ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA
CAMPUS DI FORLÌ

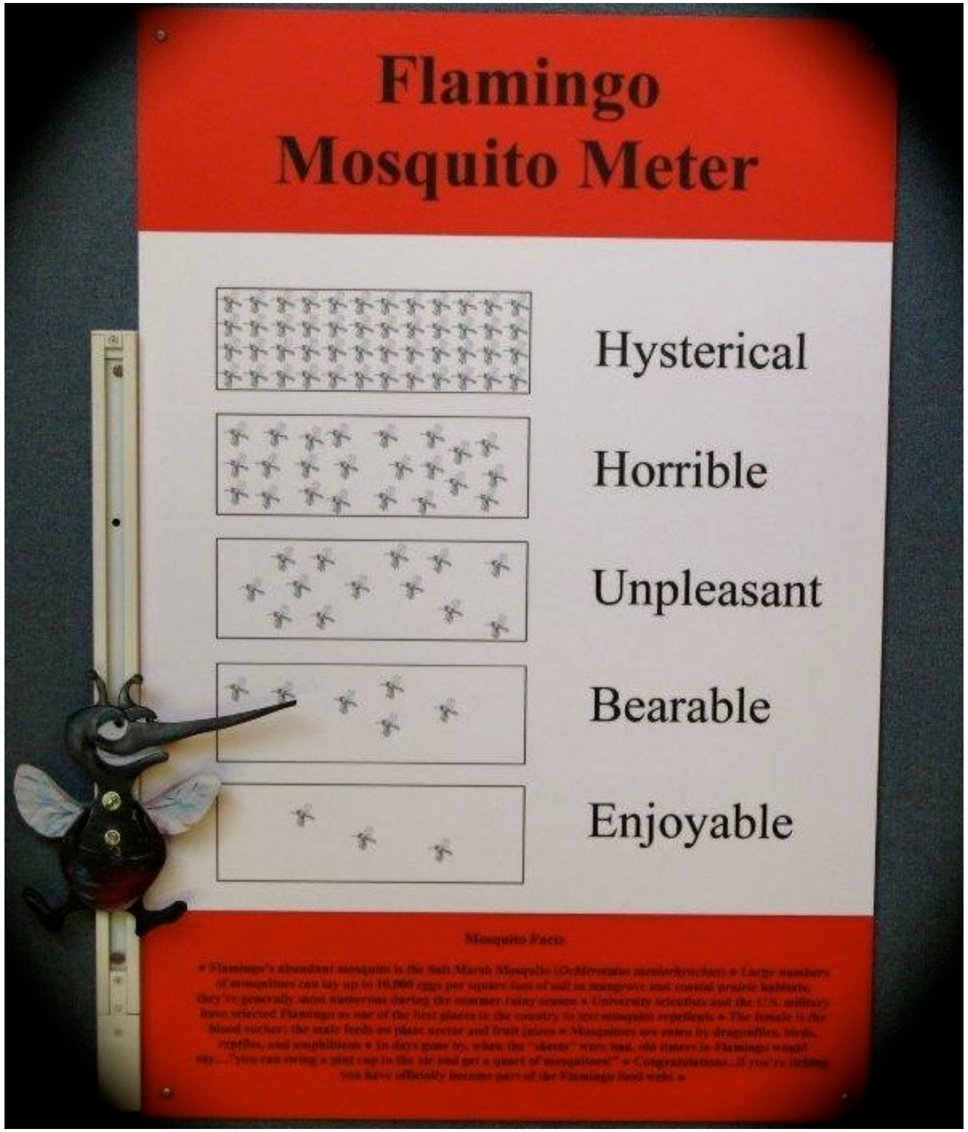
West Nile, Dengue ed altri virus trasmessi da artropodi

Vittorio Sambri

DIMEC – UOC Microbiologia
DiMeLabeT - AUSL Romagna – vittorio.sambri@unibo.it

Agenda

- Virus & arthropods
- West Nile virus (WNV)
- Dengue virus (DENV)
- Toscana virus (TOSV)



Flamingo Nat’l Park, FL.
2010

What Are Arboviruses

- ARBOvirus = ARthropod-BOrne virus
- Viruses transmitted by blood-feeding arthropods (mosquitoes, ticks, sandflies…)
- They can infect humans, arthopods and animals (mammalian & birds)

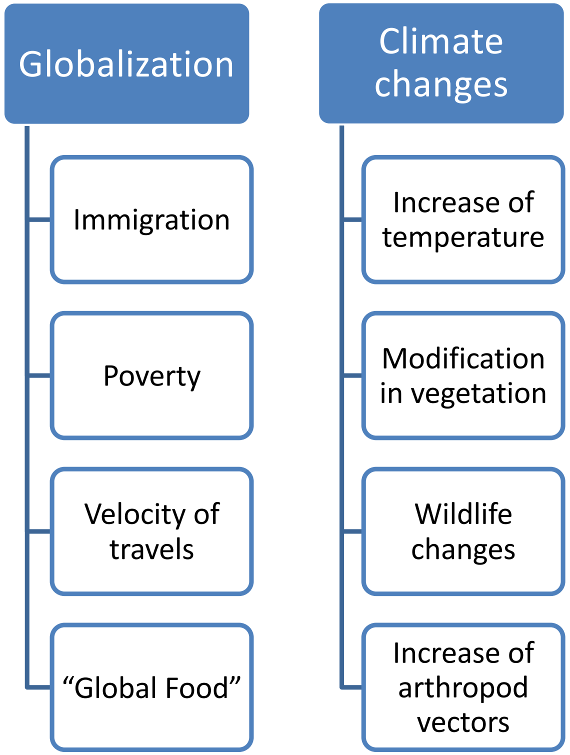
Transmission Cycle

- Arthropod vector becomes infected by feeding on an infected host
- The virus replicates within the vector
- Vector transmits the virus to a new host via bite
- Important: there is often a reservoir host (birds, Non human primates)

Main Arbovirus Families

- Flaviviridae (Dengue, Zika, Japanese Encephalitis, Tick Borne Encephalitis,Yellow Fever, West Nile)
- Togaviridae (Chikungunya)
- Bunyaviridae (Toscana virus, La Crosse)
- Reoviridae (Bluetongue virus in animals)

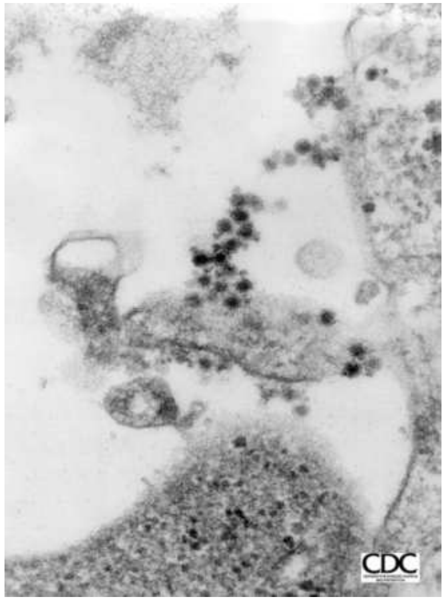
What is causing ArBo infections?



Globalization
- Immigration
- Poverty
- Velocity of travels
- "Global Food"

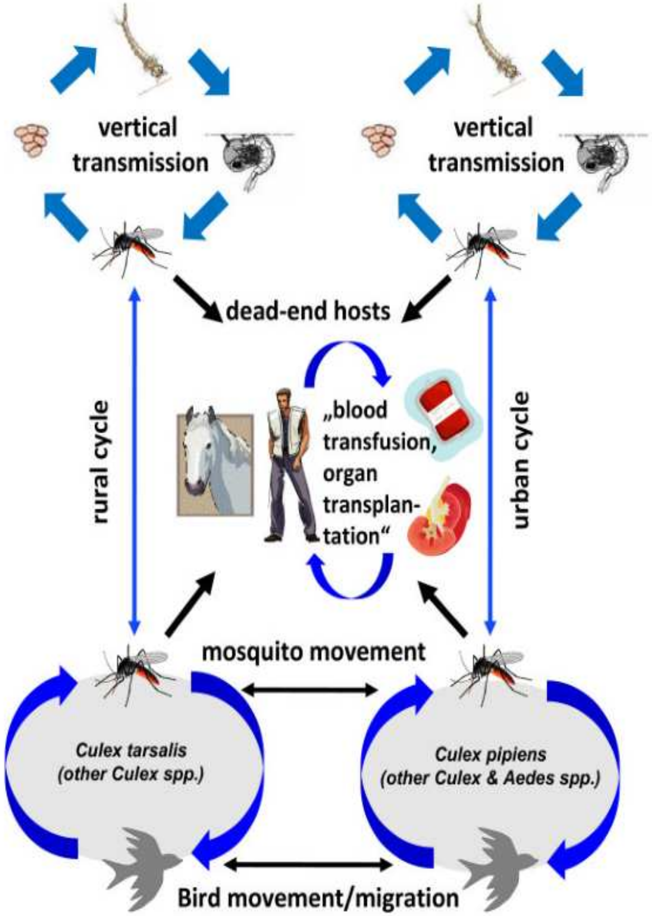
Climate changes
- Increase of temperature
- Modification in vegetation
- Wildlife changes
- Increase of arthropod vectors

West Nile Virus (WNV)



- *Flaviviridae* (*Flavivirus*)
- ss (+) RNA
- Virion (spherical shape), 40-50 nm)
- *envelope* (proteins **E** and **prM)**
- Capsid (protein **C**) 20-30 nm)

West Nile Cycle



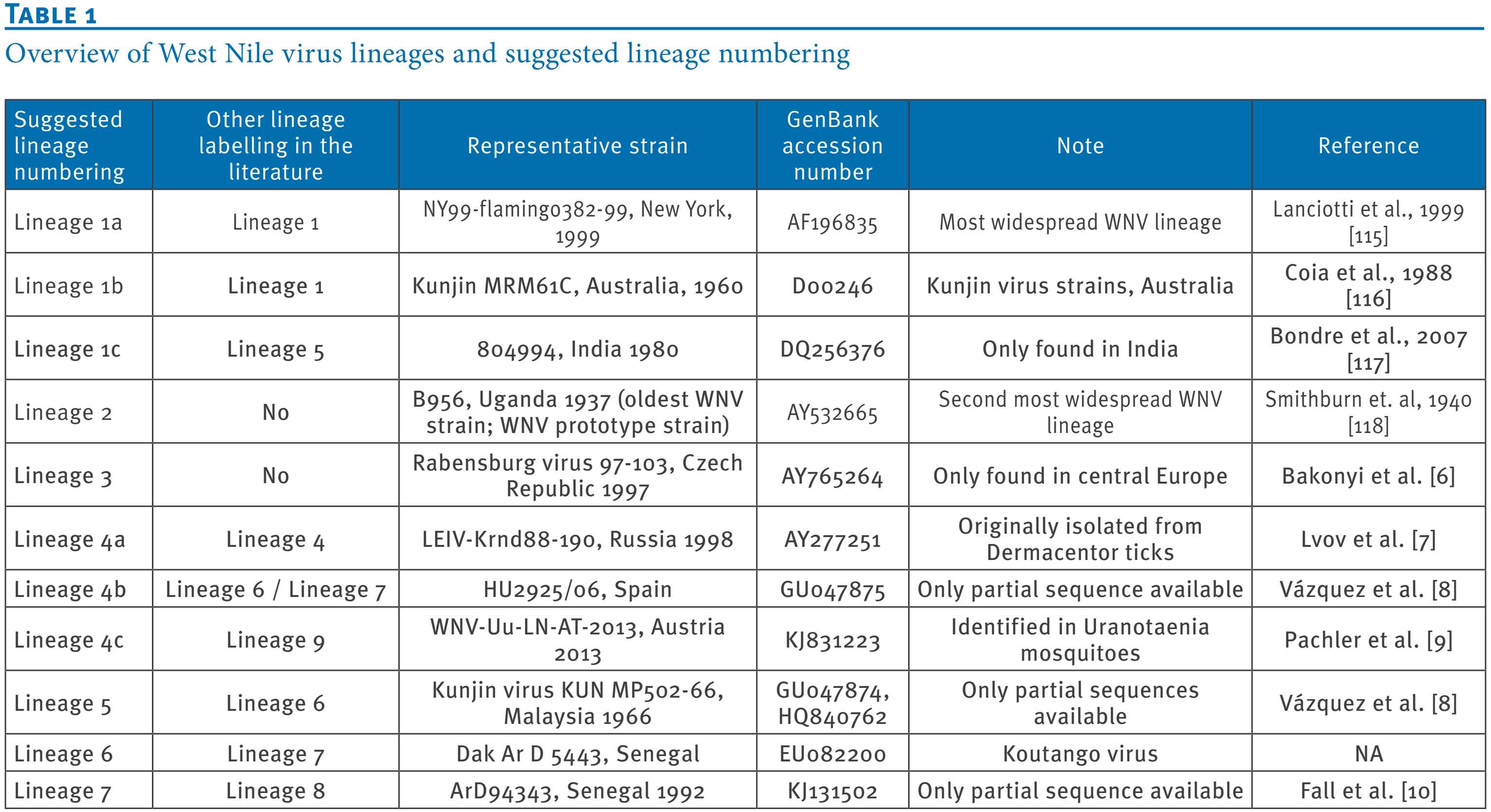


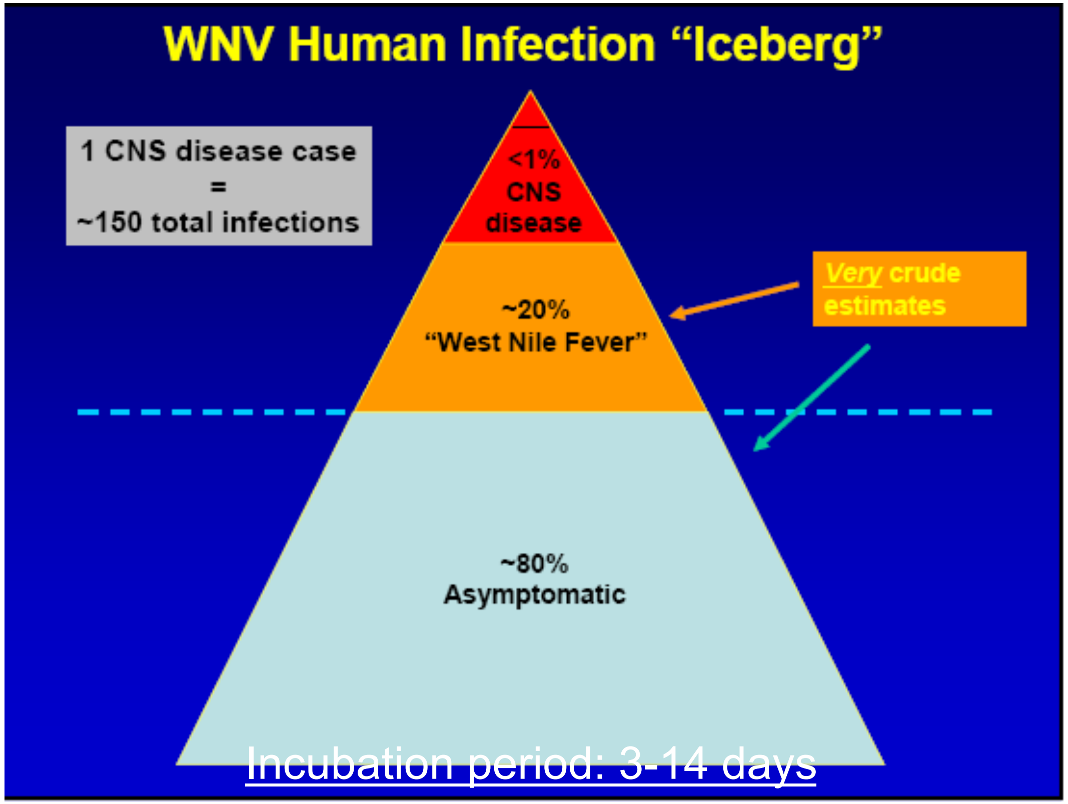
TABLE 1

Overview of West Nile virus lineages and suggested lineage numbering

Suggested lineage numbering	Other lineage labelling in the literature	Representative strain	GenBank accession number	Note	Reference
Lineage 1a	Lineage 1	NY99-flamingo382-99, New York, 1999	AF196835	Most widespread WNV lineage	Lanciotti et al., 1999 [115]
Lineage 1b	Lineage 1	Kunjin MRM61C, Australia, 1960	D00246	Kunjin virus strains, Australia	Coia et al., 1988 [116]
Lineage 1c	Lineage 5	804994, India 1980	DQ256376	Only found in India	Bondre et al., 2007 [117]
Lineage 2	No	B956, Uganda 1937 (oldest WNV strain; WNV prototype strain)	AY532665	Second most widespread WNV lineage	Smithburn et. al, 1940 [118]
Lineage 3	No	Rabensburg virus 97-103, Czech Republic 1997	AY765264	Only found in central Europe	Bakonyi et al. [6]
Lineage 4a	Lineage 4	LEIV-Krnd88-190, Russia 1998	AY277251	Originally isolated from Dermacentor ticks	Lvov et al. [7]
Lineage 4b	Lineage 6 / Lineage 7	HU2925/06, Spain	GU047875	Only partial sequence available	Vázquez et al. [8]
Lineage 4c	Lineage 9	WNV-Uu-LN-AT-2013, Austria 2013	KJ831223	Identified in Uranotaenia mosquitoes	Pachler et al. [9]
Lineage 5	Lineage 6	Kunjin virus KUN MP502-66, Malaysia 1966	GU047874, HQ840762	Only partial sequences available	Vázquez et al. [8]
Lineage 6	Lineage 7	Dak Ar D 5443, Senegal	EU082200	Koutango virus	NA
Lineage 7	Lineage 8	ArD94343, Senegal 1992	KJ131502	Only partial sequence available	Fall et al. [10]

Rizzoli A, Jiménez-Clavero MA, Barzon L, Cordioli P, Figuerola J, Koraka P, Martina B, Moreno A, Nowotny N, Pardigon N, Sanders N, Ulbert S, Tenorio A. The challenge of West Nile virus in Europe: knowledge gaps and research priorities. Euro Surveill. 2015;20(20):pii=21135. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=21135

Clinical Epidemiology of WNV infection in humans



WNV Human Infection “Iceberg”

1 CNS disease case
=
~150 total infections

<1%
CNS
disease

~20%
“West Nile Fever”

~80%
Asymptomatic

Very crude estimates

Incubation period: 3-14 days

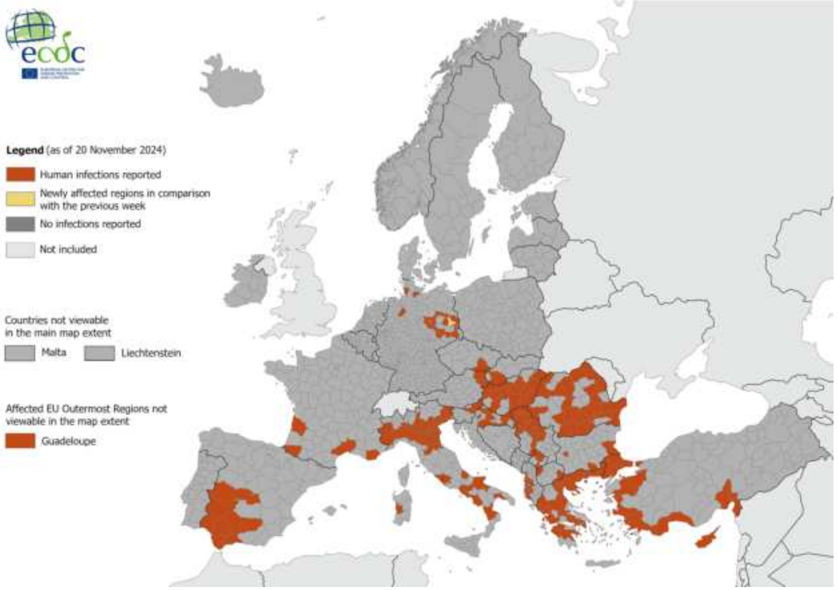
Encephalitis (meningoencephalitis)

fever, weakness, headache, myalgia, nausea, vomiting
(3 – 6 gg.)

Diagnosis of West Nile Virus Human Infections: Overview and Proposal of Diagnostic Protocols Considering the Results of External Quality Assessment Studies

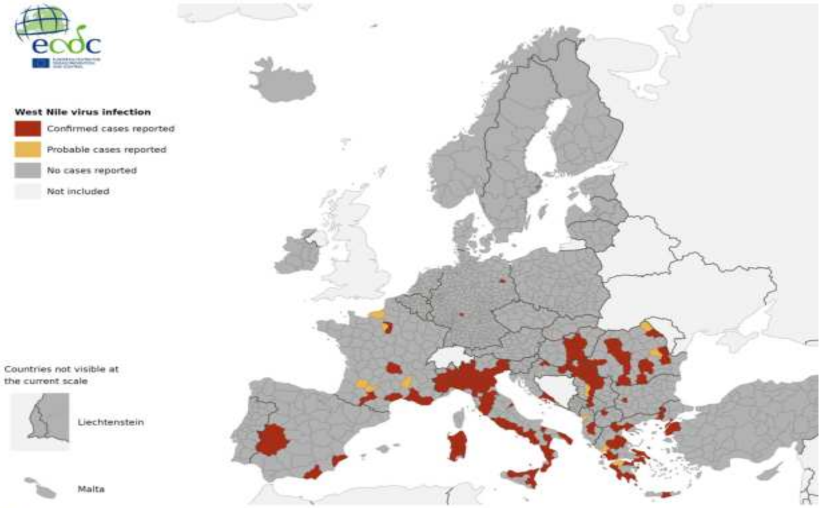
V. Sambri et al. *Viruses* **2013**, *5*, 2329-2348;

Clinical Issue (biological sample)	**Suggested laboratory tools**
Diagnosis of patients with suspected WNV infection: neuroinvasive disease and WNV fever (serum and/or plasma, whole blood, CSF, urine).	It is advisable to prioritize the order of the tests as follows: MAC-EIA, Indirect IgG EIA, IF, virus isolation, RT-PCR, Real time RT-PCR, $PRNT_{90}$
Evaluation of seroprevalence (serum and/or plasma)	$PRNT_{50}$; Indirect IgG EIA, IF; Epitope blocking EIA
Screening of blood and organ donations (serum or plasma)	NATs [MAC-EIA and IF (IgG and IgM) are suggested to perform the serologic follow up of the NATs positive donors and as early additional testing in case of not confirmed NAT positivity]
Post-mortem evaluation (tissue biopsy)	Immunohistochemistry



Geographical distribution

Figure 1. Distribution of locally acquired human West Nile virus infections in 2025 as of 29 October 2025 .



een

https://www.ecdc.europa.eu/en/west-nile-fever/surveillance-and-disease-data/disease-data-ecdc

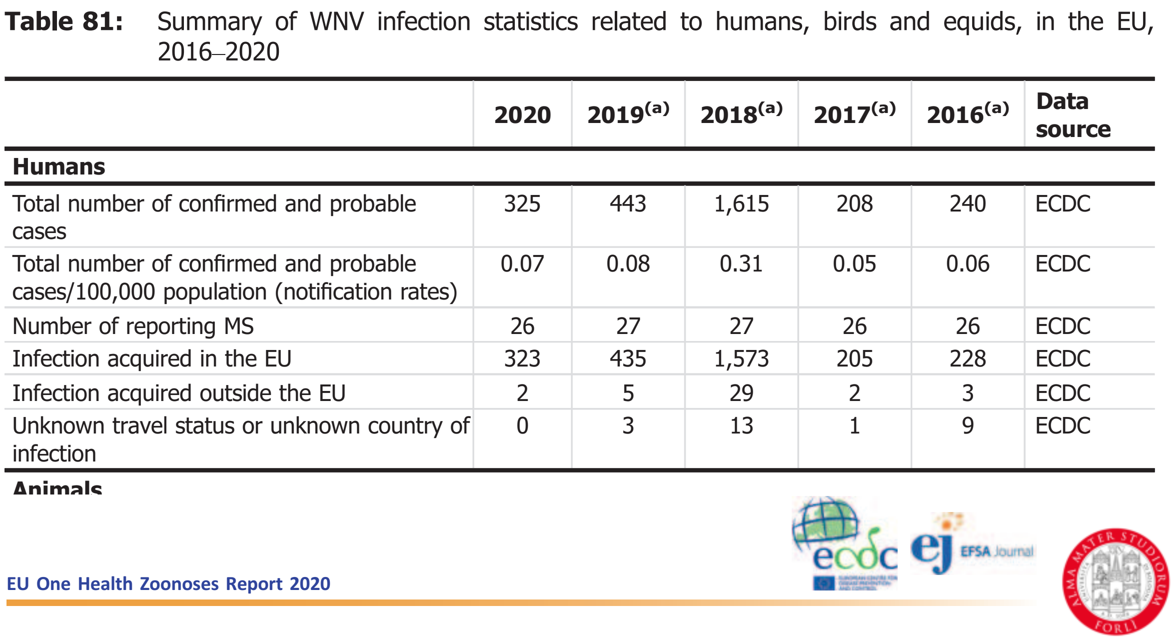
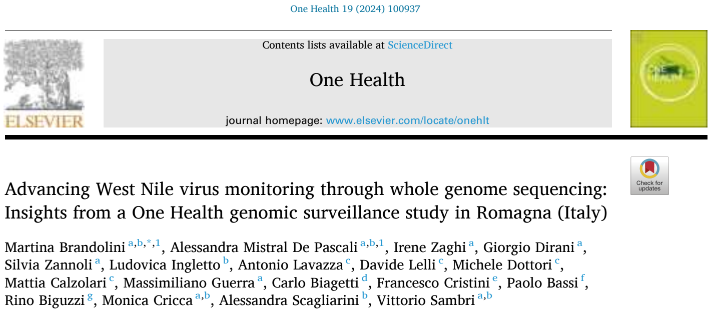


Table 81: Summary of WNV infection statistics related to humans, birds and equids, in the EU, 2016–2020

	2020	2019[(a)]	2018[(a)]	2017[(a)]	2016[(a)]	Data source
Humans						
Total number of confirmed and probable cases	325	443	1,615	208	240	ECDC
Total number of confirmed and probable cases/100,000 population (notification rates)	0.07	0.08	0.31	0.05	0.06	ECDC
Number of reporting MS	26	27	27	26	26	ECDC
Infection acquired in the EU	323	435	1,573	205	228	ECDC
Infection acquired outside the EU	2	5	29	2	3	ECDC
Unknown travel status or unknown country of infection	0	3	13	1	9	ECDC
Animals						

EU One Health Zoonoses Report 2020


One Health 19 (2024) 100937

Contents lists available at ScienceDirect

One Health

journal homepage: www.elsevier.com/locate/onehlt

Advancing West Nile virus monitoring through whole genome sequencing: Insights from a One Health genomic surveillance study in Romagna (Italy)

Martina Brandolini[a,b,*,1], Alessandra Mistral De Pascali[a,b,1], Irene Zaghi[a], Giorgio Dirani[a], Silvia Zannoli[a], Ludovica Ingletto[b], Antonio Lavazza[c], Davide Lelli[c], Michele Dottori[c], Mattia Calzolari[c], Massimiliano Guerra[a], Carlo Biagetti[d], Francesco Cristini[e], Paolo Bassi[f], Rino Biguzzi[g], Monica Cricca[a,b], Alessandra Scagliarini[b], Vittorio Sambri[a,b]

Finanziato dall'Unione europea
NextGenerationEU

Ministero dell'Università e della Ricerca

Italiadomani

INF-ACT

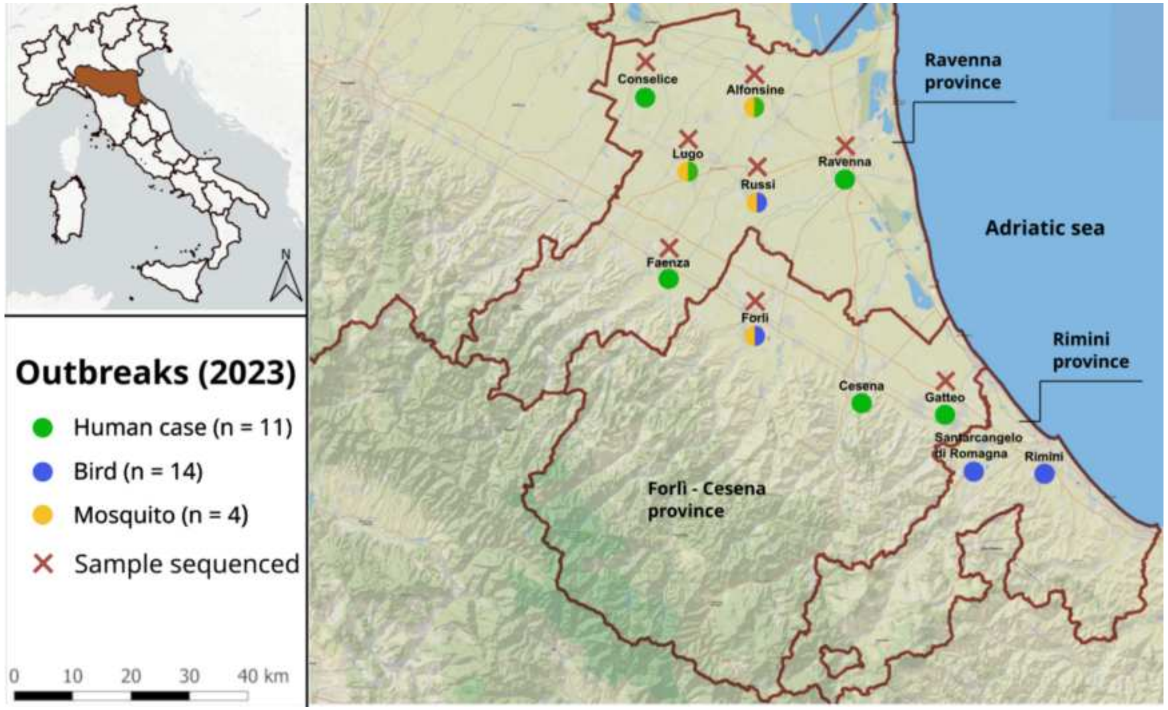
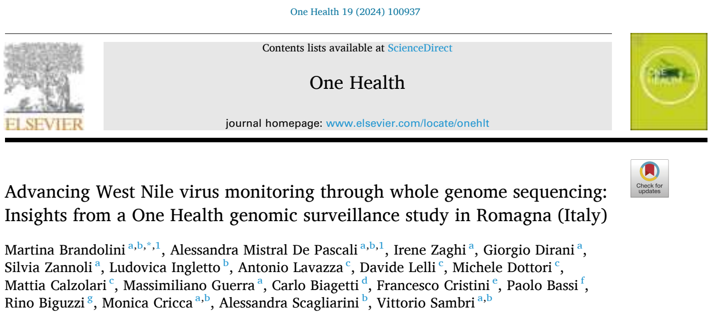


Fig. 1. Map of geo-localization sites of recorded WNV infections. In 2023, a total of 29 cases of WNV were detected in the Romagna region. These cases comprised 11 human cases (green dots on the map), 14 bird cases (blue dots), and 4 sites with WNV-positive mosquitoes (yellow dots). The primary hotspot for WNV was Ravenna province, where 9 human cases, 3 bird cases, and 3 mosquito cases were reported. Additionally, WNV circulation was observed in Forlì-Cesena province (2 human cases, 7 bird cases, 1 site with WNV-positive mosquitoes) and Rimini province (4 bird cases). Samples sequenced in this study are marked on the map with a red cross. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)



One Health 19 (2024) 100937

Contents lists available at ScienceDirect

One Health

journal homepage: www.elsevier.com/locate/onehlt

Advancing West Nile virus monitoring through whole genome sequencing: Insights from a One Health genomic surveillance study in Romagna (Italy)

Martina Brandolini[a,b,*,1], Alessandra Mistral De Pascali[a,b,1], Irene Zaghi[a], Giorgio Dirani[a], Silvia Zannoli[a], Ludovica Ingletto[b], Antonio Lavazza[c], Davide Lelli[c], Michele Dottori[c], Mattia Calzolari[c], Massimiliano Guerra[a], Carlo Biagetti[d], Francesco Cristini[e], Paolo Bassi[f], Rino Biguzzi[g], Monica Cricca[a,b], Alessandra Scagliarini[b], Vittorio Sambri[a,b]

Table 1

Whole genome sequencing results. Results of WNV-positive human and animal samples and cell culture isolates sequencing by tagmentation and hybrid-capture target enrichment using Illumina RNA Prep with Enrichment Tagmentation protocol with Viral Surveillance Panel oligos.

Sample ID	Municipality of origin (province)	Collection date	Sample matrix	RT-PCR Ct value	WNV lineage	Total number of reads	Number of WNV reads	% of WNV mapped reads	Coverage %	Mean depth	Sequence length
LU_300723	Lugo (RA)	30 Jul 2023	urine	26	2	11.117.638	90.207	0.8	99.6	420	10.920
LU_130823	Conselice (RA)	31 Aug 2023	CSF	36	2	1.840.980	166.470	9.0	98.8	77	10.841
			Vero E6 isolate	8.53	2	19.827.442	19.214.209	96.9	99.9	32.869	10.956
RA_180823	Ravenna (RA)	18 Aug 2023	urine	25.04	2	15.278.922	12.343.939	80.8	100	13.906	10.963
FA_010923	Faenza (RA)	1 Sep 2023	urine	31.41	2	1.987.836	21.385	1.1	99.8	126	10.936
FA_240823	Faenza (RA)	24 Aug 2023	Vero E6 isolate	9.08	2	11.651.273	10.937.191	93.9	100	24.971	10.995
CE_040923	Gatteo (FC)	4 Sep 2023	Vero E6 isolate	6.79	2	8.117.842	7.545.500	92.9	100	19.404	10.959
RA_040923	Russi (RA)	4 Sep 2023	urine	30.45	2	5.807.664	342.927	5.9	99	91	10.849
			Vero E6 isolate	7.07	2	12.176.029	11.601.017	95.3	100	25.823	10.957
Cx. pipiens_241,729	Forlì (FC)	3 Aug 2023	homogenate	24.58	2	11.666.237	4.938.263	42.3	99.9	5.129	10.955
Cx. pipiens_246,218	Russi (RA)	8 Aug 2023	homogenate	24.27	2	6.144.291	637.647	10.4	99.8	459	10.937
Cx. pipiens_261,861	Alfonsine (RA)	24 Aug 2023	homogenate	28.41	2	10.921.464	285.682	1.1	99.6	348	10.914
P.pica_248,896–6	Russi (RA)	10 Aug 2023	homogenate	29.35	2	7.385.939	80.153	2.6	99	136	10.853

CSF = cerebrospinal fluid.

One Health 19 (2024) 100937

One Health 19 (2024) 100937

Contents lists available at ScienceDirect

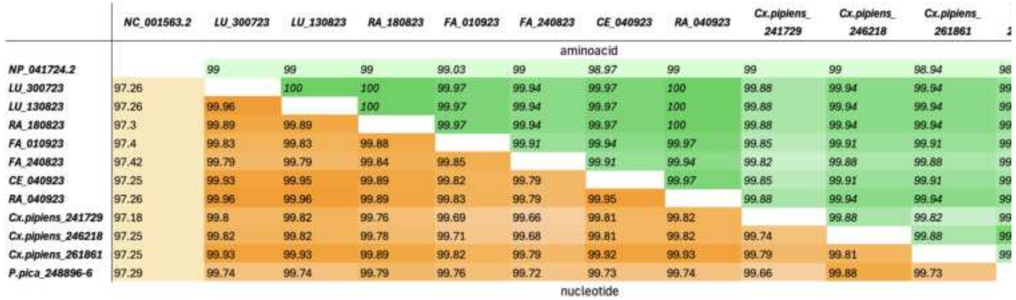
One Health

journal homepage: www.elsevier.com/locate/onehlt

Advancing West Nile virus monitoring through whole genome sequencing: Insights from a One Health genomic surveillance study in Romagna (Italy)

Martina Brandolini[a,b,*,1], Alessandra Mistral De Pascali[a,b,1], Irene Zaghi[a], Giorgio Dirani[a], Silvia Zannoli[a], Ludovica Ingletto[b], Antonio Lavazza[c], Davide Lelli[c], Michele Dottori[c], Mattia Calzolari[c], Massimiliano Guerra[a], Carlo Biagetti[d], Francesco Cristini[e], Paolo Bassi[f], Rino Biguzzi[g], Monica Cricca[a,b], Alessandra Scagliarini[b], Vittorio Sambri[a,b]

Table 2
Percentage nucleotide and amino acid identity. Identities were determined among sequenced samples and against WNV lineage 2 reference sequence (n
reference sequence accession no. **NC_001563.2**, amino acid reference sequence accession no. **NP_041724.2**). Percentage nucleotide identity is reported i
type and highlighted in orange on the left side; amino acid identity is reported in italics and highlighted in green.



	NC_001563.2	LU_300723	LU_130823	RA_180823	FA_010923	FA_240823	CE_040923	RA_040923	Cx.pipiens_ 241729	Cx.pipiens_ 246218	Cx.pipiens_ 261861	2
							aminoacid					
NP_041724.2		99	99	99	99.03	99	98.97	99	99	99	98.94	98
LU_300723	97.26		*100*	*100*	*99.97*	*99.94*	*99.97*	*100*	*99.88*	*99.94*	*99.94*	99
LU_130823	97.26	99.96		*100*	*99.97*	*99.94*	*99.97*	*100*	*99.88*	*99.94*	*99.94*	99
RA_180823	97.3	99.89	99.89		*99.97*	*99.94*	*99.97*	*100*	*99.88*	*99.94*	*99.94*	99
FA_010923	97.4	99.83	99.83	99.88		*99.91*	*99.94*	*99.97*	*99.85*	*99.91*	*99.91*	99
FA_240823	97.42	99.79	99.79	99.84	99.85		*99.91*	*99.94*	*99.82*	*99.88*	*99.88*	99
CE_040923	97.25	99.93	99.95	99.89	99.82	99.79		*99.97*	*99.85*	*99.91*	*99.91*	99
RA_040923	97.26	99.96	99.96	99.89	99.83	99.79	99.95		*99.88*	*99.94*	*99.94*	99
Cx.pipiens_241729	97.18	99.8	99.82	99.76	99.69	99.66	99.81	99.82		*99.88*	*99.82*	99
Cx.pipiens_246218	97.25	99.82	99.82	99.78	99.71	99.68	99.81	99.82	99.74		*99.88*	99
Cx.pipiens_261861	97.25	99.93	99.93	99.89	99.82	99.79	99.92	99.93	99.79	99.81		99
P.pica_248896-6	97.29	99.74	99.74	99.79	99.76	99.72	99.73	99.74	99.66	99.88	99.73	
							nucleotide					

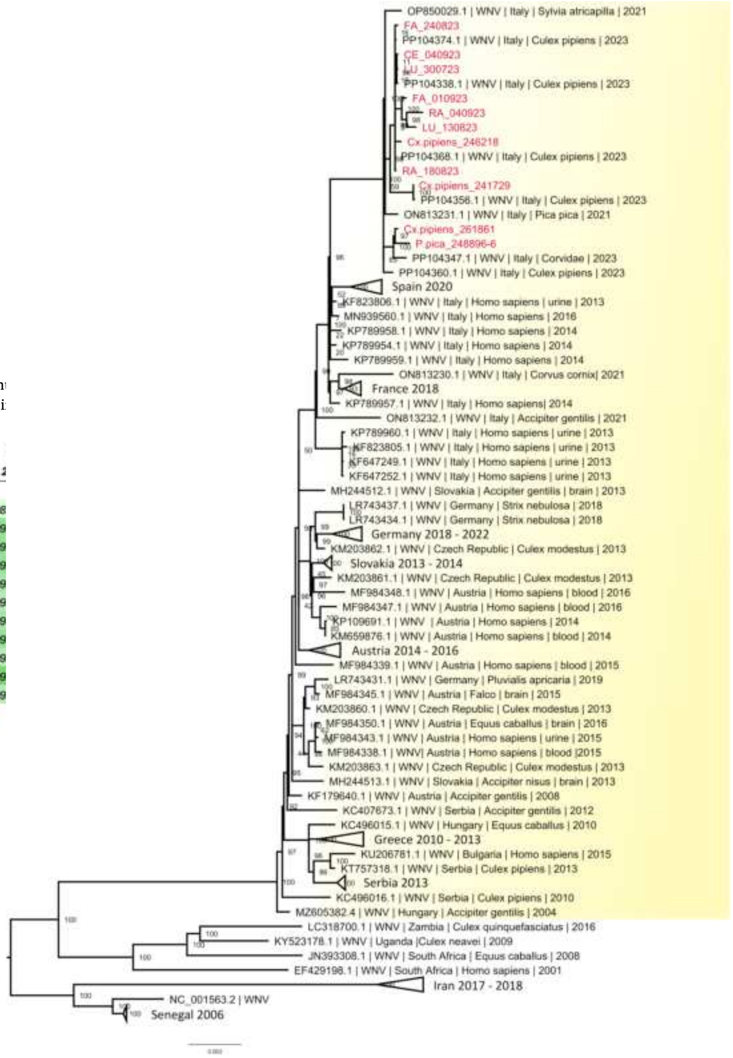
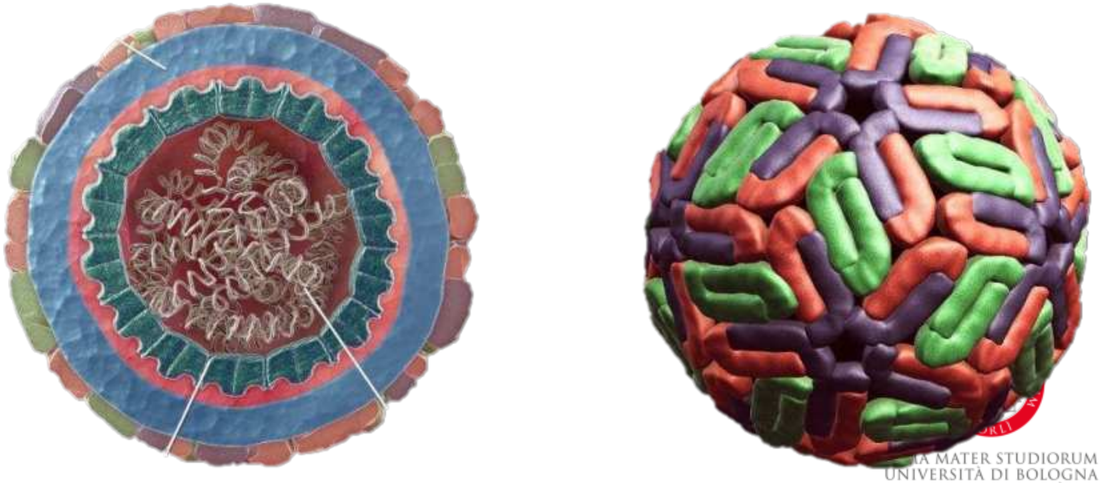


Fig. 2. Phylogenetic analysis of (nearly) complete genome nucleotide sequences of WNV lineage 2. The maximum-likelihood tree was inferred using IQ-TREE v.2.0.3, under automated model selection (GTR + F + G4) and 1000 bootstrap replicates. The viruses sequenced in this study are marked in red ($n = 11$ samples). GenBank accession numbers are indicated for each strain, with country, host, and year of isolation. Background shading distinguishes the Central-Southern European clade. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Causative agent of Dengue

- RNA Virus
- Dengue virus is a member of the viral family of Flaviviridae composed of a single-strand RNA (11000 bp)
- It is an arbovirus transmitted by mosquitoes



Dengue virus

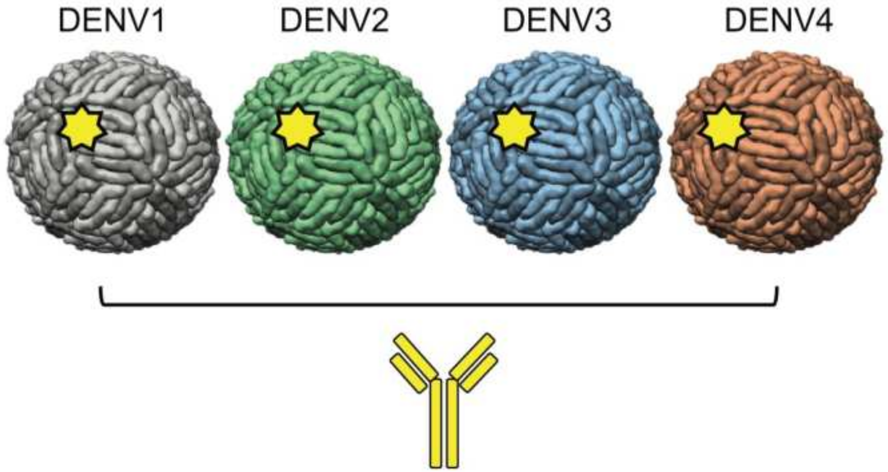
- 4 serotypes (DEN-1,2,3,4)

Each serotype provides specific lifetime immunity, and short-term cross-immunity

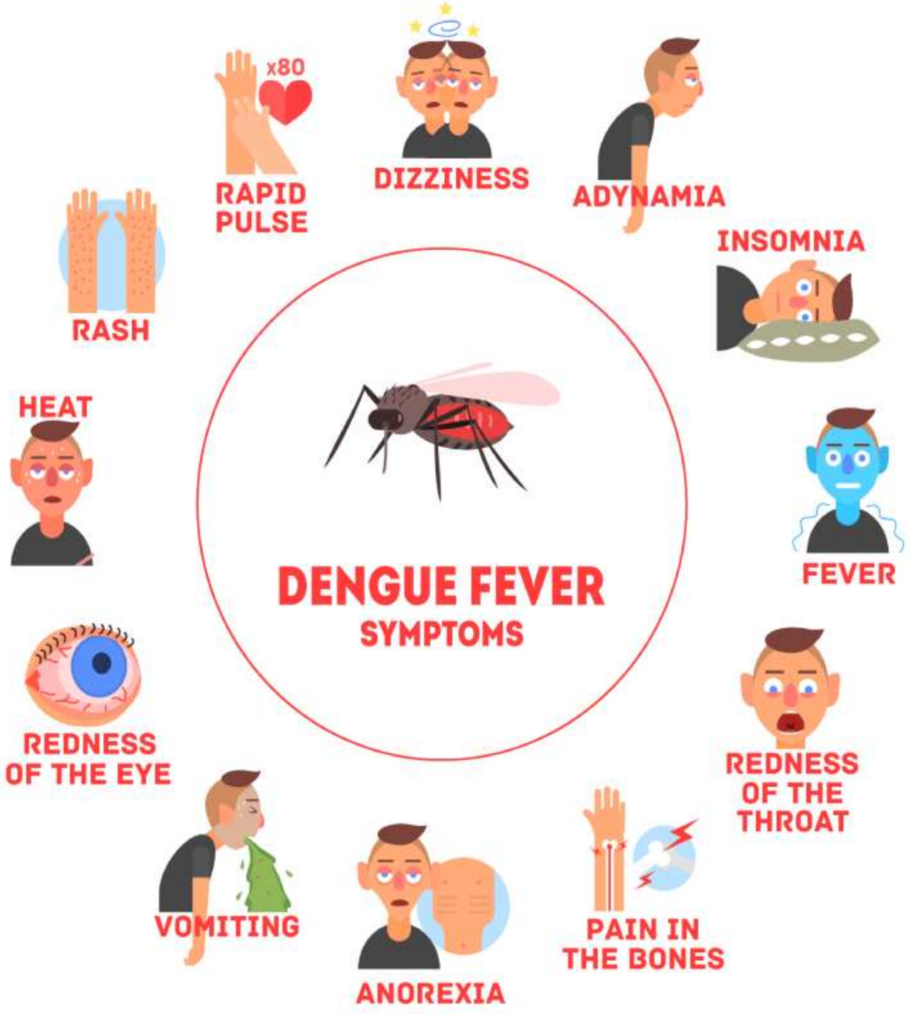
- All serotypes are cause of severe and fatal disease

- Genetic variation within serotypes

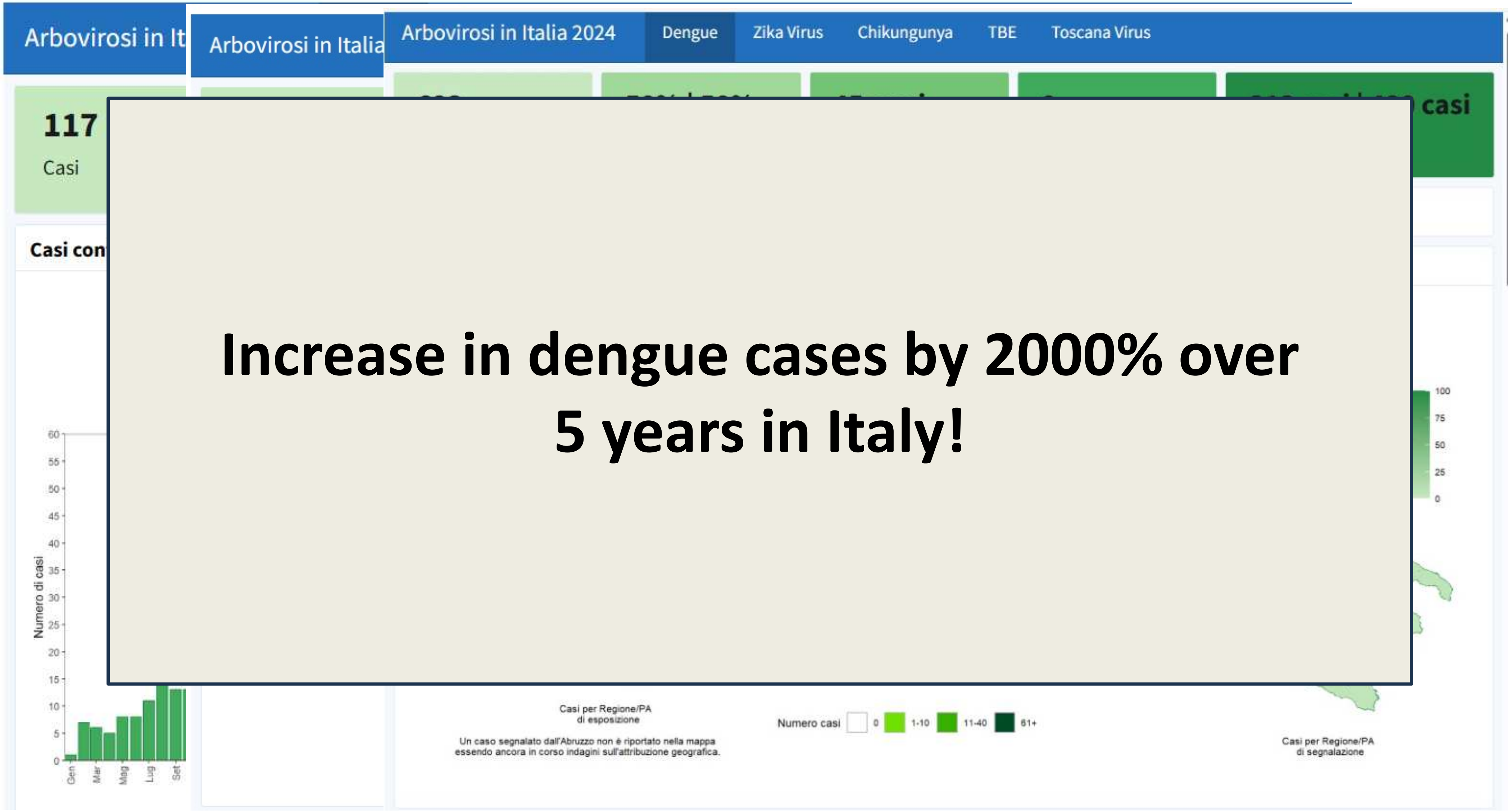
- Some genetic variant within each serotype appear to be more virulent or have greater epidemic potential



Clinical manifestations



Cases of Dengue in Italy 2022-2024



Casi per Regione/PA
di esposizione

Un caso segnalato dall'Abruzzo non è riportato nella mappa essendo ancora in corso indagini sull'attribuzione geografica.

Casi per Regione/PA
di segnalazione

Increase in dengue cases by 2000% over 5 years in Italy!

Epidemiological data of Dengue in Emilia Romagna

	2020	2021	2022	2023	2024
Imported	6	3	20	43	62
Autochtonous	0	0	0	0	**36** ⬆


Regione Emilia-Romagna

Bollettino Ufficiale della Regione Emilia-Romagna

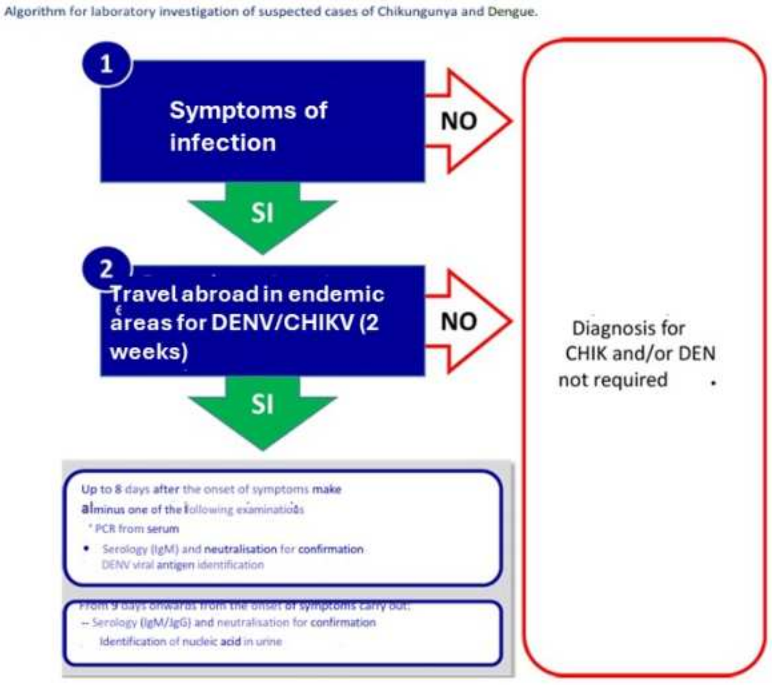
(BURERT)

/ Area Bollettini / n.94 del 28.03.2024 (Parte Seconda)

Approvazione del Piano regionale di sorveglianza e controllo delle arbovirosi - anno 2024

Algorithms for laboratory investigation of suspected cases of Chikungunya, Dengue, Zika, TBE

(Annex 15 - National Arbovirosis Plan 2020 - 2025)


Algorithm for laboratory investigation of suspected cases of Chikungunya and Dengue.

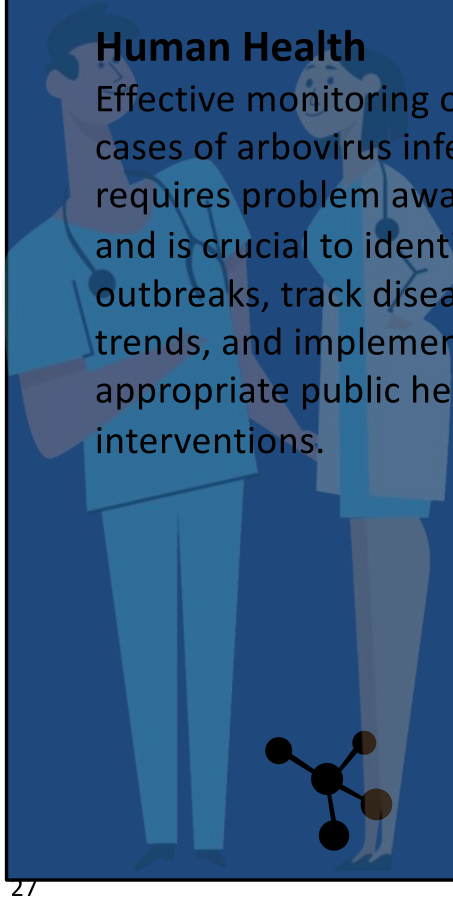
Up to 8 days after the onset of symptoms make alminus one of the following examinations

* PCR from serum
- Serology (IgM) and neutralisation for confirmation
  DENV viral antigen identification

From 9 days onwards from the onset of symptoms carry out:

- Serology (IgM/IgG) and neutralisation for confirmation
  Identification of nucleic acid in urine

Proposed methodology

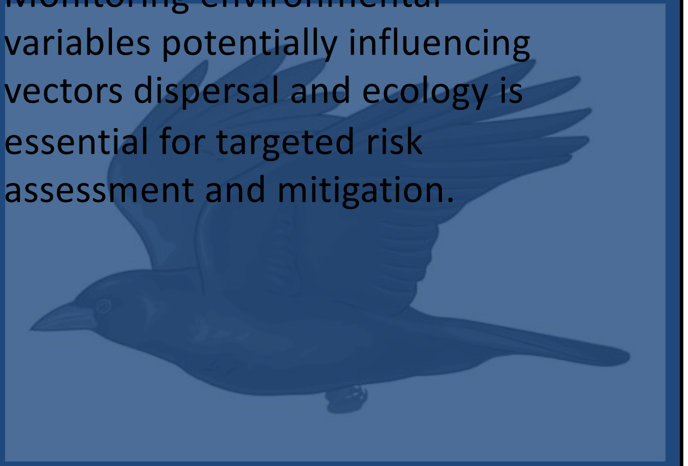


Human Health

Effective monitoring of human cases of arbovirus infections requires problem awareness and is crucial to identify outbreaks, track disease trends, and implement appropriate public health interventions.

Environmental Health

Monitoring environmental variables potentially influencing vectors dispersal and ecology is essential for targeted risk assessment and mitigation.



Animal Health

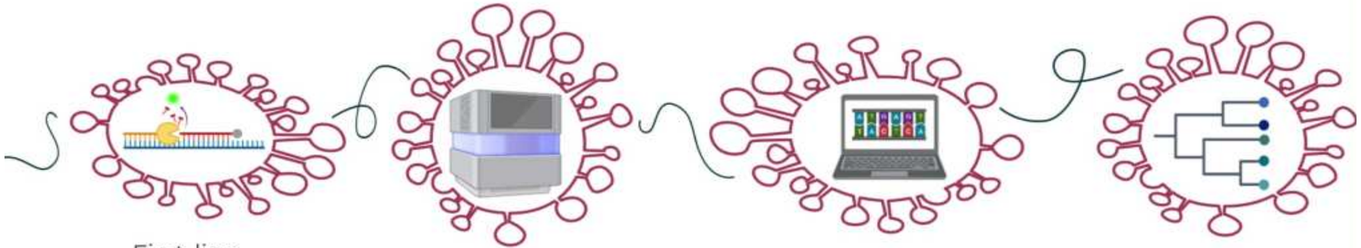
Surveillance animal populations for arbovirus infections is critical to understand the role of animals in transmission, identify potential sources of infection for humans and to track virus dispersal.

Laboratory activity of Microbiology Unit
of the Greater Romagna Area Hub Laboratory 2/2

Finanziato dall'Unione europea NextGenerationEU
Ministero dell'Università e della Ricerca
Italiadomani

One-Health Genomic Surveillance Plan Proposal for Arboviruses Tracking and Control

	Human surveillance	Reservoir/hosts and vectors surveillance
Who?	Physicians Infectious Diseases wards (AUSL Romagna Hospitals)	Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna
How?	Romagna Ethical Committee approval (**C.ARBO.SEQ**)	Regional enthomological and veterinary surveillance plan
What?	Patients enrollment Inclusion criteria: Encephalitis/meningitis cases of suspected Arboviral origin Collection of biological samples: urine, blood, CSF	Collection of: - Mosquitoes pools - Samples from birds target species (active and passive surveillance)



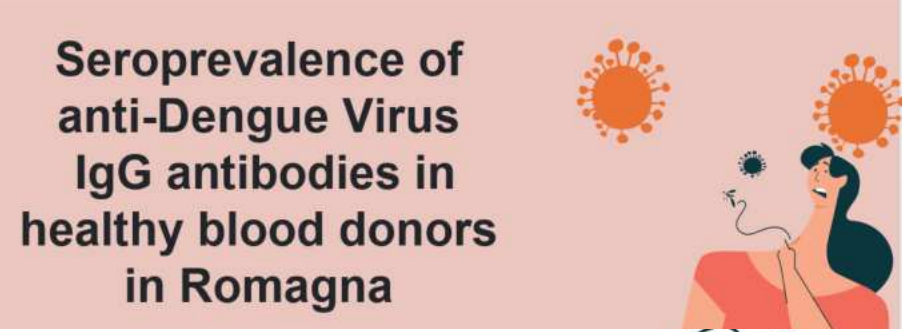
First-line molecular testing by target-specific RT-PCR

Illumina whole genome NGS by hybrid-capture-mediated target enrichment (Viral Surveillance Panel)

Data analysis. Assemblies and consensus sequences were obtained with Bowtie2 v.2.4.1 and BCFtools

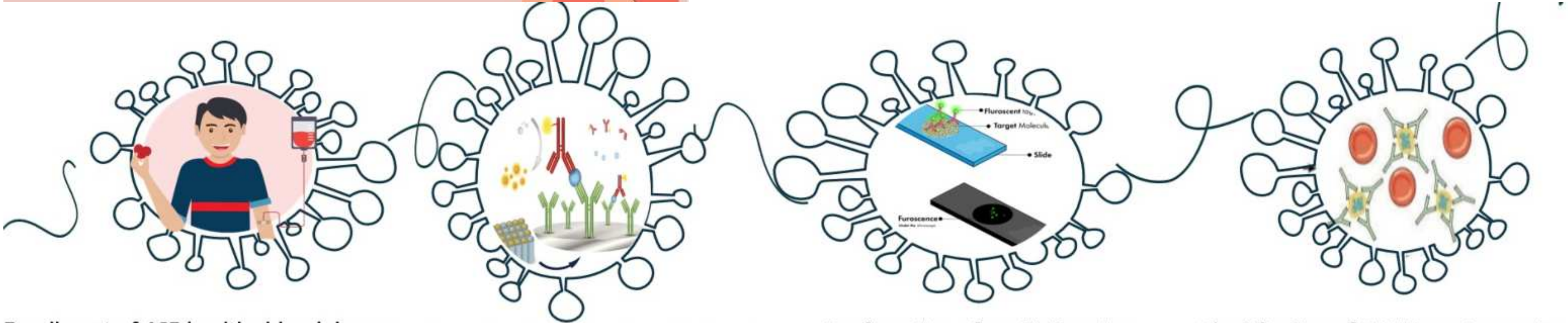
Genomic characterization and phylogeny reconstruction

Laboratory activity of Microbiology Unit of the Greater Romagna Area Hub Laboratory 1/2



Seroprevalence of anti-Dengue Virus IgG antibodies in healthy blood donors in Romagna

1 among 465 individuals enrolled (0,02%):
DENV POSITIVE (recent travel to Costa Rica)



Enrollment of 465 healthy blood donors from Romagna

Centro Regionale Sangue Emilia Romagna,
(Dr. Rino Buguzzi)

Screening for detection of IgG anti-Dengue virus using ELISA

Unità Operativa Microbiologia AUSL della Romagna

(Prof. Vittorio Sambri)

Confirmation of positivity using Immunofluorescence

Unità Operativa Microbiologia AUSL della Romagna

(Prof. Vittorio Sambri)

Identification of DENV serotype using Sero Neutralization Assay

Unità Operativa Microbiologia AUSL della Romagna

(Prof. Vittorio Sambri)

Results 1/3: Enrollment

17 April 2024

10 November 2024

How?

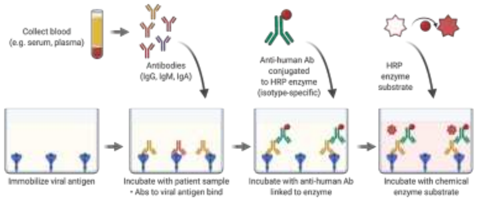
Centro Regionale Sangue Emilia Romagna, Romagna area

Who?

465 healthy blood donors

222 female (48%)
228 male (49%)
Unknown (3%)
Age range: 18-65
Mean age: 38

Results 2/4: Screening using ELISA commercial kit



SERION ELISA *classic*
Dengue Virus IgG/IgM

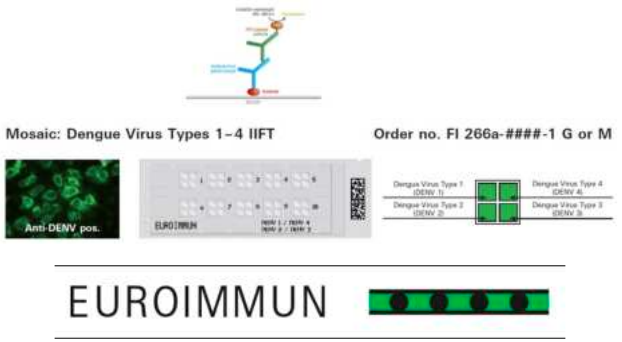
serion\diagnostics
Your Partner in Infectious Serology

12/465 (2,6%) IgG POSITIVE

7 Female (58%)
5 Male (42%)

Province	Sex	Age	Coutry of birth	Recent travels	Symptoms	ELISA IgG titre AU/ml
FC	M	39	ITALY	NO	NO	57.9
RA	F	36	ITALY	NO	NO	15.3
RA	M	54	SPAIN	NO	NO	32.8
RI	F	25	BRAZIL	YES (?)	NO	23.1
RA	M	55	ITALY	YES (COSTA RICA)	NO	16.8
RI	M	31	ITALY	NO	NO	20.4
RA	F	31	ITALY	NO	NO	17
FC	F	39	ITALY	NO	NO	16.9
FC	F	37	ITALY	NO	NO	20.5
RA	M	24	ITALY	NO	NO	21.3
FC	F	25	ITALY	NO	NO	29.5
FC	F	21	ITALY	NO	NO	16.1

Results 3/4: Confirmation using Immunofluorescence

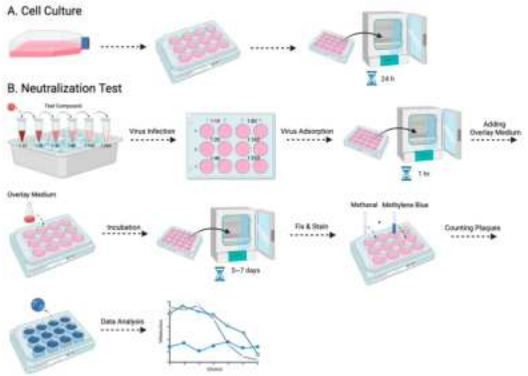


1/12 among 465 individuals enrolled
WNV POSITIVE

1/12 among 465 individuals enrolled (0,2%): DENV POSITIVE

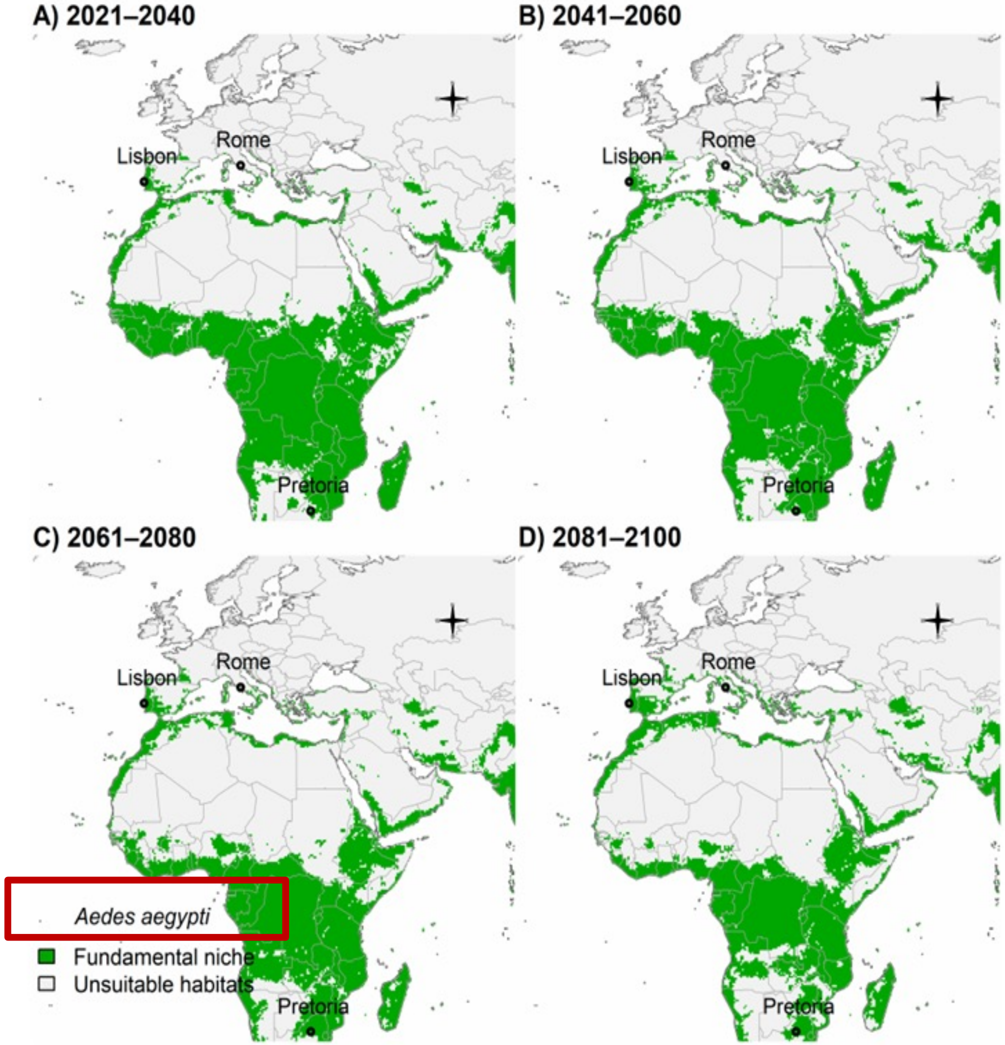
Province	Sex	Age	Coutry of birth	Recent travels	Symptoms	ELISA IgG titre AU/ml	IFA ARBOVIRUS MOSAIC
FC	M	39	ITALY	NO	NO	57.9	WNV
RA	F	36	ITALY	NO	NO	15.3	NEG
RA	M	54	SPAIN	NO	NO	32.8	NEG
RI	F	25	BRAZIL	YES (?)	NO	23.1	NEG
RA	**M**	**55**	**ITALY**	**YES (COSTA RICA)**	**NO**	**16.8**	**DENV 2 OR 3**
RI	M	31	ITALY	NO	NO	20.4	NEG
RA	F	31	ITALY	NO	NO	17	NEG
FC	F	39	ITALY	NO	NO	16.9	NEG
FC	F	37	ITALY	NO	NO	20.5	NEG
RA	M	24	ITALY	NO	NO	21.3	NEG
FC	F	25	ITALY	NO	NO	29.5	NEG
FC	F	21	ITALY	NO	NO	16.1	NEG

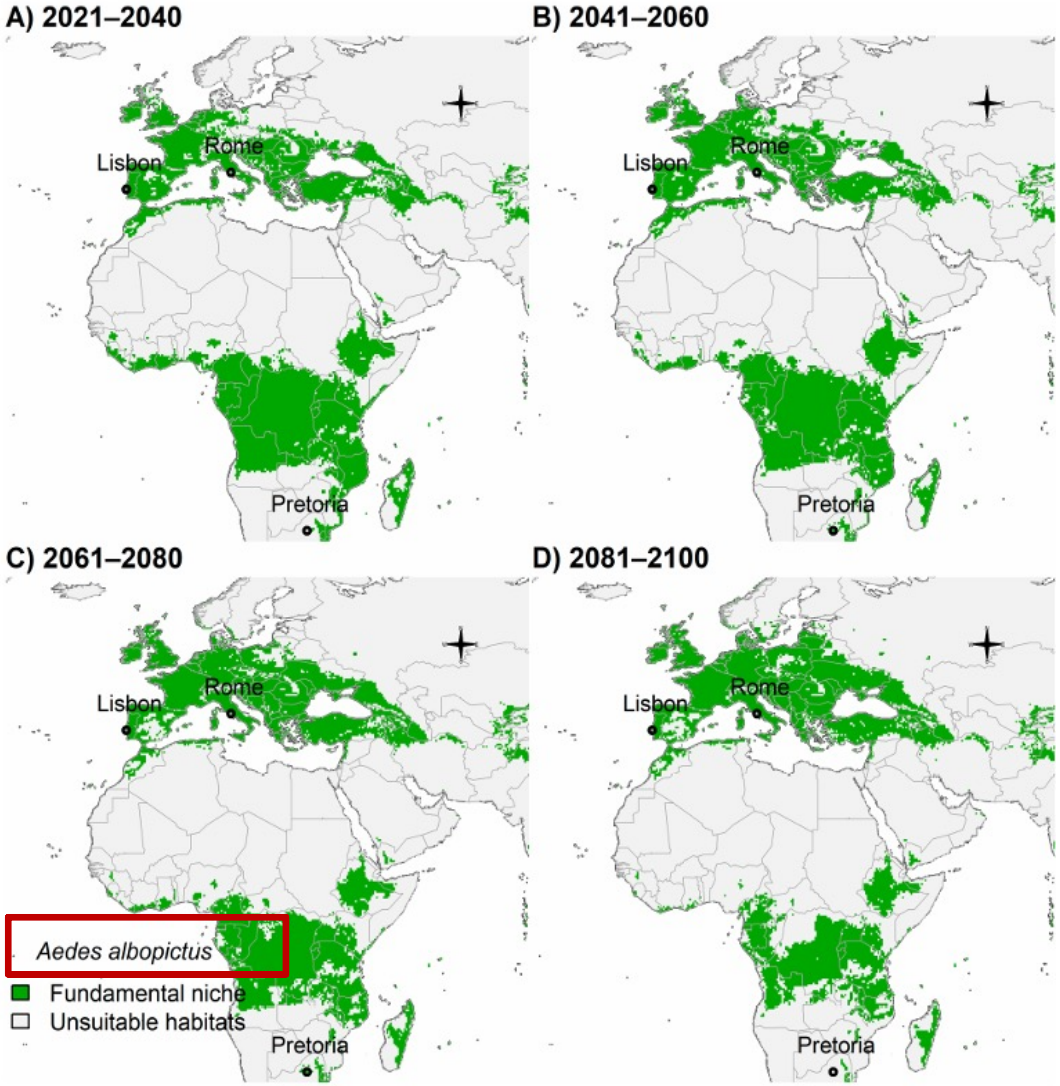
Results 4/4: Confirmation using seroneutralization assay

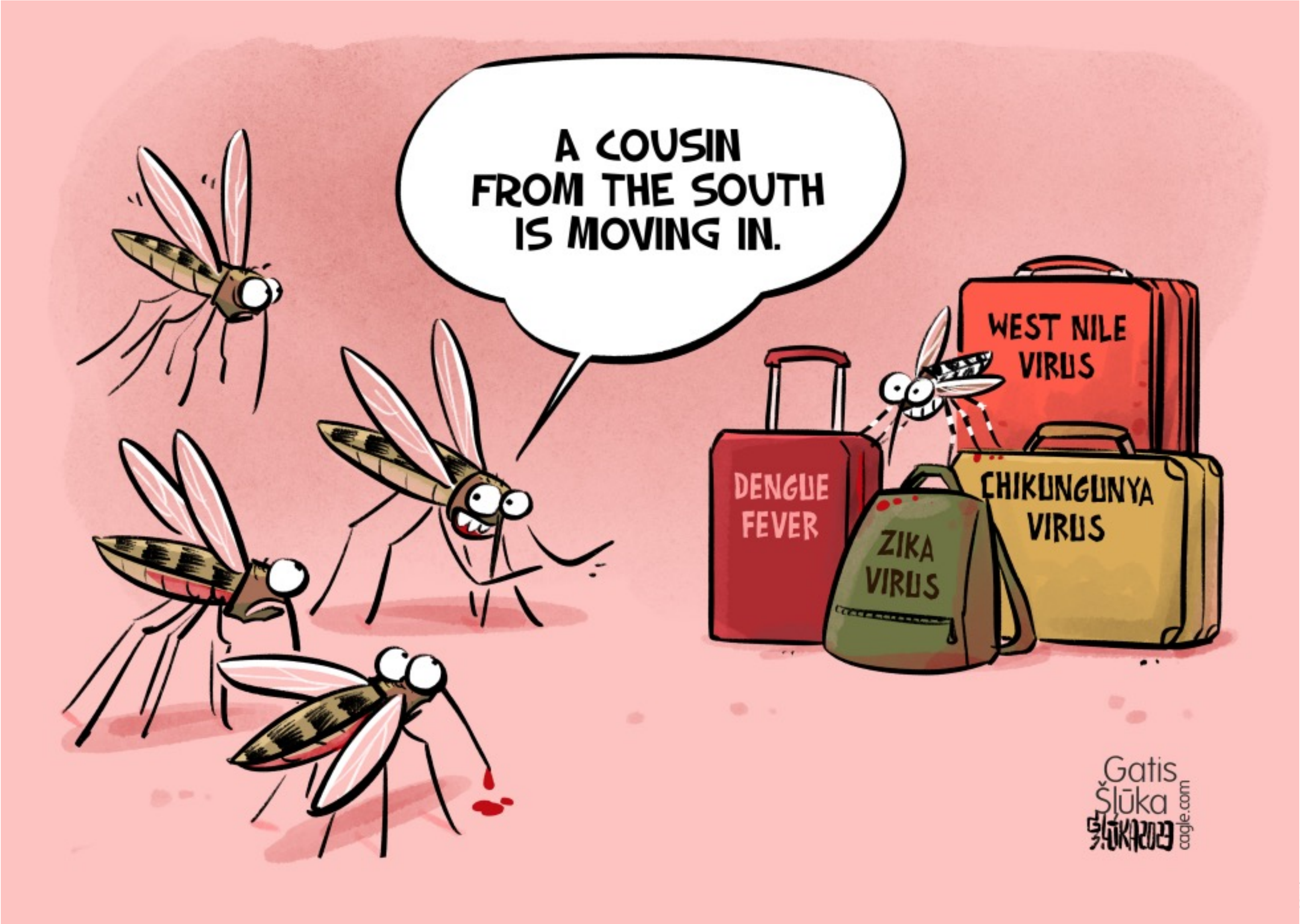


Province	Sex	Age	Coutry of birth	Recent travels	Symptoms	ELISA IgG titre AU/ml	IFA ARBOVIRUS MOSAIC	Sero neutralization
FC	M	39	ITALY	NO	NO	57.9	WNV	WNV
RA	F	36	ITALY	NO	NO	15.3	NEG	NEG
RA	M	54	SPAIN	NO	NO	32.8	NEG	NEG
RI	F	25	BRAZIL	YES (?)	NO	23.1	NEG	NEG
RA	**M**	**55**	**ITALY**	**YES (COSTA RICA)**	**NO**	**16.8**	**DENV 2 OR 3**	**DENV 2 OR 3**
RI	M	31	ITALY	NO	NO	20.4	NEG	NEG
RA	F	31	ITALY	NO	NO	17	NEG	NEG
FC	F	39	ITALY	NO	NO	16.9	NEG	NEG
FC	F	37	ITALY	NO	NO	20.5	NEG	NEG
RA	M	24	ITALY	NO	NO	21.3	NEG	NEG
FC	F	25	ITALY	NO	NO	29.5	NEG	NEG
FC	F	21	ITALY	NO	NO	16.1	NEG	NEG

1/12 among 465 individuals enrolled (0,02%):
DENV POSITIVE


A) 2021–2040
B) 2041–2060
C) 2061–2080
D) 2081–2100
Lisbon
Rome
Pretoria
Aedes aegypti
🟩 Fundamental niche
☐ Unsuitable habitats


A) 2021–2040
B) 2041–2060
C) 2061–2080
D) 2081–2100
Lisbon
Rome
Pretoria
Aedes albopictus
🟩 Fundamental niche
☐ Unsuitable habitats


A COUSIN FROM THE SOUTH IS MOVING IN.
WEST NILE VIRUS
DENGUE FEVER
ZIKA VIRUS
CHIKUNGUNYA VIRUS
Gatis Šļūka
cagle.com
ŠĻŪKA2023

General Overview TOSV

- TOSV = Toscana Virus
- Family: *Bunyaviridae*
- Genus: *Phlebovirus*
- Enveloped, segmented (3: L, M and S) negative-sense RNA virus
- First identified in Italy in the 1970s by Paola Verani at ISS

Prevention and Distribution

- No vaccine available
- Prevention based on vector control and personal protection (repellents, bed nets)
- Mainly found in:
- - Italy, Spain, France, Cyprus, Greece
- Reported in European travelers returning from endemic areas

Clinical Features

- Most infections are asymptomatic or mild
- When symptomatic:
- - Fever
- - Viral meningitis
- - Meningoencephalitis (mainly in young adults)
- Usually self-limiting, but severe cases can occur

Vectors and Transmission

- Transmitted by sandflies (*Phlebotomus* spp.), especially *P. perniciosus* and *P. perfiliewi*
- Zoonotic cycle not well understood; humans are accidental hosts
- Active during summer months
- No human-to-human transmission documented

communications biology
A Nature Portfolio journal

Article

https://doi.org/10.1038/s42003-025-08159-9

Development of a novel amplicon based whole-genome sequencing framework for improved surveillance of Toscana virus

Check for updates

Martina Brandolini [1,2], Ludovica Ingletto [2], Giorgio Dirani [1], Silvia Zannoli [1], Andreea Maria Lapusneanu [1], Massimiliano Guerra [1], Alessandra Mistral De Pascali [1,2], Davide Lelli [3], Francesco Defilippo [3], Antonio Lavazza [3], Mattia Calzolari [3], Michele Dottori [3], Laura Dionisi [2], Claudia Colosimo [2], Giulia Gatti [1,2], Anna Marzucco [1], Maria Sofia Montanari [1,2], Laura Grumiro [1,2], Monica Cricca [1,2], Alessandra Scagliarini [2] & Vittorio Sambri [1,2]

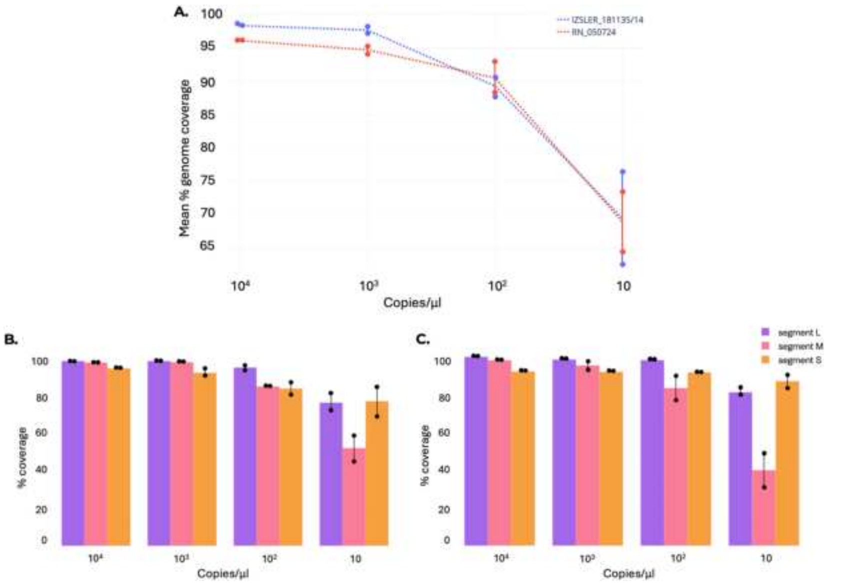


Fig. 2 | Graphical representation of mean % coverage and segment % coverage across dilutions. A Comparison of mean percentage genome coverage at varying RNA input concentrations (copies/μL) for two TOSV isolates, IZSLER_181135/14 (blue) and RN_050724 (red), with error bars representing standard deviation. Percentage genome coverage of TOSV segments (L, M, S) across varying RNA input concentrations (copies/μL) for IZSLER_181135/14 (**B**) and RN_050724 (**C**), highlighting segment-specific performance with error bars indicating standard deviation. Images are representative of two independent experiments. Error bars represent standard deviations.

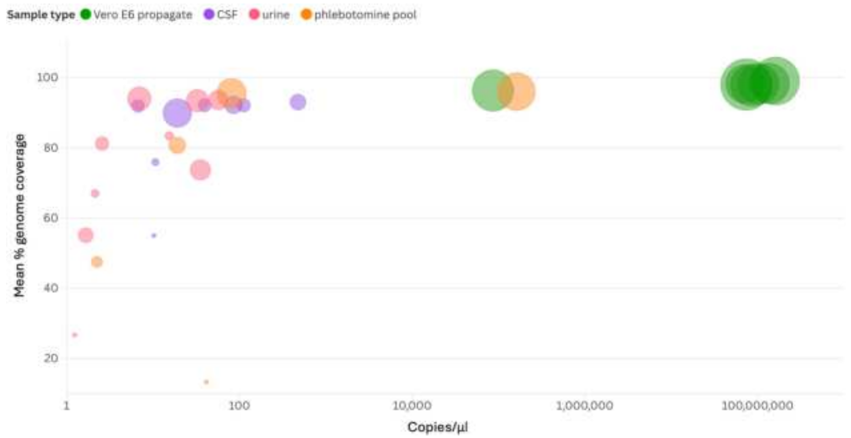


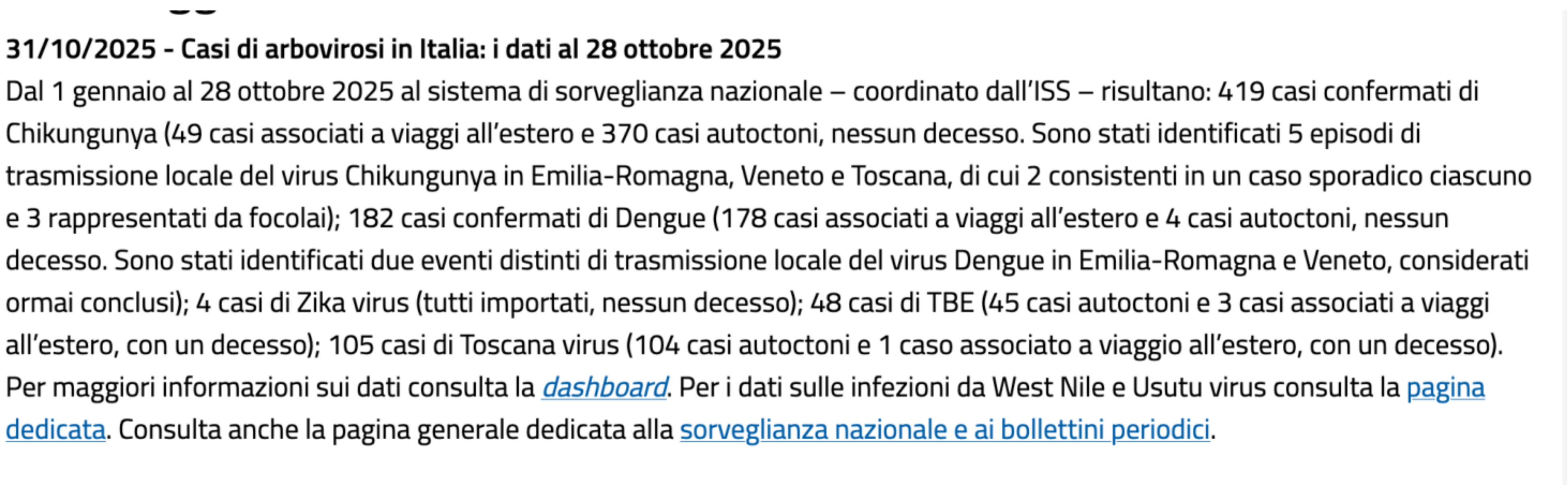
Fig. 3 | Correlation between viral genome copies/μL and mean percentage genome coverage across different sample types (Vero E6 propagates, CSF, urine, and phlebotomine pools). The size of the circles represents sequencing depth, highlighting the relationship between viral load (indicated by copies/μL), sequencing performance (mean genome coverage), and consensus sequence reliability (depth). Higher viral loads are associated with higher coverage and sequencing depth, with Vero E6 samples consistently achieving near-complete coverage. Variability is observed in clinical (CSF and urine) and vector (phlebotomine pool) samples, reflecting differences in matrix complexity and RNA quality, leading to different sequencing outputs. Vero E6 propagates are represented in green, CSF samples are represented in purple, urine samples are represented in pink, and phlebotomine are represented in orange.

Finanziato dall'Unione europea
NextGenerationEU

Ministero dell'Università e della Ricerca

Italiadomani

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA
CAMPUS DI FORLÌ



31/10/2025 - Casi di arbovirosi in Italia: i dati al 28 ottobre 2025

Dal 1 gennaio al 28 ottobre 2025 al sistema di sorveglianza nazionale – coordinato dall’ISS – risultano: 419 casi confermati di Chikungunya (49 casi associati a viaggi all’estero e 370 casi autoctoni, nessun decesso. Sono stati identificati 5 episodi di trasmissione locale del virus Chikungunya in Emilia-Romagna, Veneto e Toscana, di cui 2 consistenti in un caso sporadico ciascuno e 3 rappresentati da focolai); 182 casi confermati di Dengue (178 casi associati a viaggi all’estero e 4 casi autoctoni, nessun decesso. Sono stati identificati due eventi distinti di trasmissione locale del virus Dengue in Emilia-Romagna e Veneto, considerati ormai conclusi); 4 casi di Zika virus (tutti importati, nessun decesso); 48 casi di TBE (45 casi autoctoni e 3 casi associati a viaggi all’estero, con un decesso); 105 casi di Toscana virus (104 casi autoctoni e 1 caso associato a viaggio all’estero, con un decesso). Per maggiori informazioni sui dati consulta la *dashboard*. Per i dati sulle infezioni da West Nile e Usutu virus consulta la pagina dedicata. Consulta anche la pagina generale dedicata alla sorveglianza nazionale e ai bollettini periodici.

Grazie a tutti colleghi/Istituzioni che hanno collaborato

ISTITUTO ZOOPROFILATTICO
SPERIMENTALE DELLA LOMBARDIA E
DELL'EMILIA ROMAGNA
"BRUNO UBERTINI"

SACRO CUORE
DON CALABRIA
I R C C S

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

CINECA

EVD-LabNet

DEPARTMENT OF
MEDICAL AND SURGICAL SCIENCES

RegioneEmilia-Romagna

CENTRO
REGIONALE
SANGUE
Emilia-Romagna

Azienda Unità Sanitaria Locale
della Romagna

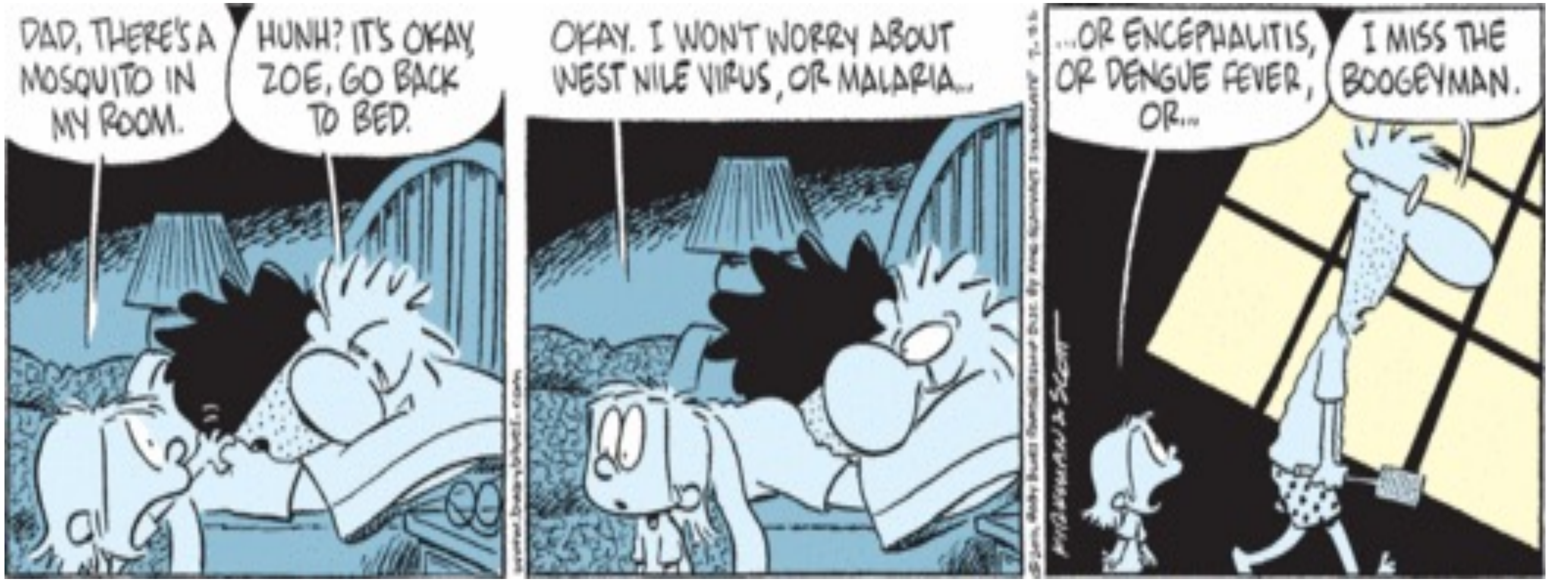
INF-ACT
Fondazione
INF-ACT

amU Faculté des sciences
médicales et paramédicales
Aix Marseille Université

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA
UNIB-OH
One Health International Study Centre

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA
CAMPUS DI FORLÌ


AIRPORT SECURITY
BIG GULP
WEST NILE


DAD, THERE'S A MOSQUITO IN MY ROOM.
HUNH? IT'S OKAY, ZOE, GO BACK TO BED.
OKAY. I WON'T WORRY ABOUT WEST NILE VIRUS, OR MALARIA...
...OR ENCEPHALITIS, OR DENGUE FEVER, OR...
I MISS THE BOOGEYMAN.
KIRKMAN & SCOTT