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The occurrence of encephalomyocarditis virus (EMCV) in European pigs from 1990 to 2001

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SUMMARY

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 programmes, 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 the 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 subclinical 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%.

INTRODUCTION

Encephalomyocarditis virus (EMCV) 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 [1]. 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

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are affected, and where and when the disease occurs is often suggestive of the cause of the disease [2] 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 [3]. 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 [4]. Pigs have been considered to be the most susceptible domestic species and clinical disease due to EMCV was first diagnosed in Panama [5]. Disease due to EMCV may take one of two main forms in pigs: an acute myocarditis, usually in young piglets [6] or reproductive failure in sows [7]. 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 [8–10]. After the initial outbreak in Panama, outbreaks were reported in Florida [6], Australia [11], Cuba [12], New Zealand [13], South Africa [14] and Brazil [15].

Although antibodies against EMCV have been reported in the United Kingdom in the 1970s [16], clinical disease was first recognized in Europe in the late 1980s, when four isolated outbreaks were reported in pig farms in Italy [17, 18] and Greece [19, 20]. In the early 1990s however, clinical disease outbreaks emerged in a number of European countries [10, 19, 21, 22]. These outbreaks were studied during two subsequent European research projects [23, 24] 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.

MATERIALS AND METHODS

Definitions

Cut-off value – antibody titre at or above which ($\geqslant$) 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.

Virology examination

Virus was detected by virus isolation (VI) on baby hamster kidney (BHK-21) cells, by monoclonal antibody (mAbs)-based sandwich ELISA [25] or by reverse transcription–polymerase chain reaction (RT–PCR) [26]. These assays were developed and/or validated during the first EU project [23].

Serology examination

Antibody to EMVC was detected by a virus neutralization test (VNT) against the ATCC reference strain [10] or by a mAbs-based competitive ELISA [25]. 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, twofold 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.

Study designs

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

Table 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 $\geqslant$1/100
Greece	Screening programme	Random	Varying	VNT	$\geqslant$1/40
Belgium	Aujeszky screening	Random	$\geqslant$15 (farm size related)	VNT	$\geqslant$1/32
Cyprus	Convenience sampling	Random	Varying	VNT	$\geqslant$1/40
France	Aujeszky screening	Bretagne, Bourgogne	1–165 (>3rd parity sows)	VNT	$\geqslant$1/32
	Slaughterhouse sampling	Nord-Pas-de-Calais	481		
Austria	Convenience sampling 1999–2001	Federal State of Upper Austria	Varying	VNT	$\geqslant$1/32
UK	Slaughterhouse sampling	UK, Ireland	Varying	VNT	$\geqslant$1/32

VNT, Virus neutralization test.

myocardial lesions or reproductive failure [27], and confirmed by virus detection by ELISA, VI or PCR.

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 [24]. On pig farms without a clinical history of EMCV, both existing screening programmes 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 1.

RESULTS

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 foetuses 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 foetuses 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 foetuses. 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.

Italy

EMCV infection and fatal myocarditis was diagnosed in Italy in 1986, followed by three further cases in 1988 [17, 18]. After almost 10 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 1 and 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

Fig. 1. Geographical distribution of clinical EMCV outbreaks and the serological status of countries participating in the European research projects.

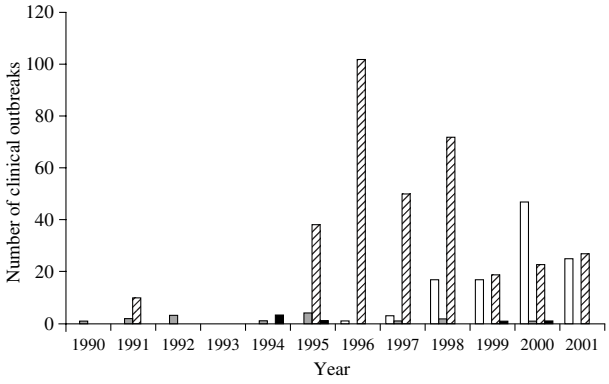

Fig. 2. Reported clinical EMCV outbreaks by year and country. □, Italy; ▩, Greece; ▨, Belgium; ■, Cyprus.

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. 3).

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).

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 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 [19]. Between 1990 and 2001, 15 new outbreaks of myocardial EMCV were recorded in pigs, of which four were re-occurrences. Eight of the 15 outbreaks were also located in the Serres region (Fig. 1). The last outbreak however was located in Loutros, Imathia, Central Macedonia.

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). 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 3).

Belgium

In Belgium, outbreaks of EMCV related to reproductive failure in sows were first reported in 1991 [22], and outbreaks of myocardial disease in 1995 [10]. Currently both forms of EMCV disease are seen in Belgium. The number of outbreaks each year varied considerably (Fig. 2). Until 2000 all the outbreaks occurred in the area of West Flanders (Figs 1 and 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. 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

Table 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 18%	6/38 15%	13/38 33%	13/38 33%	0–60
Greece	24–112	478/723	66·1	62·7–69·6	0 0	0 0	0 0	16/16 100%	47–87
Cyprus	30–152	15/182	8·2	4·2–12·2	0 0	1/2 50%	0 0	1/2 50%	5–27
Belgium	3–45	141/942	15·0	12·7–17·2	0 0	1/8 12·5%	2/8 25%	5/8 62·5%	2–67

CI, Confidence interval; Ab, antibody.

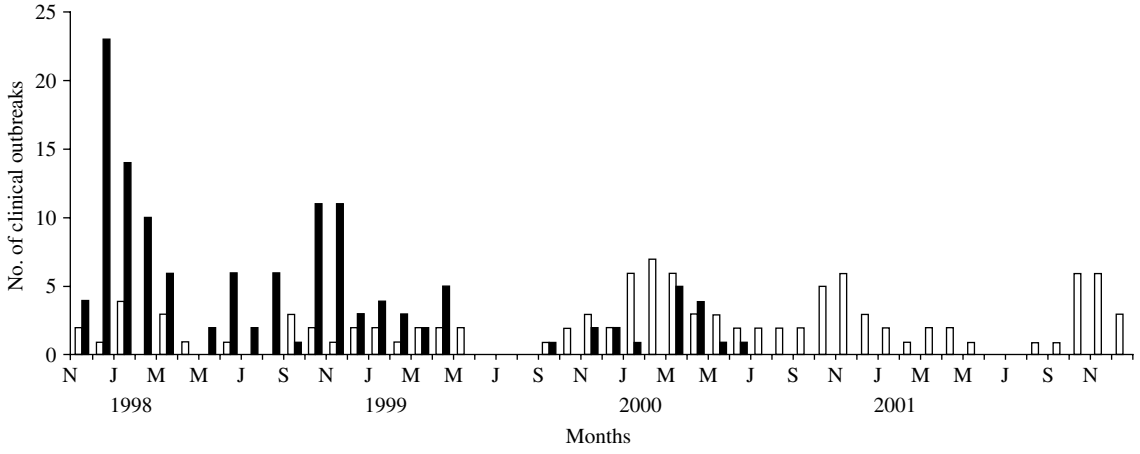

Fig. 3. Reported clinical EMCV outbreaks per month in Belgium (■) and Italy (□) (detailed Belgian data available only until June 2000).

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 programme. At a titre cut-off value of $\geqslant 1/64$, a total of 141 out of 942 samples (15%) tested seropositive.

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 [28]. 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). Since the recorded outbreaks occurred throughout Cyprus, the whole Cypriot-governed part of Cyprus was considered endemic for EMCV (Fig. 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 3. A further 30 pig sera from the farm affected in 2000 were analysed: 22 sera

Table 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

Table 4. *EMCV seroprevalence in non-clinical farms in the endemic areas of the clinical countries*

	Overall seroprevalence			Herd seroprevalence				Within-herd seroprevalence				
									Ab positive seroprevalence (%)			Within-herd *P* range (%)
Country	*N*/total	%	95% CI	Sample 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 47·7%	25/111 22·5%	26/111 23·5%	7/111 6·3%	0–60
Greece	106/172	61·6	54·4–68·9	3–24	18/18	100	—	0 0	0 0	1/18 5·6%	17/18 94·4%	14–100
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 28·9%	28/90 31·1%	26/90 28·9%	10/90 11·1%	0–62·5

CI, Confidence interval; Ab, antibody.

(73%) were considered negative (<1/40), three had a titre of 1/80 and five sera had a titre of >1/320 (Table 2).

Seroprevalence on non-clinical farms' 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 4). In total, from the 4502 sera tested, 287 (6·4%) were found positive.

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 4). The within-herd seroprevalence ranged from 14·3% (1/7) to 100% (5/5).

Table 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. positive 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 %	14·2–50·0 %	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	Abattoir	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	Abattoir	150	1/32	0/150	0				1/16

Cyprus

Of 255 samples from 51 non-clinical farms throughout Cyprus, 15 pigs (5·9 %) from 11 farms were seropositive (titre $\geqslant$1/40), giving a herd seroprevalence of 21·6 % (Table 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 of $\geqslant$1/32 (5·3 %) and 155 samples showed a titre of $\geqslant$1/64 (2·9 %), giving a herd seroprevalence of 7·0 %. A further survey of 17 farms in 2000 and 2001 resulted in 209 out of 1506 samples with a titre of $\geqslant$1/64 (13·9 %), originating from 14 farms (Table 4).

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, slaughterhouses or existing screening programmes. The results are summarized in Table 5.

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-clinical 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 3·27 % 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.

EMCV in wild boar

Italy

In total, 1412 serum samples were collected from wild boar during the hunting seasons 1999–2001 in the regions of Lombardy and Emilia (Table 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

Table 6. *EMCV seroprevalence in wild boar in Italy and France*

Country/region	Number of positive sera over years				Sero-prevalence (%)	Max. titre
	1999	2000	2001	Total		
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	

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 for virus detection tests.

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 [29], 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-Ardenne (9·4%). The maximum titres found ranged from 1/40 in Aquitaine to 1/1060 in Franche-Comte (Table 6).

Luxembourg

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

DISCUSSION

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

Local rodent populations serving as a potential virus reservoir [8, 9, 11] are often thought to be responsible for such clustering and might also explain the re-occurrence of outbreaks in the same farms. Morse [30] 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 [24], a feature often found in RNA viruses [31]. Migration due to food shortages [9] 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 [18, 32], the available infectious dose [33] 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 [10, 34]. Although other studies [21, 35] have demonstrated antigenic stability in EMCV, strain differences in biological characteristics are known. Also differences in pathogenicity within the same or between different isolates [32, 36, 37] and differences in tissue tropism are indicated between various strains [10]. 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 [19]. Experiments with mice and pigs also indicated that EMCV is more pathogenic to the myocardium of younger animals [33, 38]. This might explain the higher death rate in younger animals, especially in naive pig populations [9, 27].

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 [39] considered antibody titres of $\geqslant 1/16$ 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 [16, 40–44], 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 standardized 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 viraemic 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 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 [45]. 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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