Serodetection of Dengue Virus Infection and its Antibodies Among Saudi Donors

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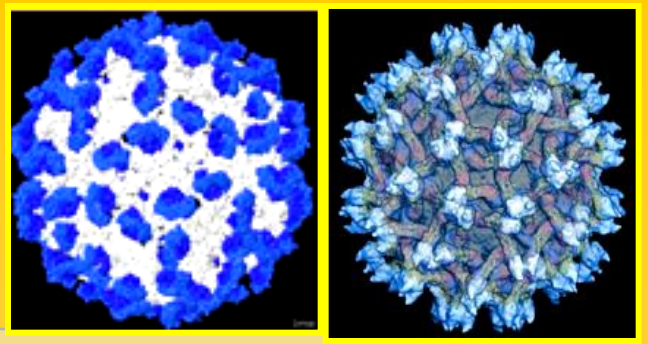
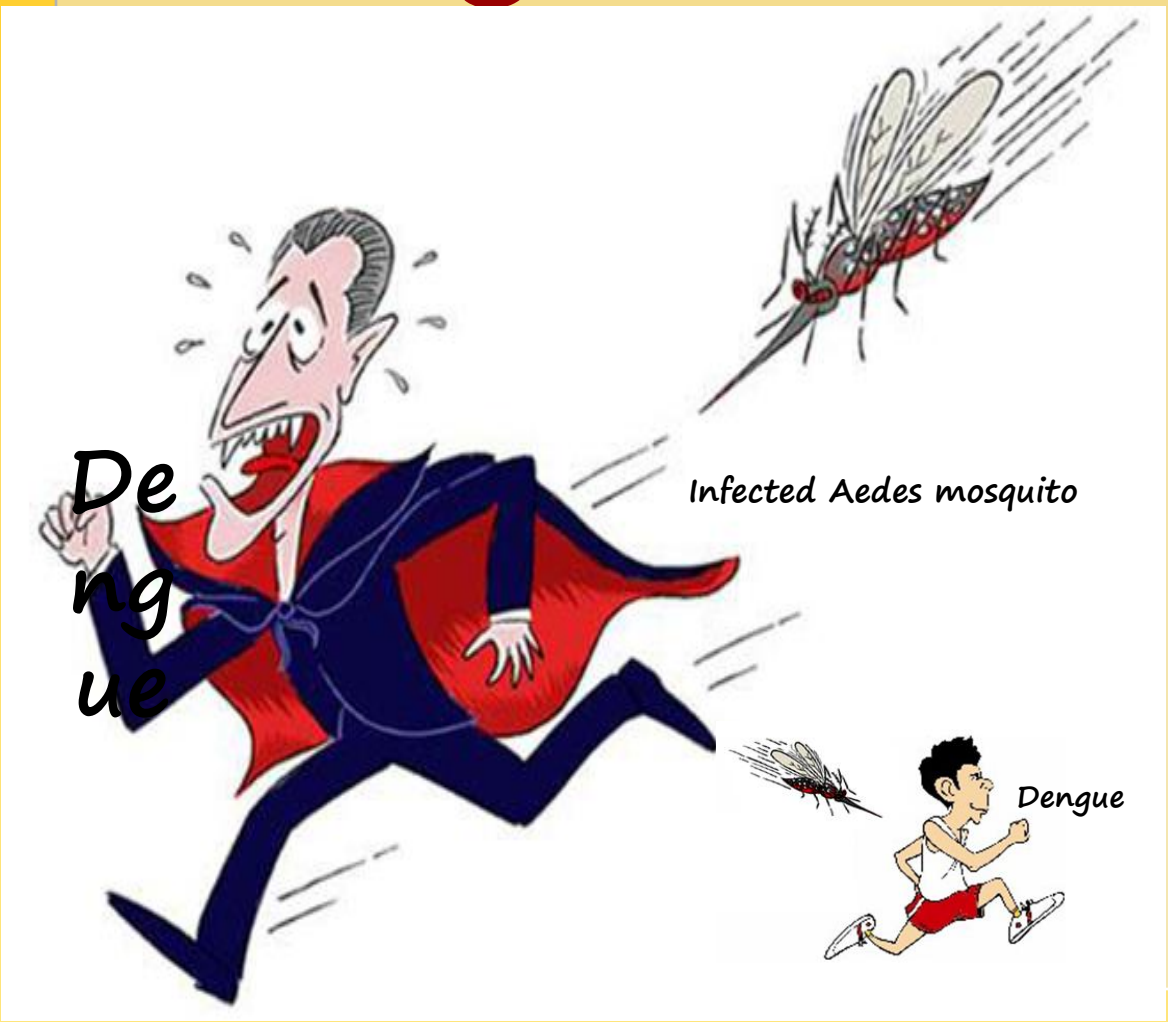
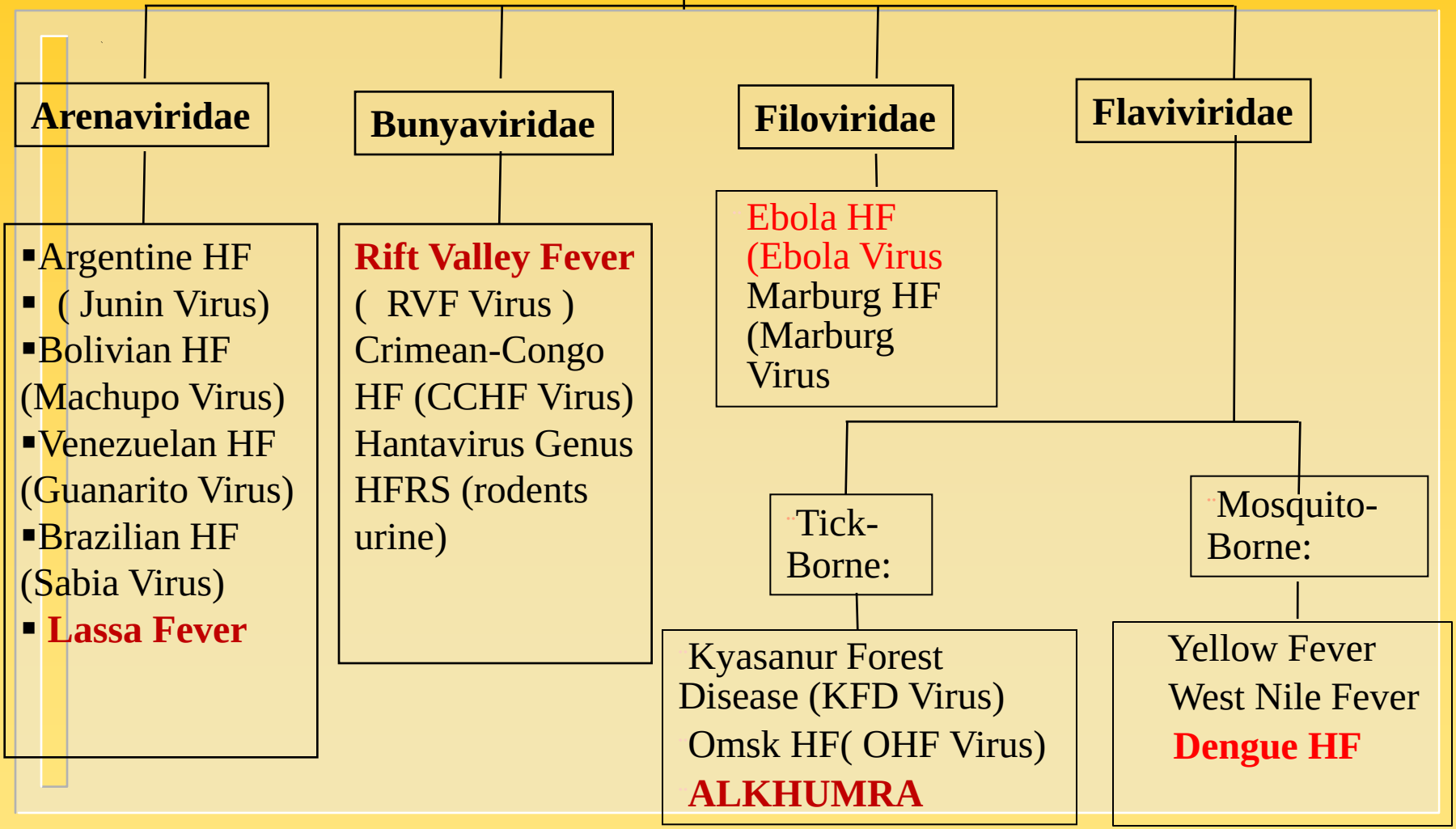
Dengue Virus & Dengue Disease





- What is Dengue?
- Mode of Transmission ?
- Why do we care about it?
- Is It Endemic In KSA?
- Lab Diagnosis of Dengue
- Background of this Study
- Aim of the Study
- Methodology
- Results & Discussion
- Conclusion & Recomm.

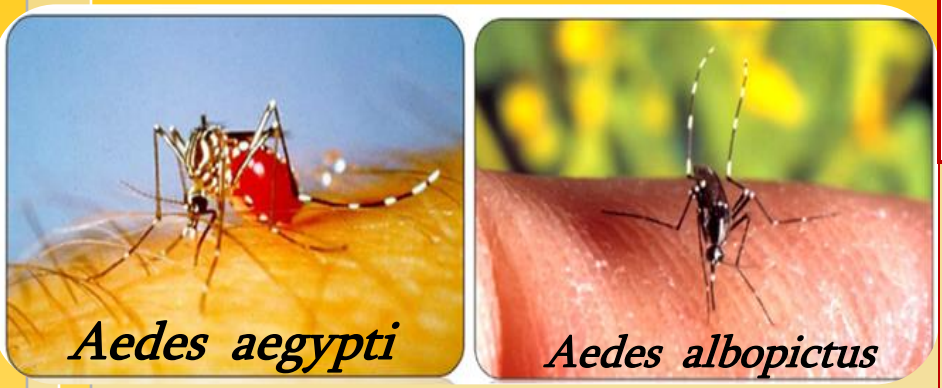
Hemorrhagic Fever Viruses: Taxonomy

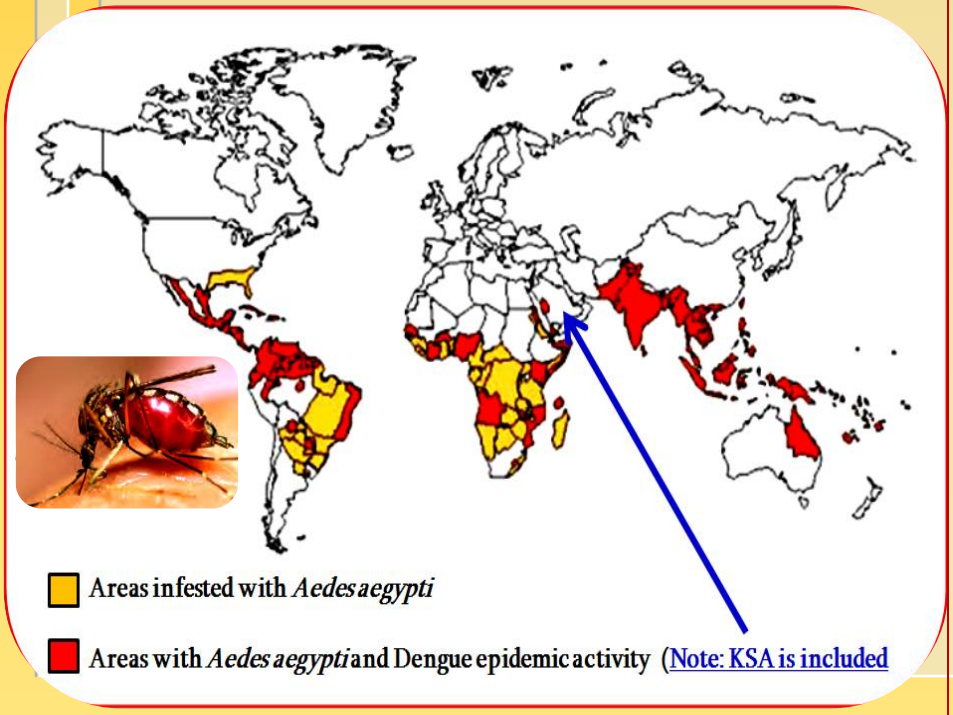


Dengue viruses

- SS-RNA arbovirus (Flavivirus)
- 5 serotypes (DEN-1, 2, 3, 4, 5)
  - Based on envelop glycoprotein
  - DEN-1 and 3 are more closely related
  - DEN-4 less closely related to others
  - **Virulent variants (genotypes) within serotype**
- Infection with any serotype confers specific lifelong immunity
- Transient cross-protection to other serotypes
- Any serotype can cause severe / fatal disease

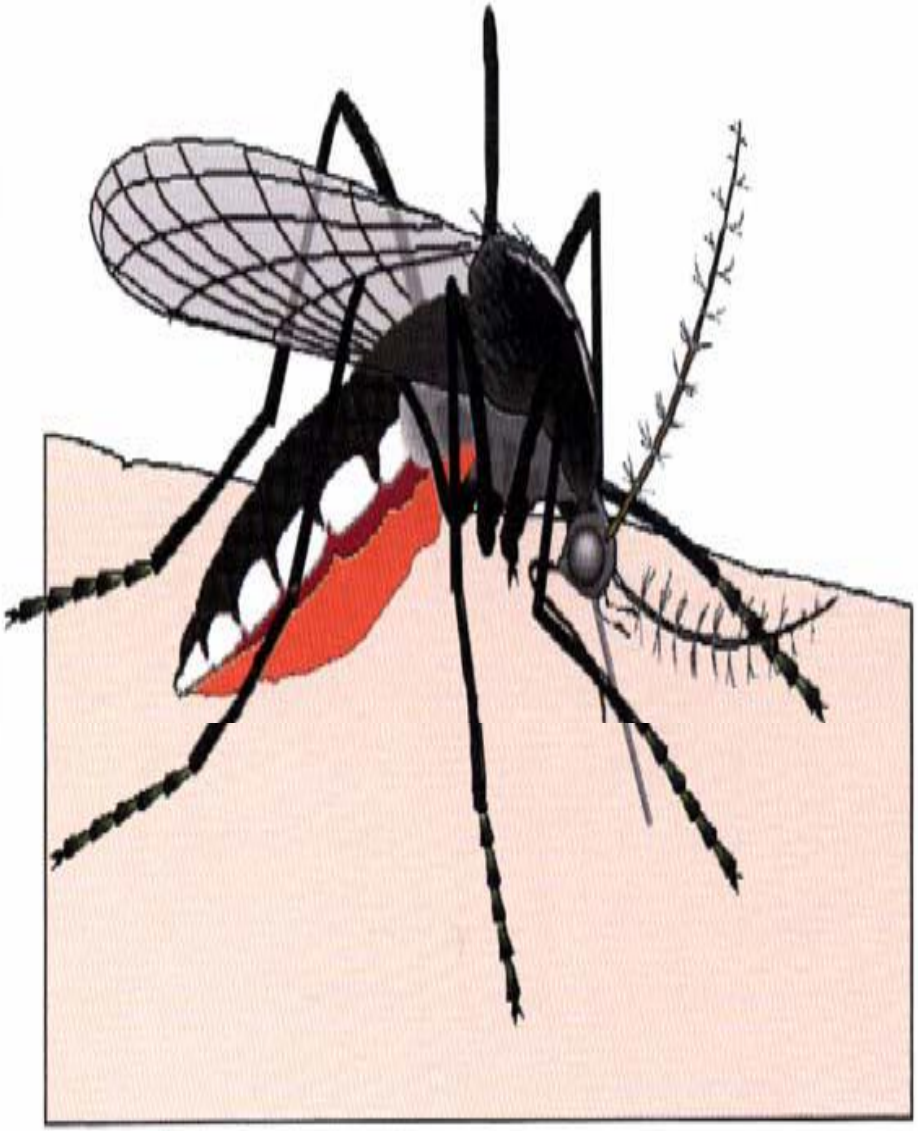
Mode of Transmission: by female Aedes mosquitoes



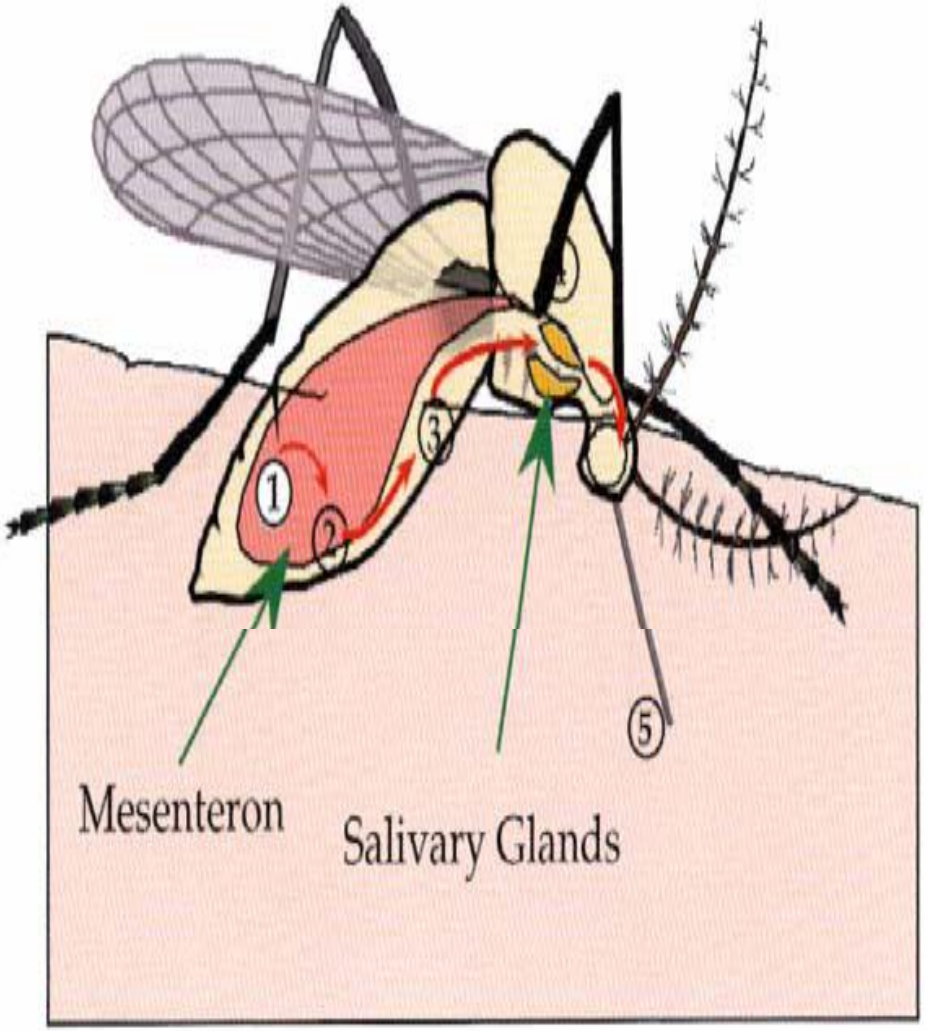


Factors Related To These Vectors That Worsen The Burden Of Dengue Endemicity:

- Once infected, they carry the virus and remain infective for their lifespan.
- Mainly fed on day time.
- Travel well between cities and countries through passengers and their merchandise &bags.
- Most of them are now resistant to the used insecticides.
- Recent research indicated that these mosquitoes secrete with their saliva specific substances that enhance virus transmission and increase its viremia levels.

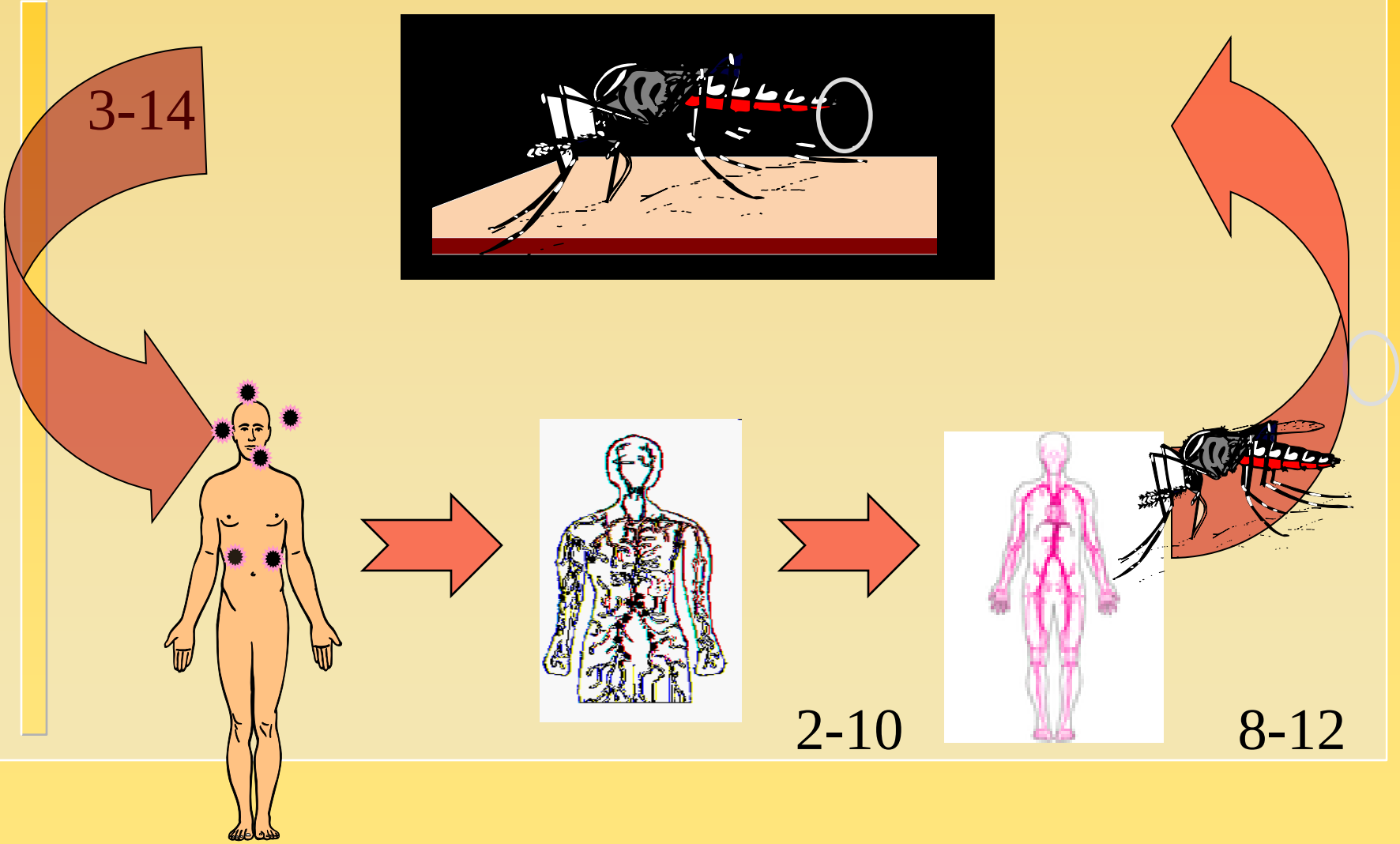


A. A female mosquito takes a blood meal



B. Cutaway view of the mosquito showing steps in the replication and transmission of an arbovirus.

Replication and Transmission of Dengue Virus (Part 1)



Why do we care about Dengue ?

- WHO estimates that dengue ranks as the most important mosquito-borne viral disease affecting the humans in the world.
- Dengue is now endemic in over 100 tropical and subtropical countries in Asia, Africa, the Eastern Mediterranean and the Western Pacific regions, and the Americas, and the infected population is about 100 million every year. Also, outbreaks of dengue has been reported more recently in Europe & Australia.
- Unfortunately, to date there is no license vaccine available for preventing dengue virus infection.

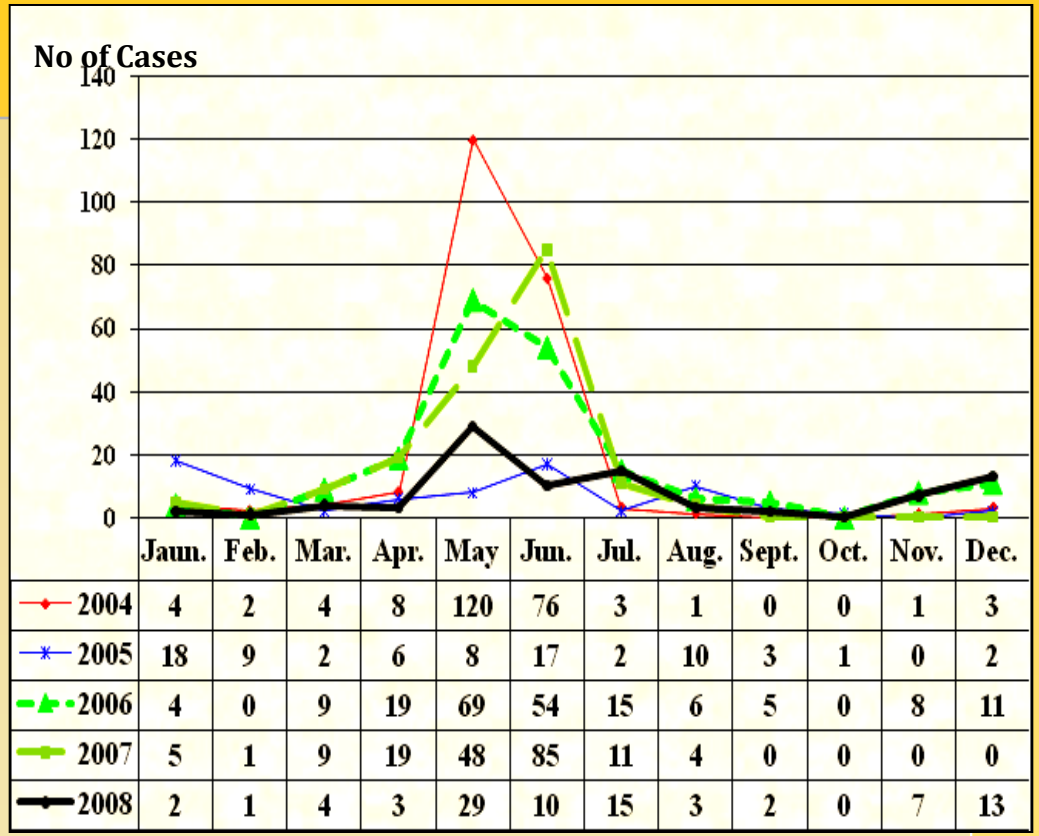
GLOBAL BURDEN OF DENGUE

The world distribution of Dengue.

As we see, the Western Region of KSA is endemic with Dengue

History of Dengue in KSA

- During the 1990s, an outbreak was reported for the first time in Jeddah, and the virus was isolated by Fakeeh and Zaki .
- 1994 to 2002, the referral laboratory in Jeddah reported 319 cases.
- Next, two peaks were reported in 2005/2006 and other two in 2008.



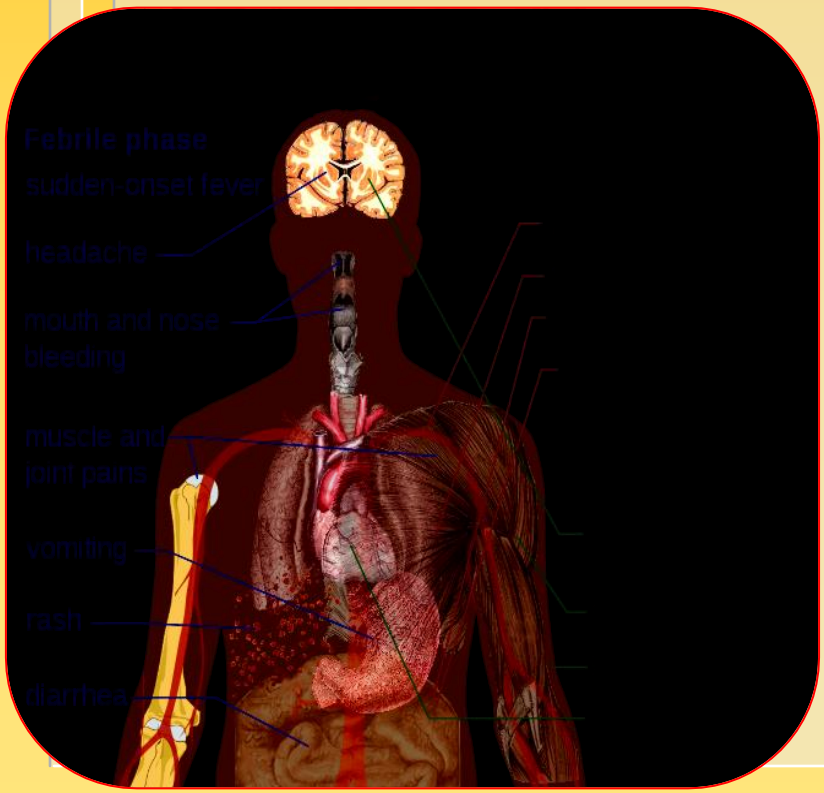
	Jaun.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sept.	Oct.	Nov.	Dec.
2004	4	2	4	8	120	76	3	1	0	0	1	3
2005	18	9	2	6	8	17	2	10	3	1	0	2
2006	4	0	9	19	69	54	15	6	5	0	8	11
2007	5	1	9	19	48	85	11	4	0	0	0	0
2008	2	1	4	3	29	10	15	3	2	0	7	13

A diagrammatic presentation of detected cases of dengue fever in Holly Makkah, KSA, from January 2004 to February 2008. (*Adopted from Central Department of Statistics and Information, KSA. 2009*).

Dengue is now endemic in the western and southern regions of KSA

Clinical Manifestations of Dengue Virus Infection

- **Asymptomatic**
- **Symptomatic**
  - Undifferentiated Fever
  - **Dengue Fever**
    - Classic Dengue
    - Dengue Hemorrhagic Fever (DHF)
    - Dengue Shock Syndrome (DSS)



With high mortality rate

Dengue Fever

Dengue fever is characterized by:

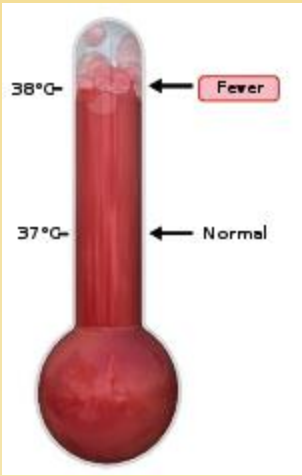
Fever

Rash (3-5 days after fever)

Muscle and joint pains

Aedes aegypti mosquito

severe frontal headache



Petechiae on chest wall in child with DHF.

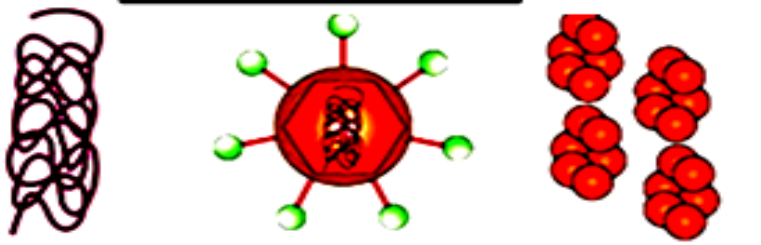
Subcutaneous hemorrhage in child with DHF

Petechiae on the arm

Hemorrhagic conjunctivitis

Laboratory Methods for Dengue Diagnosis

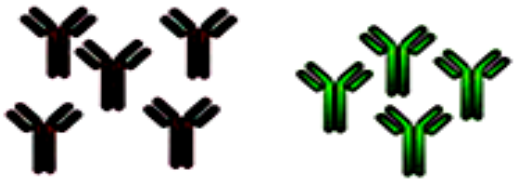
Direct Methods



Genome detection | Virus isolation | Antigen detection e.g. NS1

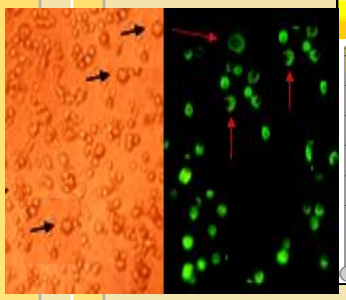
- PCR (RT-PCR)
- Virus isolation in specific cell cultures
- Serodetection of NS1 antigen by ELISA

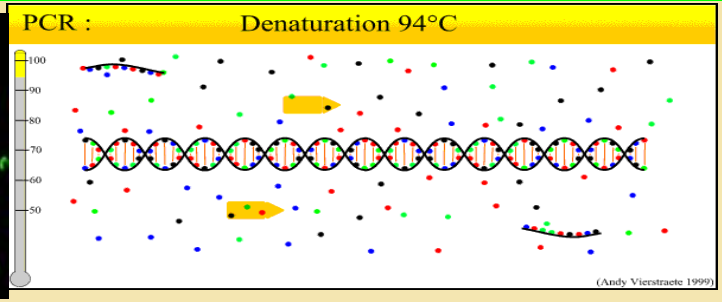
Indirect Methods



Serology IgM | Serology IgG

Serological detection of anti-DENV IgM and IgG antibodies By ELISA



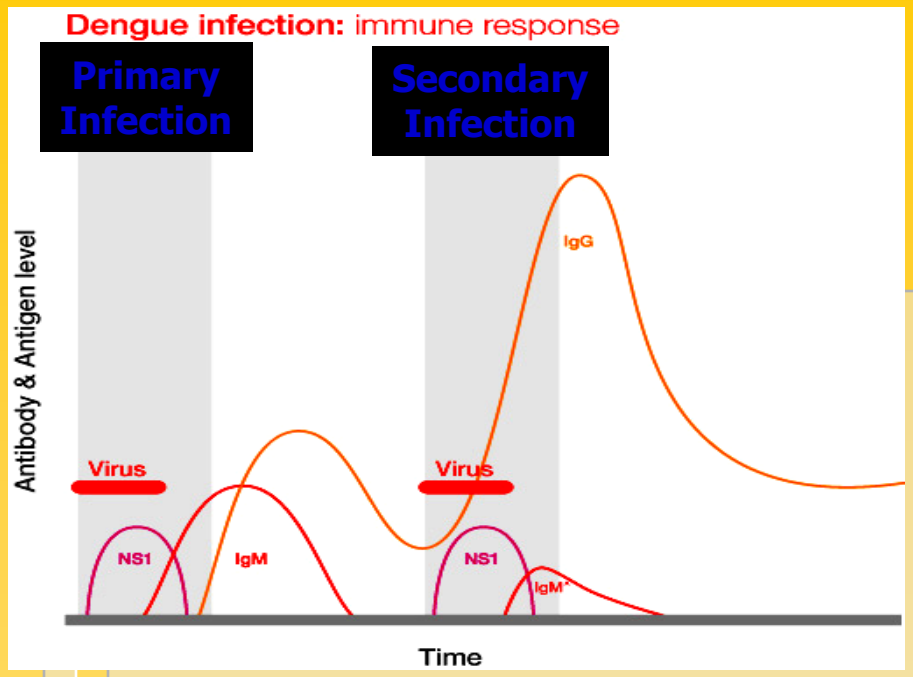


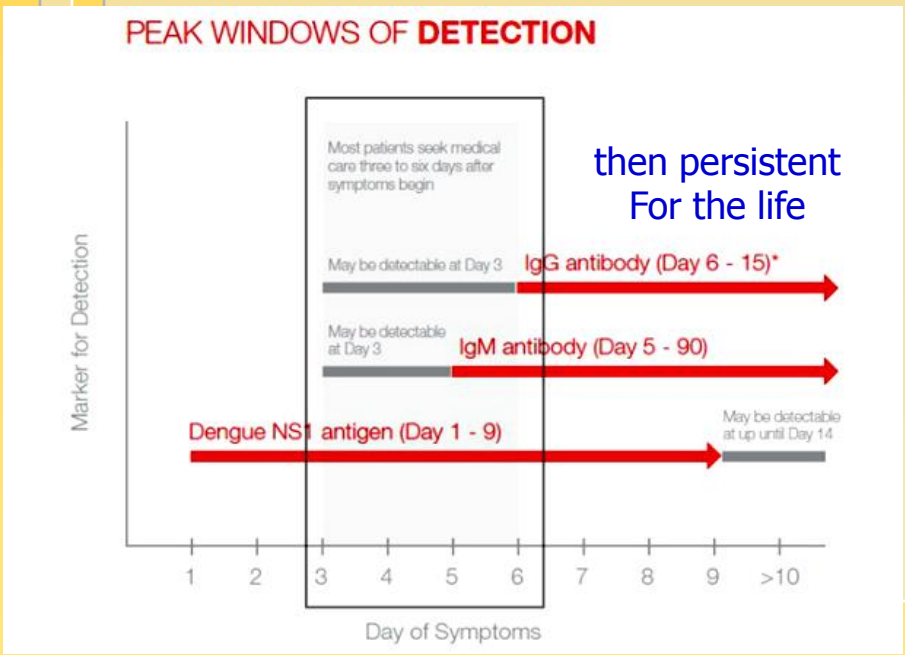
Combined Diagnostic Power

NS1 + IgM + IgG

Expensive, Not common

Dengue Infections Testing Algorithm





In addition to PCR, the major diagnostic tests of dengue infection include detection of viral NS1 antigen and IgM and IgG antibodies in patient's serum.

In most patients, NS1 antigen can be detected from day 1 to 9 of symptoms; while IgM and IgG antibodies can be detected five days after the onset of symptoms. The amounts of IgM and IgG antibodies depend on whether the infection is a primary or a secondary infection.

ALL Lab tests should be interpreted with clinical presentation.

Background of this Study

- Emerging infectious diseases still pose threats and risks in blood transfusion.
- In this aspect, a special recent attention has been paid to the significant role of blood transfusion in transmission of DENVs from asymptomatic infected donors to recipients and this in turn serve as a source of virus dissemination and endemicity.

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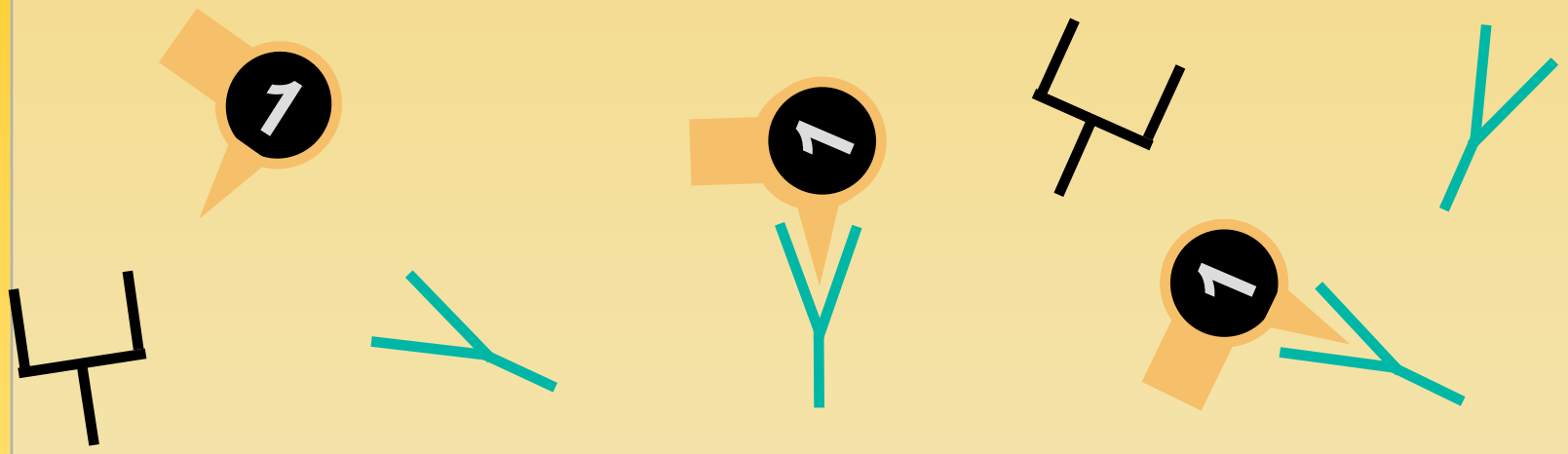
Background …continue

- Presence of anti-DENV antibodies has also facing an important concern in blood transfusion. In this concept, blood donors with positive anti-DENV antibodies may increase the susceptibility of recipients for immunology conditions, with greater risk of hemorrhagic dengue if they are infected by a second DENV serotype within six months after blood transmission.
- Further, the presence of heterophile antibodies of a previous infection may facilitate the entrance of other viral serotypes.

Therefore, The study has been designed

Homologous Antibodies Form Non-infectious Complexes

Persons who have experienced



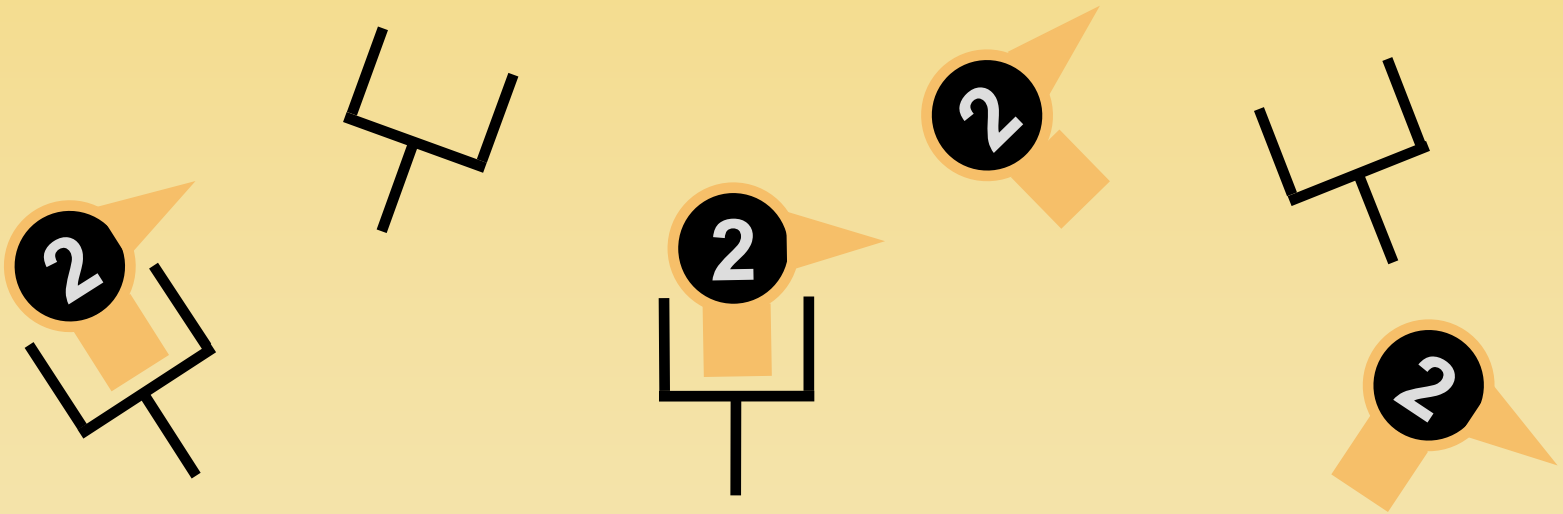
1 Dengue 1 virus

Neutralizing antibody to Dengue 1 virus

Non-neutralizing antibody

1 Complex formed by neutralizing antibody and virus

Heterologous Antibodies Form Infectious Complexes



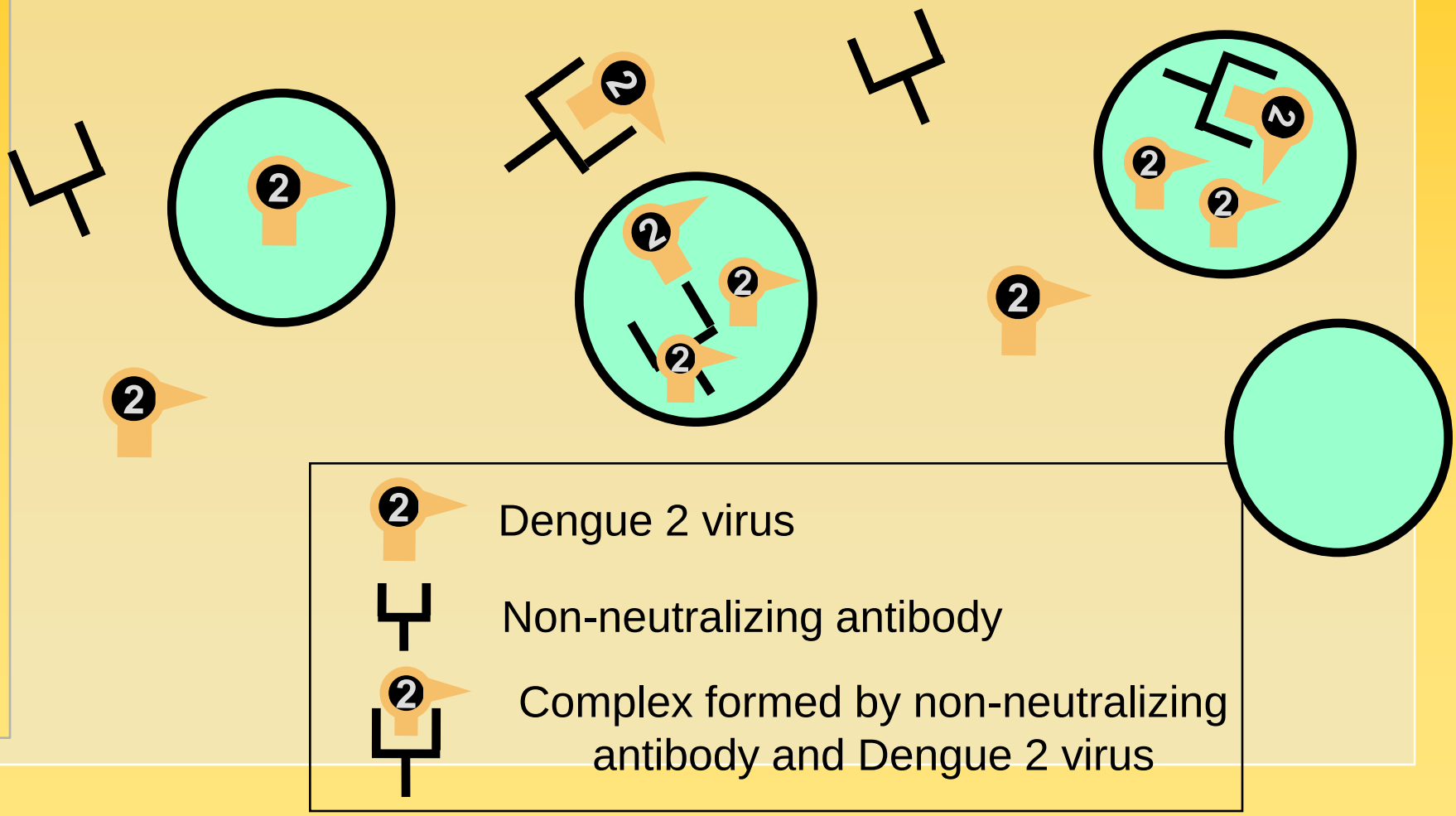
2 Dengue 2 virus

Non-neutralizing antibody to Dengue 1 virus

2 Complex formed by non-neutralizing antibody and virus

Heterologous Complexes Enter More Monocytes, Where Virus Replicates

vasoactive mediators, resulting in increased vascular permeability



Dengue in Saudi Arabia

- Still in epidemic
- Yet to have published final statistics for present epidemic

What should be done?

- Adequate bed rest
- Adequate fluid intake (Plain water alone→ **Not sufficient**)
- Analgesics/antipyretics (paracetamol + tepid sponging)

What should be avoided?

- Aspirin/NSAIDS/steroids
- Antibiotics are not indicated
- Platelets are not indicated unless there is evidence of active bleeding

Aim of The Work :

Given the absence of an approved blood screening test for dengue virus and its antibodies among the blood donors in Saudi Arabia; and in response to this emerged event, the current study is designed to highlight the seroprevalence of DENV infection and/or its antibodies among blood donors in Holy Makkah (a part of the Western Region of Saudi Arabia that endemic with DENV) to improve the safety of blood supply and blood products in blood donation services.

METHODOLOGY

Participants, Sampling & Diagnostic Assays:

A total of 100 healthy eligible Saudi male blood donors (age ranged between 25 and 50 years), who were negative for HIV/HCV/HBV infections and accepted according to the policy set up by the Kingdom of Saudi Arabia Health Ministry for blood donation, were randomly included. From each enrolled donor, 10 mL of whole venous blood was collected in tubes without anticoagulant. The tubes were centrifuged at 3000 rpm for 15 minutes to obtain the serum. The sera samples were separated and used for the following **diagnostic assays of dengue:**

1. **Direct Method** → Qualitative assay of NS1 Antigen
2. **Indirect Methods: Detection of anti-DENV-antibodies** → Qualitative assay of IgM antibodies; Qualitative assay of IgG antibodies

By using commercial ELISA kits (PanBio Diagnostics, Australia)

Data Calculation

- **In each assay, the Panbio Units** (ie, the measurement units) **of each sample were calculated according to manufacture's instructions.**

Data Interpretation

Test	Negative result (Panbio Units)	Equivocal result (Panbio Units)	Positive result (Panbio Units)
DENV-NS1 Antigen	< 9	9 – 11	> 11
Anti-DENV-IgM Ab	< 9	9 – 11	> 11
Anti-DENV-IgG Ab	< 18	18 – 22	> 22

According to the manufacture's instructions, the results were reported as positive, negative or equivocal, and not as a numerical value.

RESULTS

Table 1: Overall results of DENV-NS1 antigen and anti-DENV IgG & IgM antibodies among the tested 100 blood donors

Tested blood donors	DENV-NS1 positivity	Anti-DENV antibody positivity		
		IgM only	IgG only	IgM + IgG
100	1 (1%)	6 (6%)	7 (7%)	0 (0%)

Table 2: Instance and specificity of DENV-NS1 antigen and anti-DENV antibodies among the tested 100 blood donors.

Parameter	Panbio Unit	Interpretation*
NS1 antigen:		
1	17.30	Positive
2	10.30	Equivocal
IgM antibody:		
1	36.60	Positive
2	53.90	Positive
3	15.94	Positive
4	15.93	Positive
5	42.50	Positive
6	13.30	Positive
7	10.70	Equivocal
IgG antibody:		
1	71.00	Positive
2	182.00	Positive
3	27.90	Positive
4	73.00	Positive
5	71.30	Positive
6	23.26	Positive
7	114.00	Positive
8	20.40	Equivocal

Importance of detection of DENV-NS1 antigen

The finding of DENV-NS1 antigen suggests that these donors were actively infected with the DENV and had ongoing asymptomatic viraemia or were sub-clinical carriers of the virus. It is conceivable that blood from NS1-positive, active carriers of DENV could transmit the infection to recipients.

Importantly, some recent studies showed that recipients of blood from asymptomatic DENV-infected donors have developed fever associated with severe thrombocytopenia and hypotension 3 days after the blood transfusion.

Given that blood donations are still not routinely screened for DENV, asymptomatic DENV-infected donors may silently transmit the virus to prospective recipients, and so stake holders in blood transfusion practices should consider DENV as a potential threat to transfusion safety.

Importance of detection of Anti-DENV antibodies

Herein, both IgM and IgG anti-DENV antibodies were detected. Presence of anti-DENV antibodies in blood donation have also been confirmed in a number of worldwide recent studies.

Detection of anti-DENV IgM/IgG antibodies may indicate the presence of primary and/or secondary DENV infections depending on the antibody titers, and positivity for IgM points to an ongoing infection suggesting that the donor is in a carrier stage of infection.

Clinically, it is well known that the serious forms of Dengue disease are more likely to occur during a second infection with a different DENV serotype from that which caused the primary infection.

Importance of transfusion of Anti-DENV antibodies

The most accepted pathogenic hypothesis is related to the role of circulating non-neutralising heterotypic anti-DENV antibodies, a phenomenon known as antibody-dependent enhancement (ADE).

In ADE, both circulating neutralising and non-neutralising (or partially neutralising) antiviral antibodies are present in a person who has been infected by one DENV serotype. If a second infection by a different DENV serotype occurs, the virus may be recognized by these cross-reactive heterotypic non neutralising antibodies, resulting in antigen-antibody complex formation that enhance viral penetration and replication as well as the release of vasoactive mediators, increased vascular permeability, plasma leakage and, possibly, to the development of hypovolaemic shock.

Conclusions

- Data of the present study show for 1st time the seropositivity for DENV and its antibodies among the Saudi blood donors, and suggest the crucial need for establishment screening of DENV and/or its antibodies in blood donors in Saudi Arabia, so that the quality of blood transfusions is guaranteed and the endemicity of DENV is reduced.

- However, before drawing firm conclusions, an important limitation must be described which is: the participants were only males and the sample population was only 100 and thus less representative of the general population

- Thus, we are now expanding this study to include a large number and at different blood donation units to confirm the findings of the present study and its related recommendation.

Recommendations

- Importance of applying an effective and continuous seromonitoring of DENV infection among blood donors in countries with well know history of dengue hyperendemicity before blood donation process.
- Necessity of Future Successful Vaccine development.
- Establishment of coherent & coordinated multisectoral committee for fighting against dengue.

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Blood Transfus. 2014 Oct 29:1-4. doi: 10.2450/2014.0134-14. [Epub ahead of print]

Serodetection of Dengue virus and its antibodies among blood donors in the western region of Saudi Arabia: a preliminary study.

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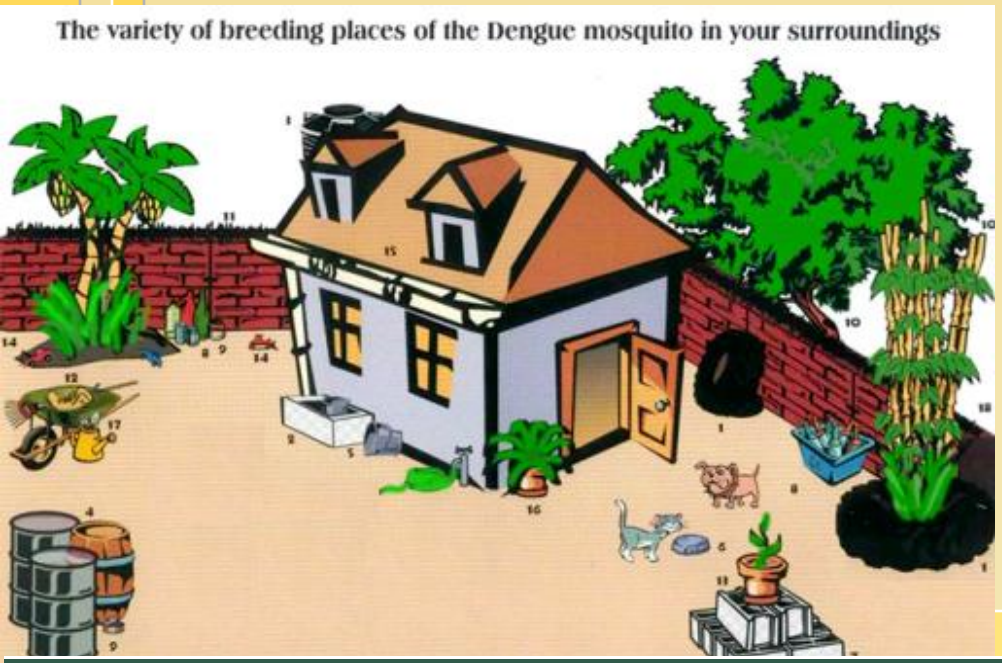
“Finally”

For Stopping Dengue

Fight Against The Bite



Fight Against Transmission by Blood Transfusion

Vaccine Development And Public Health Strategies

Stop the mosquito Stop dengue