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Encephalomyocarditis Virus Infection in Wildlife Species in Greece

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ABSTRACT: The objective of this work was to search for potential wildlife reservoir hosts of *Encephalomyocarditis virus* (EMCV) in wildlife species. From 1994 to 2006, 317 blood and tissue samples were collected from 17 wildlife species in Greece. Encephalomyocarditis virus was isolated only from two *Rattus rattus*. In addition, antibody to EMCV were detected in sera from 39 *Rattus rattus*, one *Apodemus sylvaticus*, one *Microtus arvalis*, and 13 *Sus scrofa*.

Key words: EMCV, Encephalomyocarditis virus, epidemiology, Greece, infection, wildlife.

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 to be the natural hosts, in which the virus normally persists without causing disease (Acland 1989). Several other species, both domestic and wild, are susceptible to EMCV (Zimmermann et al., 1994). In Europe, the virus was isolated from a red squirrel (*Sciurus vulgaris leucourus*) in Great Britain (Vizoso et al., 1964), from a pheasant (*Phasianus colchicus*) in Czechoslovakia (Gresikova et al., 1978), and from two dormice (*Myoxus glis*) in Italy (Amaddeo et al., 1995). An EMCV-antibody prevalence of 10.8% has been reported for wild boar (*Sus scrofa*) sampled in Italy; antibody prevalence was highest inside the endemic area. Furthermore, in France, a country without reported clinical EMCV, an overall antibody prevalence of 3.55% was recorded. Finally, in Belgium, the incidence of EMCV in tonsil samples from wild boars ranged from 1.9% to 6.8% during three different hunting seasons (Maurice et al., 2005). In Europe, EMCV has been recognized as a cause of mortality in young pigs because of acute myocarditis (Sidoli et al., 1989; Paschaleri-Papadopoulou et al., 1990; Loukaides et al., 1996; Koenen et al., 1999), with cases often clustered in so-called "endemic areas" (Maurice et al., 2005). It has also been associated with reproductive failure in sows (Koenen et al., 1997). Wildlife seems to play an important role in domestic pig infections. However, it is still unclear which wildlife species are involved and how transmission of infection to domestic pigs occurs. The objective of the current work was to search for the potential wildlife reservoir hosts of EMCV in Greece.

Three hundred and seventeen animals from 17 wildlife species were collected in Greece, both inside and outside the endemic region, between 1994 and 2006. Blood samples were collected for the presence of antibodies against EMCV. After euthanasia of wildlife animals, necropsy was performed, and samples from brain, thymus, heart, lung, liver, spleen, kidney, pancreas, and Peyer patches were collected for virus isolation.

A virus neutralization test (VNT) was performed for serologic analysis. The EMCV strain ATCC 129B, passaged on Baby Hamster Kidney (BHK-21) cells, was used. Twofold dilutions of serum were made in minimum essential medium (MEM), in 96-well flat-bottomed microtitration plates (Nunc, Thermo Fisher Scientific, Roskilde, Denmark). One hundred median tissue culture infective doses ($TCID_{50}$) of EMCV was added in equal volume. Plates were incubated at 37 C in a 5% CO_2 atmosphere for 1 hr before BHK-21 cells were added. The VNT titer was determined after incubation for 2

TABLE 1. Serologic results of 42 *Rattus rattus* captured inside five pig farms with a history encephalomyocarditis of 3–6 yr earlier.

Farm[a]	No. positive/No. tested
A	9/11
B	3/4
C	6/6
D	17/17
E	0/4

[a] The farms located inside the endemic region of encephalomyocarditis virus infection in Greece.

days. The sera were considered positive if the titer was equal or higher than 32 (Koenen et al., 2000).

Virus isolation was attempted on pooled tissue samples from each wildlife species (not from endangered species) as previously described (Billinis et al., 1999a). Genetic variability of the isolated strains was performed as previously described (Denis et al., 2006).

The Prefecture of Serres was considered to be the endemic region in Greece where clinical outbreaks of EMCV often occurred. Between 1986 and 2003, 22 outbreaks of myocardial EMCV were recorded in pigs; of which, four were reoccurrences. Fourteen of the outbreaks were located in the Prefecture of Serres, Greece.

In this region, nine brown rats (*Rattus rattus*), were trapped on a farm with clinical EMC. Virus was isolated from one rat. All the sera tested were negative. Molecular studies suggest that the rodent isolate and the pig isolates from this farm were similar (data not shown). This finding suggests a common local origin but does not indicate that the rodents are the source of the virus.

Forty-two brown rats were trapped on five pig farms with a history of clinical EMC (3–6 yr earlier) located within the endemic region. Virus was not isolated, but antibodies titers were recorded in 35 rats (Table 1).

Near another clinically affected farm that was not located within the endemic region, 10 brown rats were trapped around the farm, in a distance of 200–500 m. Virus was isolated from one rat and antibodies titer were recorded in two rats. Molecular studies suggest that the rodent isolate and the pig isolate from this farm were similar (data not shown).

A pig farm in the endemic region with a history of clinical EMC was selected and 6 yr after the last outbreak, traps were placed within 200–500 m of the farm. A total of 22 animals representing seven wildlife species were trapped. Virus was not isolated, but EMCV antibodies were detected in five sera, from two *Rattus rattus*, one *Apodemus sylvaticus*, one *Microtus arvalis*, and one *Sus scrofa.* On the same farm, we captured 17 birds (six different species). All of the sera from these birds tested negative (Table 2).

Finally, we examined 217 samples from nine species that were trapped or found dead in regions with no history of EMC (outside the endemic region). Virus was not isolated, but EMCV antibodies were detected in 12 *Sus scrofa* samples (Table 2).

From our study, it is clear that EMCV can circulate in wild boar populations, and previous studies have shown that EMCV can infect wild boars (Maurice et al., 2005). Results from serologic studies indicate that EMCV antibody prevalence is lower in free-living wild boars than in domestic pigs (Maurice et al., 2005; Kluivers et al., 2006). Several factors such as living conditions, age of infection, extent of EMCV shedding, early weaning, and vaccinations that could enhance the spread of the virus in domestic pigs may not be applicable to wild boar populations. However, the question that arises is whether wild boars play the role of hosts or carriers for EMCV infection of domestic pigs. The likely role of wild boars as temporary hosts for EMCV is in agreement with the study by Maurice et al. (2005), in which positive samples from wild boars were found in Belgium and Italy. In Belgium, the prevalence of active

TABLE 2. Serologic results of 256 wildlife species captured around pig farms located either inside or outside the prefecture of Serres, which is considered the endemic region of encephalomyocarditis virus (EMCV) infection in Greece.

Family	Species	Location[a]	
		Endemic region[b]	Nonendemic region
Mammals			
Muridae	*Mus musculus*	0/5	0/31
	Rattus rattus	2/7	0/40
	Apodemus sylvaticus	1/2	0/13
Arvicolidae	*Microtus arvalis*	1/2	0/10
Sciuridae	*Citelus citelus*	0/1	0/3
Leporidae	*Lepus europaeus*	0/4	0/97
Suidae	*Sus scrofa*	1/1	12/19
Canidae	*Vulpes vulpes*		0/3
Mustelidae	*Mustela nivalis*		0/1
Birds			
Turdidae	*Turdus merula*	0/1	
	Turdus philomelos	0/2	
Phasianidae	*Phasianus colchicus*	0/2	
	Alectoris chuckar	0/1	
Corvidae	*Pica pica*	0/1	
Alaudidae	*Galerida cristata*	0/1	
Passeridae	*Passer domesticus*	0/5	
Columbidae	*Streptopelia decaocto*	0/4	

[a] No. positive/No. samples tested.

[b] Pig farm with a history of encephalomyocarditis 6 yr earlier.

virus infection (2.5–6%) might be considered high, given the short viremic period of EMCV. However, the presence of EMCV in the tonsils of wild boars might also point to latent or persistent infection. In Italy, EMCV antibody prevalence in wild boar was considerably higher in the areas where EMCV was endemic among domestic pigs (10.8 versus 0.57%), and although this may be due to transmission between wild and domestic pigs, it probably reflects high prevalence in wild rodents (Maurice et al., 2005). Interestingly, we found antibodies in a high percentage of wild boars (12 of 19; 63%) outside the endemic region (see Table 2).

A reservoir of EMCV that would serve as common source of both wild boar and domestic pig infections has not been detected but it is possible that local rodent populations may serve as a potential virus reservoir. Currently, two routes of infection are suggested for the introduction and/or subsequent spread of the virus within domestic pigs: by ingestion of feces or carcasses of infected rodents (Littlejohns and Acland 1975; Seaman et al., 1986; Acland 1989) and by horizontal pig-to-pig transmission during the short period of viremia (Billinis et al., 1999a; Koenen et al., 1999) or after reactivation of persistent EMCV infections (Billinis et al., 1999b). However, Maurice et al. (2002) experimentally quantified pig-to-pig transmission and concluded that the spread of EMCV between pigs in most cases would be limited. Spyrou et al. (2004) highlighted the potential role of rats as a reservoir host under experimental conditions ($R_0>>1$). Further, Psalla et al. (2006) reported that EMCV spreads easily among mice under experimental conditions, which supports the potential role of mice—apart from rats—as reservoir hosts. However, the period of viral excretion in mice was shorter than in rats (Spyrou et al., 2004), in which no deaths occurred. Hence, EMCV can persist in the rodent population

by rodent-to-rodent virus transmission alone, which makes the rodent population a potential reservoir for EMCV.

In conclusion, rodents and wild boars could play a critical role in the epidemiology of EMCV infections in domestic pigs by serving as reservoir hosts. As the risk factors for EMCV introduction from the wild rodents or wild boars into the domestic pig population are not yet known, it remains difficult to predict whether new outbreaks of the disease might be expected. However, more information is needed about the infectious status and dynamics of rodent and wild boar populations to clarify their potential role in the epidemiology of EMCV in pig farms.

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