Introduction and transmission of Encephalomyocarditis virus (EMCV) in pig farms

– Studies to support prevention and control –

Huibert Maurice

Stellingen

1. Encephalomyocarditis virus (EMCV) infecties in varkensbedrijven dienen voornamelijk te worden gezien als een individueel bedrijfsprobleem.
   Dit proefschrift

2. De R_0-schattingen voor EMCV in varkens impliceren dat, naast directe varken-op-varken transmissie, ook andere introductie- en/of verspreidingsmechanismen vaak een rol zullen spelen bij het ontstaan van grote EMCV-uitbraken op varkensbedrijven.
   Dit proefschrift

3. Zeker vanuit wetenschappelijk oogpunt verdient het verzamelen van epidemiologische data tijdens een uitbraak van een (zeer besmettelijke) dierziekte nadrukkelijk een plaats in het bestrijdingsplan.

4. Het succes van landelijke dierziektebestrijdingsmaatregelen vergt niet alleen draagvlak onder veehouders, maar in toenemende mate ook onder hobbydierhouders en in de burgermaatschappij.
   In aanvulling op A. Vonk Noordegraaf, 2002.

5. Zoals het goed is voor beleidsmakers om regelmatig met de "voeten in de klei te staan", verdient het omgekeerde traject voor (agrarische) ondernemers ook aanbeveling.

6. Het ontstaan van welzijnsproblemen door het "vermenselijken" van gehouden (gezelschaps)dieren vraagt om bredere bewustwording van de behoeften van het dier.
   Geïnspireerd door G. Verburg, Minister van Landbouw, Natuur en Voedselkwaliteit, 2007.

7. De uitdrukking "Het loopt als een trein" is positief bedoeld.

8. Het gezegde 'You cannot fail, unless you quit' is geen vrijbrief tot een open einde.
   Naar Abraham Lincoln, oud-president VS.

9. Ook krimp bij de overheid vraagt om beleid.

Stellingen behorende bij het proefschrift 'Introduction and transmission of Encephalomyocarditis virus (EMCV) in pig farms; studies to support prevention and control', H. Maurice, 12 februari 2008

Introduction and transmission of Encephalomyocarditis virus (EMCV) in pig farms

Studies to support prevention and control

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Introduction and transmission of Encephalomyocarditis virus (EMCV) in pig farms

Studies to support prevention and control

Proefschrift

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op gezag van de rector magnificus
van Wageningen Universiteit,
Prof. dr. M.J. Kropff
in het openbaar te verdedigen
op dinsdag 12 februari 2008
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Introduction and transmission of Encephalomyocarditis virus (EMCV) in pig farms; studies to support prevention and control.
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Abstract

Clinical manifestations of infections by encephalomyocarditis virus (EMCV), belonging to the genus cardiovirus of the family Picornaviridae, emerged in European pig farms in the nineties. Two types of clinical disease were noticed, acute myocarditis, usually in young piglets, and reproductive failure in sows. To understand its relevance in pigs and develop hypotheses on the origin, cause and nature of infection, the occurrence of EMCV infections was studied in domestic pigs in several European countries. In addition, virus spread in pigs and rats as well as risk factors for clinical appearance of EMCV in domestic pig farms were studied to increase our knowledge of the disease and support its prevention and control. Although outbreaks appeared to be clustered in endemic areas, infection usually was restricted to individual farms. The seroprevalence varied considerably among farms, age categories and countries, while sub-clinical infection with EMCV was quite common. The variable clinical picture in pigs in literature was ascribed to the pathogenicity of the EMCV strains, the available infectious dose and/or the susceptibility of the pigs (age, breed). Local rodent populations were often suggested responsible for the observed clustering and re-occurrence in farms. A matched case-control study on risk factors inducing clinical EMCV revealed the presence of mice (OR = 8.3) as a risk factor. The transmission of a myocardial EMCV-strain in fattening piglets was quantified by the basic reproduction ratio (R_0) both from experiments (R_0=1.24, 95%-CI = 0.39 – 4.35) and field data (R_0=1.36, 95%-CI 0.93-2.23). Although these results suggested that EMCV transmission among pigs in most cases will be limited (R_0 close to 1), both studies remained inconclusive with respect to the threshold value of R_0=1. Therefore it could not be concluded whether EMCV could persist in a pig population by pig-to-pig transmission alone (i.e. R_0>1). To estimate the transmission in a compartmentalised house setting without possible interference of multiple transmission routes (field), a stochastic simulation model was developed to extrapolate the experimental results to a compartmentalised pig house setting with 22 pens. The introduction of virtual fences in itself already reduced EMCV spread by avoiding random mixing and for any R_0<1.24 the probability to observe outbreaks affecting at least 50% of the pens(major outbreaks) remained below 0.10. When contact transmission was limited by an increasing fence effect (reducing contacts between pens), the probability to observe major outbreaks was reduced to about 0.50 for any R_0<2.7. These results indicated that pigs should not be considered the main reservoir host for EMCV in compartmentalised pig farms. Additionally, EMCV transmission in rats was experimentally quantified at R_0>9.9. These findings indicated that the virus can spread and persist easily within a rat population by horizontal rat-to-rat transmission alone, which made the rat population to a potential reservoir for EMCV and a probable transmitter of EMCV into domestic pig farms. In conclusion, multiple EMCV introductions by e.g. rodents or indirect transmission routes (by manure, farmer, rodents) most probably are required to explain major outbreaks divided over many pens in commercial pig houses. Therefore the observed (temporary) emergence of EMCV in domestic pig farms most likely had a multi factorial cause mediated by the EMCV-strain type involved, the infection status of local rodent populations, the contact structure between local rodent populations and domestic pigs and individual pig factors (susceptibility due to age or breed). To prevent or control future EMCV outbreaks in pig farms, farmers should avoid contacts between potentially infected rodent populations (active rodent control), maintain high levels of bio security and isolate diseased pigs immediately.

Voorwoord

Mijn proefschrift is af! Het schrijven van dit voorwoord is letterlijk een van de laatste stappen in het afronden van mijn promotietraject. Een mooi moment ook om terug te blikken en even stil te staan bij hoe dit traject destijds begonnen is.

Na al enige tijd werkzaam te zijn geweest bij de vakgroep Agrarische Bedrijfseconomie (ABE), werd me de mogelijkheid geboden om onderzoek te gaan doen naar een virusziekte bij varkens in het kader van een Europees onderzoeksproject. Voor iemand die vroeger altijd dierenarts wilde worden een mooie gelegenheid om alsnog op het gebied van diergezondheid aan de slag te gaan. Nu, de nodige tijd en werkervaring later, kijk ik met veel plezier terug op mijn tijd bij ABE en realiseer ik me dat er door de jaren heen heel veel mensen een bijdrage hebben geleverd aan het tot stand komen van mijn proefschrift. Naast een welgemeend "dank je wel" aan alle betrokkenen, wil ik er hier graag een aantal bij name noemen.

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Huibert Maurice, Ede, 2008.

Aan mijn ouders

Contents

Chapter 1

General introduction

1.1 Introduction

Livestock disease occurrence

In livestock husbandry systems pathogens can cause disease problems among domestic farm animals. These diseases can have a highly variable direct impact on both the animal (discomfort and/or mortality among affected animals) and the farmer (production losses). In addition, livestock diseases can have indirect effects as there are the risks for human health by zoonoses (e.g. avian influenza; Claas et al., 1998),) or food safety issues (e.g. salmonella; Van der Gaag, 2004) and the loss of (inter-)national trade markets due to outbreaks of e.g. classical swine fever (Mangen, 2002) or foot and mouth disease (Tomassen et al., 2002).

Therefore, over the years epidemiological knowledge has been gathered on several livestock diseases in order to be able to react adequately. Sometimes this has led to successful eradication programs (Aujeszky's disease, Buijtels and Burrell, 2000), while in other occasions research helped to prevent introduction and/or spread of a pathogen to other animals or farms once a primary infection was diagnosed, thereby limiting the adverse effects. Still however, from time to time "new" pathogens emerge in the field, i.e. infections that either have newly appeared in a population, have rapidly increased their incidence, or have expanded their geographic range (Morse and Hughes, 1996). Examples are recent outbreaks of avian influenza or blue tongue virus in Europe. Myocardial infections due to Encephalomyocarditis virus (EMCV) which came up in commercial pig farms in Europe in the nineties, or at least were diagnosed more frequently, were taken as a model for emerging diseases especially taking into account the cross species boundaries (Koenen et al., 1997, 2002).

Disease study and control

After its recognition, those responsible (e.g. veterinarian, policy makers) subsequently have to decide on how to control such a new disease, although little is known about its origin or behaviour at the time of the initial outbreaks. As information on which animals are affected, where and when the disease occurs is often suggestive for the cause of the disease (Martin et al, 1987), its recording can be a valuable start in a disease control strategy. Although from epidemiological theory it might seem clear what should be done, e.g. towards data collection, in the field things often turn out to be more complicated. The initially collected data for example often are scattered and incomplete. Also the observed clinical signs at first can be associated with an already known or existing disease, or not be considered severe enough to take further action. So in emerging disease control people in charge are often faced with many questions towards the nature of the causative agent, possible hosts, the spread mechanisms involved or potential risk factors.

Research approach towards EMCV

After its initial diagnosis additional information on EMCV came available over time from all over the world (Joo, 1999). Nonetheless, detailed information on the epidemiology of EMCV in pig farms turned out to be lacking and became the subject of this thesis. Figure 1.1 illustrates how historical data from (clinical) disease outbreaks were combined with knowledge from experts in the field and literature to formulate basic hypotheses on the introduction and spread of the EMC virus in domestic pig farms.

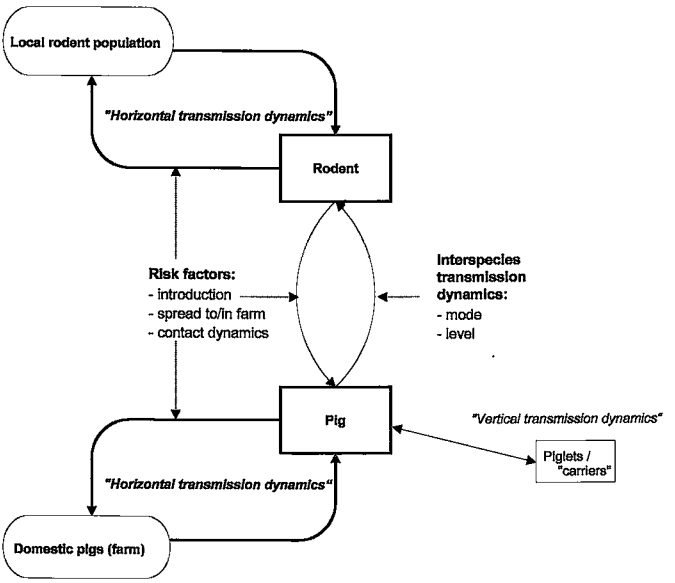

Figure 1.1 Hypothesised transmission routes and –mechanisms for EMCV based on historical data from (clinical) disease outbreaks, expert knowledge and literature.

These hypotheses and the underlying mechanisms were the starting point in the research. Although pigs were the main species of interest, rodents are generally considered the natural host of EMCV (Acland, 1989), who might introduce and spread the virus by their infected carcasses or faeces. From experiments it is known that pigs themselves are able to spread the virus both horizontally (direct pig-to-pig, Billinis et al., 1999) and vertically (transplacental, Christianson et al., 1992), but other (indirect) transmission routes/vector might as well exist. Since both level and mode of transmission between rodents, pigs and possible other host species were still unknown, this inventory (descriptive research) formed the basis for additional analytical research (risk factor study) and experimental studies on EMCV transmission.

In addition to these more or less "standard" methods in epidemiology (Noordhuizen et al., 2001; Thrusfield, 1997; Martin et al., 1987), for several diseases (e.g. bovine herpes virus 1,

classical swine fever) the use of (simulation) modelling techniques has proven to be worthwhile and helpful (De Vos, 2005; Vonk Noordegraaf, 2002).

Following the arguments by Dijkhuizen and Morris (1997), models can be helpful because they can a) create an objective basis for assessing and assimilating knowledge about a "system", b) detect where essential information is missing or inadequate and c) assist in the management control of the system under study. Therefore, although so far only limited field data was available, a basic model on EMCV spread among pigs was developed to integrate available knowledge and structure existing thoughts on the course of the disease. When proven accurate, the model subsequently could be used to test hypotheses on disease spread and evaluate potential control measures.

1.2 Encephalomyocarditis virus (EMCV)

Etiology

The EMC virus was assigned to the genus Cardiovirus within the family Picornaviridae (Van Regenmortel et al., 2000; Minor et al., 1995; Mathews, 1979), which is considered a group of antigenically indistinguishable but biologically heterogeneous agents previously described as murine enteroviruses (Mathews, 1979, Andrews and Pereira, 1967). EMCV is a ribo nucleid acid (RNA)-virus which is ether-resistant and stable over a wide range of pH-values. Although it is inactivated after 30 minutes at 60° C, some strains have shown a marked thermal stability (Joo, 1999). Like other RNA viruses EMCV has the ability to rapidly revert (Radloff, 1985; Craighead, 1966). The naming of the virus refers to the resulting (clinical) signs in rodents (brain/encephalo) and pigs (heart/myocardium).

The EMC virus is ubiquitous in nature and was first isolated from a chimpanzee with myocarditis in Florida (Helwig and Schmidt, 1945). Subsequently both virus as well as antibodies were found worldwide in a wide variety of species (e.g. horses, cattle, cats, swine and dogs) (Zimmerman, 1994; Tesh and Wallace, 1978), including man (Zimmerman, 1994; Tesh, 1978). The most important disease observed due to EMCV is encephalitis in rodents, myocarditis and reproductive failure in pigs and myocarditis in primates and African elephants (Koenen et al., 1999; Tesh and Wallace, 1978; Simpson et al., 1977; Warren, 1965). Although the observed interspecies infections make EMCV to a potential zoonotic agent (Zimmerman, 1994) and few cases of EMCV infection in humans were documented (Murane, 1981), most of the evidence of infection in humans was indirect, following detection of antibodies. No cross-neutralization was found between EMCV and 62 human enterovirus serotypes or 11 porcine enterovirus serotypes (Zimmermann, 1994). Although the virus apparently infects a wide range of species, pigs are considered the domestic animals most susceptible to clinical disease due to EMCV infection (Joo, 1999).

EMCV in pigs

Disease in pigs caused by EMCV was first reported in Panama 1958 (Murane et al., 1960), followed by reports in many other countries like e.g. Florida (Gainer, 1967), Ausralia (Seaman et al., 1986; Acland and Litteljohns, 1975), Cuba (Ramos et al., 1983), New Zealand (Sutherland et al., 1977), South Africa (Williams, 1981), Brazil (Roehe et al., 1985) and Canada (Dea et al., 1991). In Europe the first reports on (clinical) disease in pigs due to EMCV were from the late eighties and early nineties (Koenen et al., 1999; Knowles et al, 1998; Paschaleri-Papadopoulou et al., 1994, 1990; Sidoli et al, 1989), but structural information on the prevalence and epidemiology of the disease remained rather limited. In Chapter 2 the EMCV prevalence in Europe is studied in more detail.

The course of infection in pigs appears to vary considerably, possibly due to involvement of different strains in different geographical areas, infectious doses, disease history, and age and susceptibility of the individual pig (Joo, 1999; Acland, 1989). Disease due to EMCV may take one of two main forms; an acute myocarditis, usually in young piglets (Gainer, 1967) or reproductive failure in sows (Koenen et al, 1991; Christianson et al., 1990; Joo et al., 1988).
In gross pathology the majority of dead pigs have multiple discrete or coalescent foci of myocardial pallor in the heart (Acland and Littlejohns, 1975) mostly on the epicardium of the right ventricle (Joo, 1999). Definitive diagnosis is reached by virus isolation from affected pig tissues, mostly from heart or spleen. As pigs are generally found death after infection or have a short clinical course, the alternative diagnosis is based on serum antibody to EMCV, either by neutralisation test or hemagglutination test (Joo, 1999; Gard et al., 1974). An enzyme linked immunosorbent assay (ELISA) can also be used to detect EMCV antibodies (Brocchi et al., 2000). Generally serological titers >=1/16 are considered positive (Joo, 1999).

The clinical history of reproductive failure along with preweaning mortality is a useful tip in diagnosis of the reproductive variant of EMCV, although one has to be aware of the potential interference with other diseases due to the non-specific clinical signs (Joo, 1999). Interpretation of serological data in sows is sometimes difficult and/or confusing as positive titers not always have been associated with clinical disease in a farm (Joo, 1999).

Fatal disease among pigs has been found under a wide variety of management styles and standards (Acland, 1989), with oral infection as most likely route (Joo, 1999). As early attempts to demonstrate direct contact infection from pig to pig in experiments had failed (Horner and Hunter, 1979; Tesh and Wallace, 1977; Littlejohns and Acland,1975) a common source of EMCV introduction and spread into pig farms was suggested, most likely rodents (rats and mice) by either infected carcasses or contaminating pig feed with their faeces, (Fig. 1.1). More recent findings, however, indicated that the role of direct pig-to-pig transmission for myocardial EMCV shouldn't be excluded beforehand because virus excretion and contact transmission was demonstrated after experimental infection in pigs (Billinis et al., 1999; Joo, 1999; Foni et al., 1993).

1.3 Aim and objectives

Since clinical disease caused by EMCV had been demonstrated in several European countries, additional research was needed to complement and broaden currently available knowledge on the epidemiology of EMCV in domestic pig farms. To support the development of effective EMC prevention and control strategies at farm level, the research in this thesis focused on a number of key elements in the epidemiology of EMCV infections (Fig. 1.1);

1. Disease occurrence; analyze where and to what extent the virus circulates in domestic pigs to understand the relevance of (clinical) EMCV.
2. Risk factors; identify potential risk factors for the occurrence of EMCV at pig farms to focus and direct the development of EMCV prevention and control strategies.
3. Transmission dynamics at animal level; quantify transmission of EMCV within and possibly among suggested hosts (domestic pigs and rodents), to analyze and test their potential contribution to the course of EMCV outbreaks in pig farms.
4. Outbreak analysis at farm level; combine available information into an integral modelling approach in order to evaluate the role of pig-to-pig EMCV spread in a farm setting and to study the impact of potential control measures.

1.4 Outline of the thesis

To focus research efforts and better understand the (clinical) relevance of EMCV, at first data on disease occurrence at commercial pig farms during the nineties were gathered in different European countries (Chapter 2). The most recent disease outbreaks and the results of serological findings in various countries are described, specified for farms in specific areas of the countries where clinical disease occurred (Italy, Greece, Belgium, Cyprus), for affected as well as (clinical) disease free farms and for specific age groups within affected farms.

A case control study was set up in Belgium to analyse potential risk factors for the clinical appearance of EMCV infections in pig farms, the results of which are presented and discussed in Chapter 3. The data for this study were obtained by a questionnaire which was held on 58 pig farms in an affected area in Belgium, West Flanders.

Several experiments in different settings were performed to study and quantify virus transmission within and among the main suggested animal (host) species involved, domestic pigs (Chapter 4) and rats (Chapter 5). As a follow up to the experiments, the development and eventual size of an outbreak among pigs under field conditions was studied at a farm in Belgium (Chapter 6). As in the performed experiments, the level of virus transmission in the case study was quantified by means of the parameter called the reproduction ratio (R_0). The R_0 is defined as the mean number of new infections that one typical infectious individual

causes in a totally susceptible population. The R_0 has an important threshold at $R_0 = 1$, above which major outbreaks may occur.

In chapter 7 information on EMCV transmission among pigs was combined into a basic modelling concept, which was used to simulate pig-to-pig virus spread at farm level and evaluate the potential role of compartmentalisation (reduction of between pen spread) in the control of EMCV. Chapter 8 summarises and discusses the outcomes and critical elements from the various chapters of the thesis both individually and from the perspective of EMCV control. The chapter ends with the main conclusions from the research described and recommendations for the prevention and control of EMCV.

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Chapter 2

The occurrence of encephalomyocarditis virus (EMCV) in European domestic pigs from 1990-2001.

Paper by H. Maurice, M. Nielen, E. Brocchi, N. Nowotny, L. Bakkali Kassimi, C. Billinis, P. Loukaides, R.S. O'Hara, F. Koenen, 2005. *Epidemiology and Infection* (133), 547-55

Abstract

The occurrence of encephalomyocarditis virus (EMCV) among domestic pigs and wild boar in several European countries is described and discussed. From 1990 to 2001 clinical outbreaks were analysed and serum samples, partly from existing screening programs, were tested for antibodies against EMCV. Most clinical EMCV outbreaks were reported in Belgium (320), followed by Italy (110), Greece (15) and Cyprus (6). The outbreaks appeared to be clustered in "endemic areas" with an increase in outbreaks during autumn and winter months.

The within-herd seroprevalence measured in clinically affected pig farms varied considerably among farms (2-87%), with age (0-84%) and by country. Data from farms with no clinical disease showed that sub-clinical infection with EMCV was found both within (seroprevalence 6-62%) and outside (up to 17%) the endemic areas of the clinically affected countries as well as in the non-clinically affected countries Austria and France (3-5.4%).

Among wild boar, the seroprevalence varied between 0.6 and 10.8%, and a study in Belgium found a prevalence of virus infection of 3.3%.

2.1 Introduction

Encephalomyocarditis virus (EMCU) infection and disease emerged in European domestic pigs in the 1990s. Emerging infections can be defined as those infections that have newly appeared in a population, have rapidly increased their incidence, or expanded their geographic range (Morse and Hughes, 1996). Subsequent to the emergence of a new disease, those responsible have to decide on how to control the disease, although little will be known about its origin and behaviour at the time of the initial outbreaks. Information on which animals are affected, and where and when the disease occurs is often suggestive of the cause of the disease (Martin et al., 1987) and should, therefore, be recorded. Although from theory it seems clear what should be done when a "new" disease emerges, in the field things often turn out to be more complicated. For example, the observed clinical signs might at first be associated with an existing disease or not be considered severe enough to take further action, which often makes the first collected data scattered and incomplete.

EMCV belongs to the genus *Cardiovirus* of the family Picornaviridae and was first isolated from a chimpanzee with myocarditis in Florida (Helwig and Schmidt, 1945). Although the virus has been isolated from various animal species, including monkeys, elephants and squirrels, over a wide geographic range, it is generally regarded as a rodent virus (Tesh and Wallace, 1977). Pigs have been considered to be the most susceptible domestic species and clinical disease due to EMCV was first diagnosed in Panama (Murane et

al., 1960). Disease due to EMCV may take one of two main forms in pigs: an acute myocarditis, usually in young piglets (Gainer, 1967) or reproductive failure in sows (Joo et al., 1988). Currently two mechanisms of transmission are considered most important for EMCV in domestic pigs: *(a)* infection of pigs that ingest either infected faeces or the carcasses of infected rodents; or *(b)* horizontal or vertical pig-to-pig transmission (Koenen et al., 1999; Acland, 1989; Seaman et al., 1986). After the initial outbreak in Panama, outbreaks were reported in Florida (Gainer, 1967), Australia (Acland and Littlejohns, 1975), Cuba (Ramos et al., 1983), New Zealand (Sutherland, 1977), South Africa (Williams, 1981) and Brazil (Roehe et al., 1985) .

Although antibodies against EMCV have been reported in the United Kingdom in the 1970s (Sangar et al., 1977), clinical disease was first recognized in Europe in the late 1980s, when four isolated outbreaks were reported in pig farms in Italy (Knowles et al., 1998; Sidoli et al., 1989) and Greece (Paschaleri-Papadopoulou et al., 1994, 1990). In the early 1990s however, clinical disease outbreaks emerged in a number of European countries (Meroni et al., 2000; Koenen et al., 1999, 1991; Paschaleri-Papadopoulou et al., 1990). These outbreaks were studied during two subsequent European research projects (Koenen et al., 2002, 1997) in which Belgium, Greece, Italy, Cyprus, The United Kingdom, The Netherlands, France and Austria took part.

In this paper the clinical outbreak data and serological findings among pigs and wild boar resulting from these projects are described and discussed to provide a better insight in the occurrence of EMCV at both farm and country level. This information could help to develop hypotheses on the origin, cause and nature of EMCV infection in pig farms and may provide initial clues about where to expect new outbreaks.

2.2 Materials and methods

2.2.1 Definitions

Cut off value:	Antibody titre at or above which (≥) a sample is considered seropositive for EMCV.
Seroprevalence:	Percentage of tested blood samples seropositive for EMCV.
Seropositive farm:	A farm where at least one animal was seropositive.
Herd seroprevalence:	Percentage of tested herds seropositive for EMCV.
Endemic area:	Within this context an endemic area was defined as a region of a country where clinical outbreaks of EMCV occurred.

2.2.2 Virology examination

Virus was detected by virus isolation (VI) on baby hamster kidney (BHK-21) cells , by monoclonal antibody (mAbs-)based sandwich ELISA (Brocchi et al., 2000) or by reverse transcription-polymerase chain reaction (RT-PCR) (Vanderhallen and Koenen, 1997). These assays were developed and/or validated during the first EU-project (Koenen et al., 1997).

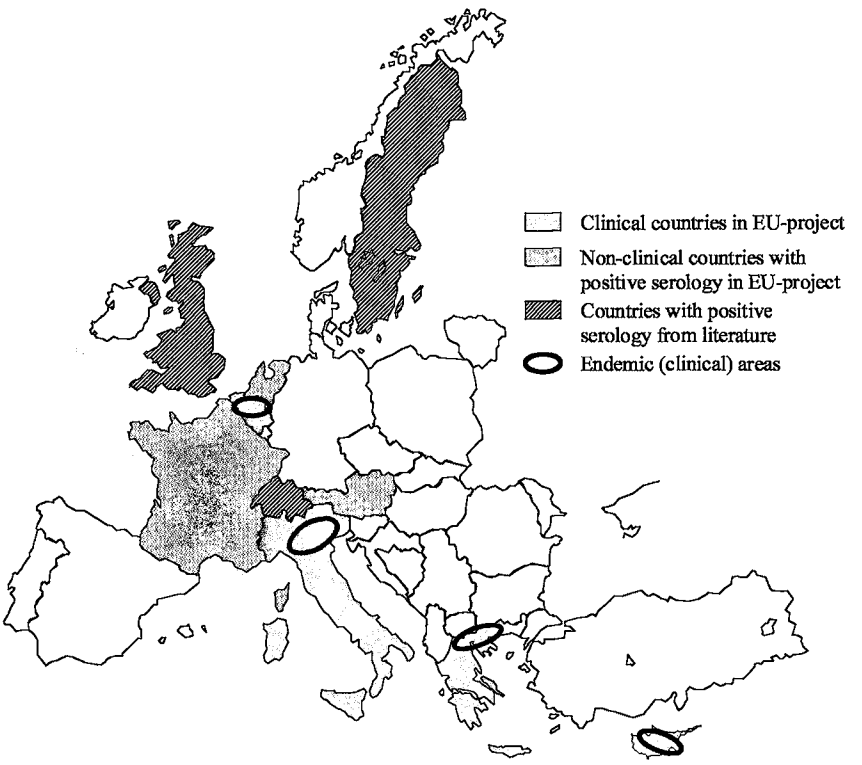

Figure 2.1: Geographical distribution of clinical EMCV outbreaks and the serological status of countries participating in the European research projects.

2.2.3 Serology examination

Antibody to EMCV was detected by a virus neutralisation test (VNT) against the ATCC reference strain (Koenen et al., 1999) or by a mAbs-based competitive ELISA (Brocchi et al., 2000). A comparison of VNT using known positive sera exchanged among the collaborating laboratories showed that the VNT applied in the different laboratories had a comparable sensitivity at the herd level.

All protocols used were very similar, which included the use of 100 $TCID_{50}$ (median tissue culture infective doses) of virus, 50 µl of serum, two-fold serum dilution, incubation of

the virus-serum mixture for 1 h at 37°C and freshly trypsinized BHK-21 or Vero-cells as indicator. The VNT titre was determined after incubation for 2 days.
Serological results on Italian samples were obtained using the mAbs-based competitive ELISA, that uses a threshold calibrated on the cut-off value of the VNT.

2.2.4 Study designs

2.2.4.1 Recording clinical outbreaks

Clinical outbreaks were studied as they occurred in the field. A clinical outbreak of EMCV was defined as when a farm had pigs with either the typical myocardial lesions or reproductive failure (Acland and Littlejohns, 1986), and confirmed by virus detection by ELISA, VI or PCR.

2.2.4.2 Measuring seroprevalence

In general, convenience sampling (with varying sample sizes) was used to collect blood samples for analysis on EMCV affected farms. Later, a standardized sampling scheme, as developed in the EU-project, was applied (Koenen et al., 2002). On pig farms without a clinical history of EMCV, both existing screening programs as well as convenience sampling were used to obtain blood samples for testing. Details on sample origin, sample size, applied tests and cut off values used in the different countries are given in Table 2.1.

Table 2.1: Sampling schemes used for serological studies in the various countries

Country	Sample origin	Area	Sample size	Test	Cut-off
Italy	Swine Vesicular Disease (SVD) survey 1998-2000	Southern Lombardy, Northern Emilia	30-60	ELISA	Equivalent to VNT >=1/100
Greece	Screening program	Random	Varying	VNT	>=1/40
Belgium	Aujeszky-screening	Random	>= 15 (farm size related)	VNT	>=1/32
Cyprus	Convenience sampling	Random	Varying	VNT	>=1/40
France	Aujeszky-screening	Bretagne, Bourgogne	1-165, (>3rd parity sows)	VNT	>=1/32
	Slaughterhouse sampling	Nord-Pas-De Calais	481		
Austria	Convenience sampling 1999-2001	Federal State of Upper Austria	Varying	VNT	>=1/32
UK	Slaugtherhouse sampling	UK, Ireland	Varying	VNT	>=1/32

ELISA, Enzyme-Linked ImmunoSorbent Assay
VNT, Virus neutralisation test

2.3 Results

2.3.1 *Description of clinical outbreaks per country and seroprevalence at clinically affected farms*

From the eight countries that were included in the study, four reported clinical outbreaks of EMCV between 1990 and 2001: Belgium, Italy, Greece and Cyprus. Italy suffered from the myocardial form of EMCV only: no evidence for the reproductive variant of EMCV was seen and 125 fetuses originating from farms with reproductive problems tested negative for EMCV. Among the countries with no clinical disease, Austria analysed 82 organ samples from aborted and stillborn fetuses and piglets showing a "sudden death syndrome", but EMCV was not isolated. In France no EMCV was isolated from heart, spleen and kidney samples from 32 aborted fetuses. The analysis of 40 paired sera from a Dutch farm with suspected clinical disease found no EMCV antibodies, as did the analysis of another 34 sera from EMCV-suspected Dutch pigs (F. Koenen, personal communication). A more detailed description of the clinical findings for each country follows.

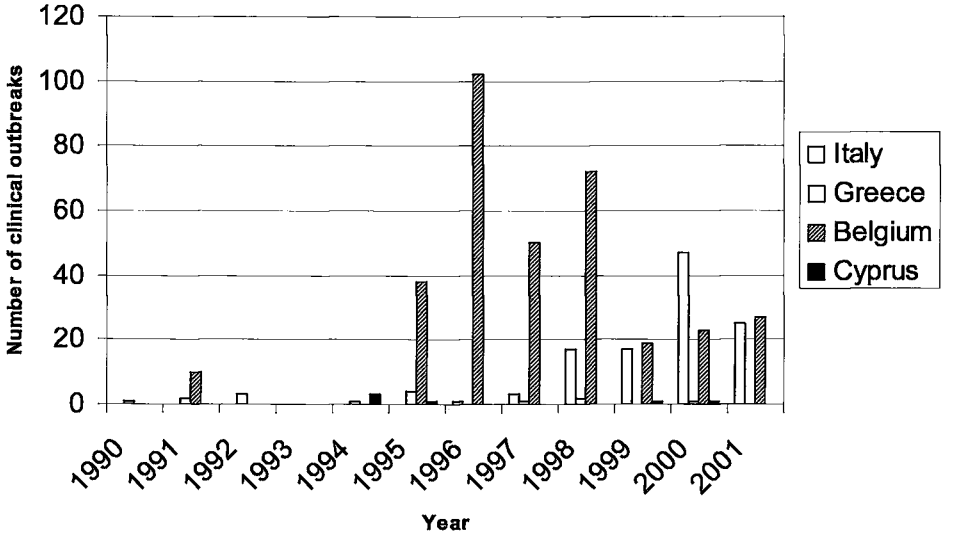

Figure 2.2: Reported clinical EMCV outbreaks by year and country.

Italy

EMCV infection and fatal myocarditis was diagnosed in Italy in 1986, followed by 3 further cases in 1988 (Knowles et al., 1998; Sidoli et al., 1989). After almost ten years of absence, the disease reappeared in pig farms in October 1996, when a severe but isolated outbreak

occurred in a large breeding farm in northeastern Italy. One year later the disease appeared endemically in a small area of Southern Lombardy, with 35 outbreaks occurring between November 1997 and December 1999. Two more outbreaks were recorded in the northeastern region, one in a farm previously affected in 1996. Re-occurrence was observed in four other farms, between 6 and 12 months, and repeatedly over 2 years on one farm.

During 2000 a total of 47 outbreaks (including four re-occurrences) and in 2001, 25 outbreaks (three re-occurrences) of fatal myocarditis were recorded in the endemic area of Southern Lombardy or the bordering regions of Veneto and Emilia (Figs 2.1 and 2.2).

The disease mostly caused low mortality, occurring as sporadic episodes of sudden death of a few (<10) suckling or weaned piglets. However, in some outbreaks the disease was more severe, resulting in 100-400 deaths. Records from 38 affected farms revealed 15 outbreaks with deaths in suckling piglets, 13 outbreaks with deaths in weaned piglets and five outbreaks with mortality in both groups. In four outbreaks, the fattening pigs were affected while in one outbreak mortality was found in both weaned piglets and fattening pigs. The duration of the more severe outbreaks was from one to several months. The frequency of EMCV outbreaks appeared to be highest during the autumn-winter period (Fig. 2.3).

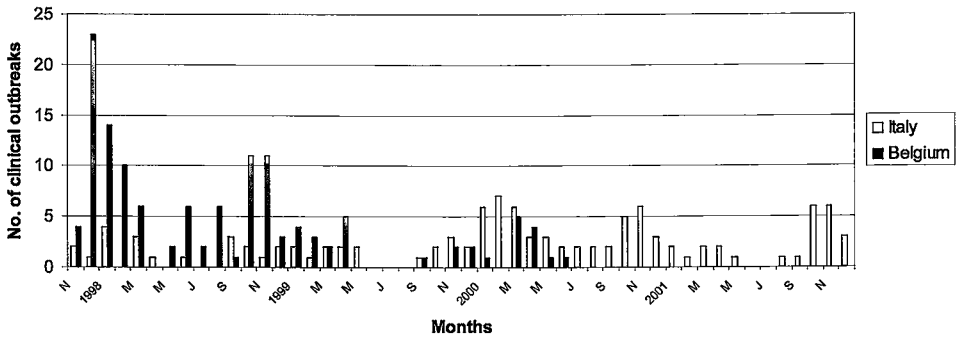

Figure 2.3: Reported clinical EMCV outbreaks per month in Belgium and Italy (detailed Belgian data available only until June 2000)

A total of 2331 sera from 38 clinically affected farms were examined using ELISA, with sample sizes per farm ranging from 10 to >100 sera. Sera collected before the clinical outbreak of EMCV were available from four farms, and in three farms positive sera were detected. No seropositive pigs were detected on six clinically affected farms, possibly because of the small number of samples tested. Antibody-positive pigs were found on 32 farms, with within-herd antibody prevalence ranging from 3 to 60%, and in one case, 100% seroprevalence among 29 sows (Table 2.2).

Overall, of 2331 sera tested, 305 (13%) were seropositive. When possible, the seroprevalence in different groups and categories of pigs was evaluated. Of 1081 sera for

which details were available, 622 were from sows, with a general seroprevalence of 11.2%. Among 459 piglets examined, the overall seroprevalence was lower (4.1 %) than among sows. A more detailed overview of the seroprevalence in the various age categories is given in Table 2.3.

Greece

In Greece, the first detected outbreak of EMCV occurred in October 1986 in a farm of approximately 100 breeding sows near Iraklia in the region of Serres, in the North of Greece. Five further clinical outbreaks were recorded before 1990, all located in the same area of Greece (Paschaleri-Papadopoulou et al., 1990). Between 1990 and 2001, 15 new outbreaks of myocardial EMCV were recorded in pigs, of which four were re-occurences. Eight of the 15 outbreaks were also located in the Serres region (Fig. 2.1). The last outbreak however was located in Loutros, Imathia, Central Macedonia.

Table 2.2: EMCV seroprevalence at clinical farms in the endemic areas

	Overall seroprevalence				Within-herd seroprevalence				
						Ab positive seroprevalence (%)			
Country	Sample size	No. Pos sera/total	%	95% CI	Ab neg	< 5	5-15	> 15	Within-herd *P*-range (%)
Italy	10-100	305/2331	12.9	11.7-14.5	6/38	6/38	13/38	13/38	0-60
					18%	15%	33%	33%	
Greece	24-112	478/723	66.1	62.7-69.6	0	0	0	16/16	47-87
					0	0	0	100%	
Cyprus	30-152	15/182	8.2	4.2-12.2	0	1/2	0	1/2	5-27
					0	50%	0	50%	
Belgium	3-45	141/942	15.0	12.7-17.2	0	1/8	2/8	5/8	2-67
					0	12.5%	25%	62.5%	

Serological investigations of 16 outbreaks in Greece, between 1986 and 2000, found at least one seropositive pig at all farms tested. The average sample size was 45 samples per farm (range 24-112, median 35), and the average within-herd seroprevalence was 66% (range 47 - 87%) (Table 2.2). The seroprevalence increased with age: 40% of the pigs <30 days old had antibody, as did 84% of 6-month-old pigs and 81% of sows (Table 2.3).

Belgium

In Belgium, outbreaks of EMCV related to reproductive failure in sows were first reported in 1991 (Koenen et al., 1991), and outbreaks of myocardial disease in 1995 (Koenen et al., 1999). Currently both forms of EMCV disease are seen in Belgium. The number of outbreaks each year varied considerably (Fig. 2.2). Until 2000 all the outbreaks occurred in the area of West Flanders (Figs 2.1 & 2.2), but in 2001 two clinical cases were seen in the southern part of Belgium. As in Italy, the highest incidence of EMCV outbreaks was seen in the autumn and winter months (Fig. 2.3). Detailed information was available for 29 outbreak farms in

2000 and 2001. Among these, EMCV resulted in clinical signs in fattening pigs in seven farms, in suckling piglets in 14 farms, while one farm only showed clinical signs among weaned piglets. Reproduction problems due to EMCV (deaths among sows, infected foetuses, premature births, etc.) were found on three farms, while two other farms also had clinical signs in suckling piglets.

For eight of the outbreak farms, sera were available from sows and fattening pigs through the Aujeszky screening program. At a titre cut-off value of ≥1/64, a total 141 out of 942 samples (15%) tested seropositive.

Table 2.3: EMCV seroprevalence for different age groups at clinical farms.

	Age class:								
Country	15 days	30 days	45 days	3 months	6 months	Sows			Overall
Greece									
No. farms	4	4	16	16	16	16			16
No. pos. / no. samples	14/34	16/41	86/170	118/183	138/164	106/131			478/723
% Positive	41.2	39.0	50.6	64.5	84.2	80.9			66.1
Italy	Suckling		weaned	Fattening	Gilts	Sows	Primi-parous	Pluri-parous	Sows with dead piglets	Overall
No. pos / no. samples	11/164		6/197	2/98	3/94	67/528	1/37	57/447	9/44	89/1081
% Positive	6.7		3.0	2.0	3.2	12.7	2.7	12.7	20.0	8.2
Cyprus									
1999-outbreak	Suckling		40 days	80 days	140 days	Sows				Overall
No. samples	0/32		4/30	0/30	3/30	0/30				7/152
% Positive	0		13.3	0	10.0	0				4.6

Cyprus

Although there were several suspected cases in Cyprus before 1994 no official diagnoses were made. In 1994 three outbreaks occurred, with considerable losses (70, 200 and 700 pigs) among 3- to 5-month old fattening pigs. On one of the farms that had an outbreak in 1994, disease re-occurred in 1995 with the death of 3200 pigs aged between 1 and 5 months old (Loukaidis et al., 1996). Between 1996 and 1999 several further, uncounted, outbreaks occurred, and vaccination was introduced on affected farms. In both 1999 and 2000 only one clinical outbreak was recorded (Fig. 2.2). Since the recorded outbreaks occurred throughout Cyprus, the whole Cypriot-governed part of Cyprus was considered endemic for EMCV (Fig. 2.1).

At the 1999 outbreak farm, 152 sera were collected from various age groups of pigs (about 30 per group) and in total seven animals were found seropositive (4.6%). No positives were found in the very young piglets (7-15 days of age), the pre-fattening piglets and the sows. The results for the weaned piglets and fatteners are given in Table 2.3. A further 30 pig sera from the farm affected in 2000 were analyzed: 22 sera (73%) were considered negative (< 1/40), three had a titer of 1/80 and five sera had a titer of >1/320 (Table 2.2).

2.3.2 Seroprevalence on non-clinical farms in endemic areas

Samples from farms without clinical EMCV disease, but in endemic regions, were investigated for EMCV antibodies.

Italy

Serological investigations for EMCV antibodies were carried out on 4502 sera from 111 farms located inside the endemic area. Approximately 50% of the farms were antibody-negative, but in the 50% with seropositive pigs, various levels of seroprevalence were detected (Table 2.4). In total, from the 4502 sera tested, 287 (6.4%) were found positive.

Table 2.4: EMCV seroprevalence in non-clinical farms in the endemic areas of the clinical countries.

								Within-herd seroprevalence				
	Overall seroprevalence			Herd seroprevalence Sample					Ab positive seroprevalence (%)			Within-herd P-range
Country	N/total	%	95% CI	size	N/total	%	95%CI	Ab neg.	< 5	5-15	> 15	(%)
Italy	287/4502	6.4	5.7-7.1	30-60	58/111	52.3	43.0-61.5	53/111	25/111	26/111	7/111	0-60
								47.7%	22.5 %	23.5 %	6.3%	
Greece	106/172	61.6	54.4-68.9	3-24	18/18	100	-	0	0	1/18	17/18	14-100
								0	0	5.6%	94.4%	
Cyprus	15/255	5.9	3.0-8.8		11/51	21.6	10.3-32.9	40/51				
								78.4%				
Belgium	490/6770	7.2	6.6-7.9	5-319	64/90	71.1	61.7-80.5	26/90	28/90	26/90	10/90	0-62.5
								28.9%	31.1%	28.9%	11.1%	

Greece

In Greece, 172 samples from 18 non-clinical farms in the endemic area were tested and 106 samples (62%) were seropositive. On average, 10 samples were tested per farm (range 3-24) and at all farms at least one seropositive animal was found (Table 2.4). The within-herd seroprevalence ranged from 14.3% (1/7) to 100% (5/5).

Cyprus

Of 255 samples from 51 non-clinical farms throughout Cyprus, 15 pigs (5.9%) from 11 farms were seropositive (titre ≥1/40), giving a herd seroprevalence of 21.6% (Table 2.4).

Belgium

In 1999 and 2000, 5264 serum samples from both sows and fattening pigs on 73 farms were tested. Of these, 281 samples from 51 farms showed a titre ≥1/32 (5.3%) and 155 samples showed a titre ≥1/64 (2.9%), giving a herd seroprevalence of 7.0%. A further survey of 17 farms in 2000 and 2001 resulted in 209 of 1506 samples with a titre ≥1/64 (13.9%), originating from 14 farms (Table 2.4).

2.3.3 Seroprevalence in a non-endemic areas

Samples from countries with no outbreaks of clinical disease, along with samples from outside the known endemic regions in the clinically affected countries, were collected from farms, slaughterhouse or existing or existing screening programmes. The results are summarized in Table 2.5.

Table 2.5: EMCV seroprevalence at non-clinical farms in the non-endemic areas or countries.

Country	No. farms/ source	Sample size	Cut-off value	No. pos. sera/total	Overall sero-prevalence	No. pos. herds/total	Herd sero-prevalence	Within-herd sero-prevalence	Max. titre
Italy	16	60	1/100	0/832	0	0/16	0	0	-
Greece	11	5-47	1/40	38/224	17.0%	5/11	45.5%	14.3 - 35.9%	-
France									
Bretagne	230	1-165	1/32	87/2507	3.5%	55/230	23.9%	1.42 - 50.0%	1/270
Bourgogne	7	5-20	1/32	5/100	5.0%	2/7	28.6%	12.5 - 20.0%	1/190
Nord-Pas-De-Calais	Abatoir	481	1/32	9/481	1.9%				1/190
Total				101/3088	3.3%				1/270
Austria	Unknown		1/32	70/1305	5.4%				1/362
UK	Abatoir	150	1/32	0/150	0				1/16

Italy

In the Emilia region in Italy, 832 sera from 16 non-clinical farms were tested, all of which were negative for EMCV antibodies.

Greece

In Greece 38 out of 224 samples (17%) tested from 11 non-affected farms were found seropositive. On average 20 samples were tested per farm (range 5 - 47) and on five farms (45%) at least one seropositive animal was found. The within-herd seroprevalence ranged from 14.3% (1/7) to 35.9% (14/39).

France

In total, 3088 pig sera from three different geographical locations in France (Bretagne, Bourgogne, Nord-Pas-De-Calais) were analysed, both from sow farms and a slaughterhouse. Overall, a seroprevalence of 3.3 % was found.

Austria

In Austria, 1305 swine sera were collected, of which the seroprevalence varied from 6.6% in 1999 to 4.6% in 2000 and 5.2% in 2001 (5.4% overall). In addition, one disease-free farm was sampled completely. In total 83 animals were sampled, but no titres >1/16 were found.

United Kingdom

Although a small proportion of the sera (10%) showed low titres (< 1/16), at a cut-off value of 1/32 none of the 150 sera tested could be considered seropositive for EMCV.

2.3.4 EMCV in wild boar

Italy

In total, 1412 serum samples were collected from wild boar during the hunting seasons 1999-2001 in the regions Lombardy and Emilia (Table 2.6). Of 545 sera from boar in Bergamo province (Lombardy), which is close to the endemic area, 59 (10.8%) were found positive. The remaining 867 samples originated from provinces in Lombardy (Varese) and Emilia (Bologna, Parma), regions located outside the endemic area. Only five (0.57%) of these were seropositive. However, no EMCV could be demonstrated from 93 wild boar tissue samples collected during 2001, mainly obtained from tonsils, but also from heart, lung, lymph node and muscle tissue, and submitted to virus detection tests.

Table 2.6: EMCV seroprevalence in wild boar in Italy and France.

	Number of positive sera over years				Sero-	Max.
Country/region	1999	2000	2001	Total	prevalence (%)	titre
Italy						
Lombardy and Emilia (outside endemic area)	0/95	5/612	0/160	5/867	0.58	-
Lombardy (Bergamo province, near endemic region)	22/125	0	37/420	59/545	10.8	-
Total	22/220	5/612	37/580	64/1412	4.5	-
France						
Bretagne				1/13	7.7	1/80
Centre				7/148	4.7	1/95
Poitou-Charentes				3/126	2.4	1/135
Aquitaine				1/77	1.3	1/40
Limousin				1/14	7.0	1/225
Languedoc-Roussillon				1/55	1.8	1/100
Franche-Comte				1/32	3.0	1/1060
Alsace				28/503	5.6	1/375
Champagne-Ardenne				6/64	9.4	1/80
Total				49/1032	4.7	

Belgium

In total, 337, 354 and 536 tonsil samples were collected for virus isolation during the hunting seasons of 1998-2001 in Luxembourg, Namur and Liege respectively. The virus prevalence was 6.8% (23/337) in the 1998-1999 hunting season, 3.1% (11/354) in the 1999-2000 season, and 1.9% (10/536) in the 2000-2001 season. Two different genotypes of EMCV, A and B (Vanderhallen and Koenen, 1998), circulated in the wild boar population.

France

Serum samples collected for a Classical Swine Fever survey were tested for antibody to EMCV and among 1380 samples, an overall seroprevalence of 3.55% was recorded. The positive samples were found in nine out of 21 tested regions, with the highest prevalence in Champagne-Ardennes (9.4%). The maximum titres found ranged from 1/40 in Aquitaine to 1/1060 in Franche-Comte (Table 2.6).

Luxembourg

In total 320 tonsils from wild boar of the Grand Duchy of Luxembourg were tested for the presence of EMCV in 2002. Only one sample was positive, and characterised as type A.

2.4 Discussion

The reported clinical EMCV outbreaks did not appear randomly over the affected countries, but seemed clustered in specific area which are now considered endemic.

Local rodent populations serving as a potential virus reservoir (Acland, 1989; Seaman et al., 1986; Acland and Littlejohns, 1975) are often thought to be responsible for such clustering and might also explain the re-occurrence of outbreaks in the same farms. Morse (1997) has described how emerging viruses already exist in nature and 'emerge' by gaining access to new host populations, often due to ecological or environmental changes. The ability of EMCV to adapt to and emerge in different environments could partly be a result of the complex quasi-species composition (Koenen et al., 2002), a feature often found in RNA viruses (Domingo and Holland, 1994). Migration due to food shortages (Seaman et al, 1986) or changes in rodent population density might mediate the transfer of EMCV from rodents to pigs and also explain the seasonal outbreak patterns observed in Belgium and Italy.

Clinical outbreaks of EMCV were reported in Italy, Greece, Cyprus and Belgium. In Italy and Belgium, the number of clinical outbreaks varied considerably in number and kind (Belgium), while in Greece the picture was more stable over the years with only a few reported clinical outbreaks each year. The high seroprevalence found both within (>60%) and outside (17%) the clinical areas in Greece, however, indicated that a lot of pig farms had been in contact with the virus in the past. In Cyprus, the picture is difficult to unravel because after the first few outbreaks vaccination was given to animals of the same age at the infected farms from 1996 onwards. However, from available information (Veterinary Services, personal communication) it could be concluded that the virus was present throughout Cyprus.

The variable clinical appearance of EMCV in domestic pigs in the various countries might be explained by differences in the pathogenicity of the EMCV strains (Billinis et al., 1999; Knowles et al., 1998), the available infectious dose (Billinis et al., 2004) and/or the susceptibility of the pigs, for example by age and breed.

The affected age categories differed among countries. In Greece, mostly 1-4 months old piglets were clinically affected with considerable losses compared to Italy, where mostly younger (suckling) piglets were affected with on average few losses per farm. In Belgium, disease was recorded in various age classes (suckling piglets, fattening pigs or reproductive failure in sows), but commonly losses were restricted to one age category per farm (Koenen et al., 1999; Love and Grewal, 1986). Although other studies (Meroni et al., 2000; Brocchi et al., 1997) have demonstrated antigenic stability in EMCV, strain differences in biological characteristics are known. Also differences in pathogenicity within the same or between different isolates (Billinis et al., 1999; Koenen and Vanderhallen, 1997; Christianson et al., 1992) and differences in tissue tropism are indicated between various strains (Koenen et al., 1999). That young piglets were always affected clinically in the early outbreaks in Greece might be due to an age susceptibility of heart tissue to the virus combined with decreased protective maternal immunity (Paschaleri-Papadopoulou et al., 1990). Experiments with mice and pigs also indicated that EMCV is more pathogenic to the myocardium of younger animals (Billinis et al., 2004; Tsui et al., 1971). This might explain the higher death rate in younger animals, especially in naïve pig populations (Seaman et al., 1986; Acland and Littlejohns, 1986).

A considerably higher seroprevalence was found in Greece than in other countries, especially in older animals, whereas the overall seroprevalence found in those countries without clinical disease (France, Austria, United Kingdom) was rather low (3-10%). In France, however, seroprevalences of up to 20% were recorded regularly at farm level.
Joo (1999) considered antibody titres of >=1/16 to be significant, so using a cut-off value of 1/32 in this study reduced the chance of non-specificity but will also probably underestimate the real seroprevalence in the field. This subclinical disease due to EMCV may be quite common, even outside the endemic regions or countries. This would be in agreement with findings from other countries (Kudo et al., 1995; Widen and Soderberg, 1994; Biner, 1992; Smith et al., 1992; Zimmerman et al., 1991; Sangar et al., 1977), although the different titre cut-off values used in the various studies, including the current study, make it hard to extrapolate or compare serological data between regions or countries. The need for a more standardised approach in studying a newly appearing disease is self-evident.

It is clear from this study that EMCV can circulate in wild boar populations. In Italy, the seroprevalence in wild boar appeared to be considerably higher in the areas where EMCV was endemic among domestic pigs and while this may be due to transmission between wild and domestic pigs, it probably reflects high prevalences in wild rodents. In Belgium, the prevalence of active virus infection (2.5 - 6%) might be considered high when given the short vireamic period of EMCV. However, the presence of EMCV in the tonsils of boar might also point to latent or persistent infection. Thus, we suggest that wild boar should be considered to be at least temporary (reservoir) hosts for the EMCV in a similar way as for domestic pigs.

The current project showed that EMCV has emerged in a number of European countries, such that some regions should be considered as having endemic infection. At the farm level, serious losses were suffered due to EMCV infections, indicating a need for further research. Since the risk factors for virus introduction, either from the wild rodents or wild boars, into the domestic pig population are not yet known, it remains difficult to predict where new outbreaks of the disease might be expected. More information about the infection status and dynamics of rodent populations might clarify their potential role in the epidemiology of EMCV on pig farms. Such information should be integrated with knowledge about other sources of infection and virus transmission characteristics in both rodents and pigs to generate a feasible control programme, a process to which simulation modelling could make a valuable contribution (De Jong, 1995). Meanwhile, the authors suggest that the monitoring of clinical cases in endemic countries and the genetic typing of strains be continued in order that any changes in incidence of EMCV infection are detected.

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Chapter 3

Factors related to clinical appearance of encephalo-myocarditis virus (EMCV) at Belgian pig farms.

Paper by H. Maurice, Nielen, M., Vyt, Ph., Frankena, K., Koenen, F., 2007. *Preventive Veterinary Medicine* (78), 24-34.

Abstract

We set up a matched case-control study of potential risk factors for clinical encephalomyocarditis virus (EMCV) in 58 pig farms in West Flanders (Belgium). In total, 29 farms experienced a clinical outbreak of EMCV confirmed by EMC virus isolation. Mortality was seen only among suckling piglets (18 case farms), in piglets and other age-groups (4 case farms), or only among fattening pigs (7 case farms). Five farms had reproductive problems among the sows. Control farms were matched geographically on farm size and farm type and were selected on the absence of clinical signs.

A questionnaire on potential risk factors for EMCV was developed to collect data at both case and control farms. The exploration of the data used clusters of factors associated with clinical EMCV infection: (a) rodents, (b) general farm set up and (c) general hygiene. The multivariable relationships between clinical appearance of EMCV and potential risk factors were tested with conditional logistic regression. The final model on all farms contained presence of mice (OR = 8.3) as a risk factor for clinical EMCV infection while the flow of manure up through the slatted floor (OR = 0.11) and movement of manure between manure pits in the pig stable (OR = 0.14) were protective.

3.1 Introduction

Encephalomyocarditis virus (EMCV) is a RNA virus and belongs to the genus Cardiovirus, within the family Picornaviridae (Minor et al., 1995). In rodents (the presumed natural host of EMCV), the virus usually persists without causing disease (Acland, 1989). Antibodies and virus have been recovered from many animal species. In pigs, disease due to EMCV takes one of two main forms: an acute myocarditis (usually causing sudden death in young pigs) and/or reproductive failure in sows (Zimmermann, 1994). Clinical disease caused by EMCV first was observed among domestic pigs in Europe in 1986 and has increased in frequency since then. Outbreaks of acute myocarditis have been reported in Italy (Sidoli et al., 1989), Greece (Paschaleri Papadopoulou et al., 1990,1994), Belgium (Koenen et al., 1996, 1999) and Cyprus (Loukaidis et al., 1996), where EMCV outbreaks often are clustered in so-called "endemic areas" (Maurice et al., 2005). Compared to clinical EMCV cases, similar seroprevalences were found in 50% of the farms without clinical signs in the endemic area in Italy—suggesting a considerable level of sub-clinical infection (Maurice et al., 2005).

Although risk-factor studies are scarce, some authors discuss factors related to the introduction and/or spread of EMC virus into pig farms. Rodents are thought to play a role in the introduction and subsequent spread of the EMC virus in pig stables by means of either their faeces or as infected carcasses (Tesh and Wallace, 1977; Acland and Littlejohns, 1986;

Seaman et al., 1986; Spyrou et al., 2004). Other potential virus-spread mechanisms at the farm level are direct pig-to-pig contact (Foni et al., 1993; Billinis et al., 1999; Maurice et al., 2002), transplacental infection (Christianson, 1992; Koenen et al., 1991,1994) or contact with infected dead pigs. Both infectious dose, virus strain and route of infection were important for the spread of the virus under experimental conditions (Littlejohns and Acland, 1975; Billinis et al., 1999, 2004).

The apparently conflicting reports about the varying clinical picture in combination with the evidence of subclinical infection (Sangar et al., 1977; Maurice et al., 2005), suggest that EMCV strains vary in pathogenicity (Koenen and Vanderhallen, 1997). However, other critical factors related to the clinical emergence of EMCV are still unknown.

Our objective was to explore and describe potential risk factors for clinical EMCV to advise farmers on disease prevention or control. A matched case-control study was set up in West Flanders, an area in Belgium where clinical EMCV outbreaks were reported often at that time (Koenen et al., 1991, 1999).

3.2 Materials and methods

3.2.1 Participating farms

From March 2000 to March 2001, all suspected clinical EMCV outbreaks (30) in the north-western part of Belgium (West Flanders) that were reported to the Regional Veterinary Investigation Centre (RVIC) in Torhout were selected as potential cases. The region of West Flanders is a densely populated pig area with ≈5000 pig farms in a 3000 km^2 area. Out of these 5000 farms, 850 farms are pure-breeding farms, 2000 are fattening farms and 2150 are mixed farms (altogether, responsible for a yearly production of 8 million fattening pigs).

The confirmation of the initial clinical diagnosis (gross pathology) was carried out by VI and RT- PCR at the Veterinary and Agrochemical Research Centre (CODA-CERVA) in Ukkel, Belgium (Koenen et al., 1999) and resulted in 29 clinical EMCV case farms. The RVIC matched each case geographically with one control farm of comparable farm type and size, which was free of clinical signs of EMCV infection. As an indirect indicator for farm set up and management (number, type and ratio of animals present), farm size also supported the match on farm type. Whenever possible, the EMCV status of the control farms (17/29) was checked by serological tests on available samples from the Belgian Aujeszky-disease control programme. In reference to Joo (1999), who considered antibody titres ≥1/16 positive, the cut-off value for the virus-neutralisation test (VNT) (Koenen et al., 1999) was set to ≥1/32 also to reduce misclassification and the effect of possible non-specific test response.

3.2.2 Questionnaire

Based on the prior findings from the field, literature, expert knowledge from partners in EU-projects (Koenen et al., 1997, 2002) and deduced hypotheses (Maurice et al., 2000), a questionnaire on potential risk factors was developed to be completed for both case and control farms. Prior to the farm visits, the questionnaire was tested for completeness and understanding of the questions by people with a (pig) farming background and the partners/experts from the EU-project. In the questionnaire, questions where organised in clusters (Table 3.1) and, where relevant, reflected on the 6 months preceding the interview.

Table 3.1: Items included in the questionnaire on ≈70 potential risk factors for clinical encephalomyocarditis virus infection, used among 58 pig farms in Belgium.

General information
Farm type, secondary enterprises, pig breed, number of animals, number of locations, shortest distance to neighbouring farm with pigs, pig farm density
Introduction and spread of EMCV
(a) Rodent control
Type of rodents present, level of rodent infestation, method of rodent control
(b/c) Bird control & pets
Accessibility of building for birds, method of control, presence of cats and dogs at the farm, access to pig housings
(d) Wild boar
Presence in the neighbourhood, reported EMCV outbreaks among wild boar
(e) Manure handling
Purchase and storage of manure, pump over of manure in pig housing, floor type, overflow of slurry pit
(f) Dead animal handling
Number of checks for dead pigs, removal of dead pigs, storage of dead animals and afterbirths
(g/h) Feed storage & feeding practice
Type of foodstuffs and by-products, method of storage, feed processing at the farm, feeding & drinking facilities
(i) Purchase of pigs
frequency of pig/semen purchase, method of transport, number of source farms
Health and hygiene at the farm
(a) Bio security: avoiding virus entrance
Presence of; hygiene gate and facilities, disinfection basins, fence, delivery room, demands on transporter
(b) Health status
Record on EMCV in past, treatment of pigs for parasites, presence of quarantine room
(c) Vaccination strategy and disease history

For the case farms, an extra section was included about the disease outbreak, referring to the affected age category, observed mortality, duration of the outbreak and the spread of the virus throughout the farm. During the interview, the questions were read out to the farmer by the interviewer and answers mostly were selected from multiple choices or otherwise written down. A full copy of the English questionnaire is available from the first author. A

veterinarian from the RVIC in Torhout visited the case farms, as soon as the clinical signs were detected. At the farm, he filled out the questionnaire and collected material to confirm the initial EMCV diagnosis. The control farms were visited for questionnaire completion by the first author during one of three short periods (18-20 December 2000, 12-13 February 2001 and 11-12 June 2001).

3.2.3 Data analysis

The ≈70 categorised potential risk factors obtained from the questionnaire were at first tested in an univariable setting for their association with the dependent variable (clinical appearance of EMCV). The analysis was done with conditional logistic regression (SPSS, 1999) and followed a stepwise procedure (Hosmer and Lemeshow, 1989). Only variables significant at $P \leq 0.25$ at the likelihood-ratio χ^2-test in the univariable analysis were selected for the next step, in which the potential risk factors were tested in a multivariable setting. Before that, bivariable correlations among the selected variables were evaluated using Fisher's exact test and when relevant, based on biological relevance, one of the two variables was excluded from further analysis.

For the second step the variables were divided in three subcategories based on biological background, also following the outline of the questionnaire. Because the variables for feeding and drinking systems were coded for different animal categories, they were recoded in new variables to apply to all farms.

Per subcategory, a multivariable conditional logistic-regression model was used with backward selection. In every step, the least-significant variable with $P > 0.10$ on the Wald's test was removed from the model, until all remaining variables were significant at $P < 0.10$. Whenever a variable was removed, the change in parameter estimates for the other variables in the model was checked to correct for possible confounders. If a regression coefficient changed >25% in magnitude after removing a factor, the factor was returned to the model and retained (Noordhuizen et al., 1997).

In the third step the factors significant in the subcategorised models were combined in a final model from which factors were removed at $P = 0.10$ in a backward-selection procedure. The overall fit of the model was assessed by the – 2 log-likelihood test (Hosmer and Lemeshow, 1989). Because most of the farms had breeding pigs and a number of variables specifically applied to sows, a separate model was fitted on the farms with sows.

3.3 Results

3.1. Characteristics of 29 case farms

In total 5 case farms were pure fattening farms (median 650 pigs, with 25th and 75th percentiles (Q1/Q3), respectively, 390 and 1315 pigs), while 2 farms had only breeding pigs (respectively 130 and 160 sows). The other 22 farms had both breeding (median 200 sows; 117/285) and fattening pigs (median 1200 pigs; 695/1700). The ratio between fatteners and sows at these farms was 5.9 (median; 4.1/6.7). EMCV infection resulted in mortality among fattening pigs in 7 farms. In 14 farms, EMCV was found among suckling piglets; another farm only showed clinical signs among weaned piglets. Two farms showed mortality among more than one age category. Reproduction problems due to EMCV (deaths among sows, infected foetuses, premature births, etc.) were found on 5 farms two of which also showed clinical signs among suckling piglets.

3.3.2 Characteristics of 29 matched control farms

The non-clinical (i.e., control) farms were matched geographically on farm size and type, which resulted in a comparable set up and excluded farm size and type from the analysis. Out of the 29 farms, 5 were fattening farms (median 700 pigs; 375/1105). The 2 matched pure-breeding farms had 115 and 110 sows, respectively, while the mixed farms had both breeding (median 180 sows; 108/225) and fattening pigs (median 800 pigs, 617/ 1425). The ratio between fattening pigs and sows at these farms was 6.2 (median; 4.2/7.3). From 17 control farms, serological results on sera from the Aujeszky-screening were available taken from sows and/or fattening pigs. From four of these 17 farms the animals tested negative for all samples taken (sample sizes from 23 to 60), while one farm had only one sample (out of 60) with a titre $\geq$1/32. All other 12 farms showed titres $\geq$1/64 in at least one sample (sample sizes from 22 to 319), indicating that they had been in previous contact with the EMC virus. The observed antibody titres varied between 1/32 and 1/128 with farm prevalences up till 35%.

3.3.3 Univariable analysis

In the univariable analyses, the presence of cows at the farm, the presence of mice, feeding automatically and group-drinking systems were significant and several factors related to sanitation, hygiene and manure handling looked protective for clinical EMCV infection (Table 3.2). In general, the different variables seemed directly or indirectly related to rodents or hygiene, which seemed to support the initial hypotheses. Due to data limitations, the variables sanitation (limited number of observations) and hygiene demands on transporter (associated with other biosecurity measures) were not included in the multivariable analysis.

The factors related to feeding and drinking systems were included in the multivariable analysis by means of recoded overall variables (Table 3.2).

Table 3.2: Overview of variables found significant in the matched univariable analysis of risk factors for the incidence of clinical encephalomyocarditis virus infection at 58 Belgian pig farms between March 2000 and June 2001 (P≤0.25).

Variable	Number of discordant (case/control) pairs with "1" only for the:		
	No. pairs	Cases (1,0)	Controls (0,1)
General information:			
Cows present at the farm; 1 = yes vs. no	29	7	3
Introduction and spread of EMCV at the farm:			
Rodent control:			
Presence of mice at night; 1 = medium or high vs. low or zero	28	15	1
Farmer controls rodents himself by sanitation; 1 = yes vs. no	21	1	14
Manure handling:			
Manure was pumped over between pits; 1 = yes vs. no	28	2	11
Manure came up through slatted floor sometimes; 1 = yes vs. no	29	1	13
Dead animal handling:			
Dead pigs were not stored apart from rodents; 1 = no vs. yes	29	3	7
Feeding practice: 1 = automated vs. by hand			
Mated sows	24	8	2
Pregnant sows	24	9	2
Farrowing sows	24	2	6
Weaners	24	7	2
Overall: 1 = automated in min. 1 category (incl fatteners) vs. all by hand	29	4	1
Drinking practice; 1 = group vs. individually			
Mated sows	24	10	2
Pregnant sows	24	9	2
Farrowing sows	24	4	1
Overall; group system in min. 1 category vs. all individually	29	10	3
Health and hygiene at the farm:			
Farm is surrounded by a fence 1 = yes vs. no	29	2	8
Farmer makes hygiene demands on transporter; 1 = yes vs. no	29	2	24
Trader comes into stable during loading 1 = yes vs. no	29	2	6

* 1=risk factor is present, 0= risk factor is absent

3.3.4 *Multivariable analysis*

Three variables remained in the overall model (Table 3.3). The presence of mice appeared to be a risk factor for clinical EMCV infection, while both variables "transport of pig manure from one pit to another" (pumpmanure) and "manure coming up through the slatted floor" (manure overflow) were protective.

Because a number of variables only applied to sows, a separate model was fitted on the farms with sows (N = 25 pairs). In the univariable analysis, the same variables were found significant as for the total dataset, except for the presence of cows at the farm. In the multivariable model, the presence of mice remained significant as a risk factor, and the flow of manure through the slatted floor again was protective (Table 3.3).

Table 3.3: Overall multivariable conditional logistic regression model on the clinical appearance of encephalomyocarditis virus infection at 58 Belgian pig farms between March 2000 and June 2001 (29 matched pairs, G=22.2, with 3 d.f., P<0.0001).

Variables	*b*	S.E. *(b)*	*P*	OR	95% CI for OR	
Overall model:						
Micepresence	2.1	1.1	0.1	8.3	1.0	67
Pumpmanure	-2.0	1.2	0.1	0.1	0.0	1.5
Manureoverflow	-2.2	1.3	0.1	0.1	0.0	1.3
Model for sow farms:						
Micepresence	1.9	1.1	0.1	7.0	0.9	57
Manureoverflow	-1.9	1.1	0.1	0.1	0.0	1.2

3.4 Discussion

3.4.1 *Factors associated with clinical EMCV infection*

That the presence of mice was an important risk factor for clinical EMCV infection seemed in agreement with various other studies, in which rats and mice were at least found associated with clinical outbreaks of EMCV (Acland and Littlejohns, 1975, 1986; Boulton, 1984; Seaman et al., 1986; Sanford et al., 1989; Koenen et al., 1999). Although none of the rat-related factors was significant in our study, their presence was indicated medium (seen at least once per week) or high (seen at least once per day) in ≈2/3 of both case and control farms. Littlejohns and Acland (1975) found piglets dying after eating infected mouse carcasses, while Seaman et al. (1986) suggested that the infection of pigs might be due to interaction between rat, mice and pig populations at the farm. In addition, Spyrou et al. (2004) indicated that EMCV spreads easily amongst rats held under experimental conditions ($R_0 >> 1$) without any mortality—highlighting their potential role as reservoir host. Although in experiments

EMCV was transmitted from rats to pigs and from mice to pigs (Littlejohns and Acland, 1975), until now it is unknown how pig infection really occurs in the field and what species are involved.

The observed protective effect of manure coming up trough the slatted floor possibly might result from contact of pigs with a low dose of the EMC virus in the manure (''vaccination''). The observed effect of this variable on the other hand seems somewhat controversial, as one might expect manure to be a source of ''contact'' infection from pig-to-pig.

3.4.2 Limitations of the study

The selection of case and control farms is one of the most-important steps in the set up of a case-control study (Martin et al., 1987). We selected cases on clinical signs (as being indicative for losses to the farmer) implying that the more-severe cases were included (because outbreaks with only mild clinical signs might be overlooked) (Littlejohns and Acland, 1975). To make them at least at risk for the disease, control farms were matched geographically with case farms in West Flanders (an endemic area for EMCV) (Koenen et al., 1999). Although the presence of antibodies against EMCV prohibited the study of risk factors related to the introduction of the EMC virus into pig farms, for the seropositive control farms it at least indicated that they really were (or had been) at risk for developing clinical EMCV infection. Because the VN-test characteristics were not yet known under field conditions, the presented results in control farms are apparent prevalences. In experimentally infected swine, SE of the VNT at a dilution of 1/32 was estimated to be 80.3% and SP 100% (Zimmerman et al., 1990). This would imply that the true prevalence at and among the control farms possibly is underestimated. Because the observed titers were comparable to results from other serological studies among apparently healthy pigs (Sangar et al., 1977; Kudo et al., 1995) and were relatively low (mainly 1/32 or 1/64) compared to titers observed after recent clinical (experimental) infection (up to 1/4096) (Koenen and Vanderhallen, 1997; Maurice et al., 2002), we did not consider these titers indicative for recent clinical infection in the control farms. In recognition of the diverse or unknown serological status of some control farms an additional univariable analysis was performed (data not shown) in which only the pairs with a serological positive control farm were included. Despite the relatively small number of observations, all variables from the overall univariable analysis remained significant albeit sometimes with somewhat higher *P*-values. Future research should aim at further understanding the biological mechanism behind the risk factors we found. Experimental work by Billinis et al. (2004) supports the hypothesis that the different risk factors mediate the infection process by influencing the infectious dose available to pigs possibly combined with the susceptibility of the pigs (due to age or breed) and the pathogenicity of the EMCV strain involved.

The effect of the limited sample size on the power of the study and the interpretation of (potential) risk factors only significant in the univariable analysis was recognised and should be taken into account. In our study, control farms were matched on farm size and type which increased similarity between cases and controls, but prohibited direct analysis of these variables (Martin et al., 1987). In a study among swine herds in Iowa (Zimmerman et al., 1991) no association was found between herd size and the prevalence of EMCV possibly questioning the need to match for this factor.

A risk in preparing a questionnaire to study a rather unknown disease is focussing too much on factors you think are important beforehand, and many questions in the current study focussed on the role of rodents in the epidemiology of EMCV. Because the questionnaire was based on available knowledge on EMCV from experts and literature and farmers did not really bring up other items during the interviews, the risk of missing other important items was considered limited. Although it remained unclear for the not-serotested control farms whether they really had been at risk for clinical EMCV infection, reduction of the number of rodents at the farm might take out one of the links in the EMCV infection chain or limit the spread of infected rodent manure in the pig stable and thus lower the infection pressure. Future research should further enlighten whether and how either one of these mechanism plays a role in the epidemiology of EMCV.

3.5 Conclusions

This study showed that odds of clinical EMCV infection were considerably higher (OR = 8.3) among farms with a reported medium or high presence of mice compared to control farms with few or no mice present, while the flow of manure up through the slatted floor (OR = 0.11) and movement of manure between manure pits in the pig stable (OR = 0.14) were protective.

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Chapter 4

Transmission of encephalomyocarditis virus (EMCV) among pigs experimentally quantified

Paper by H. Maurice, M. Nielen, J.A. Stegeman, H. Vanderhallen, F. Koenen, 2002. *Veterinary Microbiology* (88), 301-314.

Abstract

Two types of transmission experiments were performed to estimate the basic reproduction ratio R_0, indicating the level of Encephalomyocarditis virus (EMCV) transmission among pigs. In a first experimental set-up with nine separate pairs, one randomly chosen piglet per pair was inoculated with a Belgian (myocardial) EMCV strain (B279/95, 10^3 $TCID_{50}$/ml oronasally) and placed back into the pen. In the second experiment with two separate groups of five piglets, two piglets in each group were inoculated at the start. During the experiments viraemia in blood and excretions was measured as well as the serological response against EMCV antigen. After death or euthanasia, the piglets were checked for heart lesions and virus isolation was done on various tissues. In both the experiments, the majority of the inoculated piglets either died with typical heart lesions (five out of nine and three out of four resp.), or produced high levels of neutralising antibody. EMC virus was isolated from the hearts of all piglets that died during either one of the experiments. The pairwise experiment revealed a point estimate for R_0 of 2.0 (95% confidence interval (CI) = 0.37 – 10.74), while the group experiment resulted in a R_0-value of 0.71 (95% CI = 0.08 - 4.93). Combining the information from both experiments results in an estimate for R_0 of 1.24 (95% CI = 0.39 – 4.35). Since R_0 has values around the threshold value of 1, the spread of EMCV due to contacts between pigs will in most cases be limited, but due to chance processes may lead to large outbreaks as well.

4.1 Introduction

During the last several years, disease outbreaks due to severe infections with Encephalomyocarditis virus (EMCV) have been reported worldwide among pigs, like, e.g. in Florida (Gainer, 1967), Australia (Acland and Littlejohns, 1975) and New Zealand (Sutherland et al, 1977). More recently in Europe, outbreaks have been reported in, e.g. Greece (Paschaleri-Papadopoulou et al., 1992 and 1990), Italy (Sidoli et al., 1989) and Belgium (Koenen et al., 1999). The EMCV is an RNA virus that belongs to the genus *Cardiovirus* of the family Picornaviridae and is widespread among countries (Minor et al., 1995, Acland and Littlejohns, 1986). Rodents have frequently been suggested as the natural host and reservoir of EMCV, spreading the disease to a wide variety of animal species, including pigs. Antibodies to EMCV have also been found in humans (Wegscheider et al., 2000; Tesh and Wallace, 1978).

Although EMCV has been recognised as a pathogen of pigs for many years, the clinical signs of the disease appear to vary, causing mortality in piglets and/or reproductive failure among sows (Zimmermann, 1994). Sudden death of pigs due to the myocardial form of EMCV has been reported in piglets in Greece (Paschaleri-Papadopoulou et al., 1990), Italy (Sidoli et al., 1989), Cyprus (Loukaidis et al, 1996) and recently in Belgium (Koenen et al.,

1996 and 1999). For the moment reproductive failure in sows, characterised by, e.g. abortions, still- or weakborn piglets and mummification, has been described in Belgium only (Koenen et al, 1997 and 1991). From the study of Sangar et al (1977), who found antibodies to EMCV in 28% of clinical disease-free pigs tested, it can be concluded that subclinical infection might also play an important role in the epidemiology of EMCV.

At present, two routes of infection are suggested for the spread of EMCV within a pig farm. The first route is infection of pigs that ingest substances (e.g. feaces or carcasses) of infected rodents (Acland, 1989; Seaman et al., 1986; Littlejohns and Acland, 1975). The second route is horizontal or vertical pig-to-pig transmission (Foni et al., 1993; Koenen et al., 1999). If only the first route is important, than control of EMCV problems should be focussed on rodent control. However, if the second route is also important, rodent control alone is insufficient. As a consequence, it is important to quantify the transmission of EMCV between pigs. A commonly used measure of the transmission of infectious agents is the basic reproduction ratio (R_0), which is defined as the mean number of new infections that one typical infectious individual causes in a totally susceptible population (Anderson and May, 1991; Diekman et al., 1990). This transmission parameter has a threshold at the value of 1. If $R_0 < 1$ then an infection fades out after infecting only a few individuals. However, if R_0 exceeds 1, a major outbreak may occur and, also depending on the size of the population and the replacement rate, the infection may persist in the population (De Jong, 1995). The value of R_0 can be quantified in field studies (Stegeman et al., 1995) as well as in small scale transmission experiments (Nodelijk et al, 2001; Bouma et al, 1996). Thus, if pig-to-pig transmission of EMCV has an $R_0 > 1$, we can conclude that it is an important route for the within-herd transmission of the virus. For EMCV, the value of this transmission parameter has not been established yet. The goal of the present study was to experimentally quantify the horizontal pig-to-pig transmission of EMCV. In order to find out whether EMCV is able to spread in the pig population by horizontal pig-to-pig transmission, we additionally tested whether the R_0-value of the EMC virus transmission exceeded 1.

4.2 Materials and methods

4.2.1 Virus

The EMCV strain B279/95 was isolated during the first outbreak of myocardial disease due to EMCV in fattening pigs (of about 30-40 kg) in Belgium in August 1995 (Koenen et al, 1999). The virus was isolated and first passaged on Baby Hamster Kidney (BHK-21) cells (Koenen et al, 1999). The virus infectivity of the stock was 10^3 median tissue culture infective doses ($TCID_{50}$)/ml. Former animal experiments showed this virus to be virulent for weaners but non-pathogenic for sows in gestation (Koenen et al., 1999).

4.2.2 Transmission experiments

The power of transmission experiments depends among other things on the number of animals, the experimental design and the expected value of R_0. A design with S (number of susceptible animals) $\approx$ I (number of infective animals) seems an acceptable compromise between different objectives (Kroese and De Jong, 2001, Velthuis et al., 2002). Although antibody titers $\geq$ 1/16 appear to be significant (Joo, 1999), based on additional knowledge from a multi-country inter-laboratory comparison of virus neutralisation tests (VNT), in this study a contact animal was considered infected when an antibody titer $\geq$1/32 could be measured at least once (Koenen et al, 2000). In case of death of the contact animals, contact infection was confirmed by virus isolation from various organs.

4.2.2.1 Pairwise experiment

The first experiment (A) was set up with 18 conventional piglets of 25 kg, all carrying a unique ear-tag for identification. The piglets were free from classical swine fever virus (CSFV), bovine virus diarrhoea virus (BVDV), EMCV, African swine fever virus (ASFV), porcine reproductive and respiratory syndrome (PRRS) virus and Aujeszky's disease virus (pseudorabies virus, PRV). The piglets were randomly allocated to a pair and each pair was housed in a completely separated pen. At day 0 in each pen, one randomly selected piglet was moved to another pen and infected oronasally with 4 ml of the virus B279/95. After 10 h of isolation, the inoculated piglet was reintroduced into the pen.

4.2.2.2 Groupwise experiment

The second experiment (B) was set up with 10 conventional piglets of 25 kg with a unique ear-tag, free from CSFV, BVDV, EMCV, ASFV, PRRS and PRV. The 10 piglets were randomly divided into two groups (B1 and B2). In both groups, 2 randomly chosen piglets were infected oronasally with 4 ml of the virus B279/95 and reunited with their pen mates after 10 h of isolation.

4.2.3 Serological samples and analysis

Virus neutralisation was performed on VERO cells using the ATTC 129B strain (Koenen et al., 1999). In experiment A, blood samples were taken from every pig alive at 0, 6, 12, 14, 16, 21, 27 and finally at 43 days post infection (dpi). In experiment B, blood samples were taken at 0, 3, 7, 10, 14, 18, 22 or 25 dpi.

4.2.4 Virus isolation and post-mortem examination

After death during the experiments or after euthanasia at the end of the experiments, the piglets were checked for heart lesions and virus isolation was performed on various organs and in excreta as described by Koenen et al. (1999). In experiment A, contact animals without a serological reaction were not euthanised at the end of the experiment and therefore, no post-mortem VI was performed on their organs. The scoring system, as described by Billinis et al. (1999), was used to evaluate the severity of gross lesions of the heart (-: no lesions, +: mild lesions, ++: moderate lesions and +++: severe lesions). In both experiments VI was performed on the heart, tonsil, kidney, liver and lungs, while in experiment B VI was also attempted on the spleen. In both experiments, virus neutralisation was attempted at death and also virus isolation was performed on blood, faeces and urine (Koenen et al., 1999).

4.2.5 Statistical analysis

The "general epidemic model" or susceptible-infectious-removed model (SIR) was used to analyse the performed transmission experiments (De Jong, 1995). The probability distribution of the outcome of a transmission experiment, which is the total number of contact infections (final size), can be described in terms of the transmission parameter R_0 (Kroese and De Jong, 2001; De Jong and Kimman, 1994). For all experiments in this study, the probabilities of the observed outcomes were calculated for each possible value of R_0. Those individual likelihoods were aggregated into a combined likelihood $l(R \mid x_1,\ldots,x_n)$ for both types of experiments, which at their maximum value represent the maximum likelihood estimate (MLE) for R_0 (Kroese and De Jong, 2001; Bouma et al., 2000; Vanderhallen et al., 2000). The boundaries of the 95% confidence interval (CI) for R_0 were calculated, together with the p-values to test the null-hypothesis H_0: $R_0 \geq 1$ (Kroese and De Jong, 2001). The null-hypothesis was rejected when the probability was less than 0.05.

4.3 Results

4.3.1 Pairwise experiments (A)

4.3.1.1 Mortality

Out of the nine inoculated piglets, five piglets died suddenly between 2.5 and 5 dpi (Table 4.1). The four remaining piglets were killed at the end of the experiment on day 43 pi. Four out of the nine contact piglets died between 5 and 8 days after reintroduction of the

inoculated pen mate. The remaining five contact animals showed no clinical signs or serological reaction ≥1/32 and were not euthanised at the end of the experiment (Table 4.1).

4.3.1.2 Serological assays

Three of the four inoculated piglets that survived the infection showed antibody titers >1/512 (Table 4.2). The titers started to rise between day 6 and day 9 after inoculation and remained high until the end of the experiment at day 43 pi. One inoculated piglet showed a minor serological response and therefore the oronasal infection of this piglet was considered unsuccessful. None of the surviving contact piglets showed a significant antibody titer (Table 4.2).

Table 4.1: Virus isolation, day of death and detection of lesions in piglets experimentally infected with EMCV virus strain B279/95 (Exp. A)

Experiment A			Died or	Ante-mortem virus isolation [b]			Post-mortem virus isolation					Heart
Pair no.	Pig no.	Pig status [d]	killed (dpi) [a]	Blood	Faeces	Urine	Heart	Tonsil	Liver	Kidney	Lung	lesions [c]
1	45	i	43 k	-	-	-	-	-	-	-	-	-
	51	c	7 d	+	-	+	+	+	+	+	+	++
2	76	i	5 d	-	-	-	+	+	-	+	+	+++
	61	c	8 d	+	+	-	+	+	-	+	+	++
3	57	i	3 d	+	-	-	+	+	+	+	+	++
	64	c	S	-	-	-	-	-	-	-	-	nd
4	60	i	43 k	-	-	-	-	-	-	-	-	-
	56	c	S	-	-	-	-	-	-	-	-	nd
5	53	i	43 k	-	-	-	-	-	-	-	-	++
	65	c	S	-	-	-	-	-	-	-	-	nd
6	62	i	43 k	-	-	-	-	-	-	-	-	-
	59	c	S	-	-	-	-	-	-	-	-	nd
7	58	i	3 d	+	+	-	+	+	+	+	+	++
	55	c	5 d	+	+	+	+	+	+	+	+	-
8	30	i	5 d	+	+	+	+	+	+	+	+	++
	28	c	7 d	+	+	-	+	+	+	+	+	++
9	27	i	2.5 d	+	+	-	+	+	+	+	+	++
	31	c	S	-	-	-	-	-	-	-	-	nd

[a] d: died, k: killed at the end of the experiment, s: survived

[b] +: virus isolated, -: no virus isolated

[c] -: no lesions, +: mild, ++: moderate, +++: severe heart lesions, nd: not done

[d] i: inoculated piglet, c: contact piglet

4.3.1.3 Virus isolation

In the four inoculated piglets that died during the experiment VI was successful from the blood and in three animals also from faeces (Table 4.1). No virus could be isolated from blood, faeces or urine from the four inoculated piglets that survived the experiment. Only from those inoculated animals that died during the experiment, could EMCV be isolated from the heart, tonsils, kidney and lungs (Table 4.1) and in three of them also from the liver. In the four contact piglets that became infected, EMCV was isolated from the blood, heart, tonsils, kidney and lungs (Table 4.1). No virus isolation was performed in the remaining contact piglets (Table 4.1).

Table 4.2: Serological responses in piglets experimentally infected with EMCV virus strain B279/95 (Exp. A)

Experiment A			dpi[a]								
Pair no.	Pig no.	Pig status[b]	0	6	9	12	14	16	21	27	43
1	45	i	neg	>4096	>4096	>4096	>4096	>4096	2048	>4096	2048
	51	c	neg	neg	d	-	-	-	-	-	-
2	76	i	neg	d	-	-	-	-	-	-	-
	61	c	neg	neg	d	-	-	-	-	-	-
3	57	i	neg	d	-	-	-	-	-	-	-
	64	c	neg	neg	neg	neg	neg	neg	neg	neg	neg
4	60	i	neg	neg	256	512	1024	128	32	128	16
	56	c	neg	neg	neg	neg	neg	neg	neg	neg	neg
5	53	i	neg	2048	2048	>4096	2048	2048	512	512	256
	65	c	neg	neg	neg	neg	neg	neg	neg	neg	neg
6	62	i	neg	neg	neg	neg	32	neg	neg	neg	neg
	59	c	neg	neg	neg	neg	neg	neg	neg	neg	neg
7	58	i	neg	d	-	-	-	-	-	-	-
	55	c	neg	d	-	-	-	-	-	-	-
8	30	i	neg	d	-	-	-	-	-	-	-
	28	c	neg	neg	d	-	-	-	-	-	-
9	27	i	neg	d	-	-	-	-	-	-	-
	31	c	neg	neg	neg	neg	neg	neg	neg	neg	neg

[a] titers from virus neutralisation test (VNT) as described by Koenen et al. (1999), d: animal died, neg: titer <=1/16

[b] i: inoculated piglet, c: contact piglet

Table 4.3: Virus isolation, day of death and detection of lesions in piglets experimentally infected with EMCV virus strain B279/95 (Exp. B)

Experiment B			Died or	Ante-mortem virus isolation [b]			Post-mortem virus isolation						Heart
Group	Pig no.	Pig status [d]	killed (dpi)[a]	Blood	Faeces	Urine	Heart	Tonsil	Kidney	Liver	Spleen	Lung	lesions [c]
B1	57	i	1.5 d	+	+	nd	+	+	+	+	+	+	-
	60	i	2.5 d	+	-	nd	+	+	+	+	+	+	++
	27	c	25 k	-	-	-	-	-	-	-	-	-	-
	55	c	25 k	-	-	-	-	-	-	-	-	-	-
	54	c	4.5 d	-	-	nd	+	-	-	-	-	-	++
B2	30	i	25 k	-	-	nd	+	-	-	-	-	-	+
	65	i	3.5 d	-	-	nd	+	-	-	-	-	-	+++
	61	c	25 k	-	-	-	-	-	-	-	-	-	-
	62	c	25 k	-	-	-	-	-	-	-	-	-	-
	63	c	25 k	-	-	-	-	-	-	-	-	-	-

[a] d: died, k: killed at the end of the experiment

[b] +: virus isolated, -: no virus isolated, nd: not done

[c] -: no lesions, +: mild, ++: moderate, +++: severe heart lesions, nd: not done

[d] i: inoculated piglet, c: contact piglet

4.3.2 Group experiments (B)

4.3.2.1 Mortality

Out of the four inoculated piglets, three piglets died suddenly between 1.5 and 3.5 dpi. In one group, one of the three contact piglets died during the experiment at 4.5 days post contact. All surviving piglets were killed at the end of the experiment on day 25 pi (Table 4.3).

4.3.2.2 Serological assays

In experiment B, the first blood sampling was performed on day 3 pi. Only one inoculated piglet survived and showed an antibody response of 1/512 at day 7 pi (Table 4.4). The two surviving contact animals in group B1 showed no serological response. In group B2 an antibody titer of 1/256 was found in one of the contact animals, starting at day 10 pi (Table 4.4).

Table 4.4: Serological responses in piglets experimentally infected with EMCV virus strain B279/95 (Exp. B)

Experiment B			dpi[a]							
Group	Pig no.	Pig status[b]	0	3	7	10	14	18	22	At death
B1	57	i	neg[b]	d	-	-	-	-	-	neg
	60	i	neg	d	-	-	-	-	-	neg
	27	c	neg	neg	neg	neg	neg	neg	neg	neg
	55	c	neg	neg	neg	neg	neg	neg	neg	neg
	54	c	neg	neg	d	-	-	-	-	neg
B2	30	i	neg	neg	512	128	1024	512	1024	256
	65	i	neg	neg	d	-	-	-	-	neg
	61	c	neg	neg	neg	neg	neg	neg	neg	neg
	62	c	neg	neg	neg	neg	neg	neg	neg	neg
	63	c	neg	neg	neg	256	4096	8192	16384	4096

[a] titers from VNT as described by Koenen et al. (1999), d: animal died, neg: titer <=1/16

[b] i: inoculated piglet, c: contact piglet

4.3.2.3 Virus isolation

Only in two out of the four inoculated piglets the EMC virus could be isolated from the blood, but in all the inoculated piglets the virus was isolated from the heart (Table 4.3). In the six contact piglets, EMCV was only isolated in the heart of the contact piglet that died during the experiment. No EMC virus could be isolated from the euthanised contact piglets (Table 4.3).

4.3.3 Quantification of EMCV transmission by means of R_0

A first estimate of the value of R_0 was obtained from the pairwise experiment (A). Since oronasal infection of pig no. 62 was considered unsuccessful, the calculations were based on 8 pairs of animals. From the observed final size of 4 (total number of contact infections), the R_0 was estimated to be 2.0 (95% CI: 0.4 – 10.7). The null-hypothesis $R_0 \geq 1$ could not be rejected ($p = 0.91$). The probability distribution of the final size of experiment A is visualized in Fig. 4.1 for the MLE for R_0 and the borders of the accompanying 95% CI.

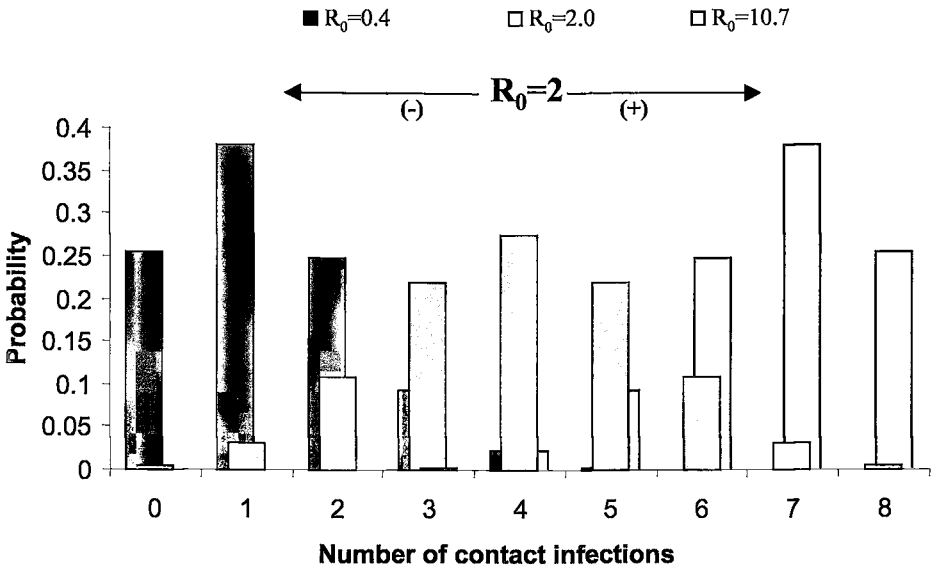

Figure 4.1: Probability distribution of the total number of contact infections for experiment A, Bin(n, $p_{infection}$), for the MLE for R_0 and the accompanying borders of the 95%-CI.

The second estimate of R_0 was based on the final number of contact infections in the group experiments B1 and B2. The pooled data of both group experiments B1 and B2 resulted in an R_0-value of 0.71 (95% CI = 0.1 - 4.9). Despite the obtained point estimate of 0.7, the null-hypothesis $R_0 \geq 1$ could not be rejected (p=0.58). The combined results of the experiments A and B, indicate an estimate for R_0 of 1.2 (95% CI = 0.4 - 4.4). Based on the combined data, the null-hypothesis $R_0 \geq 1$ could still not be rejected (p= 0.79).

4.4 Discussion

The aim of the study was to obtain insight in the level of EMC virus transmission between pigs, as measured by the reproduction ratio R_0. This parameter allows us to assess the

importance of the pig-to-pig transmission within the epidemiology of EMCV infections. If $R_0<1$, only minor outbreaks will occur and the infection will die out, but if $R_0\geq1$, large outbreaks are also possible (De Jong, 1995). Within the practical limitations, various experimental designs are possible. Besides the available number of animals, the design is also determined by the aim of the experiment and the expected value of R_0 (Kroese and De Jong, 2001). The obtained estimates for R_0 indicate values around the threshold value of 1. This means that the spread of EMCV due to contacts between pigs will in most cases probably be limited, but due to chance processes it may lead to large outbreaks as well.

Based on the data from both experiments A ($p = 0.91$) and B ($p = 0.58$), we could not reject the hypothesis that $R_0\geq1$, but based on the CIs around the point estimates for R_0, we cannot conclude whether EMCV can persist in the pig population by pig-to-pig transmission alone. The outcomes of our experiments make it doubtful whether we can establish that in transmission experiments. A next series of experiments would assume that R_0 is slightly above or below 1, but the power of experiments to detect such small differences around the value 1 is low. To overcome this problem, one would have to increase the group size per experiment or raise the total number of experiments (Kroese and De Jong, 2001).

Although various earlier efforts failed to prove contact transmission of EMCV (see, e.g. Christianson, et al, 1990; Horner and Hunter, 1979; Littlejohns and Acland, 1975), in accordance with Foni et al (1993) and Billinis et al (1999) the experiments described in this paper showed clearly that contact infection of a myocardial strain of the EMC virus among piglets is possible. Infection was detected by seroconversion or, in case of death, confirmed by virus re-isolation from various organs. In addition, in a number of contact infected piglets infection could also be proved by serum neutralization or detection of heart lesions. Since the antibody titers initially were assessed on different days, the currently presented titers of some infected animals change relatively fast over time. For those animals, from which still serum was available, the samples where re-tested afterwards on 1 day (results not shown). The newly found titers ruled out those rapid changes in antibody titer that were observed earlier.
Within the performed experiments, a variable response to infection was observed among both inoculated and contact infected piglets, varying from sudden death to a serological response only. Although the exact mechanisms behind the response to infection with the EMC virus are unknown yet, the disease is considered dependent on both viral dose and the susceptibility of the individual animal (Joo, 1999). If we in general assume a gradient of infection from no clinical signs to death as proposed by Thrusfield (1995), than the death of a piglet could be considered the most severe response to infection with EMCV.

After infection, the lymphatic tissue is considered a site of viral replication, but the highest titers were often found in the heart (Joo, 1999). Vlemmas et al. (2000) found in their experimentally infected piglets (Greek strain) that the damage to cardiac muscle cells and Purkinje fibres was related to direct action of EMCV and to hypoxia resulting from virus-induced endothelial lesions, possibly explaining the sudden death of the piglets. The ability of

a piglet to prevent or limit the EMC virus from being transmitted to the cardiac muscle cells and Purkinje fibres (possibly regulated by its immune system and the initial infectious dose) might therefore play a key role in the clinical picture observed. If we assume that the damage to the heart is somehow indicative for the level of virus replication in the animal, than the clinical signs might be an indicator for the amount of virus shed by an infected animal, a topic that is currently being studied (Gelmetti et al., 2006). If so, this could partly explain why the dying inoculated animals more often infected their contact piglets.

Compared to the studies of Foni et al. (1993), Billinis et al. (1999) and Koenen et al (1999) this is the first study in which contact piglets died due to an EMCV infection. Although these findings may partly be explained by the special set-up of the pairwise experiment, also the infectivity of the Belgian virus strain and the individual susceptibility of the animals might be of importance. The high mortality is even more remarkable since, in contrast to studies by Foni et al (1993) and Billinis et al (1999), only oronasal inoculation was applied, which is considered the natural route of infection. On the other hand, the conventional piglets of 25 kg represent an age category often showing the particular myocardial syndrome in the field (Koenen et al., 1999).

Despite the difference in EMC virus strain (Italian and Greek vs. Belgian), the results in terms of virus transmission from experiments by Foni et al. (1993) and Billinis et al. (1999) were quite comparable to the results from our experiments. A recalculation of their experimental results showed R_0 estimates of $R_0 = 0.95$ (95% CI: 0.0-9.9) and $R_0 = \infty$ (95% CI: 0.6-∞), which indicated no significant deviation from unity and large CIs.

The studies by, e.g. Billinis et al. (1999) and Foni et al. (1993) showed that virus can be isolated from blood and excreta already 1 day after inoculation, but some inoculated pigs in the current study might have died too soon to be able to pass on the infection to their contact pigs. This gives rise to the question whether these pigs must be considered "typically infectious" and therefore eligible for the R_0 calculations. However, since the R_0 is defined as the mean number of new infections caused by one typical infectious individual, these pigs might also just represent the cases where no contact infection occurs. Since virus was isolated from various organs and excreta of these animals at death, especially under normal farm conditions, these animals might still remain a source of infection due to, e.g. cannibalism. Based on the arguments of early virus shedding and the intense contact structure in experiment A, it was decided to include these two pairs in the R_0 calculations. Excluding those two animal pairs from the calculations, would have resulted in an R_0 estimate of 4.0 (95% CI: 0.57 - 44.22) and the null hypothesis $R_0 \geq 1$ would still not be rejected.

During the outbreak from which isolate B279/95 was isolated, mortality and seroprevalence was localised in some pens of the affected pig houses (Koenen et al, 1999). This phenomenon was also observed in Cyprus and in Italy, both in field and experimental infections (Foni et al, 1993). The R_0 point estimate of 2.0 from experiment A leads to the probability of a major outbreak of 50%. Populations with a R-value slightly above one have

less major outbreaks and those outbreaks appear to be smaller compared to populations with a large R_0 (Stegeman et al., 1995). Therefore the distribution of minor and major outbreaks with variable sizes related to an $R_0 = 2.0$ seems to agree with the field data (Koenen et al., 1999).

Due to the short vireamia (Billinis et al., 1999, Koenen et al. 1999 and Foni et al. 1993) infected animals might only be a potential danger to their direct neighbours shortly after their infection. Removing clinically ill and dead animals immediately from the pen might therefore be a useful tool in reducing the risk of contact infection, including from cannibalism. In the current study, virus was isolated from various excreta from both inoculated and contact infected piglets, indicating the potential role of excreta in EMC virus spread. Littlejohns and Acland (1975) observed deaths due to EMCV infection mostly at times of excitement like feeding or handling of animals, which according to Foni et al. (1993) might be the result of a damaged myocardium caused by earlier infections. Special attention for management practices in times of EMCV outbreaks could be important in avoiding high pig mortality.

4.5 Conclusion and further research

From the current study, we can conclude that EMCV transmission from pig to pig is very well possible, although the EMC virus spread at a large scale might be confined by the short vireamic period and early death of affected pigs. Since a next series of experiments would assume that R_0 has a value around 1, more small or few larger experiments are needed to prove significantly whether EMCV can persist in the population by pig-to-pig transmission alone ($R_0 \geq 1$) (Kroese and De Jong, 2001).

Although difficult to obtain, longitudinal field data on the number of (contact) infected animals, collected during or shortly after an EMCV outbreak at a farm, could also be used to estimate EMCV transmission. These data could also provide more insight in the level and role of sub-clinical EMCV infections in the epidemiology of EMCV at an infected farm. It is hypothesised that the infectious dose might play a part in causing the varying clinical picture among (contact) infected pigs. Since also the infectivity of inoculated animals is not necessarily the same as from contact infected animals (Kroese and De Jong, 2001), follow up experiments with contact infected pigs might reveal valuable information about EMCV transmission in the field. Since outbreaks of EMCV are often seen in pens at different locations at an infected farm, other infection routes (like, e.g. vertical transmission) cannot be ruled out beforehand. As indicated by Littlejohns and Acland (1975), the role of rodents in the epidemiology of EMCV deserves further attention.

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Chapter 5

Transmission and pathogenicity of encephalomyocarditis virus (EMCV) among rats

Paper byV . Spyrou, H. Maurice, C. Billinis, M. Papanastassopoulou, D. Psalla, M. Nielen, F. Koenen, O. Papadopoulos, 2004. *Veterinary Research* (35), 113-122.

Abstract

Due to the probable role played by rodents as reservoir for the transmission of the EMC virus to pigs, the experiment reported here was performed in order to assess the transmission rate of EMCV within a rat population. Twenty-five eight-week-old Wistar rats housed in individual plastic cages were experimentally infected either with a Greek myocardial EMCV strain (5 rats with a 0.2 x 10^6 $TCID_{50}$ dose per rat and 10 rats with a 0.5 x $10^{4.5}$ $TCID_{50}$ dose per rat, oronasally) or a Belgian myocardial EMCV strain (10 rats with a 0.5 x $10^{4.5}$ $TCID_{50}$ dose per rat, oronasally). Two to five days later, each inoculated rat was moved to a new clean cage and coupled with a contact rat to compare the pathogenicity of the two strains and to estimate the basic reproduction ratio R_0, indicating the level of EMCV transmission. During the experiments, faecal virus excretion was measured as well as the serological response against EMCV. After euthanasia, virus isolation was attempted from different rat tissues. Neither strains produced mortality, nor clinical signs and only low titres of neutralizing antibodies were found. All contact rats, however, were infected and the virus was isolated from their faeces and from various tissues. Both 10-pair experiments revealed a point estimate for the R_0 of ∞ (95%-CI for both the Greek and Belgian EMCV strains = 4.48 - ∞), as did the 5-pair experiment with a higher dose of the Greek strain (95%-CI = 1.83 - ∞). Combining the results from the two 10-pair experiments resulted in an estimate for R_0 of ∞ (95%-CI: 9.87 - ∞). These results indicate that the EMC virus can spread very easily within a rat population by horizontal rat-to-rat transmission ($R_0 >> 1$).

5.1 Introduction

Encephalomyocarditis virus (EMCV) is a member of the genus *Cardiovirus* of the family Picornaviridae, with a worldwide distribution (King et al., 2000). Rodents are considered as the natural host of EMCV (Acland, 1989).

In domestic pigs, EMCV has been recognised either as a cause of mortality in young pigs, due to acute myocarditis, or of reproductive failure in sows (Zimmerman, 1994). The myocardial form has been reported in young pigs in Greece (Koenen et al., 1999b; Paschaleri Papadopoulou et al., 1990), Italy (Knowles et al., 1998; Sidoli et al., 1989), Cyprus (Koenen et al., 1999b) and Belgium (Koenen et al., 1999a; Koenen et al., 1999b). The reproductive form, characterised by abortions, still- or weakborn piglets and mummification, has been reported in Belgium only (Koenen et al., 1991). These apparently conflicting reports suggest that EMCV strains may vary in pathogenicity and tissue tropism. Each form of the disease in pigs seems to be restricted to certain geographical areas, probably due to viral strains originating from local rodent populations. With respect to EMCV infections in pigs, at present time two routes of infection are suggested for the introduction and/or subsequent spread of the

virus within a pig farm. At first, pigs might get infected by the ingestion of substances (e.g. faeces or carcasses) of infected rodents (Acland, 1989; Seaman et al., 1986; Littlejohns and Acland, 1975). The second route is horizontal pig-to-pig transmission during the short period of viraemia (Billinis et al., 1999a; Koenen et al., 1999a) or after reactivation of EMCV persistence (Billinis et al., 1999b). From a recent study by Maurice et al. (2002), in which the EMCV transmission from pig-to-pig contact was experimentally quantified, it can be concluded that the spread of EMCV between pigs in most cases will be limited. The high seroprevalence levels found in the field and the observed clinical infections in separated pens and compartments of affected pig houses might therefore point to an additional spreading mechanism, for example via rodents. Although it is known that rats can be infected with the EMC virus (Acland and Littlejohns, 1975), only little is known about the spread of the virus within the rat population. More insight in the transmission of EMCV among rats might enlighten their role as a possible transmitter or a potential reservoir for the EMC virus and stress the need for an effective rodent control program at farm level. A commonly used measure of the transmission of infectious agents is the basic reproduction ratio (R_0), which is defined as the mean number of new infections that one typical infectious individual causes in a totally susceptible population (Anderson and May, 1991; Diekman et al., 1990). R_0 was used to quantify the transmission of the EMC virus among rats.

The purpose of the present experimental work was (a) to study the pathogenicity of two EMCV strains for rats and (b) to experimentally quantify the horizontal rat-to-rat transmission of EMCV. An R_0-value above one would indicate that the EMC virus is able to spread within the rat population by horizontal rat-to-rat transmission and therefore the hypothesis H_0: $R_0 < 1$ was tested.

5.2 Materials and methods

5.2.1 Animals and experimental design

Fifty eight-week-old Wistar rats were obtained from Theagenion Anticancer Institute of Thessaloniki, Greece. The rats were free of EMCV as assessed by serological and virological examinations before inoculation.

Three experiments were conducted successively, studying the pathogenicity and transmissibility of two EMC virus isolates. In each experiment, rats were randomly assigned into two groups and each rat was housed in an individual plastic cage (26 x 20 x 14 cm). In experiment A, ten rats were infected oronasally with a 0.5 x $10^{4.5}$ $TCID_{50}$ dose per rat of the Greek strain 424/90. Two days after infection each rat was transferred to a new clean cage, together with an uninfected contact rat. Infected and contact rats were euthanized 18 to 59 days post infection (p.i.) (Tab. 5.1). In experiment B, ten rats were infected oronasally with a

0.5 x $10^{4.5}$ $TCID_{50}$ dose per rat of the Belgian strain B275/95. Two days after infection each rat was transferred to a new clean cage, together with an uninfected contact rat. Infected and contact rats were euthanized 11 to 62 days p.i. (Tab. 5.2). In experiment C, five rats were infected oronasally with a 0.2 x 10^6 $TCID_{50}$ dose per each rat of the Greek strain 424/90. To quantify the rat-to-rat transmission in experiment C, each rat was transferred to a new clean cage together with an uninfected contact control rat, at predetermined (2 to 5) days after infection. Infected rats were euthanized 3 to 7 days p.i. Contact rats were euthanized 20 to 23 days post contact (p.c.) (Tab. 5.3).

In the current study a contact rat was considered infected when virus could be isolated from faeces. After euthanasia, contact infection was confirmed by virus isolation from various tissues.

In all experiments, blood samples were taken from each rat before inoculation and on the day of death. Fresh faeces were collected before inoculation and from 2 to 32 and on 58 and 59 days p.i. (experiment A), from 2 to 32 and on 43 and 62 days p.i. (experiment B) and from 2 to 23 days p.i. (experiment C), except for those rats that were killed earlier. After euthanasia, necropsy was performed and samples from brain, thymus, heart, lung, liver, spleen, kidney, pancreas and Peyer's patches were collected for virus isolation.

5.2.2 Viruses

Two EMC virus strains were used. Strain 424/90 was isolated in Greece, in 1990, from the myocardium of a three-month-old pig in a breeding farm with the typical myocardial form of EMCV (Billinis et al., 1999a). Strain B279/95 was isolated during the first outbreak of myocardial disease due to EMCV in fatteners in Belgium in August 1995 (Koenen et al., 1999a). Both were isolated and passaged on Baby Hamster Kidney (BHK-21) cells. For the preparation of the viral inocula of the Greek strain, a 4^{th} passage was performed on the same cell batch. The virus infectivity of the stock was 10^6 $TCID_{50}$/ml. In addition, for the preparation of the viral inocula of the Belgian strain the first passage was used. The virus infectivity of the stock was $10^{4.5}$ $TCID_{50}$/ml. Infected cell culture fluids were centrifuged for 5 min at 2000 *g* to remove cellular debris, mixed 1:1 with sterile glycerol and stored at -20°C.
The identification of the viruses was performed by neutralisation with a specific EMCV antiserum, electron microscopy, RNA-analysis and RT-PCR (Koenen et al., 1997). African and classical swine fever viruses, parvovirus, Aujeszky's disease virus, swine vesicular disease virus and porcine respiratory and reproductive virus were not detected in the inocula. Strain ATTC 129B was used for serological analysis.

Table 5.1: Virus isolation from tissue samples in rats experimentally infected oronasally with 0.5 ml $10^{4.5}$ $TCID_{50}$ of the EMC virus strain G424/90 (Exp.A)

Pair No.	Rat No.	Rat status[a]	Day of introduction	Killed (days p.i. or p.c.)	VNT[b]	Faeces[c]	Heart[de]	Spleen	Lung	Liver	Brain	Kidney	Pancreas	Peyer's patches	Thymus
1	31	i		59	64	2-23	-	-	-	-	-	-	-	+	+
	32	c	2	57	32	6-28	-	-	-	-	-	-	-	+	+
2	33	i		22	32	2-22	+	+	+	-	-	-	+	+	+
	34	c	2	20	16	5-19	+	+	+	+	-	+	+	+	+
3	35	i		21	16	2-21	+	+	+	+	-	-	+	+	+
	36	c	2	19	4	9-19	+	+	+	+	-	+	+	+	+
4	37	i		58	32	2-24	-	-	-	-	-	-	-	+	+
	38	c	2	56	8	6-27	-	-	-	-	-	-	-	+	+
5	39	i		22	16	3-22	+	+	+	+	-	-	+	+	+
	40	c	2	20	8	4-20	+	+	+	+	-	-	+	+	+
6	41	i		20	8	2-20	+	+	+	+	-	-	+	+	+
	42	c	2	18	4	3-18	+	+	+	+	-	+	+	+	+
7	43	i		20	16	2-20	+	+	+	+	-	-	+	+	+
	44	c	2	18	8	7-18	+	+	+	+	-	+	+	+	+
8	45	i		21	16	2-21	+	+	+	+	-	-	+	+	+
	46	c	2	19	8	4-19	+	+	+	+	-	-	+	+	+
9	47	i		58	32	3-28	-	-	-	-	-	-	-	+	+
	48	c	2	56	32	7-29	-	-	-	-	-	-	-	+	+
10	49	i		59	64	2-25	-	-	-	-	-	-	-	+	+
	50	c	2	57	16	6-29	-	-	-	-	-	-	-	+	+

[a] i: inoculated rat, c: contact rat

[b] Serum neutralisation titers on day of death

[c] Time-span of virus isolation (days p.i. or p.c.)

[d] +, virus detected

[e] -, virus not detected

Table 5.2: Virus isolation from tissue samples in rats experimentally infected oronasally with 0.5 ml $10^{4.5}$ $TCID_{50}$ of the EMC virus strain B279/95 (Exp.B)

Pair No.	Rat No.	Rat status[a]	Day of introduction	Killed (days p.i. or p.c.)	VNT[b]	Faeces[c]	Heart[de]	Spleen	Lung	Liver	Brain	Kidney	Pancreas	Peyer's patches	Thymus
1	11	i		19	32	2-19	+	+	+	-	-	-	+	+	-
	12	c	2	17	16	9-15	+	+	+	+	-	+	+	+	+
2	13	i		15	16	2-15	+	+	+	-	-	-	+	+	+
	14	c	2	13	8	9-13	+	+	+	+	+	+	+	+	+
3	15	i		62	64	2-24	-	-	-	-	-	-	-	+	-
	16	c	2	60	32	15-28	-	-	-	-	-	-	-	+	+
4	17	i		13	<2	2-13	+	+	+	+	-	+	+	+	+
	18	c	2	11	<2	2-11	+	+	+	+	+	+	+	+	+
5	19	i		62	16	4-26	-	-	-	-	-	-	-	+	+
	20	c	2	60	8	17-28	-	-	-	-	-	-	-	+	+
6	21	i		15	8	2-15	+	+	+	+	-	+	+	+	-
	22	c	2	13	<2	6-11	+	+	+	+	-	+	+	+	+
7	23	i		62	16	4-26	-	-	-	-	-	-	-	+	+
	24	c	2	60	8	17-28	-	-	-	-	-	-	-	+	+
8	25	i		62	16	2-22	-	-	-	-	-	-	-	+	-
	26	c	2	60	<2	24-28	-	-	-	-	-	-	-	+	+
9	27	i		19	16	4-19	+	+	+	-	-	+	+	+	+
	28	c	2	17	4	9-17	+	+	+	+	-	+	+	+	+
10	29	i		62	16	4-19	-	-	-	-	-	-	-	+	-
	30	c	2	60	8	20-28	-	-	-	-	-	-	-	+	+

[a] i: inoculated rat, c: contact rat

[b] Serum neutralisation titers on day of death

[c] Time-span of virus isolation (days p.i. or p.c.)

[d] +, virus detected

[e] -, virus not detected

Table 5.3: Virus isolation from tissue samples in rats experimentally infected oronasally with 0.2 ml x 10^6 $TCID_{50}$ of the EMC virus strain G424/90 (Exp.C)

Pair No.	Rat No.	Rat status[a]	Day of introduction	Killed (days p.i. or p.c.)	VNT[b]	Faeces[c]	Heart[de]	Spleen	Lung	Liver	Brain	Kidney	Pancreas	Peyer's patches	Thymus
1	1	i		3	<2	3	+	+	+	-	-	-	-	+	+
	2	c	2	20	<2	9-16	+	+	+	-	-	-	+	+	+
2	3	i		4	<2	2-4	+	+	+	-	-	-	+	+	+
	4	c	2	20	12	16	+	+	+	-	-	-	+	+	+
3	5	i		5	4	3-5	+	+	+	-	-	-	+	+	+
	6	c	3	21	<2	9-20	+	+	+	-	-	-	+	+	+
4	7	i		6	4	2-6	+	+	+	+	+	+	+	+	+
	8	c	4	22	<2	16-20	+	+	+	-	-	-	+	+	+
5	9	i		7	8	3-7	+	+	+	+	+	+	+	+	+
	10	c	5	23	<2	16-20	+	+	+	-	-	-	+	+	+

[a] i: inoculated rat, c: contact rat

[b] Serum neutralisation titers on day of death

[c] Time-span of virus isolation (days p.i. or p.c.)

[d] +, virus detected

[e] -, virus not detected

5.2.2 Viruses

Two EMC virus strains were used. Strain 424/90 was isolated in Greece, in 1990, from the myocardium of a three-month-old pig in a breeding farm with the typical myocardial form of EMCV (Billinis et al., 1999a). Strain B279/95 was isolated during the first outbreak of myocardial disease due to EMCV in fatteners in Belgium in August 1995 (Koenen et al., 1999a). Both were isolated and passaged on Baby Hamster Kidney (BHK-21) cells. For the preparation of the viral inocula of the Greek strain, a 4th passage was performed on the same cell batch. The virus infectivity of the stock was 10^6 $TCID_{50}$/ml. In addition, for the preparation of the viral inocula of the Belgian strain the first passage was used. The virus infectivity of the stock was $10^{4.5}$ $TCID_{50}$/ml. Infected cell culture fluids were centrifuged for 5 min at 2000 *g* to remove cellular debris, mixed 1:1 with sterile glycerol and stored at -20°C.

The identification of the viruses was performed by neutralisation with a specific EMCV antiserum, electron microscopy, RNA-analysis and RT-PCR (Koenen et al., 1997). African and classical swine fever viruses, parvovirus, Aujeszky's disease virus, swine vesicular disease virus and porcine respiratory and reproductive virus were not detected in the inocula. Strain ATTC 129B was used for serological analysis.

5.2.3. Serological assay

A virus neutralisation test (VNT) was performed. Two-fold dilutions of serum were made in minimum essential medium (MEM) in 96-well flat-bottomed micro-titration plates (Nunc, Denmark). One hundred $TCID_{50}$ of EMCV was added in equal volume. Plates were incubated at 37°C in a 5% CO_2 atmosphere for 1 h before BHK-21 cells were added. Results were usually read after 48 h incubation. The titres were expressed as the initial dilution of the sera at the 50% end point according to the method of Kärber (1931). The sera were considered positive if the titre was equal to or higher than 1/32 (Koenen et al., 2000).

5.2.4 Virus isolation

Virus isolation was attempted from all rats as previously described (Paschaleri Papadopoulou et al., 1990). In short, tissue supernatants were incubated on BHK-21 cell monolayers. The faeces were diluted 1/10 in MEM with the addition of 600 mg/l sulfadoxin, 120 mg/l trimethoprim and 500 000 IU/l of penicillin. After centrifugation at 3000 *g* for 10 min, supernatants were processed as for tissue homogenates. The samples that showed a cytopathic effect were submitted to a neutralisation test, using specific EMCV-antiserum to identify the isolate. Three blind passages of 3 days each were made of negative samples.

5.2.5. Statistical analysis

The performed transmission experiments were analyzed by means of the "general epidemic model" or SIR-model (Susceptible-Infectious-Removed) (De Jong, 1995). The probability distribution of the outcome of a transmission experiment, which is the total number of contact infections (final size), can be described in terms of the transmission parameter R_0 using an algorithm given by De Jong and Kimman (1994). In the case of a one-to-one or pairwise transmission experiment, the outcome of the infection process is a binary variable: contact infection occurs or not. Therefore, the total number of observed contact infections in n independent replications of a pairwise transmission experiment is binomially distributed. The maximum likelihood estimator (MLE) for the probability of infection is then simply given by the observed proportion of contact infections. If this information is combined with the formula for the final size algorithm, the MLE for R_0 can be described and calculated from the number of contact infections and the number of repetitions of the experiments (Maurice et al., 2002; Velthuis et al., 2002).

In this study, the experiments A and B can both be considered pairwise experiments with 10 repetitions, while experiment C consisted of 5 pairwise repetitions. The boundaries of the 95% confidence interval for R_0 were calculated, together with the p-values to test the null-hypothesis H_0: $R_0 \leq 1$ (Maurice et al., 2002). The null-hypothesis was rejected when the probability was less than 0.05.

5.3. Results

5.3.1 Clinical signs

None of the inoculated or contact infected rats showed any clinical signs nor died.

5.3.2 Serological assay

No neutralizing antibodies were detected in any of the rats before inoculation or contact . However, neutralizing antibodies in low titres were detected in inoculated and contact infected rats. However the titres reached the cut-off value only in few rats of experiment A and B and in no rat in experiment C (Tabs. 5.1, 5.2, 5.3).

5.3.3 Macroscopic lesions

No macroscopical lesions were observed in any organs of inoculated and contact infected rats.

5.3.4 Virus isolation

EMCV was isolated from the faeces of both inoculated and contact rats between days 2 and 29 in experiments A and B, and 2 and 20 in experiment C. In all experiments, EMCV was only isolated from the thymus and Peyer's patches from rats killed late post-infection (57-62 days post inoculation or contact), whilst EMCV was also isolated from several other tissues in rats killed sooner after infection (3-23 days) (Tabs 5.1, 5.2, 5.3).

5.3.5 Quantification of EMCV transmission by means of the R_0-value

A first estimate of the value of R_0 for the Greek EMC virus strain (G424/90) was obtained from experiment A. The R_0 was estimated to be ∞ (95%-CI = 4.48 - ∞) from the observed final size of 10 (total number of contact infections in the 10 pairwise repetitions) and the null-hypothesis $R_0 < 1$ could be rejected ($p = 0.000$).

The data of experiment B with the Belgian EMCV strain (B279/95) resulted in an R_0 value of infinity (∞) (95%-CI = 4.48 - ∞), which also resulted in rejection of the null-hypothesis $R_0 < 1$ ($p = 0.000$). When the results for both the Belgian and Greek EMCV strains (dose $10^{4.5}$) were pooled, the point estimate for R_0 did not change (∞), but the lower limit of the confidence interval was raised from 4.48 to 9.87 (95%-CI: 9.87 - ∞).

Also in the 5-pair experiment with the Greek strain (high dose, 10^6 $TCID_{50}$) all contact rats were infected, again resulting in a point estimate for R_0 of infinity (95%-CI: 1.83 - ∞) and again the null-hypothesis ($R_0 < 1$) was strongly rejected ($p = 0.004$).

5.4 Discussion

5.4.1 Pathogenicity of EMCV among rats

In this experimental work, rats were infected with two different myocardial EMCV strains. After experimental infection with either of the strains, none of the infected rats showed any clinical signs nor died. The inapparent infection of the rats was in agreement with Findlay and Howard (1951), but was in contrast with Kilham et al. (1955) who described paralysis and death after the experimental infection of albino rats with EMCV. It was confirmed that rats survive infective EMC doses that would be lethal for piglets and mice, and they infect contact rats. Transmission from rat-to-rat was slow, slower than in pigs (Billinis et al., 1999a). EMCV was isolated from the faeces of both inoculated and contact rats between days 2 and 29 in experiments A and B, and 2 and 20 in experiment C. Infected rats seem to excrete the EMC virus in faeces for a longer period than piglets do (Billinis et al., 1999a). Taking into

account the resistance of all naked viruses in the environment, the possibility of transmission from rat-to-rat and rat-to-pig during this period is evident.

Neutralizing antibodies were detectable in both inoculated and contact rats independently of the infectious dose. However, the titres were low and delayed after infection. This may partly account for the long viral excretion. In experiments A and B, the titre reached the diagnostic cut-off level of 1/32 (Koenen et al., 2000) in only ten rats. On the contrary, all rats in experiment C, which were infected with a higher dose but killed earlier, had titres lower than the diagnostic cut-off. Following euthanasia, no macroscopical lesions were observed in any organs of any of the rats.

The virus was isolated from several tissues of inoculated and contact infected rats in all experiments independently of the infectious dose. It was most frequently isolated from Peyer's patches and the thymus, and less frequently from other tissues. The number of positive tissues decreased with time. It should be noticed that in rats killed late post-infection, EMCV was isolated from the thymus and Peyer's patches only. The presence of the virus in the lymphoid tissues was in agreement with our previous work on pigs (Billinis et al., 1999b), that the macrophages of these tissues may indicate the sites of viral persistence and routes of viral shedding. In fact, the presence of virus in the Peyer's patches of most rats, in all experiments, indicates that this tissue represents a site of viral persistence after oral infection.

5.4.2 Transmission of EMCV among rats

The second target of this experimental work was to quantify the level of EMCV transmission between rats, as measured by the reproduction ratio R_0. This parameter has an important threshold; if $R_0<1$, only minor outbreaks will occur and an infection will die out, but if $R_0\geq1$, large outbreaks are also possible (De Jong, 1995). Since rats or rodents are often suggested as the potential reservoir for the EMC virus on pig farms (Acland and Littlejohns, 1975; Tesh and Wallace, 1978), information on the EMC virus transmission among rats could be used to ground this hypothesis. In many EMCV outbreaks on pig farms, plagues of rats and mice have been reported (Acland and Littlejohns, 1975; Koenen et al., 1999a; Seaman et al., 1986) and in some of these outbreaks rodents were also tested and found positive for EMCV on virus isolation from organs and/or intestines or faeces (Acland and Littlejohns, 1975; Gainer, 1967; Koenen et al., 1999a). In this study, a rat was considered infected when the EMC virus could be isolated from its faeces and contact infection was confirmed by virus re-isolation from various tissues. Based on the data from the current experiments A, B ($p = 0.000$) and C ($p = 0.004$), the hypothesis $R_0 \leq 1$ was strongly rejected for both virus strains, indicating that each infected rat would at least infect one other rat in a totally susceptible population (Anderson and May, 1991). This implies that EMCV can persist in the rat population by rat-to-rat virus transmission alone, which makes the rat population a potential reservoir for EMCV on commercial pig farms.

5.4.3 Relation to EMCV outbreaks at pig farms

Currently suggested transmission routes from rats to pigs are the following: (a) ingestion of infected faeces from rodents or (b) ingestion of infected rodent carcasses (Acland, 1989; Littlejohns and Acland, 1975; Seaman et al., 1986).

The high R_0, their survival to infection and the long period with virus excretion in the faeces could make rats a potential threat to the pigs on a pig farm. Recent findings from a case study in Belgium by Kluivers et al. (unpublished data) showed that infection among pigs was widespread throughout the affected compartment. Findings from Maurice et al. (2002), however, indicate that the pig-to-pig transmission in most cases will be limited (R_0 close to 1), suggesting that another species such as rats could be involved in the epidemiology of EMCV on pig farms.

Additional research is needed to elaborate the rat hypothesis and to test other existing theories. Seaman et al. (1986) has suggested, for example a role for mice in the infection chain for EMCV, an idea that might be supported by the finding of mice as a risk factor for clinical EMC as found by Maurice et al. (2002).

From the current study, we conclude that both EMCV strains are transmitted very effectively among rats under experimental conditions. This might imply that they could play an important role in the epidemiology of EMCV infections on pig farms by either serving as a reservoir host or as a transmitter of the virus to the pigs. More insight is needed, however, in the contact structure within the rat population and their behaviour on pig farms.

Experiments between rats and pigs, with special emphasis on the infectious dose from rats to pigs, could provide useful information on the transmission of EMCV between these species.

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Chapter 6

Transmission of encephalomyocarditis virus (EMCV) in pigs estimated from field data in Belgium by means of R_0

Paper by M. Kluivers, H. Maurice, P. Vyt, F. Koenen, M. Nielen, 2006. Veterinary Research (37), 757-766.

Abstract

Transmission of encephalomyocarditis-virus (EMCV) has been estimated in experiments, but never using field data. In this field study, a farm in Belgium was selected where the presence of EMCV was confirmed by necropsy and virus isolation. Serology was used to estimate the transmission parameter R_0. In one compartment with 630 pigs, 6 pens were fully sampled, in the remaining 38 pens, 2 randomly selected pigs were bled. The 151 pigs were bled twice and their serum was tested in a virus neutralisation test. Seroprevalence at the first and second sampling was 41 and 43% respectively, with a cut off value of 1:40. R_0 was estimated for 2 scenarios, in- and excluding mortality based on the final sizes from the serological results of the second sampling. The R_0 for the fully sampled pens was estimated between 0.6 and 1.7, the combined estimated R_0 of these 6 pens was 1.36 (95%-CI 0.93-2.23). The median of the estimated R_0 of the partially sampled pens was 1.3 and 1.4. Sampling two pigs per pen provided insight into the spread of the virus in the compartment, while the fully sampled pens provided an accurate estimation of R_0. The low R_0 strongly suggests that EMCV is not very effectively transmitted between pigs. The number of seropositive pigs in a pen and the spread in the compartment suggests that other routes of infection are more important, in this case most likely rodents. Preventing viral spread should therefore be focussed on rodent control instead of reduction of contacts between pigs.

6.1 Introduction

Encephalomyocarditis virus (EMCV) is an RNA-virus belonging to the genus *Cardiovirus* of the family Picornaviridae (Van Regenmortel et al., 2000). It was first described in the 1940's as an infection of laboratory rodents (Jungeblut and Dalldorf, 1943; Jungeblut and Sanders, 1940). Nowadays, rodents are considered the natural hosts of EMCV, in which the virus usually persists without causing disease (Acland, 1989). In pigs, EMCV was recognised as a pathogen many years ago, clinical disease resulting from an infection being first diagnosed in 1958 (Zimmerman et al., 1990). The most important sources of EMCV-infection for swine appear to be feed and water contaminated by rats, other rodents or infected carcasses (Joo, 1999). The clinical signs vary between the different age groups. In young animals and fattening pigs sudden death due to myocarditis is frequently seen, the pigs being found dead without previous signs or dying when being fed or excited (Maurice et al., 2005; Paschaleri Papadopoulou et al., 1992). In sows, reproductive failure can occur (Zimmerman, 1994). The myocardial form has been reported in Greece, Italy and Belgium, the reproductive form in Belgium mainly (Koenen et al., 1999). Subclinical infection has been reported in Europe (Maurice et al., 2005), the USA and Asia. In Britain a seroprevalence of 28% was found (Sangar et al., 1977), in Italy 69% (Gualandi et al., 1989), in the USA 8.5% (Zimmerman et

al., 1993) and in Japan 25.8% (Shibata et al., 1993). Neutralising antibodies can be detected as early as 5 days post-inoculation in experimentally infected pigs (Acland, 1989), whereas the virus can be isolated from blood and excreta already one day after inoculation (Billinis et al., 2004; Maurice et al., 2002; Foni et al., 1993). EMCV-transmission has been described from rodents to a wide variety of other species, including humans (Acland, 1989; Seaman et al., 1986; Littlejohns and Acland, 1975), while a recent study indicated that rats easily spread the virus among each other (Spyrou et al., 2004). Although EMCV-transmission from rodents to pigs is considered important, also the impact of horizontal and vertical pig-to-pig transmission needs to be known/quantified to understand their contribution to the spread of the disease on a pig farm (Maurice et al., 2002; Foni et al., 1993). This could be of great significance for control programmes, since it could indicate whether control should be focussed on rodent-to-pig transmission or, dependent on it's magnitude, also on pig-to-pig transmission. Transmission between pigs has been quantified under experimental conditions (Maurice et al., 2002), but so far never in the field. A commonly used parameter to quantify virus transmission is the basic reproduction ratio (R_0), which is defined as 'the mean number of secondary cases produced by a typical infectious individual during its entire infectious period in a completely susceptible population' (Diekman et al., 1990). This parameter is known to have an important threshold value of 1; if $R_0 < 1$, only minor outbreaks will occur and an infection will die out, but if $R_0 \geq 1$, large outbreaks are also possible (De Jong and Kimman, 1994). An important question is whether under field conditions the R_0 of EMC-virus exceeds 1, indicating that introduction of the virus on a farm may cause a major outbreak, based on pig-to-pig transmission alone. The goal of this study was to quantify the horizontal pig-to-pig transmission of EMCV from field data.

6.2 Materials and methods

6.2.1 Barn history and housing conditions

A pig farm in Belgium was selected in the autumn 2001 because myocarditis with typical EMCV lesions was found in two pigs sent in for necropsy. The pathological diagnosis was confirmed by virus isolation, but this research already started pending the result from the test. The pigs had been located at a growing and finishing farm with 2700 pigs, kept in 5 separate barns. High mortality had occurred in 2 of the barns and from one barn 2 pigs had been sent in for necropsy.

For this research, this particular barn was selected for blood sampling. The barn consisted of only one compartment and originally contained 630 pigs of the same age, in 44 pens. Additionally, 25 older pigs from a previous fattening round were present, housed in 2 pens near the entrance of the barn (Fig. 6.1). About three weeks after the pigs arrived at the farm, a

(possible) flu epidemic occurred in the compartment and the majority of the pigs was ill for a few days. During the week before this epidemic some pigs were found dead without previous signs, this mortality continued for almost two months. At the moment of our first blood sampling, two and a halve months after the arrival of the pigs, approximately 5% of the animals in the compartment had died (based on a count of the remaining animals). In the week previous to the first sampling, no pigs had died and no pigs died between the first and second sampling. The majority of the mortality was suspected to be caused by EMCV by both the farmer and veterinarian, and according to their reports only appeared in the first part of the compartment. However, no records were kept about where and when the pigs had died, and no distinct difference in the number of remaining pigs per pen in the first and second half of the compartment was found.

(a)

12	2 -0	15	2 -1
14	2 -1	13	2 -0
13	2 -0	13	2 -1
14	2 -0	14	2 -1
13	2 -0	14	2 -0
14	2 -1	13	2 -1
12	2 -1	**14***	**14 -4**
13	2 -0	**10***	**10 -1**
14	2 -1	**13***	**13 -6**
13	2 -1	14	2 -1
14	2 -1	14	2 -0
14	2 -1	13	2 -1
15	2 -0	15	2 -1
12	2 -1	13	2 -1
12	2 -1	13	2 -0
15	2 -1	15	2 -0
15	2 -1	15	2 -1
15	2 -1	15	2 -1
12*	**12 -7**	12	2 -1
14*	**14 -8**	15	2 -2
12*	**12 -6**	14	2 -1
14 (older pigs) 2-2		15	2 -2
11 (older pigs) 2-2		12	2 -2
entrance			

* = fully sampled pens

(b)

12	2 -0	15	2 -1
14	2 -1	13	2 -0
13	2 -1	13	2 -1
14	2 -0	14	2 -1
13	2 -1	14	2 -1
14	2 -1	13	2 -1
12	2 -1	**14***	**14 -4**
13	2 -0	**10***	**10 -2**
14	2 -1	**13***	**13 -5**
13	2 -1	14	2 -1
14	2 -1	14	2 -0
14	2 -2	13	2 -1
15	2 -0	15	2 -2
12	2 -1	13	2 -1
12	2 -1	13	2 -0
15	2 -0	15	2 -1
15	2 -0	15	2 -1
15	2 -1	15	2 -1
12*	**12 -7**	12	2 -2
14*	**14 -5**	15	2 -2
12*	**12 -6**	14	2 -2
empty		15	2 -2
empty		12	2 -2
entrance			

* = fully sampled pens

Figure 6.1: Floor-plan of the seropositive animals in the affected compartment at 1st (a) and 2nd (b) sampling (no. live animals in pen, sample size – no. seropositive animals).

The pens in the compartment were separated by closed partitions, but not high enough to prevent all contact between pigs in adjacent pens. The pigs had free access to water, supplied by a nipple, the water coming from a reservoir open at the top (among others for

administering drugs). The pigs were being fed ad libitum, with solid feed automatically administered once a day to dry feeders with an open top. The number of rats present in the compartment at the first farm visit was alarming. They were found dead as well as alive in the pathway, on pipes, in feeders and in pens. At the second visit, the numbers were clearly reduced because rodent control had been carried out.

6.2.2 Sampling and serological/virological examination

Two clusters of three adjacent pens were selected in which the number of pigs was clearly reduced, one cluster was located in the first half of the compartment and one in the last (Fig. 6.1). All the pigs in these 6 pens were sampled. In each of the remaining 40 pens (the 2 pens with older pigs included), 2 randomly selected pigs were bled, identified and marked, to a total of 155 pigs at first sampling. Two weeks later the same pigs were bled a second time, except for the 4 older pigs which had been sent to slaughter. A virus neutralisation test (VNT) was used to test all samples for antibodies against EMCV (Koenen et al., 1999). This test was performed on VERO cells using the ATTC 129B strain (EMCV-reference strain). According to the cut off value used in European EMCV-research (Maurice et al., 2005; Koenen et al., 2002), titres $\geq$1:40 were classified as positive. The expected sensitivity and specificity of the test are 80.3 and 100% respectively (Zimmerman et al., 1990), test characteristics were, however, not well defined but estimated. A seroprevalence estimation in The Netherlands suggested a specificity >91% at cut off 1:32, or 96% at 1:64 (Augustijn et al., 2006).

At the first farm visit feed and water samples were collected in the 2 middle pens of the fully sampled clusters, after the second farm visit 7 live rats were caught and killed. Feed, water and the hearts and spleens from the rats were tested by virus isolation (VI) and RT-PCR (Vanderhallen and Koenen, 1997).

6.2.3 Data analysis

In the analysis, the different pens were regarded as separate, independent groups of pigs. The transmission of EMCV in these groups was analysed using the "general epidemic model" or model Susceptible-Infectious-Removed (SIR)-model (De Jong, 1995). This model starts from the assumption that a susceptible (*S*) animal can become infected and infectious (*I*). The animal is infectious for a while before it is removed (R) from the process (recovered or dead). An R-animal is considered the endpoint of the SIR-process, i.e. it will not become susceptible and/or infectious again in a later stage. The total number of R-animals at the end of the infection process in each pen is called the "final size". In this case, study pigs were sampled twice to establish that the infection cycle in the compartment had ended. The final size was assessed from serology at the second sampling. The probability distribution of the final size per pen can be described in terms of the transmission parameter R_0 (reproduction ratio)

(Maurice et al., 2002; Velthuis et al., 2002; Kroese and De Jong, 2001; De Jong and Kimman 1994), where the likelihood $l(R|x)$ gives the probability of final size x if the reproduction ratio is equal to R. This probability can be calculated by the algorithm described by De Jong and Kimman (1994). In the same way, a combined likelihood can be obtained using the final sizes from independent groups (i.e. various pens). Due to the sampling strategy the prevalence in the pens where only two samples were taken could only be 0, 50 or 100% (0, 1 or 2 positive samples). In case of a prevalence of 50% in the pens with an uneven number of live animals at sampling, final size was rounded up with 0.5 animal. To account for the mortality, final sizes at the individual pen level were assessed for the two most extreme assumptions: (1) including mortality in the final size, assuming all dead pigs had died from an EMCV-infection (counted as R), and (2) excluding mortality from the final size, assuming that the dead pigs had remained susceptible and did not get infected by EMCV before death (counted as *S*).

Exact final sizes in terms of serology were known for the six fully sampled pens. For those six pens the individual probabilities for the observed final sizes at the pen level were aggregated into a combined likelihood $l(R|x_1,.., x_6)$, which at its maximum value represents the maximum likelihood estimate (MLE) for R_0 (Maurice et al., 2002; Velthuis et al., 2002; Kroese and De Jong, 2001) (see Tab. 6.1 for estimated R_0 in case of 15 animals/pen). In order to evaluate the effect of possible multiple virus introductions in the same pen at the same time or within a short period, the transmission in the 6 fully sampled pens was also estimated assuming different numbers of initially infected animals (1, 2, 3 or 5), always including mortality in the final size.

Table 6.1: Overview of R_0-estimations related to all possible final sizes in a set up with initially 1 infectious animal (I_0) and 14 susceptible (S_0) animals per pen.

No. contact infections (I_c)	Final size (I_0+I_c)	R_0-estimation (I_0=1, S_0=14)
0	1	0.0
1	2	0.6
2	3	0.8
3	4	0.9
4	5	1.1
5	6	1.2
6	7	1.3
7	8	1.4
8	9	1.5
9	10	1.7
10	11	1.9
11	12	2.1
12	13	2.5
13	14	3.2
14	15	∞

6.3 Results

6.3.1 Serological and virological results

Of the 151 pigs that were bled twice, 62 (41%) were seropositive in the VNT for EMCV at first sampling and 65 (43%) at second sampling. In the 6 fully sampled pens, 32 (43%) of the 75 pigs were seropositive at first sampling and 29 (39%) at second sampling. In the remaining 38 pens, with 2 pigs sampled, 30 (39%) of the 76 sampled animals were seropositive at first and 36 (46%) at second sampling. The frequency-distribution of the serum titres is shown in Figure 6.2. The 4 sampled older pigs that were removed after first sampling, were all positive. Seropositive pigs were found in 35 of the 44 pens, spread throughout the whole compartment (Fig. 6.1).

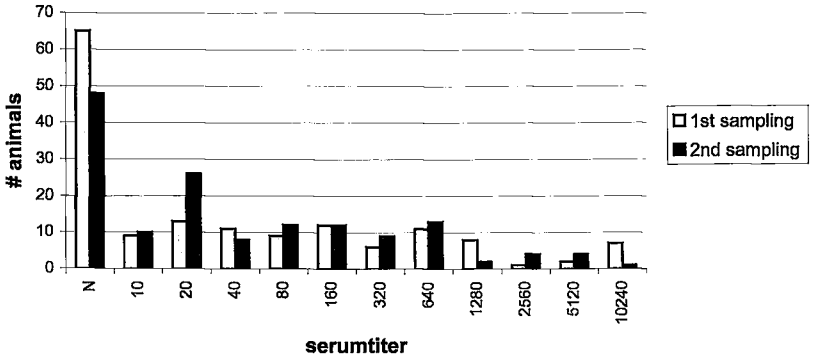

Figure 6.2: Frequency-distribution of observed serumtiters (VNT) at 1st and 2nd sampling (N=negative).

Based on serology and/or mortality 41 pens could be assumed infected. Twelve pigs seroconverted between first and second sampling (Tab. 6.2), seroconversion being defined as a fourfold increase of antibody-titre. Ten others had a lower titre at second sampling, but only 3 of them with a fourfold decrease. VI and RT-PCR performed on the feed and water samples were negative, as well as on the hearts and spleens of the 7 rats.

Table 6.2: Serological results (VNT) of the 1st and 2nd sampling (cut off value 1:40).

VN-test		1st sampling	
		neg	pos
2nd sampling	neg	76	10*
	pos	12**	53

* 3 with at least a fourfold decrease

** all with at least a fourfold increase

6.3.2 Reproduction ratio (R_0)

6.3.2.1 All pens

The frequency-distribution of the R_0 related to the observed final sizes in the pens is shown in Figures 6.3 and 6.4, for different assumptions (including or excluding mortality and cut off value 1:40 or 1:20). Pens without seropositive animals and still 15 pigs present were left out, since one cannot be sure if an initial infection occurred.

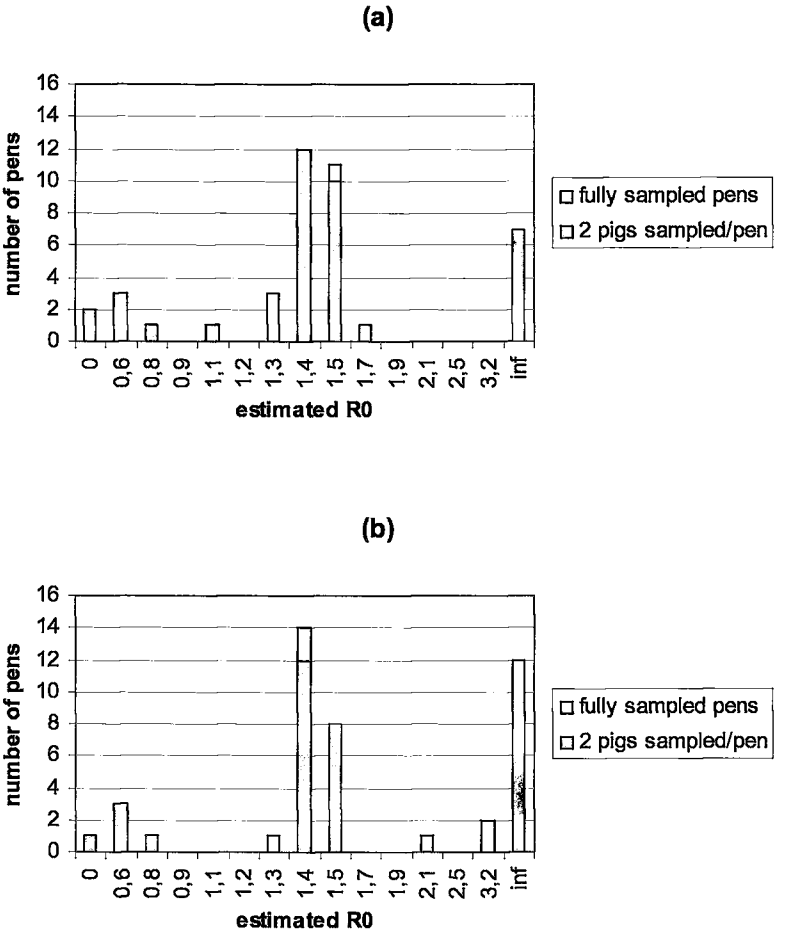

Figure 6.3: Frequency-distributions of the R0-estimations for the individual pens for the final size including mortality, using cut off values 1:40 (a, N=41) and 1:20 (b, N=43). The median values for R_0 were 1.4 and 1.5 respectively.

The median R_0 per pen varied between 1.4 and 1.3 for a cut off value 1:40 (Figs. 3a and 4a) and between 1.5 and 1.3 for a cut off value 1:20 (Figs. 6.3b and 6.4b).

6.3.2.2 Fully sampled pens

From the fully sampled pens exact final sizes were available based on the number of seropositive animals plus mortality data (Figs. 6.3 and 6.4).

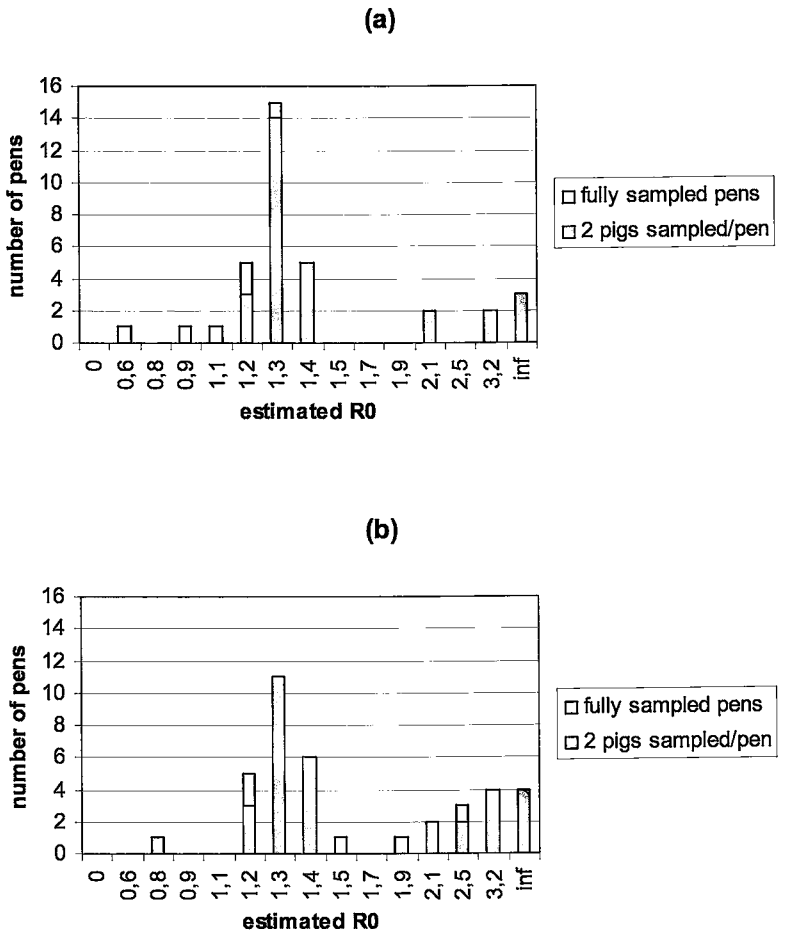

Figure 6.4: Frequency-distributions of the R_0-estimations for the individual pens for the final size excluding mortality, using cut off values 1:40 (a, *N*=35) and 1:20 (b, *N*=38). The median values for R_0 were 1.3 and 1.3 respectively.

Apart from an R_0 per pen, also a combined R_0 was calculated for the 6 pens assuming one initially infected animal (I_0=1, R_0 = 1.36 with 95%-CI 0.93-2.23) and 2, 3 or 5 initial infections (I_0) in a pen of 15 animals (Tab. 6.3).

Table 6.3: Point estimates and related 95% confidence intervals (CI) for R_0 based on combined data from the six fully sampled pens (final size including mortality), assuming different numbers of initial infections (I_0) per pen (N=number of pigs per pen).

I_0	N	R_0	95% CI
1	15	1.36	0.93-2.23
2	15	1.20	0.93-1.97
3	15	1.01	0.66-1.72
5	15	0.61	0.36-0.64

6.4 Discussion

The observed seroprevalence of around 40% is comparable to findings from a survey in Belgium, where a prevalence of 52% at slaughter was found (Koenen et al., 1999). Lower seroprevalences were found by other researchers, but in those cases samples were taken in herds where no outbreak had occurred (Zimmerman et al., 1993; Paschaleri Papadopoulou et al., 1990). A recent European study showed that seroprevalences can vary from 2 to 87% (Maurice et al., 2005). The transmission of EMC-virus in the field has never been quantified, therefore the current findings can only be compared with those from experimental studies (Maurice et al., 2002). The R_0 in experiments were around the threshold value of 1 (0.7, 1.2 and 2). The estimates in this field study are close to this range, with estimations of zero and infinite being caused by the limited sampling sizes in the partly sampled pens. The two evaluated situations, including and excluding mortality, can be considered the most extreme. The true final size and R_0 will be in between the two estimated values. The most reliable R_0-calculations are those from the 6 fully sampled pens, because in these pens exact final sizes were known. The R_0 in these pens varied between 0.6 and 1.7, while including or excluding mortality had some effect on the distribution of the R_0's, but little on the median. In calculating these R_0 values it was assumed that (a) the pens were independent, (b) only 1 animal per pen was initially infected and (c) all the within-pen transmission was pig-to-pig transmission only. Assuming multiple introductions per pen has important consequences for the estimated R_0; it decreases significantly (non-overlapping 95%-CI) when 2, 3 or 5 animals per pen are assumed initially infected (Tab. 6.3). In this case the observed total number of contact infected animals (final size) is no longer caused by just one infectious animal. Therefore the mean number of contact infected animals per initially infected animal decreases.

Reasons to suspect that multiple introductions have taken place within the compartment is that a pen prevalence of 80% (35 of the 44 pens) is not very likely with only 1 initial infection combined with a low R_0. Since the within-pen-R_0 (R_{0w}) is estimated between 1 and 2, the between-pen-R_0 (R_{0b}) can be assumed to be even lower. In experiments with Classical Swine Fever, the R_{0b} in fattening pigs was around 4-5 times lower than the R_{0w} (Klinkenberg et al., 2002). This makes it unlikely that a single introduction in the compartment would lead to the observed wide spread of the EMC-virus in the compartment. Also, with one initial infection one would expect seropositive animals to be more clustered around the pen where the initial infection took place. So, multiple introductions per pen and in the compartment are likely. Possible routes of introduction are the farmer, needles, feed, water and/or rodents. In this particular compartment, vaccination of all pigs and possible parenteral treatment of sick pigs during the flu epidemic were potential routes. Stepping from pen to pen during these actions can be a risk too. The most obvious risk factor and possibly the one responsible for EMCV introduction into various pens are the rats, which were present in abundance. The inability to isolate the virus from the captured rats does not exclude the potential role of them in the EMCV outbreak on this farm, since only very little is known about the temporal relation between an outbreak in the pigs following infection in rats.

Another assumption that was made in estimating R_0 is that the second sampling represented the final size of the outbreak. Some seroconversions occurred between the first and second sampling and may also have occurred after the second sampling. However, there was no mortality after the first sampling, which suggests that no susceptible animals were infected. The R_0 would be higher when the final size was not yet reached, but the influence of individual animals on the R_0-estimate is rather limited (Tab. 6.1) and a few more seroconversions would have had little impact.

The chance of misclassification of negative animals was reduced in this case study because of the cut off value of 1:40. Other studies used a cut off value of 1:16 (Gualandi et al., 1989). Figures 6.3 and 6.4 show that the frequency-distributions of the estimated R_0 are similar when the cut off is lowered to 1:20. Due to the imperfect sensitivity of the test, seroprevalence and consequently the R_0 may be somewhat underestimated.

In future research, to define EMCV-transmission in a field situation even more accurately, an earlier start of serological sampling should be attempted, immediately at the start of mortality. Furthermore, more sampling rounds (e.g. at slaughter) would help to ascertain that final size had been reached in a population. Although the most accurate information towards R_0-estimation was obtained from the serological data of the fully sampled pens, the partial sampling in the other pens offered valuable insights in the level and spatial distribution of EMCV infection throughout the compartment. To determine whether rodents play a role in transmission on a farm, more frequent capturing and attempts at virus isolation could give more insight.

In conclusion, data suggest that final size was reached in the barn, allowing an estimation of R_0. The MLE for R_0 from the fully sampled pens was 1.36 and the median value of the estimated R_0 per pen varied between 1.3 and 1.4 (cut off value 1:40) over all pens, suggesting that EMCV is not very effectively transmitted between pigs. The combination of this relatively low R_0 between pigs within pens and the many infected pens is considered unlikely when assuming only one initially infected pig in the barn. Most likely, multiple introductions have taken place and in this particular situation the rats could very well have been the source of these introductions.

Acknowledgments

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Chapter 7

Impact of compartmentalized housing on direct encephalomyocarditis virus (EMCV) transmission among pigs; insight from a model

Paper by H. Maurice, H.H. Thulke, J.A. Stegeman, M. Nielen (Submitted to *Preventive Veterinary Medicine* 2007).

Abstract

Although generally considered a rodent virus, pigs sometimes were suggested a potential reservoir host for encephalomyocarditis virus (EMCV), implying they can keep an infection going by pig-to-pig transmission alone (basic reproduction ratio, R_0 >1). An earlier experimental study on EMCV transmission among pigs ($R_0 \approx 1.24$; CI 0.4-4.4) was inconclusive in this respect. In this study we used a simulation model to extrapolate the experimental results to commercial, compartmentalised pig housings and tested to what extend contacts between pigs in different pens needed to be reduced in order to prevent major outbreaks in a compartment. The final size of simulated outbreaks was measured and the probability to observe outbreaks that affected at least 50 or 80% of the pens was calculated. Simulation scenarios reflect either a completely mixed pig population or increasing effect of fencing on the pig-to-pig transmission between pigs in neighbouring pens. After introduction of a fence as "physical barrier" between pens for any $R_0<1.24$ the probability to observe outbreaks affecting more than 50% of the pens remained below 10%. If fences also reduced contact transmission the probability to observe such outbreaks could be scaled down to about 50% for $R_0<2.7$. Only for $R_0>4$, major outbreaks occurred more often than by chance even if only minimal contact between adjacent pens was allowed. In conclusion the results suggested that in a compartmentalised pig housing one single EMCV introduction is unlikely to cause a major outbreak by direct pig-to-pig transmission alone, especially if the fences not only prevent random mixing but actually reduce the contacts (or their infectiousness) between pigs in adjacent pens.

7.1 Introduction

Although encephalomyocarditis virus (EMCV) is generally considered a rodent virus it has been isolated from various other mammals as well (Tesh and Wallace, 1977). In the nineties encephalomyocarditis was diagnosed more frequently, which might reflect the emergence of EMCV in domestic pigs in Europe (Maurice et al., 2002). Infection with EMCV, an RNA virus (Minor et al., 1995), may result in acute myocarditis recognised by sudden death in young pigs up to 4 months old or reproductive failure in sows (Joo, 1999; Zimmermann, 1994; Acland, 1989; Gainer, 1967). Therewith EMCV outbreaks lead to undesirable consequences for pigs and farmer (Maurice et al., 2005; Koenen et al., 1999, 1996; Paschaleri-Papadopoulou et al., 1994, 1990).

Domestic pigs even have been suggested a potential reservoir host (Smith et al., 1992) which would imply that EMCV is able to maintain itself in a pig population by pig-to-pig transmission alone. From an epidemiological point of view this means that, an infectious pig

in a population of susceptibles infects on average more than one other pig or in other words the basic reproduction ratio (R_0) exceeds one (Diekman et al., 1990). This information is important, because it would imply that the control of rodents (or any other potential non-pig transmission route) alone might not control EMCV infections on a pig farm. From experiments R_0 for EMCV infections was estimated at R_0=1.24, however, the associated confidence interval (CI) was inconclusive with respect to the threshold value of 1 (95% CI 0.39-4.35; Maurice et al., 2002). The R_0-estimate derived from a field observation was also inconclusive (R_0=1.36) and additionally is hampered by possible interference of other potential transmission routes (farmer, rodents) or multiple introductions in the stable (Kluivers et al., 2006).

Since the point estimate of R_0 is close to one, a very large animal experiment would be necessary to have sufficient power to conclude on the value of R_0, which was considered both unethical (mortality of pigs) and expensive. Moreover, adequate experimental settings should mimic farm conditions as commercial pig houses usually are subdivided in separate units (compartments), which subsequently are split up into pens. These physical barriers (fences/walls) between groups of pigs will reduce virus transmission, and consequently, it could be possible that from pig-to-pig transmission only minor outbreaks would occur within a compartment even if R_0>1 in case of random mixing. To avoid potential bias from the field we applied a simulation model to evaluate EMCV spread in a compartmentalized setting for the reported range of R_0-values (Maurice et al., 2002) and tested to what extend the number of contacts between pigs in different pens needed to be reduced compared to those with pen mates in order to prevent major outbreaks in a compartment.

7.2 Materials and methods

An individual-based, stochastic, time-discrete and spatially explicit simulation model was developed in C++.

7.2.1 Modelling unit

The infection process was modelled on an individual pig basis. An imaginary finishing pig compartment was created with two rows of 12 pens with 12 pigs each in total containing 288 pigs following a commonly applied Dutch set up (Fig. 7.1). The compartment was treated either a) as one pen, e.g. to mimic random mixing of all pigs, b) as separated pens, e.g. to simulate the spatio-temporal spread of the infection throughout the compartment.

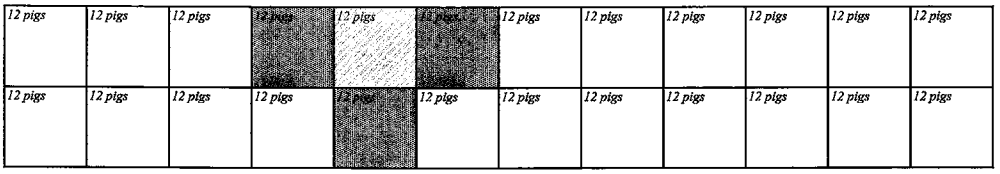


Figure 7.1: Arrangement and contact structure of pens in pig housings. A single pen (▨) was considered in contact with 3 adjacent pens (□) whilst all 4 together provide the neighbourhood of the pen. For pens at the corners of the stable, only 2 contact pens are assumed, forming a neighbourhood of 3 pens.

7.2.2 Modelling pig-to-pig virus transmission

Assuming that EMCV could only be transmitted by direct pig-to-pig contact, virus transmission was modelled according to the standard SEIR-approach, using the states Susceptible (S), Incubating/Exposed (E), Infectious (I) and Removed (R) (De Jong and Kimman, 1994; Becker, 1989; Anderson and May, 1979). With I counting the number of infectious individuals, transmission was modelled using $\left(\beta\frac{I}{N}\right)$ as force of infection with β being a transmission rate and N the number of mixing pigs. Thus the resulting probability of infection (P_{SI}) for a susceptible pig in a group of randomly mixing mates is given by:

$$P_{SI} = 1 - \exp\left(-\beta\frac{I}{N}\right) \quad (1)$$

Following Anderson and May (1979) the respective value for R_0 was approximated by:

$R_0 = \beta \bullet \gamma$, with γ = average length of the infectious period. (2)

7.2.3 Validation of the pig-to-pig transmission model

Treating the whole compartment as one unit of 288 pigs the model was checked against epidemiological theory by assuming different values for R_0, calculating the respective value of β and simulating EMCV spread after randomly infecting one pig. From the final size of the simulated outbreak (i.e. the total number of contact infected pigs) the R_0 was re-estimated - using the Maximum Likelihood Estimation (MLE) (De Jong and Kimman, 1994) - which proofed the expected agreement between the input and output value of R_0.

Fig. 7.2 illustrates the outcome of the simulations on the level of EMCV-infected individuals for different assumptions of R_0. For R_0=0.6 only minor outbreaks were observed; no outbreak affected more than 50 pigs (90% of the outbreaks resulted in less than 5 infected pigs). Following theory, major outbreaks could be expected for R_0>1 with a probability of 1-1/R and an expected final size following R=-ln(1-p_i)/p_i (with p_i= proportion of infected

animals). For R_0=3.0 this would imply an expected final size of ≈270 infected pigs (94%) with a probability of 0.67.

About ≈80% of the simulated outbreaks resulted in outbreaks affecting 260 to 280 pigs (Fig. 7.2), while ≈60% affected over 270 pigs. The minor outbreaks were limited to at most 10 infected pigs. For R_0=1.24 the expected probability to observe a major outbreak would be ≈0.20, with approximately 100 infected pigs involved (p_i=0.36). About 20% of the simulations resulted in outbreaks affecting more than 90 infected pigs (Fig. 7.2).

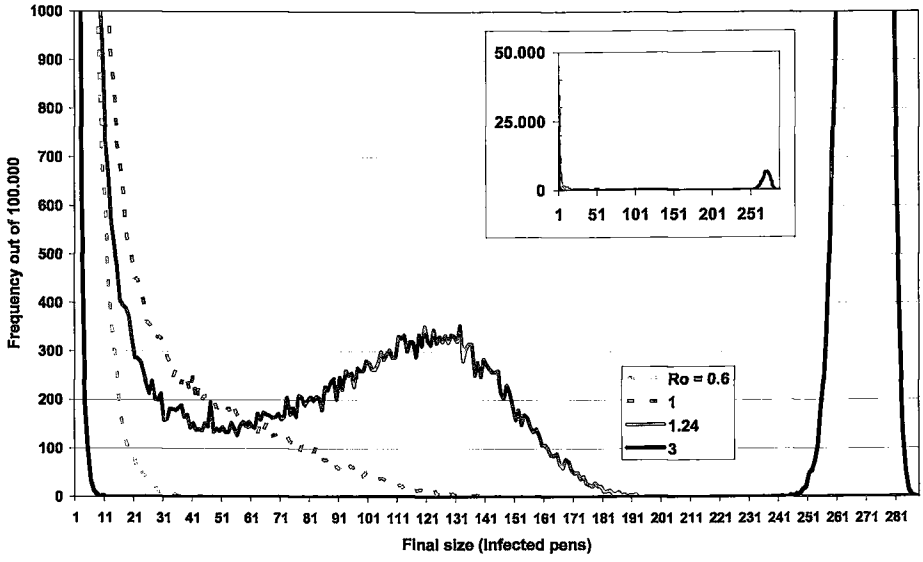


Figure 7.2: The frequency distribution of possible final size values found out of 100.000 simulated EMCV-outbreaks in a group of 288 mixing pigs. Different graphs reflect different values of R_0 around the theoretical threshold of 1. In agreement with theory the model shows only small outbreaks for R_0<1 (dotted lines). Increasing R_0 above 1 ascertains large outbreaks, more often affecting up to 288 pigs (NB: The insert shows the diagram up to scale of 50.000 repetitions while the main graph is cut at 1.000 corresponding to a maximum frequency of 1%).

7.2.4 Modelling pig-to-pig virus transmission with subdivision in pens

The imaginary compartment was subdivided into several pens by introducing fences. Initially the fence (F) was considered only a physical barrier separating the pigs into various pens and therewith avoided random mixing (F=0). Increasing the fence effect was assumed to reduce the contacts between pigs in adjacent pens up to a level where pigs in adjacent pens could be considered fully separated (F=1). Therefore, depending on the fence effect, an individual susceptible pig could be infected either by its infectious pen mates or by its infectious

neighbourhood mates (i.e. the "population" of joining pen mates and pigs of the 3 (or 2) adjacent pens). Assuming the contacts that have the potential to transmit virus keep limited per pig and per time unit (i.e. β constant) (Bouma et al., 1995), the fence effect "distributes" these contacts between the within pen population and the neighbourhood population. In line with Klinkenberg et al. (2002) the resulting probability of infection for a susceptible pig (P_{SI_total}) is thus given by:

$$P_{SI\_total} = 1 - \exp\left(-F\beta\frac{I_{in}}{N_{in}} - (1-F)\beta\frac{I_{all}}{N_{all}}\right), \text{ with;} \quad (3)$$

F = fence effect reducing transmission between pigs in adjacent pens (0 to 1),

I_{in} = number of infectious pen mates,

I_{all} = number of infectious neighbourhood mates,

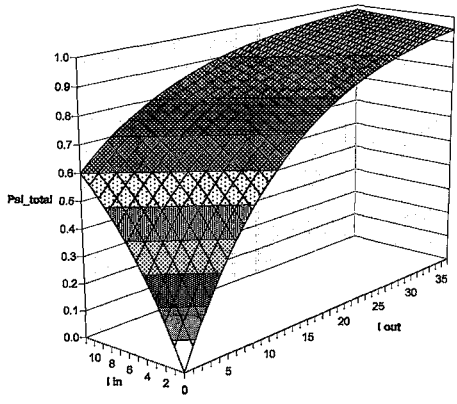
N_{in} = number of pigs in pen (12),

N_{all} = number of pigs in neighbourhood (48 or 36).

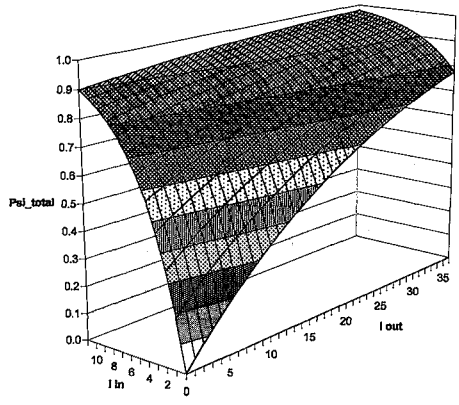
The Figures 7.3a-d visualise for an arbitrarily chosen β=4 the effect of the transmission model (3) showing $P_{SItotal}$ for all combinations of infectious pen mates (left bottom axis, I_{in}) and infectious pigs in adjacent pens (right bottom axis, I_{out}). Due to the physical subdivision of a compartment into pens any pig has contact with 11 pen mates (N_{in}–1) and 36 pigs from adjacent pens. If F=0, the 48 pigs (N_{all}) form "one" group and $P_{SItotal}$ changes with any further infectious animal in the group (Fig. 7.3a). On the contrary if maximal fence effect is assumed (F=1, Fig. 7.3d) infectious pigs in adjacent pens cannot contribute to the infection probability P_{SI_total} for the susceptible pig. Increasing the effect of fences (Fig. 7.3a-d) enlarges the contribution of the infectious pen mates to $P_{SItotal}$, whereas the impact of infectious pigs from the adjacent pens decreases (and vice versa).

7.2.5 Parameterization

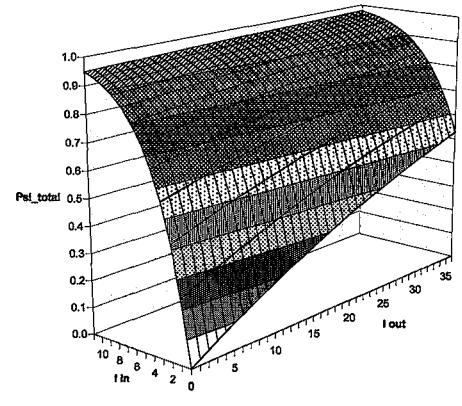
The infection of susceptible pigs followed a binomial chance process, Bin(P_{SI_total}). P_{SI_total} was calculated from (3) for any individual according to the neighbourhood of the pig's pen. For each scenario the transmission rate β was set constant in (3) applying (2) to the assumed value of R_0. The considered R_0-values (i.e. 0.3-4.8) cover the 95% -confidence interval for the R_0-point estimate from earlier experiments (Maurice et al., 2002). Additionally, the fence effect (F) was increased in steps of 0.10 from 0 to 0.9. For perfect fences (i.e. F=1, no contact between pigs in adjacent pens) (3) is collapsed into (1) which was used to validate the model against the contact experiments (Maurice et al., 2002). Stochastic simulation of the infection cycle was performed by updating the status of each individual on a daily basis using the processes given in Table 7.1. The length of incubation and infectious period (both maximal 4 days) was determined for each infected pig individually by Monte Carlo sampling from the respective empirical distributions (Billinis et al., 2004).



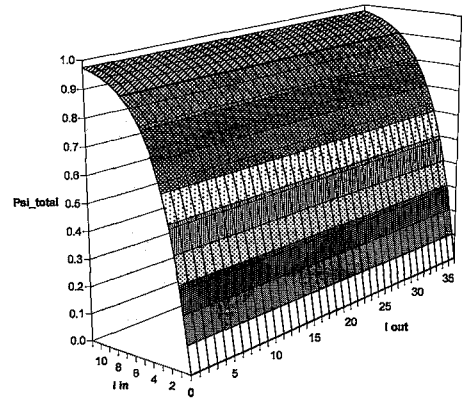
(a) F = 0 (no limiting fence effect)



F = 0.5



(c) F = 0.75



F = 1 (maximal fence effect)

Figure 7.3: Visualisation of the consequence of subdivision of the compartment on the individual probability to get infected (β=4). Depending on the fence effect the number of infectious pigs from the same (I_{in}) and adjacent pens (I_{out}) differently contribute to the total infection probability (P_{SI_total}) for a susceptible pig.

7.2.6 Simulation routine

The combinations of R_0 and F values determine 160 simulation scenarios (i.e. 16 * 10). For each scenario the simulation run was repeated 1.000 times to cover stochastic variability of the model by the outcome measures. Each run was initialised by infecting a random pig in a random pen. The simulation outcome was described on the individual pig and on the pen level. Firstly, the mean total number of pigs ever infected during an outbreak (NIPG) was

recorded to compare scenarios to those of the simulation of a non-compartmentalized pig population. Additionally, the crowding of infections in single pens was measured by the average number of infected pigs per infected pen. Secondly, also to visualise spatial spread, the number of infected pens (NIPN) was averaged over 1.000 repetitions where a pen was considered infected when at least one pig in the pen became infected. An outbreak at compartment level with NIPN $\geq$ 12 (i.e. 50%) was considered major, while outbreaks with NIPN $\leq$ 4 (i.e. at most one sixth) were considered of minor size. As the infection process has an expected bimodal outcome especially for larger R_0-values (see Fig. 7.2), results additionally were described using the probability to observe outbreaks with at least a specified number of infected pigs or pens (Fig. 7.5 and 7.6).

Table 7.1: Parameters determining the simulation of EMCV spread in the model.

Process	Parameter	Source	Distribution or Range	Reference
Infection S => E	$P_{SItotal}$ depending on:		$Bin(P_{SItotal})$	
	- R_0 (Reproduction ratio)	Data - range	(0.3 - 4.8) step 0.3	Maurice et al., 2002 (for F=1)
	- F (Fence effect)	Not known	(0.0 - 0.9) step 0.1	-
Incubation E => I	Length of incubation period	Data - fix	(25%, 25%, 75%, 100%) Cumulative frequency of transition after n days (1-4)	Billinis et al., 1999
Recovery I => R	Length of infectious period	Data - fix	(25%, 25%, 50%, 100%) Cumulative frequency of transition after n days (1-4)	Billinis et al., 1999

7.3 Simulation results

The subdivision of the compartment into pens by introducing fences prevented random mixing of all pigs in the compartment. Thus results are found by comparing simulations, either in terms of the expected number of infected pigs or pens, with different fence effect relative to the control simulation of 288 randomly mixing pigs.

Table 7.2: Modelling results of the mean number of infected pigs (NIPG) shown for representative combinations of R0 and F. The number of infected pigs per infected pen (I/pen) measures crowding of infections per pen. Values are shown after averaging all 1000 repetitions and rounding to the nearest natural.

R_0	No fence	F=0	F=0.5	F=0.9	I/pen
0.6	*3*	2	2	2	1.4 - 1.9
1.2	*29*	12	9	5	3.1 - 3.4
1.8	*136*	95	53	12	7.6 - 5.9
3.0	*232*	230	218	55	11.1 - 9.7

7.3.1 Number of infected pigs:

At F=0 (the fence is only considered a "physical" barrier, no reduction in contacts between pigs in adjacent pens) outbreaks resulting from either small or large values of R_0 on average were comparable to those for a randomly mixing population (i.e. R_0 either 0.6 or 3.0 in Table 7.2). For R_0-values fairly above 1 the average size of the outbreaks among the 288 pigs in a compartmentalised housing was considerably smaller compared to outbreaks in a randomly mixing group of 288 pigs (i.e. R_0 either 1.2 or 1.8 in Table 7.2). For increasing values of F, the expected average number of infected pigs was further reduced over the whole range of R_0-values.

To illustrate the impact of the fence on the probability to observe "major" outbreaks, Fig. 7.4 gives, in line with Fig. 7.2, the frequency distribution of the final size values (number of infected pigs per outbreak) for 1000 simulated outbreaks with R_0=3 and an increasing fence effect (resp. F=0.0, 0.5 and 0.8). The probability to observe outbreaks affecting at least 270 infected pigs (see section 7.2.3) is reduced from 0.40 at F=0 (compared to ≈0.60 at random mixing) to approximately 0.03 at F=0.8.

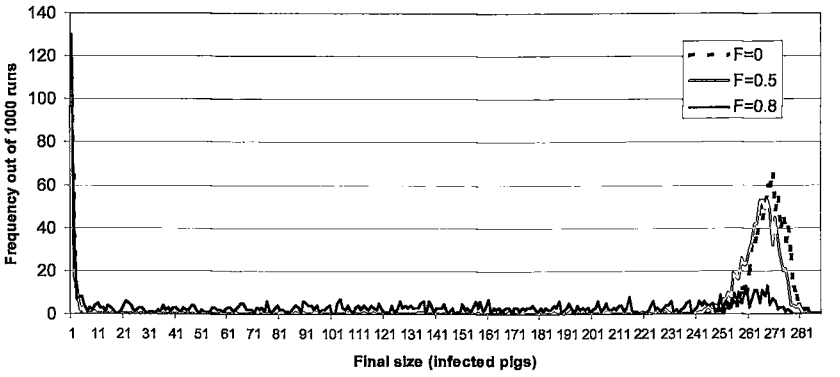


Figure 7.4: The frequency distribution of possible final size values found out of 1000 simulated EMCV-outbreaks for R_0=3 in a group of 288 pigs in a compartmentalised housing. Different graphs reflect different fence effects, resp. F=0.0, 0.5 and 0.8.

7.3.2 Number of infected pens:

Figure 7.5 shows the probability to observe major outbreaks affecting at least 50% of the pens (NIPN=12) for different values of R_0. For F=0 (background line) values of R_0 below 1.2 only have a 10% probability that resulting outbreaks affect more than 12 pens, whereas R_0 values above 1.8 reproduced such outbreaks even more certain than by chance (50%). However, increasing the fence effect up to 80% reduced the probability of major outbreaks below 50%

for values even up to R_0=2.7. Only for R_0 values beyond 4.0 outbreaks of major size are observed more often than by chance even at maximum fence effect (F=0.9). To observe outbreaks affecting at least 80% of the pens, R_0-values beyond 3.3 were required at a fence effect of 80% (F=0.8).

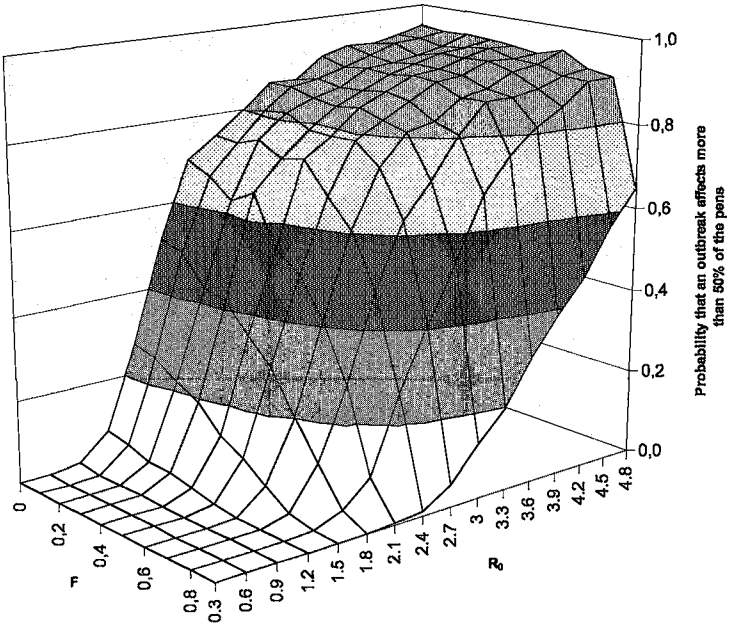


Figure 7.5: Impact of the R_0-value and the fence effect (F) on the probability to observe simulated EMCV outbreaks that affect more than 50% of the pens.

7.4 Discussion

Although estimated close to one, experimental R_0-estimates on EMCV transmission suggested potential for major outbreaks among randomly mixing pigs (Maurice et al., 2002). As finishing pigs often are kept in compartmentalised housings, the potential impact of fences on the course of EMCV outbreaks in such a setting was studied in order to prevent major outbreaks at compartment level. We used a simulation model to evaluate direct pig-to-pig EMC virus spread. Starting out from one group of 288 randomly mixing pigs, virtual fences were introduced to simulate the course of EMCV outbreaks in a realistic compartment set up. Starting point in the simulations were experimental data (Maurice et al., 2002), indicating a MLE of R_0=1.24 with a CI of 0.39-4.35. For all R_0-values in the CI the final sizes observed in the experiments (total number of infected animals) potentially could be expected, albeit with a reduced probability compared to the MLE, and therefore all these R_0-values were included in

the simulations. The stochastic modelling did not produce one single outcome but covered a broad range of potential outcomes. Simulation results were compared on their probability of occurrence as the expected bimodal outcomes prohibited comparison of means.

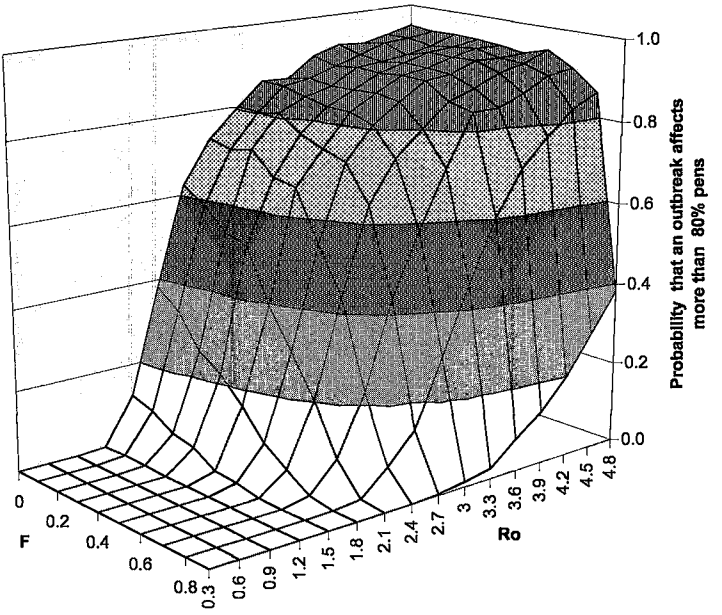


Figure 7.6: Impact of the R_0-value and the fence effect (F) on the probability to observe simulated EMCV outbreaks that affect more than 80% of the pens.

In addition, whereas the expected number of infected pigs per major outbreak can be calculated for each value of R_0 (section 2.3), the size of major outbreaks in terms of infected pens was arbitrarily set to 50% (12 pens) for all R_0-values studied. This might have led to an underestimation of the probability to observe "major outbreaks" for values below the specific (unknown) R_0, for which this cut off value would be expected as major outbreak, and an overestimation otherwise.

The introduction of fences as "physical barrier" between pens (F=0, pigs in adjacent pens still can have contact but can no longer mix randomly) in itself already reduced the final size of EMCV-outbreaks. For any $R_0<1.2$ (Maurice et al., 2002) the theoretically expected probability to observe major outbreaks (>100 infected pigs) was reduced from about 17% (see section 2.3) to about 1% due to the prohibition of random mixing of all pigs, while the probability to observe outbreaks affecting more than 50% of the pens remained below 10%. The impact of the fences on the final size of simulated outbreaks further increased as soon as

they also started to reduce the contacts, and therewith direct transmission, between pigs in adjacent pens (see i.e. Fig. 7.3 for R_0=3).

Imagining fences that, because of their height, require increasing efforts from pigs in adjacent pens to get in contact (from reaching out with the head until standing up against the wall) a reduction in contacts up to 80% was considered realistic. Model outcomes indicated that for F=0.8 the probability for an EMCV outbreak to affect at least 50% of all pens (major outbreak) could be kept below 50% for R<3. Calculating the one sided 95% CI for the experimental data (Maurice et al., 2002) narrowed down the upper limit for R_0 to a value of 3.64. Although their probability still could be reduced below 0.50, albeit with a maximal fence effect (F=0.9), especially for the R_0-values between 3 and 3.6 in the tail of the broad CI major outbreaks could not be ruled out beforehand.

However, from a recent field data set a MLE of R_0=1.36 (CI: 0.93-2.23) was obtained based on 6 fully sampled pens (with 15 pigs each) (Kluivers et al., 2006), treating pens as independent groups and assuming a single introduction per pen. When multiple introductions per pen were assumed, both the MLE and the corresponding CI for R_0 were even further reduced. Incomplete sampling in the remaining pens resulted in R_0-estimates between 0.8 and 1.7 (Kluivers et al., 2006). These available point estimates clustering around the threshold value of R_0=1 combined with the CI from Kluivers et al. (2006) tend to further narrow down the range of probable R_0-values even below R_0=3. Moreover, in the field study at least 80% of the pens were found infected (Kluivers et al., 2006). Model calculations indicated that for F=0, R_0-values beyond 2.0 would be required in order to assure reproduction of this field observation with a probability of at least 50% (Fig. 7.6). When a fence effect of 60% is considered reasonable an R_0>2.5 is needed (i.e. above the upper limit of the CI=2.23; Kluivers et al., 2006) to have a minimum parsimonious agreement with the observed 84% infected pens, while for F=0.8 only R_0-values >3.3 would be sufficient.

Both from the transmission experiments (pairwise and group experiments) as from the case study (6 fully sampled pens) the point estimates for R_0 varied between 0.7 and 1.7, while the combined R_0-estimates were 1.24 and 1.36 respectively. Model calculations indicated that, regardless of the fence effect (F), for none of these point estimates the field observation (80% infected pens) was reproduced at least as certain as by chance (50%). Hence, either all studies had jointly bad luck and underestimated the R_0 for EMCV, or single introductions are unlikely to cause the observed virus spread patterns as in the field (Kluivers et al., 2006). These results, combined with the short vireamic period and the often observed early death of infected pigs (Maurice et al., 2002; Acland, 1989), indicate that pigs should not be considered the main reservoir host for EMCV in compartmentalised pig farms and other EMCV infection mechanisms like e.g. indirect transmission (by manure, farmer, rodents) or multiple introductions by e.g. rodents (Spyrou et al., 2004; Maurice et al., 2002; Joo, 1999; Seaman et al., 1986) might need to be considered to understand reported major outbreaks divided over many pens in commercial pig houses (Kluivers et al., 2006; Koenen et al., 1999).

Consequently the model results combined with the findings by Kluivers et al (2006) also question the need for additional experiments to narrow down the CI around the point estimate of R_0=1.24 (Maurice et al., 2002) below a value of 2.2 for pig-to-pig EMCV spread in compartmentalised housings. Not only because such experiments were considered both unethical and expensive, but also since the model calculations indicated that for all values up to R_0=2.4 (Kluivers et al., 2006) the probability to observe major outbreaks already could be kept below 0.50 for F≥0.7. In addition earlier studies reported difficulties or even failures to prove contact transmission among pigs in experiments (Foni et al., 1993; Christianson et al., 1990; Horner and Hunter, 1979; Littlejohns and Acland, 1975). For randomly mixing pigs in e.g. group housing systems, it still remains important to ultimately assess whether R_0 for EMCV in pigs is above or below one.

Basic and vital assumption in the modelling approach was that EMCV transmission only resulted from direct pig-to-pig contact (no airborne spread), which allowed for the use of the same beta for both within- and between pen spread. Although multiple transmission routes might be involved for other diseases, the applied basic principle to mechanistically evaluate the within versus the between pen spread by direct contact between animals in itself remains valid and informative.

In the SIR approach the transmission rate beta (β) is constructed from "a number of contacts per time unit" times "the probability of successful transmission of infection during such a contact between a susceptible and an infectious animal", of which only the first one actively can be manipulated by zootechnical measures. In the current modelling approach the effect of the fence implicitly reduced the number of contacts per time unit between pigs in adjacent pens. Effective application of fences to prevent virus spread requires further analysis of the actual contact patterns among pigs in adjacent pens and the way they are influenced by pig-factors like age and breed.

7.5 Conclusion

In conclusion the model outcomes indicated that, combining the current estimates and information on direct pig-to-pig transmission, a single introduction of EMCV in a compartmentalised pig housing is unlikely to cause major EMCV outbreaks by direct pig-to-pig transmission alone.

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Chapter 8

General discussion

8.1 Introduction

The Encephalomyocarditis virus (EMCV), belonging to the genus cardiovirus of the family Picornaviridae (Van Regenmortel et al., 2000) came up in domestic pigs in Europe (Koenen et al, 1997). Although generally considered a rodent virus, pigs were considered the most susceptible domestic species (Joo, 1999) and clinical disease due to EMCV infection was described to take two main forms; acute myocarditis usually in young pigs (Gainer, 1967) or reproductive failure in sows (Joo et al., 1988). The research in this thesis specifically aimed at studying a number of key elements in the epidemiology of myocardial EMCV infection in domestic pig farms, to ultimately be able to support (the development of) effective EMC prevention and control strategies at farm level;

1. Disease occurrence; analyse where and to what extent the virus circulates in domestic pigs to understand the relevance of (clinical) EMC.
2. Risk factors; identify potential risk factors for the occurrence of EMC at pig farms to focus and direct the development of EMC prevention and control strategies.
3. Transmission dynamics at animal level; quantify transmission of EMCV within and possibly among suggested hosts (domestic pigs and rodents), to analyse and test their potential contribution to the course of EMCV outbreaks in pig farms.
4. Outbreak analysis at farm level; combine available information into an integral modelling approach in order to evaluate the role of pig-to-pig EMCV spread in a farm setting and to study the impact of potential control measures.

This chapter discusses how the initial research goals were met, places the obtained findings in a broader perspective and reflects on some of the methods applied. Section 8.2 discusses and integrates the results from the various chapters towards the epidemiology of EMCV infection. Section 8.3 describes control options for EMC. Section 8.4 gives an outlook on future research while paragraph 8.5 summarizes the main conclusions of this thesis.

8.2 Epidemiology of myocardial EMCV in pig farms

8.2.1 Implications from research findings

8.2.1.1 EMCV (sero)prevalence in pigs

From the prevalence study in Chapter 2 it was learned that clinical outbreaks of EMC did not appear randomly over the affected countries, but seemed clustered in so called "endemic" areas. From the perspective of outbreak control the latter forced to think why certain regions or farms indeed were exposed to the virus more often (and from what source) and why this challenge only in some cases resulted in the development of (clinical) disease. In addition, an

increase in the number of outbreaks was observed during autumn and winter months. This is in line with findings by Koenen et al. (1999), who reported the start of outbreaks in periods with very hot temperatures at the end of the summer. However, despite the clustering of infected farms, no massive spread of (clinical) infection among neighbouring farms was observed as there is for other infectious viral diseases like e.g. Classical Swine Fever (Mangen, 2002; Stegeman et al., 2000) or Foot and Mouth Disease (Thomson, 2002; Morris et al, 2001). The fact that often only few (non-adjacent) pens with clinically affected pigs were found within affected farms or compartments also suggested a rather limited direct pig-to-pig transmission of EMCV.

On the other hand the serological picture among animals and farms indicated a broader (geographical) spread of EMCV infection among pigs then expected from the clinical outbreaks only, as illustrated on farms without reported clinical disease but with a within-herd seroprevalence of 15 up to 36% in Greece (see Ch. 2). This observation of sub-clinical infection was confirmed in the case control study, where several selected control farms were serologically positive (Ch. 3). Smaller outbreaks easily might have been overlooked due to unfamiliarity with the non-typical clinical signs of EMCV infection. Also the early research efforts often concerned convenience sampling or resulted from existing monitoring programs not designed for monitoring of EMCV infections, possibly biasing the observed prevalence.
Therefore with respect to the prevention and control of myocardial EMCV infections in commercial pig farms three aspects are considered relevant; initial infection by EMCV, transmission of the virus to other pigs and clinical manifestation of the disease in pigs. The latter appeared to vary from outbreaks of only sub-clinical infection, outbreaks with only few clinically affected pigs and outbreaks clinically affecting a large number of pigs. These major clinical EMCV outbreaks cause considerable losses to individual pig farmers (Koenen, 1996, 1999, Loukaides, 1996).

8.2.1.2 The impact of direct pig-to-pig virus transmission

In most cases myocardial EMC is recognised in pig farms due to observed clinical signs, i.e. sudden death, which makes damage control (avoiding further spread in the pen, compartment or farm) most urgent. Since, also based on the prevalence study, the actual level of direct pig-to-pig spread was questioned, the contribution of this transmission route to the course of EMC outbreaks in pig farms was assessed in more detail. The R_0 for EMCV infections in pigs was estimated close to one both in the transmission experiments (R_0=1.24, 95%-CI 0.39-4.35, Ch. 4) and a field study (R_0=1.36, 95%-CI 0.93-2.23, Ch. 6). As the results were inconclusive towards the R_0-threshold value of 1, and therewith on the potential for pigs to act as an EMCV reservoir host (i.e. R_0>1, Diekman et al., 1990), the transmission data were evaluated on several other aspects.

The theoretical distribution of minor and major outbreaks in random mixing populations e.g. indicated a probability for major outbreaks of 0.20 for the point estimate R_0=1.24, affecting about 36% of the animals (Ch. 7). This figure did not seem to match with the observed (serological) response to EMCV in many farms (both within and outside the endemic areas, Ch. 2), as the level of infection within affected farms turned out to be higher than might have been expected after one single introduction in a pig farm for an R_0=1.24.

To account for the uncertainty around this point estimate for R_0 and the contact structure within a farm, the range of estimated R_0-values within the confidence interval was evaluated by the final size of the resulting outbreaks in a compartmentalised setting. The outcomes indicated that EMCV transmission is reduced after compartmentalisation while also the probability of major outbreaks was reduced for R_0 values even up to 2.7 (Ch. 7).
Based on the above findings it is concluded that the observed outbreak patterns in the field probably are the result of various introductions/small outbreaks in a farm instead of from one major outbreak after a single initial introduction followed by pig-to-pig transmission only. This is supported by the lack of severe clinical signs in most infected farms, despite the high number of (sub clinically) infected animals, as normally severe clinical signs are seen during major EMCV outbreaks (see next paragraph; Koenen et al., 1999; Paschaleri-Papadopoulou, 1994, 1990).

Rodents are considered the most likely source for the multiple virus introductions and subsequent spread of EMCV in pig farms (Joo, 1999, Seaman et al, 1986), especially since they are able to easily transmit the virus and act as a potential virus reservoir (Ch. 5).

8.2.1.3 Infection of pigs by rodents

By serving as a (local) virus reservoir rodents were suggested responsible for the observed clustering of outbreaks (Acland, 1989; Seaman et al., 1986; Acland and Littlejohns, 1975) and might as well explain the introduction of virus in different pens on affected pig farms and the (seasonal) reoccurrence of outbreaks on the same farms. More general, rodents are known to potentially carry and/or transfer contagious animal disease agents between farms (Meerburg, 2006), either as a mechanical vector or by actual virus replication. Examples are porcine parvovirus (Joo et al., 1976), Aujeszky's disease virus (Maes et al., 1979) and foot and mouth disease virus (Capel-Edwards, 1970).

Two EMCV transmission routes from rodents to pigs were suggested earlier, a) ingestion of infected faeces from rodents or b) ingestion of infected rodent carcasses (Acland, 1989; Seaman et al., 1986; Littlejohns and Acland, 1975). EMCV has been isolated from dried faeces and from intestines from rats and mice captured on farms where swine disease had previously occurred (Koenen et al, 1999; Acland and Littlejohns, 1975; Gainer, 1967), but often in practice it turned out to be rather difficult to catch the rodents in time to actually demonstrate the virus (Ch. 5). In the endemic countries Italy and Greece tested rodent species

in the direct neighbourhood of affected farms and in Italy EMCV was isolated from faeces of one rat trapped within an affected farm. In addition 3 out of 34 rat sera collected at EMCV positive farms were found antibody positive. In a study in Greece, neutralising antibodies were detected in 4 rats out of 28 rodent samples from the areas where EMCV was endemic among pigs (Koenen et al., 2002). Although Austria and France reported positive serology in their domestic pigs (Ch. 2) (Koenen et al, 2000; Nowotny et al., 1993), no positive rodent sera were found in Austria or France. No EMCV could be isolated from faeces or organ material from rodents in the United Kingdom (i.e. research in the framework of EU-project, Koenen et al., 2002). Although these negative rodent samples were not specifically linked to areas in which the seropositive pigs were found, (sero)positive findings among rodent populations without a link to (infected) pig populations on the other hand could further support their suggested role as reservoir host.

The finding of mice as a risk factor in the case control study (Chapter 3) underlined the rodent hypothesis. Although significant in the univariable analysis of this case control study only, also several other factors came up that indirectly support the rodent hypothesis. This concerned risk factors like automatic feeding- and group drinking systems (freely accessible for rodents) and protective management factors like hygiene and sanitation (immediate removal of dead, potentially infected, animals when found in the stable).

In addition, the transmission studies (Chapter 5) indicated that rats not only transmitted the virus very effectively among each other ($R_0>>1$) but also survived infection and excreted virus in their faeces for a relatively long period after infection (up to several weeks). Comparable transmission results were seen in mice (work by Billinis et al., in Koenen et al., 2002), albeit with a somewhat shorter virus excretion period in faeces and some mortality. Seaman (1986) suggested interaction between both rats and mice. His hypothesis that rats host the infection, transmit it to mice who subsequently pass it on to pigs therefore deserves further attention. Based on these results rats or mice can be considered potential reservoir hosts for EMCV in the neighbourhood of commercial pig farms. Although the proposed "link" between the two species involved (pigs and rodents) is not easily confirmed in practice (i.e. by actually demonstrating EMCV transmission from the one species to the other on a farm), an attempt was made under experimental circumstances (research by Billinis et al., in Koenen et al., 2002). In two experiments rats were experimentally infected and euthanised two days later. After being euthanised, homogenised and mixed with feed, the rats were fed to 5 pigs (one rat per pig). The first experiment resulted in 3 out of five infected pigs, while in the second even 4 out of 5 pigs got infected. Infection in pigs was confirmed by virus isolation (blood, tissue, nasal and faecal excretions) and serology. Field experience demonstrated that pigs eat rodent carcasses in a stable rather quickly (C. Billinis, pers. comm.), while such carcasses often are shared and chewed by various pigs (Littlejohns and Acland, 1975). Therefore, it was concluded that EMCV transmission from rodents to pigs by the oral route is possible.

8.2.1.4 Response to infection in pigs

Another important observation was the variable response to infection of pigs within and among age classes (Chapter 2 and Koenen et al., 1999). Such a variable response to infection, varying from sudden death to a mild serological response only, also was observed in the experiments described in Chapter 4 (in both inoculated and contact infected pigs).

In literature this variable clinical picture in farms or among age categories often was ascribed to differences in biological characteristics, pathogenicity or tissue tropism among EMCV strains (Billinis et al. 1999, , Koenen et al. 1999, Knowles et al. 1998, Koenen and Vanderhallen, 1997, Christianson et al. 1992), the available infectious dose (Billinis et al., 2004) and/or the susceptibility of the pigs (age susceptibility of heart tissue, pig breed) combined with decreased protective maternal immunity (Paschaleri Papadopoulou et al. 1990). In addition, Gelmetti et al. (2006) demonstrated that susceptible pigs developed severe myocarditis followed by sudden death, while more resistant pigs developed mild myocarditis only and remained asymptomatic.

8.2.1.5 Infectious dose

In contrast to Littlejohns and Acland (1975), who did not find substantial differences in disease patterns induced by different virus doses, Billinis et al. (2004) reported a positive association between an increase in the infectious dose and the severity of heart lesions, the mortality and the likelihood of virus isolation from various organs. Vlemmas et al. (2000) suggested that the damage to cardiac muscle cells and Purkinje fibres was related to direct action of EMCV (EMCV antigen was detected in areas showing degeneration and necrosis without inflammation) and to hypoxia resulting from virus-induced endothelial lesions. The presence of virus and the associated lesions in the Purkinje fibres are thought to lead to electromechanical dissociation, resulting in acute heart failure and death (Vlemmas et al., 2000). Gelmetti et al. (2006) concluded that acute myocarditis is strictly related to EMCV tropism in myocardiocytes. Their study indicated that EMCV is picked up by macrophages in the tonsils a few hours after exposure to the virus and distributed during the primary vireamia throughout the body to the main target organ, the heart. In the heart virus replication is, dependent on the susceptibility of a pig, followed by either severe or mild myocarditis and a secondary vireamia, potentially leading to infection of various organs and virus excretion. Assuming the clinical response in pigs (myocarditis) not only reflects the severity of infection but implicitly also the level of virus replication and excretion, especially clinically infected pigs can be expected to contribute substantially to the pig-to-pig transmission of EMCV in pig farms (see §8.3.2.1). It is hypothesised that the (clinical) development of an EMCV infection and its subsequent transmission in a group of pigs to a certain extent is mediated by the route (and therewith the dose) in which the virus is introduced in the first affected pig in a farm.

8.2.1.6 Introduction and development of EMCV infection in a pig farm

Assuming that the route (and therewith the dose) of introduction of EMCV indeed is crucial in the course of infections in pig farms, several potential introduction routes need to be considered. As severely infected pigs either will be recognised as such or quickly die, the risk of introducing myocardial EMCV into a farm by the purchase of clinically infected pigs seems fairly low. Although the introduction of sub-clinically infected pigs cannot be ruled out beforehand, the probability that a major outbreak among pigs is initiated from such pigs is now considered limited, also due to the fast and short viraemia (Billinis et al., 1999, Koenen et al., 1999, Foni et al., 1993). For the "reproductive" EMCV strains however, this introduction route might be particularly relevant as clinical signs in pregnant sows are few and mainly are seen at farrowing only (Koenen et al., 1994, 1991).

The results on both serology and virus isolation from Chapter 2 indicat that EMCV can circulate among wild boar. Extrapolating the estimates for EMCV transmission in domestic pigs, the proposed role for wild boar as a potential introduction route is considered limited, because effective contacts between (clinically) infected wild boar and domestic pigs are scarce in modern closed farms (see also 8.3.2). The positive serological and virological findings in wild boar therefore most likely reflect high EMCV prevalence in local rodent populations.

In conclusion the start of a major EMCV outbreak in a domestic pig farm is now thought to result mainly from virus introduction by infected rodents entering a stable (e.g. due to food shortage in autumn, Seaman et al., 1986). As probably a low(er) infectious dose will come available to pigs from feed or drinking water contaminated by rodent faeces (Littlejohns and Acland, 1975), this mechanism probably will account for wide spread sub-clinical infection mainly. Assuming the infectious dose indeed mediates the response to infection, the more severe (and clinical) outbreaks in pigs mainly will result from expected direct ingestion of infected rodent carcasses (or feed contaminated with infected carcasses) occasionally followed by limited direct pig-to-pig transmission. Considering the reduced pig-to-pig spread predicted in compartmentalised housings (Ch. 7) and the observed outbreak patterns in the field, it is likely that rodents often will be involved in the spread of EMCV within a stable (e.g. repeated introductions, Ch. 5).

8.2.2 EMCV transmission in and among various species

8.2.2.1 Domestic pigs

Experimental vs. field setting

The estimates for the transmission of myocardial EMCV infections among pigs as obtained from the experiments (Ch. 4) seem rather robust and trustworthy. In the experiments with pigs

(Ch. 4) the virus dose was administered oro-nasally, which is considered the natural route of infection. Also, the administered dose (10^3 $TCID_{50}$/ml) was chosen in line with virus titers observed in infected pigs, as to mimic the infectious dose a susceptible pig might receive either from another infected pig or any other source. This is specifically important as the course of infection in a pig is thought to be dependent on the received infectious dose (Billinis et al., 2004), which subsequently influences the probability that infection is passed on to other pigs. Although some limitations from the experimental setting were overcome in the field study (e.g. dosage, virus administration), especially the real life dynamics and potential interference of various transmission routes need to be considered while interpreting the results (Ch. 5). Vital in the interpretation of the field study was that the infection cycle really had ended at the moment transmission was estimated using the final size method, to avoid potential underestimation of transmission (Kroese and De Jong, 2001). Also, opposite to the experimental set up in groups, the start and development of the infection chain in the various adjacent pens could not be controlled. Since multiple contact infections among pens or infection from different sources (e.g. rodents) might have occurred, the results from the field study probably should be considered an upper estimate of pig-to-pig transmission. Chapter 5 confirmed that both the point estimate and accompanying CI were reduced as soon as multiple introductions per pen were assumed.

The transmission experiments were performed among a uniform group of pigs (breed, age) using the same infectious dose, which allowed for the model applied assuming homogeneity. In the field this situation might be different due to e.g. differences in infectivity among inoculated piglets vs. contact infected piglets or diversity in the pig population at farm level. The former might be evaluated by a different experiment (using contact infected piglets as initially infected pigs), while the latter can be dealt with by adjusting the analytical model (see also §8.4).

Virus strain type

The transmission experiments in this thesis were performed with a Belgian myocardial strain (B279/95), which was isolated during the first observed outbreak of myocardial disease due to EMCV infection in fattening pigs in Belgium (Koenen et al., 1999). Although the experiments described in Chapter 4 were the first study in which contact infected animals actually died, the results in terms of virus transmission (R_0) were quite comparable with other studies in which Greek (strain 424/90, Billinis et al., 1999; Paschaleri-Papadopoulou et al., 1990) or Italian (strain 71c/88, Foni et al., 1993) myocardial strains were used. Broader extrapolation of the current results on EMCV transmission, at least for these myocardial strains, therefore seems appropriate. The more severe clinical picture (mortality) in the experiments described in this thesis might possibly be explained by the variability in pathogenicity of the various strains, the susceptibility of the individual animals or the specific set up of the (pairwise) experiments (Ch. 4).

Reproductive failure due to EMCV is observed (e.g. Koenen et al 1999, 1991;Acland, 1989), but seems related to different strains. For these virus strains less quantitative information on transmission in pigs is available. Extrapolation of the R_0 estimates for myocardial strains is difficult as for the reproductive strains another additional transmission route, from sow to piglet, needs to be taken into account. The apparently conflicting reports on the impact of either a myocardial or a reproductive EMCV-strain, each causing their own clinical picture, suggested that EMCV strains may vary in pathogenicity and tissue tropism (Billinis et al., 1999; Koenen and Vanderhallen, 1997; Koenen et al., 1991). Serological monitoring, follow up of clinical cases and genetic typing of strains involved can be useful to detect changes in incidence and nature of EMCV infection and therewith anticipate on the risks for the various pig or age categories that are present in (farrow-to-finish) pig farms.

8.2.2.2 Rodents

The estimates on EMCV transmission among rats in Chapter 5 and among mice (work by Billinis et al. in Koenen et al., 2002), gave clear and robust outcomes with respect to their potential as a virus reservoir for EMCV (R_0>1). However, information on contact dynamics, infection status (also in areas without infection in pigs) and migration patterns among rodent populations is needed to clarify their role in the epidemiology of EMCV outbreaks in pig farms. Cleaveland and Dye (1995) formulated three conditions to test the hypothesis that a reservoir host, e.g. rats or mice, serves as a source of infection to other species; 1) the rodent population should show evidence of persistent infection, 2) infections should occur in rodents in absence of cases among domestic pigs and 3) cases among pigs should follow the cases in rodents. Collaboration with ecologists and wildlife experts is suggested to find answers to these questions.

8.3 Control of myocardial EMCV; application and outlook

8.3.1 Motivation for disease control

In the nineties, EMC presented itself as an emerging disease in commercial pig farms in Europe (Koenen et al., 1997), potentially requiring coordinated disease control programs. Based on the research described in this thesis it can be concluded that EMCV infections in pig farms primarily should be considered an individual farm problem, causing both animal health problems and related (economic) production losses. The economic impact due to myocardial EMCV outbreaks mainly consists of losses due to mortality among affected animals or growth delay due to infection (Koenen et al., 1999, 2002; Loukiades et al., 1996). Model simulations for a myocardial EMCV strain indicated that for the observed estimate of EMCV

transmission among pigs (R_0=1.24, Chapter 4) on average 12 pigs would be infected (Tabel 7.2, Ch. 7) after a single introduction in a compartmentalised housing. Preliminary calculations indicated that a suggested "minor" outbreak in a compartment of 100 fattening pigs, causing 10 dead pigs and 25 subclinically infected pigs, would result in a loss of approximately 677 Euro per fattening period (Koenen et al., 2002). Infection with a reproductive EMCV strain may result in reproductive failure in sows and early- or stillborn piglets (in 2 cases estimated at $100/inventoried sow by Christianson et al., 1990). In case of multiple introductions by rodents and considerable pig-to-pig transmission, losses may add up considerably for some infected farms. Therefore, proper control measures are required at the individual farm level.

8.3.2 Control options for myocardial EMCV

Preventive measures should generally focus on 1) the prevention of EMCV introduction into a farm or 2) the reduction of clinical disease (i.e. mortality) once the virus is introduced.

8.3.2.1 Prevention of virus introduction

Although it remains difficult to predict where and when new EMCV outbreaks could be expected, the observed prevalences (Chapter 2) indicated a seasonal pattern in outbreaks with a peak in autumn and winter in endemic regions. Therefore, especially during the risk periods in risk regions, farmers should be (made) aware of rodents migrating into their farm buildings. Farmers should take hygienic measures to avoid, or at least reduce, direct contact between pigs and rodents (both mice and rats) or their droppings. Especially in those areas where clinical outbreaks of EMCV (still) occur regularly in pig farms, (serological) monitoring might be a useful tool in order to detect circulating virus at farm or regional level and advise farmers on adjacent farms to take precautionary measures. For financial reasons one could try to make use of already existing monitoring programs in pigs.

8.3.2.2 EMCV control once present at a farm

As no effective treatment is available yet, after its introduction in a herd, EMCV control should be focused on early recognition of infection ("early warning") and reduction of subsequent spread within the farm. Early warning requires sufficient information and education on clinical signs of infection to both the veterinarian and the farmer, a task in which the (local) government or veterinary service could play a role. Mortality among pigs in the infected pens will be seen rather quickly and one should best aim at preventing the virus from spreading from the affected compartment to other compartments or buildings at the farm. As was demonstrated in Ch. 7, compartmentalisation in itself already could be a valuable tool to

reduce virus transmission and even might be applied as a preventive measure. In addition, also during the course of an outbreak, the bio-security measures should be brought or kept at a high level. The aim should be to avoid contact between healthy pigs and potentially infected pigs, rodents or their droppings (cleaning of feeding- and drinking troughs, rodent control, removal of dead animals from the pens) and to prevent rodents from further spreading the infection. Therefore, no live pigs should be moved from an affected compartment to other compartments and any indirect contacts with possibly infected pigs should be avoided (i.e. application of sterile needles for medical treatment in different pens or compartments).
Littlejohns and Acland (1975) observed mortality due to EMCV infection mostly at times of excitement like during feeding or handling the pigs, possibly caused by damaged myocardium due to earlier infection (Foni et al., 1993). Therefore, special attention for management practices during EMCV outbreaks can help to reduce losses due to mortality among pigs.
Although EMCV infection has no treatment (yet), some experience was gained on the application of vaccination in pigs during outbreaks in e.g. Cyprus (Veterinary Services, pers. comm.).

8.4 Future outlook/Research gaps

Based on the current research findings, additional (transmission) experiments don't seem necessarily required from an EMCV control point of view. From a general (scientific) interest however, a number of research questions remain towards both pigs and rodents.

8.4.1 Pigs

In § 8.2.2.1 the point estimates for EMCV transmission were discussed in relation to the suggested heterogeneity among pigs towards their infectivity. The inoculated pigs were suggested to be more infective than contact infected piglets, leading to an overestimation of transmission. This issue could be evaluated using a follow up transmission experiment in which contact infected pigs are used (as initially infected pigs) instead of inoculated pigs (see also Velthuis, 2002). The assumed difference in infectivity for clinically versus sub-clinically infected pigs can be tackled by adjusting the underlying analytical model for different subpopulations (Velthuis, 2002).

Another potential research area might be found in the suggested persistence of EMC virus in piglets. Billinis et al. (1999) re-established virus excretion in earlier infected piglets 14-21 days after the end of the initial excretion (using dexamethasone treatment), followed by transmission to contact piglets. The contact infected piglets neither showed clinical signs nor died. Their potential role in additional virus spread should be further evaluated but is probably limited also considering their relatively short presence at a farm as fattening pigs.

The research in this thesis focussed mainly on infection of pigs by myocardial EMCV strains. The risks and impact of the vertical transmission dynamics in pigs (Fig. 1.1) therefore need to be further elaborated with respect to the control of reproductive EMCV strains.

8.4.2 Rodents

Morse (1997) stated that emerging viruses often already exist in nature and emerge by gaining access to new hosts, often due to ecological or environmental changes. Although the involvement of rodents in the epidemiology of EMCV infection in pig farms would fit into this hypothesis, more information on contact dynamics and migration patterns among rodents is needed to better understand their contribution and learn where new outbreaks could be expected. Also it might be worthwhile to catch and test rodent populations apart from infected pig populations in order to further evaluate their role as reservoir host. Insight in the contact patterns among individual rodents should clarify whether experimental results on EMC virus transmission in rodents can directly be translated to the field situation and why, despite the high level of EMCV transmission among rodents, outbreaks do not seem to persist in pig farms by continuous re-infection. In this respect the hypothesis by Seaman et al. (1986) about interaction between mice and rat populations (mice get infected by rats, amplify the EMC virus and subsequently pass it on to pigs), deserves further attention. All this additional information also could be used to update and extend the model as described in Chapter 7.

8.5 Main conclusions

The main conclusions from the various studies within the framework of this thesis in relation to the research questions are;

Prevalence

- The consequences of an EMCV infection in domestic pig farms in terms of the number of (sub)clinically infected pigs vary considerably, probably due to differences in pathogenicity of the EMCV-strains, the available infectious dose and the susceptibility of the pigs (age/breed).
- Outbreaks of EMCV appear to be clustered in "endemic areas" with an increase of outbreaks during the autumn and winter months.
- EMCV infections in pigs should mainly be considered an individual farm problem as no massive farm-to-farm spread in the neighbourhood of infected farms is observed.
- Standardised sampling and laboratory protocols should be strived for in the study of newly appearing diseases as to harmonise (prevalence) data from different sources and therewith allow for comparison and extrapolation of results.

Risk factors

- Mice are considered a risk factor for clinical EMC since high or medium numbers of mice were found with a considerable higher rate among EMCV-infected (case) farms compared to control farms.
- The variables associated with an increased risk of EMCV infection directly or indirectly point at rodents and lack of hygiene as risk factors, justifying additional research in this area.

Transmission of EMCV

- The transmission of EMCV among randomly mixing pigs can probably result in a major outbreak, however in most cases minor outbreaks will occur.
- The transmission of EMCV among rats and mice is high ($R_0 >> 1$), indicating that these species have the potential to act as a reservoir host for EMCV and to introduce EMCV into pig farms.

Modelling/Course of infection

- Since extrapolation of the R_0-estimates for EMCV in pigs into a compartmentalised setting indicated only limited virus spread throughout the compartment, multiple introductions from other sources (rodents) need to be involved to explain observed major EMCV outbreaks in domestic pig farms.
- Compartmentalisation in itself already reduces the direct pig-to-pig spread of EMCV and therewith the final size of an outbreak in a domestic pig farm. Increasing the effect of fences can strengthen this effect.
- To prevent and/or contain clinical outbreaks of EMCV in pig farms, farmers should avoid contacts between their pigs and potential host populations (e.g. by rodent control) and isolate suspected pigs as soon as possible.

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Summary

Introduction

In modern livestock husbandry systems every now and then pathogens appear that cause disease problems which go along with discomfort and/or mortality among affected animals and consequently result in losses to the farmer. Although a considerable amount of knowledge was build up on several diseases over the years, still every now and then new pathogens pop up or "emerge" in the field. When such potentially harmful pathogens pop up, it is important to be able to react fast and effectively.

Infection due to Encephalomyocarditis virus (EMCV), belonging to the genus cardiovirus of the family Picornaviridae, presented itself as such in pig farms in Europe. Although generally considered a rodent virus, pigs are considered the most susceptible domestic species. Clinical disease due to EMCV was described to take two main forms, acute myocarditis usually in young pigs or reproductive failure in sows.

After its recognition veterinarians and farmers had to decide on how to deal with this disease, which required information on its origin and behaviour at the time of the initial outbreaks. To assess the relevance of EMCV in domestic pigs and to support the development of effective EMCV prevention and control strategies at farm level, the research in this thesis focused on a number of key elements in the epidemiology of EMCV in pig farms;

1. Disease occurrence; analyse where and to what extent the virus circulates in domestic to understand the relevance of (clinical) EMCV.
2. Risk factors; identify potential risk factors for EMCV at pig farms to focus and direct the development of EMCV prevention and control strategies.
3. Transmission dynamics at animal level; quantify transmission of EMCV within and possibly among suggested hosts (domestic pigs and rodents), to analyse and test their potential contribution to the course of EMCV outbreaks in pig farms.
4. Outbreak analysis at farm level; combine available information into an integral modelling approach in order to evaluate the role of pig-to-pig EMCV spread in a farm setting and to study the impact of potential control measures.

These research aims were elaborated in a stepwise approach. Historical data from (clinical) disease outbreaks were combined with knowledge from experts in the field and literature to formulate basic hypotheses on the introduction and spread of EMCV in domestic pig farms. This inventory (descriptive research) and resulting hypotheses formed the basis for additional analytical research (risk factor study) and experimental studies on the transmission of EMCV. Finally a simulation model was developed to integrate available knowledge and extrapolate experimental findings on EMCV transmission in pigs into a real farm setting.

Prevalence of EMCV

To understand its relevance in pigs and to develop hypotheses on the origin, cause and nature of infection, the occurrence of EMCV in domestic pigs and wild boar in several European countries was discussed in relation to the emerging appearance of the virus (Chapter 2). From 1990 to 2001 clinical outbreaks were analysed and serum samples, partly from existing screening programs, were tested for antibodies against EMCV. In Belgium most clinical EMCV outbreaks were reported (320) in this period, followed by Italy (110), Greece (15) and Cyprus (6). The outbreaks appeared to be clustered in "endemic areas" with an increase in outbreaks during autumn and winter months. Local rodent populations are often considered responsible for this clustering and the observed re-occurrence of outbreaks in the same farms.

The recent studies (Ch 5), indicating high transmission among rodents in experiments, demonstrated that they at least technically could serve as a potential EMC virus reservoir. The within-herd seroprevalence measured in clinically affected pig farms varied considerably among farms (2-87%), age categories (0-81%) and countries. Data from non-clinical farms showed that sub-clinical infection with EMCV is quite common, both within (seroprevalence 8-66%) and outside (7-62%) the endemic areas of the clinical countries as well as in the non-clinical countries Austria, France and The Netherlands (3-9.4%). The variable clinical picture in pigs was ascribed to differences in pathogenicity of EMCV strains involved, the available infectious dose and/or the susceptibility of the pigs due to age and breed.

Among wild boar the general seroprevalence found varied between 0.6 and 10.8% while a study in Belgium only revealed a low EMC virus prevalence (3.3%). This raised the question whether their infection status reflected either a high prevalence in wild rodents or that wild boar should also be considered a potential (temporary) host for EMCV.

In summary, the prevalence data hypothesised that (clinical) outbreaks of EMCV in domestic pig farms might occur when certain conditions are met, i.e. the presence of the virus in certain species/hosts in the neighbourhood (e.g. in rodent populations or wild boar), a cause for these species to come in contact with domestic pigs (e.g. due to food shortage) and a combination of available infectious dose and susceptibility of the pigs in order to start an infection process in a farm. To elaborate on these hypotheses both rats (Chapter 6) and pigs (Chapter 4, 5 and 7) were tested for their potential to act as an EMC virus reservoir, while risk factors for the occurrence of EMCV were studied in a case control study (Chapter 3).

Risk factors for clinical EMCV

In literature rodents (either by their infected faeces or carcasses), transplacental- and direct pig-to-pig transmission are suggested as potential routes of infection for EMCV in pig farms.

To study these and other critical factors inducing clinical emergence of EMCV, a matched case-control study was preformed among 58 pig farms in Belgium (Chapter 3). In the case control design, separate samples of units with (cases) and without (controls) the specified disease, i.e. clinical EMCV, were selected. Then the relative frequency of the potential risk factor(s) in each of these groups was compared using the odds ratio (OR).

From March 2000 onwards in total 29 farms experienced a clinical outbreak due to EMCV, confirmed by virus isolation. In most cases (62%), mortality was seen among suckling piglets, while in 4 of these farms also problems were seen in other age categories. In 24% of the cases, mortality was only among fattening pigs. Five farms showed reproduction problems in sows, while two of these farms also had disease among suckling piglets. Control farms were matched geographically on farm size and farm type and were selected on the absence of clinical signs.

A questionnaire on potential risk factors for EMCV was developed and filled out in both case and control farms to collect data, which clustered factors in 3 groups: a) rodents, b) general farm set up and c) general hygiene. Data analysis was done by conditional logistic regression and followed a stepwise procedure. In the univariable analyses, the presence of cows at a farm, the presence of mice, feeding automatically and group-drinking systems were significant and a number of factors related to sanitation, hygiene and manure handling looked protective for clinical EMCV infection. In general, the different variables seemed directly or indirectly related to rodents or hygiene, which seemed to support the initial hypotheses.
The final multivariable model including all farms contained presence of mice (OR = 8.3) as a risk factor for clinical EMCV while the flow of manure up through the slatted floor (OR = 0.11) and movement of manure between manure pits in the pig stable (OR = 0.14) were protective. It was hypothesized that the risk factors influence the infectious dose available and therewith mediate the subsequent course of infection in pigs dependent on the susceptibility of pigs and the pathogenicity of the EMCV strain.

Transmission of EMCV in pigs

From a disease control point of view it was essential to know whether attention should be focussed on avoiding (contact) transmission between pigs or that other potential transmission routes should be taken into account too. Before elaborating on transmission in e.g. rodents, the transmission of EMCV in domestic pigs was experimentally quantified to find out whether an EMCV infection could persist in a pig population by pig-to-pig transmission alone and therewith explain the observed major outbreaks in the field (Chapter 4). Epidemiologically this means that an infectious pig in a population or group of susceptible pigs infects on average more than one other pig or, in other words, that the basic reproduction ratio (R_0) exceeds one. Two types of transmission experiments were performed to estimate R_0.

In the first set up with nine separate pairs, one randomly chosen piglet per pair was inoculated with a Belgian (myocardial) EMCV strain (B279/95, 103 TCID50/ml oronasally) and placed back into the pen. In the second experiment with two separate groups of five piglets, two piglets in each group were inoculated at the start with the same strain and dose. During the experiments viraemia in blood and excretions was measured as well as the serological response against EMCV antigen. After death or euthanasia, the piglets were checked for heart lesions and virus isolation was done on various tissues. In both experiments the majority of the inoculated piglets either died with typical heart lesions (five out of nine and three out of four resp.), or produced high levels of neutralising antibody. EMC virus was isolated from the hearts of all piglets that died during one of the experiments. The results clearly indicated that contact infection of a myocardial EMCV strain among pigs is possible, while mortality was observed even in contact infected piglets. The "general epidemic model" or susceptible-infectious-removed model (SIR) was used to analyse the experiments. This allowed for the probability distribution of the outcome, i.e. the total number of contact infections (final size), to be described in terms of R_0. The pairwise experiment revealed a point estimate of R_0=2.0 (95%-CI = 0.37 – 10.74), while the group experiment resulted in an R_0 value of 0.71 (95%-CI = 0.08 - 4.93). Combining the information from both experiments resulted in an estimate for R_0 of 1.24 (95%-CI = 0.39 – 4.35). Since R_0 had values around the threshold value of R_0=1, it was concluded that the spread of EMCV due to contacts between pigs in most cases would be limited, but due to chance processes might lead to large outbreaks as well.

In addition to the experiments, EMCV was estimated from field data as well (Chapter 6). A case farm in Belgium showed 5% mortality among finishing pigs, with the presence of EMCV confirmed by necropsy and virus isolation. Serology was used to assess the final size from which the transmission parameter R_0 was estimated. In one compartment of the farm with 630 pigs in 44 pens, 6 pens were fully sampled to obtain an accurate estimate for R0. In the remaining pens 2 randomly selected pigs were bled to gain insight in the spread of EMCV in the compartment. These 151 pigs were bled twice and their serum was tested in a virus-neutralisation test to detect antibodies against EMCV. The seroprevalence in the consecutive samplings was 41 and 43% respectively (cut off value of 1:40) and seropositive animals were found in almost every pen of the compartment. Based on the final size from the second sampling, the R_0 for the 6 fully sampled pens varied between 0.6 and 1.7 per pen, while the combined estimate for these 6 pens resulted in R_0=1.36 (95%-CI 0.93-2.23). The median of the estimated R_0 values from the partially sampled pens was 1.3. These estimates confirmed the findings from the earlier experiments (Chapter 4) that EMCV is not very effectively transmitted among pigs.

Although both the transmission experiments and the case study remained inconclusive with respect to the threshold value of R_0=1, the combination of the relatively low R_0 between pigs within pens and the remarkable high percentage of affected pens in the field study (35 out of 44 pens, 80%) was considered unlikely when only one initially infected pig was

assumed in the compartment. Therefore multiple introductions most likely occurred in this field study, a scenario in which rodents were considered to be involved.

Transmission of EMCV in rodents

Infection by faeces or carcasses from infected rodents is considered one of the routes of introduction and spread of EMCV infection in pig farms. Although it is known that rats can be infected with the EMC virus, only little is known about the spread of the virus within rat populations. Insight in virus spread among rats was thought to enlighten their role as a potential transmitter or reservoir for the EMC virus and stress the need for proper rodent control at farm level. Experiments were performed in order to assess the transmission rate of EMCV within a rat population by means of R_0 (Chapter 5).

In total twenty-five eight-week-old Wistar rats housed in individual plastic cages, were experimentally infected either with a Greek myocardial EMCV strain (5 rats with dose $0.2X10^6$ $TCID_{50}$ per each rat and 10 rats with dose $0.5X10^{4.5}$ $TCID_{50}$ per each rat, oronasally) or a Belgian myocardial EMCV strain (10 rats with dose $0.5X10^{4.5}$ $TCID_{50}$ per each rat, oronasally). Two to five days later each inoculated rat was moved to a new clean cage and coupled with a control rat to study contact infection. This set up allowed for comparison of the pathogenicity of the two strains and estimation of R_0, indicating the level of EMCV transmission among rats. During the experiments, faecal virus excretion was measured as well as the serological response against EMCV. After euthanasia, virus isolation was attempted from different rat tissues.

Both EMCV strains produced neither mortality, nor clinical signs and only low titres of neutralizing antibodies were found. All contact rats however were infected and the virus was isolated from various tissues (e.g. thymus, Peyer's patches, heart, lungs). Also, the infected rats excreted the virus in their faeces for periods up to 3-4 weeks. Both 10-pair experiments revealed a point estimate for the R_0 of ∞ (95%-CI for both the Greek and Belgian EMCV strains = 4.48 - ∞), as did the 5-pair experiment with a higher dose of the Greek strain (95%-CI = 1.83 - ∞). Combining the results from the two 10-pair experiments resulted in an estimate for R_0 of ∞ (95%-CI: 9.87 - ∞). These results indicated that the EMC virus can spread very easily and persist within a rat population by horizontal rat-to-rat transmission alone ($R_0 >> 1$), which makes the rat population to a potential reservoir for EMCV in domestic pig farms.

Outbreak simulation

Both the performed transmission experiments (Chapter 4) and the field study (Chapter 6) were inconclusive with respect to the threshold value of R_0=1. Therefore from these studies it could not be concluded whether EMCV could persist in a pig population by pig-to-pig transmission alone and therewith account for observed major outbreaks in pig farms. These point estimates close to one required a large additional experiment to verify this with sufficient power, which was considered both unethical and expensive. Moreover as transmission experiments assumed random mixing of pigs, while commercial pig farms are usually subdivided in compartments and pens, and the field study possibly was hampered by interference of other transmission routes (e.g. farmer, rodents) it was questioned whether another animal experiment could easily overcome these limitations.

Alternatively in Chapter 7 a simulation model was used to extrapolate the experimental results to a compartmentalised pig house setting and to test to what extend contacts between pigs in different pens needed to be reduced in order to prevent major outbreaks in a compartment. Assuming that EMCV was only transmitted by pig-to-pig contact, virus transmission was modelled according to the standard SEIR-approach using the states Susceptible, incubating (or Exposed), Infectious and Removed. Simulation scenarios reflected either a completely mixed pig population or the increasing effect of fencing on the pig-to-pig transmission between pigs in adjacent pens. Mediated by the fence effect (F) an individual pig in a pen was infected by infectious pen mates or by infectious pigs from adjacent pens. The simulations covered all R_0-values in the CI around the R_0 point estimate from the experiments (Chapter 4) and F was increased in steps of 0.1 from 0 to 0.9. The final size of simulated outbreaks (the number of infected pigs or pens) was measured and the probability to observe outbreaks that affected at least 50 or 80% of the pens was calculated.

After introduction of fences as "physical barrier" for any R_0<1.24 the probability to observe outbreaks affecting more than 50% of the pens (major outbreaks) remained below 10%. When fences also were used to reduce contact transmission the probability to observe such outbreaks could be scaled down to about 50% for R_0<2.7 and F>0.8. Only for R_0>4, major outbreaks occurred more often than by chance even if only minimal contact between adjacent pens was allowed. From these results it was concluded that in a compartmentalised pig housing one single EMCV introduction was unlikely to cause a major outbreak by pig-to-pig transmission only, especially if the fences actually reduced the contacts (or their infectiousness) between pigs in adjacent pens.

Main conclusions

The main conclusions from the various studies within the framework of this thesis in relation to the research questions are;

Prevalence

- The consequences of an EMCV infection in domestic pig farms in terms of the number of (sub)clinically infected pigs vary considerably, probably due to differences in pathogenicity of the EMCV-strains, the available infectious dose and the susceptibility of the pigs (age/breed).
- Outbreaks of EMCV appear to be clustered in "endemic areas" with an increase of outbreaks during the autumn and winter months.
- EMCV infections in pigs should mainly be considered an individual farm problem as no massive farm-to-farm spread in the neighbourhood of infected farms is observed.
- Standardised sampling and laboratory protocols should be strived for in the study of newly appearing diseases as to harmonise (prevalence) data from different sources and therewith allow for comparison and extrapolation of results.

Risk factors

- Mice are considered a risk factor for clinical EMC since high or medium numbers of mice were found with a considerable higher rate among EMCV-infected (case) farms compared to control farms.
- The variables associated with an increased risk of EMCV infection directly or indirectly point at rodents and lack of hygiene as risk factors, justifying additional research in this area.

Transmission of EMCV

- The transmission of EMCV among randomly mixing pigs can probably result in a major outbreak, however in most cases minor outbreaks will occur.
- The transmission of EMCV among rats and mice is high ($R_0 >> 1$), indicating that these species have the potential to act as a reservoir host for EMCV and to introduce EMCV into pig farms.

Modelling/Course of infection

- Since extrapolation of the R_0-estimates for EMCV in pigs into a compartmentalised setting indicated only limited virus spread throughout the compartment, multiple introductions from other sources (rodents) need to be involved to explain observed major EMCV outbreaks in domestic pig farms.

- Compartmentalisation in itself already reduces the direct pig-to-pig spread of EMCV and therewith the final size of an outbreak in a domestic pig farm. Increasing the effect of fences can strengthen this effect.
- To prevent and/or contain clinical outbreaks of EMCV in pig farms, farmers should avoid contacts between their pigs and potential host populations (e.g. by rodent control) and isolate suspected pigs as soon as possible.

Samenvatting

Introductie

In de moderne veehouderijsectoren duiken van tijd tot tijd ziekteverwekkers (pathogenen) op die ziekteproblemen veroorzaken die gepaard gaan met ongemak en sterfte bij de getroffen dieren en mede daardoor vaak resulteren in schade voor de veehouder. Hoewel over de jaren een schat aan informatie is vergaard over diverse dierziekten, duiken van tijd tot tijd nieuwe, tot dan toe (relatief) onbekende, ziekteverwekkers op in het veld. Wanneer zulke pathogenen opduiken is het belangrijk snel en doelgericht te kunnen handelen.

Ziekte als gevolg van infectie met het Encephalomyocarditis virus (EMCV), dat behoort tot het geslacht cardiovirus van de familie Picornavirussen, presenteerde zichzelf als zodanig in de commerciële varkenshouderij in Europa. Hoewel EMCV in het algemeen wordt gezien als een knaagdiervirus, worden varkens beschouwd als de meest gevoelige gedomesticeerde diersoort. Het EMC virus veroorzaakt in varkens een tweetal klinische beelden, myocarditis (ontsteking aan het hart) in jonge dieren en voortplantings- of vruchtbaarheidsproblemen bij zeugen (o.a. abortus, doodgeboren biggen, moeilijk drachtig worden).

Na de diagnose moet de dierenarts en/of veehouder besluiten welke behandeling moet worden toegepast, hetgeen informatie vraagt over de oorsprong en het gedrag van het virus bij eerder geobserveerde uitbraken. Om de ernst van EMCV infecties in de varkenshouderij te kunnen vaststellen en controle maatregelen te kunnen ontwikkelen, richtte het huidige onderzoek zich op een aantal sleutelaspecten binnen de epidemiologie van EMCV in varkensbedrijven:

1. Het optreden van infectie (prevalentie): analyse van waar en in welke mate virus circuleert in gedomesticeerde varkens om de impact van (klinische) EMCV infecties vast te stellen.
2. Risicofactoren: analyse van mogelijke risicofactoren voor EMCV op varkensbedrijven om gerichte ontwikkeling van preventie- en controlemaatregelen te ondersteunen.
3. Virustransmissie op dierniveau: kwantificering van EMCV transmissie binnen en mogelijk tussen potentiële gastheren (varkens en knaagdieren) om hun bijdrage aan het verloop van uitbraken in varkensbedrijven vast te kunnen stellen.
4. Uitbraak analyse op bedrijfsniveau: bundeling van beschikbare informatie in een simulatiemodel om de rol van directe varken-op-varken transmissie op bedrijfsniveau te bestuderen en mogelijke controle maatregelen te evalueren.

Deze onderzoeksdoelen zijn uitgewerkt aan de hand van een aantal deelstappen. Historische gegevens van (klinische) uitbraken zijn gecombineerd met kennis van experts en beschikbare literatuur om basis hypotheses over de introductie en verspreiding van het EMC virus in varkensbedrijven te kunnen formuleren. Deze inventarisatie (beschrijvend onderzoek) en de daaruit voortkomende hypotheses vormden de basis voor aanvullend analytisch onderzoek

(studie naar risico factoren) en experimenten om de transmissie van EMCV vast te stellen. Tenslotte is een simulatiemodel ontwikkeld om beschikbare informatie te integreren en de gegevens uit experimenten te extrapoleren naar een bedrijfsmatige setting.

Prevalentie

Om de impact van EMCV infecties in varkens in te kunnen beoordelen en hypothesen te ontwikkelen over de herkomst, oorzaak en aard van de infecties, is het voorkomen van EMCV in zowel gedomesticeerde- als wilde varkens bestudeerd in relatie tot het plotselinge opkomen van de ziekte (Hfdst 2). Van 1990 tot 2001 zijn klinische uitbraken geanalyseerd en zijn serummonsters, deels uit reeds bestaande screeningsprogramma's onderzocht op antilichamen tegen EMCV. De meeste uitbraken werden in deze periode gerapporteerd in België (320), gevolgd door Italie (110), Griekenland (15) en Cyprus (6). De uitbraken bleken geclusterd inn zgn endemische gebieden met een toename in het aantal uitbraken gedurende de herfst- en wintermaanden. Veelal wordt gesteld dat infecties vanuit locale knaagdierpopulaties de reden zijn voor deze clustering, evenals voor de waargenomen her-infecties in dezelfde bedrijven.
De recente resultaten (Hfdst 5), die wijzen op een hoge verspreiding van het EMC virus onder ratten, geven aan dat deze knaagdieren in ieder geval technisch gezien de gesuggereerde rol van virusgastheer zouden kunnen vervullen.

De koppelprevalentie (%) gemeten in klinisch geïnfecteerde bedrijven varieerde aanzienlijk tussen bedrijven (2-87%), leeftijdscategorieën (0-81%) en getroffen landen. Gegevens van niet-klinische bedrijven toonden aan dat subklinische infectie regelmatig voorkomt, zowel binnen (seroprevalentie 8-66%) als buiten (7-62%) de endemische gebieden van de landen met kliniek alsook in de landen zonder gerapporteerde kliniek zoals Oostenrijk, Frankrijk en Nederland (3-9.4%). De variatie in het klinische beeld wordt toegeschreven aan verschillen in pathogeniteit van de verschillende EMCV virusstammen, de beschikbare hoeveelheid infectieus materiaal en/of the gevoeligheid van de varkens vanwege leeftijd of ras.

Onder wilde varkens varieerde de seroprevalentie tussen 0.6 en 10.8% terwijl uit onderzoek in Belgie slechts een lage virusprevalentie kon worden aangetoond (3.3%). Hierdoor rees de vraag of de bevindingen in wilde varkens slechts een weerslag vormden van de EMCV prevalentie in wilde knaagdieren of dat wilde varkens ook als een potentiële gastheer voor EMCV zouden moeten worden aangemerkt.

Concluderend riepen de prevalentie data het beeld op dat (klinische) uitbraken van EMCV in varkensbedrijven konden ontstaan wanneer bepaalde ingrediënten voorhanden waren zoals, de aanwezigheid van het EMC virus in bepaalde diersoorten/gastheren in de omgeving (bijv. in knaagdieren of wilde varkens), een reden voor het optreden van contact tussen deze gastheer en de gedomesticeerde varkens (bijv. knaagdieren trekken vanwege voedseltekort de

stal in) en een combinatie van de beschikbare infectieuze dosis versus de gevoeligheid van de varkens om een infectie aan te laten slaan. Om deze ideeën te staven is zowel voor knaagdieren (Hfdst 6) als voor de varkens zelf (Hfdst 4,5 en 7) nagegaan of deze species in potentie als gastheer voor het EMC virus zouden kunnen optreden. Tevens is een studie opgezet om risico factoren met betrekking tot het optreden van klinische EMCV te identificeren (Hfdst 3).

Risico factoren

In de literatuur worden knaagdieren, hetzij via hun besmette feaces of karkassen, verticale- en directe varken-op-varken transmissie genoemd als potentiële transmissieroutes voor EMCV in varkensbedrijven. Om deze en andere factoren die mogelijk een rol spelen bij het ontstaan van klinische EMCV te onderzoeken is een gekoppelde case/controle studie uitgevoerd bij in totaal 58 varkensbedrijven in West Vlaanderen, Belgie (Hfdst 3).

Vanaf maart 2000 werd bij 29 bedrijven een klinische uitbraak van EMCV vastgesteld (cases) en bevestigd door middel van virusisolatie. In de meeste gevallen (62%) werd sterfte onder de zuigende biggen aangetroffen, terwijl er in 4 van deze bedrijven ook ziekteproblemen waren bij andere leeftijdscategorieën. In 24% van de case bedrijven werd alleen sterfte gevonden onder de mastvarkens. Bij 5 bedrijven was sprake van vruchtbaarheidsproblemen terwijl 2 van deze bedrijven ook ziekte hadden onder de zuigende biggen. De controle bedrijven werden aan een case bedrijf gekoppeld op basis van een vergelijkbare geografische ligging, hetzelfde type en dezelfde omvang ("matching").

In het kader van de studie werd een vragenlijst betreffende potentiële risicofactoren voor EMCV opgesteld en afgenomen op zowel case- als controlebedrijven, welke vragen omvatte in een drietal clusters: a) knaagdieren, b) algemene bedrijfsopzet en c) hygiëne. Data analyse werd uitgevoerd m.b.v. conditionele logistische regressie en volgde een stapsgewijze aanpak. In de univariabele analyse ($P \leq 0.25$ in de Likelihood Ratio Chi^2-test) waren de aanwezigheid van koeien op het bedrijf, de aanwezigheid van muizen, automatische voer- en drinksystemen significant, terwijl verschillende factoren gerelateerd aan o.a. de omgang met dode dieren, hygiëne en mestbehandeling beschermend leken voor klinische EMCV. In het algemeen gold dat de diverse factoren direct of indirect gerelateerd waren aan knaagdieren of hygiëne, hetgeen de oorspronkelijke hypotheses leek te ondersteunen.

De multivariabele verbanden tussen de risicofactoren en het optreden van klinische EMCV zijn getest met conditionele logistische regressie, waarbij per stap steeds de minst significante variabele uit het model werd verwijderd ($P > 0.10$ in Wald's-test). Het eindmodel over alle bedrijven bevatte de factor " aanwezigheid van muizen" (Odds ratio-OR=8.3) als risico factor, terwijl het opkomen van varkensmest vanuit de kelder door de mestroosters (OR = 0.11) en het verplaatsen van mest tussen verschillende mestkelders (OR = 0.14)

beschermend bleken. Er wordt gesuggereerd dat de verschillende risicofactoren de beschikbaarheid van infectieus materiaal beïnvloeden en daarmee, in samenhang met de gevoeligheid van de varkens en de pathogeniteit van de diverse EMCV stammen, het infectieproces in varkens reguleren.

Verspreiding van EMCV in varkens

Vanuit ziektepreventie oogpunt is het essentieel om te weten of de aandacht moet worden gericht op het voorkomen van (contact)overdracht van het virus tussen varkens of dat er ook rekening moet worden gehouden met andere verspreidingsmechanismen.

Voor dat overgegaan werd tot het bestuderen van alternatieve transmissieroutes, werd de mate van EMCV verspreiding onder varkens bepaald om na te gaan of het virus in een populatie zou kunnen overleven door varken-op-varken transmissie alleen en daarmee grote uitbraken in varkensbedrijven zou kunnen verklaren. Epidemiologische gezien houdt dit in dat een infectieus varken in een populatie of groep van gevoelige dieren gemiddeld meer dan een ander varkens infecteert, of in andere woorden, dat de reproductie ratio (R_0) groter is dan 1.
Er werden 2 soorten transmissie experimenten uitgevoerd om R_0 te schatten. Het eerste experiment betrof een paartjesproef. In elk van de 9 paartjes werd een willekeurig gekozen varken geinoculeerd met virusmateriaal van een Belgische EMCV stam (myocarditis type B279/95, 10^3 $TCID_{50}$/ml oro-nasaal) en teruggeplaatst in het hok. Het tweede experiment betrof 2 groepen van elk 5 dieren. Per groep werden 2 dieren geïnoculeerd met eenzelfde dosis van dezelfde virusstam.

Tijdens de experimenten werd de virusuitscheiding in bloed en excreta gemeten alsmede de serologische afweerreactie tegen EMCV in het bloed. Nadat de dieren gestorven of geeuthaniseerd waren, werden ze onderzocht op de karakteristieke hartafwijkingen en werd getracht virus te isoleren uit diverse weefsels. In beide experimenten stierf het grootste deel van de geinoculeerde dieren met typische afwijkingen aan het hart (resp. 5 van de 9 en 3 van de vier), of toonde een hoge afweerreactie van neutraliserende antilichamen in het bloed.
Het EMC virus kon worden geïsoleerd uit de harten van alle varkens die stierven gedurende het onderzoek. Deze resultaten toonden duidelijk aan dat contact infectie van een myocardiale EMCV stam tussen varkens mogelijk was, waarbij zelfs sterfte optrad onder varkens die besmet waren via contact infectie.

Het algemene epidemiologische ofwel "gevoelig-infectieus-immuun"-model (Susceptible-Infectious-Removed, SIR) werd gebruikt om de experimenten te analyseren. Dit bood de mogelijkheid om de kansverdeling van de uitkomsten, nl. het aantal contactinfecties ("final size), te beschrijven in termen van de R_0. De paartjesproef resulteerde in een puntschatter R_0=2.0 (95%-CI = 0.37 – 10.74), terwijl het groepsexperiment resulteerde in R_0 schatter van 0.71 (95%-CI = 0.08 - 4.93). Indien de informatie uit beide experimenten werd gecombineerd,

kwam de schatter uit op R_0=1.24 (95%-CI = 0.39 – 4.35). Deze schattingen voor R_0 dicht bij de grenswaarde 1 suggereerden dat de verspreiding van EMCV tussen willekeurig mixende varkens in een groep in de meeste gevallen beperkt zal zijn, maar vanwege kansprocessen van tijd tot tijd ook kunnen resulteren in grote uitbraken.

Aanvullend werd de verspreiding van EMCV ook geschat uit velddata afkomstig van een uitbraakbedrijf in België waar 5% sterfte onder mestvarkens werd aangetroffen (Hfdst 6). De aanwezigheid van EMCV werd bevestigd door necropsy (?) en virus isolatie. De serologische status van dieren werd bepaald om de "final size" te schatten op basis waarvan vervolgens de R_0 kon worden berekend.

In een compartiment van het bedrijf met 630 varkens in 44 hokken, werden 6 hokken volledig bemonsterd om een nauwkeurige schatting van R_0 te verkrijgen. In de overgebleven hokken werden 2 willekeurig gekozen varkens bemonsterd om inzicht in de verspreiding van het virus door het compartiment te verkrijgen. Van deze 151 varkens werd 2 maal bloed getapt dat in een virus neutralisatie test te worden getest op antistoffen tegen EMCV. De seroprevalentie tegen EMCV in de opeenvolgende monsternames bedroeg respectievelijk 41 en 43% bij een afkapwaarde van 1:40) terwijl seropositieve dieren werden aangetroffen in bijna elk hok van het compartiment.

Gebaseerd op de final size uit de 2de monstername werd de R_0 voor de 6 individuele, volledig bemonsterde hokken geschat tussen 0.6 en 1.7 per hok, terwijl de gecombineerde R_0-schatter uitkwam op 1.36 (95%-CI 0.93-2.23). De mediaan van de geschatte R_0-waardes in de overige hokken was 1.3. Deze bevindingen bevestigden de resultaten uit de experimenten (Hfdst 4) dat EMCV niet erg effectief wordt overgedragen door contact infectie tussen varkens.

Hoewel op basis van de experimenten en de veldstudie niet kon worden geconcludeerd of R_0 significant boven of onder de drempelwaarde 1 lag, werd het met deze schattingen voor R_0 onwaarschijnlijk geacht dat de brede verspreiding van de infectie over het compartiment (35 van de 44 hokken) kan zijn veroorzaakt door 1 introductie in een hok gevolgd door alleen varken-op-varken transmissie. Daarom werd geconcludeerd dat naar alle waarschijnlijkheid meerdere introducties in het bedrijf hebben plaatsgevonden waarbij knaagdieren mogelijk een rol in hebben gespeeld.

Verspreiding van EMCV in knaagdieren

Infectie van varkens via uitwerpselen of karkassen van gesmette knaagdieren wordt gezien als een van de mogelijk introductie- en verspreidingsroutes van EMCV in varkens-bedrijven. Hoewel bekend is dat ratten besmet kunnen zijn met EMCV is er slechts weinig bekend over de verspreiding van EMCV binnen een rattenpopulatie. Inzicht in de verspreiding van EMCV tussen ratten zou inzicht kunnen verschaffen in hun gesuggereerde

rol als gastheer of verspreider van het virus en de noodzaak tot knaagdiercontrole op bedrijfsniveau kunnen onderstrepen. Hoofdstuk 5 beschrijft de paartjesexperimenten die zijn uitgevoerd om de verspreiding van het EMC virus onder ratten te kwantificeren door middel van R0.

In totaal werden 25 Wistar ratten van 8 weken oud gehuisvest in individuele kooien en geïnfecteerd met een Griekse myocardiale EMCV stam (5 ratten, dosis $0.2X10^{6}$ $TCID_{50}$ per rat en 10 ratten met een dosis van $0.5X10^{4.5}$ $TCID_{50}$ per rat, oro-nasaal) of een Belgische myocardiale EMCV stam (10 ratten, dosis $0.5X10^{4,5}$ $TCID_{50}$ per rat, oro-nasaal). Twee tot 5 dagen later werd iedere geinoculeerde rat verplaatst naar een nieuwe schone kooi samen met een controle rat om contact infectie te bestuderen.

Deze opzet maakte het mogelijk om de pathogeniciteit van de 2 stammen te vergelijken en de virusverspreiding te schatten m.b.v. R_0. Tijdens de experimenten werd zowel de virus uitscheiding in de mest als de serologische antilichaamreactie tegen EMCV gemeten. Na euthanisatie werd geprobeerd virus te isoleren van verschillende weefsels.

Geen van de 2 stammen veroorzaakte sterfte onder de ratten en er werden slechts lage afweerreacties in het bloed aangetroffen. Wel bleken alle controle ratten geïnfecteerd via contact infectie en het virus kon worden geïsoleerd van diverse weefsels (bijv. thymus, Peyer's patches, hart, longen). Tevens scheidden de ratten gedurende 3-4 weken het virus uit via hun faeces. Zowel de beide 10-paartjes experimenten resulteerden in een schatter voor R_0 van $R_0 = \infty$ (95%-CI voor zowel de Griekse als Belgische EMCV stam = 4.48 - ∞), als ook het 5-paar experiment met de hoger dosis van de Griekse stam (95%-CI = 1.83 - ∞). Combinatie van de resultaten van beide 10-paartjes experimenten leverde een schatter op van $R_0=\infty$ (95%-CI: 9.87 - ∞). Deze resultaten gaven aan dat het EMC virus zich gemakkelijk kan verspreiden en handhaven in een rattenpopulatie via horizontale rat-op-rat transmissie, waardoor ratten kunnen worden aangemerkt als een potentieel reservoir voor EMCV in en rondom varkensbedrijven.

Uitbraak simulatie

Op basis van de experimenten en de veldstudie kon niet worden geconcludeerd of R0 boven of onder de drempelwaarde $R_0=1$ lag. Daardoor kon niet worden vastgesteld of EMCV zich in de varkenspopulatie zou kunnen handhaven door middel van varken-op-varken transmissie alleen. Vanwege de waarde van de puntschatter dicht bij een zou een experiment van formaat nodig zijn om dit met voldoende power aan te kunnen tonen, wat onethisch en te kostbaar werd geacht. Aangezien er bij experimenten vanuit wordt gegaan dat de dieren vrij kunnen mengen, terwijl er in commerciële bedrijven vaak sprake is van compartimentering, en de verspreiding in de veldstudie mogelijk vertroebeld wordt door vermenging van verschillende

Curriculum vitae

Huibert Maurice werd geboren op 9 juni 1972 in Goes (Zld.). In 1990 behaalde hij zijn VWO diploma aan het Buys Ballot College in Goes. In hetzelfde jaar begon hij, na te zijn uitgeloot voor de studie Diergeneeskunde, met de studie Zootechniek aan de toenmalige Landbouwuniversiteit Wageningen. In 1995 studeerde hij af met de specialisaties Veehouderij en Agrarische Bedrijfseconomie en trad in dienst bij de Kwaliteits Controle Runderen (KCR), onderdeel van de Productschappen Vee, Vlees en Eieren (PVE). Tijdens de Varkenspestcrisis in 1997 was hij werkzaam in het Crisiscentrum Varkenspestbestrijding in Uden op de afdeling Tracering, waarna hij in 1998 werd aangesteld als toegevoegd onderzoeker bij de Vakgroep Agrarische Bedrijfseconomie van WUR tbv. een project naar de economische aspecten van verbeterde welzijnsmaatregelen in de vleeskuikensector. In 1999 werd in het kader van een Europees samenwerkingsproject bij dezelfde vakgroep gestart met het EMCV-onderzoek dat beschreven is in dit proefschrift. Tijdens zijn promotieonderzoek presenteerde hij zijn werk op diverse (inter)nationale congressen en in meetings met de Europese projectpartners. In 2003 trad hij in dienst bij de PVE waar hij o.a. werkzaam was op de dossiers Diergezondheidsfonds (herziening convenant financiering dierziektebestrijding tussen sector en overheid, DGF) en Ziekte van Aujeszky bij varkens (voorbereiding overgang "artikel 10 status"). Begin 2005 volgde een overstap naar de Directie Voedselkwaliteit en Diergezondheid van het Ministerie van Landbouw, Natuur en Voedselkwaliteit waar hij o.a. werkt aan de ontwikkeling van een strategie voor (preventieve) vaccinatie tegen vogelpest (aviaire influenza, AI) bij pluimvee.