Short Communications

West Nile virus serosurveillance in horses in Doñana, Spain, 2005 to 2008

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WEST Nile virus (WNV) activity has been recognised in southern Europe for decades, but evidence of WNV circulation in Spain has only recently been found, when serological and virological monitoring in populations of wild birds (Figuerola and others 2007, 2008, Höfle and others 2008, Jimenez-Clavero and others 2008), horses (Jimenez-Clavero and others 2007) and human beings (Bofill and others 2006, Kaptoul and others 2007) was carried out. However, the lack of observed bird mortalities and the practical absence of disease in horses and human beings suggest that the circulation of the virus is essentially silent. Current evidence supports a model in which WNV persists for a long time in a sylvatic, enzootic cycle, restricted to populations of wild birds that either remain asymptomatic or in which the disease goes unnoticed, with no (or only seldom) spillover that rarely progresses to affect peridomestic habitats (Zeller and others 2004).

The Guadalquivir marshes in Doñana National Park, south-west Spain, and adjoining wetlands constitute a favourable habitat for WNV circulation. Recently, the authors' group found relevant seroprevalences to WNV in wild bird populations from these wetlands, particularly in common coots (*Fulica atra*) (Figuerola and others 2007, 2008). During three consecutive winters (October 2003 to February 2006), a number of WNV seroconversions were detected in individual birds from this population, supporting the existence of an enzootic cycle. Moreover, the decline of WNV seroprevalence during the period examined, along with seroreversions (reduction of virus neutralisation titres by at least fourfold or disappearance of detectable virus neutralisation titres [Thrusfield 2005]) that were detected in several individuals, indicated both a decrease in the exposure of this population to WNV and a limited duration of neutralising antibodies to WNV, at least in this bird species.

In 2005, a serosurvey was conducted in 156 feral horses and 194 cattle living in the Guadalquivir marshes. The study revealed a seroprevalence of 8 per cent in horses and 0 per cent in cattle (Jimenez-Clavero and others 2007). As the feral horse population appeared to be the target for WNV infection in the area, a follow-up study was conducted in order to evaluate the evolution of the seroprevalence to WNV in the long term. This feral horse population is not considered to be peridomestic, since it lives outdoors in the marshes, far from humanised habitats, all year round. Sera taken from these horses in the summer months of 2007 and 2008 were examined using the same virus neutralisation test in microtitre format (micro-VNT) that was employed in the first survey. The horses were identified by numbered ear tags and individual microchips. In 2005, 16 of 156 horses showed neutralising antibodies to WNV (Table 1). Two years later, in 2007, 152 horses were examined, including 10 that had been VNT-positive in 2005. Of these, four remained VNT-positive and six had reverted to VNT-negative status. The remaining 142 horses gave negative results in the VNT. In 2008, none of the 68 horses examined showed neutralising antibodies to WNV in serum. Fifty-six of the samples collected in 2008 were from horses that had been examined in the previous years, although none of the horses that was found to be VNT-positive in 2007 was tested again in 2008. Ten WNV VNT-positive samples (six from 2005 and four from 2007) were examined against a second flavivirus of the same serogroup (Usutu virus) to assess whether the observed virus neutralisation was WNV-specific. In nine of these 10 cases the titres against WNV were at least fourfold higher than those observed for Usutu virus, so the serology observed could be considered to be specific to WNV. One single case was considered doubtful since it showed a low VNT titre (1:10) to both viruses (data not shown).

The serological surveillance performed between 2005 and 2008 revealed a sharp decline in neutralising antibodies to WNV in this population of feral horses living in Doñana National Park. No seroconversions were observed in this study, whereas nine seroreversions were detected in 2007. These results reveal that seroreversion of WNV neutralising antibody activity in serum is relatively frequent in horses, suggesting that this type of humoral immune response is

TABLE 1: Neutralising antibody titres to West Nile virus, expressed as the maximum reciprocal serum dilution neutralising 100 $TCID_{50}$ of the E101 strain, in feral horses examined in the Doñana National Park in three years

Horse identity	Year 2005	2007	2008*
EQ54	1:320	ND	ND
EQ55	1:160	ND	ND
EQ63	1:320	ND	ND
EQ66	1:1280	1:40	ND
EQ89	1:80	ND	ND
EQ94	1:80	1:80	ND
EQ99	1:40	1:10	ND
EQ118	1:80	ND	ND
EQ216	1:640	1:160	ND
EQ263	1:20	<1:10	ND
EQ332	1:640	<1:10	ND
EQ341	1:80	<1:10	ND
EQ383	1:20	<1:10	<1:10
EQ386	1:20	ND	ND
EQ394	1:20	<1:10	ND
EQ395	1:640	<1:10	ND
Number positive	16	4	0
Number negative	140	148	68
Total number examined	156	152	68

* Prevalence was significantly lower in 2008 versus 2005 (Fisher's exact test, P=0.02) but the difference was not significant for any of the other comparisons between years (P>0.09)
Cut-off value 1:10
ND Not determined

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probably not long-lived. Other authors have made similar observations in horses (Cabre and others 2006). In previous studies, the same phenomenon was observed in common coots (Figuerola and others 2007), suggesting that seroreversions could be more general. Another conclusion arising from these results is that the horses could have had contact with WNV in 2005 or shortly before and that contact rapidly declined in the following years. Altogether, these data suggest that virus circulation could have been more intense in 2003 to 2004 or earlier – a suggestion supported by the serological monitoring in coots (Figuerola and others 2007) – and declined in the following years. Surveillance to assess the seroprevalence of WNV in wild birds and horses from this area is ongoing.

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