

First Serological Evidence of West Nile Virus in Equine Populations from Southeastern Albania

KRISTI MORAVA¹*Department of Agri-Food, Faculty of Agriculture, Fan S. Noli University of Korçë, Albania*

REZART POSTOLI

ILIR DOVA

ENKELEDA OZUNI

DHIMITËR RAPTI

Faculty of Veterinary Medicine, Agricultural University of Tirana, Albania

KLEDIS ÇELA

National Authority for Veterinary and Plant Protection

XHELIL KOLECI

Faculty of Veterinary Medicine, Agricultural University of Tirana, Albania

Abstract

West Nile virus (WNV) is a vector-borne flavivirus with zoonotic potential, affecting a wide range of vertebrate hosts including horses, birds, and humans. Equines are considered effective sentinel species due to their sensitivity to infection and their role in signaling local virus circulation. Albania remains underrepresented in WNV surveillance studies, particularly in southeastern regions such as Korça, where environmental and climatic changes may have facilitated the introduction or expansion of the virus.

This study aimed to assess serological evidence of WNV exposure in equine populations in the southeastern region of Albania, including the area of Korça. Blood samples were collected from 137 horses across 11 administrative units between 2024 and 2025, following animal welfare guidelines for field sampling. Serum was separated and stored under controlled conditions at the fridge veterinary laboratory of Fan Noli University. For each animal, data were collected regarding sex, age, health status, and how the animals were kept and managed. Samples were analyzed using a commercial ELISA targeting WNV-specific IgG antibodies. Results showed that 12.4% of horses tested positive, while in mules 6.4%. The results provide preliminary insights into WNV circulation in southeastern Albania and reinforce the importance of active surveillance in equine populations as part of broader One Health efforts.

Key words: West Nile virus, equids, seroprevalence, ELISA, Albania, Korça, One Health

INTRODUCTION

West Nile virus (WNV) is a neurotropic, zoonotic, mosquito-borne virus belonging to the *Flaviviridae* family, genus *Flavivirus*, and is part of the Japanese encephalitis virus group. It has a broad vertebrate host range, affecting birds, mammals such as equines and humans, and various mosquito species, primarily of the genus *Culex* (Bernard *et al.*, 2001; Castillo-Olivares and Wood, 2004). Birds serve as the main amplifying hosts in a natural enzootic transmission cycle with mosquitoes, while mammals, including

¹ corresponding author: kmorava@unkorce.edu.al

horses and humans, are typically considered dead-end hosts as they usually do not develop sufficiently high viremia to infect mosquitoes (Ibarra-Juarez *et al.*, 2007; Petersen *et al.*, 2013). Nonetheless, immunosuppression in mammals may occasionally allow onward transmission.

WNV infection is endemic in Europe and has exhibited an increasing pattern of outbreaks in recent years, especially in southern, western, eastern, and central European countries (ECDPC, 2023; García-Bocanegra *et al.*, 2012). Since its first detection in North America in 1999, the virus has spread widely and caused significant morbidity and mortality in equines and humans (Petersen *et al.*, 2013). In Europe, numerous cases have been reported in several countries, including Italy, Hungary, Spain, as well as in South-Eastern Europe (e.g., Greece and Romania), including neuroinvasive disease with considerable fatalities (ECDPC, 2023; De Heus *et al.*, 2019; Calistri *et al.*, 2010). In 2018, Europe recorded more cases, with outbreaks in humans and horses higher than in 2017. (ECDPC, 2019).

Clinical manifestations in horses include encephalitis and inflammation of the brain and spinal cord, characterized by neurological signs such as ataxia, paresis, tremors, and cranial nerve deficits, which may lead to fatal outcomes or prolonged sequelae in survivors. Diagnosis relies heavily on serological testing such as IgM-capture ELISA and virus neutralization assays, combined with clinical and laboratory findings (Bernard *et al.*, 2011). The zoonotic nature of WNV, transmitted primarily via mosquito vectors, presents a public health risk, especially to vulnerable human populations including children, elderly, and immunocompromised individuals (Petersen *et al.*, 2013).

Despite its proximity to affected regions like Greece and North Macedonia, data on WNV presence and prevalence in Albania, particularly in southeastern regions such as Korça, remain scarce. Given the ecological and climatic changes influencing vector populations and migratory bird routes, there is an urgent need to assess WNV circulation locally (Hess *et al.*, 2018). Understanding the status of WNV infection in equine populations can contribute to early detection, risk assessment, and implementation of preventive measures, supporting One Health approaches in the region.

II. MATERIALS AND METHODS

This study was conducted in the southeastern region of Albania, specifically in the district of Korça, during the late summer to winter season of 2024 (August–December). A total of 153 serum blood samples were collected, consisting of 137 horses and 16 mules. Blood was obtained from the jugular vein using plain vacutainer tubes, with approximately 9 ml collected per animal under welfare-compliant conditions. After 30–60 minutes, the samples were transported to the laboratory of Fan S. Noli University, where the sera were harvested via centrifugation at 3000 rpm for 5 minutes.

Serological testing was performed using an enzyme-linked immunosorbent assay (ELISA), specifically the ID Screen® West Nile Competition Multi-species ELISA kit, designed to detect anti-pr-E antibodies in horses, donkeys, and mules (Castillo-Olivares and Wood, 2004).

The southeastern region of Albania, including the area of Korça, is characterized by a diverse geo-climatic landscape that includes lowlands, fertile plains,

and mountainous terrains, offering a variety of ecological conditions relevant to animal husbandry and disease ecology.

These zones differ in rainfall, altitude, temperature, and vegetation cover. Since mosquito-borne disease transmission is highly influenced by environmental and climatic factors, geospatial characteristics of the study area were considered critical for the assessment of West Nile Virus (WNV) exposure risk (Hess et al., 2018).

The sampled horses varied in age and sex. Animals were classified into three age categories (0–5 years, 6–20 years, and older than 21 years old), and both male and female animals were included in the sampling.

In addition to biological sampling, basic epidemiological information was recorded for each animal, including origin, body condition score, housing and feeding conditions, and application of prophylactic measures. A clinical examination of each animal was also performed. Moreover, owners were interviewed regarding the presence of wild birds and mosquitoes in the surrounding environment, as both avian and mosquito species are key components in the transmission cycle of WNV (Bernard *et al.*, 2001; Castillo-Olivares and Wood, 2004).

III. RESULTS

Serological testing using the ELISA IgG method enabled the detection of antibodies specific to West Nile Virus (WNV) in several equine samples collected across different administrative areas of the Korça region. The interpretation of test results was conducted according to the manufacturer’s criteria, which classifies samples based on the ratio between sample and negative control optical densities (S/N ratio). Based on this analysis, the study identified a limited number of positive cases among horses, indicating prior exposure to the virus.

In addition to confirmed positive cases, a small subset of samples exhibited borderline values, categorized as doubtful, suggesting possible exposure or early seroconversion that may warrant follow-up testing. The majority of tested animals, however, yielded negative results, indicating the absence of detectable levels of anti-pr-E antibodies in those individuals at the time of sampling.

Positive cases were distributed in more than one locality within the study area, and they involved both male and female horses of different ages. These findings support the assumption that WNV circulates at a low but detectable level in southeastern Albania. While the number of positive detections was low, their presence highlights the importance of ongoing surveillance in equine populations, especially considering environmental changes that may affect vector dynamics and virus transmission potential.

Tab. 1. West Nile ELISA results in equine animals

Village	% Positive	Category
Pëparimaj	0	No cases
Vëçan	0	No cases
Miras	7.7	Low (≤10%)
Broçaj	8.3	Low (≤10%)
Mënkulas	8.3	Low (≤10%)
Poroçan	8.3	Low (≤10%)
Dobrraj	10.0	Low (≤10%)

Village	% Positive	Category
Trestenik	14.3	Moderate (10–20%)
Grapsh	16.7	Moderate (10–20%)
Vlashocës	22.7	High (>20%)
Poloskë	33.3	High (>20%)

The table presents the results of the ELISA IgG diagnostic test conducted on equine species (horses and mules), categorizing them into Positive and Negative cases. Positive results were identified mainly in horses, indicating the presence of specific antibodies against West Nile Virus in some cases. Only one mule tested positive, suggesting rare serological evidence of exposure to the virus in these species.

Out of 137 tested horses, 17 (12.4%) tested positive, indicating the WN virus circulation. The majority 120 individuals (87.6 %), tested negative, indicating that they are free of the disease. Out of 16 tested mule, one tested positive indicated that the risk of mules to be infected is low.

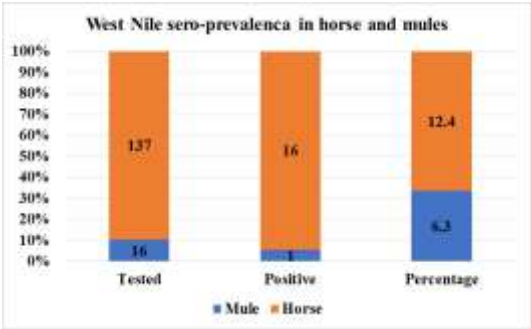


Figure 1. ELISA test results according to animal species

When adjusting for the difference in sample size, horses show a noticeably higher sero-prevalence (12.4%) than mules (6.3%). This may be due to differences in exposure risk (e.g., grazing areas, vector contact), management practices, or biological susceptibility. Only 16 mules were tested, so their prevalence estimate is less statistically robust.

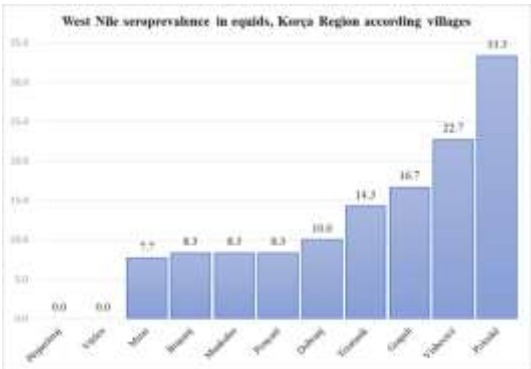


Figure 2 – West Nile sero-prevalence according villages based on ELISA test results

The villages Pëparimaj and Vëçan (0%) show no evidence of detected infection. The villages Miras, Broçaj, Mënkulas, Poroçan, and Dobrraj (7.7–10%) have a low risk, but continuous surveillance is needed. The villages Trestenik and Grapsh (14.3–16.7%) have moderate infection levels, and active surveillance is recommended, while the villages Vlashocës and Poloskë (22.7–33.3%) represent hotspot areas. It is suggested to focus control measures and reduce contact with vectors. The distribution of West Nile virus is not uniform in the Korça district. The villages Poloskë and Vlashocës have the highest prevalence and may represent high-risk zones, while some villages have shown no positive cases. Surveillance and control measures should focus on villages with high prevalence to prevent the spread of the virus.

Statistical Analysis

The ELISA test results for West Nile Virus (WNV) antibodies in equine species were analyzed using both the Chi-square test and Fisher’s exact test to evaluate statistical significance.

Sex	Tested	Positive	Positivity Rate (%)
Horse (Male)	88	11	12.5%
Mare (Female)	49	6	12.2%
Total Horses	137	17	12.4%

Sex	Tested	Positive	Positivity Rate (%)
Mule Male	6	0	0.0%
Mule Female	10	1	10%
Total Mules	16	1	4.3%

The overall seroprevalence in horses was 12.4% (17/137), whereas in mules it was 4.3% (1/23), indicating a higher exposure rate in horses. When comparing sexes, male horses (n=88) had a positivity rate of 12.5%, while female horses (mares, n=49) had a similar rate of 12.2%, suggesting no significant sex-related difference in WNV exposure within horses.

In contrast, among mules, male individuals (n=6) showed 0% positivity, whereas female mules (n=10) showed 10% positivity, suggesting a potential sex-related variation in exposure. However, given the small number of mules tested, these observations require cautious interpretation.

To assess the significance of the difference in WNV seroprevalence between horses and mules, a 2x2 Chi-square test was conducted. The expected values met the assumptions for this test. Although the observed difference in seroprevalence between horses (12.4%) and mules (4.3%) appeared notable, it was not statistically significant at $p < 0.05$.

Due to the small sample size in the mule group, Fisher’s exact test was also applied for greater accuracy. The contingency table used is presented below:

Species	Positive	Negative
Horses	17	120
Mules	1	15

Results indicate that Odds Ratio is 2.12 and p-value 0.695.

The odds ratio indicates that horses were more likely to test positive for WNV antibodies compared to mules. However, the p-value of 0.695 confirms that the observed difference is not statistically significant. These findings indicate that WNV is circulating at a moderate level in the horse population, with lower prevalence observed in mules. The absence of seropositivity among male mules and the higher positivity in females may suggest a sex-related exposure pattern, although this requires further investigation with larger sample sizes.

ELISA S/P% Distribution Analysis

To further validate the ELISA test performance and differentiate between seropositive and seronegative individuals, S/P% values were analyzed using boxplot distribution (Figure 3). Results demonstrated a clear separation between seropositive and seronegative groups. The seronegative group exhibited significantly higher S/P% values, with a median of 83.7%, and an interquartile range (IQR) of 78.8% to 88.4%. Several outliers extended beyond 100%, reaching up to 118.5%, indicating a strong negative antibody response or potential nonspecific reactivity. The seropositive group, by contrast, had markedly lower S/P% values, with a median of 16.8% and an IQR of 13.4% to 19.9%, further confirming successful detection of WNV-specific antibodies.

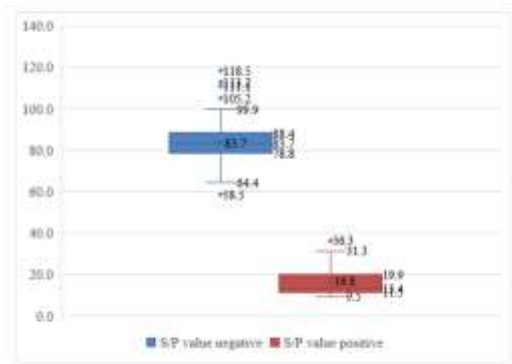


Figure 3 – Distribution of ELISA S/P% Values in Seropositive and Seronegative Equids

Boxplot comparison of S/P% (Sample-to-Positive control ratio percentage) values in equids tested for West Nile Virus using competitive ELISA. The seronegative group (blue) exhibited higher S/P% values (median: 83.7%), while the seropositive group (red) showed significantly lower S/P% values (median: 16.8%). This inverse pattern confirms the expected performance of the competitive ELISA format, where lower S/P% reflects higher antibody levels. Outliers are represented as individual data points.

This inverse relationship between S/P% and serostatus supports the use of a competitive ELISA format, where lower S/P% values reflect higher antibody presence due to inhibition of antigen-antibody binding. The distinct non-overlapping distribution between groups indicates that the test has good discriminatory power, with minimal ambiguity in classification.

While outliers were noted in both groups, the low variability within the positive group suggests a consistent antibody response in confirmed WNV-exposed individuals. The higher dispersion in the negative group may reflect biological

variation, subclinical exposure, or nonspecific binding, and should be interpreted with caution in borderline cases.

IV. DISCUSSION

The detection of antibodies against West Nile Virus (WNV) in horses provides preliminary evidence for the presence of the virus in Albania, indicating that the pathogen exists in multiple areas of the country and emphasizing the importance of further surveillance and comprehensive epidemiological research to clarify its distribution and potential impact on both animal and public health. Although only a limited number of seropositive cases were identified, their existence marks a significant finding for Albania, where WNV circulation has not been previously confirmed through serological evidence in equine populations. The positive cases were not associated with observable clinical symptoms at the time of sampling, reinforcing previous observations that WNV infections in horses are often asymptomatic or present with non-specific, mild symptoms (Petersen *et al.*, 2013).

The southeastern Albanian region, including the Korça district, although not traditionally considered a favorable habitat for large mosquito populations due to its altitude and climatic features, appears to be experiencing ecological changes potentially linked to climate change. These changes, including increased humidity and temperature during the summer months, may be enhancing the breeding potential of mosquito species, particularly *Culex* spp., which are recognized vectors of WNV (Ibarra-Juarez *et al.*, 2012; ECDPC, 2023). A field study conducted in Albania by Rogozi *et al.*, (2012) provided valuable insight into the seasonal dynamics of mosquito populations across multiple regions of the country. The researchers found a marked increase in mosquito density during the summer months, particularly in areas with favorable breeding conditions such as stagnant water and high humidity. The southeastern region, including Korça, was highlighted as one of the locations with significant mosquito activity during the warmer season. These findings align with field observations reported in the current study, reinforcing the hypothesis that the risk of vector-borne transmission may be elevated during the summer due to increased mosquito presence.

Seropositivity in horses, even in the absence of clinical signs, is of particular importance for public health surveillance. Horses, as dead-end hosts, serve as useful sentinels for WNV circulation in a given region. The fact that equines may harbor the virus without displaying symptoms necessitates the implementation of broader and systematic sero-surveillance programs. The current findings suggest that the virus may be circulating silently in the environment, highlighting the need for integrated surveillance strategies that also include wild and domestic birds, which are the natural reservoirs of WNV (Petersen *et al.*, 2013; García-Bocanegra *et al.*, 2012).

The presence of WNV in neighboring countries, such as Greece and North Macedonia, both of which have documented outbreaks in humans and animals (De Heus *et al.*, 2016) raises concerns for cross-border transmission. Given Albania's geographical position and active trade relations, especially in the livestock sector, transboundary movement of animals and vectors cannot be ruled out as a potential route for virus introduction or spread (Calistri *et al.*, 2010). This suggests that WNV may have been introduced into southeastern Albania through such exchanges.

Similar patterns of silent WNV circulation have been observed in other European and Mediterranean countries where the disease later emerged with greater epidemiological significance. According to the European Centre for Disease Prevention and Control (ECDPC), there has been a progressive increase in the number and distribution of WNV outbreaks within the EU, particularly in southern and central Europe, including Spain, Italy, France, Greece, and Hungary (ECDPC, 2019, 2023). This shows why proactive measures are important, even in regions where no clinical cases have been reported.

In Albania, further research is needed to clarify the epidemiological status of WNV. Serological testing in more equine and avian species, along with broader geographic sampling and mosquito surveillance, is essential to understand WNV ecology in the country. Establishing such a knowledge base will also enable the identification of potential risk factors and support the development of evidence-based strategies for disease prevention and control.

The findings of this study provide a scientific basis for raising awareness among veterinary and public health authorities regarding the potential emergence of WNV in Albania. They also emphasize the importance of collaboration across sectors, following the One Health approach that links human, animal, and environmental health.

V. CONCLUSION

The results of this study provide preliminary evidence for the presence of West Nile Virus (WNV) in the equine population of southeastern Albania. Although few cases were seropositive, these results show the need for improved monitoring and follow-up testing in horses and birds, as birds play a key role in WNV transmission.

Given the zoonotic nature of WNV and the growing geographic spread of the virus across Europe, proactive measures must be taken to assess and mitigate potential risks to animal and public health. This includes integrated mosquito control programs and educational campaigns aimed at veterinarians, farmers, and the general public.

Future studies with broader geographic coverage, larger sample sizes, and a One Health approach are essential to better understand WNV dynamics in Albania. These efforts will offer important information for developing focused monitoring and effective prevention measures for animals and humans.

In summary, surveillance should be strengthened in horses during peak mosquito seasons, and mule sampling increased for better statistical reliability. Differences between male and female mules should be monitored using structured sampling, while mosquito numbers and species should be monitored to better understand WNV transmission risk. Regular serological surveys should also be conducted to monitor changes in virus exposure among equids.

Acknowledgments

We kindly acknowledge the support and expertise provided by the Laboratory of Infectious Diseases at the Faculty of Veterinary Medicine, University of Agriculture of Tirana. We also express our gratitude to the local veterinary office in the Korça district, whose veterinarians showed great cooperation and assistance during the sampling phase of this study.

REFERENCES

1. Benjelloun, A., Ladjel, S., & Sadek, A. (2017). Seroprevalence of West Nile virus in horses in different Moroccan regions. *Veterinary Medicine and Science*, 3(2), 198–207. <https://doi.org/10.1002/vms3.59>
2. Berxholi, K., Ziegler, U., Rexhepi, A., Schmidt, K., Mertens, M., Korro, K., Cuko, A., Angenvoort, J., & Groschup, M. H. (2013). Indigenous West Nile virus infections in horses in Albania. *Transboundary and Emerging Diseases*, 60 (Suppl. 2), 45–50. <https://doi.org/10.1111/tbed.12141>
3. Bernard, K. A., Maffei, J. G., Jones, S. A., Kaufman, E. M., Ebel, G. D., Dupuis, A. P. II, Ngo, K. A., Nicholas, D. C., Young, D. M., Shi, P. Y., Kulasekera, V. L., Eidson, M., White, D. J., Stone, W. B., & Kramer, L. D. (2001). West Nile virus infection in birds and mosquitoes, New York State, 2000. *Emerging Infectious Diseases*, 7(4), 679–685. <https://doi.org/10.3201/eid0704.010402>
4. Calistri, P., Giovannini, A., Hubalek, Z., Ionescu, A., Monaco, F., Savini, G., Lelli, R. Epidemiology of West Nile virus in Europe and in the Mediterranean Basin. *Veterinary Research*, 2010; 41(6): 69. PMID: 20517490. doi:10.1051/vetres/2010027
5. Castillo-Olivares, J., & Wood, J. (2004). West Nile virus infection of horses. *Veterinary Research*, 35(4), 467–483. <https://doi.org/10.1051/vetres:2004022>
6. De Heus, P., Kolodziejek, J., Camp, V., Dimme, K. L., Bagó, Z., Hubálek, Z., Van den Hoven, R., Nowotny, N. Emergence of West Nile virus lineage 2 in Europe: Characteristics of the first seven cases of West Nile neuroinvasive disease in horses in Austria. *Transboundary and Emerging Diseases*, 2020; 67(2): 773–781. doi:10.1111/tbed.13452
7. European Centre for Disease Prevention and Control. (2023). Weekly updates: 2023 West Nile virus transmission season. <https://www.ecdc.europa.eu/en/publications-data/weekly-updates-2023-west-nile-virus-transmission-season>
8. European Centre for Disease Prevention and Control. West Nile virus in Europe in 2019 - equine cases, updated 27 September 2019. (Online). Available at: <https://www.ecdc.europa.eu/en/publications-data/west-nile-virus-europe-2019-equine-cases-updated-27-september-2019> (Accessed August 2025).
9. García-Bocanegra, I., Arenas-Montes, A., Napp, S., Jaén-Téllez, J. A., Fernández-Morente, M., & Arenas, A. V. (2012). Seroprevalence and risk factors associated to West Nile virus in horses from Andalusia, Southern Spain. *Veterinary Microbiology*, 160(3–4), 341–346. <https://doi.org/10.1016/j.vetmic.2012.05.011>
10. Hess, A., Davis, J. K., & Wimberly, M. C. (2018). Identifying environmental risk factors and mapping the distribution of West Nile Virus in an endemic region of North America. *GeoHealth*, 2(12), 395–409. <https://doi.org/10.1029/2018GH000161>
11. Ibarra-Juarez, L., Eisen, L., Bolling, B. G., Beaty, B. J., Blitvich, B. J., Sanchez-Casas, R. M., Ayala-Sulca, Y. O., & Fernandez-Salas, I. (2012). Detection of West Nile virus-specific antibodies and nucleic acid in horses and mosquitoes, respectively, in Nuevo Leon State, northern Mexico, 2006–2007. *Medical and Veterinary Entomology*, 26(3), 351–354. doi:10.1111/j.1365-2915.2012.01014.x
12. Lufo, L., Bizhga, B., Moka, G., & Berxholi, K. (2023). Serosurvey for detection of equine's West Nile infection in Albania. *Albanian Journal of Agricultural Sciences*, 22(Special Issue), 44–50. Agricultural University of Tirana.
13. Petersen, L. R., Brault, A. C., & Nasci, R. S. (2013). West Nile Virus: Review of the literature. *JAMA*, 310(3), 308–315. <https://doi.org/10.1001/jama.2013.8042>
14. Rogozi E., Velo E., Bino S., Severini F., & Schaffner F. (2012). An update of the mosquito fauna of Albania, based on a country-wide field survey 2011–2012 (Poster presentation). 18th International Conference “From Biology to Integrated Control in a Changing World”, ESOVE (October 8–11), Montpellier, France; Abstract p. 141.