_{k-1} = i)$ for all i, until $P(D|X_0 = i)$ is obtained. In the last computation step from $P(D_2^+|X_1 = i)$ to $P(D|X_0 = i)$, a correction has to be made to take account of

Table 6.2. Summary [a] of the $\hat{\rho}_2$ by both the original and the adjusted ML method

Current week m	(ρ_2, μ_2)	$\hat{\rho}_2$ by original method	$\hat{\rho}_2$ by adjusted method
5	(1.1, 0.15)	1.1 [0.8 – 1.7]	1.0 [0.5 – 1.2]
5	(1.1, 0.5)	1.0 [0.9 – 1.7]	1.0 [0.9 – 1.4]
5	(1.3, 0.15)	1.2 [0.8 – 2.5]	1.2 [0.6 – 1.4]
5	(1.3, 0.5)	1.4 [0.8 – 1.9]	1.2 [0.6 – 1.5]
10	(1.1, 0.15)	1.1 [0.9 – 1.3]	1.1 [0.9 – 1.1]

[a] Median of 20 estimates [second lowest, second highest]

the definition of D, which states that $d_1 > 0$. This correction was not made in Meester et al. (2002), but hardly affects the estimation results in most cases. As it is impossible to do the recursions for all i, an upper boundary i_{max} has to be chosen beforehand, so that the number of infected herds can never exceed i_{max}.

Two problems arose with programming and running the estimation procedure. The first problem had to do with i_{max}. The calculation of $P(D)$ does not take into account the excess probability $P(X_k > i_{max} | Z_k = d_k \cap X_{k-1} = j)$, which can become important if i_{max} is too low in relation to D and the parameter values. If the excess probability is ignored, the value of the likelihood will be too low. On the other hand, if it is included into $P(X_k = i_{max})$, the likelihood value can become too high for some parameter values, as $P(X_k = i_{max})$ can become almost 1, which causes the underlying development of (large) X_k's to become more or less deterministic. Therefore, ignoring $P(X_k > i_{max})$ appeared to be the best option.

The second problem had to do with the search procedure on the parameter grid. Instead of the (λ, μ) grid used by Meester et al. (2002), we decided to use a $(\log\rho, \text{logit}\mu)$ grid, where $\text{logit}\mu = \log(\mu/(1-\mu))$. The new grid made location of the maximum easier, because the (λ, μ) grid appeared to contain a diagonal 'likelihood ridge', which disappeared in the $(\log\rho, \text{logit}\mu)$ grid. Furthermore, the log and logit transformations removed the boundaries of the grid and decreased the step sizes near small ρ and μ. The mesh width we used was 1/64 in the $\log\rho$ direction and 1/32 in the $\text{logit}\mu$ direction.

6.4.2 The estimation performance on simulations

The four sets of 20 simulated epidemics were used to estimate ρ_1, μ_1, ρ_2, and μ_2. We will treat the estimates as functions of i_{max} and refer to them as $\hat{\rho}_1(i_{max})$, $\hat{\mu}_1(i_{max})$,

$\hat{\rho}_2(i_{max})$, and $\hat{\mu}_2(i_{max})$. We define $\hat{\rho}_1 = \lim_{i_{max}\to\infty} \hat{\rho}_1(i_{max})$; $\hat{\mu}_1$, $\hat{\rho}_2$, and $\hat{\mu}_2$ are defined likewise.

Table 6.3. Relation between $\hat{\rho}_2$, $\hat{\lambda}_2$, and $\hat{\mu}_2$, as obtained with the original ML method

	$\hat{\rho}_2 < 1$	$\hat{\rho}_2 \geq 1$
$\hat{\lambda}_2 =$	0	$\hat{\rho}_2 - 1$
$\hat{\mu}_2 =$	$1 - \hat{\rho}_2$	0

We determined $\hat{\rho}_1(i_{max})$, $\hat{\mu}_1(i_{max})$, $\hat{\rho}_2(i_{max})$, and $\hat{\mu}_2(i_{max})$ with $D = \{d_1,\ldots,d_5\}$ of all 80 simulations, and with $D = \{d_1,\ldots,d_{10}\}$ of the 20 simulations with $\rho_2 = 1.1$ and $\mu_2 = 0.15$. By using at least two values of i_{max}, 50 and 100, the dependence of the estimates on i_{max} was checked.

It very soon appeared that $\hat{\rho}_1(i_{max})$ and $\hat{\mu}_1(i_{max})$ did not have any relation to ρ_1 and μ_1 and often changed if i_{max} was varied, as was the case with $\hat{\mu}_2(i_{max})$. Only ρ_2 could be estimated quite well (Table 6.2). By increasing the number of weeks in the datasets from 5 to 10, $\hat{\rho}_2$ was generally closer to the real ρ_2, as shown in Table 2. It was possible to approximate $\hat{\rho}_2$ by means of a Generalised Linear Model (GLM), e.g. in Genstat (1998). The GLM fits D_2^+ to the following model:

$$E(d_k) = C\rho_2^k.$$

By using a log link function and assuming d_k to be Poisson-distributed, an estimate of ρ_2 is obtained.

Close inspection of the estimates from the simulations revealed that all estimates could be derived by simple rules in the following order, which are applied to the dataset $D = \{3,5,2,6,5\}$ as an illustration:

1. $\hat{\rho}_2$ can be approximated with a GLM. With the example dataset, the GLM estimate is $\hat{\rho}_2 = 1.09$, whereas the ML gives $\hat{\rho}_2 = 1.02$ if $i_{max} = 50$, and $\hat{\rho}_2 = 1.03$ if $i_{max} = 100$.
2. $\hat{\mu}_2$ (the limit of $\hat{\mu}_2(i_{max})$) = 0 if $\hat{r}_2 \geq 1$, whereas $\hat{\mu}_2 = 1 - \hat{\rho}_2$ if $\hat{\rho}_2 < 1$, which corresponds to $\hat{\lambda}_2 = 0$ (Table 6.3). Because the combination of $\hat{\mu}_2 = 0$ and the dataset D reflect very large X_k's, $\hat{\mu}_2(i_{max})$ decreases with increasing i_{max}. With the example dataset, $\hat{\rho}_2 \geq 0$, so $\hat{\mu}_2 = 0$. With the i_{max} values of 50 and 100, the estimates were $\hat{\mu}_2(50) = 0.14$ and $\hat{\mu}_2(100) = 0.07$.
3. The link between the first and second epidemic stage is formed by X_1, which is the result of the first stage and the starting point of the second stage. The

estimates $\hat{\rho}_2(i_{max})$ and $\hat{\mu}_2(i_{max})$, together with the dataset D, can be used to calculate $E(X_1|D \cap \hat{\rho}_2(i_{max}) \cap \hat{\mu}_2(i_{max}))$ as follows [for notational convenience, we define $\xi_k = E(X_k|D \cap \hat{\rho}_2(i_{max}) \cap \hat{\mu}_2(i_{max}))$]: first, the recurrence relation $E(X_k|X_{k-1} = x_{k-1}) = (x_{k-1}-d_k)(1+\hat{\lambda}_2)+d_k\hat{\lambda}_2/2$ is used to express $\xi_2, \xi_3, \ldots, \xi_{m-1}$ in terms of ξ_1; second, the equation $\hat{\mu}_2 = (d_2+d_3+\ldots+d_m)/(\xi_1+\xi_2+\ldots+\xi_{m-1})$ is used to express ξ_1 in terms of D, $\hat{\rho}_2(i_{max})$, and $\hat{\mu}_2(i_{max})$. With the example dataset, $\xi_1 = 31.8$ if $i_{max} = 50$ and $\xi_1 = 61.4$ if $i_{max} = 100$.

4. $\hat{\rho}_1$ and $\hat{\mu}_1$ together are estimated such that the average outcome of the first epidemic stage will be ξ_1, as computed in the previous step. The exact values of $\hat{\rho}_1$ and $\hat{\mu}_1$ are always such that they reflect the shortest history possible. Therefore, $\hat{\mu}_1 = 1$ and $\hat{\rho}_1 = \xi_1$ if $d_1 = 1$, which means that the epidemic must have started in week 0. If $d_1>1$, then $\hat{\mu}_1(i_{max})$ takes a value reflecting that $X_{-1} = 1$, so that the epidemic must have started in week –1. With the example dataset, the resulting estimates were $\hat{\rho}_1(50) = 5.0$ and $\hat{\mu}_1(50) = 0.38$, which lead to $E(X_1) = 31.8$; and $\hat{\rho}_1(100) = 7.2$ and $\hat{\mu}_1(100) = 0.30$, leading to $E(X_1) = 61.4$.

The predictability of the estimates indicates that the method can only extract ρ_2 from the data. Two problems appear to occur. First, it is impossible to obtain a good estimate of μ_2, which suggests that the data, if used with the ML method, do not contain information on the magnitude of the X_k values, but only on their relative change by $\hat{\rho}_2$. Second, it is impossible to estimate both $\hat{\rho}_1$ and $\hat{\mu}_1$, which is probably due to the fact that only one observation d_1 is available for the first epidemic stage.

In order to check whether the problems of the first epidemic stage also cause the problems of estimating μ_2, we analysed the simulations with an adjusted ML method. In the adjusted method, estimation of ρ_1 and μ_1 was replaced by estimation of x_1, the realised number of infected herds at the start of the second stage. Instead of $P(D)$, $P(D_2^+|X_1 = x_1)$ had to be maximised.

As with the original ML method, $\hat{\rho}_2$ was always in reasonable agreement with ρ_2 (Table 6.2), whereas the other parameters were not. Now, the ML estimates could not be derived by one single procedure, but three different types of maxima were observed:

– Maxima in which $\hat{\mu}_2 = 0$ and $\hat{x}_1 \to \infty$. This is similar to what happened with the original ML method and is only observed with $\hat{\rho}_2 \geq 1$.

– Maxima in which $\hat{\mu}_2 = 1$ (or almost 1) and $\hat{x}_1 = d_2$. Sometimes, $\hat{\mu}_2$ was not exactly 1, namely, if for some k, $d_k = 0$ and $d_{k+1} > 0$. This type of maximum is observed with both $\hat{\rho}_2 < 1$ and $\hat{\rho}_2 \geq 1$.
– Maxima in which $0 < \hat{\mu}_2 < 1$ and $\hat{x}_1 \approx \sum d_k$. Although at first $\hat{\mu}_2$ seems to have a more realistic value, it is completely determined by the interplay between $\hat{x}_1$ and $\hat{\rho}_2$. Also this type of maximum is observed with $\hat{\rho}_2 < 1$ and $\hat{\rho}_2 \geq 1$.

The difference between the adjusted and the original ML method is that the estimates with the adjusted ML method are not completely predictable. However, for all three types of maxima, the observation that the data, analysed with the ML method, do not contain information on the magnitude of the X_k values still holds: the first type implies that all X_k's are infinitely large, the second type implies that all X_k's are as small as possible, and the third type gives an estimate of x_1 near $\sum d_k$. In conclusion, only ρ can be accurately estimated by maximum likelihood if a sequence of detections is known, but that can also be done by a much faster GLM. The failure to estimate both ρ and μ from simulated epidemics leads to the conclusion that the ML method that only uses D is not suitable for the branching process model.

6.5. The distribution of X_m

Before seeking methods to improve the estimations of ρ and μ, we considered it useful to test the prediction capacities of the model. Predictions by the model consist of distributions of the X_k conditioned on D, of which the distribution of X_m is the most crucial, because it reflects the current situation and is the base for prediction of the future. Therefore, we tested the accuracy of the distributions of X_m with the 80 simulated epidemics, with 5-weeks and 10-weeks datasets.

The distribution of X_m is determined as described in Meester et al. (2002). In short, $P(D)$ is first calculated as described in section 4. Then, $P(D)$, all $P(D|X_0 = i)$, and all $P(X_0 = i)$ are used to obtain the distribution of X_0 conditioned on D, where the fact that $d_1 > 0$ has to be taken into account as was the case with the computation of $P(D)$. Subsequently, the distributions of X_1, X_2, etc. are computed recursively, until finally, the distribution of X_m conditioned on D is obtained.

To determine X_m distributions with the model, values for the parameters ρ_1, μ_1, ρ_2, and μ_2 are needed, which were originally supposed to be estimated by the ML method. Because the ML method does not work properly, we adjusted it by

assuming that μ_1 and μ_2 were known, so only the accompanying ρ_1 and ρ_2 were estimated with μ_1 and μ_2 at their simulation value. We assumed knowledge of μ_1 and μ_2, because they might be estimated through alternative methods (as will be explained in the Discussion). We let X_{max} be 200 for estimation of ρ_1 and ρ_2 and 350 for determination of the X_m distribution, and did not use the result if those values appeared too low. Because X_{max} was too low for 22 of the 40 10-weeks simulations with $\rho_2 = 1.3$, only the 10-weeks datasets with $\rho_2 = 1.1$ were used.

We used Pearson's p_λ statistic (Rao, 1973), p.168) to test whether the observed simulated values of X_m, denoted by x_m, can be regarded as random draws from the distributions of X_m conditioned on D. Pearson's p_λ is equal to:

$$p_\lambda = \sum_{j=1}^{n} -2\log\left[P\left(X_{m,j} \le x_{m,j}\right)\right] = \sum_{j=1}^{n} -2\log\left[\sum_{k=0}^{x_{m,j}} P\left(X_{m,j} = k \middle| D_j\right)\right],$$

in which j refers to the jth simulation, n is the number of simulations, and the $P(X_{m,j} = k|D_j)$ make up the distribution of $X_{m,j}$, conditioned on D_j. If the distributions are correct, the $P(X_{m,j} \le x_{m,j})$ are uniformly distributed on [0,1], and p_λ is χ^2 distributed with $2n$ degrees of freedom. We computed p_λ separately for all X_m distributions of the 5-weeks datasets, and for the 40 X_m distributions of the 5-weeks and 10-weeks datasets from the simulations with $r_2 = 1.1$. Subsequently, the P-values corresponding to the p_λ's were determined, and interpreted as a quality measure for the X_m distributions.

The P-value for all 5-weeks datasets was 0.003, which indicates that many of the X_m distributions largely deviated from the real x_m. By observing the individual X_m distributions, it appeared that as much as 26 of 77 $P(X_{m,j} \le x_{m,j})$ were smaller than 0.025 or larger than 0.975 (for 3 distributions, i_{max} was too low), which means that the 95% CI of the accompanying X_m distributions did not include the simulated x_m. If the datasets were made 5 weeks longer, the X_m distributions tended to improve if only the datasets of the 40 simulations with $\rho_2 = 1.1$ are considered: the P-value increased from 0.04 to 0.15 and the number of X_m distributions of which the 95% CI did not include the simulated x_m decreased from 12 to 5.

Closer inspection of all X_m distributions and x_m realisations reveals that the 'bad' X_m distributions are mostly due to bad ρ_2 estimates, i.e. estimates that differ from the real ρ_2 by more than 0.15. This explains why longer datasets give better results: the ρ_2 estimates are generally better. Whereas 33 of the 40 ρ_2 estimates based on 10 weeks of data are within the range (0.95-1.25), only 15 of the 40 ρ_2 estimates are, if 5 weeks of data of the same simulations are used.

6.6. Discussion

Summarising, the maximum likelihood procedure is well able to provide a useful estimate of ρ, if a sequence of detections is known. From the simulations, the median estimates were always close to the simulation parameters and a longer dataset improved $\hat{\rho}_2$. It should be noted, however, that $\hat{\rho}$ can be very well approximated by a GLM, by fitting the sequence of detections to an exponential curve. Therefore, if $\hat{\rho}$ would be the only interesting result, the time-consuming estimation procedure of the model is not needed.

To be able to predict the current number of infected farms, however, estimates of μ and of parameters of previous stages are also necessary. Unfortunately, with none of the simulations it appeared possible to obtain a reasonable $\hat{\mu}_2$. Some simple rules, irrelevant to the branching process model, determined the outcome of $\hat{\mu}_2$, and of $\hat{\rho}_1$, $\hat{\mu}_1$, and $\hat{x}_1$. The conclusion is that the maximum likelihood method is not suitable to obtain information about μ_2. The maximum always appears to lie in an area of the multidimensional parameter space which has nothing to do with the meaning of μ_2: either $\hat{\lambda}_2$ is close to 0, $\hat{\mu}_2$ is close to 0 or 1, or $\hat{x}_1$ is near $\sum d_k$.

These results lead to questions concerning the theoretical background of the observations, and concerning the analysis of CSFV epidemics if the virus would enter the pig population in the near future.

An important theoretical question that needs to be answered is whether it is the ML *method* that cannot extract information on μ_2 (or the magnitude of the X_k's) from a series of detections, or whether it is the data *itself* that do not contain any information on μ_2. A related question concerns the mechanism behind the predictable estimation results of our model; if we understand the mechanism we may have an answer to the first question.

In order to answer the theoretical questions, it might be useful to formulate and study a simpler model, which contains the key features of our epidemic model, i.e. a discrete-time branching process with two parameters, of which only the individuals with zero offspring are observed. An example is a model with the following offspring distribution:

$$P(X_{k+1} = 0|X_k = 1) = \mu + (1-\mu)e^{-\lambda}$$
$$P(X_{k+1} = k|X_k = 1) = (1-\mu)e^{-\lambda}\lambda^k/k!$$

which means that there is a probability $1-\mu$ that an individual does have offspring, which is Poisson-distributed with parameter λ. The simple models should be used to

study the relation between the input parameters and the output distributions, and to get insight into the behaviour of ML methods for estimation of the model parameters. If it would appear that it is the ML *method* and not the *data* that make estimation of μ impossible, an estimation method that does provide estimates for both ρ and μ should be developed.

Apart from the above theoretical questions, there is also the practical problem of analysis of CSFV epidemics in the near future. A reasonable and simple practical adjustment of the method seems to lie in putting *constraints* on the parameters, by use of other data. For example, a distribution of X_1 could be used as a constraint before use of the ML method. Such a distribution might be available from tracing data during an epidemic. Alternatively, the detection rate μ could be estimated with data on the average time between detection and infection, which is equal to $1/\mu$ according to the model. Of the 1997-1998 epidemic, for 82 herds the times of infection could be determined, which could be used to estimate the average infection-detection time at 32 days (Stegeman et al., 1999a), which would mean that $\hat{\mu} = 0.22$ in our model. During each epidemic, new data on μ can be obtained, which could make $\hat{\mu}$ more specific for that epidemic. When $\hat{\mu}$ is obtained, the ML method can be used to estimate ρ. Because the model can give a reasonable distribution of X_m, and because $\hat{\rho}$ is estimated reasonably well, providing the model with an estimate of μ looks promising.

Concluding, the ML method as described by Meester et al. (2002) cannot be used directly to estimate both ρ and μ from a series of detections. The mechanism is yet unclear and should be studied by use of simpler branching process models. For practical purposes, the estimation method might be adjusted by use of additional data, as the branching process model provides an accurate distribution of the current number of infected farms if the detection parameter would be known beforehand.

Acknowledgements

The authors gratefully acknowledge financial support from STW (Technology Foundation), Utrecht, The Netherlands

Chapter 7

Towards better control of classical swine fever epidemics: vaccination with an E2 marker vaccine

D. Klinkenberg, M.C.M. de Jong

Submitted to *Vaccine*

Abstract

This paper discusses the effectiveness of emergency vaccination with two E2 subunit vaccines during epidemics of classical swine fever virus (CSFV). Results of animal experiments indicate a sufficient reduction of horizontal transmission (reproduction ratio $R_i < 1$), but not of vertical transmission. This can lead to problems with clinical detection of herds with persistently infected piglets, which might be overcome by a control strategy in which sows are not vaccinated. This strategy effectively limits the epidemic size and duration, as observed in a mathematical model of between-herd CSFV transmission, reflecting the circumstances of the Dutch 1997-1998 epidemic without spatial structure. Furthermore, potential endscreening problems with the discriminatory E^{rns} ELISA's can be solved by declaring a herd infected only if the number of seropositive samples is above some 'positivity threshold', provided that no other pestiviruses are prevalent. In conclusion, E2 marker vaccines can be effectively employed in CSFV epidemics.

7.1. Introduction

In 1980, a common classical swine fever (CSF) policy came into force in the European Union (EU), which urged all member states to first eradicate CSF virus (CSFV) and then stop vaccination (Anonymous, 1980; Vandeputte and Chappuis, 1999). Since then, CSFV epidemics have been controlled by EU-prescribed control measures, which involve hygiene measures, contact tracing and inspection, and a transport prohibition in the affected area (Anonymous, 1980; Elbers et al., 1999; Koenen et al., 1996; Vandeputte and Chappuis, 1999; Williams and Matthews, 1988). However, these control measures appeared insufficient in pig farm dense areas like the area of the Dutch 1997-1998 epidemic. Only when preventive culling of traced contacts and of herds within a radius of 1 kilometre was applied as an extra control measure (Elbers et al., 1999; Stegeman et al., 1999b), the reproduction ratio between herds R_h – defined as the average number of herds infected by one typical infectious herd – decreased below the threshold value of 1, below which epidemics certainly die out (Stegeman et al., 1999b).

Preventive culling resulted in the killing and destruction of 1.1 million pigs in possibly infected herds, although a safe and highly effective C-strain vaccine was available, which was known to accomplish CSFV eradication when applied in a mass-vaccination campaign (Terpstra and Wensvoort, 1987; Terpstra et al., 1990;

Van Oirschot, 1994; Vandeputte and Chappuis, 1999). However, emergency vaccination was not applied, since the C-strain vaccine evokes an immune response just like the wild-type CSFV, which interferes with the serological endscreening (Blaha, 1995; Moennig, 2000). An endscreening has to be carried out 30 days after the last detected case to establish if the pig population in the previously affected area is CSFV free (Anonymous, 1980; Anonymous, 2001).

In the early 1990's, it was realised that the difficulty with diagnosis in vaccinated animals might be solved by so-called 'marker vaccines' (Blaha, 1995; Van Oirschot, 1994). Marker vaccines are vaccines that are accompanied by a discriminatory serological test to diagnose infection with the wild-type virus in a vaccinated population. At present, two commercial CSF marker vaccines are available, each with its own discriminatory test: Bayer's Bayovac® CSF marker is accompanied by the Ceditest E^{rns} ELISA and Intervet's Porcilis® Pesti by the Chekit CSF marker E^{rns} ELISA. Both vaccines contain the E2 protein of the virus and both ELISA's test for antibodies against the E^{rns} protein of the wild-type virus (Hulst et al., 1993; Moormann et al., 2000).

This paper discusses the possibility of using the E2 vaccines in an emergency vaccination campaign. First, an overview of relevant studies regarding the effectiveness of the vaccines against virus transmission and the performance of the E^{rns} ELISA's is given. Then, an effective control strategy with emergency vaccination is proposed and suggestions are done for an effective endscreening.

7.2. Effectiveness of the E2 vaccines against virus transmission

Studies into the effectiveness of CSF vaccines have to aim at two modes of virus transmission. Besides the usual horizontal virus transmission through direct or indirect contacts between animals, CSFV can also be transmitted from sows to unborn foetuses by vertical transmission (Van Oirschot, 1992). Vertical transmission often leads to mummification, stillbirth, or birth of diseased piglets, but so-called 'carrier sows' sometimes give birth to persistently infected piglets (Van Oirschot, 1992; Van Oirschot and Terpstra, 1977). Persistently infected piglets excrete large amounts of virus, do not develop an immune response, and are initially clinically healthy until mild symptoms can arise after a few months. Therefore, they are infectious during their entire life and will not be easily detected (Van Oirschot, 1992).

Many horizontal transmission studies have been conducted, from which can be concluded that the E2 vaccines have their greatest effect on the infectiousness of infected pigs, and not so much on the susceptibility of uninfected pigs (Bouma et al.,

1999; De Smit et al., 2001a; Dewulf et al., 2001; Dewulf et al., 2000; Chapter 3). The reproduction ratio between individual animals R_i, which is – analogous to R_h – the average number of animals infected by one typical infectious animal in a virus free population, can be brought significantly below 1, and therefore the vaccines can prevent major outbreaks in herds (Bouma et al., 2000; Chapters 3 and 4). In conventional animals, complete protection against transmission is reached in three weeks and lasts for at least six months (Chapters 3 and 4; Uttenthal et al., 2001). Therefore, both vaccines are capable of limiting an outbreak on the herd level to a few infected pigs, even if the herd would be infected only a week after vaccination (Chapter 3). Maternal antibodies in piglets reduce the serological response upon vaccination, because the maternal antibodies interfere with the vaccines, but R_i is still estimated smaller than 1 (Chapter 4).

Vertical transmission studies show that both vaccines are effective at the sow level, which means that either a sow does not transmit virus, or a sow does transmit virus and infects most of its piglets. Neither vaccine does completely prevent vertical transmission (Ahrens et al., 2000; De Smit et al., 2000a; Depner et al., 2001; Dewulf et al., 2001). Double vaccination gives a higher probability of uninfected litters than single vaccination and Bayovac® appears to induce a greater reduction of vertical virus transmission. After a single vaccination, Porcilis® Pesti did not induce any reduction, while a double vaccination variably affected virus transmission: in three experiments together, 16 out of 25 litters were virus free (Ahrens et al., 2000; Depner et al., 2001; Dewulf et al., 2001). Single vaccination with Bayovac® prevented CSFV transmission in 10 out of 17 sows in two experiments, whereas double vaccination resulted in 14 virus negative litters out of 15 (De Smit et al., 2000a; Depner et al., 2001).

In conclusion, both vaccines can prevent CSFV outbreaks on herds without breeding sows, as R_i due to horizontal transmission is reduced to below 1. However, vertical transmission cannot be blocked completely, which may lead to the birth of persistently infected piglets, which can act as continuous virus sources.

7.3. Characteristics of the E^{rns} ELISA's

The discriminatory E^{rns} ELISA's are designed for serological testing of vaccinated animals. The sensitivities of the Ceditest ELISA (provided with Bayovac®) and of the Chekit ELISA (with Porcilis® Pesti) were determined from reference sera to be 73.5% and 94.1%, respectively (Floegel Niesmann, 2001). The specificities of the tests depend on the choice of reference sera. If reference sera with antibodies against other pestiviruses (bovine viral diarrhoea virus and border disease virus) are used,

the specificities of the Ceditest and Chekit ELISA's were estimated to be 91.8% and 70.6%, respectively (Floegel Niesmann, 2001). However, with random CSF-negative field sera from the Dutch 1999 pig population, the specificity of the Ceditest is 98.7% (Moormann et al., 2000). This is in accordance with the specificities of both tests, measured from recent European CSF-negative field sera and sera from pigs vaccinated with the marker vaccine: 100% and 98% with Ceditest and Chekit, respectively (Floegel Niesmann, 2001).

Serological tests are carried out during CSFV epidemics and in endscreenings after CSFV epidemics "in order to detect the possible presence of classical swine fever virus" (Anonymous, 2001). Notwithstanding the importance of the test characteristics, the sensitivity and specificity of the complete testing procedure at the herd level also depend on the expected seroprevalence, the sample size, and the use of confirmation tests. In animals vaccinated with the E2 vaccine, however, serological confirmation tests are not (yet) available.

During CSF epidemics, it is important to detect as many CSFV-infected herds as possible, so herd sensitivity has to be very high. Generally, herd sensitivity can be improved by increasing the sample size, which will decrease herd specificity if no confirmation tests are applied. However, during epidemics a very high herd specificity is not crucial, because occasional false positive herds do hardly affect the further epidemic course.

After CSFV epidemics, however, when endscreenings are conducted, a low herd specificity can become a real burden, as false-positive results can continuously delay declaring an area CSFV free. Moreover, a low herd specificity can become costly if herds have to be sampled again and confirmation tests need to be conducted. Therefore, the major problem of the E^{rns} ELISA's seems to be the specificity, or rather the absence of serological confirmation tests, and not the low sensitivity, which can be adjusted for by increasing the sample size.

7.4. The E2 vaccine in an emergency vaccination campaign

As described above, the E2 vaccines are very effective in reducing horizontal transmission, and the E^{rns}-ELISA's can be applied during epidemics. However, emergency vaccination may lead to some problems involving vaccinated sows, and it may lead to problems with herd specificity during an endscreening.

7.4.1. Problems with vaccinated sows

Regarding the vaccination of sows, there are some uncertainties. First, the level of virus transmission between newborn maternally immune piglets is unknown. Second, the effectiveness of vaccination of maternally immune piglets may be reduced. Third, vertical transmission can still occur and lead to the birth of persistently infected piglets. Whereas herds with persistently infected piglets are usually detected through the contact infections they cause, detection is prevented if the herd is completely vaccinated. All these problems of an emergency vaccination campaign might be avoided by omitting vaccination of breeding sows.

Omitting the vaccination of breeding sows is only legitimate if the resulting control strategy is effective, i.e. if R_h can still be brought below 1. The effectiveness of this control strategy, as of many more strategies, was studied with a mathematical model by Klinkenberg et al. (Chapter 5).

The model was a very basic description of CSFV transmission within and between two types of pig herds, herds with and herds without breeding sows. It was fitted to data of the Dutch 1997-1998 epidemic, which means that the model described CSFV epidemics in a pig dense area with a moderately virulent CSFV strain. The model did not take account of local differences with respect to herd density and the proportions of both herd types. All virus transmission routes except animal transport were subject to the EU-prescribed control measures and were joined into one transmission parameter. Furthermore, assumptions were made regarding the effectiveness of vaccination. Because the vaccines mainly reduce the infectiousness of infected pigs, it was assumed that major outbreaks within herds do not occur if the first infected pig is vaccinated. Therefore, vaccination affects the probability that virus entry into a herd leads to a major outbreak, e.g. if 60% of the pigs in a herd is vaccinated, then the probability of a major outbreak is reduced by 60%. Vaccination was assumed not to affect detection of herds nor virus transmission within herds with major outbreaks (Chapter 5).

Two conditions that are necessary to ensure extinction of a CSFV epidemic could be derived from the model: (i) no virus transmission through transport and (ii) reduction of the number of major outbreaks by at least 50%. The proposed control scenario, with vaccination of all piglets and finishing pigs but not the breeding sows, amply meets these conditions. If only piglets and finishing pigs in herds with breeding sows are vaccinated, R_h is still above 1, whereas vaccination of only the herds without breeding sows does reduce R_h sufficiently, but can lead to very long-lasting epidemics with many infected herds (Table 7.1) (Chapter 5).

Table 7.1. Expected effectiveness of vaccination scenarios [a]

Vaccination of all herds without breeding sows	Vaccination of all piglets and finishing pigs in herds with breeding sows	Duration (days)	95% CI [b]	Size (infected herds)	95% CI [b]
–	–		ND [c]		ND [c]
+	–	226	63 - 1070	42	13 - 452
–	+		ND [c]		ND [c]
+	+	103	47 - 239	16	10 - 35

[a] The model (Chapter 5) simulated CSFV epidemics under the circumstances of the Dutch 1997-1998 CSFV epidemic without spatial structure. It contained two types of herds, viz. herds with and herds without breeding sows. The results have been obtained by simulating epidemics with a starting condition of 5 undetected infected herds of both types (10 infected herds in total). The shaded scenario is the scenario, as proposed in this paper

[b] Confidence interval.

[c] Not determined, because $R_h > 1$.

7.4.2. Problems with herd specificity

The specificity of the E^{rns} ELISA's, or rather the absence of confirmation tests, was recognised as a potential problem during endscreenings. It is, however, possible to achieve a high herd specificity with only the E^{rns} ELISA, by declaring a herd to be infected only if the number of positively tested samples is above some 'positivity threshold'. Table 2 gives some positivity thresholds to achieve a herd specificity of 99.9% with the Ceditest ELISA (test specificity of 99.4%, mean of two publications (Floegel Niesmann, 2001; Moormann et al., 2000)) and with the Chekit ELISA (test specificity of 98% (Floegel Niesmann, 2001)).

Of course, adjusting the positivity threshold to increase the herd specificity leads to a decrease in herd sensitivity. However, because the herd sensitivity strongly depends on the expected seroprevalence, it is positively affected by the 30 day delay between the last detection and the endscreening. Namely, if CSFV infected herds are still present during an endscreening, the seroprevalence on those herds will be reasonably high, which will increase the herd sensitivity. As an example for both ELISA's, Table 2 shows the herd sensitivity in a herd of 1000 animals with different sample sizes, if the herd specificity is set at 99.9% by adjusting the positivity threshold and the expected seroprevalence is 5%. Table 7.2 indicates that both E^{rns} ELISA's can be used to design an endscreening procedure with high herd sensitivity and high herd specificity.

Table 7.2. Positivity threshold with 99.9% herd specificity, and resulting herd sensitivity in a herd of 1000 pigs [a]

	Ceditest ELISA [b]		Chekit ELISA [c]	
Sample size	Positivity threshold	Herd sensitivity	Positivity threshold	Herd sensitivity
100	4	42.1%	7	33.8%
200	6	76.6%	11	69.0%
500	10	99.9%	21	99.6%
1000	15	>99.9%	35	>99.9%

[a] Expected seroprevalence of 5%, calculation method as in Cameron and Baldock (1998)
[b] Test sensitivity at animal level is 73.5%, test specificity is 99.4%
[c] Test sensitivity at animal level is 94.1%, test specificity is 98.0%

7.5. Discussion

Previous publications on the E2 marker vaccines often concluded that the vaccines reduced virus transmission rather late after vaccination. For instance, Dewulf et al. (2000) state that "a 2 weeks interval between vaccination and protection will be an important hindrance for the use of the vaccine in emergency vaccination programmes" since about half of the outbreaks on herds would occur within the first two weeks after the nearest neighbouring outbreak. This reasoning overlooks the fact that vaccination contributes to a reduction of R_h not by preventing that herds become infected upon CSFV uptake, but by preventing that vaccinated herds do develop major outbreaks and spread the virus to other herds. In short, vaccination affects herd infectiousness more than herd susceptibility, and that is already achieved if virus enters a herd one week after vaccination (Chapter 3).

A second often-expressed objection against the E2 vaccines is their effect on vertical transmission, which cannot be prevented even after double vaccination (Depner et al., 2001; Dewulf et al., 2001). Vertical transmission can lead to the birth of persistently infected piglets, which will result in an infectious herd that cannot be clinically detected. This problem is avoided in a CSFV control strategy in which the EU-prescribed control measures are accompanied by vaccination of all piglets and finishing pigs, but not the sows. Under the circumstances of the Dutch 1997/1998 epidemic, it appears that the strategy results in an R_h smaller than 1, with limited outbreak size and duration (16 herds in 3 months, if 10 infected herds are yet undetected at time of vaccination).

The final and major objection against the E2 vaccines of many authors was not the vaccines themselves, but the limited performance of the accompanying E^{rns}

ELISA's. To cite Floegel-Niesmann (Floegel Niesmann, 2001), "the limitation of the two discriminatory ELISA's is the major factor that would prevent the use of these two marker vaccines under emergency field conditions." In contrast, in this paper it was argued that the ELISA's can be effectively applied during CSF epidemics by increasing the sample size. In an endscreening procedure, high herd specificity is important to avoid unnecessary long epidemics. By increasing the sample size and adjusting the positivity threshold, both herd sensitivity and herd specificity can become very high, provided that no other pestivirus infections are present in the population. Then, a serological confirmation test is needed.

Aside from adjusting the sample size and the positivity threshold, the endscreening procedure might also be optimised by adjusting the test characteristics at the laboratory level, i.e. by increasing the test specificity, which decreases the test sensitivity. It is also possible to perform an endscreening in two steps: a first quick screening with small sample sizes and low herd specificity, and a second screening with larger samples in the positive herds of the first screening.

The most important remaining question about the effectiveness of the E2 vaccines is whether maternal immunity reduces virus transmission between newborn piglets. Although the proposed control strategy without vaccination of sows does not need an answer to this question, extra research is needed to fill this gap if vaccination of sows would be considered.

The decision to use emergency vaccination will hinge on economic and ethical considerations, which have to be weighed in a political process influenced by scientific knowledge and public opinion. Studies into the economic effects of control scenarios concluded that emergency vaccination can be useful as a buffer in case of restricted destruction capacity, and that it works out very positive if vaccinated animals can be traded after the epidemic (Mangen et al., 2001; Saatkamp et al., 2000).

In conclusion, the positive results of experiments and models have made that, from epidemiological perspective, emergency vaccination with E2 marker vaccines is a good alternative to preventive culling.

Acknowledgements

We gratefully acknowledge financial support from STW (Technology Foundation), Utrecht, The Netherlands.

References

Aitkin, M., Anderson, D., Francis, B., Hinde, J., 1989. Survival data, Statistical modelling in GLIM, Oxford.

Anderson, R.M., May, R.M., 1979. Population biology of infectious diseases: Part I. Nature 280, 361-367.

Anderson, R.M., May, R.M. (Eds.), 1991. Infectious diseases of humans. Oxford University Press, New York.

Anonymous, 1980. EU Council Directive 80/217/EEC on Community measures for the control of Classical Swine Fever.

Anonymous, 2001. EU Council Directive 2001/89/EC on Community measures for the control of Classical Swine Fever.

Bailey, N.T.J., 1975. General Epidemics. In: Bailey, N.T.J. (Ed.), The mathematical theory of infectious diseases and its applications. Hafner Press, New York, pp. 81-133.

Beaumont, G.P. (Ed.), 1980. Intermediate mathematical statistics. Chapman and Hall, London.

Becker, N.G. (Ed.), 1989. Analysis of infectious disease data. Chapman and Hall, London.

Beynon, A.G., 1969. The eradication of swine fever from Great Britain. Vet Rec 84, 623-624.

Birkes, D., 1998. Likelihood ratio tests. In: Armitage, P., Colton, T. (Eds.), Encyclopedia of biostatistics. John Wiley & Sons Ltd, Chichester, West Sussex, pp. 2245-2248.

Bironf, P., Leunen, J., Vandeputte, J., 1987. Inhibition of virus replication in the tonsils of pigs previously vaccinated with a Chinese strain vaccine and challenged oronasally with a virulent strain of classical swine fever virus. Vet Microbiol 14, 105-113.

Bouma, A., De Jong, M.C., Kimman, T.G., 1997. The influence of maternal immunity on the transmission of pseudorabies virus and on the effectiveness of vaccination. Vaccine 15, 287-294.

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

Bouma, A., De Smit, A.J., De Jong, M.C.M., De Kluijver, E.P., Moormann, R.J.M., 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.

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

Campbell, A.D., 1969. Swine fever. Proc R Soc Med 62, 824-826.

Chenut, G., Saintilan, A.F., Burger, C., Rosenthal, F., Cruciere, C., Picard, M., Bruyere, V., Albina, E., 1999. Oral immunisation of swine with a classical swine fever vaccine (Chinese strain) and transmission studies in rabbits and sheep. Vet Microbiol 64, 265-276.

Colijn, E.O., Bloemraad, M., Wensvoort, G., 1997. An improved ELISA for the detection of serum antibodies directed against classical swine fever virus. Vet Microbiol 59, 15-25.

Cox, D.R., Miller, H.D. (Eds.), 1965. The theory of stochastic processes. Chapman and Hall Ltd., London.

Crauwels, A.P.P., De Koning, R., Nielen, M., Elbers, A.R.W., Dijkhuizen, A.A., Tielen, M.J.M., 2001. A concept for a decision support system based on practical experiences from a national disease emergency - the Dutch experience. Acta Vet Scand Suppl. 94, 61-69.

Crauwels, A.P.P., De Koning, R., Nielen, M., Tielen, M.J., 2000. Decision support in animal disease control. In: Tielen, M.J.M., Voets, M.T. (Eds.), Proceedings of the Xth International Congress on Animal Hygiene. Maastricht, pp. 362-365

Dahle, J., 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.M., 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., Moormann, R.J.M., 2001a. Duration of the protection of an E2 subunit marker vaccine against classical swine fever after a single vaccination. Vet Microbiol 78, 307-317.

De Smit, A.J., Bouma, A., van Gennip, H.G.P., de Kluijver, E.P., Moormann, R.J.M., 2001b. 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.

De Smit, A.J., Van de Wetering, G., Colijn, E.C., Hulst, M., Kramps, J.A., Van den Blink, A., Moormann, R.J.M., 2000. Evaluation of an ELISA for the detection of antibodies against the E^{rns} envelope protein of classical swine fever virus. In: de Smit, A.J. (Ed.), Classical Swine Fever. Efficacy of marker vaccines and laboratory diagnosis. Thesis University Utrecht, Utrecht, pp. 117-131.

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

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.

Diekmann, O., Heesterbeek, J.A.P. (Eds.), 2000. Mathematical epidemiology of infectious diseases. Model building, analysis and interpretation. John Wiley & Sons Ltd, Chichester, West Sussex.

Dunne, H.W., 1966. The control of pig diseases by immunization. Cornell Vet 56, 293-299.

Edwards, S., Fukusho, A., Lefevre, P.-C., Lipowski, A., Pejsak, Z., Roehe, P., Westergaard, J., 2000. Classical swine fever: The global situation. Vet Microbiol 73, 103-119.

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

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

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

Genstat, 1998. 5 ed. Rothamsted: Lawes Agricultural Trust.

Greiser Wilke, I., Depner, K., Fritzemeier, J., Haas, L., Moennig, V., 1998. Application of a computer program for genetic typing of classical swine fever virus isolates from Germany. J Virol Meth 75, 141-150.

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

Jalvingh, A.W., Nielen, M., Maurice, H., Stegeman, A.J., Elbers, A.R.W., Dijkhuizen, A.A., 1999. Spatial and stochastic simulation to evaluate the impact of events and control measures ion the 1997-1998 classical swine fever epidemic in The Netherlands. I. Description of simulation model. Prev Vet Med 42, 271-295.

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

Kaden, V., Lange, E., Fischer, U., Strebelow, G., 2000. Oral immunisation of wild boar against classical swine fever: evaluation of the first field study in Germany. Vet Microbiol 73, 239-252.

Kermack, W.O., McKendrick, A.G., 1991. Contributions to the mathematical theory of epidemics--I. 1927 [classical article]. Bull Math Biol 53, 33-55.

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

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

Kroese, A.H., De Jong, M.C.M., 2001. Design and analysis of transmission experiments. In: Menzies, F.D., Reid, S.W.J. (Eds.), Proceedings of the Society for Veterinary Epidemiology and Preventive Medicine. Noordwijkerhout, the Netherlands, pp. xxi - xxxvii

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

Laevens, H., Koenen, F., Deluyker, H., Berkvens, D., deKruif, A., 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., 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.

Lehmann, E.L. (Ed.), 1959. Testing Statistical Hypotheses. John Wiley & Sons, Inc., New York.

Lipowski, A., Drexler, C., Pejsak, Z., 2000. Safety and efficacy of a classical swine fever subunit vaccine in pregnant sows and their offspring. Vet Microbiol 77, 99-108.

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.

Mangen, M.-J.J., 2002. Economic welfare analysis of simulated control strategies for Classical Swine Fever epidemics, Department of social sciences. Wageningen: Wageningen University.

Mangen, M.-J.J., Nielen, M., Burrell, A.M., 2002. Simulated effect of pig-population density on epidemic size and choice of control strategy for classical swine fever epidemics in The Netherlands. Prev Vet Med 56, 141-163.

Meester, R., De Koning, J., De Jong, M.C.M., Diekmann, O., 2002. Modeling and Real-Time Prediction of Classical Swine Fever Epidemics. Biometrics 58, 178-184.

Meuwissen, M.P.M., Horst, S.H., Huirne, R.B.M., 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., 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. J Vet Med B Infect Dis Vet Public Health 48, 143-149.

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

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

Moormann, R.J.M., de Smit, A.J., Van Rijn, P., Wensvoort, G., Gielkens, A., Hulst, M., van Gennip, R., Miedema, C., de Kluijver, E.P., Terpstra, C., 1997. The potential use of marker vaccines for classical swine fever. In: Edwards, S., Paton, A., Wensvoort, G. (Eds.), Proceedings of the 3rd ESVV symposium on pestivirus infections. CVL, Weybridge, UK, pp. 180-187

Nielen, M., Jalvingh, A.W., Meuwissen, M.P.M., Horst, S.H., 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.

OIE, 2002. International Animal Health Code. 11th ed.

Pensaert, M., 1978. Bestrijdingsmogelijkheden van virale ziekten bij het varken nu en in de toekomst. II. Specifieke toepassingen. Tijdschr Diergeneesk 103, 523-531.

Pittler, H., 1986. Bekämpfung der Europäischen Schweinepest unter Berücksichtigung neuer EG-Vorschriften. Tierärztl Prax 14, 55-65.

Precausta, P., Kato, F., Brun, A., 1978. Peste porcine classique. immunisation active conferee par la souche chinoise CL aux porcelets issus de truies immunes. [Hog

cholera: active immunity conferred by the Chinese strain vaccine to young pigs born to immune sows]. Dev Biol Stand, 41367-41379.
Precausta, P., Kato, F., Brun, A., 1983. Swine fever immunisation of piglets. Comp Immunol Microbiol Infect Dis 6, 281-289.
Rao, C.R. (Ed.), 1973. Linear statistical inference and its applications. John Wiley & sons, New York.
Ressang, A.A., Den Boer, J.L., 1967. A comparison between the cell culture, frozen tissue section and mucosal smear technique for fluorescent antibody in the diagnosis of hog cholera. Tijdschr Diergeneesk 92, 567-586.
Robijns, K.G., 1971. Swine fever and swine fever eradication in the Netherlands. Bull Off Int Epiz 95, 532-552.
Saatkamp, H.W., Berentsen, P.B.M., Horst, S.H., 2000. Economic aspects of the control of classical swine fever outbreaks in the European Union. Vet Microbiol 73, 221-237.
Saulmon, E.E., 1973. Hog cholera eradication - dream or reality. J Am Vet Med Assoc 163, 1103-1105.
Sharpe, K., Gibbens, J., Morris, H., Drew, T., 2001. Epidemiology of the 2000 CSF outbreak in East Anglia: preliminary findings. Vet Rec 148, 91.
Siegrist, C.-A., 2001. Neonatal and early life vaccinology. Vaccine 19, 3331-3346.
Siva software B.V., 2001. Bedrijfsvergelijking 2000. Wageningen, The Netherlands.
Sokal, R.R., Rohlf, F.J. (Eds.), 1981. Biometry. W.H. Freeman and company, New York.
Soos, P., Mojzis, M., Pollner, A., Sumeghy, L., 2001. Evaluation of vaccine-induced maternal immunity against classical swine fever. Acta Vet Hung 49, 17-24.
Staubach, C., Teuffert, J., Thulke, H.H., 1997. Risk analysis and local spread mechanisms of classical swine fever. Epidemiol Sante Animale, 31-32.
Stegeman, A., Elbers, A., de Smit, H., Moser, H., Smak, J., Pluimers, F., 2000. The 1997-1998 epidemic of classical swine fever in the Netherlands. Vet Microbiol 73, 183-196.
Stegeman, A., Elbers, A.R.W., Bouma, A., de Smit, H., de Jong, M.C.M., 1999a. Transmission of classical swine fever virus within herds during the 1997-1998 epidemic in The Netherlands. Prev Vet Med 42, 201-218.
Stegeman, A., Elbers, A.R.W., Smak, J., de Jong, M.C.M., 1999b. 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.
Taylor, D.J., 1995. Classical swine fever (hog cholera). In: Taylor, D.J. (Ed.), Pig Diseases. Burlington Press, Cambridge.
Terpstra, C., 1978. Aantonen van C-virus in varkens na vaccinatie tegen varkenspest. Tijdschr Diergeneesk 103, 678-684.
Terpstra, C., 1987. Epizootiology of swine fever. Vet Q 9 Suppl 1, 50s-60s.
Terpstra, C., 1988. Epizootiology of hog cholera. In: Liess, B. (Ed.), Classical swine fever and related viral infections. Martinus Nijhoff publishing, Boston, pp. 201-216.
Terpstra, C., Bloemraad, M., Gielkens, A.L., 1984. The neutralizing peroxidase-linked assay for detection of antibody against swine fever virus. Vet Microbiol 9, 113-120.

Terpstra, C., de Smit, A.J., Stegeman, A., Elbers, A., de Smit, H., Moser, H., Smak, J., Pluimers, F., 2000. The 1997/1998 epizootic of swine fever in the Netherlands: control strategies under a non-vaccination regimen. Vet Microbiol 77, 3-15.

Terpstra, C., 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., Tielen, M.J., 1976. Antibody response against swine fever following vaccination with C-strain virus. Zentralbl Veterinarmed [B] 23, 809-821.

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

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

Vagsholm, I., 1996. Benefit-cost analysis and simulation models: Tools in the decision making process whether starting a vaccination programme or not. Acta Vet Scand Suppl. 90, 17-24.

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

Van Oirschot, J.T., 1992. Hog cholera. In: Leman, A.D., Straw, B.E., Mengeling, W.L., D'Allaire, S., Taylor, D.J. (Eds.), Diseases of swine. Iowa State University Press, Ames, Iowa, pp. 274-285.

Van Oirschot, J.T., 1994. Vaccination in food animal populations. Vaccine 12, 415-418.

Van Oirschot, J.T., 1999. Diva vaccines that reduce virus transmission. J Biotechnol 73, 195-205.

Van Oirschot, J.T., Terpstra, C., 1977. A congenital persistent swine fever infection, I. Clinical and virological observations. Vet Microbiol 2, 121-132.

Van Rijn, P.A., Van Gennip, H.G.P., Moormann, R.J.M., 1999. An experimental marker vaccine and accompanying serological diagnostic test both based on envelope glycoprotein E2 of classical swine fever virus (CSFV). Vaccine 17, 433-440.

Vandeputte, J., Chappuis, G., 1999. Classical swine fever: the European experience and a guide for infected areas. Rev Sci Tech 18, 638-647.

Wensvoort, G., Terpstra, C., Boonstra, J., Bloemraad, M., Van Zaane, D., 1986. Production of monoclonal antibodies against swine fever virus and their use in laboratory diagnosis. Vet Microbiol 12, 101-108.

Williams, D.R., Matthews, D., 1988. Outbreaks of classical swine fever in Great Britain in 1986. Vet Rec 122, 479-483.

Wolfram, S. (Ed.), 1999. The Mathematica Book. University Press, Cambridge.

Summary

The thesis describes epidemiological research carried out to improve the control of classical swine fever virus (CSFV) epidemics. The research was part of an STW (Technology Foundation) funded project which also covered the management and the economic consequences of CSFV control.

Two important aspects of improvement of CSFV control have been addressed. The first is investigation of control measures and strategies in order to enable quick and adequate action upon CSFV detection; a good preparation to virus entry. The second is the development of epidemiological tools to make it possible to measure the effectiveness of CSFV control during an epidemic; with such tools, it becomes possible to steer the control strategy if it appears to be insufficient.

Regarding classical swine fever (CSF), the European Union (EU) has a non-vaccination policy. Also during epidemics, vaccination has never been applied, because vaccinated pigs could not be guaranteed to be virus free as the vaccines evoked the same antibody response as the virus. To overcome the detection problems, E2 subunit marker vaccines have been developed with accompanying discriminatory tests, which react positive only after infection with the virus and not after vaccination. Hence, it has become possible, also after vaccination, to guarantee that an area is virus free.

The thesis describes CSFV transmission experiments and a mathematical model of CSFV transmission between herds, used to assess the effectiveness of the marker vaccines with respect to CSFV transmission. Quantification of the effectiveness was done with the 'basic reproduction ratio' R, defined as the number of individuals that is infected by one typical infectious individual in a completely susceptible population. Only if R exceeds 1, a major epidemic is possible. For the analysis of the transmission experiments, R was used at the level of the individual animal (R_i: the number of animals infected by one animal), whereas the mathematical modelling study made use of R at the herd level (R_h: the number of herds infected by one herd).

The transmission experiments pointed out that R_i decreases to below 1 as of three weeks after vaccination until at least six months after vaccination. Already one week after vaccination of a pig herd, virus entry will lead to only minor outbreaks. It appeared that the presence of maternal antibodies at the time of vaccination may reduce the antibody levels at later age, but these lower levels still keep R_i below 1.

Vertical CSFV transmission can lead to the birth of persistently infected piglets, which shed large amounts of virus and show only few clinical symptoms after some months. Therefore, clinical detection of a herd with persistently infected piglets

occurs through the contact infections of those piglets. If a herd is completely vaccinated, however, clinical detection has become impossible, and because vaccination does not prevent vertical transmission, completely vaccinated herds can become a risk. This risk might be avoided by omitting the vaccination of breeding sows, which leads to the continuous presence of some unvaccinated young piglets. These piglets can show clinical disease upon infection and hence make detection of the herd possible.

The strategy with omitting the vaccination of breeding sows is one of the strategies tested in a mathematical model of CSFV transmission. The model results point out that an effective control strategy ($R_h < 1$) requires a complete prohibition of transport of unvaccinated animals. Moreover, in addition to the control measures that are obliged by EU legislation (like the tracing of infectious contacts and hygiene measures), the virus transmission between herds should be halved, e.g. by vaccinating 50% of all pig herds. If all animals but the breeding sows would be vaccinated, the demands for a successful control strategy are well met. If ten undetected, infected herds are present at the time the strategy is implemented, the last infected herd is expected to be detected after three months. The total number of infected herds will then be 16, including the 10 infected herds at the start.

For correct interpretation of the results, it is essential to realise that the model was based on the Dutch CSFV epidemic of 1997/1998, with a moderately virulent virus strain in a pig dense area with relatively many multiplier herds. Moreover, the model described only the most essential elements of virus transmission within and between herds ands it did not include local spatial effects.

In addition to the investigation of control strategies, a tool was tested, which had been developed to analyse the effectiveness of the control strategy in an ongoing epidemic. The tool consisted of a very simple model for CSFV transmission between herds and was designed to use data of an ongoing epidemic for estimation of R_h and the number of infected, but yet undetected herds. The only data needed for the calculations are the numbers of detected herds in each week of the epidemic. Unfortunately, the only result the model could generate was whether R_h was smaller or larger than 1.

In order to understand why the model cannot give more precise estimates and why it cannot estimate the present number of undetected infected herds, it will be necessary to thoroughly study simpler versions of the model. To be able to analyse ongoing epidemics until a better model is available, some extra data might be used, e.g. an estimate of the number of infected herds at the day of the first detection, or an estimate of the average time between infection of a herd and detection.

Samenvatting

Doel van het onderzoek

Als klassieke-varkenspestvirus (KVPV), dat klassieke varkenspest (KVP) veroorzaakt, binnenkomt in de Nederlandse (gedomesticeerde) varkenspopulatie, dan kan dat tot grote KVP-epidemieën leiden. Een voorbeeld hiervan was de KVP-epidemie in 1997 en 1998, die 12 miljoen varkens het leven kostte en resulteerde in een economische schade van ongeveer 2,3 miljard euro. Naar aanleiding van deze epidemie is er discussie ontstaan over de effectiviteit en de ethische aanvaardbaarheid van de gebruikte bestrijdingsmaatregelen, met name het preventief slachten van op het oog gezonde varkens.

Schade als gevolg van KVP kan worden beperkt door te werken aan preventie, dus voorkomen dat het virus Nederland binnenkomt, en door effectievere bestrijding van epidemieën als het virus eenmaal binnen is. In een door STW (Stichting Technische Wetenschappen) gefinancierd project is vanaf 1998 onderzoek gedaan naar voornamelijk het tweede onderdeel, bestrijding van epidemieën. Het werk in dit proefschrift maakt deel uit van dat project, en beslaat het epidemiologische aspect van KVPV-bestrijding. Andere aandachtspunten in het project waren de organisatie van KVPV-bestrijding en de economische gevolgen van KVP-epidemieën.

Epidemiologisch onderzoek ter verbetering van KVPV-bestrijding kan zich richten op twee belangrijke doelen. In de eerste plaats is het gewenst om maatregelen en strategieën te onderzoeken om snel tot adequaat ingrijpen te kunnen overgaan zodra het virus wordt gedetecteerd; een goede voorbereiding op virusintroductie dus. Ten tweede is het nuttig om tijdens de bestrijding te beschikken over epidemiologische tools, hulpmiddelen die het mogelijk maken om gedurende de epidemie de effectiviteit van de bestrijding te meten. De bestrijding kan dan snel worden bijgestuurd als het mis gaat. Aan beide doelen, onderzoek van bestrijdingsmaatregelen en ontwikkelen van tools, wordt in dit proefschrift aandacht besteed.

Onderzoek aan bestrijdingsstrategieën

Achtergrond

Met betrekking tot KVP wordt er in de Europese Unie (EU) een non-vaccinatiebeleid gevoerd, waarmee de internationale handelsregels worden gevolgd zoals die zijn opgesteld door de OIE (Office Internationale des Epizooties). Deze

regels stellen dat een varkenspopulatie met de hoogste gezondheidsstatus (wat varkenspest betreft) bestaat uit niet-besmette, niet-gevaccineerde dieren. Landen of gebieden die deze status hebben, kunnen import van varkens of varkensproducten weigeren uit gebieden zonder deze status, en dus biedt de status een economisch voordeel. Het gevolg van dit beleid is dat eventuele introducties van KVPV tot grote problemen kunnen leiden omdat de volledige varkenspopulatie vatbaar is. Het is zaak om binnen dit beleid tot optimale bestrijdingsstrategieën te komen.

Hoewel vaccinatie als bestrijdingsmaatregel *tijdens* epidemieën volgens de EU-wetgeving nooit verboden is geweest, is het nooit toegepast. Immers, om de hoogste gezondheidsstatus weer te bereiken hadden in zo'n geval alle gevaccineerde dieren weer moeten worden geruimd, wat de zin van vaccinatie op zijn minst twijfelachtig zou hebben gemaakt. Ook een alternatieve regeling waarin gevaccineerde dieren alleen binnen de EU worden geconsumeerd, werd als een te groot risico beschouwd, omdat vaccinatie de KVP-detectie zou bemoeilijken. De ELISA (antilichaamtest) die gebruikt werd om gebieden infectievrij te verklaren reageert namelijk ook positief op serummonsters van gevaccineerde dieren, waardoor een gedegen screening op aanwezigheid van KVPV onmogelijk werd.

Om dit detectieprobleem te omzeilen, is in de jaren '90 gewerkt aan de ontwikkeling van zogenaamde markervaccins. Dit zijn vaccins waarin één of meer (stukjes van) eiwitten van het virus ontbreken, waardoor een infectie met het werkelijke virus kan worden opgespoord door te testen op antilichamen tegen deze ontbrekende stukjes. Dat gebeurt met een discriminerende ELISA-test. Twee markervaccins zijn momenteel voorhanden, beide bestaand uit alleen het E2-eiwit van het virus. De bijbehorende discriminerende test (de E^{rns}-ELISA) reageert op antilichamen tegen het E^{rns}-eiwit.

Het gebruik van een E2-markervaccin

Het inzetten van vaccinatie als bestrijdingsmaatregel is natuurlijk alleen maar nuttig als het de verspreiding van het virus voldoende remt. Een zinvolle maat voor de virusverspreiding is het begrip 'basaal reproductiegetal' of 'basic reproduction ratio', dat wordt weergegeven door de letter *R*. De *R* is gedefinieerd als het aantal individuen dat gemiddeld door één typisch besmet individu wordt geïnfecteerd in een volledig vatbare populatie. Alleen als *R* groter is dan 1, dus als elk individu gemiddeld het virus naar meer dan één ander individu doorgeeft, is een epidemie mogelijk. De *R* kan worden gedefinieerd op verschillende niveaus, bijvoorbeeld op individueel dierniveau (R_i: het aantal dieren dat wordt geïnfecteerd door één dier) of op bedrijfsniveau (R_h: het aantal bedrijven geïnfecteerd door één bedrijf). Een

effectieve bestrijdingsstrategie zal in staat moeten zijn de R_h onder de 1 te brengen, want alleen dan zal de epidemie uiteindelijk doodlopen.

Als een vaccin in staat is de R_i onder de 1 te brengen, dan wordt voorkomen dat KVPV kan spreiden in een gevaccineerd bedrijf, wat ook de R_h onder de 1 zal brengen. Het is mogelijk te toetsen of R_i daalt als gevolg van vaccinatie met transmissie-experimenten met KVPV, dierexperimenten waarin geïnfecteerde en vatbare dieren worden samengebracht en waarin de transmissie van het virus tussen dieren wordt gevolgd met verschillende diagnostische testen. Deze experimenten kunnen ook worden gebruikt om R_i te schatten. Dit proefschrift beschrijft eerst een methode om R_i te schatten en vervolgens enkele transmissie-experimenten waarmee de effectiviteit van de E2-vaccins is getest.

Het blijkt dat beide vaccins de virustransmissie reduceren, waarbij vanaf drie weken tot tenminste zes maanden na vaccinatie de reductie maximaal is. De R_i is dan statistisch significant tot onder de 1 gedaald, wat dus betekent dat op gevaccineerde varkensbedrijven geen uitbraken meer kunnen plaatsvinden en R_h kleiner dan 1 zal zijn. Hoewel de vaccins pas na drie weken de R_i onder de 1 brengen, wordt een grote uitbraak op een bedrijf al voorkomen als het virus slechts één week na vaccinatie wordt geïntroduceerd. Dat is het gevolg van de tijdsvertraging die optreedt doordat het virus zich eerst moet vermenigvuldigen binnen een dier voordat het dier nieuwe infecties kan veroorzaken. Hierdoor kan het eerst geïnfecteerde dier nog wel meer dan één contactinfecties veroorzaken, maar kunnen deze contactgeïnfecteerde dieren het virus nauwelijks meer verder verspreiden.

De effectiviteit van vaccinatie van jonge dieren wordt bij veel infectieziekten gehinderd door de aanwezigheid van maternale antilichamen, antilichamen die de jongen van de (gevaccineerde) moederdieren krijgen via de melk. Deze antilichamen kunnen het vaccin 'wegvangen', waardoor de reactie van het immuunsysteem van de jonge dieren vermindert. In een transmissie-experiment, beschreven in dit proefschrift, kon worden aangetoond dat, ook in het geval van het E2-vaccin tegen KVP, maternale antilichamen tegen het E2-vaccin de immuunreactie beïnvloeden bij vaccinatie op twee weken leeftijd: de hoeveelheid antilichamen in het bloed op latere leeftijd, drie en zes maanden, is lager in dieren van gevaccineerde zeugen dan in dieren van niet-gevaccineerde zeugen. Vaccinatie blijft echter voldoende effectief, want ook in maternaal immuun gevaccineerde dieren daalt R_i tot onder de 1.

Omdat vaccinatie van alle varkens op een bedrijf het ontstaan van een grote uitbraak voorkomt, zal volledige vaccinatie van alle varkensbedrijven zeker leiden tot een R_h onder de 1, en zal dat dus een effectieve strategie zijn. Er is echter ook een belangrijk nadeel aan volledige vaccinatie en dat heeft te maken met verticale transmissie: virusoverdracht van de moederzeug naar de ongeboren biggen. Uit

experimenten blijkt dat vaccinatie verticale transmissie niet volledig kan voorkomen. Een eigenschap van varkenspestvirus is dat verticale transmissie kan leiden tot de geboorte van persistent geïnfecteerde biggen, die immuuntolerant zijn voor KVPV. Dat betekent dat het immuunsysteem van deze biggen niet reageert op de infectie, waardoor de dieren gedurende lange tijd veel virus kunnen uitscheiden. Omdat ze pas na enkele maanden lichte klinische symptomen vertonen, is detectie op basis van kliniek niet waarschijnlijk. Echter, omdat ze zich normaal gesproken bevinden tussen veel dieren die wél ziek worden van het virus, zal het getroffen bedrijf gedetecteerd worden via de contactinfecties die de persistent geïnfecteerde biggen veroorzaken. Op volledig gevaccineerde bedrijven zijn alle dieren klinisch beschermd, waardoor geïnfecteerde bedrijven onopgemerkt zouden kunnen blijven.

Het probleem van het niet detecteren van bedrijven met persistent geïnfecteerde biggen kan worden vermeden door de moederzeugen niet te vaccineren, maar R_h zou dan nog altijd onder de 1 moeten blijven. Dit is één van de bestrijdingsstrategieën die getest zijn met een mathematisch model dat de verspreiding van KVPV tussen varkensbedrijven beschrijft. Het model is een sterke vereenvoudiging van de werkelijkheid, waarbij alleen de meest essentiële elementen van virustransmissie zijn opgenomen. Een belangrijke aanname is dat er twee bedrijfstypes zijn, ten eerste vermeerderingsbedrijven die meer biggen produceren dan afmesten en derhalve dieren verkopen aan andere bedrijven (mestbedrijven), en ten tweede alle andere bedrijven, zoals mestbedrijven en gemengde bedrijven. Het eerste type is een veel groter risico voor verdere virusspreiding dan het tweede type.

Het model geeft aan dat voor een effectieve strategie ($R_h < 1$) geen vervoer van niet-gevaccineerde dieren tussen bedrijven mag worden toegestaan, wat in de huidige bestrijdingsstrategie ook verboden is. Bovendien moet, bovenop alle maatregelen die door de EU worden opgelegd (zoals hygiënemaatregelen en het traceren van infectieuze contacten), de transmissie tussen bedrijven tenminste worden gehalveerd, bijvoorbeeld door minimaal de helft van de bedrijven te vaccineren. Indien, met uitzondering van de moederzeugen, alle varkens worden gevaccineerd, dan wordt ruimschoots aan deze minimumeisen voldaan. Wanneer er tien ongedetecteerde, geïnfecteerde bedrijven aanwezig zijn op het moment dat de strategie wordt ingezet, dan wordt naar verwachting drie maanden later het laatste geïnfecteerde bedrijf ontdekt. Het totaal aantal besmette bedrijven komt dan op 16, inclusief de 10 die er al waren.

Om de resultaten te kunnen gebruiken voor beslissingen in toekomstige epidemieën, is het nodig te beseffen op welke aannames ze gebaseerd zijn en waar de essentiële verschillen tussen werkelijkheid en model zitten. Een belangrijke aanname is de al eerder aangegeven verdeling van de varkenspopulatie in twee bedrijfstypes waar er eigenlijk meer zijn. Ook is van belang dat alle parameters in

het model, die de verspreiding van KVPV binnen en tussen bedrijven kwantificeren, zijn geschat uit de Nederlandse epidemie in 1997 en 1998. Dat betekent dat de resultaten met name gelden voor gemiddeld varkensdichte gebieden met relatief veel vermeerderingsbedrijven, en dat ze betrekking hebben op een mild-virulente virusstam zoals die tijdens de epidemie voorkwam. Tenslotte kunnen in de werkelijkheid lokale concentraties van varkensbedrijven maken dat de R_h lokaal wat verhoogd of verlaagd is.

Onderzoek aan een epidemiologische tool

Behalve onderzoek aan bestrijdingsmaatregelen beschrijft dit proefschrift ook het onderzoek aan een tool, die was ontworpen om tijdens een epidemie te analyseren hoe het met de bestrijding loopt. Deze tool bestaat uit een zeer eenvoudig model voor de verspreiding van KVPV tussen bedrijven. Het zou kunnen worden gebruikt om, met behulp van gegevens uit de lopende epidemie, R_h te schatten en om het aantal nog niet gedetecteerde geïnfecteerde bedrijven te schatten. De gebruikte gegevens bestaan enkel uit de aantallen bedrijven, die in elke week van de epidemie tot dan toe gedetecteerd zijn. Met behulp van gesimuleerde epidemieën op basis van het verspreidingsmodel is onderzocht hoe goed het model R_h en het aantal geïnfecteerde bedrijven kan schatten.

Helaas bleek dat het model alleen in staat is om in te schatten of R_h groter danwel kleiner dan 1 is, maar een preciezere schatting bleek onmogelijk, laat staan dat het aantal nog geïnfecteerde bedrijven kon worden ingeschat. Om te begrijpen waarom het model deze schattingen niet kan geven, zal het noodzakelijk zijn wiskundige studies te doen naar eenvoudiger versies van het model. Om tot die tijd toch nog lopende epidemieën te kunnen analyseren, zal gebruik moeten worden gemaakt van extra gegevens, bijvoorbeeld een inschatting van het aantal geïnfecteerde bedrijven op het moment van de eerste detectie, of een schatting van de gemiddelde tijd tussen infectie en detectie van een bedrijf.

Dankwoord
Curriculum Vitae
Publicaties

Dankwoord

Tegen het dankwoord heb ik het langst opgezien van alle stukken tekst in het proefschrift. Ik hoop dan ook dat iedereen die op enigerlei wijze tot het totstandkomen van dit proefschrift heeft bijgedragen zich realiseert dat ik die bijdrage zeer waardeer.

Mart, zeer intensief is de begeleiding niet altijd geweest, maar meestal vond ik dat wel prettig. Je gaf namelijk altijd de mogelijkheid tijd op te eisen als ik wilde. Door jou ben ik ervan overtuigd dat echte wetenschappelijke interesse nooit hoeft te verdrinken in grote hoeveelheden managementtaken: als het om de inhoud ging, was je enthousiasme aanstekelijk, en dat maakte je begeleiding echt belangrijk.

Rob, in het maternale-antilichamenexperiment ligt onze duidelijkste samenwerking, maar tijdens het hele project heb ik via discussies van jouw inzichten mogen profiteren. Tevens heb jij me betrokken bij al het overige varkenspestonderzoek op het instituut, wat de breedte van mijn kennis erg goed heeft gedaan. Het bloedtappen op de Tolakker zal ik niet gauw vergeten.

Jos, buiten het regelmatige overleg heb ik je weinig bij mijn werk betrokken, maar je oprechte interesse en je vermogen snel de essentie van problemen te benoemen maakten je begeleiding waardevol.

Jan, ook jou wil ik graag bedanken voor je bijdragen in de totstandkoming van dit proefschrift.

Met plezier kijk ik terug naar mijn vrijdagochtenden en –middagen in de Leeuwenborch, voor het STW-overleg. Alle STW-ers wil ik graag bedanken voor de samenwerking en jullie inbreng in mijn onderzoek, en natuurlijk voor jullie geduld als ik weer eens te laat was. Mirjam, jij dwong mij af en toe het overzicht over mijn werk te bewaren, een zeer welkome stimulans. Bij deze dank ik tevens de STW-gebruikercommissie voor de getoonde belangstelling en het gestelde vertrouwen.

Ook bij QVE hebben veel mensen mij geholpen. Joop en Bas, met veel problemen en vragen ging mocht ik jullie hulp inschakelen, bedankt. Eerst Arjan en later Annemarie, met vele praktische vragen over de KVP-epidemie, over experimenten en over varkenspest in het algemeen kon ik bij jullie terecht, waarvoor dank. Annemarie, bedankt ook voor je hulp bij mijn schrijfwerk en natuurlijk alle ‘trein’-gesprekken, zowel in als over. Annelie, onze samenwerking heeft mij veel geleerd en ik kijk er nog graag op terug, dank je wel.

Met mijn transmissieproef hebben me enorm veel mensen geholpen. Dierverzorgers op de Tolakker, bij DB aan de Edelhertweg en bij DB aan de Houtribweg, enorm bedankt voor jullie zorg en inzet. De mensen van het

varkenspestlab wil ik bedanken voor al het werk aan mijn proef, en met name voor het mij aanleren van diverse technieken. Ook jullie gezelschap tijdens al mijn dagen 'binnen' heb ik plezierig gevonden.

Nog verscheidene mensen hebben me in de loop van de tijd geholpen. De afdeling AIV, Fred van Welie, de bibliotheek en Marian Jonker vanuit Wageningen, allen hartelijk dank.

De hele QVE-groep wil ik graag bedanken voor een ontzettend leuke werktijd. De dagelijkse trip naar Lelystad werd een stuk draaglijker met het vooruitzicht dat in elk geval de pauzes het vroege opstaan al waard waren. Als dan het werk ook nog een beetje opschoot, was het helemaal mooi. Ook de QVE'ers in Wageningen en andere ID'ers, o.a. van IPE en I&K, hebben trouwens bijgedragen aan een leuke periode, waarvoor dank.

De dagelijkse rit naar Amsterdam terug was de moeite waard vanwege het thuiskomen, eerst bij Marije en later bij Rutger. Ik vertel niet veel over mijn werk, maar de alledaagse gesprekjes met jullie bij thuiskomst heb ik altijd erg prettig gevonden. Ook Yuri en vele anderen in Amsterdam bedankt: jullie hebben mij elke dag weer drie uur doen reizen, simpelweg door niet in Lelystad te wonen.

Nu ik na ruim 28 jaar ook eindelijk de schooltas aan de vlaggenstok mag hangen, dank ik nog Maurice, Lucienne, Joy en andere vrienden en familie die mijn leven compleet maken. En tenslotte, lieve vader en moeder, jullie dank ik voor alles wat ik heb mogen leren; jullie steun was en is onmisbaar.

Curriculum Vitae

Ik ben geboren op 28 februari 1975, in Apeldoorn. Ik heb in Apeldoorn gewoond tot mijn studietijd en heb van 1987 tot 1993 mijn VWO-opleiding gedaan aan het Gymnasium Apeldoorn, in Apeldoorn. Vervolgens ben ik (in 1993) Medische Biologie gaan studeren in Amsterdam, aan de UvA-faculteit der Biologie. In 1998 slaagde ik voor mijn doctoraalexamen. In datzelfde jaar ben ik begonnen als onderzoeker in opleiding (OIO), in dienst bij de Stichting Technische Wetenschappen en werkend bij het Instituut voor Dierhouderij en Diergezondheid (ID-DLO, thans ID-Lelystad), bij het cluster Kwantitatieve Veterinaire Epidemiologie. Tijdens dit dienstverband heb ik het onderzoek gedaan dat beschreven is in dit proefschrift.

Publicaties

Olde Scheper, T., Klinkenberg, D., Pennartz, C., Van Pelt, J., 1999. A mathematical model for the intracellular circadian rhythm generator. J Neurosci 19, 40-47.

Olde Scheper, T., Klinkenberg, D., Van Pelt, J., Pennartz, C., 1999. A model of molecular circadian clocks: multiple mechanisms for phase shifting and a requirement for strong nonlinear interactions. J Biol Rhythms 14, 213-220.

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.

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

Klinkenberg, D., De Bree, J., Laevens, H., De Jong, M.C.M., 2002. 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, A.J., Bouma, A., De Jong, M.C.M., 2002. Influence of maternal antibodies on efficacy of a subunit vaccine: transmission of classical swine fever virus between pigs vaccinated at 2 weeks of age. Vaccine 20, 3005-3013.

Submitted

Klinkenberg, D., Bouma, A., Floegel-Niesmann, G., De Jong, M.C.M. E2 subunit marker vaccines reduce transmission of classical swine fever virus sufficiently to halt epidemics. Prev Vet Med.

Klinkenberg, D., de Jong, M.C.M. Towards better control of classical swine fever epidemics: vaccination with an E2 marker vaccine. Vaccine.

Klinkenberg, D., Everts-van der Wind, A., Graat, E.A.M., de Jong, M.C.M. Quantification of the effect of control strategies on classical swine fever epidemics. Math Biosci.