

First Serological Assessment of West Nile Virus Activity in Horses in Serbia

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West Nile virus (WNV), the most widely distributed *Flavivirus* worldwide, has lately re-emerged in Europe causing worrisome outbreaks in humans and horses. Serological analysis by enzyme-linked immunoassay (ELISA) and plaque reduction neutralization test (PRNT) showed for the first time in Serbia that 12% of 349 horses analyzed presented specific neutralizing WNV antibodies, which in one case also cross-neutralized Usutu virus (USUV). This is the first time that anti-USUV high neutralizing antibody titers are reported in horses. All these data indicate that WNV and USUV are circulating in the region and advice on the convenience of implementing surveillance programs.

Samples

Sera from 349 horses were randomly collected during 2009–2010 (29.5%, 39.2%, 28.4%, and 2.9% during winter, spring, summer, and autumn, respectively) in stables of the Belgrade district area, of the municipality of Sabac and of 26 municipalities of the Vojvodina province of northern Serbia, which is bordered to Croatia, Hungary, and Romania (Fig. 1).

Vojvodina presents more than 50% of Serbian water surfaces and raises more than one-third of the country's horses.

Almost half of the animals (48.4%) analyzed were racing horses, 35.8% Lipizzaner breed horses, 10.3% ponies, 1.7% Arabian horses, and 3.7% mixed breed horses (Table I). Around one-third of the horses were in large stables and the remaining were from small private stables at their owners.

Mean age of the animals was 7.9 years (range: 3–19), 59.3% being mares and 40.7% stallions. Animals had not been vaccinated or presented signs of neurological disorders.

Methods

Anti-WNV IgG antibodies were detected by an ELISA based on recombinant envelope E (rE) protein expressed in *Trichoplusia ni* larvae (Alonso-Padilla et al., 2010).

Plaque reduction neutralization tests (PRNT) were conducted on Vero cells using two fold serial dilutions with either WNV NY-99 or USUV SAAR 1776 strains (Alonso-Padilla et al., 2009)

To detect WNV, samples were assayed by culture on Vero cells (up to 3 consecutive blind passages) and real-time RT-PCR (Lanciotti et al., 2000).

Results

Forty (12%) of the sera were IgG positive (average P/N = 4.2, range: 2–10.6).

All 42 serum neutralized WNV infectivity (Fig. 2) (average PRNT₉₀ = 120, range: 42–650), but, with one exception, none of them neutralized USUV (PRNT₉₀ < 40). The only sera that neutralized USUV (PRNT₉₀ = 90) also presented high PRNT and ELISA titers against WNV (PRNT₉₀ = 140 and P/N = 10.5, respectively). On the other hand, none of the 30 randomly selected IgG-negative sera tested were PRNT positive for either virus

No virus could be detected by cell culture or real-time reverse transcriptase–polymerase chain reaction in any of the samples analyzed.

No differences were observed among the proportion of positive animals detected in the different seasons and a similar proportion of positive animals were observed among mare (13%) and stallions (9.8%).

Serum neutralizing antibodies were present in 23% of the mixed breed horses, 12.8% of Lipizzaner breed horses, 13% of sport animals, and 2.8% of the ponies tested (Table I).

Positive horses were found in 14 of the 28 municipalities studied, which are up to 200km distant (Fig. 1).

Two of the positive horses were imported from Hungary and Croatia, respectively, and were bled while in quarantine.

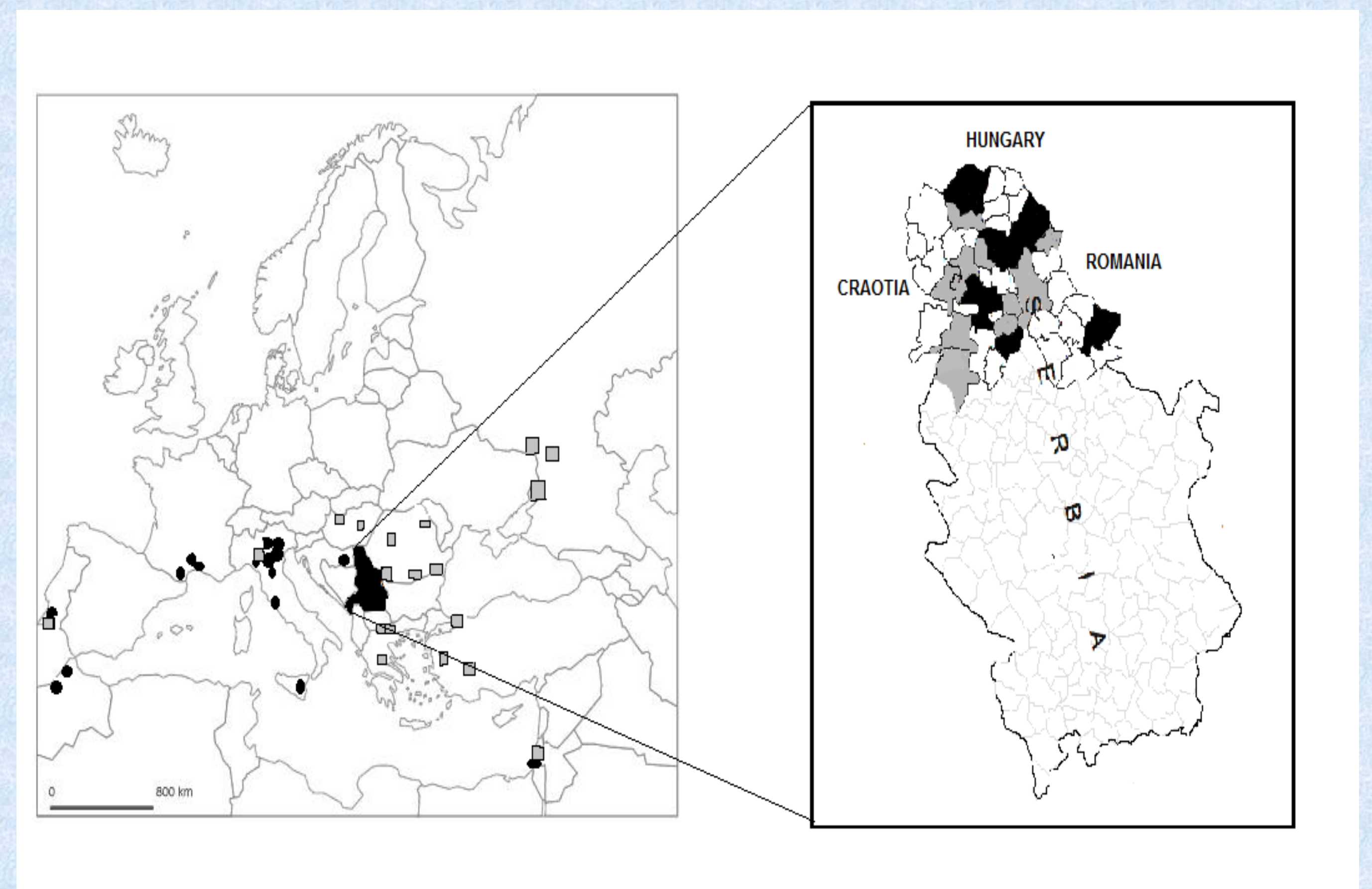


Figure 1. WNV activity in Serbia. Left panel shows recent reported WNV activity in Europe and the Mediterranean basin in humans (grey squares) and horses (black circles); Serbia is shadowed in black. Right panel displays the municipalities (in grey) from Serbia's Vojvodina province where samples were collected and in black those where anti-WNV IgG positive horses were detected.

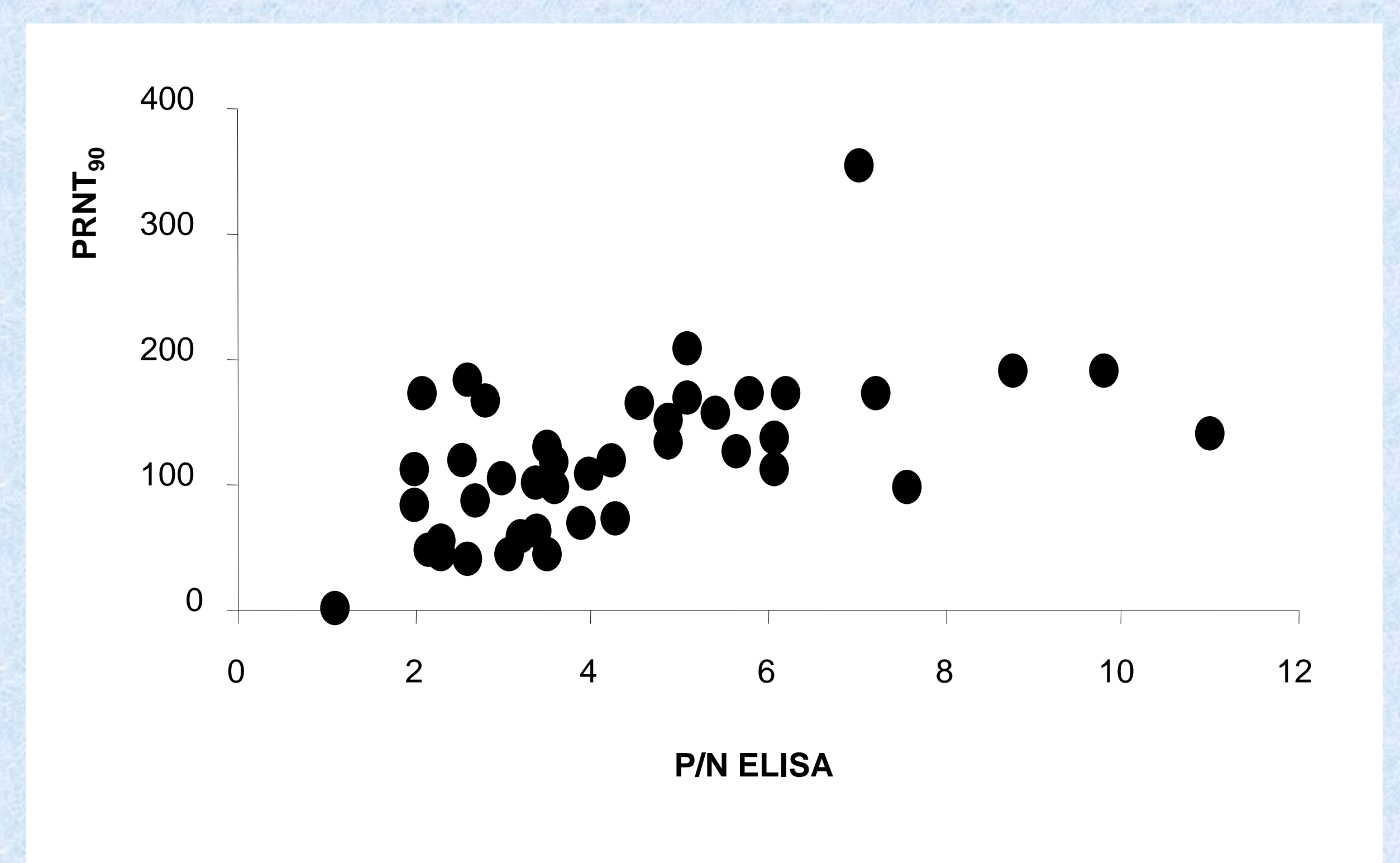


Figure 2. P/N and PRNT₉₀ values in WNV positive sera.

Table I

Animals	Nº	Nº of anti-WNV IgG & PRNT ₉₀ positive
Racing	169 (48.4%)	22 (13%)
Lipizzaner Breed	125 (35.8%)	16 (12.8%)
Ponies	36 (10.3%)	1 (2.8%)
Arabian	6 (1.7%)	0 (0%)
Mixed Breed	13 (3.7%)	3 (23%)
TOTAL	349 (100%)	42 (12%)

Conclusions

- WNV is circulating in Serbia, as 12% of the 349 horses tested were anti-WNV IgG positive
- USUV neutralizing antibodies were detected for the first time in a horse.
- Coordinated surveillance programs for Flavivirus activity across Europe should be implemented.