

MINISTRY OF RURAL DEVELOPMENT AND FOOD
ATHENS VETERINARY CENTER – DEPARTMENT OF MOLECULAR DIAGNOSTICS, FMD,
VIROLOGICAL, RICKETTSIAL AND EXOTIC DISEASES, ATHENS, GREECE

WNV 2018-2020 outbreaks in Greece

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Workshops of the European Reference Laboratories for EVA
and arthropod-borne encephalitis viruses


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DEPARTMENT OF MOLECULAR DIAGNOSTICS, FMD, VIROLOGICAL, RICKETTSIAL AND EXOTIC DISEASES

 National Reference Laboratory for 15 animal diseases including:

- ✓ West Nile Fever in Equidae
- ✓ *Capripox* viruses (LSD, sheep pox, goat pox)
- ✓ Rabies
- ✓ Bluetongue
- ✓ Foot and Mouth Disease
- ✓ Peste des petites ruminants

 Responsible for the application of national control and surveillance programs against viral diseases such as Bluetongue, West Nile Fever in Equidae and Rabies.

 Responsible for the detection of other viral pathogens in animals by serological, virological and molecular methods, such as Parapox, Maedi-Visna, Myxomatosis etc.



WNF SURVEILLANCE PROGRAMME

The Animal Health Directorate of the Ministry of Rural Development and Food in cooperation with local veterinary authorities at prefecture level implements:

A) Passive surveillance: To notify every suspect case in equidae, birds and other animal species susceptible to WNV and to update the census of the equidae population throughout the country.

B) Active surveillance: in equine and wild avian populations.

EQUINES

i. *The clinical examination and laboratory evaluation of equines from holdings:* a. within a zone of 20km from any positive human or animal case and b. in neighboring Regional Units (RUs) in a distance of 20 km from the borders with affected RUs.

ii. *The periodic serological examination of sentinel horses that are selected in a strategic manner so as to obtain surveillance results that are representative of the whole country.*

ACTIVE SURVEILLANCE

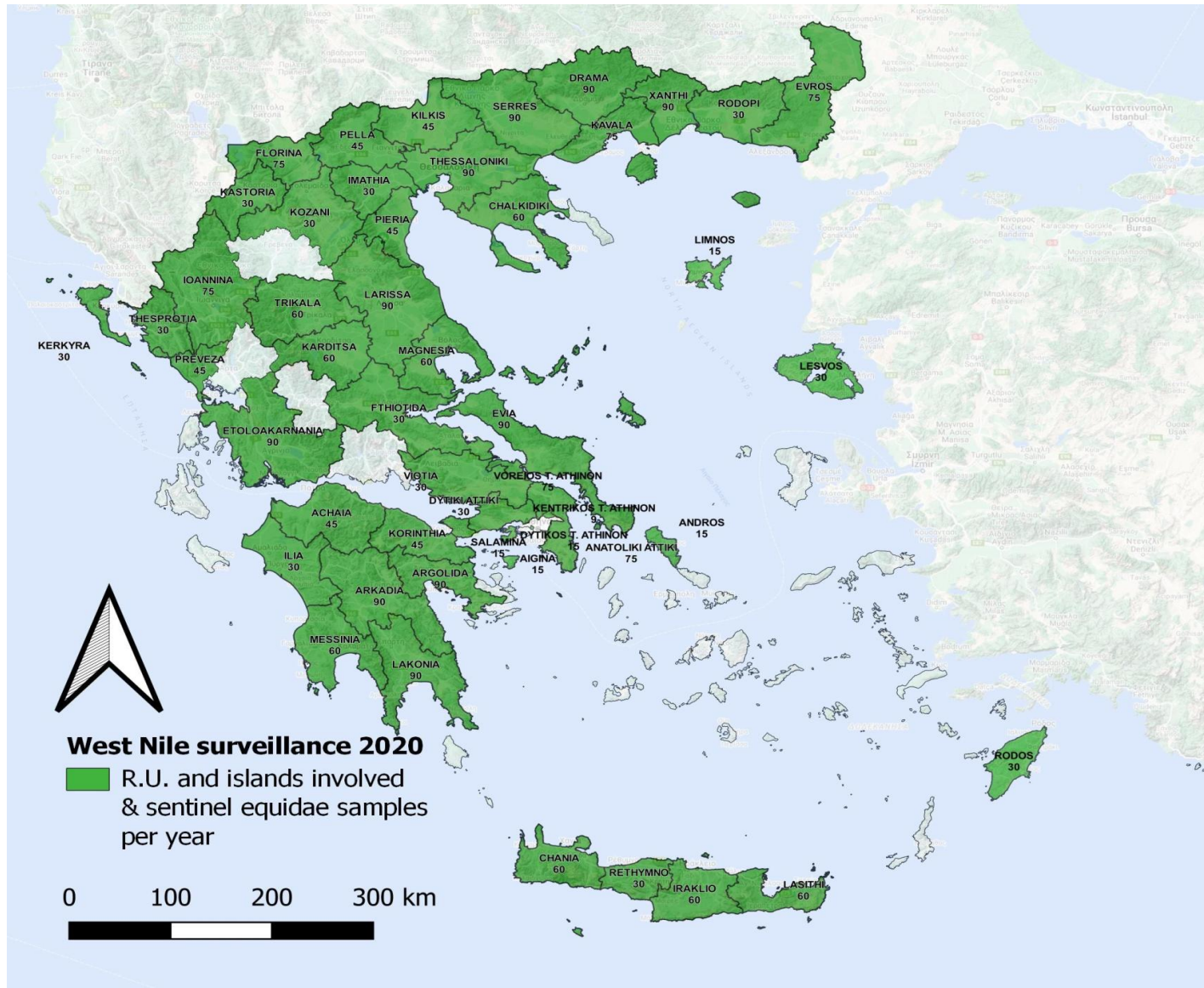
- ✓ The criteria for the selection of sentinel horses include:
 - a. geographical criteria
 - b. holdings and animals with no application of insecticides or insects repellents are selected
 - c. horses that are free to move only within a radius of 20km from their holdings are selected
- ✓ 3 samplings in 2021 : 01/05 until 30/06
01/07 until 31/07
01/08 until 30/09
- ✓ Predicted number of serum samples to be tested: 2574/year.

WILD BIRDS:

- ❖ It consists of blood sampling of wild avian populations from selected areas of the Greek mainland (specified number of wild birds per species and sampling site).
- ❖ The samples are dispatched to the Department of Pathology of Birds, Bees and Aquaculture, Directorate of Veterinary Center of Thessaloniki.



WEST NILE SURVEILLANCE 2020



LABORATORY DIAGNOSIS

i) Serological techniques

- ✓ From 2001 until 2006 serum samples were tested in our laboratory by Seroneutralisation test (according to Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise G., Teramo, Italy method).
- ✓ Since 2007 serum samples are examined with ID Screen West Nile Competition, IDVET ELISA which is c-ELISA and INgezim West Nile Compact ELISA.
- ✓ Since 2010 all seropositive in c-ELISA samples are tested with an IgM Antibody Capture ELISA (MAC).

ii) Molecular techniques

- ✓ Conventional and Real-time RT-PCR according to the protocols of EURL
- ✓ Commercial kit (VetMAX™ West Nile Virus Kit)

i) Virological techniques

- ✓ Inoculation of Vero E6 cells



LABORATORY RESULTS IN EQUIDAE: 2018-2020

	2018	2019	2020
Total number of samples tested in equidae by c-ELISA	1455	1082	1062
c-ELISA positive	356	258	322
IgM positive	19	24	8
No of samples tested by PCR	33	53 (1 positive)	17

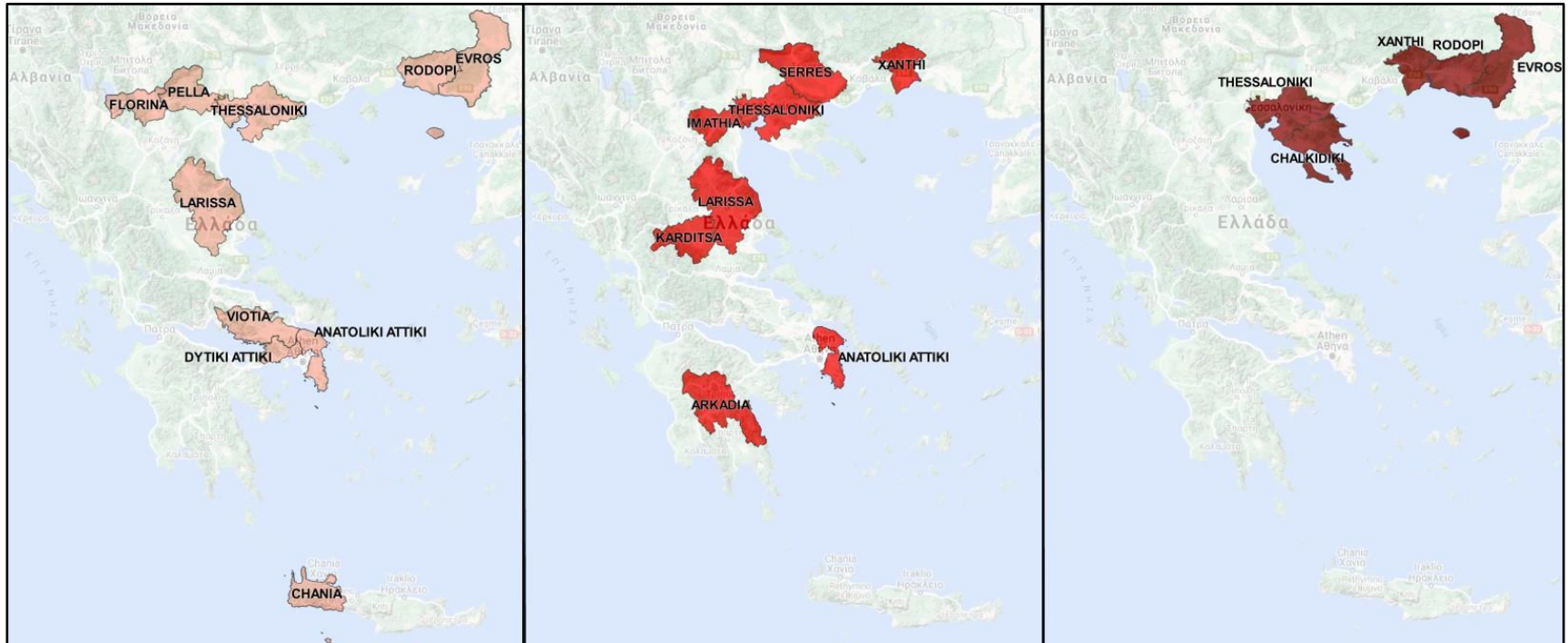


NUMBER OF OUTBREAKS IN EQUIDAE (2018-2020)

	2018	2019	2020
Number of confirmed horse cases (WNND or WNF)	15	21	8
Number of suspect cases (e.g. horses with neurological symptoms)	2	4	1



OUTBREAKS IN EQUIDAE (2018-2020)



2018

9 affected Regional Units
15 equidae cases (plus 1
canine case in Rodopi)

2019

8 affected Regional Units
21 cases

2020

5 affected Regional Units
8 cases

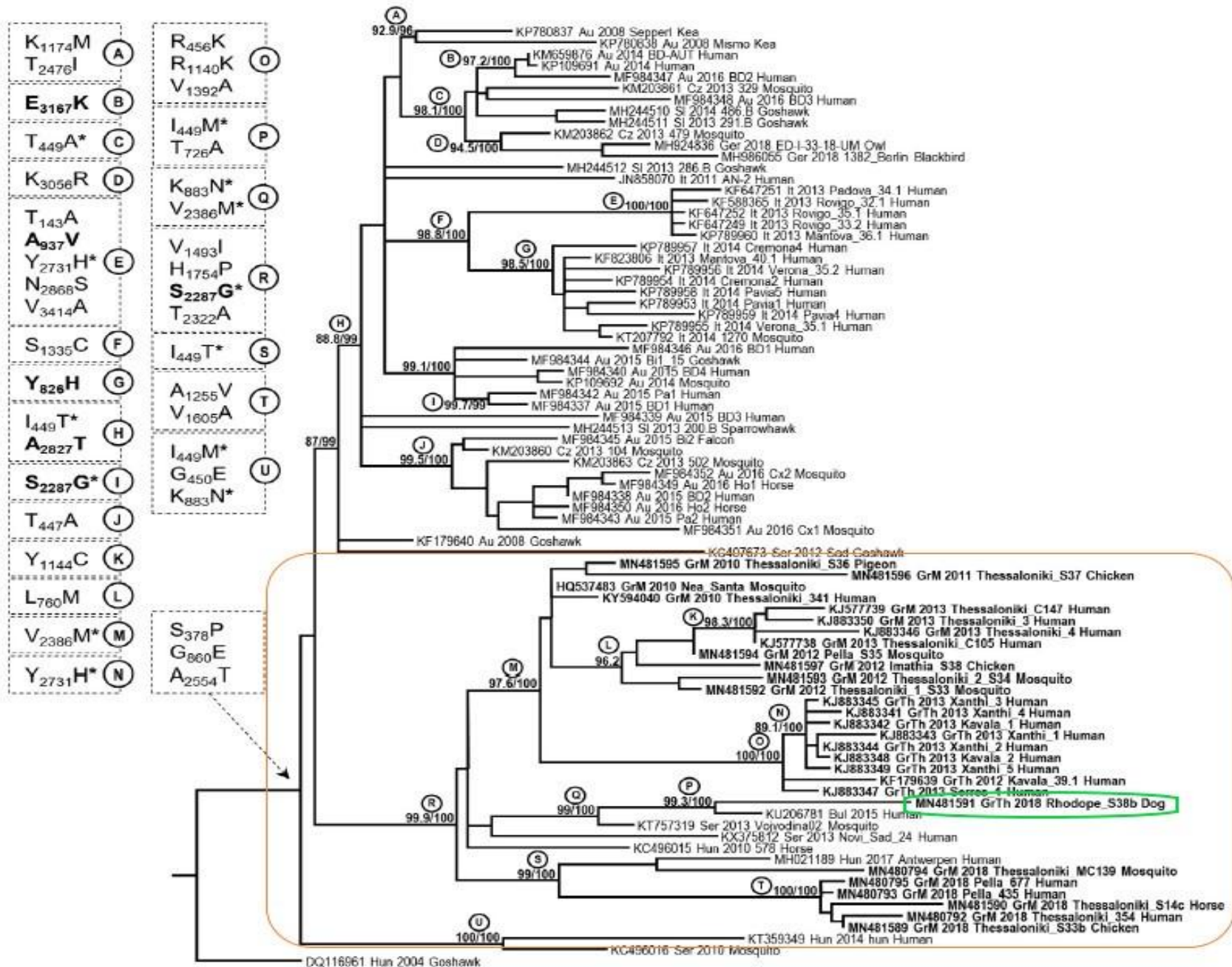


WNF IN DOGS (2018)

- ✓ Female, 1 year old dog in a public kennel with 420 dogs in Trace region of Greece (Rodopi RU).
- ✓ The dog presented neurological signs (epileptic seizure, loss of conscience, hemorrhagic diarrhea and emaciation).
- ✓ Blood sample was dispatched to the Faculty of Veterinary Medicine in Thessaloniki (Real-time PCR for CDV → **POS**, Real-time PCR for WNV → **POS**).
- ✓ The head of the dog was dispatched to our laboratory for rabies testing (Fluorescent of Antibody Test and Real-time PCR) → **NEG**.
- ✓ The internal organs of the dog (liver, heart, kidney, lung, spleen) were tested in our laboratory by Real-time PCR for WNV and CDV → **POS**.

Detection of the genome of both viruses (CDV and WNV)

- ✓ Molecular testing (real-time RT-PCR for WNV) from blood samples collected by other dogs (N = 40) of the same kennel revealed three positive samples.



Chaintoutis et al, (2019) "Evolutionary dynamics of lineage 2 West Nile Virus in Europe: 2004-2018: Phylogeny, selection pressure and phylogeography", *Molecular Phylogenetics and Evolution* 141. 106617.

CONCLUSIONS

- ❑ Evidence from mosquitoes, wild birds and chickens demonstrated that the WNV epidemics in Greece were caused by WNV lineage 2 (*Papa et al 2011, Valiakos et al 2014, Chaintoutis et al, 2013*).
- ❑ According to a recent publication by *Chaintoutis et al. (2019)*, Greek WNV strains do not comprise a monophyletic group. The strains obtained in Northern Greece during the 2018 were associated with novel, independent introductions, which were not associated with the “Nea Santa-Greece-2010” subgroup.
- ❑ Detection of seroconversions in horses has been suggested as a sentinel system to detect risk of WNV transmission to people (early detection system).
- ❑ In some areas:
 - ✓ equidae WNV cases precede or coincide with human WNV cases.
 - ✓ equidae WNV cases follow human WNV cases.
 - ✓ equidae WNV cases were the only indicators of the circulation of the virus without human WNV cases.
 - ✓ human WNV cases were the only indicators of the circulation of the virus without equidae WNV cases.





Thank you for your attention!

