Using Biotechnology to Study the Dengue Fever Virus

At a Glance

This lesson is designed to be implemented over a period of several weeks. Module 1 can be implemented early in the school year as a supplement to instruction on the nature of scientific inquiry. Module 2 can be used as a culminating activity for the biochemistry unit.

Students will be introduced to dengue fever as an emerging pathogen in Florida and conduct research to learn more about dengue. They will conduct a field study to determine if the vectors for the dengue viruses are present in the community.

Later, students will be given case studies for fictional subjects at risk for dengue virus exposure. Students will be responsible for accurately recording information about each patient, performing an ELISA to test patients’ sera for antibodies to dengue, and producing a written explanation of the results.

The Focus

Students will engage in scientific inquiry to determine if the vectors of an emerging viral pathogen are present in their community. They will be introduced to ELISA as a tool to detect the presence of antibodies to a virus, an indication of exposure to that pathogen.

Major Concepts

- Students will recognize that scientists conduct research in a variety of ways.
- Students will know that exposure to a pathogen leads to an immune response by the host.
- Students will realize that a pathogen may cause disease symptoms of varying severity.
- Students will understand the role of vectors in the spread of disease.
- Students will understand the multiple roles of proteins.
- Students will appreciate the role of biotechnology as a tool in the fields of research and medicine.

Objectives

- Students will conduct research using reliable Internet resources to increase their knowledge about a vector-borne disease.
- Students will engage in inquiry to determine whether or not disease vectors are present in their community and if it is possible for the pathogen to become endemic.
- Students will be able to illustrate and explain the steps of the ELISA antibody test including a description of how different proteins function as antigens, antibodies, or enzymes.
- Students will successfully complete the procedures of ELISA antibody tests and accurately report and interpret their results.
- Students will apply their knowledge about vector-borne diseases and act to eliminate vector habitats in their community.

Prerequisite Knowledge

Module 1

Students should be introduced to various methods of data collection and the difference between quantitative and qualitative data. They should know how to organize data into tables, create and interpret graphs, and know that data must be analyzed in order to be meaningful.
Module 2

Students should be familiar with the basic structure and functions of proteins. They should understand that enzymes are catalysts for biochemical reactions. Students should know that viral proteins serve as antigens, and that antibodies form as part of the immune response after exposure to a virus. They should understand that detection of antibodies to a viral antigen indicates a patient has been exposed to that virus.

**Overall time estimate**

Module 1 requires one 45-minute class and two 90-minute block periods. In addition, students must spend some time at home collecting water samples. The total time span required depends on the maturation of mosquito larvae into adults.

Module 2 requires three 45-minute classes and one 90-minute class. The total time span depends only on the placement of the activities within the regular biology curriculum.

**Vocabulary**

- antibody: a protein produced by the immune system when it detects a foreign substance
- antigen: a substance that causes the immune system to produce antibodies
- circulatory failure: the inability of the circulatory system to function well enough to sustain health
- ELISA: enzyme-linked immune-sorbent assay, commonly used laboratory test to detect antibodies
- endemic disease: a disease that is common and occurs at a predictable rate within an area
- enzyme: a protein that is a catalyst for a specific biochemical reaction
- hemorrhagic: bleeding, usually severe bleeding
- larva/larvae: a developmental stage in the life cycle of some insects
- pathogen: a disease-causing agent
- risk factor: a condition or behavior that make someone susceptible to contracting a disease
- subtropical: a climate characterized by hot, humid summers and mild winters
- symptom: a condition that is an indication of disease
- tropical: a climate characterized by warm, humid weather year-round
- vaccine: a preparation that stimulates the immune system to produce antibodies against a pathogen
- vector: an organism that delivers a pathogen to a host organism

**National Science Education Standards**

**Next Generation Florida Science Standards**

SC.912.N.1.1 Define a problem based on a specific body of knowledge, for example: biology, chemistry, physics, and earth/space science, and do the following:

1. pose questions about the natural world,
2. conduct systematic observations,
3. examine books and other sources of information to see what is already known,
4. review what is known in light of empirical evidence,
5. plan investigations,
6. use tools to gather, analyze, and interpret data (this includes the use of measurement in metric and other systems, and also the generation and interpretation of graphical representations of data, including data tables and graphs),
7. pose answers, explanations, or descriptions of events,
8. generate explanations that explicate or describe natural phenomena (inferences),
9. use appropriate evidence and reasoning to justify these explanations to others,
10. communicate results of scientific investigations,
11. and evaluate the merits of the explanations produced by others.

SC.912.N.1.4 Identify sources of information and assess their reliability according to the strict standards of scientific investigation.

SC.912.N.1.6 Describe how scientific inferences are drawn from scientific observations and provide examples from the content being studied.

SC.912.N.1.7 Recognize the role of creativity in constructing scientific questions, methods and explanations.

SC.912.L.14.6 Explain the significance of genetic factors, environmental factors, and pathogenic agents to health from the perspectives of both individual and public health.

SC.912.L.14.52 Explain the basic functions of the human immune system, including specific and nonspecific immune response, vaccines, and antibiotics.

SC.912.L.18.4 Describe the structures of proteins and amino acids. Explain the functions of proteins in living organisms. Identify some reactions that amino acids undergo. Relate the structure and function of enzymes.

SC.912.L.16.10 Evaluate the impact of biotechnology on the individual, society and the environment, including medical and ethical issues.

HE.912.C.1.3 Evaluate how environment and personal health are interrelated.

HE.912.C.1.8 Analyze strategies for prevention, detection, and treatment of communicable and chronic diseases.

LA.910.2.2.3 Organize information to show understanding or relationships among facts, ideas, and events (e.g., representing key points within text through charting, mapping, paraphrasing, summarizing, comparing, contrasting, or outlining)

The Basic Science-Health Connection

Using articles from the local newspaper, students will be introduced to dengue fever, a disease that they probably believe is exotic and far removed from them. The articles will illustrate that, in an age when world travel is common for humans and cargo, pathogens are able to move from one area of the world to another. Field research will reveal that, although the locally diagnosed cases were contracted elsewhere, the vectors for the viral pathogen can readily be found in the students’ neighborhoods.

Case studies of people at risk for contracting dengue will introduce students to a range of dengue fever symptoms. ELISA testing will expose students to a biochemical assay that detects an immune response in individuals who have been exposed to a pathogen. The results of the assay coupled with the case study symptoms will help students understand that a disease may be mild and remain undetected unless diagnostic testing is performed. They should see that undiagnosed disease can allow a pathogen to quietly become established in an area.
Finally, students will see the importance of preventive measures in limiting the spread of disease. While health departments recommend the “5Ds” for prevention of mosquito-borne illness, these recommendations are widely ignored. Hopefully, students will see that, in the absence of a vaccine for dengue fever, the only way to prevent the disease is to act to limit the vectors that carry the dengue virus.

**The Introduction**

This lesson plan is designed to incorporate the use of different types of scientific research to investigate the appearance of an emerging pathogen in the community.

To peak their interest, students are given newspaper articles about the presence of dengue fever in Florida, including reports of dengue fever diagnosed in the county. Students are then guided to reliable Internet sources to discover the symptoms of dengue, identify the viral vectors, locate the regions of the world where dengue is found, and explain why dengue is considered an emerging pathogen in Florida.

Following a lecture about mosquito-borne diseases, students discuss the conditions required for dengue to become endemic to an area. The diagnosis of disease implies the presence of the virus, however dengue virus cannot become established in an area unless a disease vector is present. Students are tasked with collecting water samples with mosquito larvae from locations near the school or their homes and bringing the samples to class. Water containing larvae is transferred into mosquito breeders. Once the larvae mature into adults and die in the breeder apparatus, students identify the mosquitoes and determine if the vector species for dengue viruses can be found in local standing water.

Students are given the opportunity to learn to use micropipettes, adjusting volumes, fitting and removing the pipette tips, and reading coordinates to deliver solution to a 96-well plate accurately. Following this practice activity, students are introduced to the ELISA for antibody detection. They are given fictional scenarios of people presenting symptoms of illness, possibly of dengue fever. Students must transfer information accurately, perform an ELISA simulation on the patient samples, and analyze the results to confirm or rule out a diagnosis of dengue fever for each patient. The final assignment is preparation of a written report that describes and explains the ELISA results.

**Materials and Preparation**

Mosquito breeders are available from BioQuip online catalog. Adult mosquitoes can be fixed to microscope slides using either Permount or clear nail polish. Hand lenses and photographs of mosquitoes will be needed for mosquito identification.

The Micropipette Coordinates Activity and ELISA Simulation were provided by the University of Florida Center for Precollegiate Education and Training.

**Procedure**

**Module 1: Dengue fever in Marion County**

BEFORE THE LESSON

Students are introduced to various methods of data collection and the difference between quantitative and qualitative data. They are able to organize data into tables, create and interpret graphs, and know that data must be analyzed in order to be meaningful.
Students are given newspaper articles reporting dengue as having become established in Key West and the diagnoses of cases of dengue fever in Marion County.

**ACTIVITY 1: DENGUE FEVER RESEARCH**

Using a webquest, students conduct research to
- describe the symptoms of dengue and consequences of repeat exposure to the virus,
- name *Aedes aegypti* and *Aedes albopictus* as the vectors that carry the viruses,
- locate the regions of the world where dengue is found,
- explain why dengue is considered an emerging pathogen in Florida.

**ACTIVITY 2: THINK/WRITE/PAIR/SHARE**

Following a presentation about *A. aegypti* and *A. albopictus* habitats and life cycles, students will address the following questions in a think/write/pair/share organizer:
- What factors must be present in order for dengue to become established in an area?
- If dengue virus vectors are in Marion County, could dengue become endemic here?
- How can we determine if the vector mosquitoes are here?

**ACTIVITY 3: FIELD RESEARCH**

Students collect standing water from around their homes and examine it for the presence of mosquito larvae. Water samples with larvae are placed in mosquito breeders in the lab and held until the mosquito larvae mature into adults and die. Students examine the adults to determine if they are *A. aegypti* or *A. albopictus*.

**Module 2: ELISA Testing to confirm a diagnosis**

**BEFORE THE LESSON**

Students learn that viral proteins can serve as antigens; antibodies form as part of the immune response to them. They understand that detection of antibodies to an antigen indicates exposure to the pathogen. Students know that enzymes are protein catalysts for biochemical reactions.

**SET**

Students are given a scenario in which fictional subjects presented symptoms which might indicate dengue fever. Patient sera are tested to confirm or rule out a dengue fever diagnosis.

**ACTIVITY 1: MICROPIPETTE COORDINATES ACTIVITY**

Given a set of instructions and a 96-well microplate, students practice the use of micropipettes to accurately deliver colored solutions to prescribed wells.

**ACTIVITY 2: ELISA ANTIBODY TEST ANIMATION**

Students watch an ELISA Antibody Test animation to see how ELISA uses the various functions of proteins to detect the presence of antibodies in a patient’s serum.

**ACTIVITY 3: MEDICAL RECORDS**

Students are given case studies describing scenarios in which fictional patients have been in situations that may have exposed them to dengue virus. Personal and medical information are given about each patient in descriptive form. Students must determine if the symptoms described for each person are indicative of dengue fever.

**ACTIVITY 4: ELISA ANTIBODY TEST**

Students perform a simulated ELISA antibody test on patient samples and interpret assay results.
ACTIVITY 5: INTERPRETING AND REPORTING DATA
Taking on the role of lab pathologist, students examine the symptoms of each patient, the ELISA results, and information from the WHO and the CDC. Students prepare a report explaining their observations and conclusions to the referring physician.

Assessment

Module 1 Assessments
A pre-test will determine students’ prior knowledge of dengue fever, disease vectors, and mosquito-borne disease.

ACTIVITY 1: DENGUE FEVER RESEARCH
Students will complete a worksheet to show they are able to locate and interpret information about dengue.

ACTIVITY 2: THINK/ WRITE/ PAIR/ SHARE
Completion of the graphic organizer summarizing individual responses to the questions and the results of pair discussions will be followed by class discussion. Class discussion will reveal if the students understand the role of the two specific mosquito species in the spread of dengue. Students may suggest various ways to capture, examine, and identify adult mosquitoes. They will probably need to be guided toward the possibility of collecting larvae and examining the adults that result.

ACTIVITY 3: FIELD RESEARCH
Students will examine the adult mosquitoes to determine if they are A. aegypti or A. albopictus. Written lab reports will demonstrate student proficiency in collecting and organizing qualitative and quantitative data. Reports will also show students’ ability to analyze results and form logical conclusions based on data analysis.

At the end of Module 1, a post-test will allow students to evaluate their pre-test responses and indicated if their knowledge about dengue fever and disease vectors has increased.

Module 2 Assessments

ACTIVITY 1: MICROPIPETTE COORDINATES ACTIVITY
Successful reading of the instructions and accurate pipetting will result in a recognizable design in each microplate allowing for student self-assessment.

ACTIVITY 2: ELISA ANTIBODY TEST ANIMATION
Students will produce a diagram illustrating and explaining the steps of the ELISA antibody test.

ACTIVITY 3: MEDICAL RECORDS
Students will accurately record patient information on a laboratory reporting form. They will be able to identify symptoms of dengue fever from a variety of symptoms in the narrative.

ACTIVITY 4: ELISA ANTIBODY TEST
Students will follow a protocol to complete the ELISA simulation then correctly interpret the assay results. The results for each patient sample will be accurately recorded on the lab report form.
Students will prepare a report explaining their assay results and their interpretation of them. Each report requires explanations of several situations: positive ELISA for a patient with severe symptoms of dengue, positive ELISA for a patient with mild symptoms, negative ELISA for a patient with flu-like symptoms, negative ELISA for a patient without indications of any disease, or with symptoms unlike dengue symptoms.

**Potential Extensions**

Students may participate in a community outreach to increase awareness of the dangers of mosquito-borne diseases and how they may be prevented by reducing vector habitats.

**Resources**


Bio-Rad, Inc. ELISA antibody test [animated tutorial]. Available at www.bio-rad.com


Grady D, Skipp C. 2010 July 23. Key West concerned about dengue fever, its impact on the island’s tourism. Ocala Star-Banner; section B, 7.

Lawrence, Charles. ELISA simulation kit. University of Florida Center for Precollegiate Education and Training.


Star-Banner Staff. 2010 July 15. Dengue fever confirmed; mosquito spray requested. Ocala Star-Banner; section B, 2.


**Supporting materials**
Dengue pre- and post-test
Dengue news articles
Dengue webquest
Think/Write/Pair/Share chart
Dengue case studies 1-5
Subject information form
CDC Dengue Outbreak Notice
WHO Dengue Fact Sheet
Mosquito-borne Disease PowerPoint (separate file)
Instructions for ELISA
WHAT DO YOU KNOW ABOUT DENGUE?

1. In what climates or areas of the world would you expect there to be cases of dengue fever?
   Pre-test
   Post-test

2. What do you think causes dengue – a bacterium, a virus, a fungus, or something else?
   Pre-test
   Post-test

3. What is a disease vector?
   Pre-test
   Post-test

4. What is the vector for dengue?
   Pre-test
   Post-test

5. What are some symptoms of dengue fever?
   Pre-test
   Post-test

6. How does someone get dengue hemorrhagic fever?
   Pre-test
   Post-test

7. How can dengue be prevented?
   Pre-test
   Post-test

8. Is dengue in Florida?
   Pre-test
   Post-test
WHAT DO YOU KNOW ABOUT DENGUE?

1. In what climates or areas of the world would you expect there to be cases of dengue fever?
   Dengue is found in tropical and subtropical climates.

2. What causes dengue?
   Dengue is caused by viruses.

3. What is a disease vector?
   A disease vector carries a pathogen from one host to another.

4. What is the vector for dengue?
   Mosquitos carry dengue viruses. *Aedes aegypti* and *Aedes albopictus* are dengue vectors.

5. What are some symptoms of dengue fever?
   Symptoms of dengue include fever and headaches, muscle, bone and joint pain, and pain behind the eyes. There may be a rash, nausea and vomiting, and bleeding from the gums or nose.

6. How does someone get dengue hemorrhagic fever?
   Being infected by more than one dengue virus may lead to dengue hemorrhagic fever.

7. How can dengue be prevented?
   Dengue is prevented though prevention of mosquito bites: empty containers of water around the home, wear insect repellent, avoid being outside at dusk and dawn, community spraying of mosquito breeding areas.

8. Is dengue a disease that is found in Florida?
   Dengue has been found in Florida.
Key West concerned about dengue fever, it’s impact on the island’s tourism dollars

By Denise Grady
and Catharine Siippp

Thursday, May 10, 2012

A woman planning a Florida vacation in Key West called the health department there last week to ask if it were true that the city was being evacuated because of an epidemic of dengue fever.

"No!" Chris Titel, a spokesman for the Monroe County Health Department, says he told her. "No, no, no, no, no."

Dengue is a viral illness, spread by mosquitoes, that can cause fever, headaches, body aches and a rash. Symptoms range from mild to severe, although some people have no symptoms.

Without a doubt, there is dengue in Key West, although at 27 known cases last year and 18 so far this year, it is hardly what most people would call an epidemic. Those cases are the first outbreak in Florida since 1954, and some medical experts fear that the disease, once rampant on the Eastern Seaboard, could take hold again.

News of the disease has apparently unsettled a few potential visitors. Tourism officials and business owners in Key West are even more unsettled by the Centers for Disease Control and Prevention’s recommendation that the public avoid the island.

On July 13, the Centers for Disease Control and Prevention recommended that travelers avoid Key West because of a threat of dengue fever. The recommendation was based on reports from the Florida Keys and the neighboring islands of Cuba and Puerto Rico.

"I don’t know if the CDC understands what it potentially has done here," said Andy Newman, the director of media relations for the Florida Keys and the Key West tourism council.

Robert Eadie, assistant administrator of the health department, called the disease centers' report "very alarming."


Kris Hail Inspects standing water for mosquito larvae at the Key West Cemetery in Key West on Wednesday.

branch in Puerto Rico, said it was statistically valid to extrapolate from the 240 people tested. "Somehow the virus is getting there," Margolis said.

An infected visitor may have passed the virus to local mosquitoes, or a mosquito carrying dengue may have arrived on an airplane or cruise ship. Key West has plenty of Aedes aegypti, a type of mosquito that can carry dengue. People are worried about being stigmatized, especially those with businesses. A restaurant owner who was infected a year ago agreed to be interviewed only if his name was not published, because he thought fear of the disease might keep customers away, even though the virus is not spread by food or personal contact. He said he had had a mild flu-like illness for about five days. He had no idea it was dengue until health workers asked him to be tested. Then they urged him to avoid being exposed again, because there are five strains, and people who have had one strain and later contract another can develop a dangerous form of the disease that can cause hemorrhaging and even death.

Dr. Peter J. Hotez, a tropical medicine expert at George Washington University, said he thought the potential was "pretty high" for dengue to spread up the Gulf Coast, where another species of Aedes aegypti that can carry the virus is common. If the disease does get there, it will strike poor people hardest, he predicted, because many of them lack screens and air-conditioning. There is no vaccine.

"I believe the threat is very real," he said. "And we understand that the CDC is about to close its dengue branch. Can you imagine anything so stupid? This is the worst time possible."

The disease centers confirmed that the 2011 budget does estmate $100,000 for the "vector borne" disease branch, which tracks dengue, West Nile virus, plague, encephalitis and other illnesses carried by insects.

In October, a group calling itself Dengue Night Fever included a John Travolta look-alike and followers sporting giant mosquito wings.

In Key West, residents have been taking it all in stride. At a parade in honor of the island’s centennial, a float featured a giant mosquito. The wasp control inspectors have been dispatched to neighborhoods with suspected cases. Sometimes they have to deal with vacant houses because Key West, like many cities, is dotted with foreclosures. The inspectors have also told landscapers to stock ponds with minnows, which feeds on mosquito larvae.

At the Key West Cemetery, where the gravestone of B.J. Roberts (who died in 1979) reads, "I told you I was sick," dozens of "virtrups" — black plastic cups laced with poison to kill female mosquitoes and their eggs — mingled among concrete urns and vases of water ride with spewing larvae. Plans for next year include providing sterile male mosquitoes.

Key West residents have been taking it all in stride. At a parade in honor of the island’s centennial, a float featured a giant mosquito.
Marion County Health Department

For more information, call the health department at 629-0137.

Avoid standing water where mosquitoes may breed. Use repellents and DEET to protect exposed skin.

Symptoms include headache, fever, rash and muscle pain. Seek medical attention.

The Marion County Health Department in a press release.

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Marion County Health Department
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Officials urge spraying after new dengue fever case.
Dengue fever confirmed; mosquito spray requested

The Marion County Health Department has asked the Marion County Board of County Commissioners to conduct a mosquito spray in the northeast area of Citra after receiving confirmation of a case of Dengue fever in the county.

The fever is caused by a mosquito-borne virus usually seen in subtropical and tropical regions. Symptoms include headache, fever, exhaustion, severe joint and muscle pain, swollen glands, mild bleeding and rash. There is no specific treatment.

Because the virus can be transmitted to a human from a mosquito that has bitten a person with the virus, citizens should protect themselves from mosquito bites.

The department advises following the “five D’s”:

■ Dusk and Dawn — Avoid being outdoors when mosquitoes are most active.
■ Dress — Wear clothing that covers most of the skin.
■ DEET — Use repellents containing DEET (N,N-diethyl-meta-toluamide, or N,N-diethyl-3-methylbenzamidade). Picaridin and oil of lemon eucalyptus are options.
In what types of climate is dengue found? ______________________________________________________

What type of infective agent causes dengue? ______________________________________________________

What type of insect carries the infective agent of dengue? ____________________________________________

What are some symptoms of dengue? ______________________________________________________________

How does dengue hemorrhagic fever differ from dengue fever? ________________________________________

What is the most severe form of dengue? __________________________________________________________

Why is prevention of dengue fever important? _______________________________________________________
http://www.who.int/mediacentre/factsheets/fs117/en/

How many different viruses cause dengue? __________________________

What happens to a person’s immune system after infection by one of these viruses? ______________

_____________________________________________________________________________________

What may happen if a person recovers from dengue fever, but is infected by another dengue virus or viruses later?

_____________________________________________________________________________________
_____________________________________________________________________________________
_____________________________________________________________________________________

http://edis.ifas.ufl.edu/in699

What two insects serve as vectors (carriers) of dengue? (Give the scientific names.)

_____________________________________________________________________________________


Where in the world is dengue found? ______________________________________________________
_____________________________________________________________________________________
_____________________________________________________________________________________
_____________________________________________________________________________________

http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5919a1.htm

Why is dengue considered an emerging pathogen in Florida? ________________________________
_____________________________________________________________________________________
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http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5919a1.htm
In what types of climate is dengue found? Dengue is found in tropical and subtropical climates.

What type of infective agent causes dengue? Dengue is caused by four related viruses.

What type of insect carries the infective agent of dengue? Mosquitos carry dengue viruses.

What are some symptoms of dengue? Some people with dengue may have no symptoms. Symptoms of dengue include fever and headaches, muscle, bone and joint pain, and pain behind the eyes. There may be a widespread rash, nausea and vomiting, and minor bleeding from the gums or nose.

How does dengue hemorrhagic fever differ from dengue fever? Dengue hemorrhagic fever results in bleeding from the nose and mouth, bleeding under the skin, severe abdominal pain, persistent vomiting, and problems with the lungs, liver, and heart.

What is the more severe form of dengue? Dengue hemorrhagic fever is the more severe form of dengue.

Why is prevention of dengue fever important? There is no vaccine available to protect against dengue, and there are no specific medications to treat it.

How many different viruses cause dengue? Four different, but related, viruses cause dengue.

What happens to a person’s immune system after infection by one of these viruses? Infection by one of the viruses leads to immunity to that virus, but only limited protection from the others.

What may happen if a person recovers from dengue fever, but is infected by another dengue virus or viruses later? Evidence suggests that infection by more than one virus increases the risk of developing dengue hemorrhagic fever.

What two insects serve as vectors (carriers) of dengue? (Give the scientific names.) Aedes aegypti and Aedes albopictus are dengue vectors.
Where in the world is dengue found? Dengue is found in Africa, the Americas, the Caribbean, Eastern Mediterranean, Southeast Asia and the Western Pacific regions.

Why is dengue considered an emerging pathogen in Florida? Dengue has been acquired by people living and traveling in Key West, Florida. These cases were contracted within the state.
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<thead>
<tr>
<th>Question</th>
<th>What I thought</th>
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<tr>
<td>What factors must be present in order for dengue to become endemic?</td>
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<td>If dengue virus vectors established in an area?</td>
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<td>Could dengue become endemic in Marion County?</td>
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<td>If dengue virus vectors are in Marion County, could dengue become endemic here?</td>
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<td>How can we determine if the vector mosquitoes are here?</td>
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<td>What I thought</td>
<td>What my partner thought</td>
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<td>What we will share</td>
<td>What I thought</td>
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CASE STUDY 1

The Carson family held their family reunion in Key West, Florida during the first week of June, 2010. Upon their return to Delaware, Jeffery Carson fell ill. Knowing that dengue fever has become a problem in Key West, the family physician tested Jeffery Carson’s wife and children, and his parents, for dengue.

Patient

A  Alfred Carson is 67 years of age and complained of being very tired and having a headache soon after arriving in Key West. He felt fine for the rest of the reunion and was healthy upon his return to Delaware.

B  Mildred Carson is 64 and had no medical complaints either during her stay in Key West or upon her return home.

C  Jeffrey Carson is 37 years old. He developed a persistent headache, severe joint pain, and a fever of 102°, two days after returning to Delaware.

D  Annette Carson, also 37, reported feeling nauseous and tired during the plane ride home, but had no other medical complaints.

E  Karen Carson is 15 years old and had no medical complaints.

F  Bradley Carson is 12 years old and complained of being tired, having a headache and a stomach-ache three days after returning home. His mother measured his temperature at 98.6°.

G  Jonathan Carson, 9, also started to complain three days after returning home. He reported feeling tired and sick to his stomach, having a headache, and aching bones. His mother measured his temperature at 101°.

H  Rosemary Carson’s mother reported that she was fussy during the trip home. She has a rash on her legs and arms. She is 18 months old.
CASE STUDY 2

In May of 2009, BioSys, Inc. executives and their staff traveled to West Africa to explore business opportunities. One team traveled to the Cape Verde islands while the other team went to Senegal. The BioSys employees spent two weeks meeting with government officials and touring various medical facilities. On the trip home, two of the four members of the Cape Verde team became ill.

Patient

A  Paul Anderson is 52 and led the Cape Verde team. He became nauseous and feverish on the plane trip back to New York. Once home, he developed a headache, pain behind the eyes, and severe joint and bone pain.

B  Barbara Sutton, the 45-year-old executive assistant to Mr. Anderson, became feverish and nauseous the day before the team left Cape Verde. She developed headaches and bone pain at home, and a rash on her legs.

C  Martin Alexander, 38, a junior executive, came down with a mild case of the flu during the second week in Cape Verde. He had a low fever, a headache, and body aches for three days. He felt fine during the flight to New York and in the weeks after his return home.

D  Charlotte Edwards, a junior executive on the Cape Verde team, has no medical complaints. She is 37 years old.

E  Abigail Norman is the 50-year-old senior executive who led the Senegal team. She has no medical complaints.

F  Donna McGill is the 49-year-old executive assistant to Ms. Norman. She felt nauseous and slightly feverish upon her arrival in Africa, but felt fine for the remainder of the trip and had no medical complaints after returning to New York.

G  Tabitha Marshall, 30, is a junior executive on the Senegal team with no medical complaints.

H  Charlie Drayton, 35, is a junior executive who became nauseous on the plane trip from New York to Senegal, but has no other medical complaints.
CASE STUDY 3

In May of 2010, a Miami resident was diagnosed with dengue fever shortly after returning from a trip to visit relatives in Malaysia. Employees associated with the Malaysia flight reported illnesses, so airline executives and Miami Airport officials decided to test employees for dengue.

Patient

A Aaron Fisher, 48, piloted the flight from Malaysia and had no medical complaints.

B Elizabeth Porter, 39, served as co-pilot for the flight, and came down with symptoms of food poisoning one week after the Malaysia flight. She had abdominal pain, vomiting, diarrhea, and fatigue that lasted for two days.

C Carla Westcott is 37 years old. She has been flying transcontinental flight for 10 years and served as chief flight attendant for the Malaysia flight. She recalled having a headache during the last 45 minutes of the trip, but had no other medical complaints.

D Frank Roberts, 28, served as a flight attendant for the Malaysia trip on his first overseas assignment. He had no medical complaints.

E Sharon Lister was a flight attendant on the Malaysia flight and has been flying transcontinental routes for 6 years. Eight days after the Malaysia flight she suddenly developed a high fever, joint pain, muscle pain, pain behind the eyes, and headache. After a few days of fever, her temperature dropped, and she began to exhibit signs of circulatory failure. She was admitted to the hospital where she was treated for her symptoms, and recovered from her illness. She is 32.

F Gary Beckman, 45, was the ground crew supervisor when the Malaysia flight landed. He complained of back pain in the weeks following the arrival of the flight, and still suffers from muscle pain in the lower back.

G Troy Newport, a 25-year-old baggage handler for the Malaysia flight, developed symptoms of the flu three days after unloading the plane’s cargo hold. He had a high fever, headache, muscle and joint pain, and fatigue. He was sick for four days.

H Terry Markham, 21, was also a baggage handler for the Malaysia flight. He developed symptoms of the flu 7 days after the plane arrived in Miami. He also had a high fever, headache, muscle and joint pain, and fatigue. He was sick for 6 days.
CASE STUDY 4

Following reports of dengue fever in visitors to Key West, public health workers asked Key West residents to participate in a study to determine if they had been exposed to dengue. Researchers asked residents to fill out a survey regarding their health over the past year. Residents whose survey results indicated that they may have had an undiagnosed case of dengue fever had their blood drawn to test for antibodies to dengue.

Participant

A  Calvin Gardiner is 42 and has been living in the Old Town section of Key West his entire life. He owns and works in a souvenir store near the Seaport Boardwalk. He reported having the flu some time during the previous summer, with a high fever, muscle and joint pain, and headache. He remembered being sick for about 3 days.

B  Joe Brightman is 45 and has been living in Old Town for 10 years. He runs a fishing charter boat. He had flu-like symptoms in July: fever, headache, nausea and vomiting. His symptoms lasted for 5 days.

C  Margaret Proctor is the manager of the Best Western motel near where the Cow Key Channel enters the Atlantic Ocean. In early September, she had a fever, and severe headache with nausea and vomiting. She developed a rash on her legs, and had to miss work for 10 days. She is 32 years old.

D  Manuel Perez works for the Keys Film Commission and had persistent headaches during the month of October. He also had a sore throat, cough, and aching neck. He is 25.

E  Tarisha Brown, 24, works in the Hemingway Home and Museum. She reported having a mild case of the flu for about 3 days in July. She had a fever, aching muscles, and fatigue.

F  Gordon Bramston, 31, operates a ferry that travels between Key West, Marco Island, and Fort Meyers. He reported feeling sick in September, with fever, headache, and nausea. He thinks he was sick for about 2 days.

G  Irene O’Connor is a 23 year old mother of two living in the Bahama Village area of Key West. She reported having a fever, headache, and pain in her joints and muscles in August. She was tired and weak for several days after the worst of the symptoms went away.

H  Bethany O’Connor, 11 months old, became sick two days after her mother. Bethany was taken to the pediatrician when her fever reached 101°F. Her grandmother reported that Bethany either just cried or slept for three days, not wanting to be fed or to play.
CASE STUDY 5

In April of 2010, missionaries from the Orlando and Kissimmee areas traveled to Piura in northwestern Peru. They spent two weeks working in the city and the surrounding coastal areas. Several of the missionary team members reported various symptoms of illness while in Peru and after their return to Florida. After discovering that there had been a dengue outbreak while the team was in Piura, church sponsors of the trip asked team members to be tested for antibody to dengue fever.

Team Member

A  Annette Chalmers organized the trip to Peru. She suffered from nausea, vomiting, and diarrhea shortly after arriving in Piura. She also ran a slight fever. Symptoms lasted about a day. She is 41 years old.
B  Peter Chalmers, Annette’s son, is 16 and home-schooled. He had no health complaints either during the trip or after.
C  Harvey Armstrong, 38 years old, is a youth pastor of a Kissimmee church. He became ill on the flight from Peru to Miami with nausea and vomiting.
D  Beth Li is a 19 year old student at Valencia Community College. She reported no illness during or after the mission trip.
E  Raymond Hernandez, 20, is also a student at Valencia Community College. The day before leaving Piura, Raymond suddenly developed a high fever and a severe headache. During the return trip he began to feel pain in his muscles and joints. A rash had appeared on his arms and chest by the time the plane landed in Miami. He was sick for five days.
F  Karl Heinzelman is a salesman in Kissimmee. Two days after his return to Orlando he developed muscle pain and a slight fever that lasted three days. He continued to feel tired for another two days. He is 28 years old.
G  Jodi Carpenter is a 19 year old student at Columbia College in Orlando. Two weeks after her return from Peru, Jodi became ill with a fever, headache, and muscle aches. Her illness lasted for five days, and she continued to feel tired and weak for another seven days.
H  Deshawn Williams is a 23 year old student at Florida Christian College in Kissimmee. He complained of headache and a sore throat the week following his return to Florida. He may have had a slight fever, but he is not sure.
CASE STUDY 1

The Carson family held their family reunion in Key West, Florida during the first week of June, 2010. Upon their return to Delaware, Jeffery Carson fell ill. Knowing that dengue fever has become a problem in Key West, the family physician tested Jeffery Carson's wife and children, and his parents, for dengue.

Patient

A Alfred Carson is 67 years of age and complained of being very tired and having a headache soon after arriving in Key West. He felt fine for the rest of the reunion and was healthy upon his return to Delaware.

B Mildred Carson is 64 and had no medical complaints either during her stay in Key West or upon her return home.

C **Jeffrey Carson** is 37 years old. He developed a persistent headache, severe joint pain, and a fever of 102\(^\circ\), two days after returning to Delaware.

D Annette Carson, also 37, reported feeling nauseous and tired during the plane ride home, but had no other medical complaints.

E Karen Carson is 15 years old and had no medical complaints.

F Bradley Carson is 12 years old and complained of being tired, having a headache and a stomach-ache three days after returning home. His mother measured his temperature at 98.6\(^\circ\).

G **Jonathan Carson**, 9, also started to complain three days after returning home. He reported feeling tired and sick to his stomach, having a headache, and aching bones. His mother measured his temperature at 101\(^\circ\).

H **Rosemary Carson**'s mother reported that she was fussy during the trip home. She has a rash on her legs and arms. She is 18 months old.
CASE STUDY 2

In May of 2009, BioSys, Inc. executives and their staff traveled to West Africa to explore business opportunities. One team traveled to the Cape Verde islands while the other team went to Senegal. The BioSys employees spent two weeks meeting with government officials and touring various medical facilities. On the trip home, two of the four members of the Cape Verde team became ill.

Patient

A Paul Anderson is 52 and led the Cape Verde team. He became nauseous and feverish on the plane trip back to New York. Once home, he developed a headache, pain behind the eyes, and severe joint and bone pain.

B Barbara Sutton, the 45-year-old executive assistant to Mr. Anderson, became feverish and nauseous the day before the team left Cape Verde. She developed headaches and bone pain at home, and a rash on her legs.

C Martin Alexander, 38, a junior executive, came down with a mild case of the flu during the second week in Cape Verde. He had a low fever, a headache, and body aches for three days. He felt fine during the flight to New York and in the weeks after his return home.

D Charlotte Edwards, a junior executive on the Cape Verde team, has no medical complaints. She is 37 years old.

E Abigail Norman is the 50-year-old senior executive who led the Senegal team. She has no medical complaints.

F Donna McGill is the 49-year-old executive assistant to Ms. Norman. She felt nauseous and slightly feverish upon her arrival in Africa, but felt fine for the remainder of the trip and had no medical complaints after returning to New York.

G Tabitha Marshall, 30, is a junior executive on the Senegal team with no medical complaints.

H Charlie Drayton, 35, is a junior executive who became nauseous on the plane trip from New York to Senegal, but has no other medical complaints.
CASE STUDY 3

In May of 2010, a Miami resident was diagnosed with dengue fever shortly after returning from a trip to visit relatives in Malaysia. Employees associated with the Malaysia flight reported illnesses, so airline executives and Miami Airport officials decided to test employees for dengue.

Patient

A Aaron Fisher, 48, piloted the flight from Malaysia and had no medical complaints.

B Elizabeth Porter, 39, served as co-pilot for the flight, and came down with symptoms of food poisoning one week after the Malaysia flight. She had abdominal pain, vomiting, diarrhea, and fatigue that lasted for two days.

C Carla Westcott is 37 years old. She has been flying transcontinental flight for 10 years and served as chief flight attendant for the Malaysia flight. She recalled having a headache during the last 45 minutes of the trip, but had no other medical complaints.

D Frank Roberts, 28, served as a flight attendant for the Malaysia trip on his first overseas assignment. He had no medical complaints.

E Sharon Lister was a flight attendant on the Malaysia flight and has been flying transcontinental routes for 6 years. Eight days after the Malaysia flight she suddenly developed a high fever, joint pain, muscle pain, pain behind the eyes, and headache. After a few days of fever, her temperature dropped, and she began to exhibit signs of circulatory failure. She was admitted to the hospital where she was treated for her symptoms, and recovered from her illness. She is 32.

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<td>☑ Male</td>
<td>☐ Fever ☐ Headache ☐ Pain behind eyes ☐ Joint/muscle pain</td>
<td>☐ Rash ☐ Nausea/vomiting ☐ Bleeding ☐ Circulatory failure</td>
</tr>
<tr>
<td>E</td>
<td>Sharon Lister</td>
<td>☑ Female ☑ Female 32 yr</td>
<td>☑ Fever ☑ Headache ☐ Pain behind eyes ☐ Joint/muscle pain</td>
<td>☐ Rash ☐ Nausea/vomiting ☐ Bleeding ☑ Circulatory failure</td>
</tr>
<tr>
<td>F</td>
<td>Gary Beckman</td>
<td>☑ Male</td>
<td>☑ Fever ☑ Headache ☐ Pain behind eyes ☐ Joint/muscle pain</td>
<td>☐ Rash ☐ Nausea/vomiting ☐ Bleeding ☐ Circulatory failure</td>
</tr>
<tr>
<td>G</td>
<td>Troy Newport</td>
<td>☑ Male</td>
<td>☑ Fever ☑ Headache ☐ Pain behind eyes ☐ Joint/muscle pain</td>
<td>☐ Rash ☐ Nausea/vomiting ☐ Bleeding ☐ Circulatory failure</td>
</tr>
<tr>
<td>H</td>
<td>Terry Markham</td>
<td>☑ Male</td>
<td>☑ Fever ☑ Headache ☐ Pain behind eyes ☐ Joint/muscle pain</td>
<td>☐ Rash ☐ Nausea/vomiting ☐ Bleeding ☐ Circulatory failure</td>
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</table>
Subject Information and ELISA Results

<table>
<thead>
<tr>
<th>Serum Sample</th>
<th>Name</th>
<th>Sex / Age</th>
<th>Dengue Symptoms</th>
<th>ELISA Results for dengue antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Calvin Gardiner</td>
<td>☑ Male, ☐ Female, Age 42 yr</td>
<td>☑ Fever, ☑ Headache, ☑ Pain behind eyes, ☐ Joint/muscle pain</td>
<td>☑ Positive, ☐ Negative</td>
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<tr>
<td>B</td>
<td>Joe Brightman</td>
<td>☑ Male, ☐ Female, Age 45 yr</td>
<td>☑ Fever, ☑ Headache, ☐ Pain behind eyes, ☐ Joint/muscle pain</td>
<td>☐ Positive, ☑ Negative</td>
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<tr>
<td>C</td>
<td>Margaret Proctor</td>
<td>☐ Male, ☑ Female, Age 32 yr</td>
<td>☑ Fever, ☑ Headache, ☑ Pain behind eyes, ☑ Joint/muscle pain</td>
<td>☑ Positive, ☐ Negative</td>
</tr>
<tr>
<td>D</td>
<td>Manuel Perez</td>
<td>☑ Male, ☐ Female, Age 25 yr</td>
<td>☐ Fever, ☑ Headache, ☐ Pain behind eyes, ☑ Joint/muscle pain</td>
<td>☐ Positive, ☑ Negative</td>
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<tr>
<td>E</td>
<td>Tarisha Brown</td>
<td>☐ Male, ☑ Female, Age 24 yr</td>
<td>☑ Fever, ☑ Headache, ☑ Pain behind eyes, ☑ Joint/muscle pain</td>
<td>☑ Positive, ☐ Negative</td>
</tr>
<tr>
<td>F</td>
<td>Gordon Bramston</td>
<td>☑ Male, ☐ Female, Age 31 yr</td>
<td>☑ Fever, ☑ Headache, ☐ Pain behind eyes, ☑ Joint/muscle pain</td>
<td>☐ Positive, ☑ Negative</td>
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<tr>
<td>G</td>
<td>Irene O’Connor</td>
<td>☐ Male, ☑ Female, Age 23 yr</td>
<td>☑ Fever, ☑ Headache, ☐ Pain behind eyes, ☑ Joint/muscle pain</td>
<td>☑ Positive, ☐ Negative</td>
</tr>
<tr>
<td>H</td>
<td>Bethany O’Connor</td>
<td>☐ Male, ☑ Female, Age 11 mos</td>
<td>☑ Fever, ☑ Headache, ☓ Pain behind eyes, ☑ Joint/muscle pain</td>
<td>☑ Positive, ☐ Negative</td>
</tr>
<tr>
<td>Serum Sample</td>
<td>Name</td>
<td>Sex / Age</td>
<td>Dengue Symptoms</td>
<td>ELISA Results for dengue antibody</td>
</tr>
<tr>
<td>--------------</td>
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<td>----------------------------------</td>
</tr>
<tr>
<td>A</td>
<td>Annette Chalmers</td>
<td>Female 41 yr</td>
<td>Fever, Headache, Pain behind eyes, Joint/muscle pain</td>
<td>Positive Negative</td>
</tr>
<tr>
<td>B</td>
<td>Peter Chalmers</td>
<td>Male 16 yr</td>
<td>Fever, Headache, Pain behind eyes, Joint/muscle pain</td>
<td>Positive Negative</td>
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<tr>
<td>C</td>
<td>Harvey Armstrong</td>
<td>Male 38 yr</td>
<td>Fever, Headache, Pain behind eyes, Joint/muscle pain</td>
<td>Positive Negative</td>
</tr>
<tr>
<td>D</td>
<td>Beth Li</td>
<td>Female 19 yr</td>
<td>Fever, Headache, Pain behind eyes, Joint/muscle pain</td>
<td>Positive Negative</td>
</tr>
<tr>
<td>E</td>
<td>Raymond Hernandez</td>
<td>Male 20 yr</td>
<td>Fever, Headache, Pain behind eyes, Joint/muscle pain</td>
<td>Positive Negative</td>
</tr>
<tr>
<td>F</td>
<td>Karl Heinzelman</td>
<td>Male 28 yr</td>
<td>Fever, Headache, Pain behind eyes, Joint/muscle pain</td>
<td>Positive Negative</td>
</tr>
<tr>
<td>G</td>
<td>Jodi Carpenter</td>
<td>Female 19 yr</td>
<td>Fever, Headache, Pain behind eyes, Joint/muscle pain</td>
<td>Positive Negative</td>
</tr>
<tr>
<td>H</td>
<td>Deshawn Williams</td>
<td>Male 23 yr</td>
<td>Fever, Headache, Pain behind eyes, Joint/muscle pain</td>
<td>Positive Negative</td>
</tr>
</tbody>
</table>
Outbreak Notice
Update: Dengue in Tropical and Subtropical Regions

This information is current as of today, August 20, 2011 at 15:10 EDT

Updated: May 02, 2011

Situation Information

Dengue fever is the most common cause of fever in travelers returning from the Caribbean, Central America, and South Central Asia. Dengue is reported commonly from most tropical and subtropical countries of Oceania, Asia, the Caribbean, the Americas, and occasionally Africa. This disease is caused by four similar viruses (DENV-1, -2, -3, and -4) and is spread through the bites of infected mosquitoes.

Dengue virus (DENV) transmission occurs in both rural and urban areas; however, dengue infections are most often reported from urban settings. For the most up-to-date information on dengue worldwide, see the Dengue Map on the CDC website.

Africa and the Indian Ocean Islands

Dengue infection occurs throughout Africa and the Indian Ocean Islands. Dengue has been diagnosed in travelers returning from these areas, including countries where the disease has not been officially reported.

South Pacific

Dengue infection is endemic in the South Pacific, where cyclical epidemics also occur. Even outside the typical dengue transmission season, travelers are at risk for the disease. Malaysia, Philippines, Singapore, and Thailand are among the countries reporting dengue activity in 2011. Australia also continues to report sporadic dengue activity in endemic areas of northern Queensland.

The Americas and the Caribbean

Many countries in Central and South America, as well as in the Caribbean, are reporting dengue activity. According to the Pan American Health Organization, in 2010, more than 1,800,000 dengue cases were reported throughout the Americas. In the first 2 months of 2011, over 200,000 dengue cases were reported in this region, including 2,744 cases of severe dengue. These cases were reported in many countries throughout the region, including Argentina, Bolivia, Brazil, Colombia, Paraguay, and Peru.

Middle East

Dengue activity is reported sporadically throughout the Middle East, including areas popular with travelers, such as Jeddah in Saudi Arabia.

Advice for Travelers

Travelers can reduce their risk of infection with dengue fever by protecting themselves from mosquito bites. The mosquitoes that spread dengue usually bite at dusk and dawn but may bite at any time during the day, especially indoors, in shady areas, or when the weather is cloudy.
Travelers should follow the steps below to protect themselves from mosquito bites:

- Where possible, stay in hotels or resorts that are well screened or air conditioned and that take measures such as spraying with insecticide to reduce the mosquito population.
- When outdoors or in a building that is not well screened, use insect repellent on uncovered skin. If sunscreen is needed, apply before applying insect repellent.
  - Look for a repellent that contains one of the following active ingredients: DEET, picaridin (KBR 3023), Oil of Lemon Eucalyptus/PMD, or IR3535. Always follow the instructions on the label when you use the repellent.
  - In general, repellents protect longer against mosquito bites when they have a higher concentration (percentage) of any of these active ingredients. However, concentrations above 50% do not offer a marked increase in protection time. Products with less than 10% of an active ingredient may offer only limited protection, often no longer than 1-2 hours.
  - The American Academy of Pediatrics \(^\text{©}\) approves the use of repellents with up to 30% DEET on children more than 2 months old.
  - Protect babies less than 2 months old by using a carrier draped with mosquito netting with an elastic edge for a tight fit. For more information about the use of repellent on infants and children, please see the “Insect and Other Arthropod Protection” section in Traveling Safely with Infants and Children and the “Children” section of CDC’s Frequently Asked Questions about Repellent Use.
  - For more information on the use of insect repellents, see the information on the Mosquito and Tick Protection webpage.
- Wear loose, long-sleeved shirts and long pants when outdoors.
  - For greater protection, clothing may also be sprayed with a repellent containing permethrin or another EPA-registered repellent. (Remember: don't use permethrin on skin.)

### Symptoms and Treatment

Symptoms of dengue include:

- fever
- severe headache
- pain behind the eyes
- joint and muscle pain
- rash
- nausea/vomiting
- hemorrhagic (bleeding) manifestations

Usually dengue fever causes a mild illness, but it can be severe and lead to dengue hemorrhagic fever (DHF), which can be fatal if not treated. People who have previously been infected with dengue fever are more at risk of getting severe dengue.

No vaccine is available to prevent dengue, and there is no specific medicine to cure illness caused by dengue. Those who become ill with dengue fever can be given medicine to reduce fever, such as acetaminophen, and may need oral rehydration or intravenous fluids and, in severe cases, treatment to support their blood pressure. Aspirin (acetylsalicylic acid), aspirin-containing drugs, and other nonsteroidal anti-inflammatory drugs (e.g., ibuprofen) should be avoided because of the possibility of bleeding. Early recognition and treatment of severe dengue (e.g., signs and symptoms consistent with impending blood pressure failure) can reduce the risk of death.

If you return from a trip abroad and get sick with a fever, you should seek medical care right away. Be sure to tell the doctor or other health care provider about your recent travel.
Dengue and dengue haemorrhagic fever

Key facts

- Dengue is a mosquito-borne infection that causes a severe flu-like illness, and sometimes a potentially lethal complication called dengue haemorrhagic fever.
- Global incidence of dengue has grown dramatically in recent decades.
- About two fifths of the world's population are now at risk.
- Dengue is found in tropical and sub-tropical climates worldwide, mostly in urban and semi-urban areas.
- Dengue haemorrhagic fever is a leading cause of serious illness and death among children in some Asian countries.
- There is no specific treatment for dengue, but appropriate medical care frequently saves the lives of patients with the more serious dengue haemorrhagic fever.
- The only way to prevent dengue virus transmission is to combat the disease-carrying mosquitoes.

Dengue is a mosquito-borne infection that in recent decades has become a major international public health concern. Dengue is found in tropical and sub-tropical regions around the world, predominantly in urban and semi-urban areas.

Dengue haemorrhagic fever (DHF), a potentially lethal complication, was first recognized in the 1950s during dengue epidemics in the Philippines and Thailand. Today DHF affects most Asian countries and has become a leading cause of hospitalization and death among children in the region.

There are four distinct, but closely related, viruses that cause dengue. Recovery from infection by one provides lifelong immunity against that virus but confers only partial and transient protection against subsequent infection by the other three viruses. There is good evidence that sequential infection increases the risk of developing DHF.
Global burden of dengue

The incidence of dengue has grown dramatically around the world in recent decades. Some 2.5 billion people – two fifths of the world’s population – are now at risk from dengue. WHO currently estimates there may be 50 million dengue infections worldwide every year.

In 2007 alone, there were more than 890,000 reported cases of dengue in the Americas, of which 26,000 cases were DHF.

The disease is now endemic in more than 100 countries in Africa, the Americas, the Eastern Mediterranean, South-east Asia and the Western Pacific. South-east Asia and the Western Pacific are the most seriously affected. Before 1970 only nine countries had experienced DHF epidemics, a number that had increased more than four-fold by 1995.

Not only is the number of cases increasing as the disease is spreading to new areas, but explosive outbreaks are occurring. In 2007, Venezuela reported over 80,000 cases, including more than 6,000 cases of DHF.

Some other statistics:

- During epidemics of dengue, infection rates among those who have not been previously exposed to the virus are often 40% to 50%, but can reach 80% to 90%.
- An estimated 500,000 people with DHF require hospitalization each year, a very large proportion of whom are children. About 2.5% of those affected die.
- Without proper treatment, DHF fatality rates can exceed 20%. Wider access to medical care from health providers with knowledge about DHF - physicians and nurses who recognize its symptoms and know how to treat its effects - can reduce death rates to less than 1%.

The spread of dengue is attributed to expanding geographic distribution of the four dengue viruses and their mosquito vectors, the most important of which is the predominantly urban species *Aedes aegypti*. A rapid rise in urban mosquito populations is bringing ever greater numbers of people into contact with this vector, especially in areas that are favourable for mosquito breeding, e.g. where household water storage is common and where solid waste disposal services are inadequate.

**DengueNet: WHO surveillance**

**Transmission**

Dengue viruses are transmitted to humans through the bites of infective female *Aedes* mosquitoes. Mosquitoes generally acquire the virus while feeding on the blood of an infected person. After virus incubation for eight to 10 days, an infected mosquito is capable, during probing and blood feeding, of transmitting the virus for the rest of its life. Infected female mosquitoes may also transmit the virus to their offspring by transovarial (via the eggs) transmission, but the role of this in sustaining transmission of the virus to humans has not yet been defined.

Infected humans are the main carriers and multipliers of the virus, serving as a source of the virus for uninfected mosquitoes. The virus circulates in the blood of infected humans for two to seven days, at
approximately the same time that they have a fever; *Aedes* mosquitoes may acquire the virus when they feed on an individual during this period. Some studies have shown that monkeys in some parts of the world play a similar role in transmission.

**Characteristics**

Dengue fever is a severe, flu-like illness that affects infants, young children and adults, but seldom causes death.

The clinical features of dengue fever vary according to the age of the patient. Infants and young children may have a fever with rash. Older children and adults may have either a mild fever or the classical incapacitating disease with abrupt onset and high fever, severe headache, pain behind the eyes, muscle and joint pains, and rash.

Dengue haemorrhagic fever (DHF) is a potentially deadly complication that is characterized by high fever, often with enlargement of the liver, and in severe cases circulatory failure. The illness often begins with a sudden rise in temperature accompanied by facial flush and other flu-like symptoms. The fever usually continues for two to seven days and can be as high as 41°C, possibly with convulsions and other complications.

In moderate DHF cases, all signs and symptoms abate after the fever subsides. In severe cases, the patient's condition may suddenly deteriorate after a few days of fever; the temperature drops, followed by signs of circulatory failure, and the patient may rapidly go into a critical state of shock and die within 12 to 24 hours, or quickly recover following appropriate medical treatment (see below).

**Treatment**

There is no specific treatment for dengue fever.

For DHF, medical care by physicians and nurses experienced with the effects and progression of the complicating haemorrhagic fever can frequently save lives - decreasing mortality rates from more than 20% to less than 1%. Maintenance of the patient's circulating fluid volume is the central feature of DHF care.

**Immunization**

There is no vaccine to protect against dengue. Although progress is underway, developing a vaccine against the disease - in either its mild or severe form - is challenging.

- With four closely related viruses that can cause the disease, the vaccine must immunize against all four types to be effective.
- There is limited understanding of how the disease typically behaves and how the virus interacts with the immune system.
- There is a lack of laboratory animal models available to test immune responses to potential vaccines.

Despite these challenges, two vaccine candidates have advanced to evaluation in human subjects in countries with endemic disease, and several potential vaccines are in earlier stages of development. WHO provides technical advice and guidance to countries and private partners to support vaccine research and evaluation.
Prevention and control

At present, the only method of controlling or preventing dengue virus transmission is to combat the vector mosquitoes.

In Asia and the Americas, *Aedes aegypti* breeds primarily in man-made containers like earthenware jars, metal drums and concrete cisterns used for domestic water storage, as well as discarded plastic food containers, used automobile tyres and other items that collect rainwater. In Africa the mosquito also breeds extensively in natural habitats such as tree holes, and leaves that gather to form "cups" and catch water.

In recent years, *Aedes albopictus*, a secondary dengue vector in Asia, has become established in the United States, several Latin American and Caribbean countries, parts of Europe and Africa. The rapid geographic spread of this species is largely attributed to the international trade in used tyres, a breeding habitat.

Vector control is implemented using environmental management and chemical methods. Proper solid waste disposal and improved water storage practices, including covering containers to prevent access by egg-laying female mosquitoes are among methods that are encouraged through community-based programmes.

The application of appropriate insecticides to larval habitats, particularly those that are useful in households, e.g. water storage vessels, prevents mosquito breeding for several weeks but must be re-applied periodically. Small, mosquito-eating fish and copepods (tiny crustaceans) have also been used with some success.

During outbreaks, emergency vector control measures can also include broad application of insecticides as space sprays using portable or truck-mounted machines or even aircraft. However, the mosquito-killing effect is transient, variable in its effectiveness because the aerosol droplets may not penetrate indoors to microhabitats where adult mosquitoes are sequestered, and the procedure is costly and operationally difficult. Regular monitoring of the vectors’ susceptibility to widely used insecticides is necessary to ensure the appropriate choice of chemicals. Active monitoring and surveillance of the natural mosquito population should accompany control efforts to determine programme effectiveness.

For more information contact:

WHO Media centre
Telephone: +41 22 791 2222
E-mail: mediainquieries@who.int
ELISA Antibody Test Procedures

1) Place the ELISA 96-well plate in the table. Note that rows 10, 11 and 12 will not be used.

2) Place a paper towel unfolded and flat on the table. Mark it “USED PIPETTES”.

3) Find the centrifuge tube labeled “Dengue Antigen”. Using one disposable pipette, place one drop of Dengue Antigen into wells 1 through 9 in rows A through H.
4) Place the disposable pipette on the towel marked “USED PIPETTES”.

**What Did We Just Do?**
You just placed dengue antigens into the wells where some attached to the walls of the well.

5) Find the tube labeled “Wash” and using one NEW disposable pipette, place one drop of the Wash solution into wells 1 through 9 in rows A through H. **Set this pipette aside, you will use it again for the Wash Solution.**

6) Take the ELISA tray in the palm of your hand and quickly turn it upside down into the sink to empty the wells. Tap the tray several times, and then place it back on the table right side up.

**What Did We Just Do?**
You washed away any dengue antigens that were not attached to the walls of the wells.

7) Find the tube labeled “Blocker” and using one NEW disposable pipette, place one drop of the Blocker Protein solution into wells 1 through 9 in rows A through H. Place the pipette tip on the towel marked “USED PIPETTES”.

8) Take the ELISA tray in the palm of your hand and quickly turn it upside down into the sink to empty the wells. Tap the tray several times, and then place it back on the table right side up.

9) Find the tube labeled “Wash” and using the disposable pipette you used for the last wash, place one drop of the Wash solution into wells 1 thru 9 in rows A through H. Set this pipette aside, you will use it again for the Wash Solution.
10) Take the ELISA tray in the palm of your hand and quickly turn it upside down into the sink to empty the wells. Tap the tray several times, and then place it back on the table right side up.
What Did We Just Do?
You added a solution containing a blocker protein that will stick to any surface of the well that is not coated with dengue antigen. You then washed away any extra blocker proteins that were not attached.

11) Find the tube labeled “+ Control” and using the micropipette, place 20 l of the Positive Control solution into wells 1 through 3 in rows A thru H. Place the pipette tip on the towel marked “USED PIPETTES”.

What Did We Just Do?
You added serum that we know contains antibodies to dengue virus. The antibodies attach to the antigens in the wells.

Antibody to dengue antigen
12) Find the centrifuge tube labeled “A” and using a micropipette, place 20 l drop of the Subject A serum into wells 7, 8, and 9 in row “A”. Place the pipette tip on the towel marked “USED PIPETTES”.

13) Using a new pipette tip, place 20 l drop of the Subject B serum into wells 7, 8, and 9 in row “B”. Place the pipette tip on the towel marked “USED PIPETTES”.

14) Remembering to use a new pipette tip for each different serum sample, repeat this procedure for the tubes marked C, D, E, F, G, and H.

What Did We Just Do?
You added the blood plasma for testing to the wells. If a person has been exposed to dengue virus, he would have had an immune response to it and antibodies will be in the blood. Antibodies will attach to the dengue antigen in the well. (figure 1)
If a person has not been exposed to dengue, there will not be antibodies to it. No antibody will attach to the dengue antigen in the well. (figure 2)

15) Take the ELISA tray in the palm of your hand and quickly turn it upside down into the sink to empty the wells. Tap the tray several times, and then place it back on the table right side up.
16) Find the large tube labeled “Wash” and using the disposable pipette you used for the last wash, place one drop of the Wash solution into wells 1 through 9 in rows A through H. \textit{Set this pipette aside, you will use it again for the Wash Solution.}

17) Take the ELISA tray in the palm of your hand and quickly turn it upside down into the sink to empty the wells. Tap the tray several times, and then place it back on the table right side up.

\textbf{What Did We Just Do?}
You washed away any antibodies that had not attached to antigens.
18) Find the tube labeled “F Tag” and using one NEW disposable pipette, place one drop of the Fluorescent Tagged Antibodies into wells 1 through 9 in rows A through H.

19) Take the ELISA tray in the palm of your hand and quickly turn it upside down into the sink to empty the wells. Tap the tray several times, and then place it back on the table right side up.

20) Find the large tube labeled “Wash” and using the disposable pipette you used for the last wash, place one drop of the Wash solution into wells 1 through 9 in rows A through H.
21) Carefully take the ELISA tray in the palm of your hand and quickly turn it upside down into the sink to empty the wells. Tap the tray several times, and then place it back on the table right side up. Place the pipette on the “USED PIPETTES” towel.

**What Did We Just Do?**
You added horse antibodies that attach to human antibodies. These anti-human antibodies have a fluorescent molecule attached to them. Then you washed away any anti-human antibodies that did NOT attach to something in the well.

![Diagram of ELISA process](image)

22) Obtain an ultraviolet light and shine it over the ELISA plate.

**What Did We Just Do?**
The ultraviolet light stimulated the fluorescent molecules attached to the horse antibodies that were attached to the human antibodies that were attached to the dengue antigens in the well. These fluorescent molecules then gave off a visible light.
If no light was seen, there were no horse antibodies in the wells because there were no human antibodies to dengue for them to attach to.

![Diagram of ELISA process](image)

23) Record which wells fluoresced using a + sign on the ELISA Test Data Sheet. The subjects whose blood serum was placed in these wells have tested positive for the presence of antibodies to dengue.

24) Record which wells did not fluoresce with a − sign on the ELISA Test Data Sheet. The subjects whose blood serum was placed in these wells do not have antibodies to the dengue virus.

25) Record the ELISA results for each subject on the “Subject Information and ELISA Results” form.