

WOAH Reference Laboratory Reports Activities 2023

Activities in 2023

This report has been submitted : 12 juin 2024 12:15

Laboratory Information

Name of disease (or topic) for which you are a designated WOAHO Reference Laboratory:	West Nile fever
Address of laboratory:	campo boario
Tel.:	+39 0861 33.22.05
E-mail address:	f.monaco@izs.it
Website:	www.izs.it
Name (including Title) of Head of Laboratory (Responsible Official):	Nicola D'Alterio, General Director, Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise, "G. Caporale"
Name (including Title and Position) of WOAHO Reference Expert:	Federica Monaco, Head of the diagnosis and surveillance of exotic viral diseases of animals laboratory Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale"
Which of the following defines your laboratory? Check all that apply:	Governmental

TOR1: DIAGNOSTIC METHODS

1. Did your laboratory perform diagnostic tests for the specified disease/topic for purposes such as disease diagnosis, screening of animals for export, surveillance, etc.?
(Not for quality control, proficiency testing or staff training)

Yes

Diagnostic Test	Indicated in WOAHO Manual (Yes/No)	Total number of test performed last year	
		Nationally	Internationally
Indirect diagnostic tests			
c-ELISA - IgG		170	0
ELISA - IgM		134	0
Virus Neutralization (microtitre)		123	0
Direct diagnostic tests			
Virus isolation (C6/36 and Vero cells)		275	0
Real-time RT-PCR WNV lineage 1 and Lineage 2		2567	1
Whole genome sequencing		167	0

TOR2: REFERENCE MATERIAL

2. Did your laboratory produce or supply imported standard reference reagents officially recognised by WOAHO?

No

3. Did your laboratory supply standard reference reagents (nonWOAH-approved) and/or other diagnostic reagents to WOAHO Members?

Yes

TYPE OF REAGENT AVAILABLE	RELATED DIAGNOSTIC TEST	PRODUCED/ PROVIDE	AMOUNT SUPPLIED NATIONALLY (ML, MG)	AMOUNT SUPPLIED INTERNATIONALLY (ML, MG)	NO. OF RECIPIENT WOAHO MEMBER COUNTRIES	COUNTRY OF RECIPIENTS
WNV lineage 1 reference strain Eg101	RT-PCR	Produced and provided	21 ml	0	1	ITALY,

WNV lineage 2 reference strain B956	RT-PCR	Produced and provided	21 ml	0	1	ITALY,
Purified RNA from 23 WNV L1 field strain	RT-PCR	Produced and provided	0	200 ul/strain (4.6 ml)	1	SERBIA,
Purified RNA from 23 WNV L2 field strain	RT-PCR	Produced and provided	0	200 ul/strain (4.6 ml)	1	SERBIA,
Non Purified MoAb vs WNV	Immunofluorescence, ELISA	Produced	4400 ml	0	1	ITALY,

4. Did your laboratory produce vaccines?

No

5. Did your laboratory supply vaccines to WOAHA Members?

No

TOR3: NEW PROCEDURES

6. Did your laboratory develop new diagnostic methods for the designated pathogen or disease?

No

7. Did your laboratory validate diagnostic methods according to WOAHA Standards for the designated pathogen or disease?

No

8. Did your laboratory develop new vaccines for the designated pathogen or disease?

No

9. Did your laboratory validate vaccines according to WOAHA Standards for the designated pathogen or disease?

No

TOR4: DIAGNOSTIC TESTING FACILITIES

10. Did your laboratory carry out diagnostic testing for other WOAHA Members?

Yes

NAME OF WOAHA MEMBER COUNTRY SEEKING ASSISTANCE	DATE	WHICH DIAGNOSTIC TEST USED	NO. SAMPLES RECEIVED FOR PROVISION OF DIAGNOSTIC SUPPORT	NO. SAMPLES RECEIVED FOR PROVISION OF CONFIRMATORY DIAGNOSES
TUNISIA	2023-11-05	RT-PCR	1	0

11. Did your laboratory provide expert advice in technical consultancies on the request of an WOAHA Member?

Yes

NAME OF THE WOAHA MEMBER COUNTRY RECEIVING A TECHNICAL CONSULTANCY	PURPOSE	HOW THE ADVICE WAS PROVIDED
ITALY	In the framework of the national surveillance plan for WNV and Usutu virus for 2023, the laboratory has been in charge for: - defining the surveillance activities in animals and vectors; - harmonizing and assessing the diagnostic capabilities of the regional laboratories network through proficiency tests; - the collection and management of the data generated by the surveillance activities in animals and vectors.	In loco and remote assistance

TOR5: COLLABORATIVE SCIENTIFIC AND TECHNICAL STUDIES

12. Did your laboratory participate in international scientific studies in collaboration with WOAHA Members other than the own?

Yes

Title of the study	Duration	PURPOSE OF THE STUDY	PARTNERS (INSTITUTIONS)	WOAHA MEMBER COUNTRIES INVOLVED OTHER THAN YOUR COUNTRY

Senegal – Italy Arbovirus Introduction and Emergence (SIAIE)	3 years	Characterization of WNV and USUV, geographically and within specific host and vector populations, between Senegal and Italy	- Institute Pasteur, Senegal - Fondazione Edmund Mach, Italy	SENEGAL
European network of medical and veterinary entomology (VectorNet)	5 years	Developing a network of medical and veterinary experts and organisations to maintain a common database on the presence and distribution of vectors and pathogens in vectors across Europe and the Mediterranean basin	ECDC EFSA Experts and organisations from the medical and veterinary domains	ITALY
Ecology of Wild-life, Livestock, huMan and Infectious Diseases in changing environments — WiLiMan-ID	5 years	The main objective of WiLiMan-ID is to identify key factors allowing five animal infectious diseases to spread and persist, in changing environments. The five diseases are: Avian influenza, African swine fever, West-Nile fever, African horse sickness and Chronic wasting disease	Austria-Biofaction Belgium- Sciensano Denmark-The University of Copenhagen France-ANSES France-Ecole Nationale Vétérinaire (ENV) France-National Research Institute for Agriculture, Food and Environment (INRAE) Germany-Friedrich-Loeffler-Institut (FLI) Morocco-The Hassan II Agronomic and Veterinary Institute (IAV) Norway-The Norwegian Veterinary Institute (vetinst) Spain-The Central Veterinary Laboratory of the Spanish Ministry of Agriculture, Fisheries and Food. Sweden-Swedish Veterinary Agency (SVA) The Netherlands-Wageningen University & Research (WUR)	AUSTRIA BELGIUM DENMARK FRANCE GERMANY MOROCCO NORWAY SPAIN SWEDEN THE NETHERLANDS

13. In exercising your activities, have you identified any regulatory research needs* relevant for WOH?

No

TOR6: EPIZOOLOGICAL DATA

14. Did your Laboratory collect epidemiological data relevant to international disease control?

Yes

IF THE ANSWER IS YES, PLEASE PROVIDE DETAILS OF THE DATA COLLECTED:

- Italian data are collected from the Sistema Informativo Nazionale Malattie Animali (SIMAN) <https://www.vetinfo.it/Italy>
- Data about human outbreaks in EU Member States and EU neighbouring countries are collected from the European Surveillance System (TESSy) database while worldwide animal data are collected through the Animal Disease Information System (ADIS) of the European Commission and the World Animal Health Information System (WAHIS) database. <https://wahis.woah.org/#/home>
- Outbreak data referred to human cases in Greece are retrieved from the National Public Health Organization (NPHO) Report_WNV_20220906_ENG.pdf (eody.gov.gr)

15. Did your laboratory disseminate epidemiological data that had been processed and analysed?

Yes

IF THE ANSWER IS YES, PLEASE PROVIDE DETAILS OF THE DATA COLLECTED:

A Web Geographic Information System application has been developed to collect and disseminate disease data, and full genome sequences of selected isolated strains of WNV. The tool (Disease Monitoring Dashboard) compiles multiple datasets through user-friendly web tools for epidemiological analysis (<https://netmed.izs.it/networkMediterraneo/>)

WNV data are disseminated through a public web site (www.izs.it) where information and data on WN is continuously updated in order to have:

- weekly bulletins summarizing the current (2023) epidemiological situations in Italy and Mediterranean Basin;
 - maps on entomological, virological and serological surveillance activities;
- the past epidemiological situations in Italy (2008-2022) and the Mediterranean Basin (2010-2022);

- the latest on the Italian and European Regulations;
- scientific documents on-line.

Since 2018 surveillance activities are summarized in the interactive Story Maps available on the public web site and provide a description of the disease and the complete data from the human, animal and entomological surveillance activities (https://westnile.izs.it/j6_wnd/home).

A predictive model to identify area at risk for WNV circulation has been developed and is freely accessible (https://mapserver.izs.it/gis_wn_predictions/#). The model uses EO data with the WNV detection in mosquitoes, birds and horses since 2017 to train an Extreme Gradient Boosting model to automatically predict in space and time WNV circulation

16. What method of dissemination of information is most often used by your laboratory? (Indicate in the appropriate box the number by category and list the details in the box)

a) Articles published in peer-reviewed journals:

12

- Constant O, Maarifi, G., Barthelemy, J., Martin, M., Tinto, B., Savini, G., Van de Perre, P., Nisole, S., Simonin, Y., Salinas, S. 2023. Differential effects of usutu and west nile viruses on neuroinflammation, immune cell recruitment and blood-brain barrier integrity. *Emerging Microbes and Infections*, 12(1) doi:10.1080/22221751.2022.2156815
- Diagne M. M., Ndione, M. H. D., Mencattelli, G., Diallo, A., Ndiaye, E. H., Di Domenico, M., Diallo, D., Kane, M., Curini, V., Top, N. M., Marcacci, M., Mbanne, M., Ancora, M., Secondini, B., Di Lollo, V., Teodori, L., Leone, A., Puglia, I., Gaye, A., Sall, A. A., Loucoubar, C., Rosà, R., Diallo, M., Monaco, F., Faye, O., Cammà, C., Rizzoli, A., Savini, G., & Faye, O. 2023. Novel amplicon-based sequencing approach to West Nile virus. *Viruses*, 15(6) doi:10.3390/v15061261
- Jurisic L., Malatesta, D., Zaccaria, G., Di Teodoro, G., Bonfini, B., Valleriani, F., Teodori, L., Bencivenga, F., Leone, A., Ripà, P., D'Innocenzo, V., Rossi, E., Lorusso, A. 2023. Immunization with usutu virus and with a chimeric west nile virus (WNV) harboring usutu-E protein protects immunocompetent adult mice against lethal challenges with different WNV lineage 1 and 2 strains. *Veterinary Microbiology*, 277 doi:10.1016/j.vetmic.2022.109636
- Mencattelli G., Ndione, M. H. D., Silverj, A., Diagne, M. M., Curini, V., Teodori, L., Di Domenico, M., Mbaye, R., Leone, A., Marcacci, M., Gaye, A., Ndiaye, E., Diallo, D., Ancora, M., Secondini, B., Di Lollo, V., Mangone, I., Bucciacchio, A., Polci, A., Marini, G., Rosà, R., Segata, N., Fall, G., Cammà, C., Monaco, F., Diallo, M., Rota-Stabelli, O., Faye, O., Rizzoli, A., Savini, G. 2023. Spatial and temporal dynamics of west nile virus between africa and europe. *Nature Communications*, 14(1), 6440. doi:10.1038/s41467-023-42185-7
- Molini U., Franzo, G., Bonfini, B., de Villiers, L., de Villiers, M., Khaiseb, S., Monaco, F., Savini, G., & D'Alterio, N. 2023. Low seroprevalence of WNV in namibian dogs suggests a limited effectiveness as sentinels for infection monitoring. *Tropical Medicine and Infectious Disease*, 8(4) doi:10.3390/tropicalmed8040203
- Kholoud Khalid Ben-Mostafa, Savini G., Di Gennaro A., Teodori L., Leone A., Monaco F., Masoud A. Alaoqib M., Rayes AA., Dayhum A., Eldaghayes I. Evidence of West Nile virus circulation in horses and dogs in Libya. *Pathogens* Dec 31;13(1):41. doi: 10.3390/pathogens13010041.
- Bonicelli L., Porrello A., Vincenzi S., Ippoliti C., Iapaolo F., Conte A., Calderara S. 2023. Spotting Virus from Satellites: Modeling the Circulation of West Nile Virus Through Graph Neural Networks / - In: *IEEE TRANSACTIONS ON GEOSCIENCE AND REMOTE SENSING*. - ISSN 1558-0644. - 61:(2023), pp. 1-1. [10.1109/TGRS.2023.3293270]
- Gil P., Exbrayat A., Loire E., Rakotoarivony I., Charriat F., Morel C., Baldet T., Boisseau M., Marie A., Frances B., L'Ambert G., Bessat M., Otify Y., Goffredo M., Mancini G., Busquets N., Birnberg L., Talavera S., Aranda C., Ayari E., Mejrji S., Sghaier S., Bennouna A., El Rhaffouli H., Balenghien T., Chlyeh G., Fassi Fihri O., Reveillaud J., Simonin Y., Eloit M., Gutierrez S. 2023. Spatial scale influences the distribution of viral diversity in the eukaryotic virome of the mosquito *Culex pipiens*. *Virus Evol.* Aug 24;9(2):vead054. doi: 10.1093/ve/vead054. PMID: 37719779; PMCID: PMC10504824.
- Ascentis M., Quaglia, M., D'alesio, S. G., Iapaolo, F., Pizzurro, F., Ruggeri, F., Rossi, N., Blandi, M., Ippoliti, C., Cioci, D., Portanti, O., Piscicella, M., Di Lorenzo, A., Ciarrocchi, E., Irelli, R., Conte, A., Morelli, D., Monaco, F., Savini, G., Goffredo, M. 2023. Species of mosquitoes present in Abruzzo and Molise and their possible role as vectors of Usutu and West Nile viruses. *Veterinaria Italiana*, 58(4), 435-445. doi:10.12834/VetIt.3046.20276.1
- de Martinis C., Cardillo L., Pesce F., Viscardi M., Cozzolino L., Paradiso R., Cavallo S., De Ascentis M., Goffredo M., Monaco F., Savini G., D'Orilia F., Pinto R., Fusco G. 2023. Reoccurrence of West Nile virus lineage 1 after 2-year decline: first equine outbreak in Campania region. *Front Vet Sci.* 2023 Nov 30;10:1314738. doi: 10.3389/fvets.2023.1314738. eCollection 2023.
- Ben Mostafa K., Savini, G., Dayhum, A., & Eldaghayes, I. 2023. First detection of west nile virus antibodies in animals in libya. *International Journal of Infectious Diseases*, 130, S76. doi:10.1016/j.ijid.2023.04.188
- Vouillon A., Barthelemy, J., Lebeau, L., Nisole, S., Savini, G., Lévêque, N., Simonin, Y., Garcia, M., & Bodet, C. 2023. Skin tropism during usutu virus and west nile virus infection: An amplifying and immunological role. *Journal of Virology*, e0183023. doi:10.1128/jvi.01830-23

b) International conferences:

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- Morelli D., Monaco F. "WNV Surveillance system in EU". WOA meeting on Vector-borne zoonoses affecting equines: Japanese Encephalitis and West Nile fever in Asia and Pacific regions. Oral presentation. 29 March 2023. Webinar.
- Savini G. "West Nile virus a virtual bridge that links Africa to Europe". WSV2023 One Health - One World - One Virology. Oral presentation. 15-17 June 2023, Riga (Latvia)
- Iapaolo F. "2022 WNV transmission season" 15th Workshop of the EURL for equine diseases- arthropod-borne equine encephalitis viruses. 13 October 2023 Maison Alfort (France).
- Morelli D. "WNV surveillance system in Italy". ArboFrance WN meeting. Oral presentation. 29 November 2023. Webinar.

c) National conferences:

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- Monaco F. "Sorveglianza e gestione di West Nile virus e Usutu virus. L'importanza di un approccio One Health". *Malattie trasmesse da vettori: L'importanza dell'approccio*

One Health, Focus su West Nile ed Usutu. Naples, 12 April 2023.

- Goffredo M. "L'entomologia in Sanità Pubblica. I principali vettori presenti in Italia e nell'area del bacino del Mediterraneo: metodi di sorveglianza e controllo". *Malattie trasmesse da vettori: L'importanza dell'approccio One Health, Focus su West Nile ed Usutu. Naples, 12 April 2023.*

- Savini G. "Tipologia e diffusione delle principali malattie trasmesse da vettori (importate e non) in sanità pubblica". *Malattie trasmesse da vettori: L'importanza dell'approccio One Health, Focus su West Nile ed Usutu. Naples, 12 April 2023.*

- D'Alessio S.G., Goffredo M. "Ecologia degli artropodi vettori di infezioni umane in Italia". *Microbiology and Infections. Francavilla al Mare, 19 October 2023*

- Iapaolo F. "Arbovirosi e approccio One Health. Focus su West Nile e Usutu". *Sassari, 14 July 2023*

- Iapaolo F., Di Donato G. "Sorveglianza integrata per i virus West Nile e Usutu: un solido esempio di approccio One Health". *I Centri ed i Laboratori di Referenza nell'ottica One Health. Rome, 6 December 2023*

d) Other (Provide website address or link to appropriate information):

4
Please refer to the answer provided for the question n.15 for the details related to the links listed below:
Epidemiological situation in Italy and the Mediterranean region: www.izs.it
Disease Monitoring Dashboard: <https://netmed.izs.it/networkMediterraneo/>
StoryMaps 2018-2021 (https://westnile.izs.it/j6_wnd/home)
WNV predictive model (https://mapserver.izs.it/gis_wn_predictions/#)

TOR7: SCIENTIFIC AND TECHNICAL TRAINING

17. Did your laboratory provide scientific and technical training to laboratory personnel from other WOAHA Members?

No

TOR8: QUALITY ASSURANCE

18. Does your laboratory have a Quality Management System?

Yes

Quality management system adopted	Certificate scan (PDF, JPG, PNG format)	
ISO 17025	Accreditation certificate	Accreditation certificate.pdf

19. Is your quality management system accredited?

Yes

Test for which your laboratory is accredited	Accreditation body
i-ELISA - IgG	ACCREDIA
c-ELISA - IgG	ACCREDIA
ELISA IgM	ACCREDIA
Plaque Reduction neutralization test (PRNT)	ACCREDIA
Virus neutralization (microtitre format)	ACCREDIA
Real-time RT-PCR WNV lineage 1 and lineage 2	ACCREDIA

20. Does your laboratory maintain a "biorisk management system" for the pathogen and the disease concerned?

Yes

The biosecurity policy integrates aspects related to safety, security and environment, in fact risks associated with all our activities are assessed and managed to ensure the safety of workers and of the environment in accordance with international standards. In particular, IZS- Teramo has developed its biosecurity manual in accordance with the WHO standards "The WHO Laboratory Biosafety Manual (LBM) 4th ed.) as well as the specific procedures for safe handling and containment of infectious microorganisms and hazardous biological material. Furthermore, to reduce or eliminate the exposure of the environment (air, water, soil) to potentially infectious or hazardous agents IZSAM obtained the certification according to the ISO 14001 Lastly, a rigorous management of biologicals, chemicals and their associated waste is in place and information and communication to personnel done on a routine bases. To ensure the safety handling and movement of goods, the IZS-Teramo has developed protocols and procedures according to the World Health Organization standards (WHO/WHE/CPI/2019.20 Guidance on regulations for the Transport of infectious Substances" - 2019-2020; pag.1-29.). The laboratory is officially authorised by the Italian Ministry of Health to import biological materials and biological reagents of any origin through the airports of Rome (Fiumicino) and Milan (Malpensa and Linate) Transport by air of biological materials considered as infectious substances is done according to the international regulations guidelines developed by IATA (Infectious Substances Shipping Guidelines-1 January 2006- 7th Edition p.1-41). The IZS - Teramo also complies with ADR (European Agreement concerning the International Carriage of Dangerous Goods by Road and Directive 2014/103/UE) regulations to guarantee the safe road transportation of dangerous goods and owns vehicles properly equipped for the purpose. Traceability of biological material for research purposes is provided by

the use of MTA, and dispatch and receipt are regulated by Standard Operating procedures

TOR9: SCIENTIFIC MEETINGS

21. Did your laboratory organise scientific meetings related to the pathogen in question on behalf of WOAHP?

No

22. Did your laboratory participate in scientific meetings related to the pathogen in question on behalf of WOAHP?

Yes

Title of event	Date (mm/yy)	Location	Role (speaker, presenting poster, short communications)	Title of the work presented
WOAH meeting on Vector-borne zoonoses affecting equines: Japanese Encephalitis and West Nile fever in Asia and Pacific regions	2023-03-29	Webinar	Speaker	WNV Surveillance system in EU
WSV2023 One Health - One World - One Virology	2023-06-15	Riga, Latvia	Speaker	West Nile virus a virtual bridge that links Africa to Europe
15th Workshop of the EURL for equine diseases- arthropod-borne equine encephalitis viruses	2023-10-13	Maison Alfort, France	Speaker	2022 WNV transmission season in Italy
ArboFrance WN meeting	2023-11-29	Webinar	Speaker	WNV surveillance system in Italy

TOR10: NETWORK WITH WOAHP REFERENCE LABORATORIES

23. Did your laboratory exchange information with other WOAHP Reference Laboratories designated for the same pathogen or disease?

No

24. Do you network (collaborate or share information) with other WOAHP Reference Laboratories designated for the same pathogen?

No

25. Did you organise or participate in inter-laboratory proficiency tests with WOAHP Reference Laboratories designated for the same pathogen?

No

26. Did your laboratory collaborate with other WOAHP Reference Laboratories for the same disease on scientific research projects for the diagnosis or control of the pathogen of interest?

No

TOR11: OTHER INTERLABORATORY PROFICIENCY TESTING

27. Did your laboratory organise or participate in inter-laboratory proficiency tests with laboratories other than WOAHP Reference Laboratories for the same pathogen?

Yes

Purpose for inter-laboratory test comparisons ¹	Role of your reference laboratory (organizer/participant)	No. participating laboratories	Name of the Test	WOAHP Member Countries
Determining a laboratory's capability to conduct molecular assays for WNV and USUV detection and WNV Lineage identification	Organizer	14	Real time RT-PCR	ITALY, SERBIA,
Determining a laboratory's capability to conduct serological assays to detect WNV and USUV infection	Organizer	11	ELISA IgG, ELISA IgM	ITALY,
Molecular diagnostic tools for equine infections by arthropod-	Participant	23	Real time RT-PCR	AUSTRIA, BELGIUM, BULGARIA, CROATIA, CYPRUS, DENMARK, FINLAND, FRANCE, GERMANY, GREECE, HUNGARY, IRELAND, ITALY,

borne encephalitis viruses (WNV, JEV, EEEV, WEEV and VEEV)

LATVIA, POLAND, PORTUGAL, ROMANIA, SLOVAKIA, SLOVENIA, SPAIN, SWEDEN, THE NETHERLANDS,

Serology of equine infections by arthropod-borne encephalitis viruses (WNV, JEV, EEEV, WEEV and VEEV)

Participant

23

ELISA IgG, ELISA IgM

AUSTRIA, BELGIUM, BULGARIA, CROATIA, CYPRUS, DENMARK, FINLAND, FRANCE, GERMANY, GREECE, HUNGARY, IRELAND, ITALY, LATVIA, POLAND, PORTUGAL, ROMANIA, SLOVAKIA, SLOVENIA, SPAIN, SWEDEN, THE NETHERLANDS,

TOR12: EXPERT CONSULTANTS

28. Did your laboratory place expert consultants at the disposal of WOA?H?

Yes

KIND OF CONSULTANCY	Location	SUBJECT (FACULTATIVE)
Overview of WNV surveillance activities in Europe	on line	WOAH webinar on Vector-borne zoonoses affecting equines: Japanese Encephalitis and West Nile fever in Asia and Pacific regions

29. Additional comments regarding your report:

No