Prima Sessione:
La Sorveglianza delle Arbovirosi

Bologna, 26 Novembre 2018

Diagnosi sierologica e molecolare di arbovirosi: esperienza del Centro di Riferimento Regionale per le Emergenze Microbiologiche (CRREM)

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POLICLINICO DI SANT'ORSOLA

Dipartimento della Medicina diagnostica e della prevenzione
Unità Operativa Microbiologia

SERVIZIO SANITARIO REGIONALE
EMILIA-ROMAGNA
Azienda Ospedaliero - Universitaria di Bologna

Policlinico S. Orsola-Malpighi

Centro Riferimento Regionale Emergenze Microbiologiche (CRREM)

Bologna

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Research and diagnostic activities on emerging and re-emerging infections

- Arbovirus
- Malaria
- Leishmania
- Influenza/ MERS-CoV
- Invasive bacterial infections
- Multidrug resistant bacteria

Ministero della Salute
DIREZIONE GENERALE DELLA PREVENZIONE SANITARIA
Ufficio V – Prevenzione delle malattie trasmissibili e profilassi internazionale
DIREZIONE GENERALE DELLA SANITÀ ANIMALE E DEI FARMACI VETERINARI
Ufficio III – Sanità animale e gest. oper. Centro Naz. di lotta ed emergenza contro le malattie animali e unità centrale di crisi

Piano Nazionale di sorveglianza e risposta alle arbovirosi trasmesse da zanzare invasive (*Aedes sp.*) con particolare riferimento ai virus Chikungunya, Dengue e Zika - 2018.

Piano nazionale integrato di sorveglianza e risposta al virus West Nile e Usutu - 2018

Human surveillance of arbovirus infections in Emilia-Romagna region, 2013 - 2018

	2013-2017		2018	
	Suspected cases	% Pos	Suspected cases	% Pos
Chikungunya	120 - 330	1 – 9 %	220	0%
Dengue	120 - 310	5 - 20%	220	7%
Zika	310	3,5%	220	-
West Nile virus	260 - 450	3 – 10%	867	**21%**
Toscana virus	260 - 450	8 – 19%	867	**6%**
Usutu virus	450	0%	867	**0,2%**

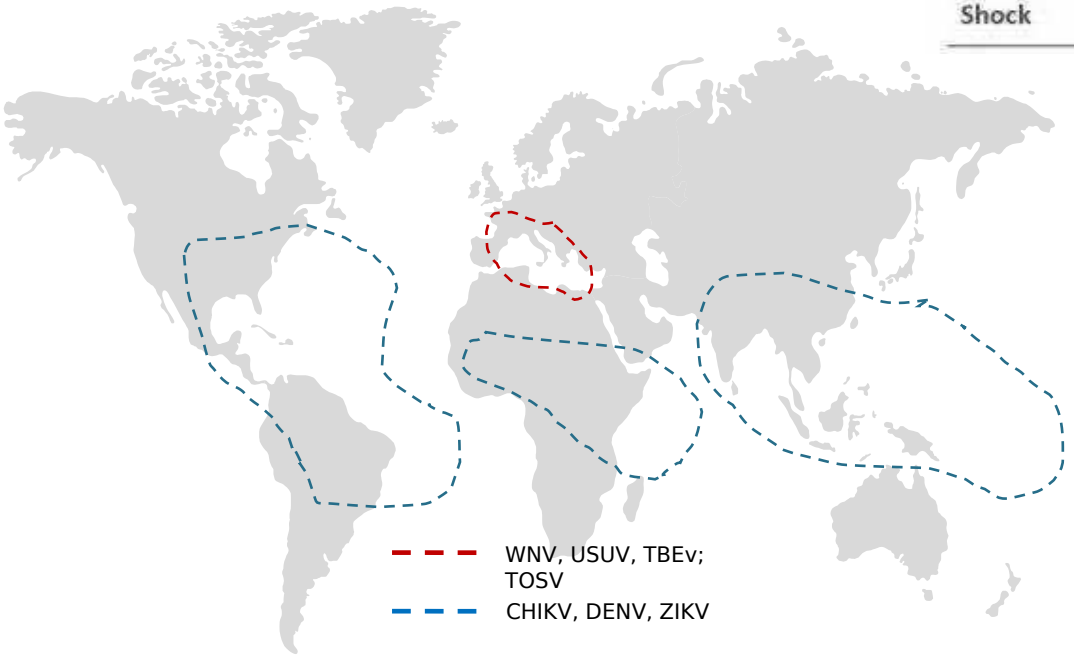
Many arboviruses display overlapping symptoms and geographical distribution

clinical manifestation

- sindrome febbrile
- artralgia, artrite, rash
- sindrome emorragica
- sindrome neurologica

Features	Zika	Dengue	Chikungunya
Fever	++	+++	+++
Rash	+++	+	++
Conjunctivitis	++	-	-
Arthralgia	++	+	+++
Myalgia	+	++	+
Headache	+	++	++
Hemorrhage	-	++	-
Shock	-	+	-

geographical distribution



Ministero della Salute

Multiplex diagnostic algorithms - syndromic approach -

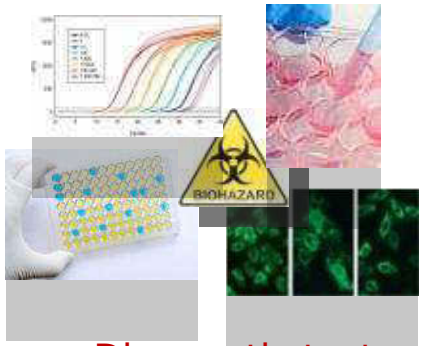
Piano Nazionale di sorveglianza e risposta alle arbovirosi trasmesse da zanzare invasive (*Aedes sp.*) con particolare riferimento ai virus Chikungunya, Dengue e Zika - 2018.

Piano nazionale integrato di sorveglianza e risposta al virus West Nile e Usutu - 2018

Viaggio zone endemiche (o caso sospetto autoctono) + sintomi

- **Dengue**
- **Zika**
- **Chikungunya**

→



Diagnostic test results within 24h

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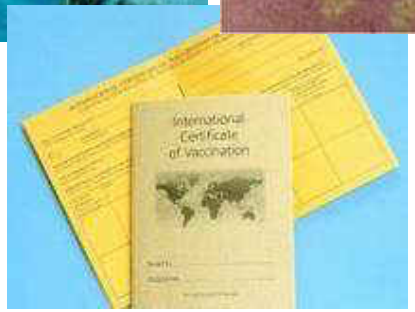
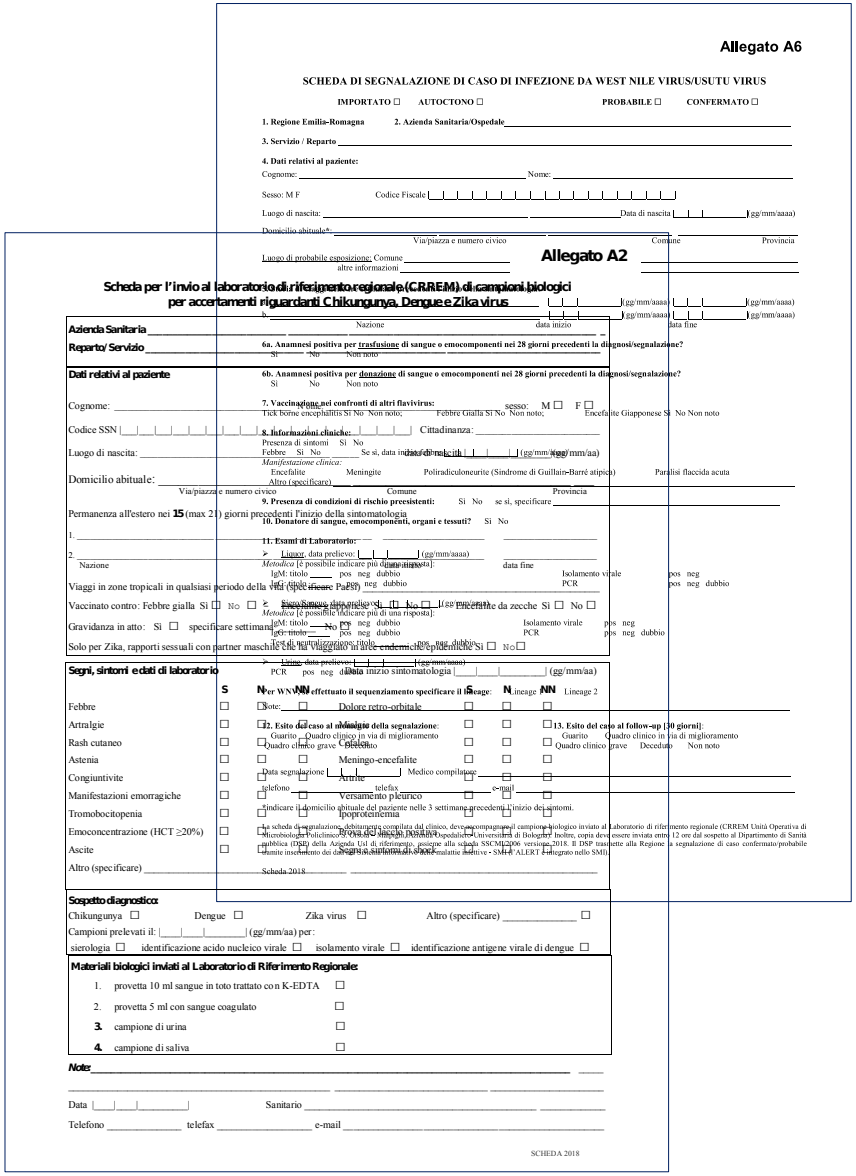
Febbre e/o manifestazioni neurologiche

- **West Nile**
- **Usutu**
- **Toscana**

Arbovirus diagnostics - important aspects

- ✓ **Travel history**
  - Where?
  - How long?
  - Date of return?
- ✓ **Onset of symptoms**
- ✓ **Clinical presentation**
- ✓ **Other risk factors** (medical conditions, pregnancy, immunosuppression, ….)
- ✓ **Vaccination history**





Allegato A6

SCHEDA DI SEGNALAZIONE DI CASO DI INFEZIONE DA WEST NILE VIRUS/USUTU VIRUS

IMPORTATO ☐ AUTOCTONO ☐ PROBABILE ☐ CONFERMATO ☐

1. Regione Emilia-Romagna 2. Azienda Sanitaria/Ospedale
3. Servizio / Reparto
4. Dati relativi al paziente:
Cognome: Nome:
Sesso: M F Codice Fiscale
Luogo di nascita: Data di nascita (gg/mm/aaaa)
Domicilio abituale*: Via/piazza e numero civico Comune Provincia
Luogo di probabile esposizione: Comune
altre informazioni

Allegato A2

Scheda per l'invio al laboratorio di riferimento regionale (CRREM) di campioni biologici per accertamenti riguardanti Chikungunya, Dengue e Zika virus

Azienda Sanitaria
Reparto/Servizio

Dati relativi al paziente

Cognome:
Codice SSN
Luogo di nascita:
Domicilio abituale:
Via/piazza e numero civico Comune Provincia
Permanenza all'estero nei 15 (max 21) giorni precedenti l'inizio della sintomatologia
1.
2.
Nazione data inizio data fine
Viaggi in zone tropicali in qualsiasi periodo della vita (Specificare Paesi)
Vaccinato contro: Febbre gialla Si ☐ No ☐ Encefalite Giapponese Si ☐ No ☐ Encefalite da zecche Si ☐ No ☐
Gravidanza in atto: Si ☐ specificare settimana
Solo per Zika, rapporti sessuali con partner maschile che ha viaggiato in aree endemiche/epidemiche Si ☐ No ☐

Segni, sintomi e dati di laboratorio

	S	N	NN
Febbre	☐	☐	
Artralgie	☐	☐	
Rash cutaneo	☐	☐	
Astenia	☐	☐	
Congiuntivite	☐	☐	
Manifestazioni emorragiche	☐	☐	
Tromobocitopenia	☐	☐	
Emoconcentrazione (HCT ≥20%)	☐	☐	
Ascite	☐	☐	
Altro (specificare)			

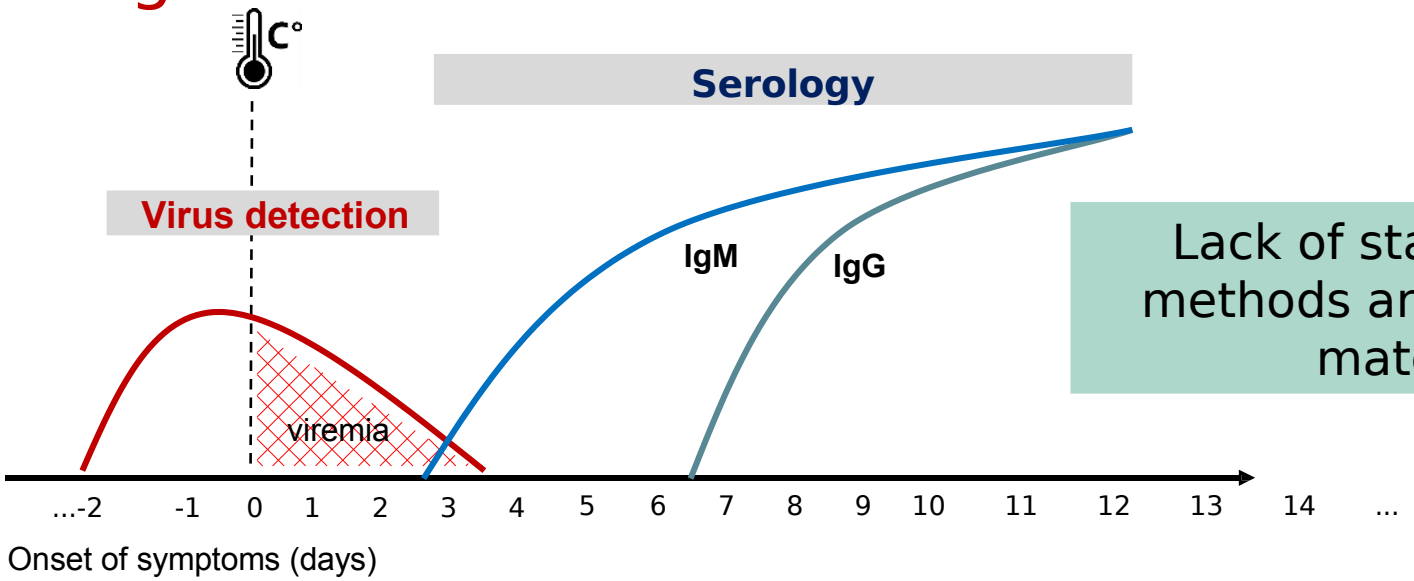
Sospetto diagnostico:
Chikungunya ☐ Dengue ☐ Zika virus ☐ Altro (specificare) ☐
Campioni prelevati il: (gg/mm/aa) per:
sierologia ☐ identificazione acido nucleico virale ☐ isolamento virale ☐ identificazione antigene virale di dengue ☐

Materiali biologici inviati al Laboratorio di Riferimento Regionale:
1. provetta 10 ml sangue in toto trattato con K-EDTA ☐
2. provetta 5 ml con sangue coagulato ☐
3. campione di urina ☐
4. campione di saliva ☐

Note:
Data Sanitario
Telefono telefax e-mail

SCHEDA 2018

Opportunities, limitations & pitfalls in arbovirus diagnostic



Lack of standardized methods and reference materials

Combination of Molecular and Serological tests

- **Direct virus detection**
- Viremic period of most arboviruses is short
- Viral load is low
- Peak for viremia before symptoms

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Testing multiple specimens can increase sensitivity

- **Serology**
- Cross-reactivity among flaviviruses and with vaccine Abs
- IgM is not always a reliable marker of acute infection

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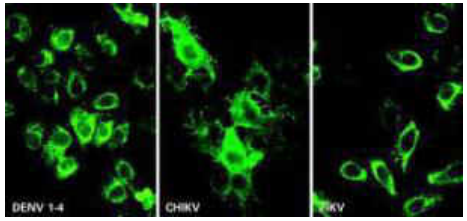
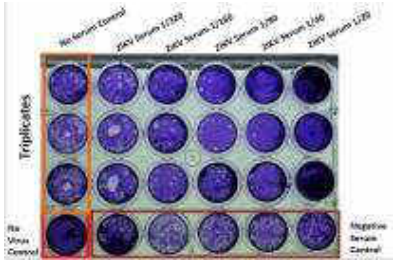
Sequential sampling

Arboviruses serology



IFA

ELISA



PRNT

Flavivirus serology – a diagnostic dilemma
Definitive serological diagnosis is not always possible!

Confirmed acute Zika infection in a patient with previous Dengue infection

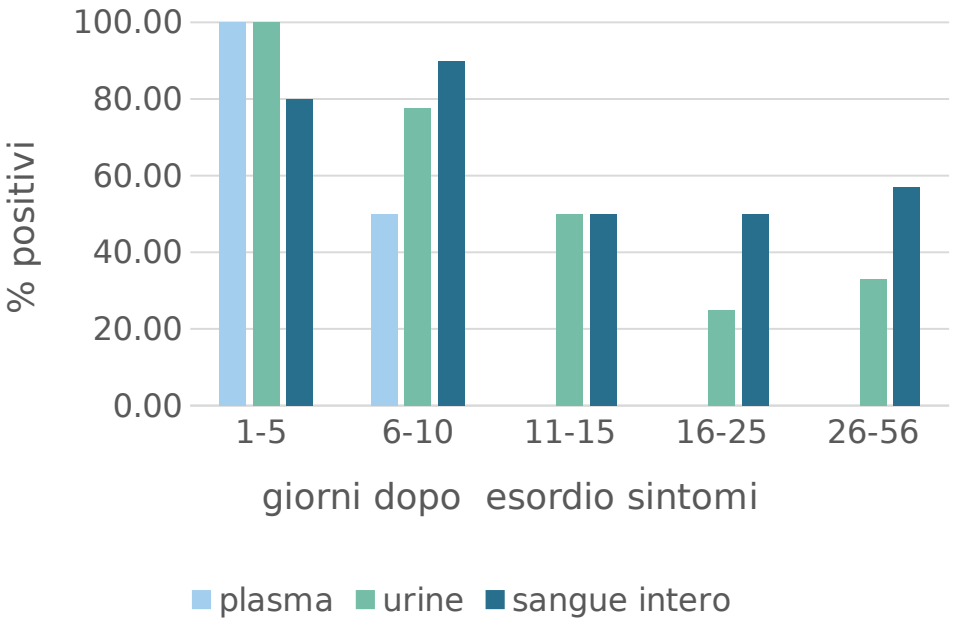
IgM Zika	IgM Dengue	PRNT-90 Zika	PRNT-90 Dengue 1	PRNT-90 Dengue 2
POS	POS	1:160	1:5120	1:320

courtesy from R.Lanciotti, CDC, 2017

cross-reattività è minima in pazienti senza precedenti infezioni da flavivirus
cross-reattività elevata in pazienti con precedenti infezioni da flavivirus (o vaccinazioni)

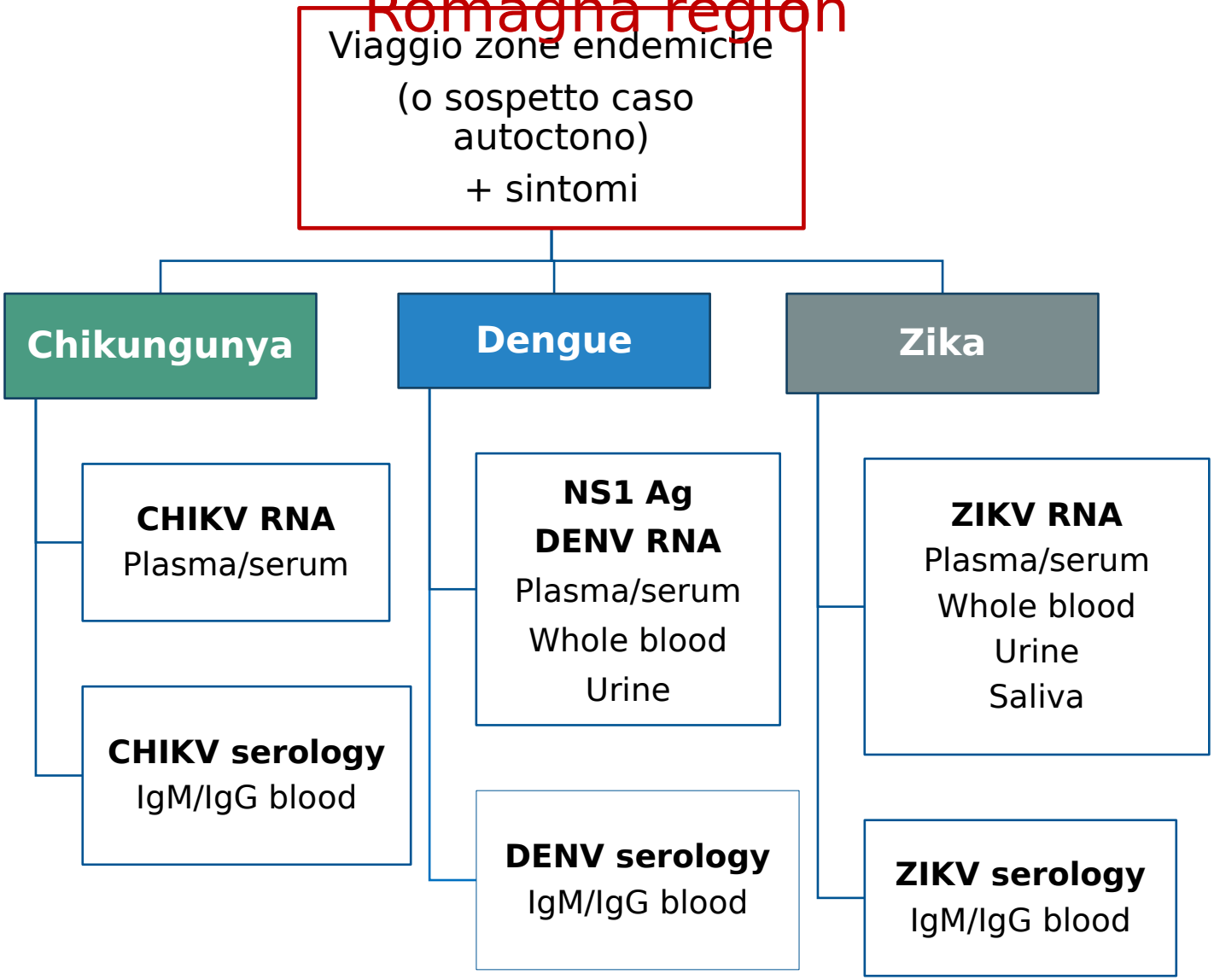
Testing multiple specimens for ZIKV RNA → Overcoming the interpretation of serological assays

Laboratory testing	N.pos/N.tested (%)
RNA plasma +	12/21 (57)
RNA urine +	15/21 (71)
RNA whole blood +	16/21 (76)
RNA saliva +	5/11 (45)
Plasma Urine + Whole blood	**19/21 (90)**
Time from symptoms onset to diagnosis, days, median (range)	*6 (2-48)*

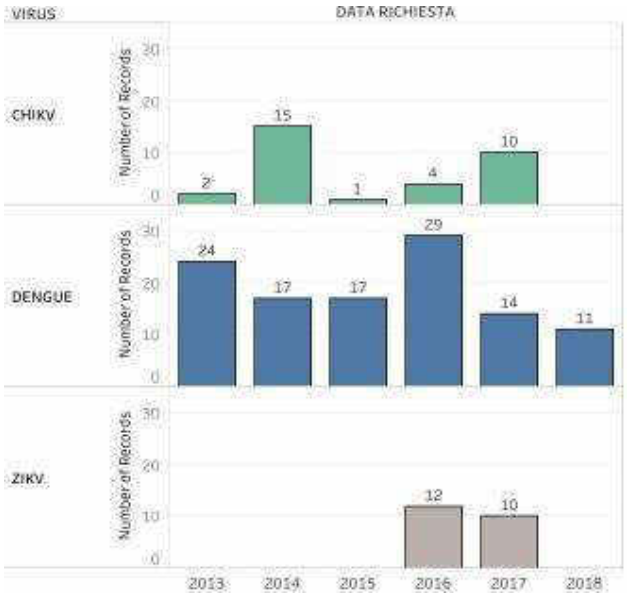


RNA + in plasma max 10 days
RNA + in urine e whole blood max 56 giorni

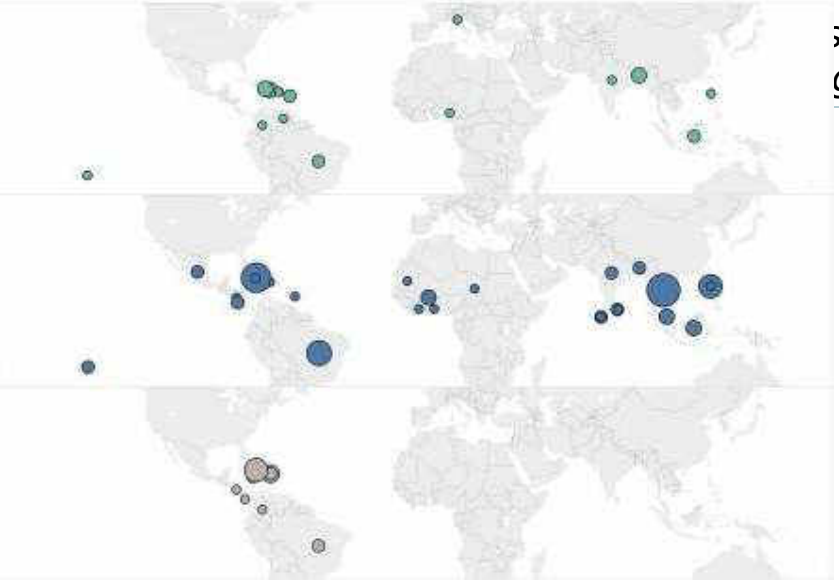
Diagnostic algorithm in Chikungunya, Dengue and Zika surveillance in Emilia Romagna region

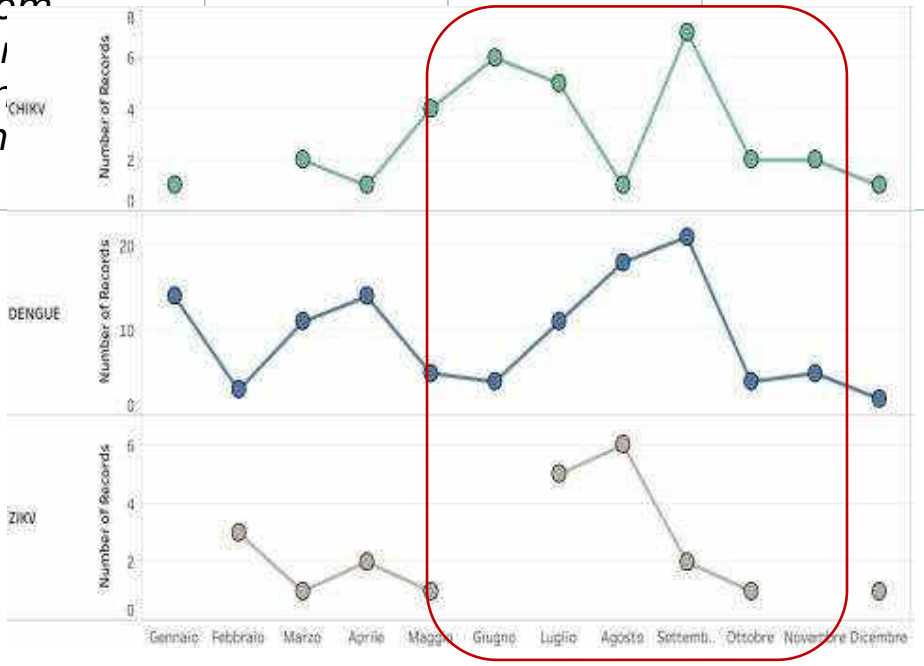


Chikungunya, Dengue and Zika imported infections, ER 2013-18: virological and serological findings



	CHIKV	DENV	ZIKV
RNA plasma +	15/32 (**47%**)	70/106 (**66%**)	12/22 (**55%**)
Antigen NS1 +	-	99/116 (85%)	-
Serology +	23/32 (72%)	68/113 (61%)	18/22 (81,8%)
Time fro… symptoi… to diagr… s, m… ge)			





Zika infection in pregnancy, Emilia-Romagna 2017

Donna gravida / partner
Donna / partner pre-concezionale

↓

Esposizione all'infezione

(viaggio zona affetta o rapporto sessuale con persona che vive/ha viaggiato in zona affetta)

- Donna italiana
- 5° settimana gravidanza (feb '17)
- Panama 23 dic '16 – 10 gen '17
- 30/12/16: febbricola
- Vaccinata per YFV

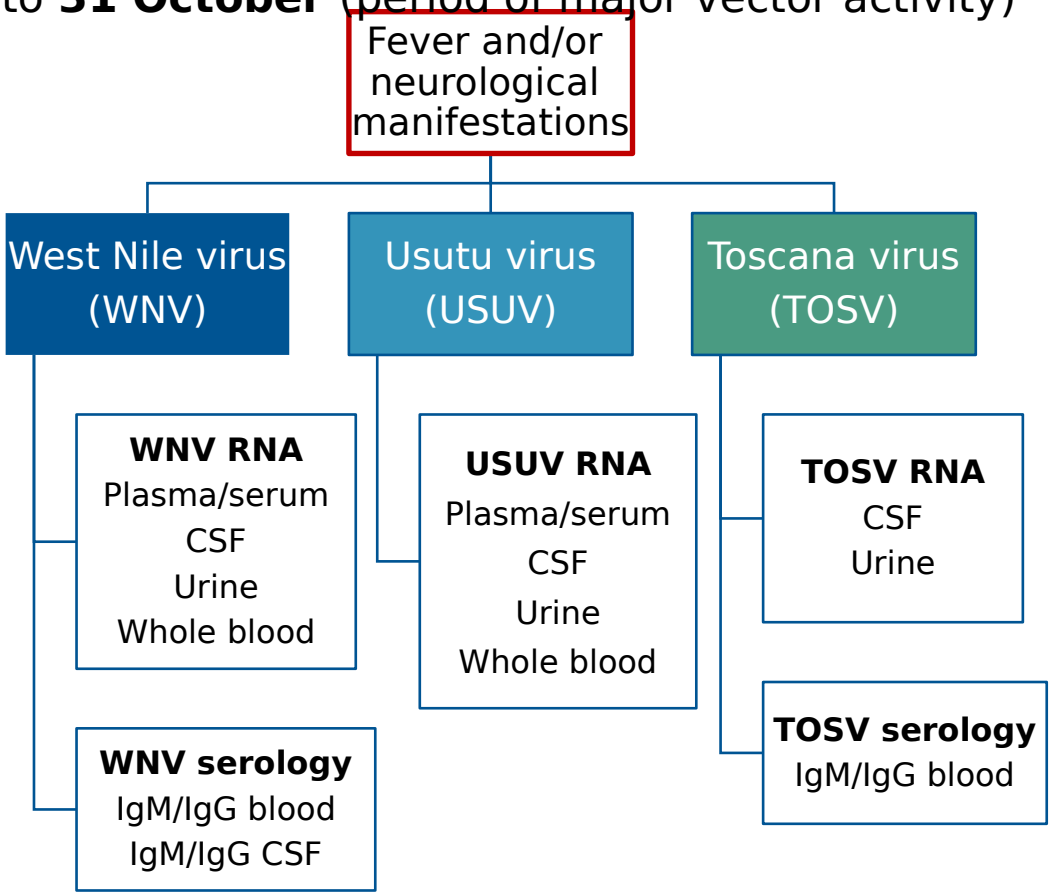
	d +38 (post symptoms onset)	**d +46** (post symptoms onset)	**d +56** (post symptoms onset)
ZIKV IgM	**POS**	**Reatt. Debole**	Neg
ZIKV IgG	**POS**	**POS**	**POS**
ZIKV MNT	1:80	1:160	na
DENV IgM	Neg	Neg	Neg
DENV IgG	POS	POS	POS
ZIKV RNA sangue	**POS**	**POS**	**POS**
ZIKV RNA urine	Neg	**POS**	**POS**
ZIKV RNA saliva	Neg	Neg	Neg
ZIKV tamp. vaginale	na	Neg	na

Diagnostic algorithm in human WNV/USUV surveillance in Emilia-Romagna region

The surveillance of human cases is based on the clinical forms of **neuroinvasive disease (WNND)**

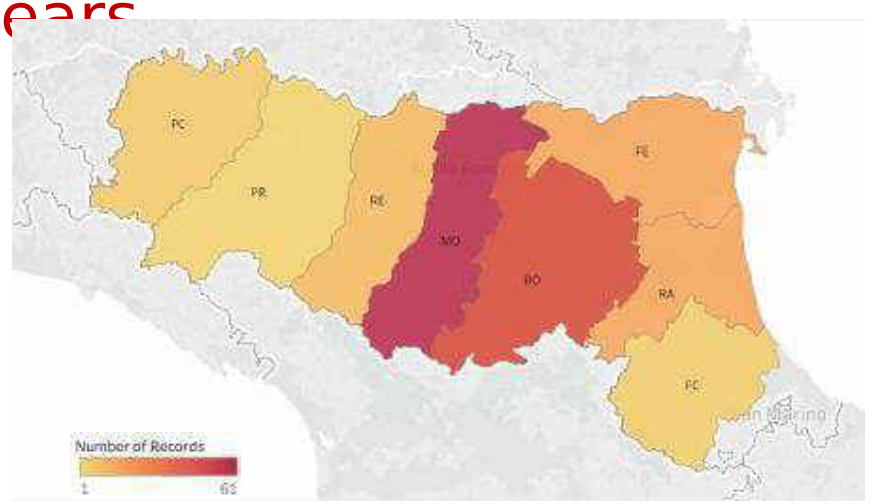
- **Fever** and **Neurological manifestations**: encephalitis, meningitis, polyradiculoneuritis (Guillain-Barré-like Syndrome) or acute flaccid paralysis.

- from **15 June** to **31 October** (period of major vector activity)

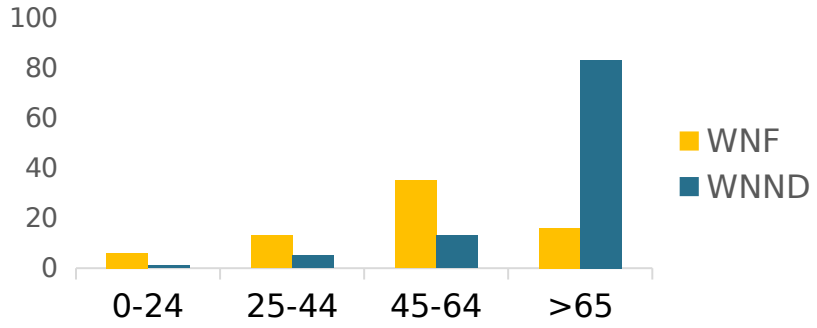


WNV in ER region, 2018: Higher number of WNND cases than in previous years

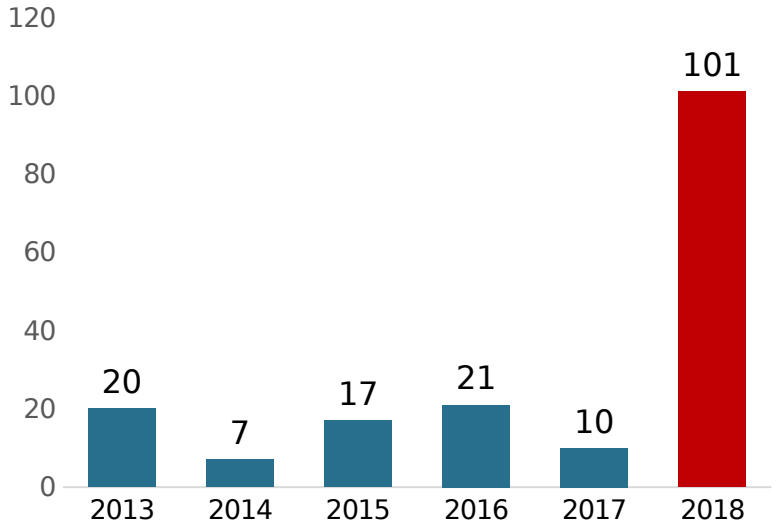
2018	WNND	WNF
Cases	**101**	**70**
Fatal cases	21 (20,8%)	-
Sex, male (%)	63,3%	43,3%
Age, years, median (range)	77 (17-94)	55 (19-91)



e distribution of human WNV cases, ER 2018



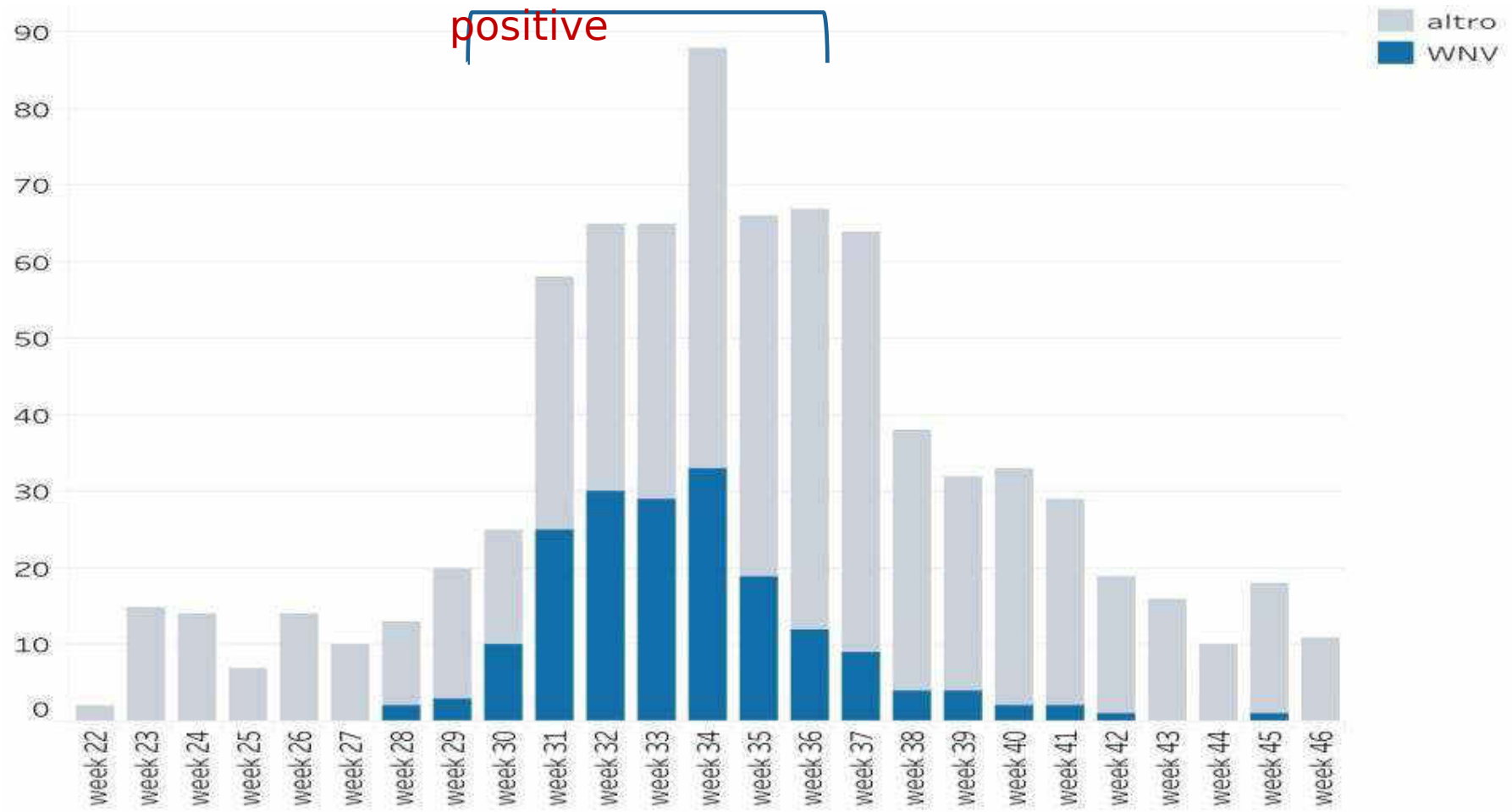
WNND cases, ER 2013-18



Human WNV infection and laboratory workload, ER region 2018

1st June – 15th November: **800** requests

19% - **46**% WNV positive

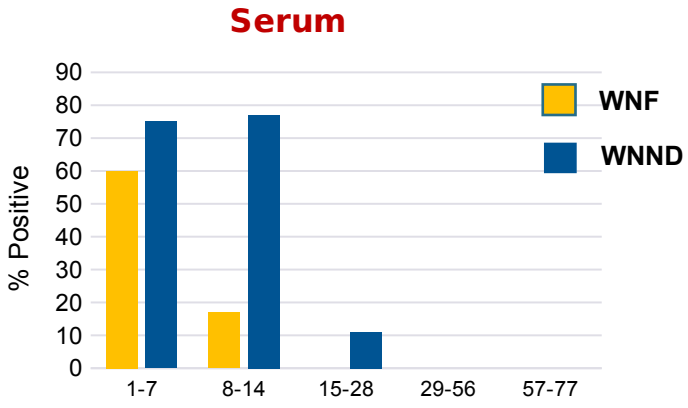


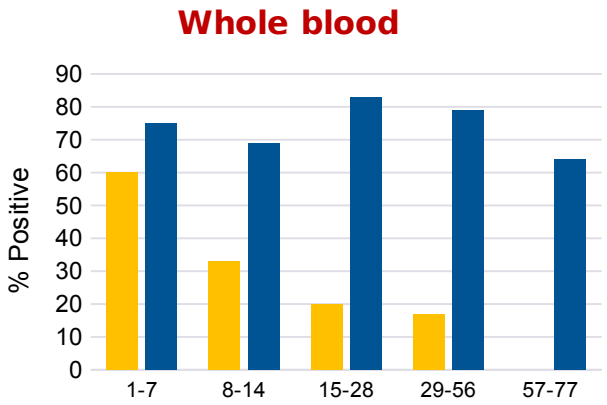
WNV RNA in body fluids during acute infection

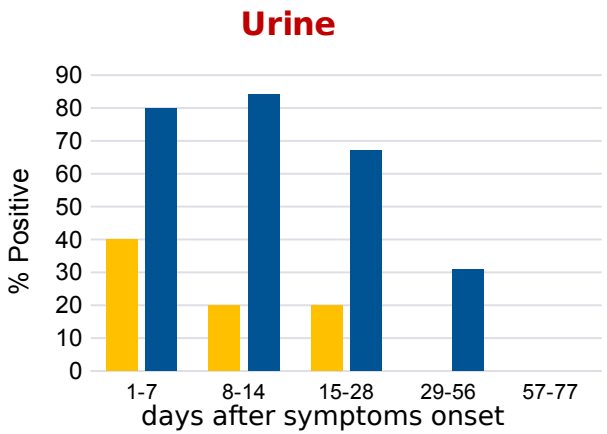
	WNF **N. Pos /N. tested (%)**	**WNND** **N. Pos /N. tested (%)**
Serum	78/121 (64)	100/180 (55)
Urine	68/97 (70)	113/153 (74)
Whole blood	37/57 (65)	64/89 (72)
CSF	-	35/138 (25)
Parallel testing multiple specimens		
Plasma / Whole blood / Urine	47/54 (**87**)	64/79 (**81**)
Time to diagnosis, days median (range)	*5,5 (0-35)*	*6,5 (0-36)*

Parallel testing of multiple specimens greatly improves the overall clinical sensitivity of molecular methods in acute stage of WNV infection

Kinetics of WNV RNA in body fluids







18 WNND and **6 WNF** patients
plasma, urine and whole blood samples collected longitudinally at 7, 15, 30, 60 and 180 days after initial diagnosis

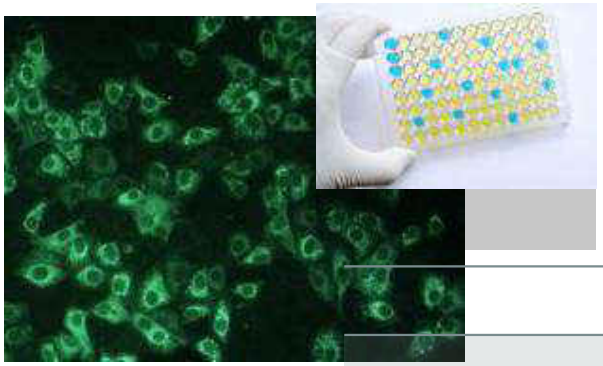
Testing WNV RNA in whole blood and urine
→ widening the window for viral RNA detection
→ direct diagnosis at late times after symptoms onset

days

	WNF	WNND
plasma	10	18
urine	25	44
whole blood	40	74

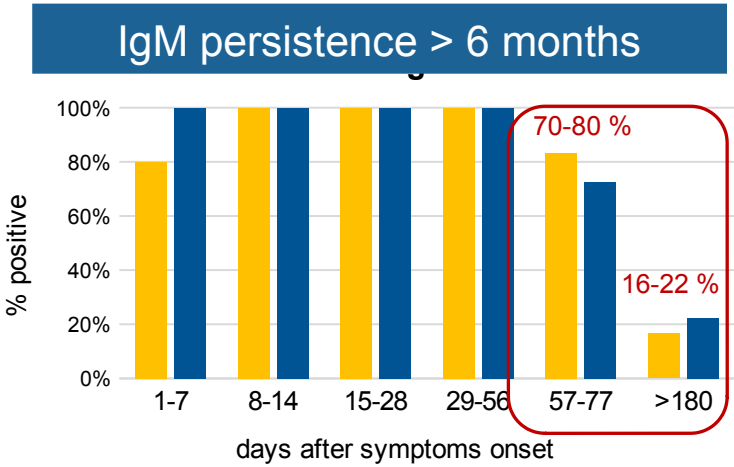
In each body fluid:
- the detection rate of WNV RNA was higher in WNND than in WNF patients
- WNV RNA persisted longer in WNND than WNF patients

→ viral RNA persistence in body fluids might be associated with the clinical outcome of WNV infection

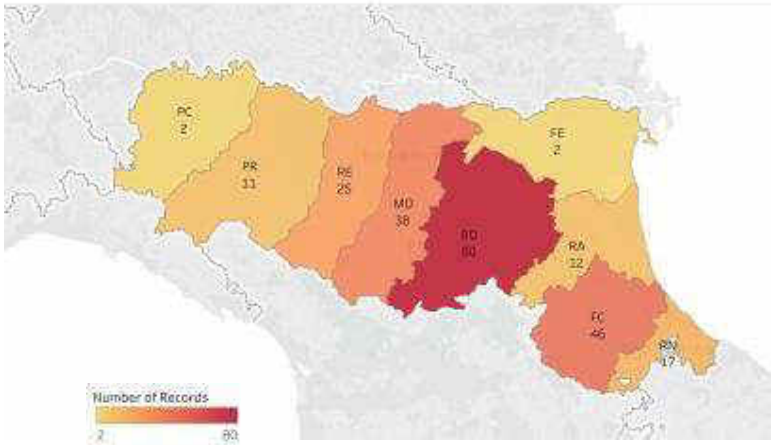


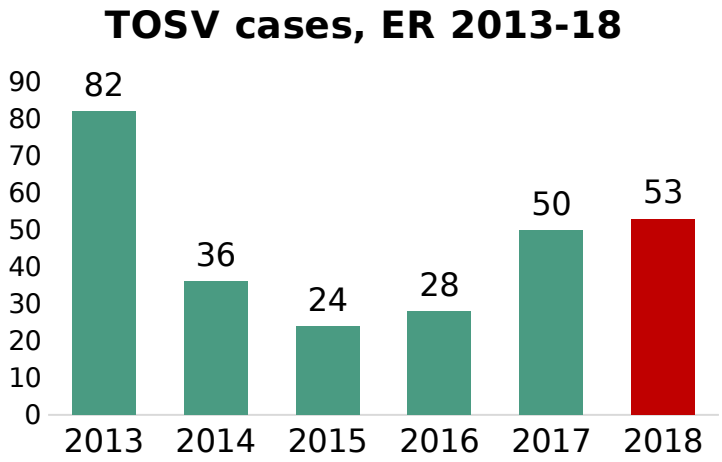
WNV-IgM and -IgG during acute infection

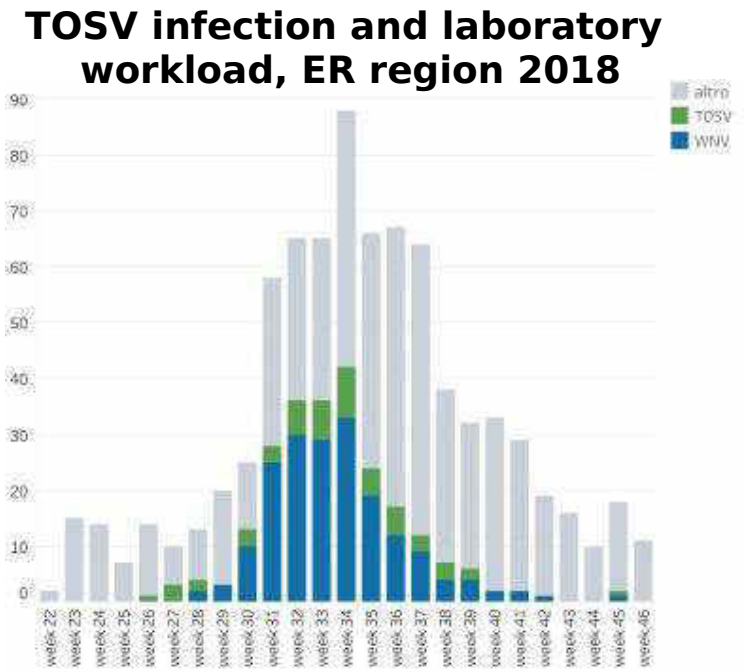
	WNF	WNND	
	blood	blood	CSF
IgM - / IgG -	42/122 (34%)	18/180 (10%)	23/137 (17%)
IgM + / IgG -	5/122 (4%)	8/180 (4%)	8/137 (6%)
IgM + / IgG +	68/122 (**56**%)	149/180 (**83**%)	95/137 (**70**%)
Time to diagnosis, median days (range)	*5,5 (0-35)*	*6,5 (0-36)*	



Toscana virus infection in ER region, 2013-18







Virological and serological findings in acute TOSV infection

	No./No. tested (%)
RNA+ in CSF	**221/231 (95,6%)**
IgM+/IgG-	6/265 (2,3%)
IgM+/IgG+	**239/265 (90,2%)**
IgM-/IgG-	12/265 (4,5%)
RNA+ in Urine	**56/129 (43,4%)**

Human Usutu virus infection, ER 2018

	Case N. 1	**Case N. 2**
Province	Modena	Forlì
Sex	F	M
Age	41	26
Clinic	Encephalitis	Fever
USUV RNA blood	**POS**	NEG
USUV RNA CSF	NEG	-
USUV RNA urine	NEG	**POS**
WNV IgM/IgG	NEG	**POS**

Arbovirus: Diagnostic Challenges

- Sovrapposizione distribuzione geografica e presentazione clinica
- “Multiplexing diagnostic”
- viremia è di breve durata e spesso già non rilevabile al momento della diagnosi

→ testare differenti materiali biologici

- Interpretazione dei test sierologici è complessa

- Scarsa standardizzazione delle procedure
- Difficoltà di reperimento di materiale di riferimento
- Network nazionali e internazionali

Continuo aggiornamento delle metodiche diagnostiche
Continuo aggiornamento sull’epidemiologia
Necessità di ampliare la capacità diagnostica sulle arbovirosi

http://www.evd-labnet.eu

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Regione Emilia-Romagna