Epidemiol. Infect. (2006), **134**, 826–829. © 2005 Cambridge University Press
doi:10.1017/S0950268805005492 Printed in the United Kingdom

SHORT REPORT
Serological evidence of West Nile Virus (WNV) in mammalian species in Turkey

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(*Accepted 30 August 2005, first published online 29 November 2005*)

SUMMARY

In this study, the sera collected from a variety of mammalian species (ass-mules, cat, cattle, dog, horse, human and sheep) in 10 representative provinces of Turkey, were surveyed for the presence of neutralizing antibodies to West Nile virus (WNV). Overall, 1 of 40 (2·5%) ass-mules, 4 of 100 (4%) cattle, 43 of 114 (37·7%) dogs, 35 of 259 (13·5%) horses, 18 of 88 (20·4%) humans and 1 of 100 (1%) sheep, tested positive for WNV-neutralizing antibodies. The results indicate that a wide range of mammals are exposed to a West Nile-related virus and this could contribute to the long-term survival of this virus in the absence of overt disease.

West Nile virus (WNV) is a single-stranded, positive-sense RNA virus belonging to the genus *Flavivirus* (family Flaviviridae). Until 1999, the geographical distribution of the virus was limited to Africa, the Middle East, India, and western and central Asia with occasional epizootics and epidemics in Europe [1]. Since the summer of 1999, the distribution of WNV has expanded to include 46 states of the United States, seven Canadian provinces, plus Mexico, and probably a number of the Caribbean islands [2–4]. As WNV has spread across North America, the number of human and veterinary cases and deaths has continued to rise, resulting in the largest recorded epidemic of arboviral encephalitis in the western hemisphere during 2003 [5]. The recent outbreaks in Europe, Israel, and North America involving humans, equines, and birds have been associated with significant rates of neurological disease [6, 7]. Three genetic variants of WNV have been demonstrated in Russia before the outbreak of the most recent epidemic. WNV isolates showed genetically high similarity to those reported from the United States and Israel [8]. The latest epizootic in the Mediterranean basin was reported in Morocco in horses with fatal encephalitis [9].

The first serological evidence of WNV infection in Turkey was reported in the 1970s based on the detection of haemagglutination-inhibiting (HI) antibodies [10–12]. One study reported WNV seroprevalence in the western region of Turkey as 6% and 1·5%, in humans and sheep respectively [10]. Five years later, seroprevalence as high as 40% was reported in randomly selected human sera collected in southeast Turkey, using the HI assay [11].

Globally, in addition to humans, WNV has been isolated from a wide range of birds [5, 7, 13], and mammals including horses [7, 13, 14], llamas, alpacas

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Table. *West Nile Virus* (*WNV*) *seroprevalence in mammalians by locations*

Group of species*	Province	Geographical position	Number of		Mean antibody titres†			
			Serum samples	Seropositives (%)	1:10	1:20	1:40	>1:40
Ass-mules	Hatay	36° 37′ N, 36° 07′ E	40	1 (2·5)	–	–	1	–
Cat	Mugla	37° 12′ N, 28° 22′ E	63	–	–	–	–	–
Cattle	S. Urfa	37° 08′ N, 38° 46′ E	100	4 (4)	3	1	–	–
Dog 1	Izmir	38° 25′ N, 27° 09′ E	46	9 (19)	–	2	1	6
Dog 2	Mugla	37° 12′ N, 28° 22′ E	68	34 (50)	1	4	12	17
Horse 1	Adana	37° 01′ N, 35° 18′ E	200	11 (5·5)	3	3	5	–
Horse 2	Bursa	40° 11′ N, 29° 04′ E	59	24 (40·6)	13	10	1	–
Human 1	Ankara	39° 56′ N, 32° 52′ E	48	14 (29·1)	6	3	5	–
Human 2	Antalya	36° 53′ N, 30° 42′ E	40	4 (10)	–	3	1	–
Sheep	Izmir	38° 25′ N, 27° 09′ E	100	1 (1)	–	1	–	–
Total			764	102 (13·3)				

* In alphabetical order.

† Indicates reciprocal of serum dilutions yielding a ⩾50% reduction in plaque number.

[13, 15], sheep, [16] dogs, cats [13, 17, 18], squirrels [5, 13], primates [19], alligators [20], and crocodiles [21] with or without clinical disease.

The objective of this study was to survey sera collected from a variety of mammalian species (ass-mules, cat, cattle, dog, horse, human and sheep) in 10 representative provinces of Turkey, for the presence of neutralizing antibodies to WNV. It was hoped that this might provide the basis for further investigations concerned with the risk of WNV epidemics in the Mediterranean basin. Furthermore, the data might indicate which particular animal species might provide the highest risk to humans in rural areas.

A total of 764 blood samples taken from eight species were screened for the presence of WNV-neutralizing antibodies. Human serum samples were obtained from collections held in the virology and microbiology laboratories of the Refik Saydam Hygiene Center and the Medical School of Akdeniz University respectively. Human sera were obtained either from patients with unidentified encephalomyelitis or, from transplantation patients with no evidence of microbiological infection. The animal samples were randomly collected from individuals >1 year old and reared in various provinces of Turkey (Table). The feline and canine samples were obtained by local veterinarians. Alternatively, the dogs in Mugla were housed in the regional animal shelter for unclaimed animals (Table).

All sera were tested for virus-neutralizing antibodies to WNV (NY99-4132) using the plaque reduction neutralization assay (PRNA) in Vero cell culture as previously described [14]. Initially all sera were screened at a single final dilution of 1:10. The procedure was as follows. Diluted serum (1:5) was heat inactivated at 56 °C for 30 min and then mixed with an equal volume (0·1 ml) of virus diluted in Dulbecco's modified Eagle's medium (DMEM) containing 5% fetal calf serum, to produce an estimated 100 plaque-forming units of virus per 0·2 ml. The virus-antibody mixtures were then inoculated, as 0·2-ml volumes onto Vero cell monolayers grown in six-well plates and overlaid with 3·2% carboxymethyl celulose in 2× DMEM for the plaque assay. Plaques were scored on day 4 following incubation at 37 °C. All sera that neutralized >70% of the challenge virus were subsequently re-tested in duplicate by titration in serial twofold dilutions. The neutralization titre was expressed as the highest dilution yielding a ⩾50% reduction in plaque numbers. Sera with titres higher than 1:40 dilution were expressed as >40.

On the basis of this antibody survey our preliminary studies confirmed that either WNV or a very closely related virus is circulating over a wide region of Turkey and a wide range of species is being exposed to the virus, presumably as the result of the bites of infected mosquitoes. Overall, 1 of 40 (2·5%) ass-mules, 4 of 100 (4%) cattle, 43 of 114 (37·7%) dogs, 35 of 259 (13·5%) horses, 18 of 88 (20·4%) humans and 1 of 100 (1%) sheep, tested positive for WNV-neutralizing antibodies. Perhaps surprisingly, all 63 cats were negative in these tests. Reciprocal titres ranged between 10 and >40 for all seropositive

species (Table). Based on their locations there were variations in the seroconversion rates of some species. For example, there was a much higher proportion of WNV-seropositive dogs in Mugla than in Izmir, and a higher proportion of positive horses in Bursa than in Adana. However, this is a preliminary investigation and clearly far greater numbers of samples from each region will be required before the significance of these virus distributions amongst different species can be analysed in detail.

This study reports the detection of neutralizing antibodies against WNV or a closely related virus for the first time in mammalian species in Turkey. The widespread presence of WNV has been recognized in the Mediterranean basin [1] and the Balkans [22] for a long time. However, all previous serological tests in Turkey utilized the HI test which is generally considered to be an indicator of Flavivirus group-reactive antibodies rather than virus-specific antibodies [10–12]. In contrast, the PRNA is generally regarded as being relatively specific for the virus against which it is tested. However, appropriate control tests with closely related flaviviruses such as Usutu virus, Yaounde virus, Koutango virus or Israel Turkey meningoencephalomyelitis virus, will ideally be required in more extensive investigations.

In these preliminary studies, the overall percentage of WNV seropositivity for humans was similar to that reported in the Czech Republic [22]. Local variations detected in our tests could reflect the particular sources of the samples examined. WNV causes fever and neurological disease and, therefore, higher seroprevalence rates might be expected in the patients with encephalomyelitis in this study (human 1 group) as has been reported elsewhere [4, 23]. In the second group of patients, i.e. the transplant recipients that showed no clinical disease, the lower seroprevalence rate could simply reflect the immunosuppression given to reduce the risk of graft rejection.

Among the three equine species screened in the study, the proportion WNV-seropositive in the ass-mules group was much lower than that in horses even though they were located in neighbouring provinces (Adana and Hatay). However, this might reflect the fact that the horses were stabled a short distance from a lake in Bird Heaven National Park (40° 20′ N, 27° 58′ E), which is an important central area of Turkey for migrating water birds. Thus, as in many other areas of the world, migrating birds could be the source for transmission of WNV to these horses by mosquitoes.

Turkey has an enormous population of ruminants farmed intensively, and also reared in households throughout the rural areas. The low proportion of WNV seropositivity in cattle and sheep may indicate low prevalence and low sensitivity to WNV in some areas as reported elsewhere [24, 25]. It is of note that in cattle reared in southeastern Turkey (S. Urfa) the proportion of positive sera was higher (4%). This is possibly related to the region's environmental features, i.e. having the biggest artificial water reserves which provide suitable conditions for all-year-round breeding of the vector mosquitoes.

Strikingly, WNV seroprevalence in dogs was higher than it was in other animal species. Despite the overall occurrence in dogs of 37·7%, the neutralizing antibodies were found to be more prevalent (50%) in the Mugla region. This might be due to a combination of poor hygiene standards, intensive mosquito biting activity and high densities of animals within the shelter. The relatively high serum antibody titres in the positive dogs could imply that they are very unlikely to serve as amplifying hosts for WNV. In contrast, no detectable antibody response was monitored in cats used in the study. Clearly, increased sample numbers from different habitats will be needed before robust conclusions can be drawn. Nevertheless, significant viraemias have been observed in experimentally infected cats suggesting that they could support infection of mosquitoes, probably with low efficiency relative to many avian hosts [17].

The recent surprising observation that transmission of WNV can occur directly between mosquitoes co-feeding on non-viraemic hosts [26] will also have to be considered in any future investigations since this might partly explain how WNV and possibly other arboviruses are able to circulate efficiently amongst mammalian populations without causing overt disease. Transmission studies of Bunyaviruses [27] and Flaviviruses [28] between ticks co-feeding on non-viraemic rodent hosts may provide valuable information for detailed future investigations on this area.

Indeed, whilst the mammalian species surveyed in this study are not recognized natural hosts for WNV, they do represent very large numbers of mammals in Turkey and if non-viraemic transmission is a significant factor in mosquito-borne virus transmission these mammals could contribute to virus dispersal.

ACKNOWLEDGEMENTS

The authors thank Professor E. A. Gould (Centre for Ecology and Hydrology, Oxford, UK) for his helpful comments in the preparation of this paper.

DECLARATION OF INTEREST

None.

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