Assessment of a seroprevalence study of West Nile, Usutu, and Tick-borne encephalitis viruses in a sample of the equine population in Gironde in 2023 – *Margaux De Mas, 2025*

Abstract :

A seroprevalence study of West Nile virus (WNV), Usutu virus (USUV), and Tick-borne encephalitis virus (TBEV) was conducted in Gironde in the spring of 2023, following the emergence of WNV in equids in this department in 2022. The serological status of 494 horses, located in three geographical areas (Confluence, Intermediate Zone, and Arcachon Basin), was assessed through blood sampling for these three viruses. The results showed an overall seroprevalence of 14% for the three orthoflaviviruses, with the highest rates observed in the Confluence zone : 9% for WNV and 5% for USUV. Housing type (exclusive pasture) and the distance to the nearest Special Protection Area (SPA) for birds were identified as significant risk factors for WNV seropositivity. This study, the first of its kind on the French Atlantic coast, demonstrates active circulation of WNV in this region as well as the presence of USUV in equids.

Key-words : Orthoflavivirus, West Nile, Usutu, TBEV, seroprevalence, One Health

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Background and Issues

The West Nile virus (WNV), Usutu virus (USUV), and Tick-borne encephalitis virus (TBEV) are zoonotic arboviruses from the *Flaviviridae* family. These viruses are often responsible for asymptomatic infections, but they can sometimes cause serious symptoms, especially neurological disorders. In Europe, they represent a growing public health concern. Since 2021, WNV has been classified under Category E of the European Animal Health Law, due to its implications in both humans and animals, which justifies reinforced surveillance measures. Given that most infections are asymptomatic, passive surveillance is often insufficient. Climate change, as well as increasing mobility of human and animal populations and their vectors, facilitates the silent spread of these viruses into new territories. In this context, the One Health approach—which integrates human, animal, and environmental health—is considered essential. Horses, which are susceptible to both WNV and USUV, are particularly suited to act as epidemiological sentinels due to their outdoor exposure, wide geographic distribution, and status as dead-end hosts (i.e., they do not contribute to further transmission of the virus). In October 2022, the first autochthonous cases of WNV were diagnosed in equines in Gironde, a department that had not previously been affected by this disease. In response, a seroprevalence study was launched in spring 2023, in collaboration with several institutions (ANSES, DDPP33, Equine Clinic of Conques, and local veterinarians), to better understand the extent of viral circulation and to identify associated risk factors.

Study Objectives

This thesis continues the work of Dr. Noémie Chevalier (Resident in Equine Internal Medicine) and pursues two main goals:

1. Literature review: To present the current state of knowledge on zoonotic orthoflaviviruses of veterinary interest in France (WNV, USUV, TBEV), including their epidemiology, pathogenesis, diagnosis, prevention, treatment, and surveillance.
2. Experimental section: To present the results of a novel epidemiological study conducted in Gironde in 2023, following the emergence of WNV in the region in 2022. The study aimed to assess the intensity of viral circulation, identify risk factors for infection, establish a baseline seroprevalence of orthoflaviviruses in the local equine population.

Materials and Methods

Population and Study Area

This study was conducted in collaboration with the Conques Equine Clinic, RESPE, ANSES, DDPP 33, and several local veterinarians. It was approved by the Ethics Committee of the National Veterinary School of Alfort (n°2023-06-23). Blood samples were collected anonymously and free of charge from April 15 to May 31, 2023, a period chosen to reflect exposure to viruses during summer 2022, prior to the resumption of summer vector activity. A total of 494 equines (horses, ponies, donkeys), aged over 2 years, were recruited from 39 equine establishments located in three distinct zones:

- Confluence zone: Where the 2022 WNV cases were detected (junction of the Garonne and Dordogne rivers).
- Intermediate zone: Located between the Confluence area and the Arcachon Basin.
- Arcachon Basin: A humid area along the East Atlantic migratory bird flyway.

To be included, the horses had not been vaccinated against WNV, had resided in the study zone during 2022, had not traveled to endemic areas in the past six years, and were asymptomatic at the time of sampling.

Sampling and Analysis

Blood samples were drawn from the jugular vein, centrifuged, and sera stored at +4°C before being analyzed at ANSES (WNV National Reference Laboratory, Maisons-Alfort). Initial detection of IgG antibodies against flaviviruses was performed using the Pan-Flavivirus Competitive ELISA test *(ID Screen® Flavivirus competition, Innovative Diagnostics)*. This test detects antibodies directed against WNV, USUV, TBEV, as well as other flaviviruses (e.g., JEV, ZIKV, DENV), but cannot differentiate between them. Samples that tested positive or doubtful in ELISA were further tested using virus neutralization tests (VNT), the gold standard for identifying specific neutralizing antibodies to WNV, USUV, and TBEV. The serological titer was defined as the inverse of the highest dilution that showed inhibition of the cytopathic effect.

Epidemiological Data and Analysis

A questionnaire was used to collect both individual data: age, sex, coat color, activity, medical history, and environmental data: type of housing, mosquito control measures, perceived mosquito nuisance score, proximity to wetlands and Special Protection Areas (SPAs). Data were analyzed using mixed-effects logistic regression models, with the establishment as a random effect. A non-parametric analysis was also conducted to assess the independence of WNV and USUV infections, using the Vaumourin et al. method (2014).

Results

Orthoflavivirus Seroprevalence and VNT Results

Of the 494 equids tested, 70 animals (14.2%) were positive on the Pan-Flavivirus ELISA, with most positives located in the Confluence zone, showing a seroprevalence of 21% (95% CI: 16–26%), against 5% (95% CI: 2–10%) in the intermediate zone, and only 1 case (1%, 95% CI: 0–7%) in the Arcachon Basin. These results indicate localized viral circulation, mainly in the Confluence area. Higher concentrations were observed near Special Protection Areas and the Entre-Deux-Mers plateau. The structures with the highest seroprevalence all reported maximum mosquito nuisance scores (5/5), although this association wasn't always consistent. Notably, the two equine centers that had recorded clinical WNV cases in 2022 did not show higher seroprevalence than the others. Among the 81 ELISA-positive/doubtful samples, VNTs identified the specific antibodies as follows :

- WNV: 9% in Confluence zone (95% CI: 6–13%), 3% in Intermediate zone, 1% in Arcachon.
- USUV: 5% in Confluence, 1% in Intermediate, 0% in Arcachon.
- TBEV: 2% in Confluence, 1% in Intermediate, 0% in Arcachon.

A total of 35 samples (43%) tested negative for all three viruses in VNT despite positive ELISA results. This suggests either cross-reactivity with other untested orthoflaviviruses, or low neutralizing antibody titers below the VNT detection threshold. Several cases of co-seropositivity to WNV and USUV were also confirmed.

Risk Factors

No individual characteristics (age, sex, coat color, activity) were significantly associated with seropositivity, even after geographic stratification. A multivariate mixed-effects logistic regression was carried out specifically in the Confluence zone, including only complete datasets and the two most prevalent viruses (WNV and USUV). For WNV, two environmental factors showed significant associations: the type of housing, as horses exclusively kept on pasture had a markedly increased risk (Odds Ratio [OR] = 3.64), and distance to the northern SPA: Risk decreased with distance (OR = 0.93 per km increase). No significant risk factors were identified for USUV. The non-parametric analysis revealed a statistically significant association between WNV and USUV seropositivity ($p = 0.006$).

Discussion

The study highlights active circulation of both WNV and USUV in 2022, particularly in the Confluence zone. These findings point to the emergence of a new viral focus outside of the traditionally endemic Camargue region. For reference, Camargue showed from 2016–2020 : 13.2% seroprevalence for WNV, and 3.8% for USUV. The Confluence zone shows comparable numbers as the ones in Camargue revealed in 2003, suggesting a potential trend toward endemicity in the area. Regarding TBEV, the low seroprevalence (1.4%) is difficult to interpret. Horses are not proved to be optimal sentinels for this tick-borne virus. Future studies should include rodents, ticks, and possibly cattle for more relevant surveillance. Additionally, the high number of ELISA-positive/VNT-negative samples suggests known cross-reactions between flaviviruses, or undetected viruses in the region (e.g., Bagaza virus or divergent WNV/USUV strains). This underscores the need for improved diagnostic tools. The risk factor analysis confirmed the dominance of environmental over individual

factors, which aligns with previous European studies. However, literature on age, breed, and coat color as risk modifiers remains inconsistent and contradictory.

Study Limitations

Several methodological limitations must be acknowledged: the sampling was not representative of the entire Gironde region, being targeted to high-risk areas. Moreover, some data were incomplete or subjective (e.g., mosquito nuisance score, imprecise distances). Besides, the protocol was not optimized for TBEV, and horses are a poor model for its surveillance. Finally, the serological tests, especially ELISA, lack specificity, increasing the risk of false positives.

Perspectives and Recommendations

Future investigations would benefit from standardized investigator training to ensure consistent data collection, and improved questionnaires, including for example precise grazing schedules, movement history, and objective mosquito exposure metrics. Besides, we would recommend broader, more targeted sampling for TBEV, including cattle and small mammals. To go further, we could integrate the study of underexplored individual variables (e.g., blood type, phenotypic traits), and viral sequencing to detect divergent or novel strains. The Confluence zone may be on a trajectory similar to the Camargue, with increasing risk of endemic WNV circulation. Strengthened surveillance using a One Health approach is essential to mitigate the rising threat of emerging arboviruses.

Conclusion

This first epidemiological investigation on orthoflaviviruses in the Gironde department highlights the active circulation of West Nile virus (WNV) and Usutu virus (USUV) in a region not previously considered endemic. Although the data do not allow firm conclusions regarding Tick-borne encephalitis virus (TBEV), the study reveals risk areas and key environmental factors associated with equine infection. Horses have proven to be valuable sentinel indicators of viral circulation, both for their own health and for public health. The results of this study have already led to the implementation of preventive measures (vaccination, surveillance), and lay the groundwork for enhanced, multidisciplinary surveillance in the face of a rapidly expanding epidemic threat. This study underscores the need to continue and expand research into the individual, environmental, and behavioral factors influencing infection susceptibility, as well as into vector biology, host-pathogen interactions, and the still poorly explored viral diversity. The development of more specific diagnostic tools and a deeper understanding of eco-epidemiological cycles are major challenges for early detection and effective prevention. Finally, in a context of increasing arboviral emergence, this study fully illustrates the value of a One Health approach, integrating human medicine, animal health, and disease ecology. It also confirms the urgent need to coordinate human, veterinary, and ecological efforts to anticipate future health crises. This strategy is part of a broader, global public health challenge.