

# Neutralising antibodies to West Nile virus detected in horses in Windhoek, Namibia

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West Nile virus (WNV) is a vector-borne virus maintained in nature by a bird-mosquito cycle. However, it can occasionally and accidentally infect horses and human beings, leading to sometimes severe or even fatal outcomes in these species. Therefore, the monitoring of its circulation and disease occurrence is of relevance. Unfortunately, it is underdiagnosed or not diagnosed in several African counties, including Namibia, where no data is currently available for horses. In this study, 98 horses in three different stables in the Windhoek city area were investigated. They were found to have a seroprevalence of approximately 7%. Positive reactions were seen at all three stables, suggesting a greater than expected prevalence of the virus. This is the first report of serological evidence for the presence of the virus in horses in Namibia. Even though clinical signs were not reported in any of the stables from which the sera were derived, the seroprevalence to the virus suggests that horses with high genetic and/or economic value could benefit from vaccination against WNV. Because of the zoonotic potential of the virus, these findings are also of significance to human health authorities.

**Keywords:** West Nile virus, Windhoek, Namibia, antibody, horses, cELISA

## Introduction

West Nile virus (WNV) is a zoonotic vector-borne virus of the family of *Flaviviridae* genus *Flavivirus*, and a member of the Japanese Encephalitis complex (Petersen et al. 2013). West Nile virus was isolated for the first time in 1937 from a human patient in the West Nile district in Uganda (Baba et al. 2014). The virus is maintained in nature in a mosquito-bird transmission cycle from which it can be transmitted to humans and horses by a bite of an infected mosquito. WNV has the broadest geographical distribution among encephalitic flaviviruses and has caused outbreaks in the Americas, Africa, Europe and Asia. Moreover, it remains underreported in Africa (Steyn et al. 2019). This emerging disease in equids and birds has been present in southern Africa since 1958 (Gurthrie et al. 2003), but reports of neurovirulent WNV have increased since 2007 (Bertram et al. 2020). Published studies indicate that 20% of infected horses may show symptoms ranging from fever to severe neurological signs (90%), while mortality rates in unvaccinated horses range from 30–40% (Venter et al. 2017). Human cases of WNV fever have been diagnosed in South Africa, while the detection of antibodies against WNV in humans has been recorded in South Africa and Zambia (Bertram et al. 2020; Mweene-Ndumba et al. 2015). A study performed in South Africa between 2011 and 2012 demonstrated a 7.9% WNV seroprevalence among veterinarians (Van Eeden et al. 2014).

Little is known about the current prevalence of WNV in horses around Namibia. Since independence in 1990, only two surveys

on mosquito-borne arbovirus and a WNV serological surveillance study in donkeys were conducted in Namibia (Guggemos et al. 2021; Molini et al. 2021; Noden et al. 2014).

The objective of this study was to determine the seroprevalence of WNV in a selected population of horses in Windhoek, Namibia.

## Materials and methods

### Sample collection

In August 2020, blood samples were collected by private veterinarians from 98 horses located at three different stables in Windhoek city areas.

To estimate the WNV seroprevalence in the Namibian donkey population, the sample size was calculated assuming a prevalence of 10% and aiming at achieving a 5% precision with 90% confidence level.

Sixty-four geldings, 33 mares and one stallion, ranging between two and 27 years of age, were sampled. Sixteen of these were Arab, nine Quarter Horse and 56 Warmbloods. The other animals belonged to eight breeds or crossbreeds. Four of the 98 horses under study had been recently imported from South Africa, while the others were born and raised in Namibia and during the time they were sampled had not travelled outside the Windhoek district. None of the horses sampled had been vaccinated against WNV. Serum was separated by centrifugation at 3 000 rpm for 5 min, refrigerated, and sent to the Central Veterinary Laboratory (CVL) in Windhoek for testing.

**Table I:** Seroprevalence of WNV in Windhoek horses

Stable/Location	n Horses	cELISA	VNT WNV
A	19	6/19 (31.58% [95% CI = 12.58–56.55])	1/19 (5.26% [95% CI = 0.13–26.03])
B	31	10/31 (32.26% [95% CI = 16.68–51.37])	3/31 (9.68% [95% CI = 2.04–25.75])
C	48	22/48 (45.83% [95% CI = 31.37–60.83])	3/48 (6.25% [95% CI = 1.31–17.20])
<b>Total</b>	<b>98</b>	<b>38/98 (38.77% [95% CI = 29.10–49.15])</b>	<b>7/98 (7.14% [95% CI = 2.92–14.16])</b>

cELISA – competitive enzyme-linked immunosorbent assay, WNV – West Nile virus, VNT – virus neutralisation test

### Serological tests

Serum samples were screened using a commercial competitive ELISA (cELISA) (ID Screen® West Nile Competition Multi-species, IDvet, Grabels, France), which targets the antibodies against one epitope of the Pr-E protein of Flavivirus. WNV ELISA positive samples were subjected to a confirmatory test using a virus neutralisation test (VNT) as described by Di Gennaro et al. (2014) which is more specific than the ELISA. The VNT was performed at the OIE Reference Laboratory for West Nile Disease, Istituto Zooprofilattico Sperimentale dell' Abruzzo e del Molise "G. Caporale", Teramo, Italy.

### Statistical analyses

The association between WNV serology results and animal sex, breed, age, and origin (imported or not) was evaluated using the Fisher's exact test as implemented in the *gmodels* library (Warnes et al. 2015) of the R statistical software, while the association with animal age was tested using the non-parametric Mann–Whitney test since the age data was proven to be non-normally distributed by the Shapiro–Wilk test. Since several breeds were underrepresented, the main designated groups were Arab, Quarter Horse and Warmbloods; the others were recorded in a single group designated Other Breeds. The statistical significance level was set to  $p$ -value < 0.05.

### Results

A total of 38 out of 98 (38.75% [95% CI = 29.10–49.15%]) analysed horses tested positive by ELISA, and in seven of these, the presence of WNV neutralising antibodies was confirmed by VNT (Table I). The WNV seroprevalence in horses was therefore 7.14% (95% CI = 2.92–14.16%) (7/98). Neutralising titres of 1:10, 1:20 and > 1:20 were found in two, three, and two animals, respectively. No significant association was found between WNV serological data and sex ( $p = 0.39$ ) or animal age ( $p = 0.19$ ). On the other hand, a significant association was found with the breed ( $p = 0.003$ ), the Arab breed being overrepresented among positives and Warmbloods underrepresented. None of the confirmed positive WNV horses had current or previous neurological signs related to WNV.

### Discussion

This study provides the first report of WNV seropositivity in horses in Namibia with a prevalence of 7.14% (95% CI = 2.92–14.16%). Surveys from other African countries have detected variable WNV infection rates in healthy equines: South Africa 11.1%, Egypt 28.3%, Morocco 29.52%, Tunisia 27.1%, Djibouti 9.0%, Cote d'Ivoire 28.0%, Democratic Republic of Congo 30.0%,

Nigeria 25%, and Gabon 3.0% (Benjelloun et al. 2016; Cabre et al. 2006; Guthrie et al. 2003; Selim et al. 2020). A previous study conducted on donkeys in Namibia showed a WNV prevalence of 18.07% (95% CI = 13.59–23.30%) (Molini et al. 2021). The lower WNV prevalence in the Windhoek horses compared to horses in neighbouring areas/countries and to the prevalence obtained from the Namibian donkeys in the region could be related to the management of the horses that are regularly treated with insecticides, repellents and held in vector-proof establishments to prevent African Horse Sickness. Antibodies to WNV were detected in horses from all the three stables in the city with seroprevalence rates ranging from 5.26% to 9.68%, indicating that the virus is present in the Windhoek district. The large number of samples positive by ELISA (38/98) and negative by VNT (31/38) may have been due to a limited specificity of the ELISA Kit (95%) (Beck et al. 2017) which cross-reacts with other antigenically-related flaviviruses (Pérez-Ramírez et al. 2020).

### Conclusion

This report provides the first published evidence (or data) on WNV antibodies among local horses in Windhoek, Namibia. Based on this finding, the use of the equine WNV vaccine is recommended especially for horses with high genetic, economic or sentimental value to avoid the risk of developing the clinical form of the disease. The presence of WNV in a highly-populated area could have public health implications for humans also.

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### Conflict of interest

The authors declare no conflict of interest.

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### Ethical approval

Serum samples previously collected for an annual check-up of horses by private veterinarians were used for the research. Therefore, no ethical approval specific to this study was needed.

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