



Short communication

West Nile virus in horses during the summer and autumn seasons of 2015 and 2016, Portugal



Sílvia C. Barros^{a,*}, Fernanda Ramos^a, Teresa Fagulha^a, Margarida Duarte^a, Ana Margarida Henriques^a, Helga Waap^a, Tiago Luís^a, Teresa Costa^b, Rita Amador^c, Sofia Quintans^c, Miguel Fevereiro^a

^a Instituto Nacional de Investigação Agrária e Veterinária (INIAV), Avenida da República, Quinta do Marquês, 2780-157 Oeiras, Portugal

^b Direção de Serviços de Alimentação e Veterinária da Região do Algarve, Braciais – Patacão, 8005-424 Faro, Portugal

^c Direção Geral de Alimentação e Veterinária, Campo Grande 50, 1700-162 Lisboa, Portugal

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ABSTRACT

West Nile fever (WNF) is an emergent disease in Europe, under surveillance in the European Union. Following a 5-year period of apparent silence (autumn 2010 to summer 2015), West Nile virus (WNV) reemerged in the South of Portugal, in July 2015.

Here we present data from the onset, geographic location within mainland Portugal, and outcome of clinical cases of WNV infection in horses in 2015 and 2016. During the transmission seasons of 2015 and 2016, twenty-seven horses, most symptomatic ($n = 20$) were found positive to IgM, pr-E immunoglobulins and VNT, leading to the subsequent report to Animal Disease Notification System of the European Commission (ADNS) by the Portuguese National Authority for Animal Health.

Outbreaks occurred in the middle summer (August) and early/mid autumn (October/November) of 2015 and 2016, in the southern regions of the country (Alentejo and Algarve).

Compared with the previous WNV transmission seasons of 2004 and 2010, a higher number of cases were reported in 2015 and 2016.

The results of our study contribute to increase information concerning the geographic areas affected and time period for WNV transmission risk in Portugal.

1. Introduction

West Nile virus (WNV) is an arthropod-borne, single-stranded positive-sense RNA virus that belongs to the Japanese encephalitis virus (JEV) serocomplex within the *Flaviviridae* family. WNV transmission cycle includes a wide range of bird species as natural reservoirs, mosquitoes as biological vectors, and humans and equines as dead-end hosts. Horses can become infected when bitten by a mosquito-carrying virus, but they do not contribute to the spread or amplification of WNV in natural cycle, as the low level viremia is insufficient to contribute to the amplification cycle (Bunning et al., 2002). Furthermore, this short viraemic phase (around 4–6 days) occurs during the incubation period which may vary from 3 to 15 days. Seroconversion occurs 5 to 7 days post-infection (Bunning et al., 2002). Most horses seroconvert without clinical disease. Only around 10% of the infected horses show clinical symptoms (Ostlund et al., 2000). The first symptoms are mostly unspecific and include fever, depression, loss of appetite and colic. When

infection proceeds symptoms usually include encephalitis with ataxia as well as limb weakness, recumbency and muscle fasciculation (Ostlund et al., 2000).

Acute WNV infections in equines can be diagnosed by the detection of the virus genome by RT-PCR or presence of IgM specific antibodies by ELISA. However, the detection of viral RNA by RT-PCR is difficult, due to the virus' low-level, short term viraemia in horses. Therefore, diagnosis of WNV in horses is commonly achieved by serological tests, demonstrating the presence of IgM antibodies.

Since 2010, WNV infections among horses were repeatedly reported in the Mediterranean basin (OIE disease information). In Portugal, in the summer of 2004, two linked WNV cases were reported in Irish tourists that had participated in a bird watching tour in Algarve (Connell et al., 2004). After these human cases, the Portuguese National Authority for Animal Health (Direção-Geral de Alimentação e Veterinária- DGAV) set up a regional surveillance program, implemented in risk areas (wetlands and bird sanctuaries located in Algarve region),

* Corresponding author.

E-mail address: silvia.santosbarros@iniav.pt (S.C. Barros).

Table 1

Horse serum samples from all over the country received at the laboratory (INIAV) for diagnosis of WNV in the years 2015 and 2016 (Clinical suspicions of WNF and cohabitants).

Serological Results										
Year 2015										
Location NUTS II ^a	Municipality	Date of collection	N°. of Premises	IgMs	prE-IgGs	VNT	Titre range	Neurol. signs/ Confirmed Clinical cases	Case fatality	Outbreaks (ADNS) ^b
				Pos/Tested	Pos/Tested	Pos/Tested				
Algarve	Loulé ^c	Aug–Nov	13	7/45	23/45	22/23	> 10–640	5/2		2 (31 Aug 2015)
	Faro	Aug–Oct	3	1/10	1/10	1/1	40	3/1		1 (31 Aug–2015)
	Olhão	Sept	1	1/15	1/15	1/1	160	2/1		1 (28-Sept-2015)
	Albufeira	Sept	1	0/1	0/1	–	–	0		–
	Lagos	Sept–Oct	3	3/19	9/19	8/9	> 10–160	2/1	1	1 (18-Sept-2015)
	Silves	Oct	1	0/2	0/2	–	–	1/0		–
Alentejo	S. do Cacém	Sept–Oct	2	0/7	0/7	–	–	2/0		–
	Á. do Sal	Sept–Oct	4	3/8	4/8	4/4	40–160	6/3	1	2 (24-Sept-2015 14-Oct-2015)
	Arronches	Oct	1	1/1	1/1	1/1	160	1/1		1(15–10-2015)
	Alpiarça	Oct	1	1/1	1/1	1/1	320	1/1		–
	Évora	Oct	1	0/1	0/1	–	–	1/0		–
	Benavente	Nov	1	0/1	0/1	–	–	1/0		–
A.M.Lisboa	Portalegre	Nov	1	0/1	0/1	–	–	1/0		–
	Seixal	Dez	1	0/1	0/1	–	–	1/0		–
Total			34	17/113	40/113	38/40		27/10	2	8
Year 2016										
Algarve	Loulé	Fev–Oct	3	1/5	2/5	2/2	20	4/1	1	1 (30-Aug-2016)
	Lagos	July–Dec	2	1/2	1/2	1/1	160	2/1	1	1 (14-Nov-2016)
	Silves	Oct–Dec	3	2/3	3/3	3/3	80	3/2	1	2 (13-Oct 2016 18-Oct-2016)
Alentejo	Odemira	May	1	0/5	1/5	1/1	80	5/0		
	S. de Magos	Jun–Sept	2	0/2	1/2	1/1	320	2/0		
	Portalegre	Sept–Nov	2	2/4	2/4	2/2	20	4/2		
	Álter-do-Chão	Oct	1	1/1	1/1	1/1	40	1/1	1	
	Benavente	Nov	1	1/1	1/1	1/1	160	1/1	1	
	Elvas	Nov–Dec	1	1/2	1/2	1/1	80	2/1		1(8-Nov-2016)
	Évora	Nov	2	0/2	0/2	–	–	2/0		
	Beja	Nov–Dec	2	1/3	1/3	1/1	40	1/1		
A.M.Lisboa	Seixal	Apr	1	0/1	0/1	–	–	1/0		
	Moita	Dec	1	0/1	0/1	–	–	1/0		
Centro	Alcanena	Apr	1	0/1	0/1	–	–	1/0		
Norte	Fafe	Sept	1	0/1	0/1	–	–	1/0		
Total			24	10/34	14/34	14/14		33/10	5	5

^a NUTS – Nomenclature of territorial units for statistics (Eurostat-GISCO 2014).^b ADNS- Animal Disease Notification System of the European Commission (ADNS). Notifications sent by DGAV.^c Municipality where the human case was registered. Pos- positive samples.

including testing horse sera, and checking for increased mortality in susceptible bird species. Through this program, neutralizing antibodies were detected in nine horses and two birds, although neither symptomatic horses nor bird mortality were found (Barros et al., 2011). From 2005 to middle 2010, no human or horse clinical cases were reported in the country. In 2010, another WNV human case was identified (Alves et al., 2012) and horse clinical cases were also reported (Barros et al., 2011).

After a five years period of epidemiological silence, in the summer of 2015, a case of West Nile Neuroinvasive Disease (WNND) was diagnosed in a man living in the Algarve region (Ze-Ze et al., 2015).

Here we presented data from the onset, geographic location within mainland Portugal, and outcome of clinical cases of WNV infection in horses in 2015 and 2016. Also, data from the laboratory serosurvey, conducted at the National Institute for Agrarian and Veterinary Research (INIAV, I.P.) between 2011 and April 2015, comprising the serological testing of 989 horse serum originated all over the country is divulged.

2. Materials and methods

2.1. Case definition

For the purposes of this study, a horse case was defined according with OIE by (1) the presence of one or more of signs of WNV clinical disease (ataxia, recumbency, paresis, paralysis or death) and (2) positive to IgM ELISA or RNA WNV by RT-PCR. Once a clinical case was identified, the regional services of the veterinarian authorities (DGAV) visited the holding to collect samples and to conduct an epidemiological survey concerning the animal's travel history, the exact location and the vaccination status. Information on the cohabitants was also gathered. In addition, any occurrence of death birds on the surroundings was investigated.

2.2. Serological tests

Horse sera were tested for WNV antibodies. IgMs and total antibodies against the viral envelope protein E (pr-E), mainly IgGs (therefore hereafter referred to as pr-E IgGs), but also other immunoglobulin classes were investigated with two commercial competition enzyme linked immunosorbent assays (ELISAs), following the manufacture

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