

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

Marion KLUIVERS^a, Huibert MAURICE^b, Philip VYT^c, Frank KOENEN^d,
Mirjam NIELEN^{a,*}

^a Department of Farm Animal Health, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 7, 3584 CL Utrecht, The Netherlands

^b Department of Social Sciences, Business Economics Group, Wageningen University, Hollandseweg 1, 6706 KN Wageningen, The Netherlands

^c Animal Health Care Flanders, Torhout, Belgium

^d Department of Virology, Section of Epizootic Diseases, CODA-CERVA-VAR, Groeselenberg 99, 1180 Ukkel, Belgium

(Received 19 May 2005; accepted 26 April 2006)

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 contact between pigs.

encephalomyocarditis virus / pigs / transmission / R_0 / field data

1. INTRODUCTION

Encephalomyocarditis virus (EMCV) is an RNA-virus belonging to the genus *Cardiovirus* of the family Picornaviridae [26]. It was first described in the 1940's as an infection of laboratory rodents [10, 11].

Nowadays, rodents are considered the natural hosts of EMCV, in which the virus usually persists without causing disease [1]. In pigs, EMCV was recognised as a pathogen many years ago, clinical disease resulting from an infection being first diagnosed in 1958 [28]. The most important sources of EMCV-infection for swine appear to be feed and water contaminated by

* Corresponding author: m.nielen@vet.uu.nl

rats, other rodents or infected carcasses [9]. 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 [18, 20]. In sows, reproductive failure can occur [30]. The myocardial form has been reported in Greece, Italy and Belgium, the reproductive form in Belgium mainly [13]. Subclinical infection has been reported in Europe [18], the USA and Asia. In Britain a seroprevalence of 28% was found [21], in Italy 69% [8], in the USA 8.5% [29] and in Japan 25.8% [23]. Neutralising antibodies can be detected as early as 5 days post-inoculation in experimentally infected pigs [1], whereas the virus can be isolated from blood and excreta already one day after inoculation [3, 7, 17]. EMCV-transmission has been described from rodents to a wide variety of other species, including humans [1, 16, 22], while a recent study indicated that rats easily spread the virus among each other [24]. 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 [7, 17]. This could be of great significance for control programmes, since it could indicate whether control should be focussed on rodent-to-pig transmission or, depending on its magnitude, also on pig-to-pig transmission. Transmission between pigs has been quantified under experimental conditions [17], 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" [6]. This parameter is known to have an important threshold value of 1; if $R_0 < 1$, only mi-

nor outbreaks will occur and an infection will die out, but if $R_0 \geq 1$, large outbreaks are also possible [4]. An important question is whether under field conditions the R_0 of the EMC-virus exceeds 1, indicating that the 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.

2. MATERIALS AND METHODS

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. 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 half 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

(a)		(b)	
12	2-0	12	2-0
14	2-1	14	2-1
13	2-0	13	2-1
14	2-0	14	2-0
13	2-0	13	2-1
14	2-1	14	2-1
12	2-1	12	2-1
13	2-0	13	2-0
14	2-1	14	2-1
13	2-1	13	2-1
14	2-1	14	2-1
15	2-0	15	2-2
12	2-1	12	2-1
12	2-1	12	2-1
15	2-1	15	2-0
15	2-1	15	2-1
15	2-1	15	2-1
12*	12-7	12*	12-7
14*	14-8	14*	14-5
12*	12-6	12*	12-6
14 (older pigs)	2-2	empty	
11 (older pigs)	2-2	empty	
entrance		entrance	

* = fully sampled pens

Figure 1. Floor-plan of the seropositive animals in the affected compartment at 1st (a) and 2nd (b) sampling (# live animals in pen, sample size – # seropositive animals).

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.

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.

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. 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 [13]. This test was performed on VERO cells using the ATCC 129B strain (EMCV-reference strain). According to the cut off value used in European EMCV-research [14, 18], titres $\geq 1:40$ were classified as positive. The expected sensitivity and specificity of the test are 80.3 and 100% respectively [28], 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 [2].

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 [25].

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 Susceptible-Infectious-Removed (SIR)-model [5]. 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

Table I. 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	∞

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) [4, 15, 17, 27], 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 [4]. 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

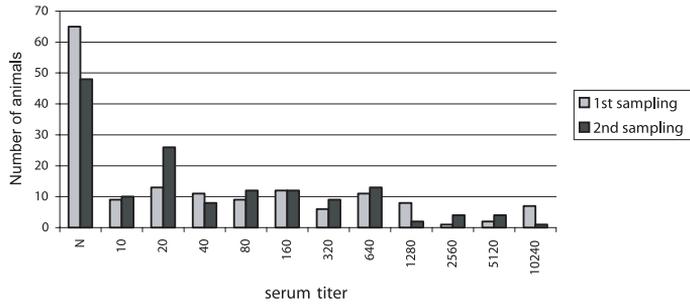


Figure 2. Frequency-distribution of observed serum titres (VNT) at 1st and 2nd sampling (N = negative).

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 $l(R|x_1, \dots, x_6)$, which at its maximum value represents the maximum likelihood estimate (MLE) for R_0 [15, 17, 27] (see Tab. I 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.

3. RESULTS

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%)

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

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

* 3 with at least a fourfold decrease.

** All with at least a fourfold increase.

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 2. The four sampled older pigs that were removed after the first sampling, were all positive. Seropositive pigs were found in 35 of the 44 pens, spread throughout the whole compartment (Fig. 1). Based on serology and/or mortality 41 pens could be assumed infected. Twelve pigs seroconverted between the first and second sampling (Tab. II), seroconversion being defined as a fourfold increase of antibody-titre. Ten others had a lower titre at second sampling, but only three 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.

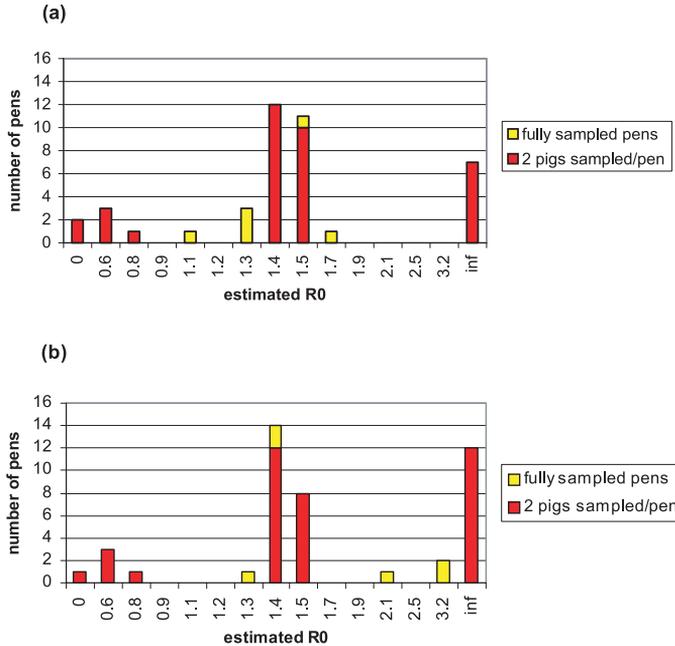


Figure 3. Frequency-distributions of the R_0 -estimations for the individual pens for the final size including mortality, using cut off values 1:40 (a, $N = 41$) and 1:20 (b, $N = 43$). The median values for R_0 were 1.4 and 1.5 respectively.

3.2. Reproduction ratio (R_0)

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 3 and 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. 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. 3b and 4b).

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. 3 and 4). 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. III).

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 [13]. Lower seroprevalences were found by other researchers, but in those cases samples were taken in herds where no outbreak had occurred [19, 29]. A recent European study showed that seroprevalences can vary from 2 to 87% [18]. The transmission of EMC-virus in the field has never been quantified,

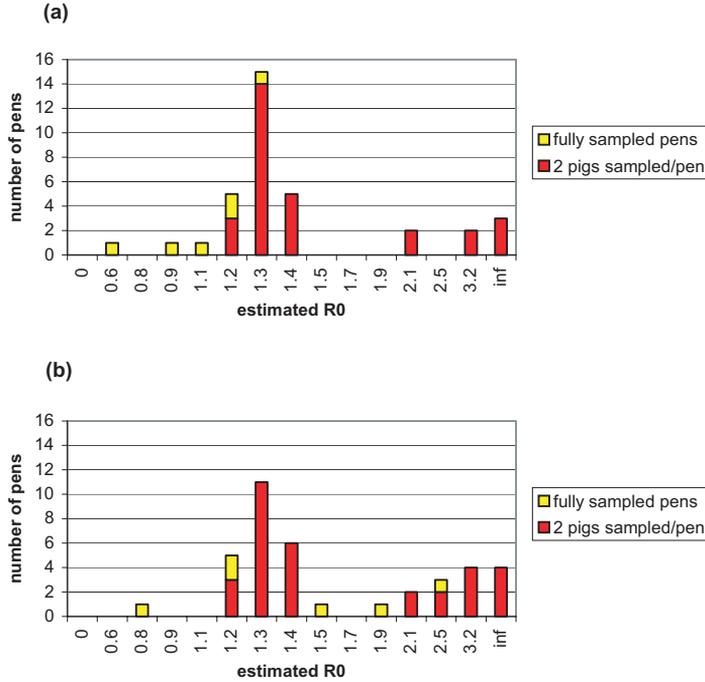


Figure 4. Frequency-distributions of the R_0 -estimations for the individual pens for the final size excluding mortality, using cut off values 1:40 (a, $N = 35$) and 1:20 (b, $N = 38$). The median values for R_0 were 1.3 and 1.3 respectively.

Table III. 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

therefore the current findings can only be compared with those from experimental studies [17]. 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. III). 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.

The 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} [12]. This makes it unlikely that a single introduction in the compartment would lead to the observed wide spread of the EMCV 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. I) 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 [8]. Figures 3 and 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, the 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.

ACKNOWLEDGEMENTS

The authors like to thank the family Bossuyt for making this study possible at their farm and P. Dop, M. Houben and W. Kuller for their assistance during sampling. The contribution to and feedback from A. Velthuis and Prof. J.A. Stegeman on respectively the R_0 -calculations and the article are very much appreciated.

REFERENCES

- [1] Acland H.M., Encephalomyocarditis virus infection, in: Pensaert M.B. (Ed.), *Virus Infections of porcines*, Elsevier Science Publishers, Amsterdam, 1989, pp. 259–264.
- [2] Augustijn M., Elbers A.R., Koenen F., Nielen M., Estimation of seroprevalence of encephalomyocarditis in Dutch sow herds using the virus neutralization test, *Tijdschr. Diergeneeskd.* 131 (2006) 40–44.
- [3] Billinis C., Leontides L., Psychas V., Spyrou V., Kostoulas P., Koenen F., Papadopoulos O., Effect of challenge dose and age in experimental infection of pigs with encephalomyocarditis virus, *Vet. Microbiol.* 99 (2004) 187–195.
- [4] De Jong M.C.M., Kimman T.G., Experimental quantification of vaccine-induced reduction in transmission, *Vaccine* 12 (1994) 761–766.
- [5] De Jong M.C.M., Mathematical modelling in veterinary epidemiology: why model building is important, *Prev. Vet. Med.* 25 (1995) 183–193.
- [6] Diekmann O., Heesterbeek J.A.P., Metz J.A.J., On the definition and computation of the reproduction ratio R_0 in models for infectious diseases in heterogenous populations, *J. Math. Biol.* 28 (1990) 362–382.
- [7] Foni E., Barigazzi G., Sidoli L., Marcato P.S., Sarli G., Della Salda L., Spinaci M., Experimental encephalomyocarditis virus infection in pigs, *J. Vet. Med. B* 40 (1993) 347–352.
- [8] Gualandi G.L., Cammi G., Cardetti G., A serologic survey of encephalomyocarditis virus infection in pigs in Italy, *Microbiologica* 12 (1989) 129–132.
- [9] Joo H.S., Encephalomyocarditis Virus, in: Straw B.E. (Ed.), *Diseases of swine*, Oxford, Blackwell Scientific Publications, 1999, pp. 139–144.
- [10] Jungeblut C.S., Sanders M., Studies of a murine strain of poliomyelitis virus in cotten rats and white mice, *J. Exp. Med.* 72 (1940) 406–436.
- [11] Jungeblut C.S., Dalldorf G., Epidemiological and experimental observations on the possible significance of rodents in a suburban epidemic of poliomyelitis, *Am. J. Public Health* 33 (1943) 169–172.
- [12] Klinkenberg D., De Bree J., Laevens H., De Jong M.C.M., Within- and between-pen transmission of classical swine fever virus: a new method to estimate the basic reproduction ratio from transmission experiments, *Epidemiol. Infect.* 128 (2002) 293–299.
- [13] Koenen F., Vanderhallen H., Castryck F., Miry C., Epidemiologic, pathogenic and molecular analysis of recent encephalomyocarditis outbreaks in Belgium, *J. Vet. Med. B* 46 (1999) 217–231.
- [14] Koenen F., Nowotny N., Brocchi E., Cruciere C., Papadopoulos O., Nielen M., Knowles N., Loukaides P., Molecular characterisation and epidemiology of EMCV: a model for emerging diseases, Ukkel, Belgium, CODA-SERVA-VAR Report no. FAIR6 CT98–4146, 2002.
- [15] Kroese A.H., De Jong M.C.M., Design and analysis of transmission experiments. Annual Conference of the Society for Veterinary Epidemiology and Preventive Medicine (SVEPM), Noordwijkerhout, 28–30 March 2001, pp. xxi–xxxvii.
- [16] Littlejohns I.R., Acland H.M., Encephalomyocarditis virus infection of pigs. 2. Experimental disease, *Aust. Vet. J.* 51 (1975) 416–422.
- [17] Maurice H., Nielen M., Stegeman J.A., Vanderhallen H., Koenen F., Transmission of encephalomyocarditis virus (EMCV) among pigs experimentally quantified, *Vet. Microbiol.* 88 (2002) 301–314.

- [18] Maurice H., Nielen M., Brocchi E., Nowotny N., Bakkali Kassimi L., Billinis C., Loukaides P., O'Hara R.S., Koenen F., The occurrence of encephalomyocarditis virus (EMCV) in European pigs from 1990–2001, *Epidemiol. Infect.* 133 (2005) 547–557.
- [19] Paschaleri Papadopoulou E., Axiotis I., Lapidis C., Encephalomyocarditis of swine in Greece, *Vet. Rec.* 126 (1990) 364–365.
- [20] Paschaleri Papadopoulou E., Anastasiadis G., Skoufos J., Kyriakis S.C., Papadopoulos O., Encephalomyocarditis of swine in Greece: a follow-up, International Pig Veterinary Society Congress, The Hague, The Netherlands, 1992, p. 106.
- [21] Sangar D.V., Rowlands D.J., Brown F., Encephalomyocarditis virus antibodies in sera from apparently normal pigs, *Vet. Rec.* 100 (1977) 240–241.
- [22] Seaman J.T., Boulton J.G., Carrigan M.J., Encephalomyocarditis virus disease of pigs associated with a plague of rodents, *Aust. Vet. J.* 63 (1986) 292–294.
- [23] Shibata I., Ogawa M., Samegai Y., Onodera T., A serological survey of encephalomyocarditis virus infection in pigs in Japan, *J. Vet. Med. Sci.* 55 (1993) 117–118.
- [24] Spyrou V., Maurice H., Billinis C., Papanastassopoulou M., Psalla D., Nielen M., Koenen F., Papadopoulos O., Transmission and pathogenicity of encephalomyocarditis virus (EMCV) among rats, *Vet. Res.* 35 (2004) 113–122.
- [25] Vanderhallen H., Koenen F., Rapid diagnosis of encephalomyocarditis virus infections in pigs using a reverse transcription-polymerase chain reaction, *J. Virol. Methods* 66 (1997) 83–89.
- [26] Van Regenmortel M., Fauquet C., Bishop D., Carstens E., Estes M., Lemon S., Maniloff J., Mayo M., Mc Geoch D., Pringle C., Wickner R., *Virus Taxonomy*, 7th ed., Academic Press, USA, 2000, pp. 667–669.
- [27] Velthuis A.G.J., De Jong M.C.M., De Bree J., Nodelijk G., Van Boven M., Quantification of transmission in one-to-one experiments, *Epidemiol. Infect.* 128 (2002) 193–204.
- [28] Zimmerman J.J., Hill H.T., Beran G.W., Meetz M.C., Serologic diagnosis of encephalomyocarditis virus infection in swine by the microtiter serum neutralization test, *J. Vet. Diagn. Invest.* 2 (1990) 347–350.
- [29] Zimmerman J.J., Schwartz K., Hill H.T., Meetz M.C., Simonson R., Carlson J.H., Influence of dose and route on transmission of encephalomyocarditis virus to swine, *J. Vet. Diagn. Invest.* 5 (1993) 317–321.
- [30] Zimmerman J.J., Encephalomyocarditis, in: Beran G.W., Steele J.H. (Eds.), *Handbook of Zoonoses*, Section B Viral, 2nd ed., CRC Press, Boca Raton, 1994, pp. 423–436.