

Chikungunya

WRAIR- GEIS 'Operational Clinical Infectious Disease' Course

WRAIR

Walter Reed Army
Institute of Research

Soldier Health • World Health



Chikungunya Virus: Overview

- Virus
 - Family Togaviridae, Genus Alphavirus
- Mosquito-transmitted
 - *Aedes aegypti*, *Aedes albopictus*
- Causes an acute febrile illness with polyarthralgias
 - Potential for chronic joint and tendon pain and disability
- No vaccine, no specific antiviral therapeutic

Lindsay Lohan Suffering From Incurable Virus During Holiday Vacation



The actress contracted Chikungunya, a mosquito transmitted viral disease which causes fever, joint pain, fatigue and a rash, during her holiday in French Polynesia

Transmission: Vectors

Aedes aegypti

- Urban areas of tropics and subtropics
- Prefers humans for bloodmeal
- Flowerpots, trash, cups
- Will bite another if feeding is interrupted

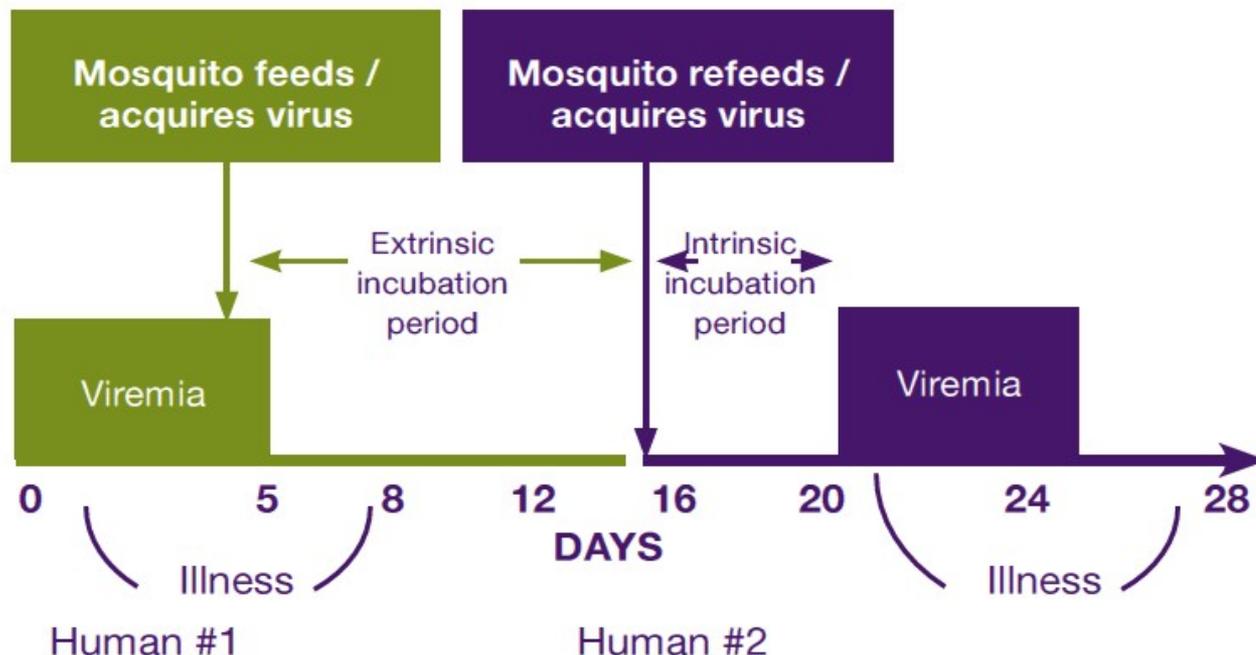


Aedes albopictus

- Asian tiger mosquito
- Bites more hosts vs. *Ae.aegypti*
- Wider distribution vs. *Ae.aegypti*
 - Found in temperate climates
- Dengue / CHIK co-infection documented

Transmission: Other Considerations

CHIKV mutations may enhance transmission



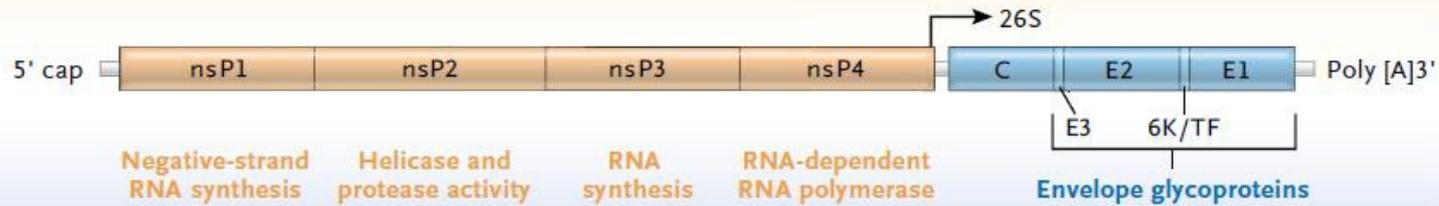
Extrinsic incubation period ~ 10 days, less in warmer climates.

Aedes mosquitoes live 2-4 weeks, longer in warmer climates.

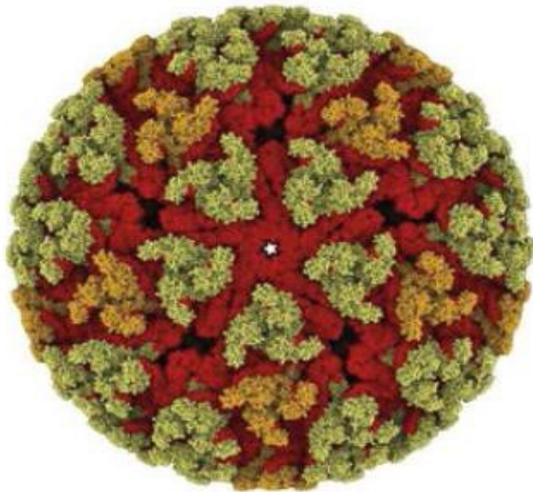
Transmission: Other Methods

- Rare But Documented
 - Intrapartum from mother to child
 - *In utero* transmission resulting in miscarriage
 - Percutaneous needlestick
 - Laboratory exposure
- Theoretical: Blood transfusion, Organ Transplantation
- No Evidence of Breast Milk Transmission

A Genome structure



B Virion structure



C Envelope glycoprotein spike structures

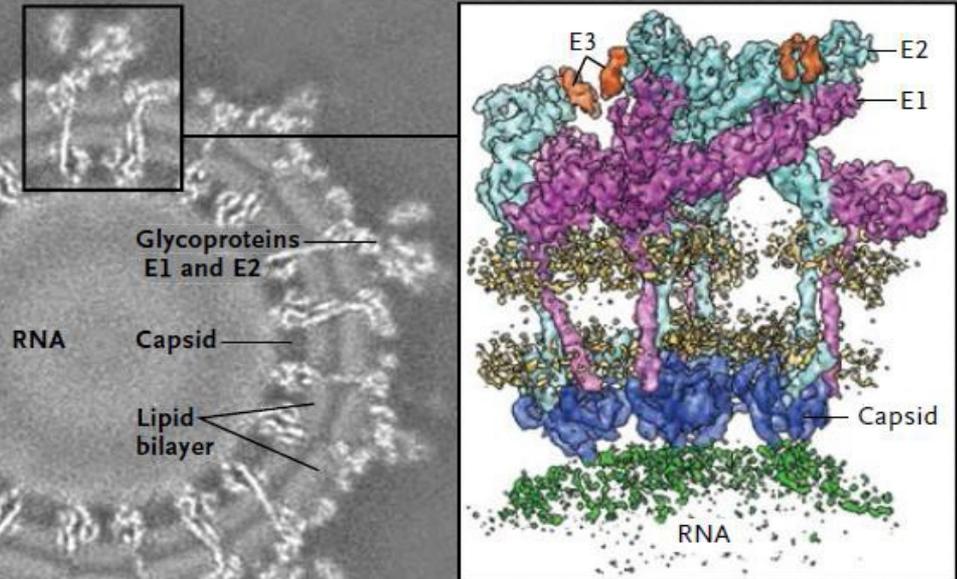


Figure 1. Chikungunya Virus Genetic and Physical Structure.

Panel A shows the organization of the chikungunya virus genome, including its nonstructural proteins 1 through 4 (nsP1–nsP4) and structural proteins C (capsid), E1–E3 (envelope glycoproteins), and 6K/TF (6K and TF [transframe] are alternative translation products of the same gene). Panel B shows the structure of the virion (image courtesy of Felix Rey, Institut Pasteur, Centre National de la Recherche Scientifique). Panel C shows spike-protein predicted structures based on atomic resolution structures of the envelope glycoproteins² and high-resolution cryoelectron microscopic reconstructions of chikungunya virus and other alphavirus particles.³

Chikungunya Virus (CHIKV)

- Single serotype
 - Immunity presumed to be life-long following infection
 - Some cross-reactivity with related alphaviruses
- Multiple genotypes
 - Asian;
 - East/Central/South African;
 - Indian Ocean Lineage
 - West African, Indian Ocean

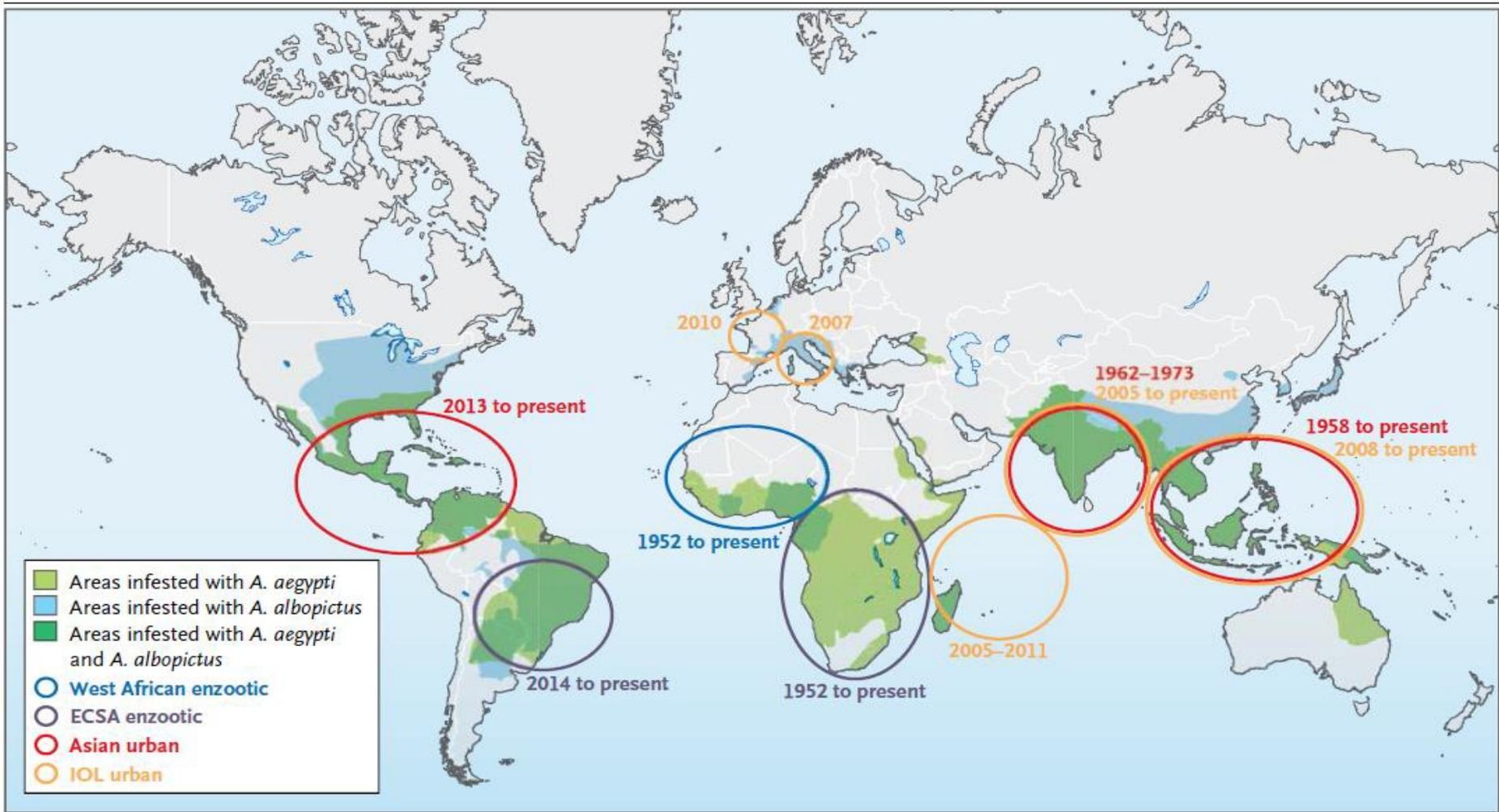


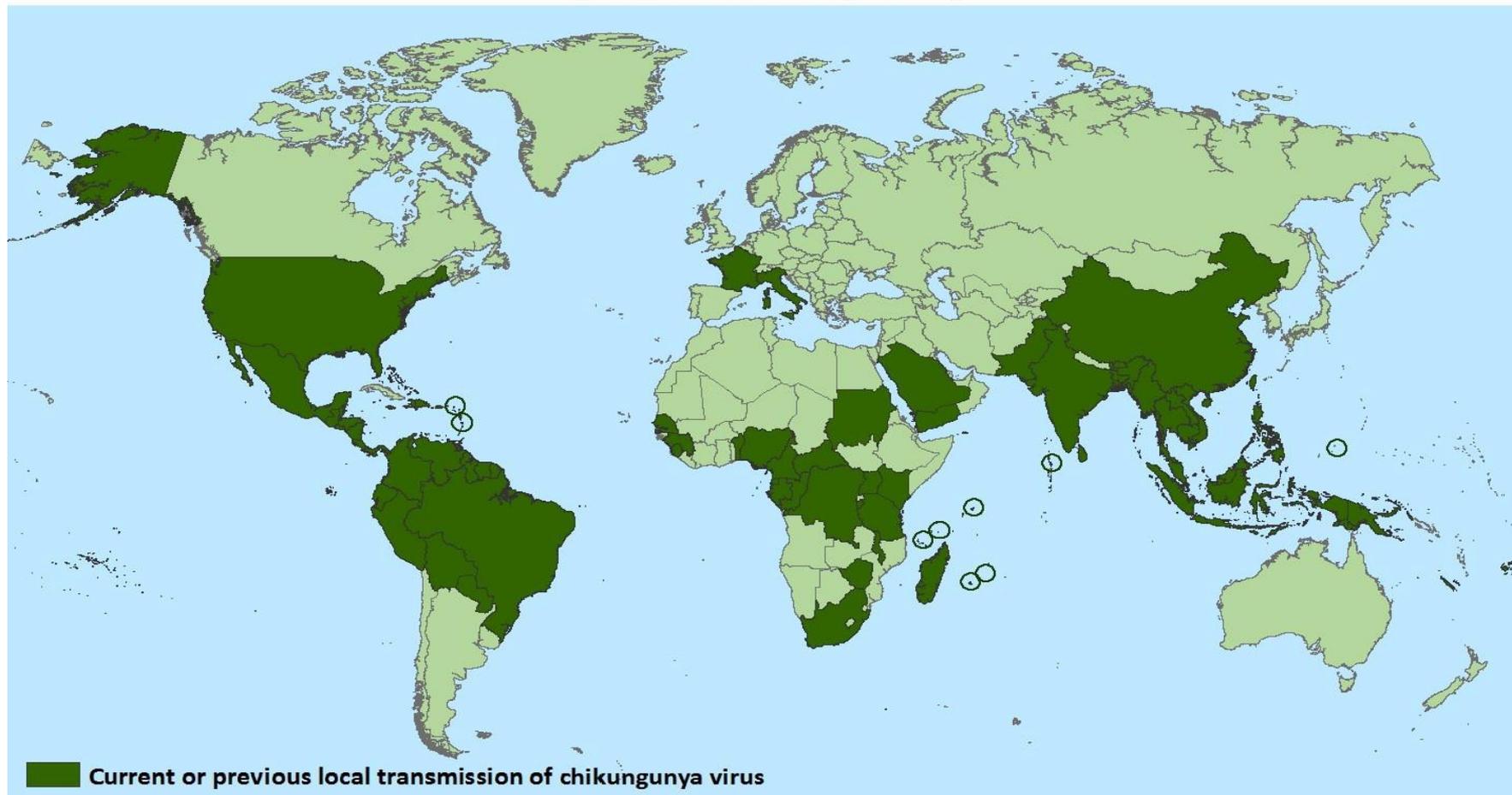
Figure 2. Origin, Spread, and Distribution of Chikungunya Virus and Its Vectors.

The map shows the African origins of enzootic chikungunya virus strains and the patterns of emergence and spread of the Asian lineage and Indian Ocean lineage (IOL) of the virus during epidemics since the 1950s, based on phylogenetic studies.^{4,5} The distributions of the peridomestic vectors, *Aedes aegypti* and *A. albopictus*, are also shown. ECSA denotes eastern, central, and southern African.

Global Distribution of CHIKV

<http://www.cdc.gov/chikungunya/map/index.html>

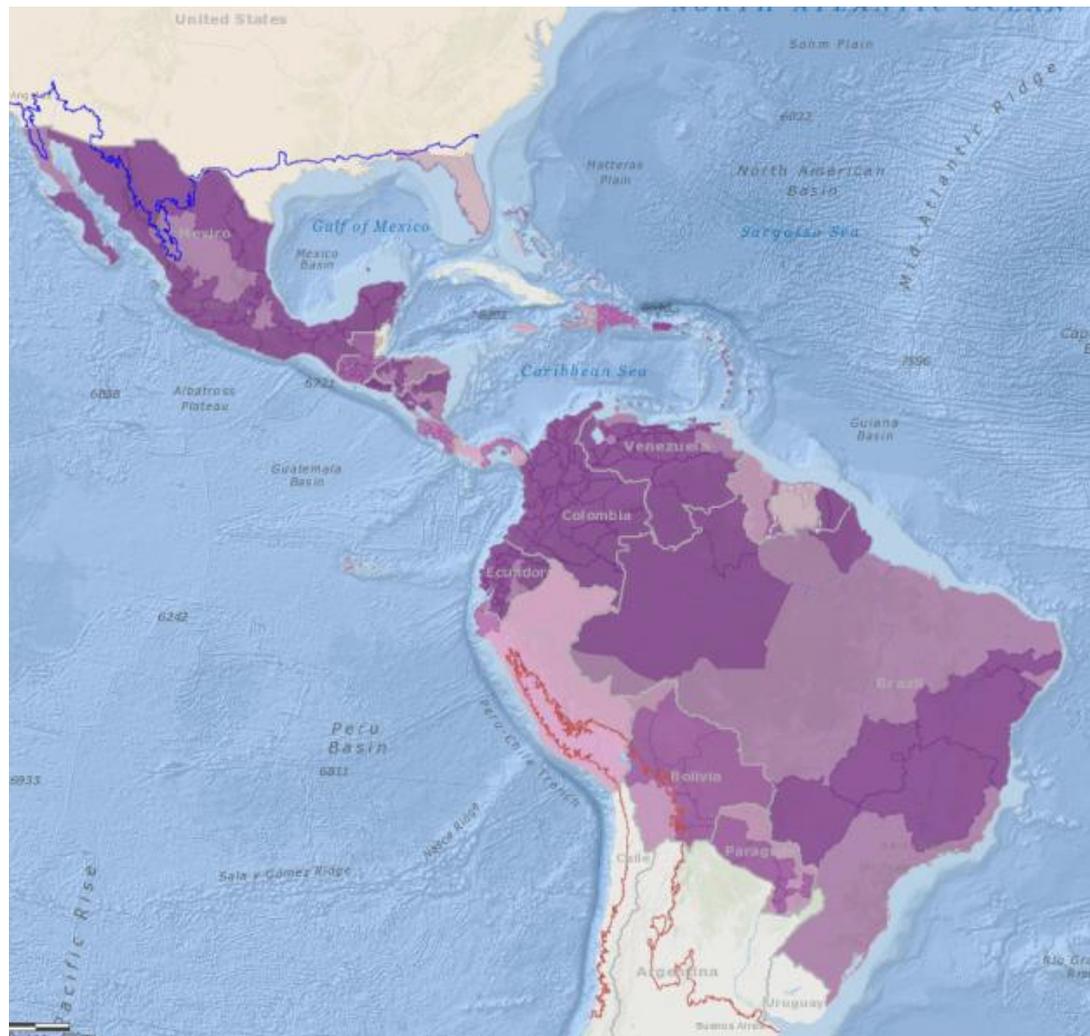
Countries and territories where chikungunya cases have been reported
(as of October 20, 2015)



CHIKV in the Americas, 2013-15

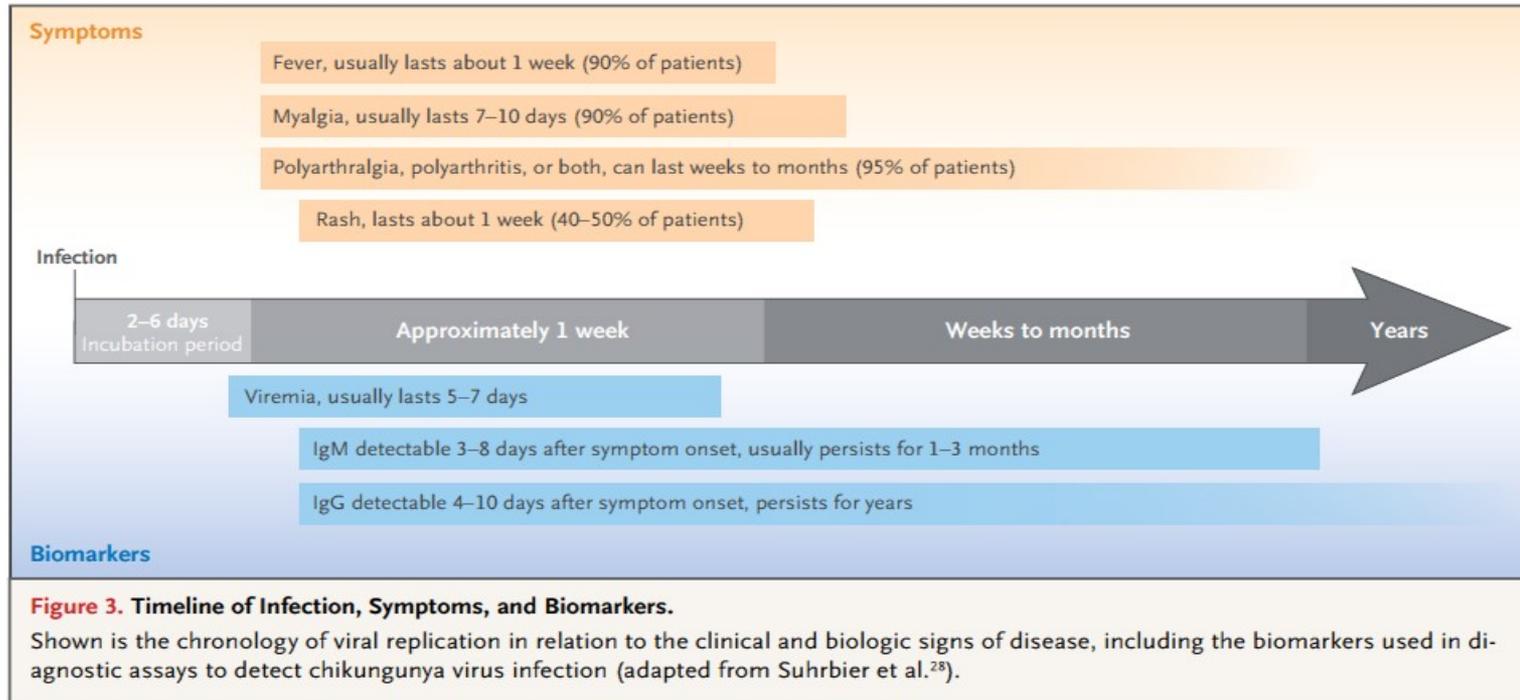
<http://www.paho.org> , AFHS Chikungunya in the Americas Surveillance Summary #52, 12 Nov 2015

- Total cases:
 - 2014: 1,072,000
 - 2015: 617,000
- CONUS-acquired cases:
 - 2014: 11
 - 2015: 0
- Deaths:
 - 2014: 169
 - 2015: 72



Clinical features

Chikungunya virus (CHIKV) infection causes a characteristic triad of fever, arthritis, and rash, although many cases are asymptomatic (Gay, et al, 2015).



Weaver, NEJM, March 2015

Gay N, Rousset D, Huc P, et al. Seroprevalence of Asian lineage chikungunya virus infection on Saint Martin island, 7 months after the 2013 emergence. Am J Trop Med Hyg. Dec 7, 2015. doi: 10.4269/ajtmh.15-0308

Clinical Features: Rash



Acute Joint Manifestations

- Symmetric
- Distal
- Edematous



<http://vir.sgmjournals.org>

CHIK Clinical Features

Symptom or sign	Frequency range (% of symptomatic patients)
Fever	76–100
Polyarthralgias	71–100
Headache	17–74
Myalgias	46–72
Back pain	34–50
Nausea	50–69
Vomiting	4–59
Rash	28–77
Polyarthritis	12–32
Conjunctivitis	3–56

Atypical CHIK Manifestations

System	Clinical manifestations
Neurological	Meningoencephalitis, encephalopathy, seizures, Guillain-Barré syndrome, cerebellar syndrome, paresis, palsies, neuropathy
Ocular	Optic neuritis, iridocyclitis, episcleritis, retinitis, uveitis
Cardiovascular	Myocarditis, pericarditis, heart failure, arrhythmias, hemodynamic instability
Dermatological	Photosensitive hyperpigmentation, intertriginous aphthous-like ulcers, vesiculobullous dermatosis
Renal	Nephritis, acute renal failure
Other	Bleeding dyscrasias, pneumonia, respiratory failure, hepatitis, pancreatitis, syndrome of inappropriate secretion of antidiuretic hormone (SIADH), hypoadrenalism

Adapted from Rajapakse et al. ²⁰

Chikungunya: A Potentially Emerging Epidemic?

Michelle M. Thiboutot^{1,2}, Senthil Kannan², Omkar U. Kawalekar², Devon J. Shedlock², Amir S. Khan³, Gopalsamy Sarangan⁴, Padma Srikanth⁴, David B. Weiner², Karupppiah Muthumani^{2*}

Table 1. Comparison of clinical features of Chikungunya and Dengue virus.

Clinical Features	Chikungunya Virus (CHIKV)	Dengue Virus (DENV)	Reference
1) Fever, asthenia	Common	Common	[6,8]
2) Myalgia	Possible	Very common	[6]
3) Polyarthritits	Very Common, edematous	None	[56]
4) Tenosynovitis	Yes	None	[57]
5) Leukopenia	None	Yes	[58]
6) Thrombocytopaenia	None	Yes	[59]
7) Rash	Days 1–4, important skin edema	Days 3–7	[6,35,58]
8) Retro-orbital pain	Rare	Common	[60]
9) Hypotension	Possible	Common, Days 5–7	[60,61]
10) Minor bleeding	Chronic polyarthritits up to 1 year	Common	[17,56]
11) Second stage	Possible; Tenosynovitis at M2–M3 Raynaud's syndrome at M2–M3	Fatigue up to 3 mo	[6,56,57,58,62,63]

doi:10.1371/journal.pntd.0000623.t001

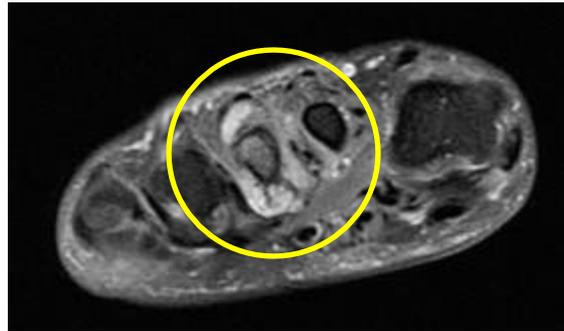
Persistent Chikungunya



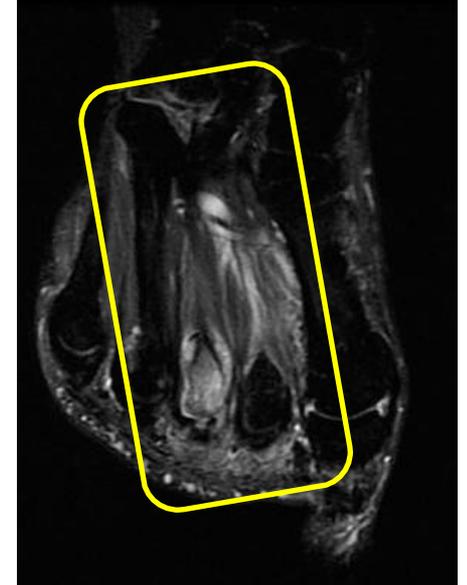
Persistent Chikungunya



Calcifications in shoulder tendon 18 months after infection



Inflammatory osteoarthritis, foot, 5 years after infection



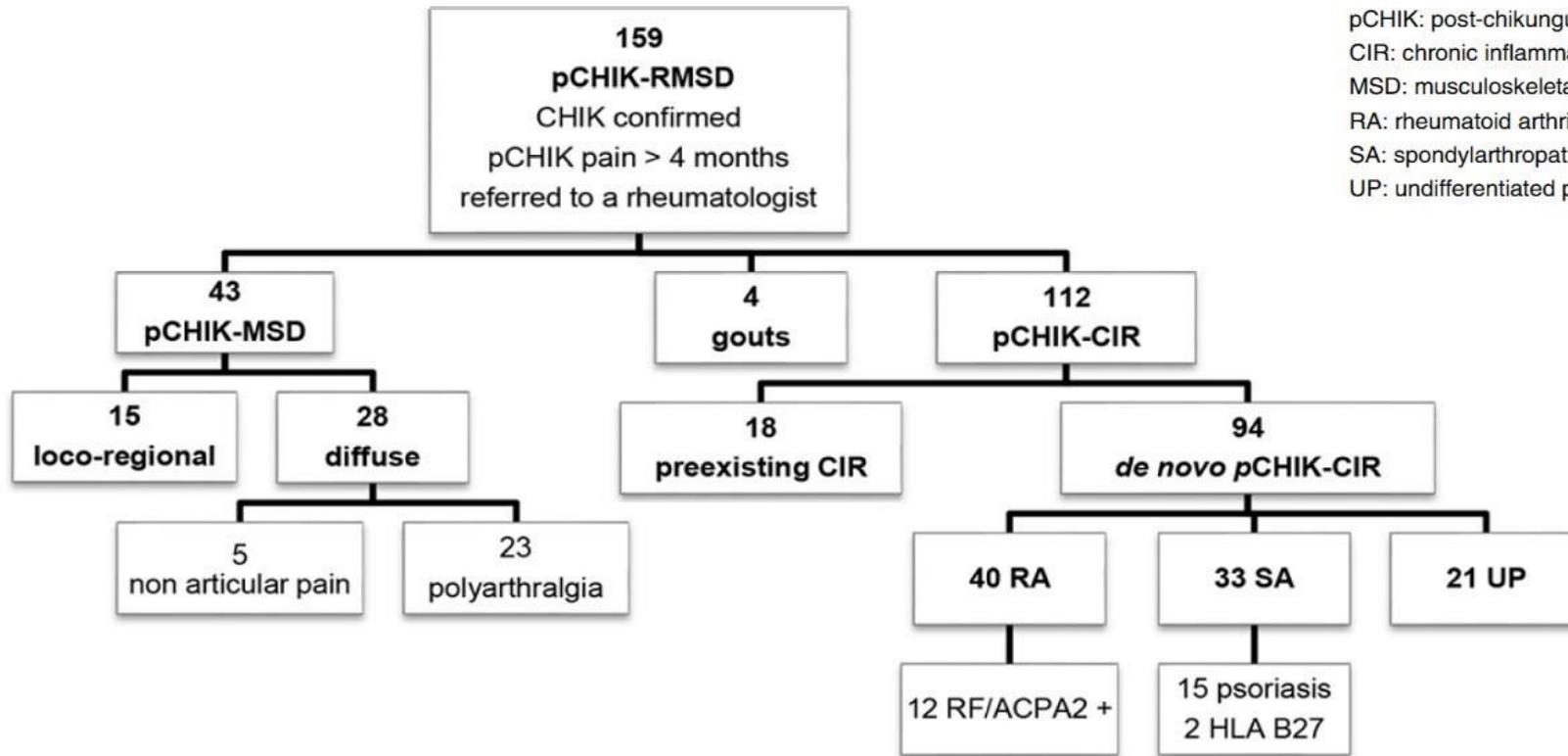
Persistent Chikungunya

Three clinical components, singly / in combination:

1. Distal polyarthrititis / monoarthrititis improved with NSAIDs;
2. Frequent tenosynovitides in the hands, wrists, or ankles, sometimes responsible for carpal or tarsal tunnel syndromes, highly sensitive to short-term systemic corticotherapy; and
3. Exacerbation of pain in previously injured joints and bones requiring painkillers.

Simon F, Parola P, Grandadam M, et al. Chikungunya infection: an emerging rheumatism among travelers returned from Indian Ocean islands. Report of 47 cases. *Medicine (Baltimore)*. 2007;86:123–37.

Post-chikungunya rheumatic disorders

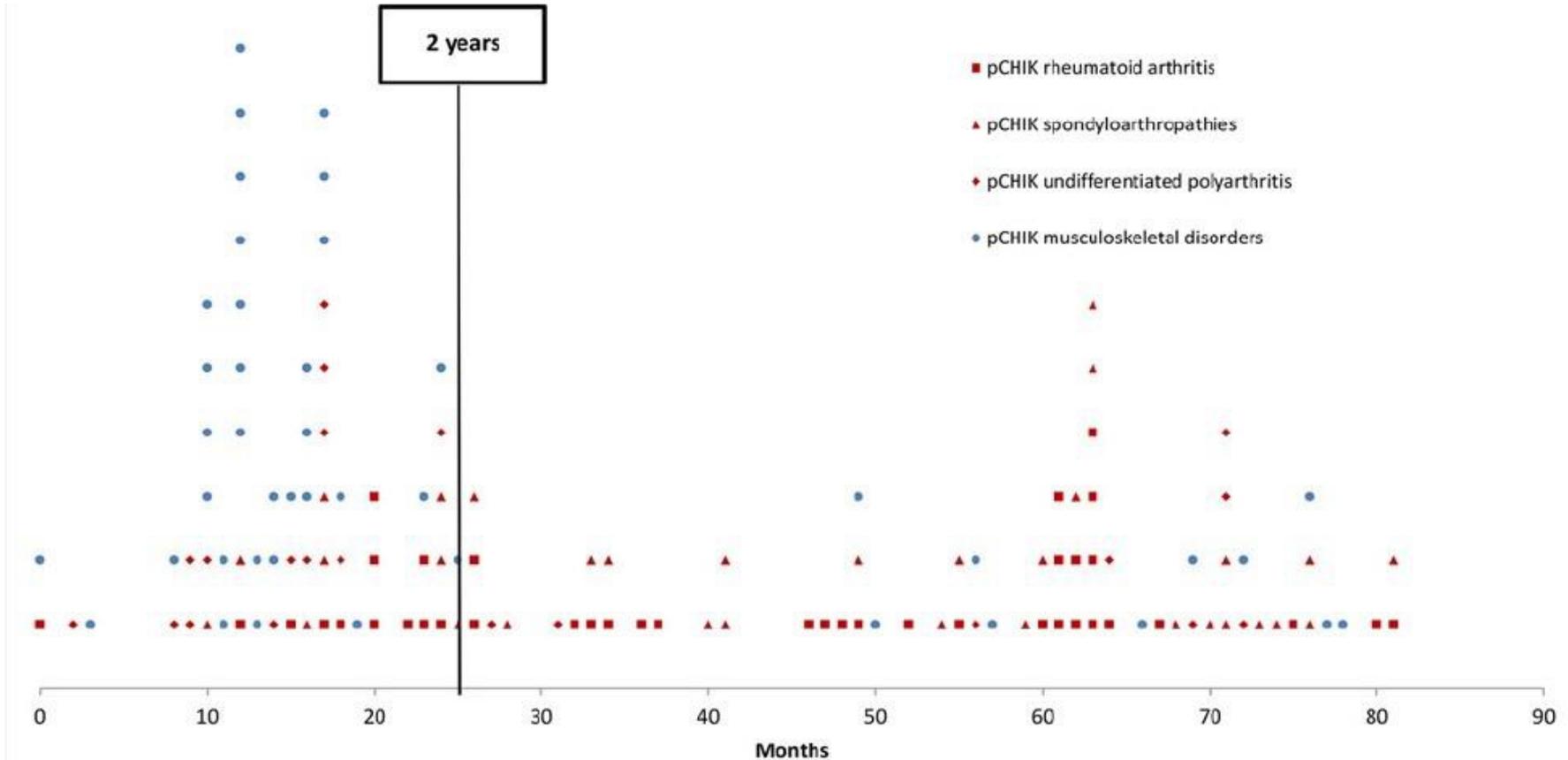


pCHIK: post-chikungunya;
 CIR: chronic inflammatory rheumatism,
 MSD: musculoskeletal disorders,
 RA: rheumatoid arthritis,
 SA: spondylarthropathy,
 UP: undifferentiated polyarthritis.

Nosologic flow-chart of patients referred to a rheumatologist for post-chikungunya (pCHIK) persistent rheumatic musculoskeletal pain, Saint-Denis, Reunion Island, 2006–2012.

Javelle, et al, PLoS Negl Trop Dis, 2015

Post-chikungunya rheumatic disorders



Time elapsed between chikungunya (CHIK) infection and the first visit to a rheumatologist for rheumatic or musculoskeletal disorders, Saint-Denis, Reunion Island, 2006–2012: musculoskeletal disorders versus chronic inflammatory rheumatisms.

Javelle, et al, PLoS Negl Trop Dis, 2015

Diagnosis

- **Isolation** (BL3) of CHIKV, confirm by IFA, RT-PCR, sequencing
- Detection of CHIKV **RNA** by real time RT-PCR.
- Identification of a positive **IgM** result + acute **symptoms**, followed by the demonstration of CHIKV-specific antibody determined by **PRNT**
- **Seroconversion** or a four-fold rise in PRNT, HI, or ELISA titers

Figure 2. Viremia and immune response following Chikungunya virus infection.

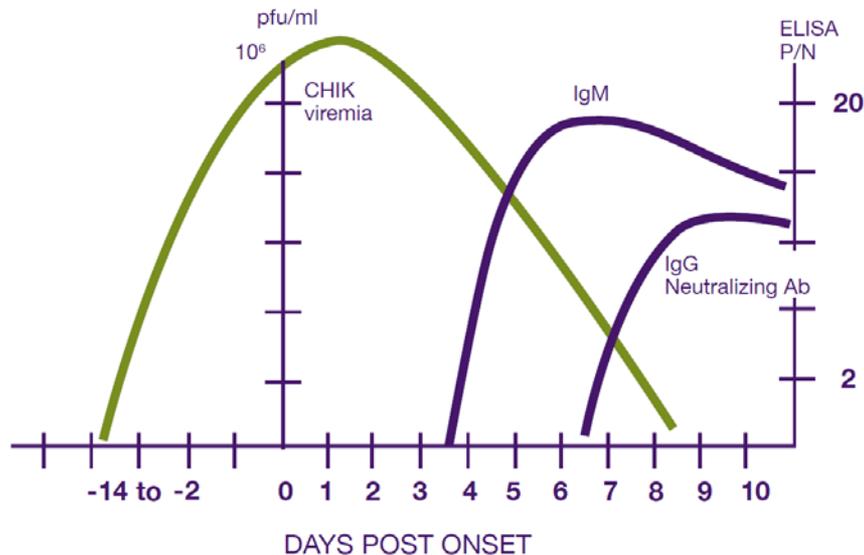


Table 6. Typical results of samples tested at various time points post-infection.

Days post illness onset	Virus testing	Antibody testing
Day 1-3	RT-PCR = Positive Isolation = Positive	IgM = Negative PRNT = Negative
Day 4-8	RT-PCR = Positive Isolation = Negative	IgM = Positive PRNT = Negative
>Day 8	RT-PCR = Negative Isolation = Negative	IgM = Positive PRNT = Positive

Treatment – Acute Infection

- Symptomatic,
- Supportive,
- Collect specimens for diagnostic testing,
- Acetaminophen for initial fever and pain,
- Consider using narcotics,
- If dengue possible, do not use aspirin or NSAIDs (e.g., ibuprofen, naproxen, toradol) until they have been afebrile ≥ 48 hours and have no warning signs for severe dengue

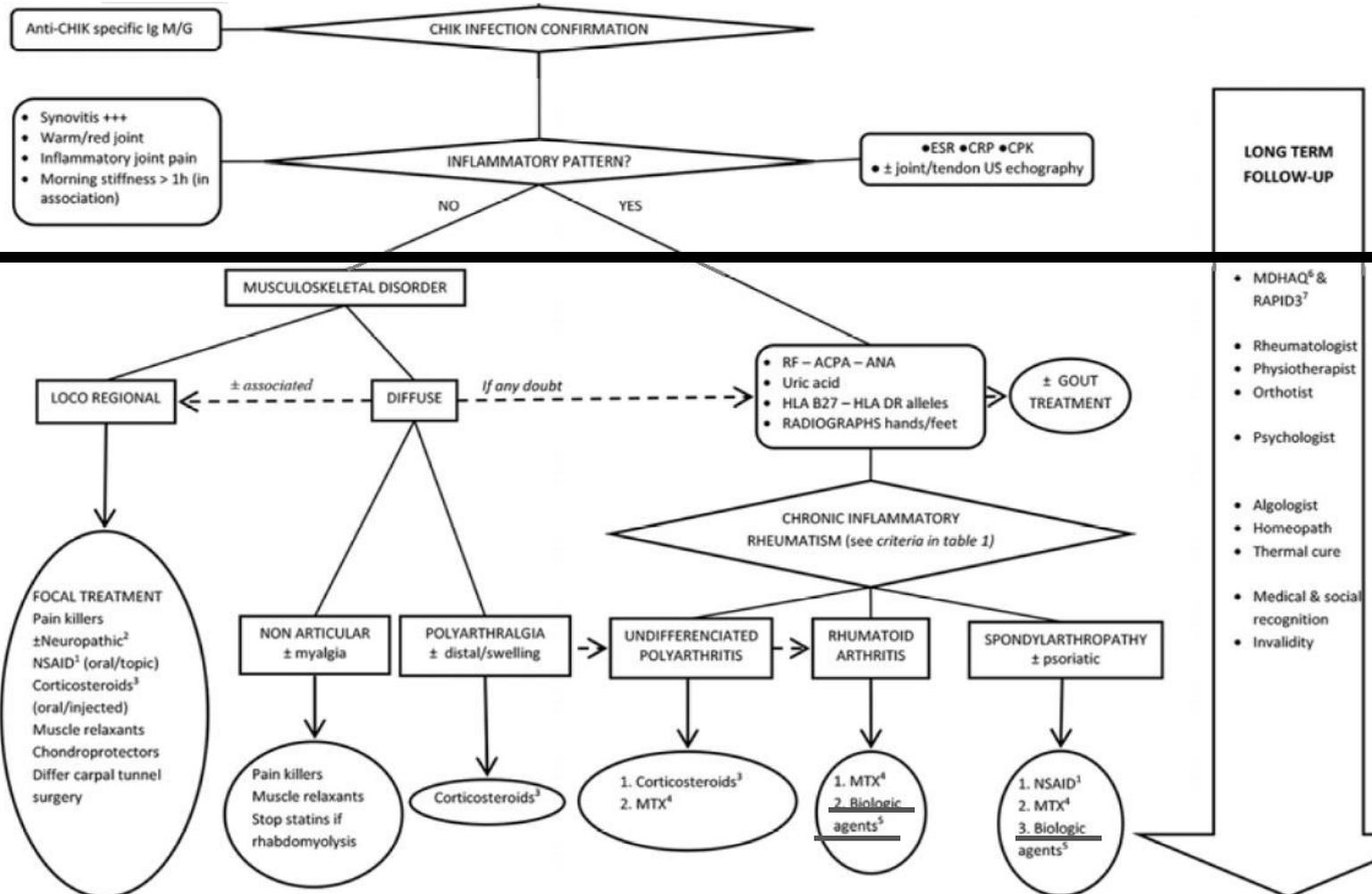
Treatment - Acute Infection

Direct-acting antivirals against chikungunya virus.

Antiviral agent	Mechanism of action	<i>In vitro</i> efficacy	<i>In vivo</i> efficacy
Chloroquine	Inhibition of fusion of the viral E1 protein with the endosomal membrane by raising the endosomal pH	Inhibition of CHIKV infection in Vero A cells	<u>No significant efficacy</u> in a macaque model or <u>clinical trials</u> in CHIKV infected patients
Ribavirin	Inhibition of viral genome replication, mostly via GTP pools depletion	Inhibition of CHIKV replication in Vero cells (EC50 = 341 μM). <u>Synergistic inhibitory effect in combination with IFN-α2b and doxycycline</u>	Reduced the viral load and inflammation in infected ICR mice when combined with doxycycline
6-Azauridine	Inhibition of orotidine monophosphate decarboxylase enzyme (depletion of UTP pools)	Inhibition of CHIKV replication in Vero cells (EC50 = 0.82 μM)	Not determined
Favipiravir (T-705)	Inhibition of viral genome replication	Inhibition of CHIKV-induced CPE in Vero A cells (EC50 = 25 μM)	Reduction of the mortality rate in infected AG129 mice with >50% and protection from neurological disease
Monoclonal antibody C9	Interaction with CHIKV E2 glycoprotein	Neutralization of CHIKV pseudovirions in HEK293T cells and replication-competent CHIKV in Vero cells	As prophylaxis: complete protection of infected C57BL/6 mice from arthritis and viremia As therapy: 100% survival of CHIKV infected mice when given at 8 or 18 h post infection

Abdelnabi, Antiviral Research 121 (2015) 59–68

Treatment - Chronic Symptoms



etanercept, infliximab, adalimumab, golimumab, abatacept, tocilizumab

Military Implications

An Analysis of Fevers of Unknown Origin in American Soldiers in Vietnam

JOHN J. DELLER, JR., LT. COL., MC, USA, and PHILIP K. RUSSELL, MAJ., MC, USA
Long Binh, South Vietnam

TABLE 1. Final Diagnoses on 110 Cases, (81 Confirmed, 29 Remaining "Fevers of Unknown Origin" (FUOs))

Disease	Test*	Diagnoses Confirmed	
		no.	%†
Dengue	HI	31	28
Chikungunya	HI	10	9
Scrub typhus	Fluorescent antibody, Weil-Felix	9	8
Malaria	Smear	8	7
Scrub—malaria	(Above)	2	2
Miscellaneous diseases		11	10
Enteric diseases		10	9
Remaining FUOs		29	26

* HI = hemagglutination inhibition.

† Approximate percentage.

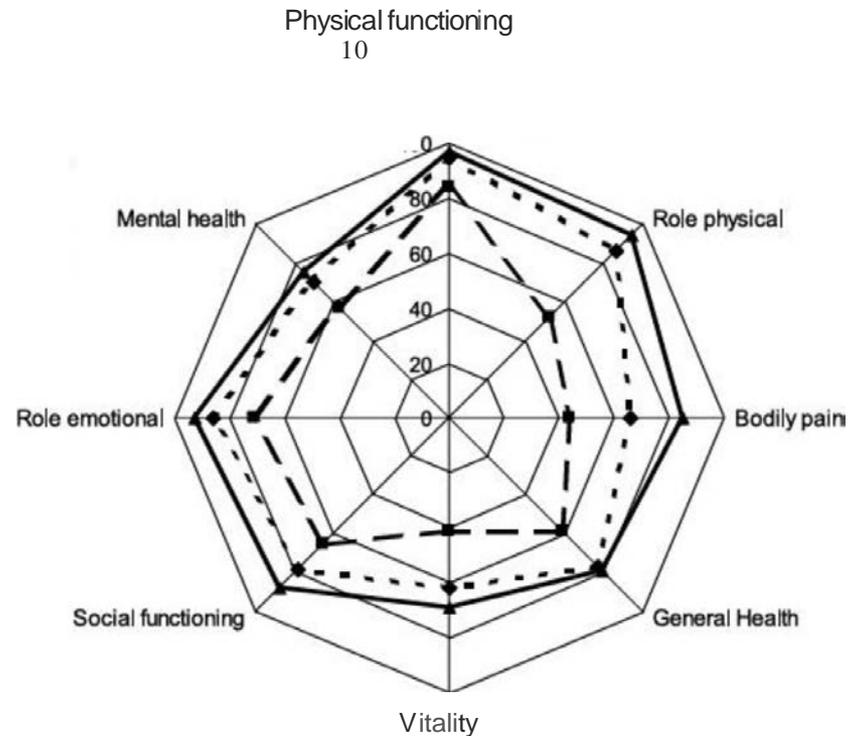
Morbidity and Impaired Quality of Life 30 Months After Chikungunya Infection

Comparative Cohort of Infected and Uninfected French Military Policemen in Reunion Island

Catherine Marimoutou, MD, PhD, Elodie Viviel MD, Manuela Oliver, PharmD, Jean-Paul Boutin, MD, and Fabrice Simon, MD, PhD

• Not healed CHIKV+ • • • Healed CHIKV+ CHIKV-

FIGURE 5. Quality of life in June 2008 according to the 8 components of the MOS-SF36 scale at 30 months among 382 gendarmes exposed to the 2006 chikungunya outbreak in Reunion Island.



Chikungunya in the US military

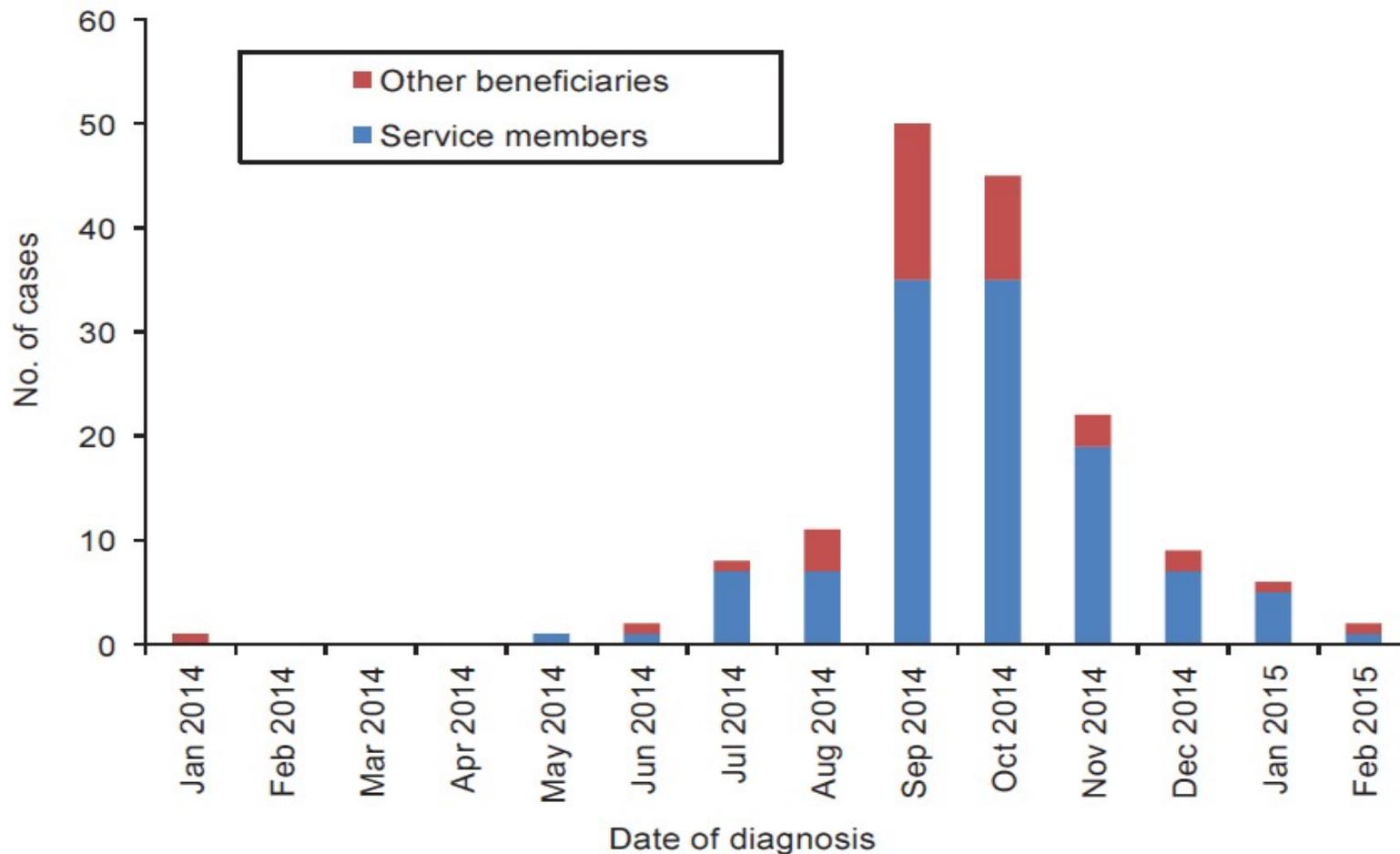
TABLE 2. Age and sex distribution of all DoD chikungunya cases among service members and other Department of Defense beneficiaries identified between 1 January 2014 and 28 February 2015

	Service members			Total
	Active component	Reserve component	Other beneficiaries	
Age				
0–20	0	0	7	7
21–25	5	2	3	10
26–30	12	11	1	24
31–35	14	12	7	33
36–40	10	14	3	27
41–45	8	11	3	22
46–50	1	7	4	12
51+	0	11	11	22
Sex				
Female	5	18	20	43
Male	45	50	19	114

Writer, MSMR. 2015 Oct;22(10):2-6.

Chikungunya in the US Military

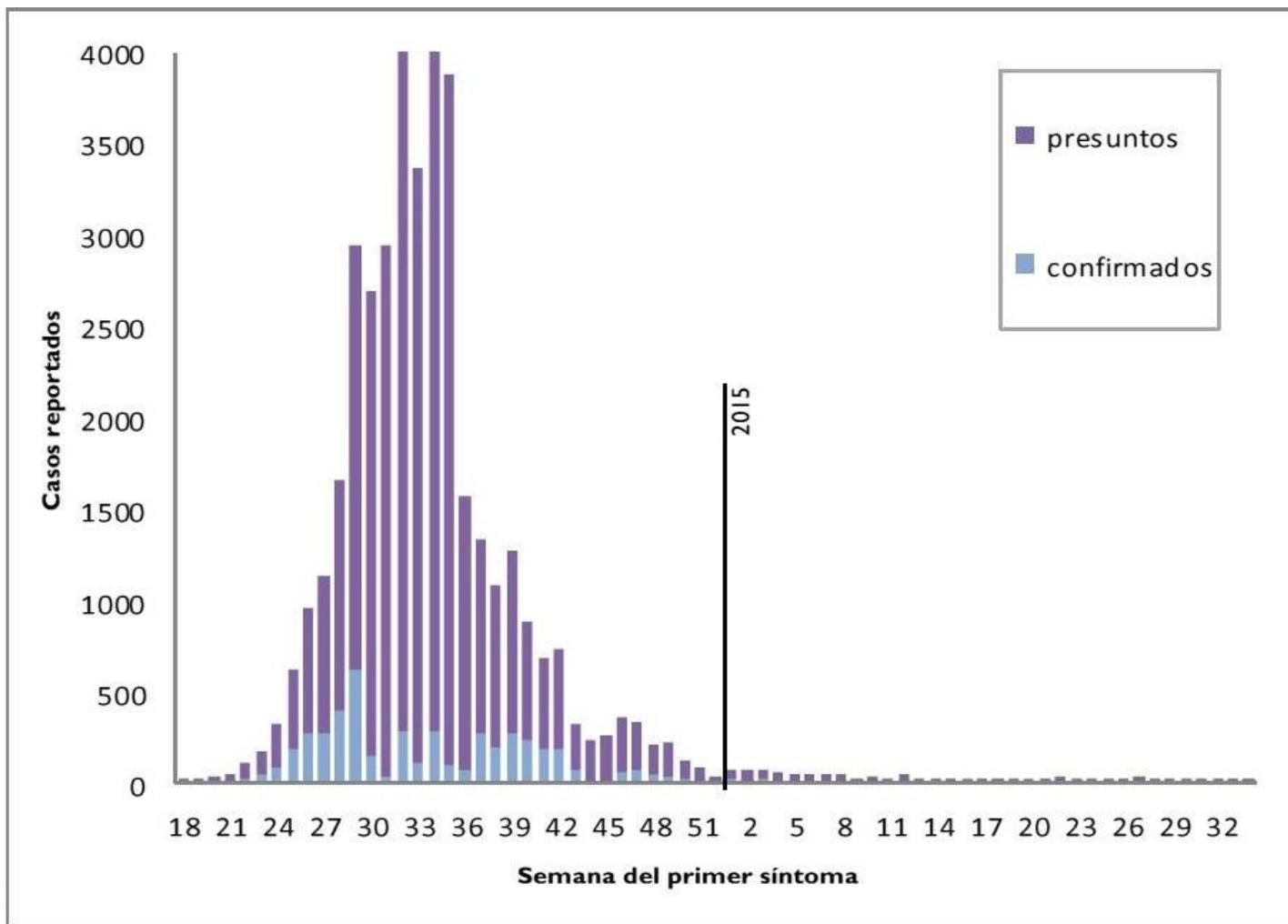
FIGURE. Month of diagnosis for all DoD chikungunya cases among service members and other DoD beneficiaries identified between 1 January 2014 and 28 February 2015



Chikungunya in the US Military



Chikungunya Epidemic in Puerto Rico

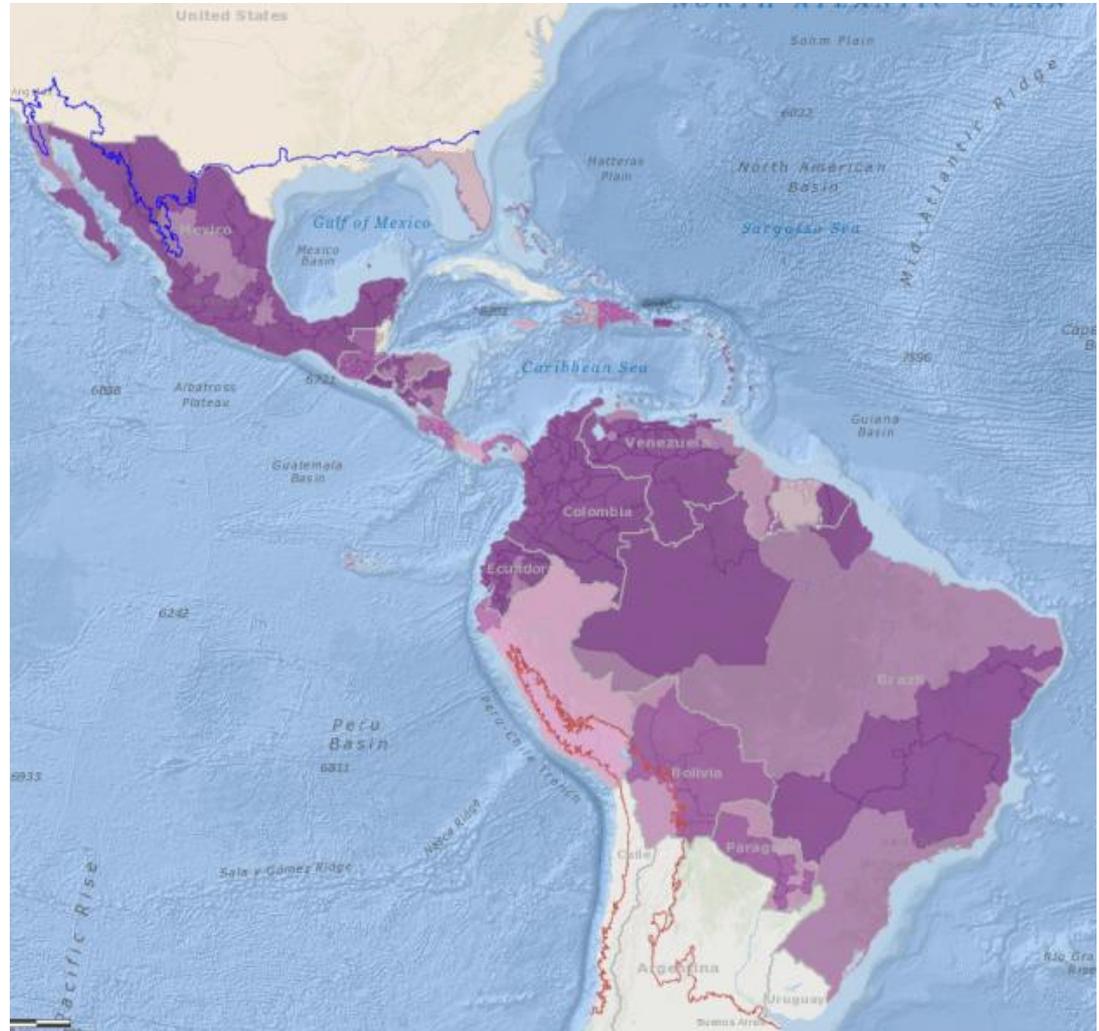


<http://www.salud.gov.pr/Estadisticas-Registros-y-Publicaciones/Pages/Chikungunya.aspx>

CHIKV in the Americas, 2013-15

<http://www.paho.org> , AFHS Chikungunya in the Americas Surveillance Summary #52, 12 Nov 2015

- Total cases:
 - 2014: 1,072,000
 - 2015: 617,000
- DoD cases:
 - 2014: 92
 - 2015: 12

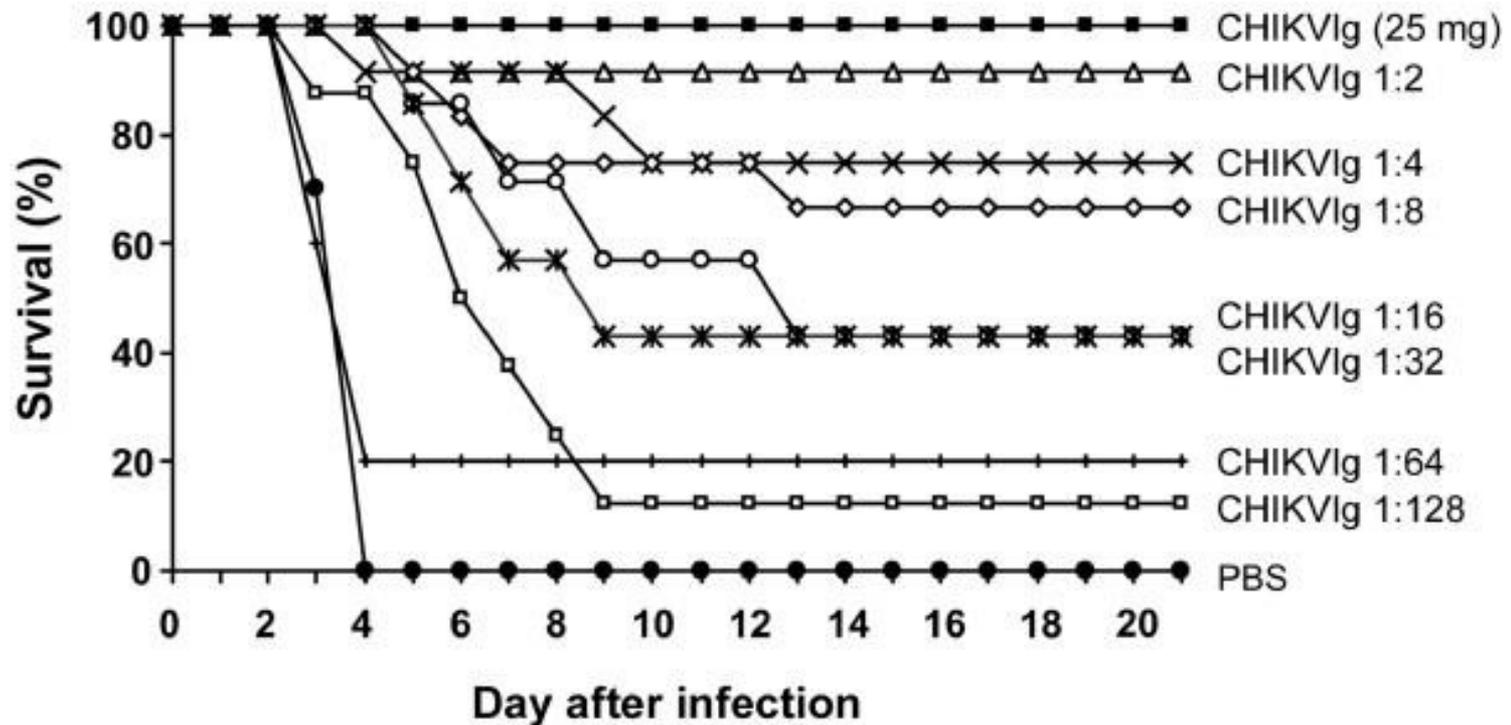


Prevention

- PPMs, avoidance
- Vector control
- Polyclonal and monoclonal antibodies
- Vaccines
 - Challenges
 - Sustaining commercial interest
 - Demonstrating efficacy

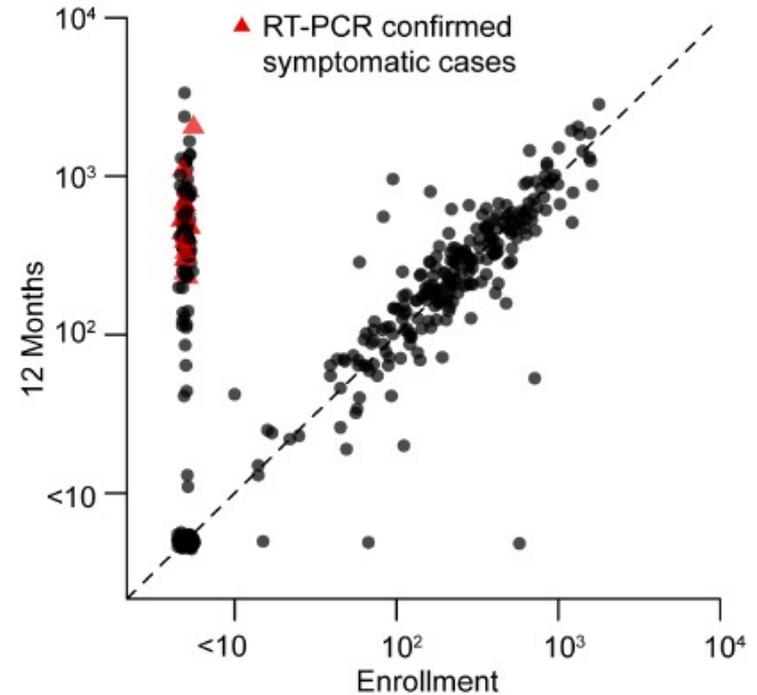
Neutralizing Antibody Correlates with Protection

- Transfer of anti-CHIKV antibodies protects susceptible mice in a dose-dependent fashion.



Neutralizing Antibody Correlates with Protection

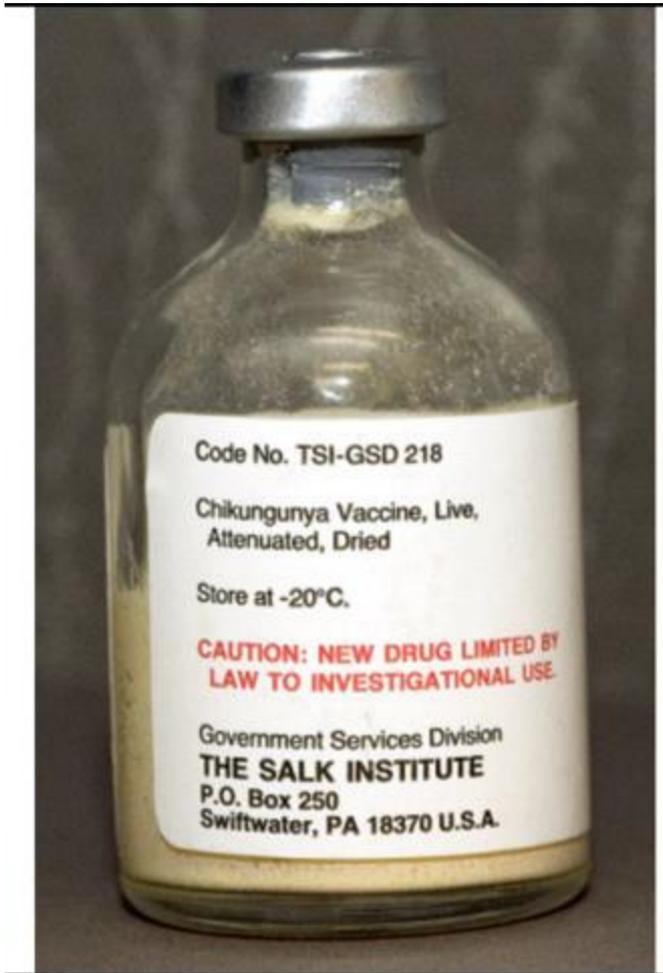
- Seroepidemiology studies in endemic areas support PRNT as a marker of protection
- “All 19 symptomatic and 87 subclinical CHIKV infections had enrollment PRNT titer <10.” Yoon, PLoS NTD, 2015



Relationship between symptomatic CHIKV infection and baseline enrollment CHIKV PRNT status.^a

Baseline CHIKV PRNT titer ^b	Symptomatic CHIKV infection present, n (%)	Symptomatic CHIKV infection absent, n (%)	Total, n (%)	% Protection when CHIKV PRNT ≥10 (95% CI)	P-value
<10	19 (3.1)	595 (96.9)	614 (100)	100.0 (46.1, 100.0)	0.003
≥10	0 (0)	239 (100)	239 (100)		

Chikungunya Vaccine Candidates



Code No. TSI-GSD 218

Chikungunya Vaccine, Live,
Attenuated, Dried.

Store at -20 C or lower

**CAUTION: NEW DRUG LIMITED BY
FEDERAL LAW TO INVESTIGATIONAL USE.**

THE SALK INSTITUTE
Government Services Division
P.O. Box 250
Swiftwater, PA 18370 U.S.A.

Chikungunya virus (CHIK 181/Clone 25), prepared in MRC-5 cell culture. After reconstitution, the vaccine contains less than 0.02 µg Neomycin base and 0.25g% human serum albumin U.S.P. per mL.

For reconstitution: Add 21 mL of Sterile Water for Injection, U.S.P.

Dose: 0.5 mL subcutaneously.

After reconstitution: Store at +4 C and use within 3 hours.

CAUTION: This vaccine vial contains live virus. Autoclave before discarding.

Table 4

Proportion of alphavirus naïve recipients of chikungunya vaccine that developed plaque reduction neutralizing antibody (PRNT_{50 or 80})^b antibody, and geometric means of maximum titers.

Study	Number with antibody/number of alphavirus naïve recipients	% Developing neutralizing antibody between days 15 and 32	Geometric mean of maximum titer on days 11–38 (95% CI)
A	29/30	97	305 (189–494)
C	19/19	100	297 ^(a)
D	3/3	100	640 (114–3580)
F	21/21	100	110 ^(a)
G	57/58	98	582 ^(a)
Total	131	98	

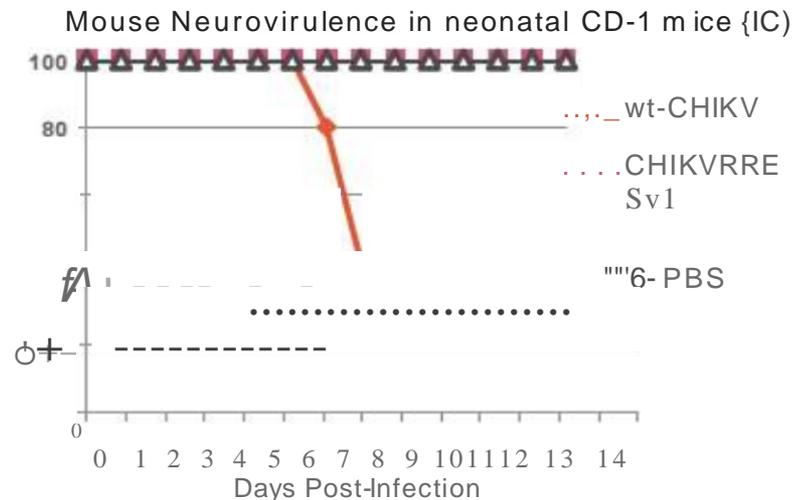
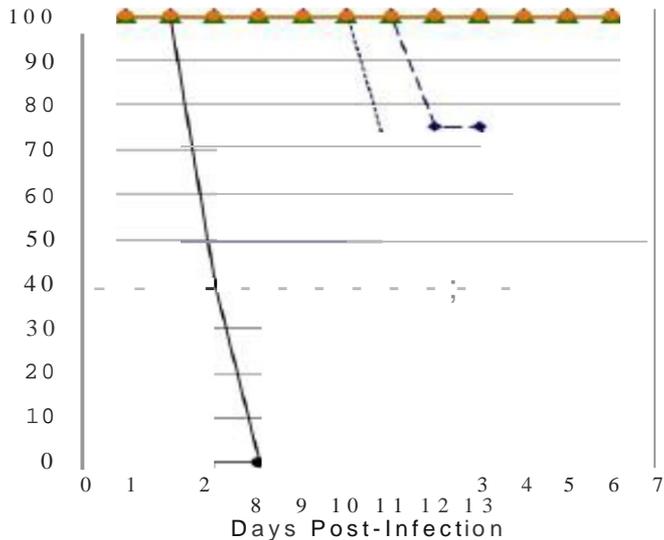
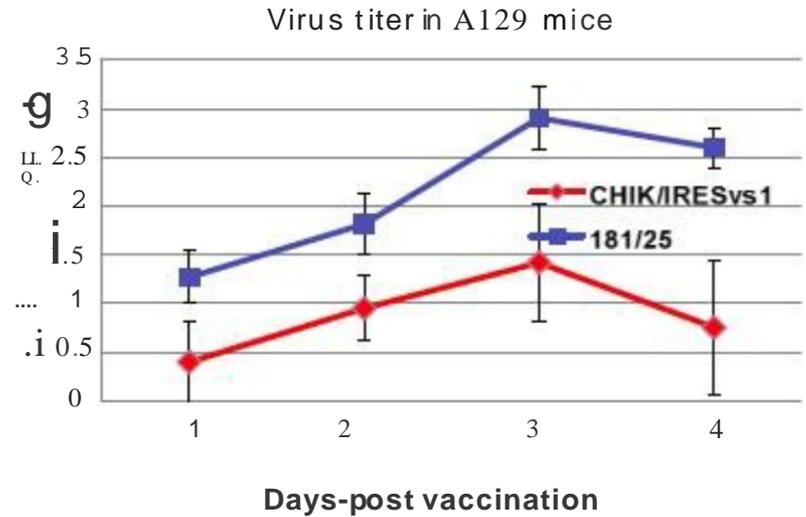
^a Not available because only GMT data (not individual data) reported.

^b PRNT 80 for trials A, C, D, and F, and PRNT 50 for trial G.

Preclinical Safety data in the A129 mouse model

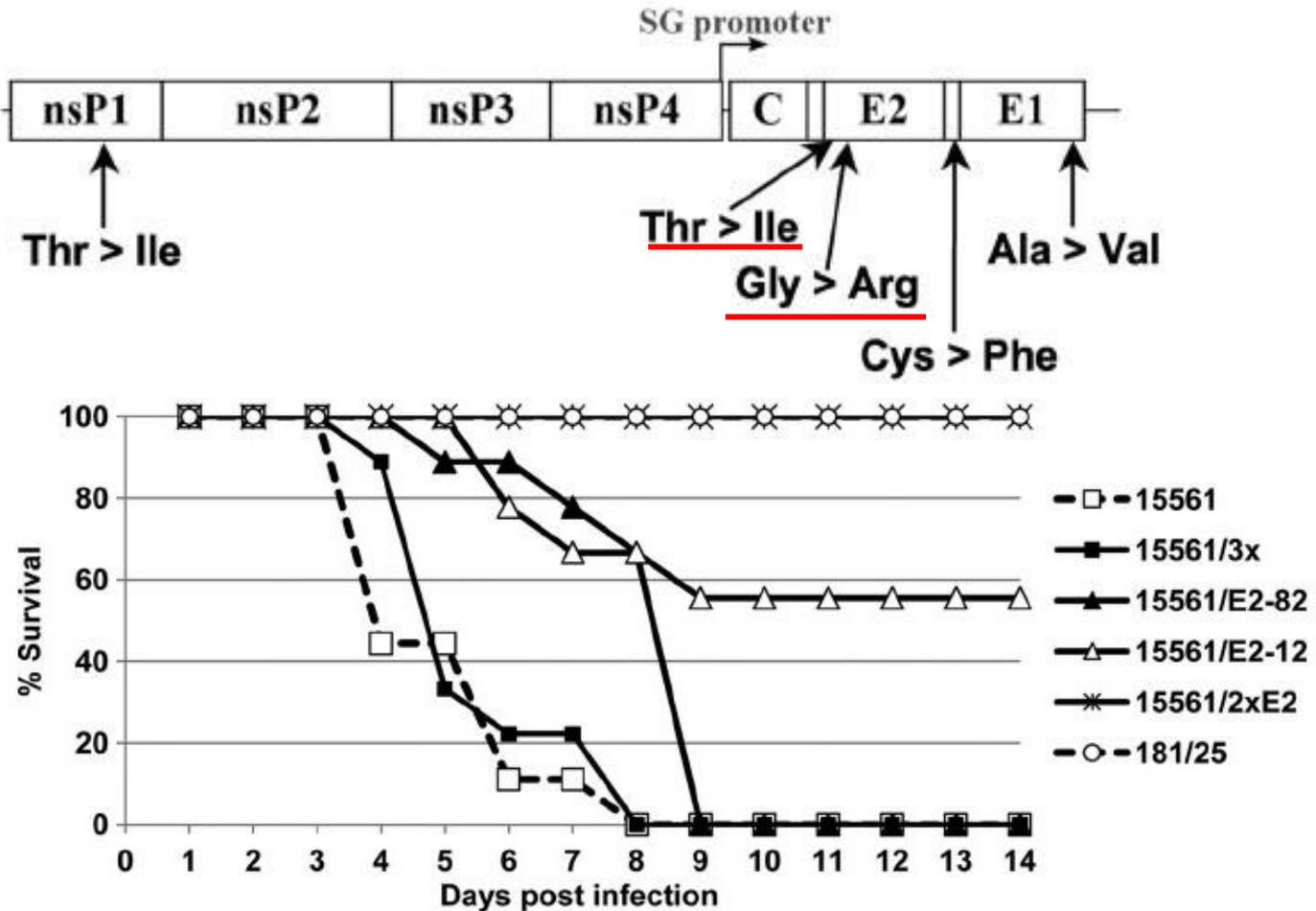


- A129 mice immunized in footpad with 10^4 PFU 181/25 or candidate Chikungunya vaccine
- Chikungunya vaccine remained stable after 5 serial mouse brain passages in 2 parallel lines.
- No consensus mutations found
In contrast, 181/25 obtained several mutations and developed a lethal phenotype



Takeda Data on file: unpublished

Attenuation of 181/25 Rests on Two Mutations



Survival of 5- to 8-week-old A129 mice ($n = 9$) after ID infection with 10^4 PFU of CHIKV strain AF15561, mutants, or vaccine strain 181/25.

The Fate of 181/25 as a Vaccine

- “Further development was discontinued because of side effects in 8% of volunteers...” Ahola, 2015.
- “The 181/25 strain is attenuated by only two point mutations, and reversions in vaccinated mice suggest that genetic instability explains its underlying reactogenicity...” Weaver, 2012.
- “Although a few vaccinated individuals developed transient arthralgia, the vaccine was thought to be generally safe and efficacious. However, further assessment of this vaccine was discontinued by the US Army in 2000 because research priorities changed.” Burt, Lancet, 2012.

Chikungunya Vaccine Pipeline Assessment

courtesy David Beasley, UTMB

Bharat Biotech	Inactivated (various strains, various methods)	X		
Indian Immunological	Inactivated (formalin-treated 181/25 from US Army)	X	2016?	
DRDE, India	Inactivated (formalin treated Indian 2006 isolate)	X		
Nanotherapeutics Inc. (from Baxter)	Inactivated	X		
Medigen	DNA (plasmid-launched 181/25 live attenuated)	X		
DRDE, India	Recombinant subunit (E. coli expressed E1/E2)	X		
National Inst. Virology, India	Recombinant subunit (E. coli expressed E2)	X		
Valneva/Karolinska Inst.	Live, attenuated (CHIKV-Δ5nsP3)	X		
Takeda/UTMB	Live, attenuated (CHIK/IRES)	X		
Arbovax/NC State Univ	Live, attenuated (transmembrane deletion)	X		
UTMB	Live, attenuated chimeric (various alphavirus backbones)	X		
Themis Bioscience/Inst. Pasteur	Live, vectored (measles virus)		X	
Profectus/Yale/UTMB	Live, vectored (VSVΔG-CHIKV)	X		
Karolinska Inst./CSIC Madrid	Live, vectored (MVA-CHIKV E1E26KE3)	X		
Univ. Wisconsin/Takeda	Live, vectored (MVA-CHIKV E2E3)	X		
NIAID/Leidos Biomed	Virus-like particle		X	
TI Pharma/Wageningen Univ.	Virus-like particle	X		
Merck	Virus-like particle	X		
Bharat Biotech	Inactivated (various strains, various methods)	X		

Formalin Inactivated Vaccine

Serologic responses of volunteers without prior CHIK experience to administration of two doses of CHIK vaccine

Group (Dosage Schedule)		Mean Responses by Day after First Dose (Range)			
		0	14	28	42
<i>ml</i> I (0.5 + 0.5)	HI ^a	<10	18 (<10-80)	15 (<10-40)	49 (<10-320)
	LNI ^b	0	1.8 (0.7-2.7)	1.9 (1.3-3.0)	2.7 (2.0-3.5)
	CF ^a	2 (<4)	2 (<4-8)	2 (<4-4)	7 (<4-16)
II (1.0 + 1.0)	HI	<10	11 (<10-20)	9 (<10-10)	26 (10-80)
	LNI	0	1.9 (1.3-2.5)	1.9 (1.0-3.0)	1.7 (2.0-3.5)
	CF	2 (<4)	2 (<4-8)	3 (<4-8)	6 (<4-8)

^a HI and CF test results are reciprocals of geometric mean titers.

^b Log₁₀ serum neutralization index.

Formalin-Inactivated Vaccine

- The Army vaccine was an inactivated virus, a well-tested approach used for many other vaccines...
- “There’s no reason that it couldn’t be done fairly easily; it’s a proven technology.” Weaver says. “But I think there’s an emphasis now on newer technologies.” Also, a protocol published decades ago can probably no longer be patented, rendering commercial backing unlikely.

Alan Dove, Nature Medicine 21, 1107 (2015)

Formalin Inactivated Chikungunya

Asian Journal of Infectious Diseases (1979) 3 : 119-124

Original Paper.

Persistence in Humans of Antibody After Immunization with Four Alphavirus Vaccines*

JOSEPH L DeMEIO, ARMAND N DeSANCTIS and WILLIAM J THOMAS

The Salk Institute, Government Services Division, P O Box 250, Swiftwater, Pennsylvania 18370, U S A

Virus	CHIK.	
	Pre serum @7 yrs. 9 mos.	Post serum @7 yrs. 11 mos.
VEE	70	50
EEE	<10	10
WEE	<10	<10
CHIK.	<10	<10

Chikungunya Vaccine Pipeline Assessment

courtesy David Beasley, UTMB

Bharat Biotech	Inactivated (various strains, various methods)	X			
Indian Immunological	Inactivated (formalin-treated 181/25 from US Army)	X	2016?		
DRDE, India	Inactivated (formalin treated Indian 2006 isolate)	X			
Nanotherapeutics Inc. (from Baxter)	Inactivated	X			
Medigen	DNA (plasmid-launched 181/25 live attenuated)	X			
DRDE, India	Recombinant subunit (E. coli expressed E1/E2)	X			
National Inst. Virology, India	Recombinant subunit (E. coli expressed E2)	X			
Valneva/Karolinska Inst.	Live, attenuated (CHIKV- Δ 5nsP3)	X			
Takeda/UTMB	Live, attenuated (CHIK/IRES)	X			
Arbovax/NC State Univ	Live, attenuated (transmembrane deletion)	X			
UTMB	Live, attenuated chimeric (various alphavirus backbones)	X			
Themis Bioscience/Inst. Pasteur	Live, vectored (measles virus)		X		
Profectus/Yale/UTMB	Live, vectored (VSV Δ G-CHIKV)	X			
Karolinska Inst./CSIC Madrid	Live, vectored (MVA-CHIKV E1E26KE3)	X			
Univ. Wisconsin/Takeda	Live, vectored (MVA-CHIKV E2E3)	X			
NIAID/Leidos Biomed	Virus-like particle		X		
TI Pharma/Wageningen Univ.	Virus-like particle	X			
Merck	Virus-like particle	X			
Bharat Biotech	Inactivated (various strains, various methods)	X			

Immunogenicity, safety, and tolerability of a recombinant measles-virus-based chikungunya vaccine: a randomised, double-blind, placebo-controlled, active-comparator, first-in-man trial

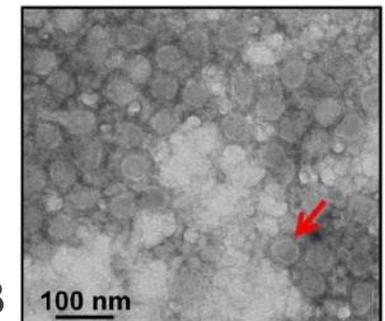
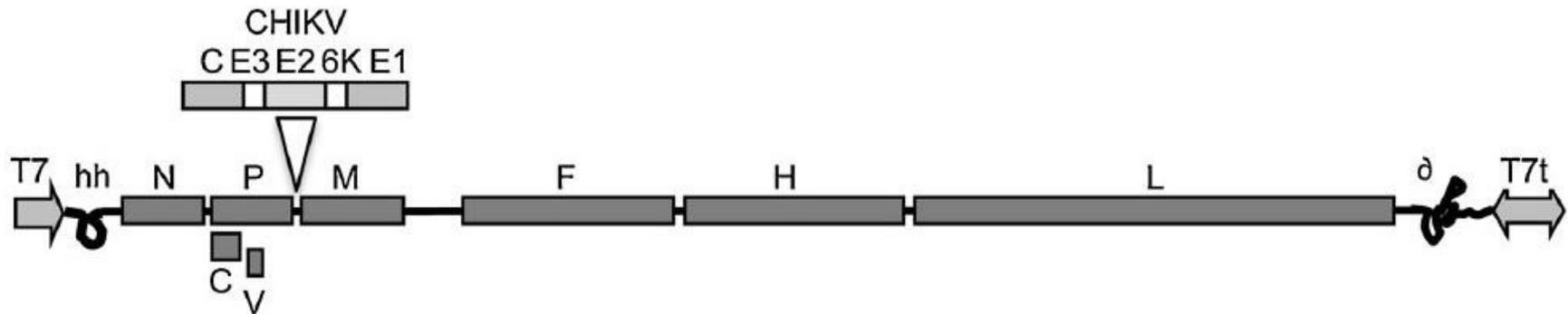
Katrin Ramsauer¹, Michael Schwameis², Christa Firbas³, Matthias MÖHNER⁴, Robert Putnaf.⁵; Stephen Thomas, Philippe Despres, Erich Tauber, Bernd JilmU Frederic Tangy

Findings Between Nov 22, 2013, and Feb 25, 2014, we randomly assigned 42 participants to receive the low dose (n=12), the medium dose (n=12), or the high dose (n=12) of the measles-virus-based candidate vaccine, or Priorix (n=6), of whom 36 participants (86%; n=9, n=12, n=10, n=5, respectively) were included in the per-protocol population. The candidate vaccine raised neutralising antibodies in all dose cohorts after one immunisation, with seroconversion rates of 44% (n=4) in the low-dose group, 92% (n=11) in the medium-dose group, and 90% (n=10) in the high-dose group. The immunogenicity of the candidate vaccine was not affected by pre-existing anti-measles immunity. The second vaccination resulted in a 100% seroconversion for all participants in the candidate vaccine groups. The candidate vaccine had an overall good safety profile, and the rate of adverse events increased with vaccine dose and volume. No vaccination-related serious adverse events were recorded.

Interpretation The live recombinant measles-virus-based chikungunya vaccine had good immunogenicity, even in the presence of anti-vector immunity, was safe, and had a generally acceptable tolerability profile. This vaccine is the first promising measles-virus-based candidate vaccine for use in human beings.

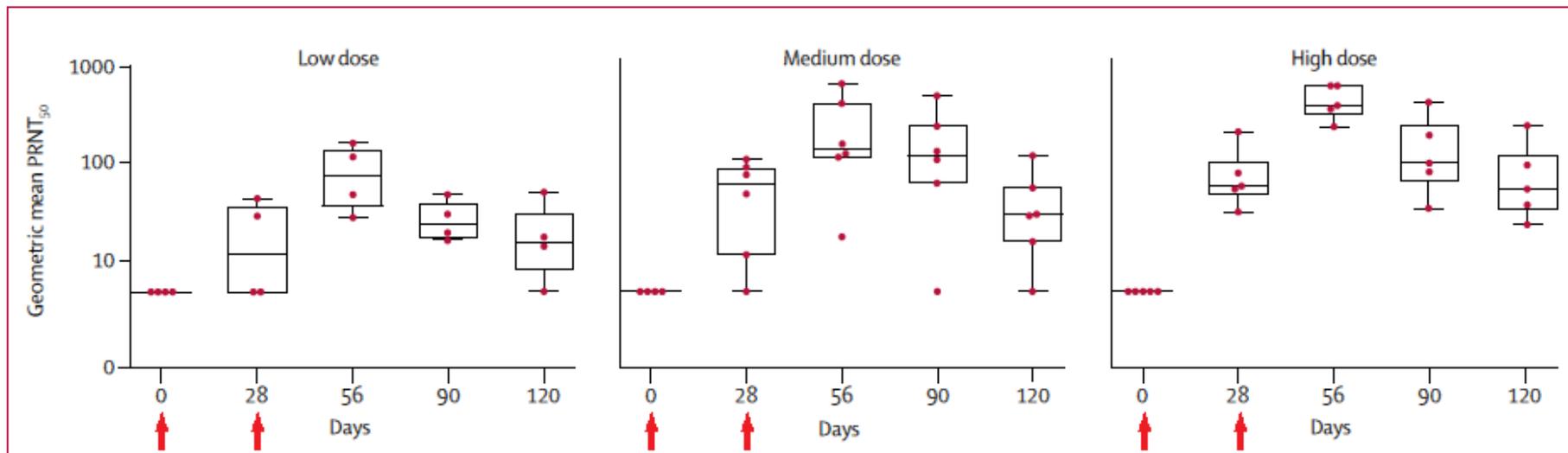
Measles-Vectored CHIK Vaccine

- Chikungunya structural genes inserted into measles vaccine strain backbone.
- Replication and VLP production occur after immunization.



Brandler et al, Vaccine, 2013

Immunogenicity of MV-CHIK



Dose-dependent increases in geometric mean titers (95%CI) from days 0 to 120 in the per-protocol population

Ramsauer, 2015

Immunogenicity of MV-CHIK

- MV-CHIK APPEARS TO BE SAFE AND TOLERABLE WHEN GIVEN IN LESS THAN 1 ML
 - MV-CHIK VACCINE INDUCED NEUTRALIZING ANTIBODIES AGAINST CHIKUNGUNYA AFTER A SINGLE SHOT
 - INCREASE IN DOSE IMPROVED THE SEROCONVERSION RATE AND GMT
-
- SECOND IMMUNIZATION BOOSTED CHIKUNGUNYA NEUTRALIZING TITER IN ALL TREATMENT GROUPS
 - PRE-EXISTING ANTI-VECTOR (MEASLES) IMMUNITY DID NOT INTERFERE WITH RESPONSE TO THE CHIKUNGUNYA ANTIGENS

Chikungunya Vaccine Pipeline Assessment

Bharat Biotech	Inactivated (various strains, various methods)	X			
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Merck	Virus-like particle	X			
Bharat Biotech	Inactivated (various strains, various methods)	X			

Safety and tolerability of chikungunya virus-like particle vaccine in healthy adults: a phase 1 dose-escalation trial

Lee-jah Chang, Kimberly A Dowd*, Horeliz H Mendoza, Jamie G Saunders, Sandra Sitar, Sarah H Plummer, Galina Yamshchikov, Uzma N Sarwar, Zonghui Hu, Mary E Enama, Robert T Bailer, Richard A Koup, Richard M Schwartz, Wataru Akahata, Gary J Nabel, John R Mascola, Theodore Pierson, Barney S Graham, Julie E Ledgerwood, and the VRC311 Study Team*

Findings 25 participants were enrolled from Dec 12, 2011, to March 22, 2012, into the three dosage groups: 10 µg (n=5), 20 µg (n=10), and 40 µg (n=10). The protocol was completed by all five participants at the 10 µg dose, all ten participants at the 20 µg dose, and eight of ten participants at the 40 µg dose; non-completions were for personal circumstances unrelated to adverse events. 73 vaccinations were administered. All injections were well tolerated, with no serious adverse events reported. Neutralising antibodies were detected in all dose groups after the second vaccination (geometric mean titres of the half maximum inhibitory concentration: 2688 in the 10 µg group, 1775 in the 20 µg group, and 7246 in the 40 µg group), and a significant boost occurred after the third vaccination in all dose groups (10 µg group $p=0.0197$, 20 µg group $p<0.0001$, and 40 µg group $p<0.0001$). 4 weeks after the third vaccination, the geometric mean titres of the half maximum inhibitory concentration were 8745 for the 10 µg group, 4525 for the 20 µg group, and 5390 for the 40 µg group.

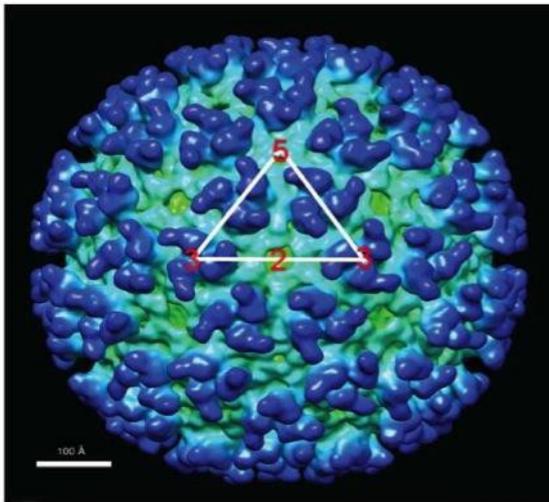
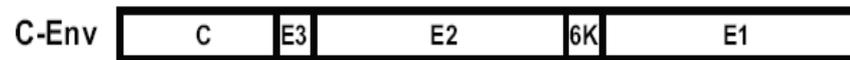
Interpretation The chikungunya VLP vaccine was immunogenic, safe, and well tolerated. This study represents an important step in vaccine development to combat this rapidly emerging pathogen. Further studies should be done in a larger number of participants and in more diverse populations.

CHIK Virus-like Particles (VLPs)

CHIKV Genome

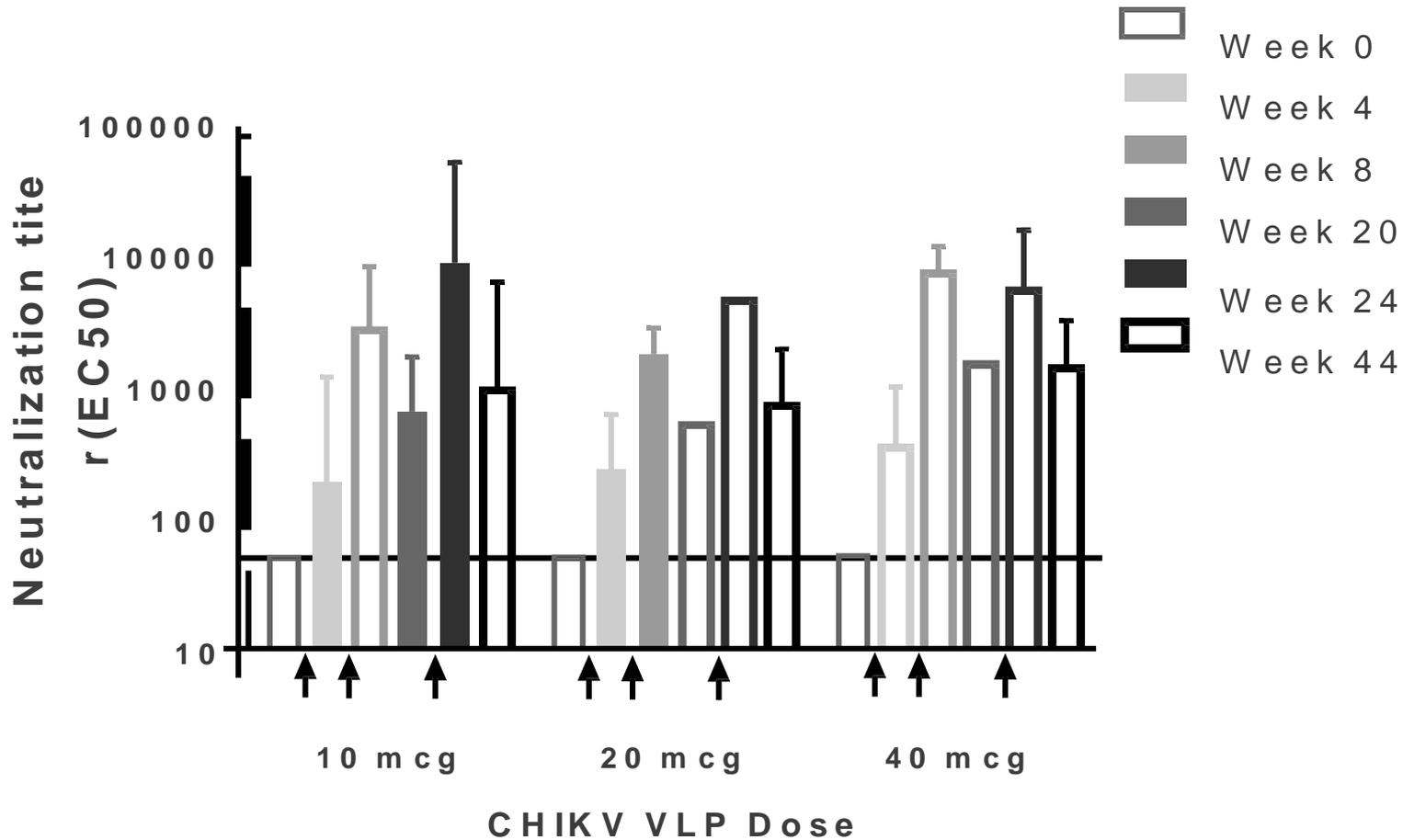


VLP construct:



- Advantages of the VLP vaccine platform
 - Express fraction of genome as a particle
 - Highly symmetric and \resembles wild type virus
 - Efficiently recognized by immune system
 - Elicits high titer neutralizing antibody
 - Safety
 - Other VLPs licensed and safe in humans
 - VLP does not replicate

VLP Immunogenicity



Antibody Titers

	ELISA titre (strain 37997)			Neutralisation IC ₅₀ titre (strain OPY1)		
	10 µg group (n=5)	20 µg group (n=10)	40 µg group (n=10)	10 µg group (n=5)	20 µg group (n=10)	40 µg group (n=10)
0*†	50 (50-50)	51 (49-52)	52 (50-54)
4*	160 (19-1317)	278 (98-788)	424 (134-1338)	188 (30-1179)	236 (90-614)	346 (120-999)
8	3378 (358-31856)	5881 (2026-17077)	20480 (12144-34539)	2688 (885-8166)	1775 (1129-2791)	7246 (4512-11637)
20*	2560 (758-8645)	1114 (557-2229)	4740 (1852-12133)	650 (251-1680)	510 (288-901)	1485 (831-2655)
22	31042 (14378-67021)	13512 (4852-37626)	14482 (4362-48073)	NA	NA	NA
24	40960 (40960-40960)	15521 (6058-39763)	34443 (22862-51890)	8745 (1514-50516)	4525 (2252-9093)	5390 (1865-15573)
44	4457 (442-44860)	5881 (2026-17077)	8611 (2730-27161)	940 (141-6254)	717 (267-1927)	1385 (605-3171)

Data are the geometric mean titre (95% CI). All available samples were used for each reported result. IC₅₀=half maximum inhibitory concentration. NA=not assessed. *Visit at which vaccine was administered. †For ELISA, week 0 values were used to background correct titres for subsequent weeks.

Convalescent IC₅₀ titers:

- 7057
- 4227

VLP Phase 1 Summary

VRC CHIKV VLP vaccine was safe and immunogenic

- Well tolerated
- No arthralgia
- Immunogenic after 1st injection at all 3 doses
 - Boosted with 2nd and 3rd injections
- High titer Ab responses at all doses
- Neutralized outbreak strain OPY-1
 - more divergent from the vaccine strain than Asian/Caribbean strain
- Comparable responses to convalescent human sera
 - suggesting responses are in the protective range

Phase II Trial in Endemic Population

VRC 704: CHIK VLP Vaccine

- 6 Caribbean sites
- 400 healthy adults (18-60 yrs)
- 2 IM injections (Day 0 and 28)
 - n=200 20 mcg vaccine
 - n=200 PBS placebo
- 7 follow-up visits over 72 weeks
- Screening began Oct 2015



Demonstrating Vaccine Efficacy

- “Vaccine developers are required to make inferences about a candidate's potential for clinical benefit based on its ability to protect a nonhuman primate from viremia after wild-type challenge and by measuring immunogenicity in small to moderate-sized phase 1 and 2 studies.
- “Vaccinologists require development tools, which more accurately predict an immune profile's ability to prevent or significantly attenuate disease after natural infection.”

Cassetti MC, Thomas SJ,
Dengue human infection model: introduction.
J Infect Dis. 2014.

Human Infection Models

- Dengue
 - Under-attenuated vaccine strains given to reproduce mild disease.
 - – Given high rate of asymptomatic viremia, may want a model that produces disease.
 - Potential for severe disease?
- Chikungunya
 - Under-attenuated vaccine strain given to reproduce viremia.
 - – Given low rate of asymptomatic viremia, may not need a model that produces disease
 - Potential for long-term side effects?

Re-enter 181/25

- Given to >200 volunteers with no acute SAE's.
- Transient arthralgias seen in some studies.
- More than 1/3 with detectable viremia by cell culture.
- Use of PCR will further increase sensitivity.
- Lower doses will likely also produce viremia and can increase the margin of safety.
- Concerns about long-term pathology?

Chikungunya Vaccines Summary

- Four vaccine candidates have been in phase 1 clinical trials.
 - 181/25 safe and immunogenic, but viremia, arthralgia, and potential for reversion to wild-type are legitimate concerns.
 - Mixed data on formalin-inactivated CHIKV.
 - Further studies are planned in 2016.
 - Virus-like particle vaccines appear safe, immunogenic.
 - MV-CHIK to be evaluated at higher doses.
 - Both VRC CHIKV VLP and MV-CHIK to be evaluated in endemic areas in 2016.

CHIKV Human Challenge Model Summary

- A human challenge model has the potential to accelerate our understanding of immune protection and our ability to demonstrate vaccine efficacy.
- A live attenuated vaccine strain, 181/25, is available.
 - Given to >200 volunteers without acute SAE.
 - Reliably produces viremia.
 - Arthralgia, potential for reversion to wild-type are legitimate concerns.
 - Risks versus benefits.

Questions?

