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Understanding the immune response to West Nile and Usutu Viruses

Submission date
06/07/2025

Recruitment status
Recruiting

Registration date
13/07/2026

Overall study status
Ongoing

Last Edited
13/07/2026

Condition category
Infections and Infestations

[_] Prospectively registered
[_] Protocol
[_] Statistical analysis plan
[_] Results
[_] Individual participant data
[X] Record updated in last year

Plain English summary of protocol

Background and study aims
West Nile virus (WNV) and Usutu virus (USUV) are members of the Orthoflavivirus genus, primarily transmitted by Culex mosquitoes. These pathogens circulate among avian hosts but can occasionally infect mammals, including humans. WNV has been present in Europe since the 1950s and has achieved global distribution, while USUV was first identified in Europe in 1996. Both viruses can cause neuroinvasive disease, with WNV posing a particularly significant threat to elderly and immunocompromised individuals. USUV rarely causes such severe outcomes. Since 2008, WNV has become endemic in several European regions, with a notable increase in epidemic occurrences. Although most human infections with WNV and USUV are asymptomatic, symptomatic cases can range from influenza-like illness to severe neurological complications. Diagnosis relies on serological and molecular tests, but can be challenging due to cross-reactivity among orthoflaviviruses.

The immune response, both innate and adaptive, plays a crucial role in controlling these viral infections. Neutralizing antibodies are essential for defense, as demonstrated in murine models. CD8+ T lymphocytes are key in controlling infection but may also contribute to central nervous system damage. CD4+ T lymphocytes stimulate specific antibody production, support CD8+ T cell function, and directly attack infected cells. The immune response to USUV is less well-studied and requires further investigation. Given the increasing prevalence and potential health impacts of these viruses, continued research into their pathogenesis, immune responses, and potential therapeutic interventions is warranted. This study aims to characterize the immune response induced by West Nile Virus (WNV), Usutu Virus (USUV), and other Orthoflavivirus infections. The goal is to obtain knowledge useful for developing new vaccines against these viruses. It is part of the YELLOW4FLAVI research project funded by the European Commission.

Who can participate?
Healthy male or female adults aged 18-60 years, tested positive for WNV or USUV infection through blood donor screening, without other acute infections or active chronic diseases, willing to provide informed consent, and available for at least two study visits.

What does the study involve?
This is an in vitro study on biological fluids (blood and urine samples) collected from blood

donors who tested positive for WNV or USUV during follow-up visits scheduled at 1, 7, 21, 40 days, and 6 and 12 months after the positive donation. No additional blood draws or visits are required beyond those already scheduled for the follow-up of donors who tested positive for West Nile virus. The study will analyze the innate and adaptive immune responses, including antibody levels, T-cell responses, viral replication dynamics, and host genetic factors.

What are the possible benefits and risks of participating?
Study participants will benefit from close monitoring for any symptoms that may arise due to WNV or USUV infection, and will receive appropriate support and advice. Additionally, study participants can receive free results of tests on antibody titers induced by the infection, the presence of the virus in blood and urine, and any previous infections by related viruses. By participating, study subjects will also contribute to improving the understanding of immune response mechanisms induced by WNV, USUV, and similar viruses, thus aiding research for vaccine development against these viruses.

A potential risk related to study participation involves blood draws, which will be performed by qualified personnel at the Transfusion Medicine Unit clinics. Although simple, this procedure may cause some discomfort, such as pain or swelling at the needle insertion site. However, no special protective measures are necessary beyond those normally adopted for blood sampling. It is important to note that medical and nursing staff will follow all standard procedures to ensure safety and comfort during this phase of the study. In any case, no additional blood draws will be performed beyond those already scheduled for monitoring subjects who tested positive for WNV NAT.

Where is the study run from?
UOC di Medicina Trasfusionale (DIMT) Padova - Azienda ULSS6 Euganea, Italy

When is the study starting and how long is it expected to run for?
January 2024 to December 2028

Who is funding the study?
HORIZON EUROPE Framework Programme, European Union.

Who is the main contact?
Prof Luisa Barzon, luisa.barzon@unipd.it

Contact information

Type(s)
Public, Scientific, Principal investigator

Contact name
Prof Luisa Barzon

ORCID ID
https://orcid.org/0000-0003-0720-8456

Contact details
Department of Molecular Medicine, University of Padova
Padova
Italy

35121
+390498218946
luisa.barzon@unipd.it

Additional identifiers

Protocol serial number
Protocol number 123964

Study information

Scientific Title
Virological characterization and analysis of the immune response in subjects with West Nile virus and Usutu virus infection

Acronym
Y4F-WNV-USUV

Study objectives
Characterize the immune response induced by WNV, USUV, and other Orthoflavivirus infections to obtain knowledge useful for the development of new vaccines.

Ethics approval required
Ethics approval required

Ethics approval(s)
Approved 10/07/2025, Comitato Etico Territoriale Area Centro - Est Veneto (USTS CET-ACEV) (Azienda Ospedale Università di Padova, via Giustiniani 2, Padova, 35128, Italy; +39 049 8212342; ce.sperimentazione@aopd.veneto.it), ref: 6350/U6/25

Primary study design
Observational

Study design
Single-centre longitudinal observational study

Study type(s)
Diagnostic

Health condition(s) or problem(s) studied
Innate and adaptive immune response in subjects with West Nile virus and Usutu virus infections

Interventions
This is an in vitro study on biological fluids to investigate the adaptive humoral and cell-mediated immune responses in blood donors with West Nile virus (WNV) and Usutu virus (USUV) infections. The study will encompass a minimum of 10 subjects per virus, with a maximum of 80 participants, followed up at 1, 7, 40 days, and 6 and 12 months post-infection.

The study involves healthy adult subjects presenting at the Transfusion Medicine Unit of ULSS6 Euganea Company, DIMT Padua, for blood donation and who have shown reactivity in the WNV

NAT screening test. Subjects who tested positive for WNV NAT in previous years and who consent to the use of their biological samples stored at the Reference Laboratory for Arboviruses of the Veneto Region, headed by Prof. Luisa Barzon, PI of this study, may also be enrolled.

Blood donors who have given their written informed consent for additional tests on blood samples collected or already collected for routine investigations will be enrolled. These tests will be aimed at characterizing the innate and adaptive humoral and cell-mediated immune responses, and possibly identifying host-associated factors predictive of infection progression. Each study subject will be associated with a pseudonymized code, and the results obtained from additional tests will be anonymized and therefore not traceable to the individual.

Intervention Type
Other

Primary outcome(s)
1. Quantification of neutralizing antibody titers by PRNT and MNT at 1, 7, 40 days, and 6 and 12 months post-infection
2. Detection of WNV- and USUV-specific antigen antibodies by EIA at 1, 7, 40 days, and 6 and 12 months post-infection
3. Assessment of cross-reactivity against other orthoflaviviruses by EIA and neutralization assays at 1, 40 days, and 12 months post-infection
4. Analysis of CD4+ and CD8+ T lymphocyte responses measured using flow cytometry at 1 day and 12 months post-infection

Key secondary outcome(s)
There are no secondary outcome measures

Completion date
31/12/2028

Eligibility

Key inclusion criteria
1. Male or female individuals aged between 18 and 60 years
2. WNV/USUV infection detected through WNV NAT testing as part of blood donor screening
3. Individuals without other acute infections or active chronic diseases, apart from WNV or USUV infection
4. Willingness to provide written informed consent after being adequately informed about the risks and benefits of the study
5. Availability to participate in at least two scheduled study visits

Participant type(s)
Healthy volunteer, Patient

Healthy volunteers allowed
No

Age group
Adult

Lower age limit
18 Years

Upper age limit
60 Years

Sex
All

Total final enrolment
0

Key exclusion criteria
1. Any individual who, in the investigator's opinion, does not comply with the study objectives and protocol
2. Anticipated absence from the study center during the study period, for example, due to a planned vacation

Date of first enrolment
15/07/2025

Date of final enrolment
31/12/2028

Locations

Countries of recruitment
Italy

Study participating centre
UOC di Medicina Trasfusionale (DIMT) Padova - Azienda ULSS6 Euganea
Via P. Cosma, 1
Camposampiero PD
Italy
35012

Sponsor information

Organisation
University of Padua

ROR
https://ror.org/00240q980

Funder(s)

Funder type
Government

Funder Name
HORIZON EUROPE Framework Programme

Alternative Name(s)
Horizon Europe, Horizon Europe Programme, Framework Programme, Horizon Europe, EU Framework Programme, Horizon, Horizonte Europa

Funding Body Type
Government organisation

Funding Body Subtype
National government

Location

Results and Publications

Individual participant data (IPD) sharing plan
The datasets generated during and/or analysed during the current study will be available upon request from Prof. Luisa Barzon, luisa.barzon@unipd.it. Anonymized individual participant data that underlie the results reported in publications (e.g., text, tables, figures, and appendices) will be available beginning 6 months and ending 24 months following article publication, as well as the study protocol. Consent from participants about data sharing was required and obtained.

IPD sharing plan summary
Available on request