

DENGUE RESEARCH IN INDONESIA

Prof.dr. Pratiwi P Sudarmono, Sp.MK(K), PhD
dr Herman Kosasih

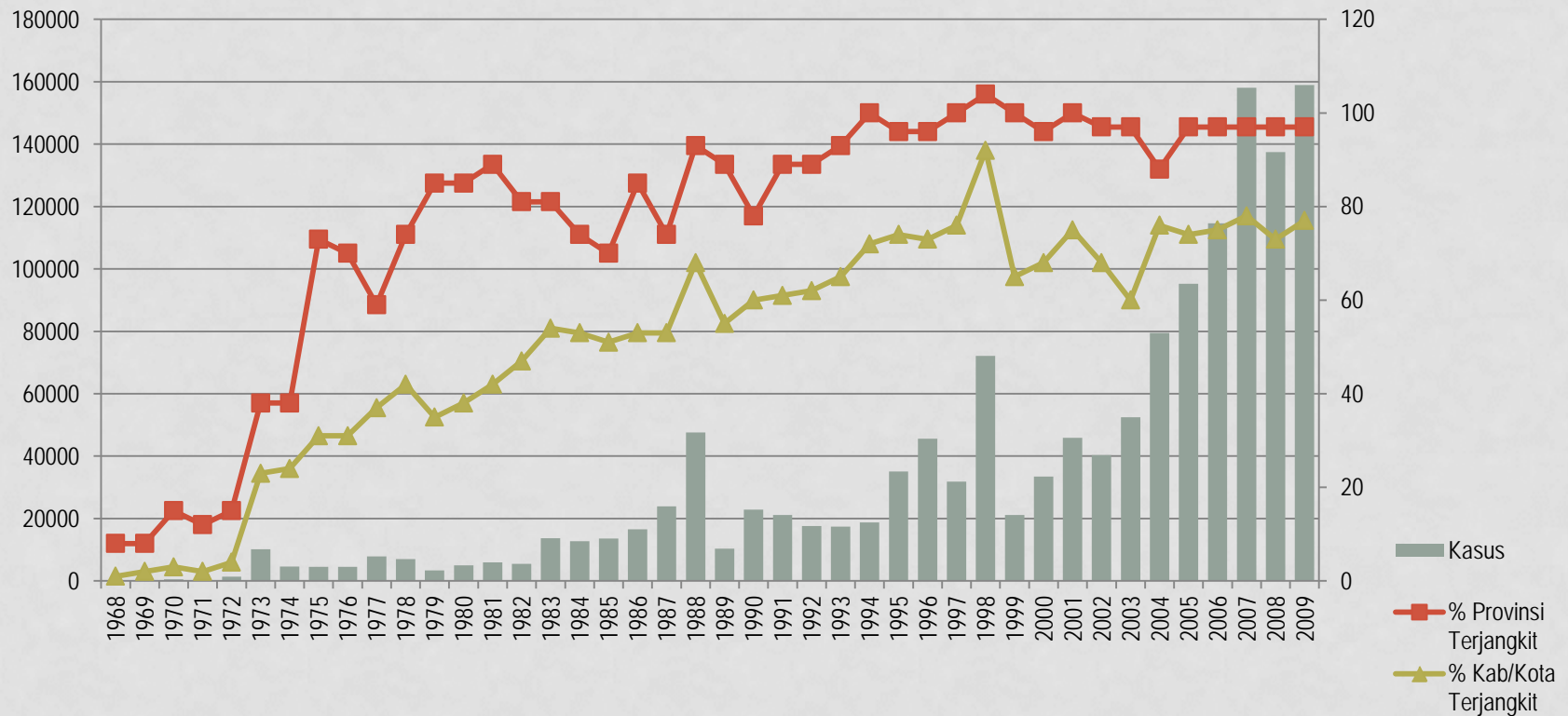
UNIVERSITAS INDONESIA
INDONESIA RESEARCH PARTNERSHIP ON INFECTIOUS DISEASE
(INA-RESPOND)
Jakarta, 30 April 2015



Dengue Situation in Indonesia

CASES INCREASED RAPIDLY IN THE LAST 20 YEARS, ALMOST ALL PROVINCES AND DISTRICTS.

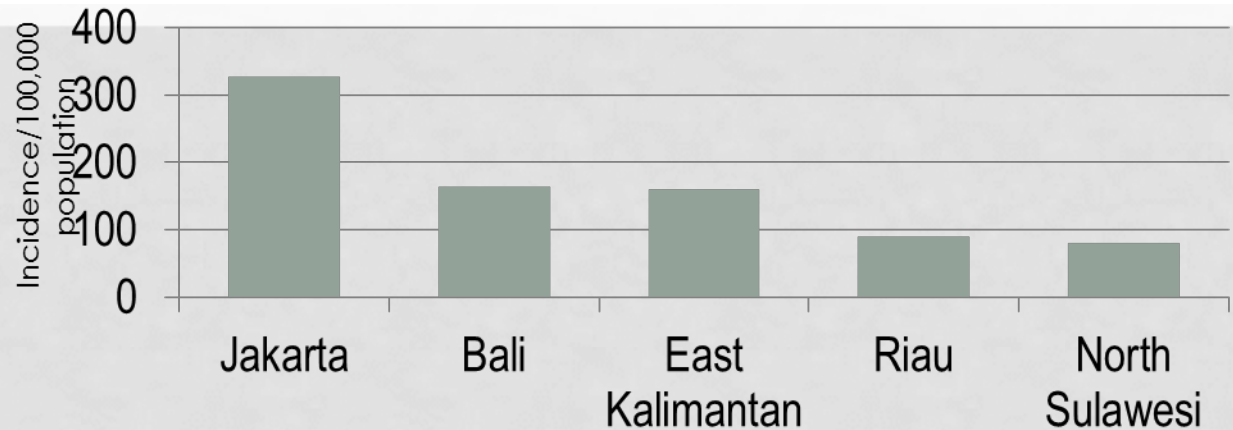
SEVERAL PEAKS OF DENGUE CASES, AFFECTED DISTRICTS AND PROVINCES OCCURRED DURING "LA NINA" YEAR



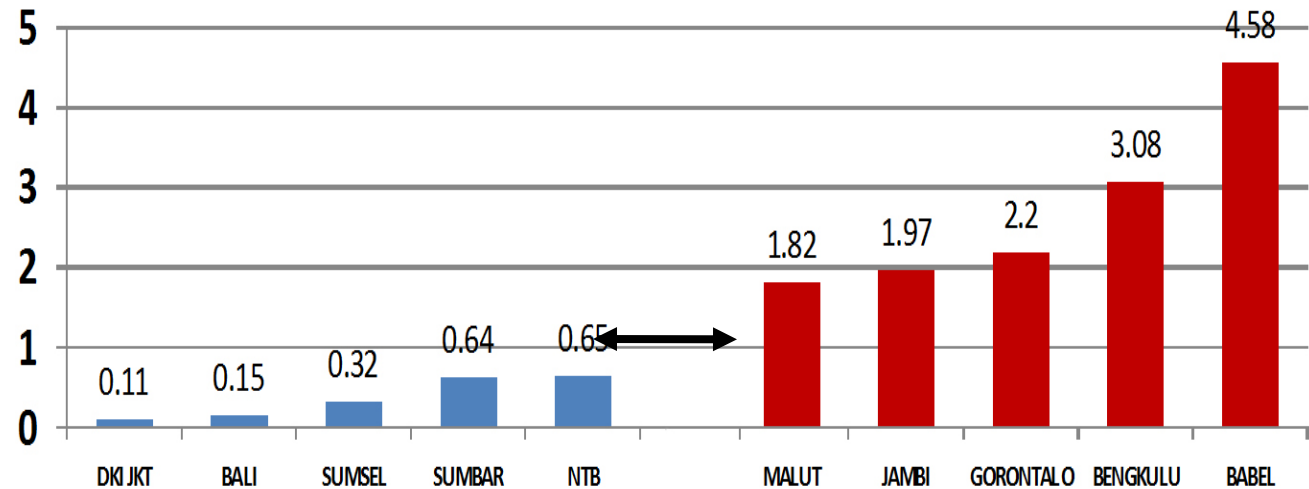
Source : Ditjen PP & PL Depkes RI,
2009 (BJE(2),2010)

PROVINCES WITH HIGHEST INCIDENCE (2005-2009) & MORTALITY RATE (2009)

Highest incidence
in urban areas
(2005-2009)



Highest
Mortality
rates

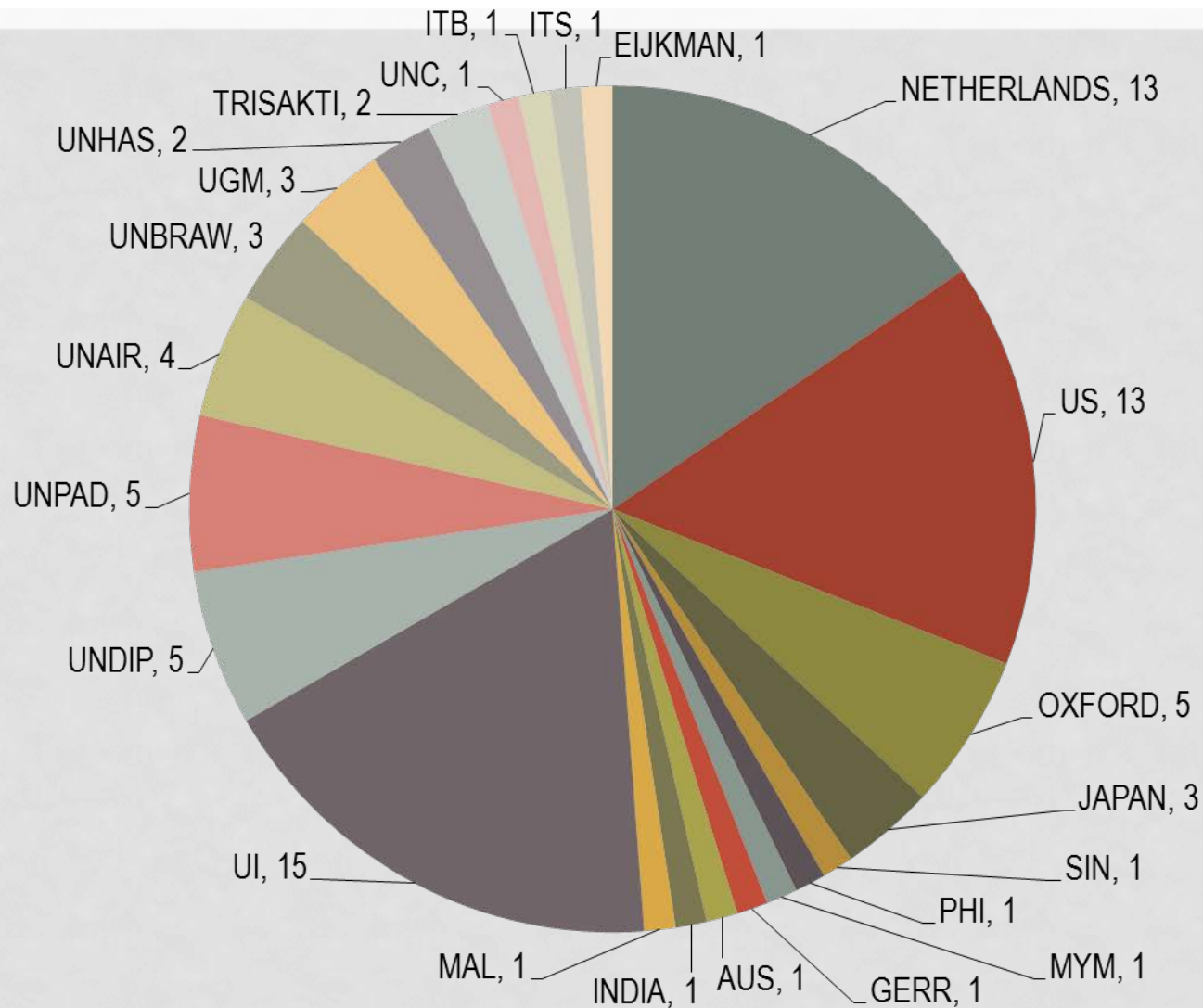


DENGUE RESEARCH IN INDONESIA*

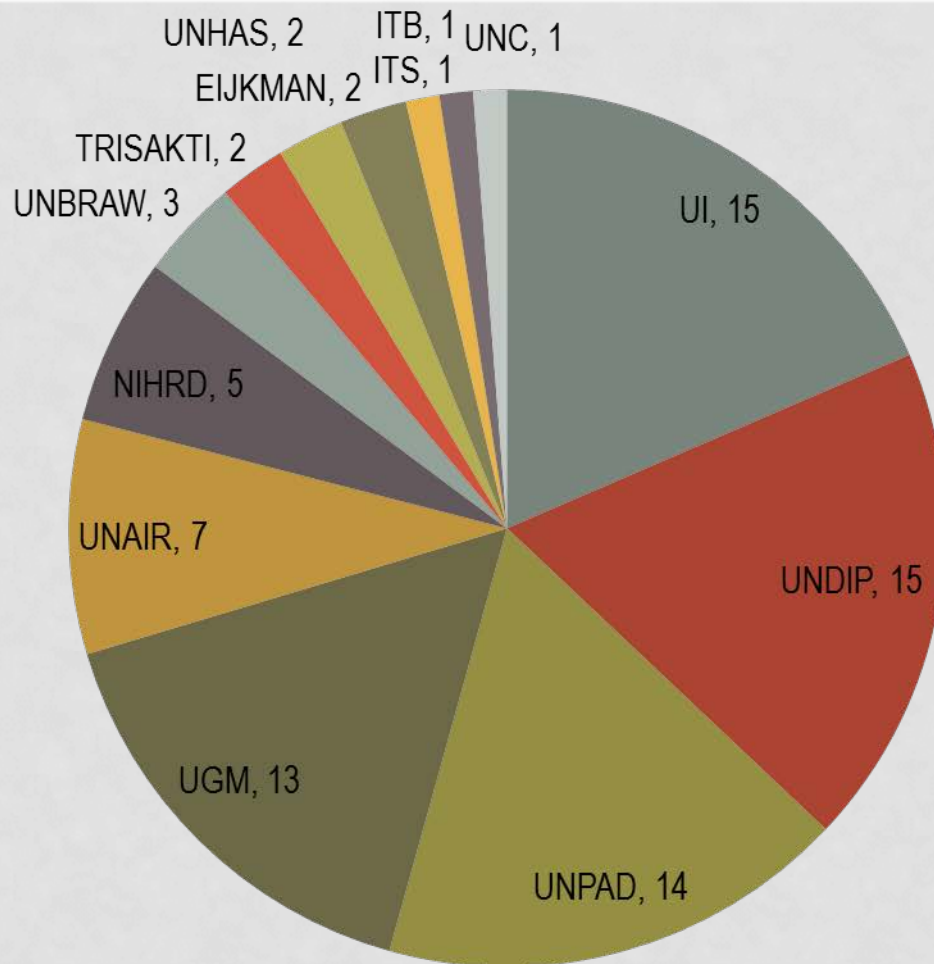
- Entomology (7)
- Epidemiology (16)
- Molecular Biology (6.5)
- Clinical Aspects (13)
- Lab Assays and Diagnosis (6)
- Pathogenesis (24.5)
- Treatment (7.5)
- Prevention (3.5)

*pub med and scopus searching: 'dengue' title, 'Indonesia' affiliation

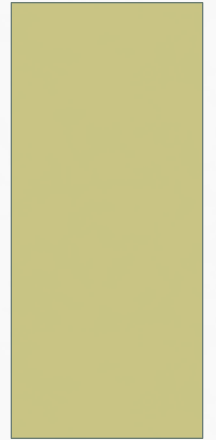
DENGUE RESEARCH IN INDONESIA



DENGUE RESEARCH IN INDONESIA



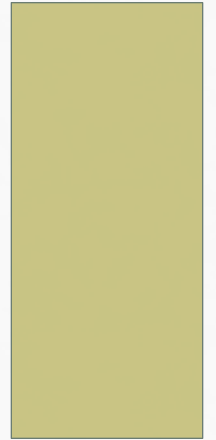
ENTOMOLOGY



ENTOMOLOGY

- **Transmission thresholds and pupal/demographic surveys** in Yogyakarta, Indonesia for developing a dengue control strategy was conducted in dry and wet seasons. From 3000 water containers associated with 320 residences, 6% had pupae and *Aedes aegyptii* was 10x more frequent. The **ratio of pupae per person was 0.57 and transmission threshold was 0.43.** (Focks,2007)
- **A multi-country study** in twelve to twenty urban and peri-urban neighborhoods of **six Asian countries** reconfirmed the **association between rainfall and dengue cases** and underlined **the importance of determining through pupal productivity surveys** the most productive containers types. Pupal productivity surveys conducted during the wet season will identify almost all of the most productive container types for both the dry and wet seasons and will therefore facilitate cost-effective targeted interventions **(Wai, 2012)**

EPIDEMIOLOGY



MATHEMATICAL MODELING

- Model of dengue transmission when vaccination is applied (Supriatna, 2008)
- Model of dengue transmission in vaccinated population and considering immigration and emigration factors (Tasman, 2012)
- Model to determine strategies to optimally control dengue transmission (Aldila, 2013)

MATHEMATICAL MODELING

- IR DHF are influenced by people's behavior, rainfall and temperature, the highest level in Klojen, Lowokwaru, and Pakis. (Adhisuwignjo, 2012)
- The spreading pattern of the *Aedes aegypti* in East Java have spatially and temporally positive correlation (Astutik, 2011 and 2012)
- Two basic reproduction numbers (host-to-vector and host-to-host) are derived to estimate dengue transmission during 2002-2007 outbreaks in Bandung, Indonesia (Supriatna, 2009)

OUTBREAK INVESTIGATIONS

- **DHF Epidemic in Merauke, Papua** Indonesia in 2001 was mostly in children, 1.2% CFR and caused by **DENV-3**. No history of DHF before (**Sukri,2003**)
- The **2004 outbreak** of dengue in **Jakarta**, Indonesia, was characterised by the circulation of **multiple virus serotypes (DENV-3 predominant)** and resulted in a relatively high percentage of hospitalised patients with DHF (**Suwandono,2006**)

EPIDEMIOLOGY: CLIMATE FACTORS

- The apparent **associations of entomological and climatic effects (ENSO)** that **precipitated the epidemic** before the influx of reported human cases in Palembang, Indonesia (**Bangs, 2006**)
- Climate factors and recorded dengue cases may be used to predict DHF epidemics (**Halide 2008 and 2009**)
- The **influence of climate** (optimum: rainfall of 1500 to 3670 mm, temperature of 22 to 27 C, humidity of 82 to 87%), **low income** per capita and populations below 15 years to the increase of **dengue incidences** (**Salamah,2012**)

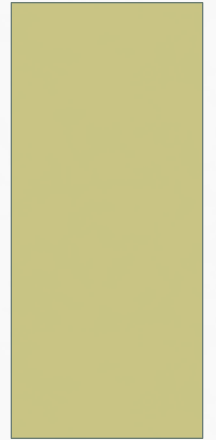
PROSPECTIVE EPIDEMIOLOGY STUDY (1)

- A two-week **virological and serological investigation** in 53 **communities** where dengue virus was currently transmitted in West Jakarta, to **detect dengue cases prior to onset** of clinical illness.
- During observation, 28 dengue infections occurred, 8 asymptomatic, 15 DF and 5 DHF.
- This study provided **a better estimation of dengue transmission in a community** from every hospitalized case. The design can be utilized for a study that seek **to define early immunologic events** following dengue infections that contribute to the development of DHF **(Beckett, 2005)**

PROSPECTIVE EPIDEMIOLOGY STUDY (2)

- A **prospective study** of dengue infections in adult volunteers from textile factories located in Bandung, West Java. **Volunteers were actively followed for the occurrence of dengue infection.**
- The **incidence of symptomatic** dengue was 18 cases per 1,000 person-years and **asymptomatic/ mild infection** was 8-56 cases per 1,000 person-years. All four serotypes were detected **(Porter, 2005)**
- The incidence rate was **~200x higher than the national incidence rate during the corresponding years**

MOLECULAR BIOLOGY

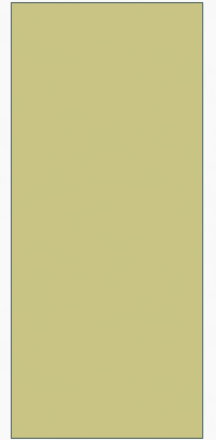


MOLECULAR EPIDEMIOLOGY

Study	Year , Place	Serotype	Genotypes Clades
Sjatha (2012)	1988 1988	DENV-1 DENV-3	IV, 2 clades I
Raeki Ansyah (2005)	1973, 1994 1998	DENV-3, clinically different	I II
Ong (2008)	2004, Jakarta	DENV-3	I
Fahri (2013)	2012, Semarang	DENV-1 DENV-2 DENV-3	I, II [^] cosmopolitan I
Yama naka (2010)	Ap-Sep 2009, Srby Sep-Dec 2010, Srby	DENV-1 DENV-1 DENV-1	IV I I new clade

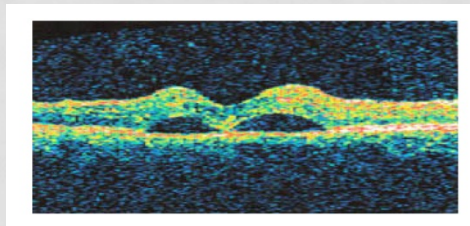
[^]old genotype ,never been reported in the last three decades , discovered in an area 1,001 meters , suggesting the silent transmission

CLINICAL MANIFESTATIONS

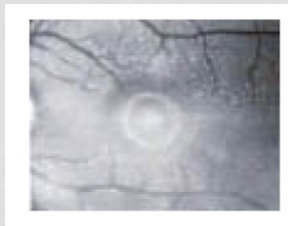


UNCOMMON ORGAN INVOLVEMENT

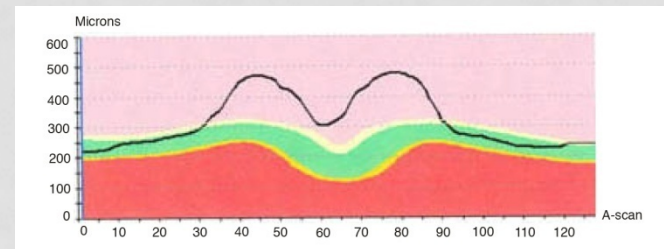
- A 23-year old female with confirmed dengue infection experienced **retinal detachment** on the 7th day of fever. Her CBC showed leukopenia and thrombocytopenia (16.800 mm³) Retinal detachment, although rare, was probably **an ophthalmic complication of DHF and was associated with nadir platelet** (Sumardi,2011)



A) Optical coherence tomography



B) Funduscopy



C) Retinal thickness showing a hemorrhage

UNCOMMON ORGAN INVOLVEMENT

- A 59 year old male with **acute pancreatitis as the complication DHF** was reported. Although rare, this may cause more severe fatal condition, and difficulties in treatment. Early diagnosis and treatment ASAP is important (**Simadibrata,2012**)

REPEATING DENGUE INFECTIONS

- In a prospective study of in Bandung from 2000 until July 2004, **four volunteers with evidence of previous dengue exposure, experienced two consecutive episodes of dengue infections.**

Volunteer	Previous	2 nd infection	3 rd infection
1	DENV-1	DENV-3	unknown
2	DENV-2	DENV-3	DENV-1
3	DENV-2	DENV-4	DENV-3
4	multiple	DENV-4 (DHF)	DENV-3

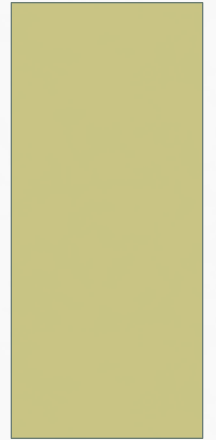
- Humans can experience **three sequential heterologous dengue infections.** Importantly, the occurrence of a second and third infection in individuals with pre-illness antibodies against multiple dengue serotypes indicates that **neutralizing antibodies are cross-reactive in vitro but not cross-protective in vivo** (Kosasih,2006)

WHO 1997 VS 2009 CLINICAL CATEGORIES

Study	Criteria	Severe dengue	Severity Gold standard	Sen	Spec
Setiati (2007)	WHO 97	DSS	Circulatory failure	86%	N/A
	Combination clinical signs			88% -99%	N/A
Basuki (2010)	WHO 97	DHF 3&4	Clinical intervention	74%	100%
	WHO 2009			87%	99%
V d Weg (2012)	WHO 97	all DHF	Intensive intervention	89.9%	25%
	WHO 2009			70.5%	70.4%

Different and contradictive results indicate larger studies using standardized criteria are needed

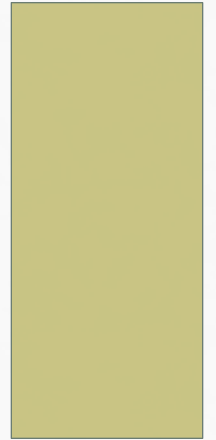
LABORATORY ASSAYS AND DIAGNOSTICS



NEUTRALIZING ANTIBODIES

Study	Purpose	Results / Conclusion
Martin (2006)	To evaluate an immunocytometric assay to measure neutralizing antibodies rapidly using a human cell line transfected to express a putative natural dengue receptor, DC-SIGN (CD209),	The DC-SIGN FACS neutralization assay is both rapid and amenable to automation, making it capable of the high-throughput required for analysis of samples from large vaccine clinical trials.
Yama-naka (2012)	to measure the balance of enhancing and neutralizing activities, which provides useful information for estimating in vivo antibody status.	A relatively high proportion of endemic children possessed complement-independent enhancing antibodies against some DENV types.
Konishi (2010)	To establish a simple assay system for infection-enhancing and -neutralizing antibodies to dengue type 2 virus using layers of semi-adherent K562	Neutralization tests using K562 cells reveal a more accurate in vivo status than using Vero cells. Human sera showed neutralizing and enhancing activities

PATHOGENESIS



TREATMENT



IV TREATMENT USING POLYGELINE

- Compared to baseline levels, IV infusion using **polygeline** decreased hematocrite in the first 6 hours during treatment and persisted in 48 hours of observation. Polygeline colloid (haemaccel) was a safe initial fluid treatment and can be used for maintaining fluid adequacy in adults with stage I-II of DHF (Pohan,2009)

PLATELET TRANSFUSION

- Platelet transfusions: **do not reduce the incidence of severe bleeding**. Avoiding unnecessary platelet transfusion may reduce hospitalization cost. (Chairulfatah,2003)
- There is currently no evidence to support prophylactic platelet transfusions . **A global survey** conducted in 20 countries highlights **the differences in the use of platelets in dengue and lacks of evidence base**. (Whitehorn,2012)

IN VITRO STUDY ON THE INHIBITORY EFFECT OF QUERCUS LUSITANICA

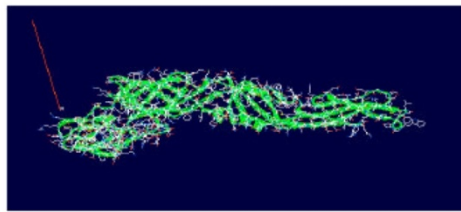
- Quercus lusitanica (majuphal, sky-fruit, **tunjuk langit**) **extract may inhibit dengue virus type 2 replication** by down-regulating NS1 protein expression of infected C6/36. The antiviral activity showed dose-dependent inhibition. (**Muliawan,2006**)



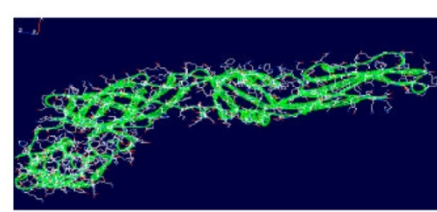
PREVENTION

DENGUE VACCINE

- **In silico study** of dengue virus vaccines, by using envelope (E) protein of DENV-2 and DENV-3 as their backbones found that among six E DENV-3 **petides** and six E DENV-2 petides, HMM4; HMM6; ANN1; ANN3; ANN4; ANN5; and ANN6 were **the best in silico vaccine design, based on their similarity with native E DENV Proteins**. This research could be applied for the wet laboratory and computerised vaccine design (Tambunan, 2009).



ANN1 vaccine design



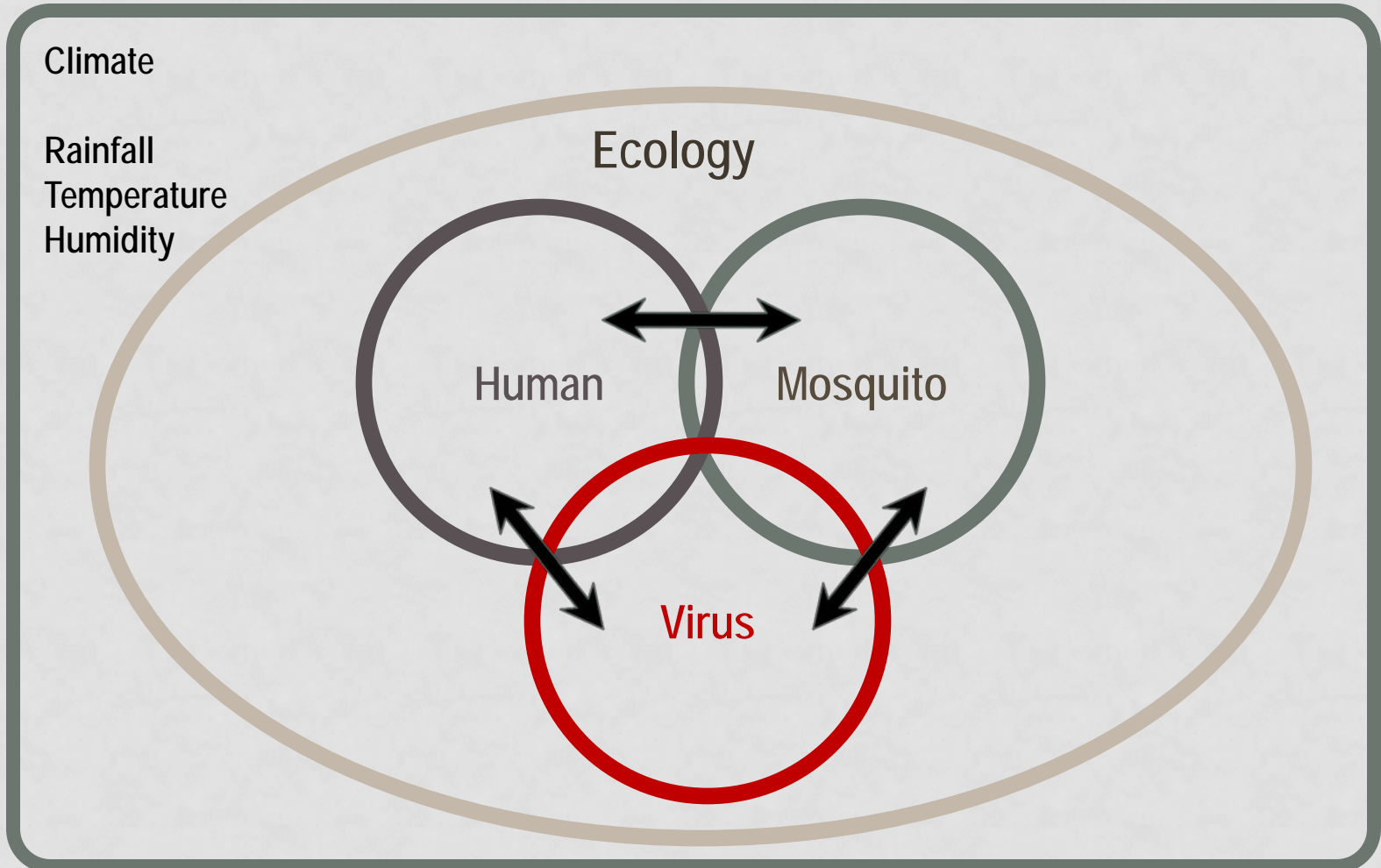
HMM1 vaccine design

Current report (Halstead, 2012)

Dengue vaccine development: a 75% solution?

DENV2 genotype incorporated into this vaccine might not have raised protective antibodies against the different DENV2 genotype circulating in Thailand in 2009–11.

CONCLUSION



CONCLUSION

- Dengue research in Indonesia covers all fields, but not well-coordinated → dengue consortium is required
- Needs to focus on research that may provide significant public health impact
- These reports are just parts of dengue research in Indonesia → many unpublished
- International collaboration is encouraged

RESEARCH GAP AND NEEDED

- **Reducing disease severity and case fatality**
 - Optimization of Clinical management
 - A better understanding of dengue pathogenesis
- **Transmission control through improved vector management**
 - Vector control tools and strategies
 - Surveillance and response
- **Primary and secondary prevention**
 - Vaccines
 - Drugs

REDUCING DISEASE SEVERITY AND CASE FATALITY

- **Optimization of clinical management**
 - Guidelines for triage and outpatient care
 - Validity and accessibility of new diagnostics for dengue
 - Predictive value of prognostic markers (host/viral early warning signs)
 - Management of patient in pregnancy or with co-morbidities (DM, hypertension, obesity, CHD, etc)

REDUCING DISEASE SEVERITY AND CASE FATALITY

- **A better understanding of dengue pathogenesis**
 - Molecular and pathophysiological changes underlying endothelial permeability
 - dengue virus diversity (heterogeneity: virulence, epidemic potential)
 - Mechanisms of antibody-mediated enhancement and protection
 - Mechanisms of virus entry and cellular/tissue tropism
 - T and B cell responses → immunopathology of 1st/2nd infections

TRANSMISSION CONTROL THROUGH IMPROVED VECTOR MANAGEMENT

- **Vector control tools and strategies**
 - New vector control tools and strategies in different contexts
 - Effectiveness, cost, community acceptance of new and/or existing tools, integrated vector management and ecosystems interventions
 - Scaling up of pilot projects to state or national level
 - e.g. Wolbachia study?
- **Surveillance and response**
 - Entomological surveys as indicators of risk for outbreak
 - Dengue virus diversity
 - Early warning response system
 - The contribution of information technology

PRIMARY AND SECONDARY PREVENTION

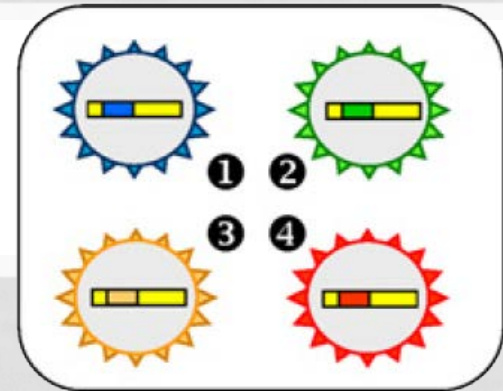
- **Vaccine**

- Discovery and pre-clinical research of vaccines, candidates, adjuvant and vaccination strategies
- Several candidates in clinical development
- Safe but broadly immunogenic

VACCINE TRIAL DEVELOPMENT

- DENVax:
 - based on the backbone of the attenuated DENV-2 and replacing prM and E genes from other serotypes to the attenuated DENV-2
 - phase I, 96% sero-converting to ≥ 3 serotypes
- US-NIH TV-005 phase I trial:
 - Live attenuated tetravalent vaccine
 - Single subcutaneous dose is safe and induces a tetravalent response in 90% of vaccinees
- UI/Kobe:
 - DNA vaccine based on prM/E regions of DENV-2
Cosmopolitan, pre-clinical study in mice
 - Induced humoral immune responses that could neutralize other DENV-2 genotype

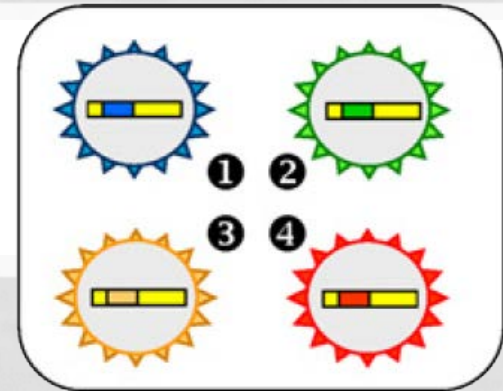
VACCINE CYD-TDV



- Sanofii Pasteur :

- In 10,275 children observed for 25 months: incidence was 1.8% (treat) and 4.1% control → 56.5%
- To DENV 1,2,3,and 4: 50%, 35%, 65%, 72% → overall depends on serotype distribution at any given time
- The absence of severe disease due to ADE is reassuring, but **observation should be continued** (Cuba epidemic: 4-20 y interval)
- Efficacy in younger, which have higher incidence of dengue and higher risk for more severe, was lower than older groups (33.7% vs.74.4%) →boosts and broadens pre-existing immunity rather than raising protective immunity
- Efficacy 56% justify this vaccine for immunization program?

VACCINE CYD-TDV



- **Questions to study:**

- Efficacy 56% justify this vaccine for immunization program?
- Epidemiological threshold of dengue activity upon which national dengue vaccination are justified and cost effective?
- What's about epidemiological setting with high dominance of serotype 2?
- Population: high-risk age groups or groups with highest vaccine efficacy?
- With 56% efficacy, continued support for the development of other novel strategies (vaccine, drugs, case management, vector control is needed)

PRIMARY AND SECONDARY PREVENTION

- **Drugs**

- Anti-dengue drugs may have:
 - Prophylactic use (e.g. outbreak prevention)
 - Therapeutic use (prevention of severe disease)
- Drug discovery has accelerated due to the knowledge of 'drugable' targets in the virus
- Further elucidation of:
 - The structure of viral encoded proteins to aid rational drug
 - New (including natural) products or existing licensed drugs with good safety profile

DRUG TRIALS

- Celgosivir
 - In mice showed enhanced survival, reduced viremia and robust immune response (Rathore, 2011, Watanabe, 2012)
 - a phase 1b, randomised, double-blind, placebo-controlled, proof-of-concept trial (Low, 2014)
 - Mean viral log reduction and AUC was greater in celgosivir (n=24) than in placebo (n=26), $p=0.203$ and $p=0.973$, faster NS1 clearance → **underpowered, further clinical trials needed**
 - Adverse events between groups: safe and well-tolerated → possible to alter the dosage in future studies

DRUG TRIALS

- Balapiravir failed to achieve efficacy (Farrar, 2013)
 - Randomized, double-blind placebo controlled trial
 - Adverse event profile in balapiravir (n=32) and placebo (n=32) groups was similar .
 - Daily viremia and NS1 evaluation indicated balapiravir did not alter these virological markers, nor did it reduce the fever clearance time.
- Possibly explanation of the discrepancy between in vitro and invivo results of balapiravir (Chen, 2014)
 - Balapiravir lost 125-fold potency when it was used to treat PBMCs that were pre-infected with DENV
 - should be calculated when estimating the efficacious dose

TERIMA KASIH

