West Nile and Dengue virus

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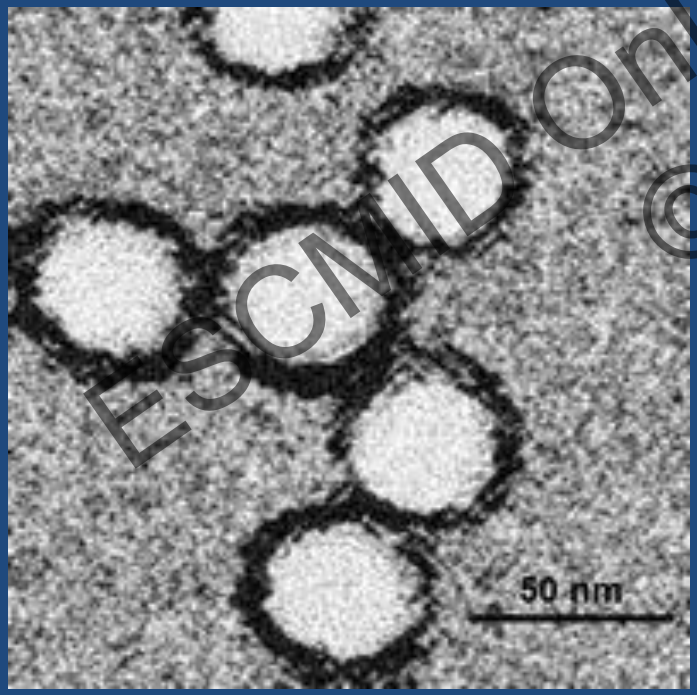
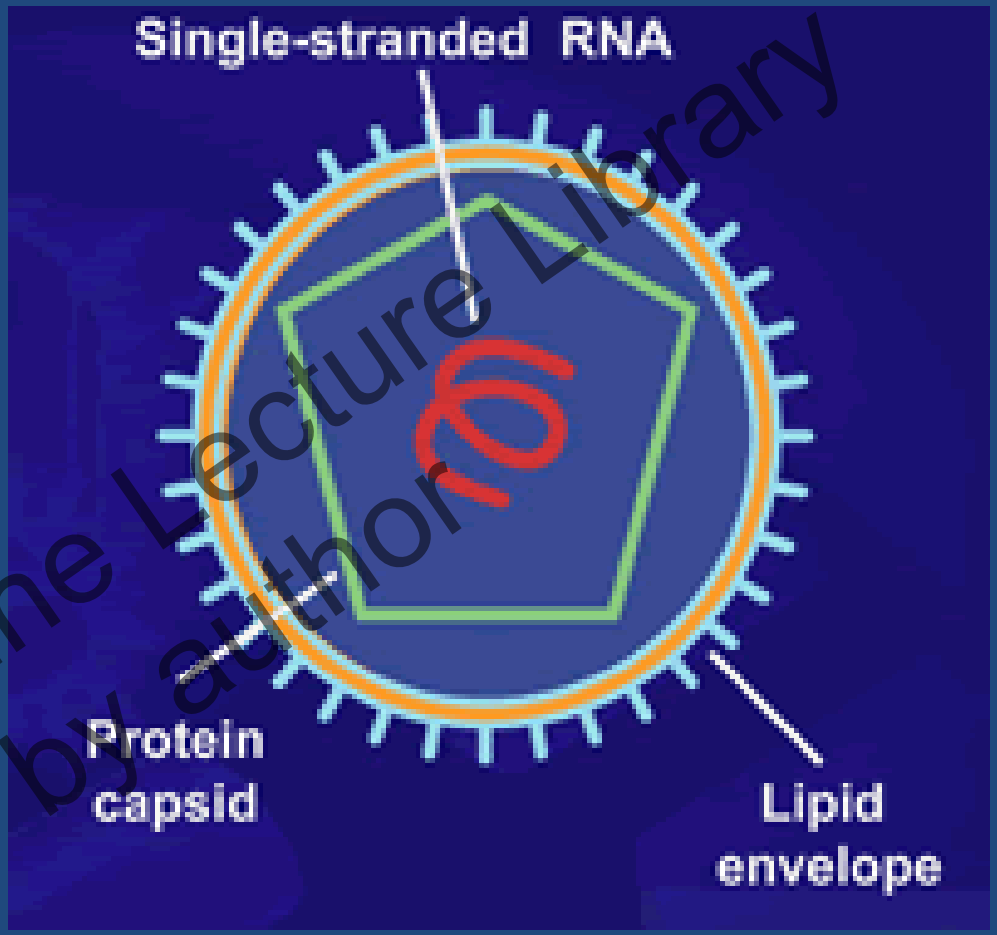
Flaviviruses





West Nile Virus

"Arbovirus" (*ar*thropod-*bo*rne virus) that is transmitted by a mosquito vector

WNV: Culex sp.

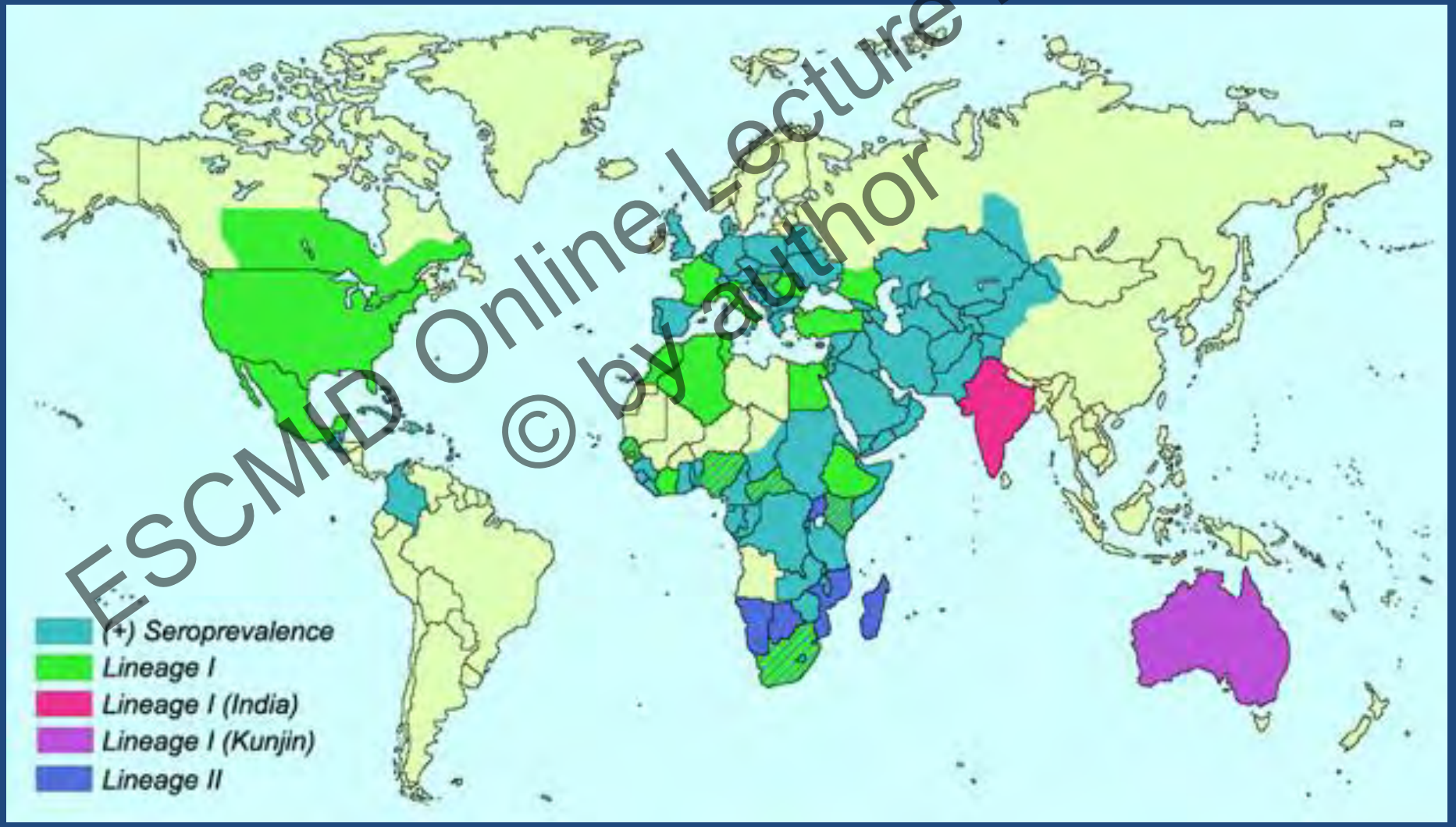
Transmission cycle

- *Culex* mosquitoes are the primary global transmission vector
- Cycle between the mosquito, animal host (most commonly birds) and humans as dead-end hosts
  - *Culex* mosquitoes feed on birds and humans (shift during summer months)
- Currently no transmission between humans
  - Potentially possible if Aedes mosquitoes, which feed primarily on humans, become primary transmission vectors for WNV
- Rare modes of transmission: blood transfusion, organ transplantation, transplacental infection, breast-feeding, and laboratory-acquired infection

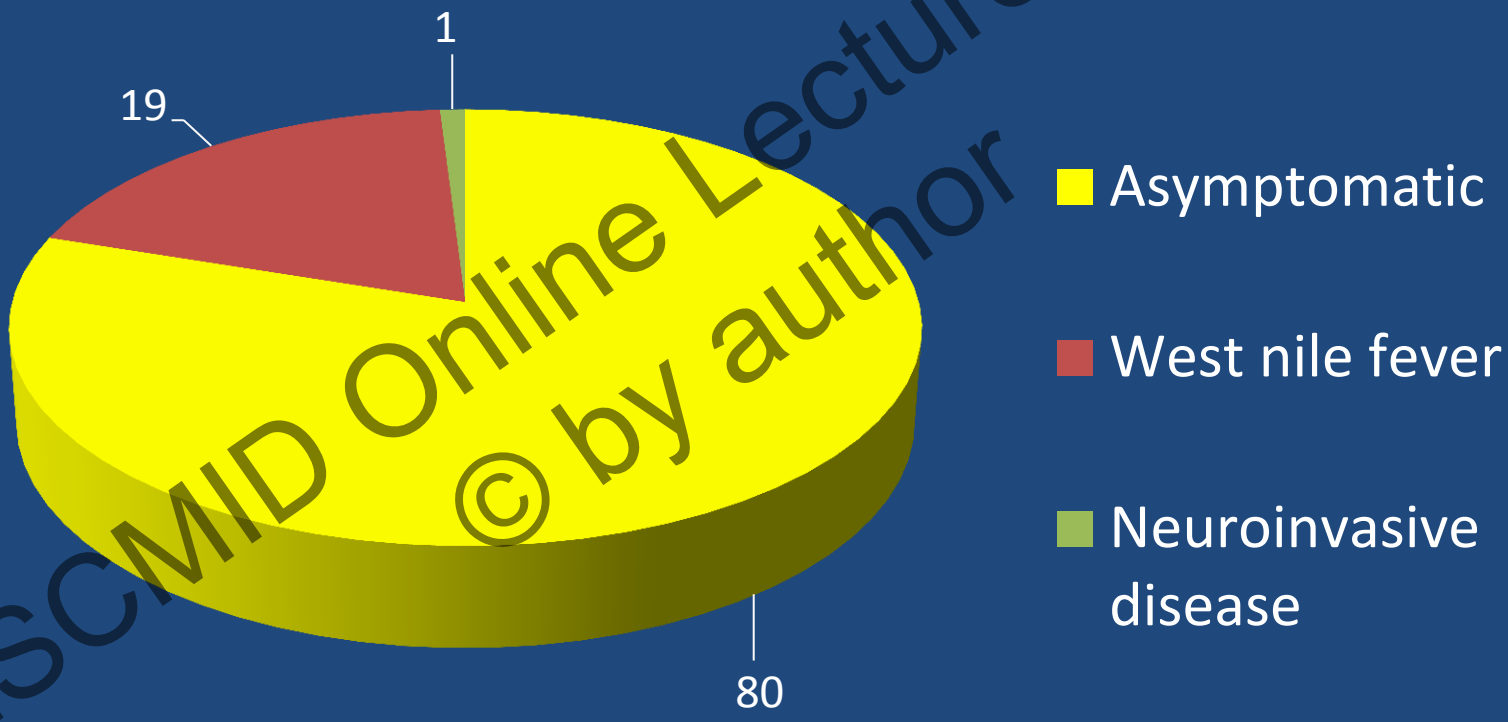
The poor birds

- Birds may have viremia that lasts for more than 100 days
- Highest titer viremias have been reported in jays, grackles, finches, crows, sparrows
  - transmission to more than 80% of biting mosquitoes
- In 1998, a change has been observed in Israel, with birds starting to die of WNV
- Sudden die-off of crows or other passerine species can serve as a sentinel event presaging subsequent human epidemics
  - might explain why human cases often decrease sharply in a particular year after a major WNV outbreak

Epidemiology



Clinical features



Clinical manifestations - WNF

- Fever (abrupt onset)
- Headache
- Fatigue, with variable malaise, anorexia, nausea,
- Myalgia
- Lymphadenopathy
- Nonpruritic generalized maculopapular rash (rare)

Neuro-invasive WNV disease

- Meningitis ~40%
- Encephalitis ~60%
  - Myoclonus
  - Visual problems (fundoscopic examination)
- Acute flaccid paralysis /poliomyelitis ~5-10%
  - Asymmetric
  - Younger patients

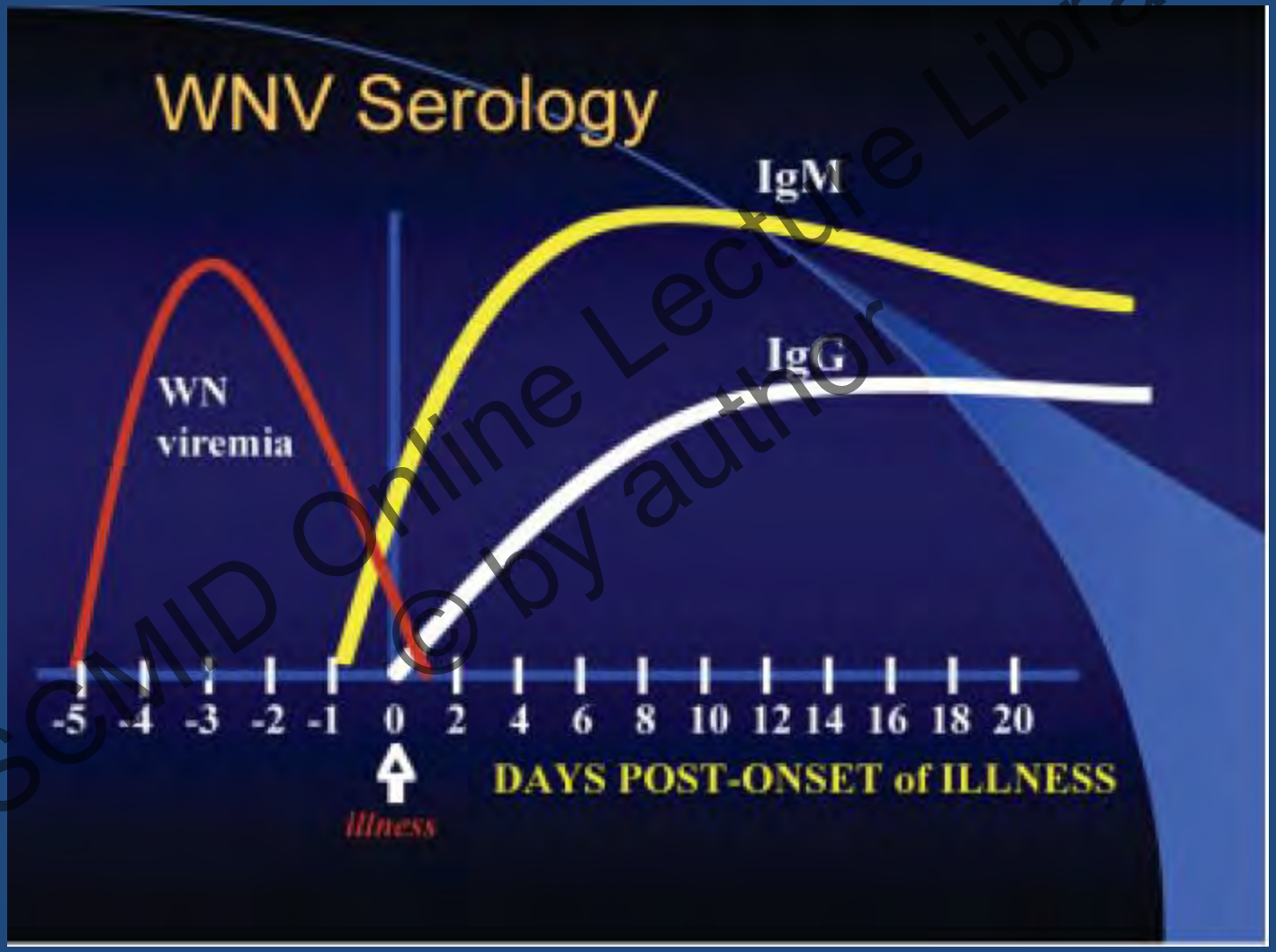
Usually features are combined

Risk factors for neurological complications

- **Older age**
  - Age >70 yrs. case-fatality rate ranges from 15% to 29%
- **Immunosuppression**
  - Solid organ transplantation
- Alcohol abuse
- Diabetes
- Chronic renal disease
- CCR5 deficiency (associated with decreased for HIV infection after exposure)

Diagnosis

- Viremia within 1 to 2 days of a mosquito bite, typically persists for up to a week
  - Low level, difficult to detect
  - Usually absent at the time of symptomatic illness
  - Blood product screening performed through nucleic acid testing of plasma (US)
- IgM seroconversion is observed as viremia ends (may persist for >2 months)
- IgG seroconversion ~ 8 days later (remains elevated for a long time after infection >1 year)
- IgA appears in serum in between IgM and IgG



CSF features

- Mild pleocytosis, mean cell count 227/mm^3, predominantely lymphocytic (~60%)
- Normal glucose
- Elevated protein: 76mg/dl in meningitis and 101mg/dl in encephalitis

Diagnosis of neuroinvasive disease

- WNV IgG can cross the blood–brain barrier, thus its presence in the CSF is not diagnostic
- WNV IgM is produced in-situ in CSF during WNV encephalitis, thus its detection in CSF is diagnostic for WNV encephalitis
  - WNV IgM can persist in CSF for up to 199 days
- IgA in CSF is also specific for WNV encephalitis
- Identification of WNV in CSF
  - viral culture (biosafety!)
  - WNV polymerase chain reaction (sensitivity 57-70% compared to WNV IgM)

Test interpretation

WNV IgA, IgG and IgM qualitative reaction patterns for 139 paired CSF and serum samples collected during the 2005 season

# Paired samples	CSF			Serum		
	IgA	IgG	IgM	IgA	IgG	IgM
119	–	–	–	–	–	–
6	+	–	+	+	–	+
3	+	+	+	+	+	+
1	–	+	+	–	+	+
1	–	+	–	–	+	–
6	–	–	–	–	+	–
1	+	+	+	+	–	+
1	–	+	–	–	–	–
1	+	–	–	–	–	–

Nixon et al. West Nile virus immunoglobulin A (WNV IgA) detection in cerebrospinal fluid in relation to WNV IgG and IgM reactivity. J Clin Virology 37 (2006) 174–178

False positive WNV serology

- Cross-reacting antibodies with other Flaviruses
  - St Louis encephalitis
  - Japanese encephalitis
  - Dengue serotype 2
  - Yellow fever
  - Powassan viruses
- Rheumatoid arthritis/ other autoimmune for IgM

Suggested diagnostic criteria

- Fever AND
- Acute systemic signs AND
- Neurological symptoms/ signs/ CSF abnormality with no alternative diagnosis AND at least one of:

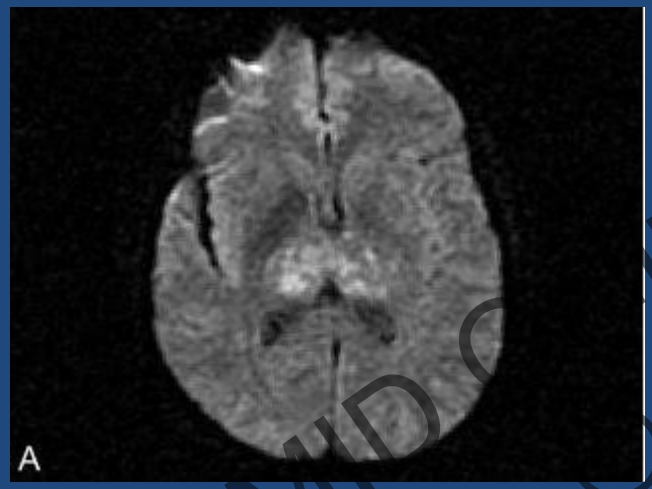
A. Demonstration of WNV IgM antibody in serum without vaccination with yellow fever or Japanese B viral vaccines in past 5 years or recent infection with other flavivirus, such as St Louis encephalitis virus. **Note:** If WNV has occurred in region in prior years, criterion B is needed because previously infected individuals may have prolonged persistence of IgM in serum.
B. Fourfold or greater increase in serum WNV IgG or IgM antibody titer between acute and convalescent samples taken 10 to 28 days apart.
C. Demonstration of WNV IgM antibody in CSF
D. Identification of WNV in CSF by viral culture or of WNV nucleic acid by polymerase chain reaction

Davis et al. West Nile virus neuroinvasive disease. Ann Neurol. 2006 Sep;60(3):286-300

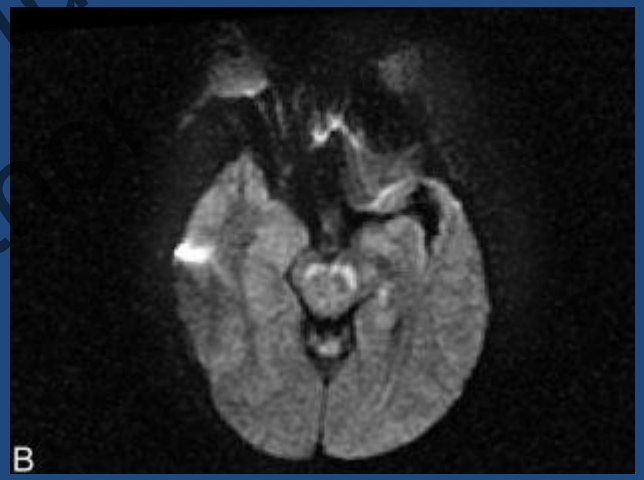
Brain imaging

- CT normal
- MRI usually normal (20 to 70% of patients with WNV neurological disease)
  - Observed more early-on
  - When present, predilection for deep gray matter structures including the basal ganglia, thalami, brainstem and cerebellum

MRI neuroimaging WNV



Thalamus



Substantia nigra

Neurological recovery

- Prolonged!
- Age is the most important risk factor for adverse long-term outcomes
- New York City outbreak in 1999: only 37% had achieved a full recovery at 1 year after illness
  - Cognitive impairments: memory loss (44%), depression (44%), irritability (39%), lightheadedness (37%), loss of concentration (33%), confusion (31%)
- Recovery unrelated to severity of initial encephalitis

Treatment

- No proven antiviral
- Interferon alpha suggested, not proven, no RCTs
- Ribavirin reported as associated with worst outcome
- IVIG containing high titers of WNV IgG was associated with variable improvement in outcomes in case reports and case series in Israel
  - RCTs of IVIG or monocloncal antibodies terminated due to slow accrual

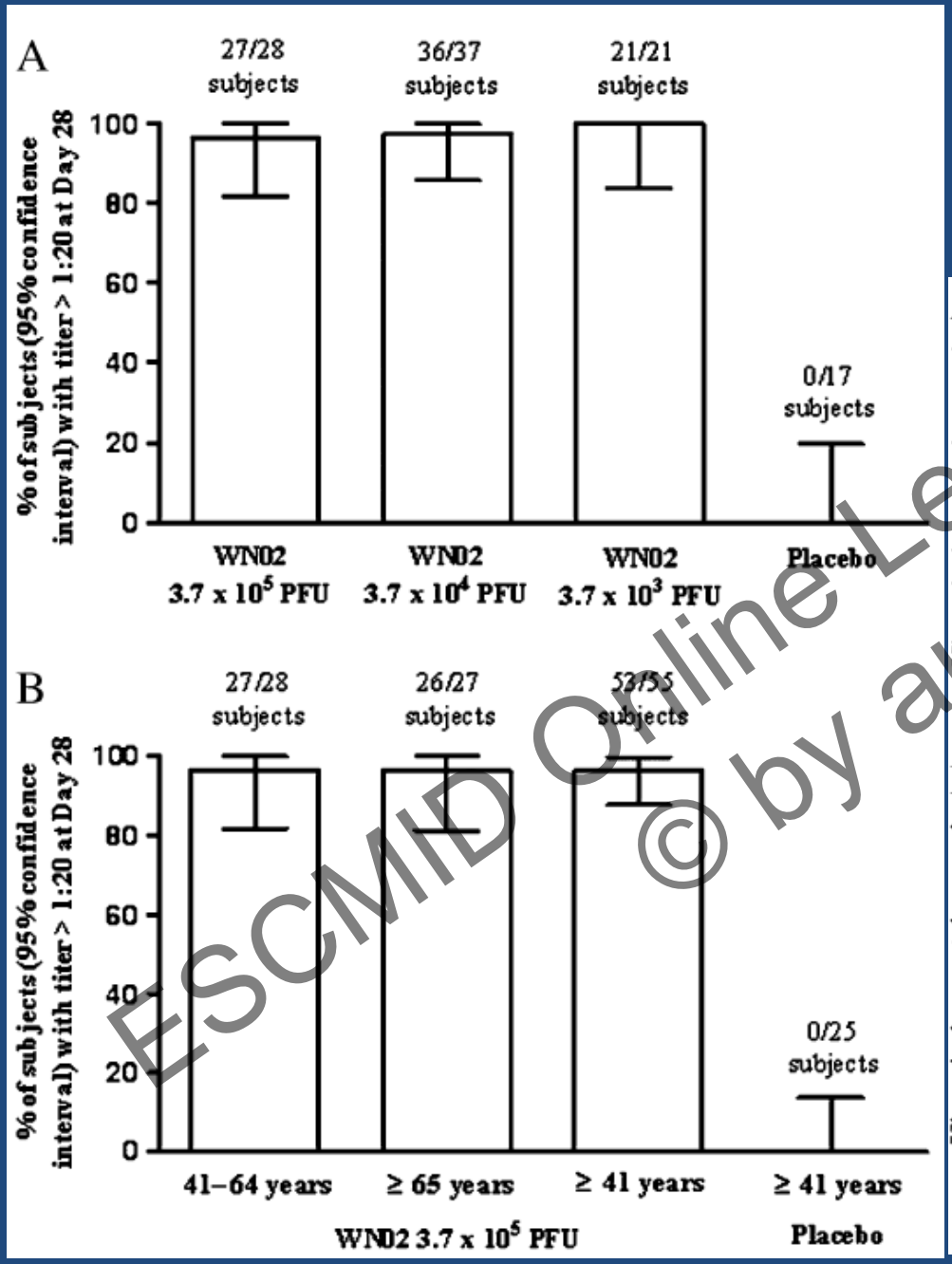
Prevention

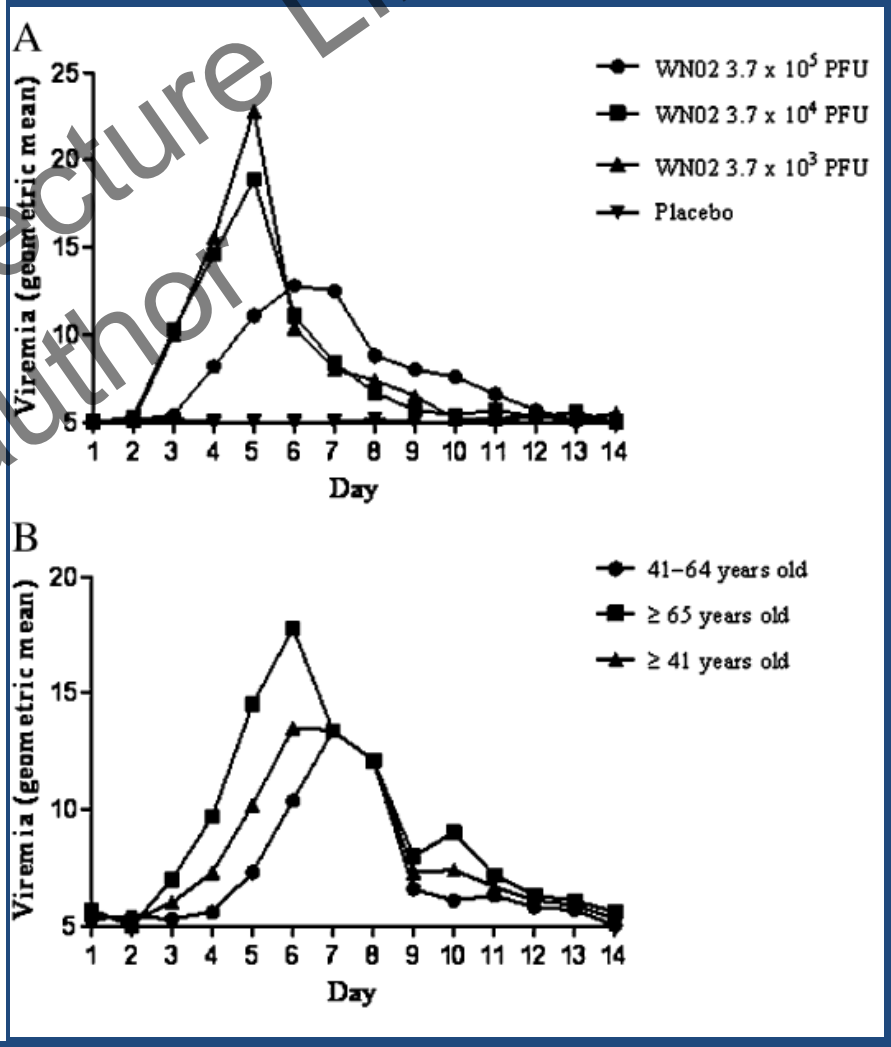
- Reduction in risk for exposure to infected mosquitoes
  - Personal
  - Public
- Human vaccine: live attenuated vaccine in phase II trials, proven safe and immunogenic in healthy adults and the elderly (ChimeriVax-WN02, Sanofi Pasteur)
  - Chimeric vaccine: produced by insertion of the genes encoding the pre-membrane and envelope E proteins of WNV (strain NY99) into the yellow fever vaccine clone., with the E gene mutated at 3 sites to reduce

Vaccine trial outcome measurements

- Immunogenicity: plaque reduction neutralization test to determine the neutralizing antibody levels (ChimeriVax-WN02 vaccine virus and OrVax-Vero cells). Examined at day 0 and 28 post-injection and defined as four-fold or greater rise in titer
- IgM antibodies: measured qualitatively by ELISA
- Viremia: analysed for a subset of patients. Blood taken every second day. Plaque assay on duplicate Vero cell monolayers. Detectable at ≥20 pfu/mL and <60 pfu/mL and quantifiable at ≥60 pfu/mL

Viremia followed by seroconversion rates at day 28





Biedenbender et al. J Infect Dis 2011;203:75–84. Phase II, double blind, dose ranging, 3 age ranges

Viremia followed by seroconversion rates at day 28

Summary of viremia.

	WN02 4×10^3 (A)	WN02 4×10^4 (B)	WN02 4×10^5 (C)	Placebo	*P*-values		
	N = 80	*N* = 82	*N* = 73	*N* = 80	Overall[a]	A vs. B[b]	A vs. C[b]
	M Mean [95% CI]						
Log10 (Cmax in pfu/mL)	49 1.63 [1.55; 1.72]	50 1.74 [1.63; 1.84]	45 1.71 [1.61; 1.81]	1 1.94 [NC]	0.398	0.211	0.288
Log10 (AUC)	48 2.40 [2.43; 2.47]	49 2.46 [2.38; 2.54]	45 2.43 [2.37; 2.49]	1 2.47 [NC]	0.546	0.281	0.450
Duration (days)							
Subjects with detectable viremia 20 pfu/mL and <60 pfu/mL	34 2.53 [1.80; 3.26]	33 2.70 [1.88; 3.51]	29 2.45 [1.66; 3.23]	0	0.969	0.827	0.975
Subjects with quantified viremia 60 pfu/mL	15 5.93 [3.93; 7.94]	17 5.24 [3.79; 6.68]	16 4.00 [2.60; 5.40]	1 1.00 [NC]	0.203	0.521	0.104
Days viremic							
Subjects with detectable viremia 20 pfu/mL and <60 pfu/mL	34 1.53 [1.28; 1.78]	33 1.64 [1.33; 1.94]	29 1.62 [1.31; 1.93]	0	0.927	0.750	0.738
Subjects with quantified viremia 60 pfu/mL	15 3.07 [2.14; 399]	17 2.88 [2.18; 3.58]	16 2.25 [1.68; 2.82]	1 1.00 [NC]	0.282	0.787	0.160

Geometric mean titer and seroconversion rates by plaque reduction neutralization test.

	WN02 4×10^3 (A)	WN02 4×10^4 (B)	WN02 4×10^5 (C)	Placebo	*P*-values			
	N = 114	N = 118	N = 108	N = 114	Overall	A vs. B	A vs. C	B vs. C
	Geometric mean titer [95% CI]							
Day 0 (pre-vaccination)	5.00 [NC]	5.76 [4.92; 6.74]	5.06 [4.94; 5.20]	5.22 [4.80; 5.68]	0.085	0.048	0.308	0.207
Day 28	688 [453; 1047]	600 [405; 890]	674 [464;978]	5.93 [4.96; 7.08]	0.870	0.623	0.940	0.683
	n/*N* [%], [95% CI]							
Seroconversion	105/114 [92.1] [85.5; 96.3]	110/118 [93.2] [87.1; 97.0]	103/108 [95.4] [89.5; 98.5]	3/114 [2.6] [NC]	0.600	0.805	0.411	0.575
<1:10 at baseline and 1:20 on Day 28	105/114 [92.1] [85.5; 96.3]	109/118 [92.4] [86.0; 96.5]	102/108 [94.4] [88.3; 97.9]	3/114 [2.6] [NC]	0.749	1.000	0.596	0.600
1:10 at baseline and fourfold on Day 28	0/114 [0.0] [NC]	1/118 [0.8] [NC]	1/108 [0.9] [NC]	0/114 [0.0] [NC]	0.767	1.000	0.486	1.000

Dayan et al. Vaccine 2012; 30: 6656– 6664. Phase II, double blind, dose ranging, >50 years

Dengue virus

Aedes aegypti

Virology

- Four antigenically distinct serotypes (DENV 1-4)
- Infection by a specific serotype confers lifelong immunity against the specific serotype but not to the remaining three
- Antibody dependent enhancement (ADE): an increased risk for severe disease (dengue hemorrhagic fever) with secondary infection of a different serotype. Binding of crossreactive, neutralizing antibodies at subneutralizing concentration that enhances the infection of monocytes and dendritic cells
- Epidemics of severe disease are usually reported from areas where two or more serotypes circulate in succession or simultaneously.

Epidemiology

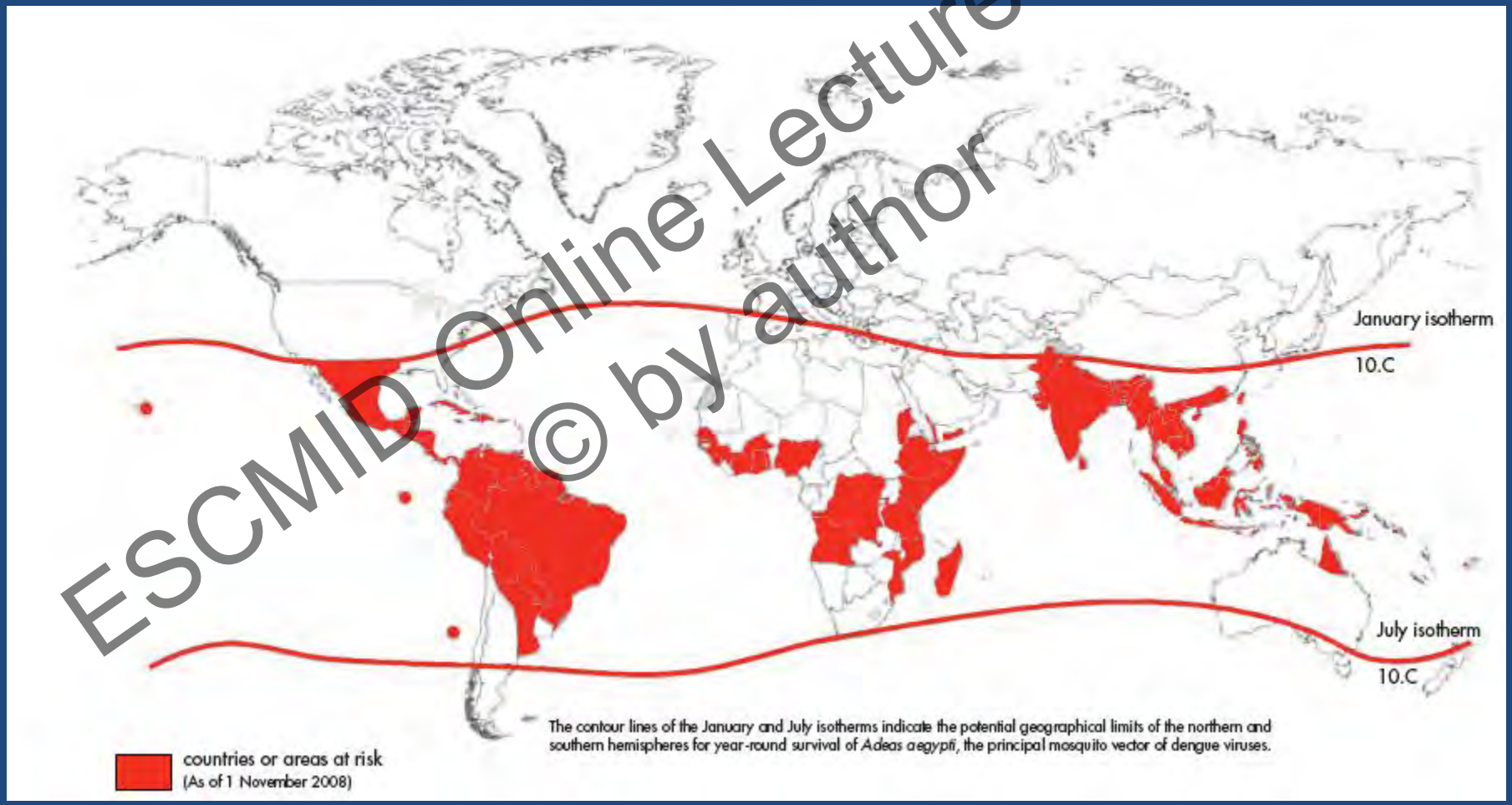
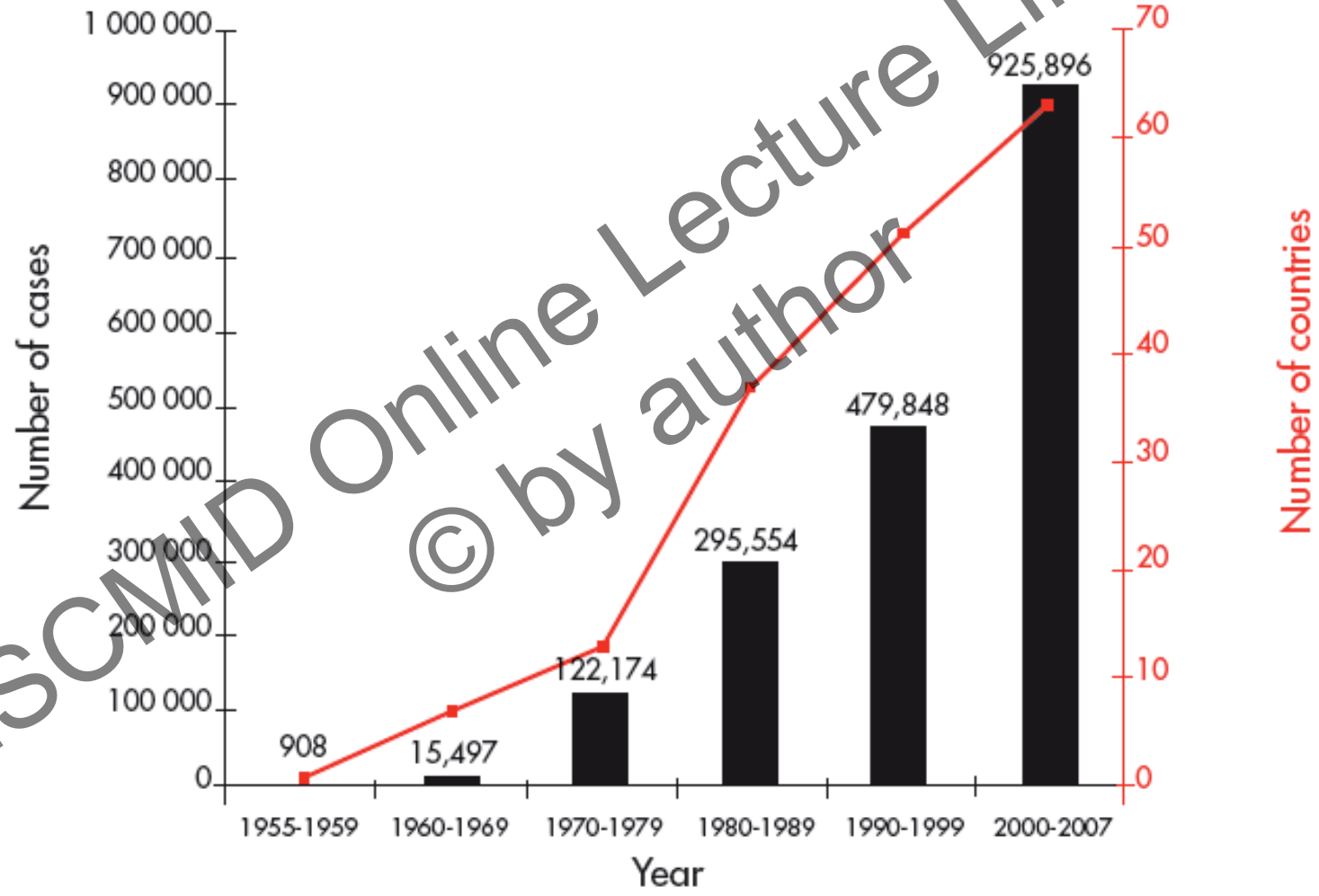


Figure 1.2 Average annual number of dengue fever (DF) and dengue haemorrhagic fever (DHF) cases reported to WHO, and of countries reporting dengue, 1955–2007



Recent outbreaks

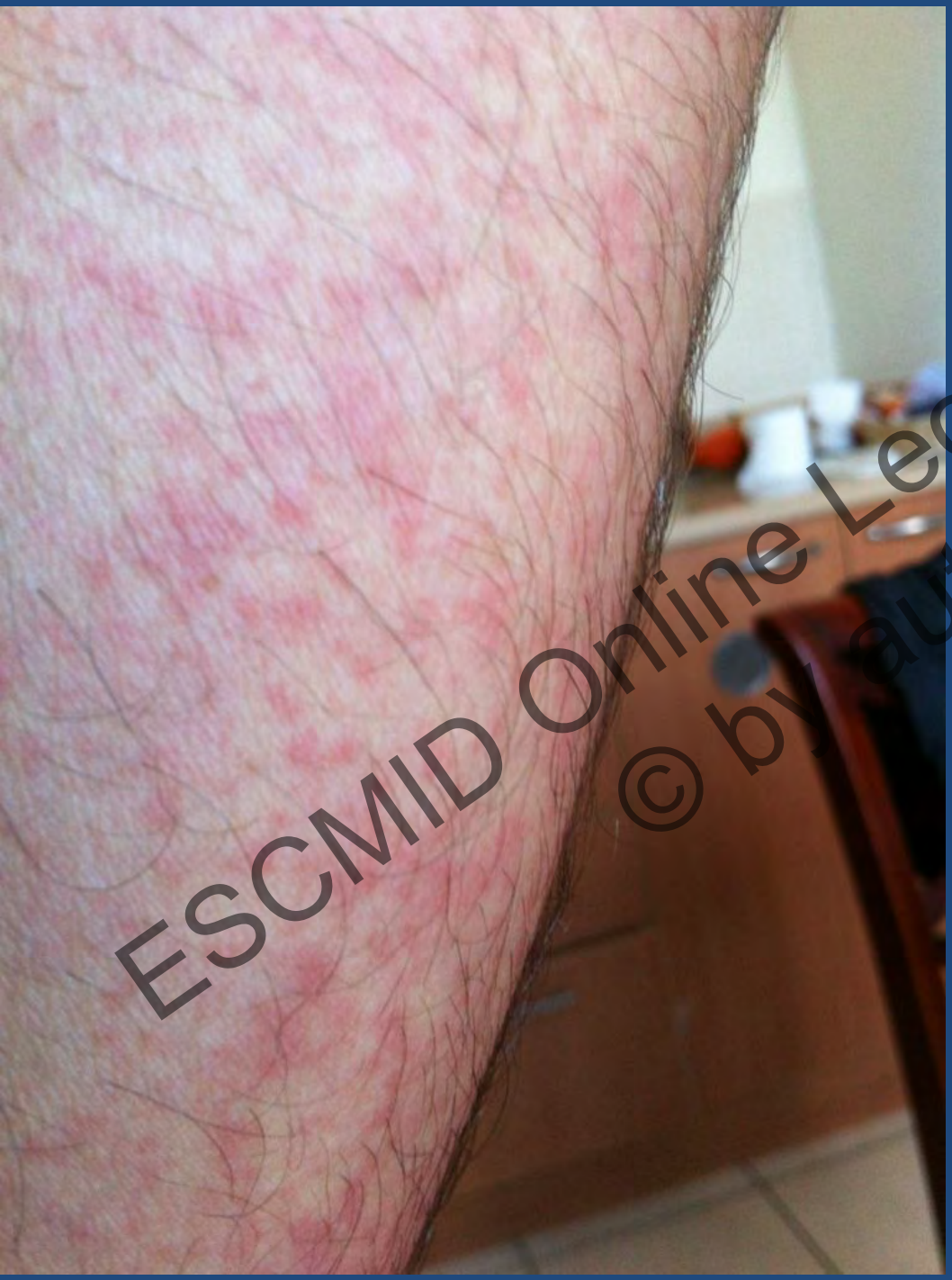
Travel-related dengue infections acquired in Luanda, Angola, reported from GeoSentinel sites, March–May 2013 (n=10)

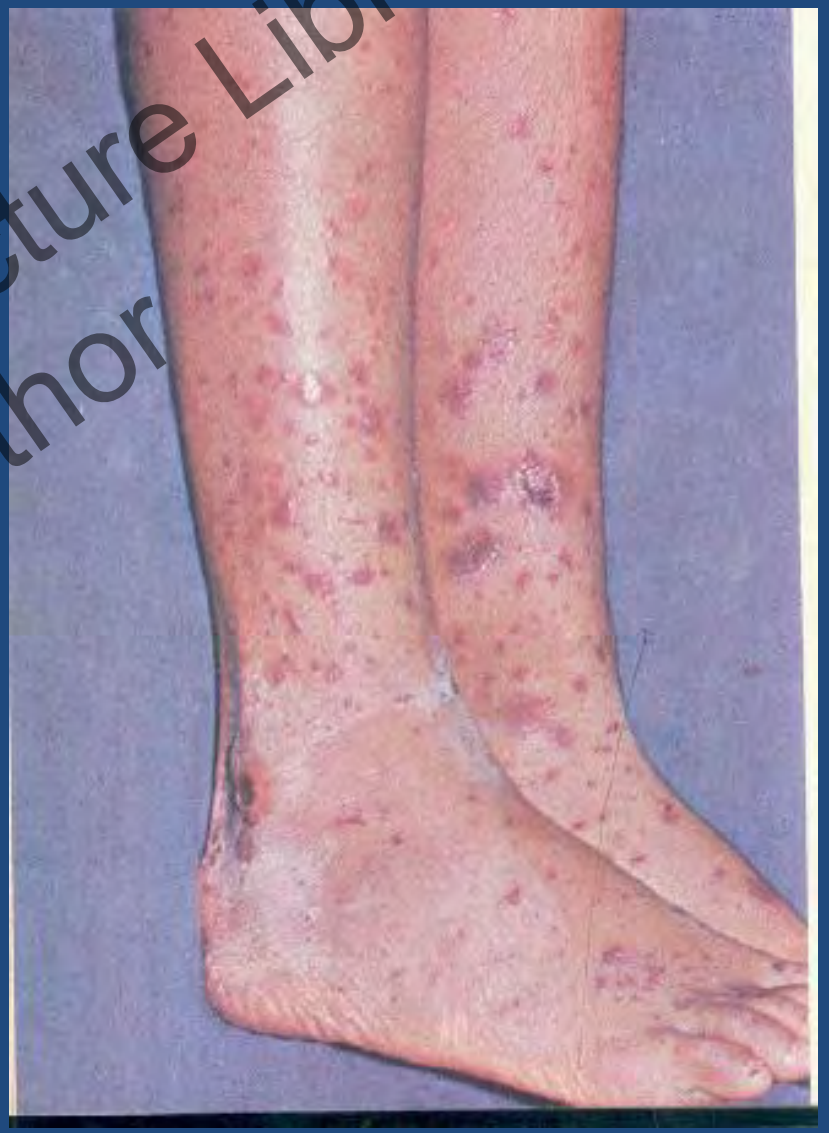
Country of origin of the case	Fever onset date	Time from fever onset to test (days)	NS1	Serology-IgM	Qt-PCR
Germany	30 March	4	Positive	Positive	ND
Canada	3 April	10	Negative	Positive	ND
France	5 April	12	Negative	Positive	Negative
Germany	7 April	14	Negative	Positive	ND
South Africa	10 April	7	ND	Positive	Negative
Israel	11 April	14	ND	Positive	ND
Israel	17 April	7	Positive	Positive	ND
Israel	18 April	4	Positive	Positive	DENV-1
Israel	25 April	5	Positive	Positive	DENV-1
Israel	2 May	6	Positive	Positive	ND

Schwartz et al. Detection on four continents of dengue fever cases related to an ongoing outbreak in Luanda, Angola, March to May 2013 .Euro Surveill. 2013;18(21):

Table 1. Symptoms differentiating dengue infection from other febrile illnesses.

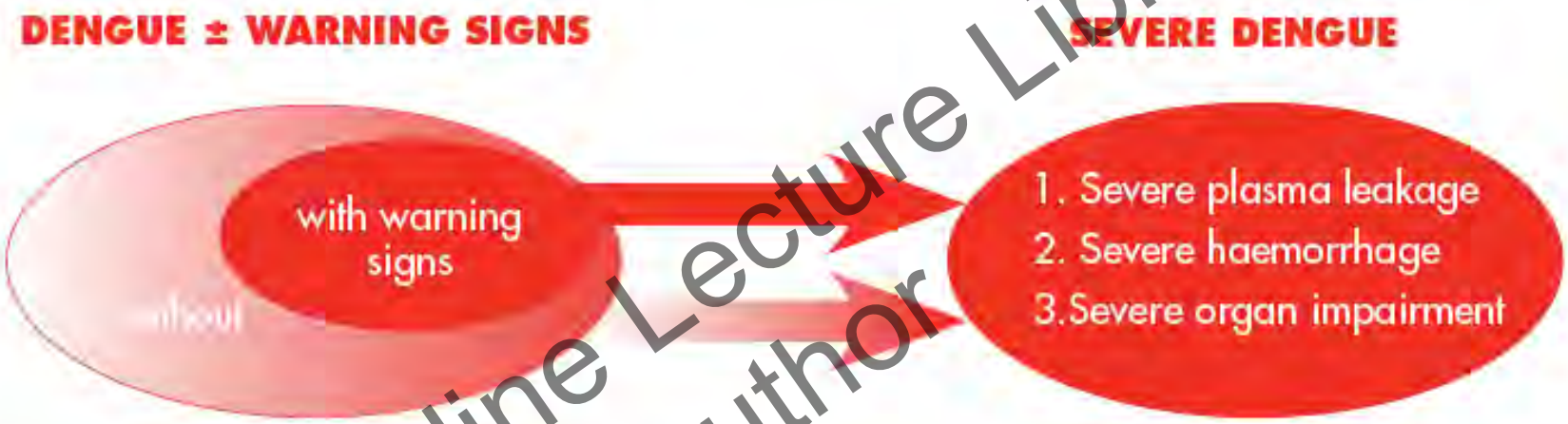
Symptoms	Den|OFIs	p-value	Children (<15 years)|adults (>15 years)	p-value
Nausea	50.0|28.9% 68.0|49.0%† 51.3|30.5%	<0.00001 <0.05 <0.001	50.2|76.4%	<0.001
Vomitting	16.4|8.4% 16.2|8.5% 57.0–64.0|31.0–46.0%† 70.0|52.0%†	<0.00001 0.03 <0.01 <0.05	50.2|76.4%	<0.001
Retro-orbital pain	26.0|15.9% 26.6|13.5% 10.01§	<0.00001 0.003 0.001	8.7|29.1%	<0.001
Aches/pains	1.4§	<0.0001	20.3|36.4%	0.012
Rash	11.2–41.2|3.0–6.4%	<0.003/0.007	NA	NA
Tourniquet test positive	34.0|19.0% 42.0|5.0%† 43.0–65.0|21.0–39.0% 1.86§	0.02 <0.01 <0.1 <0.001	NA	NA
Leukopenia	3.8×10^3|7.3×10^3/µl 4.5×10^3|8.1×10^3/µl <4.5×10^3/µl: 72.1|11.5%	<0.0001 <0.1 <0.001	NA	NA
Thrombocytopenia (platelet/mm³)	16|4% (≤100,000)† 16|82%‡ (≤100,000) 66|95%‡ (≤100,000) 14.9|1.5% (≤100,000) 32,000|96,500 163,500|239,000 70,000|104,000¶	NA NA <0.01 <0.001 <0.001 <0.0001 NA	NA	NA





Tourniquet test

WHO suggested dengue case classification and levels of severity



CRITERIA FOR DENGUE ± WARNING SIGNS

Probable dengue

live in /travel to dengue endemic area.

Fever and 2 of the following criteria:

- Nausea, vomiting
- Rash
- Aches and pains
- Tourniquet test positive
- Leukopenia
- Any warning sign

Laboratory-confirmed dengue

(important when no sign of plasma leakage)

Warning signs*

- Abdominal pain or tenderness
- Persistent vomiting
- Clinical fluid accumulation
- Mucosal bleed
- Lethargy, restlessness
- Liver enlargment >2 cm
- Laboratory: increase in HCT concurrent with rapid decrease in platelet count

*(requiring strict observation and medical intervention)

CRITERIA FOR SEVERE DENGUE

Severe plasma leakage

leading to:

- Shock (DSS)
- Fluid accumulation with respiratory distress

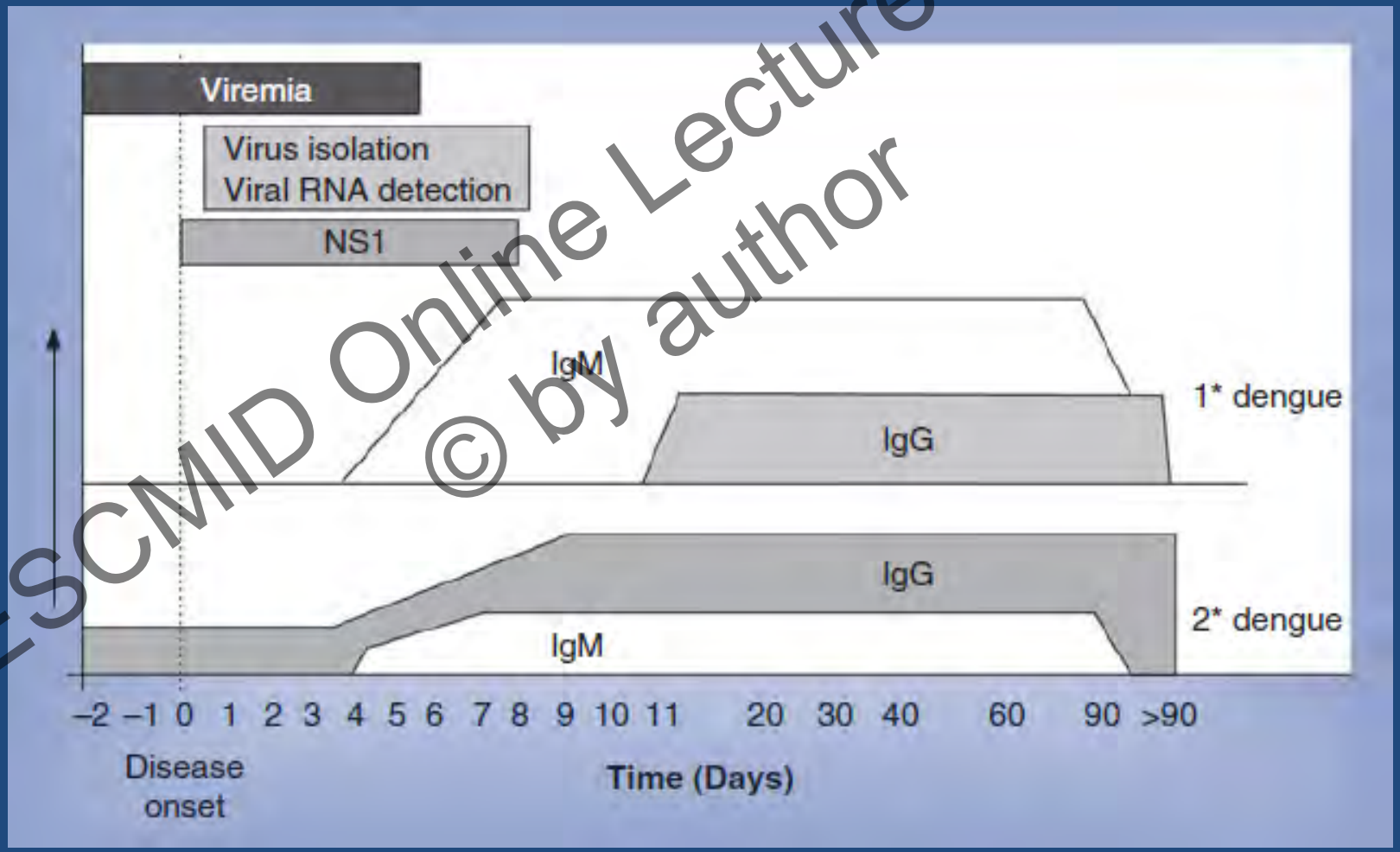
Severe bleeding

as evaluated by clinician

Severe organ involvement

- Liver: AST or ALT >=1000
- CNS: Impaired consciousness
- Heart and other organs

Dengue diagnosis



Serology interpretation

Table 7
Interpretation of serologic assays

Diagnosis of Dengue	Serology Results
Highly suggestive	Positive IgM in single serum sample Positive IgG in a single sample with an HI titer of ≥1280
Confirmed diagnosis	IgM seroconversion in paired sera IgG seroconversion in paired sera or ≥4-fold IgG titer in paired sera
Primary infection	Negative IgG in the acute-phase serum and a positive IgG in the convalescent-phase serum Ratio of IgM and IgG in single serum sample ≥1.2
Secondary infection	Positive IgG in the acute-phase serum and a 4-fold rise in IgG titer in the convalescent-phase serum sample Ratio of IgM and IgG in single serum sample ≤1.2

Dengue virus vaccine

- Must be equally protective against each of the four DENV serotypes to reduce the risk of ADE
- Vaccine candidates under development
  - live attenuated virus
  - live chimeric virus most advanced (Sanofi Pasteur ChimeriVax Dengue tetravalent vaccine). Low and unequal effectiveness (~30%)
  - inactivated virus
  - live recombinant DNA
  - subunit vaccines
- No licensed vaccine to date. The future…

Recommended reading

- Colpitts et al. West Nile Virus: Biology, Transmission, and Human Infection. Clin. Microbiol. Rev. 2012, 25(4):635.
- Davis et al. West Nile virus neuroinvasive disease. Ann Neurol. 2006 Sep;60(3):286-300.
- WHO. DENGUE: Guidelines for Diagnosis, Treatment, Prevention and Control – New Edition. WHO, Geneva, Switzerland (2009)
- Tang et al. Diagnosis of dengue: an update. Expert Rev Anti Infect Ther. 2012;10(8):895-907
- Watta et al. Infectious disease emergencies in returning travelers: special reference to malaria, dengue fever, and chikungunya. Med Clin North Am. 2012 Nov;96(6):1225-55.
- Schiøler KL, McCarty CW. Vaccines for preventing dengue infection. Cochrane Database of Systematic Reviews 2003, Issue 4. Art. No.: CD004613. DOI: 10.1002/14651858.CD004613.