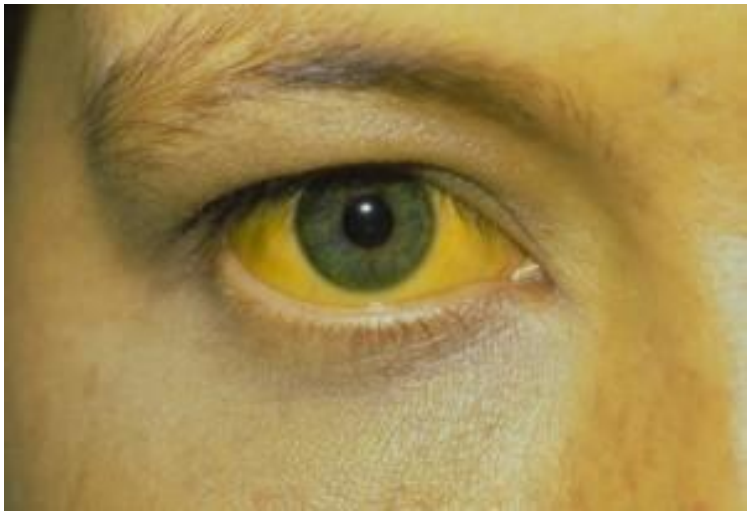

YELLOW FEVER

Medical Virology

STUDENT

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1- Introduction:

1.1-History of the disease :

Yellow fever virus (YFV) YFV was first isolated in Ghana, West Africa, from the blood of a male patient with fever, headache, backache and prostration.

There are two epidemio logical patterns: in the sylvatic cycle, virus is transmit ted among monkeys by mosquitoes (Haemagogus and Sabethes species in South America and Aedes species in Africa). Man is infected incidentally when entering the area, e.g. to work as foresters. In the urban cycle, human-to-human transmission is via Ae. aegypti, which breeds close to human habitation in water, in pits and scrap containers such as oil drums.(1)

1.2- Introduction of virus:

The flaviviruses were classified as arthropod-borne viruses because they are usually spread by arthropod vectors. These viruses have a very broad host range including vertebrates (e.g., mammals, birds, amphibi ans, reptiles) and invertebrates (e.g., mosquitoes, ticks). Diseases spread by animals or with an animal reservoir are called zoonoses. Examples of pathogenic alpha vi ruses and flaviviruses The hepatitis C and G viruses are classified as flaviviruses on the basis of their genomic structure. They are not likely to prove to be arboviruses.(2)

1.3-The distribution of this disease:

Yellow fever is caused by a mosquito-borne flavivirus that is found in tropical South America and Africa The disease is characterized by the sudden onset of headache and fever with temperatures exceeding 39°C, and is accompanied by generalized myalgia, nausea and vomiting , after an incubation period of 3-6 days,

YFV is found mostly in tropical Africa and tropical South America.

No urban YF has been reported in South America since 1954 In the Americas, the majority of YF cases are reported in Peru and Bolivia and involve males aged 15-45 years who are agricultural and forest workers.

YF usually occurs from December to May and peaks during March and April, when populations of Haemagogus mosquitoes are highest during the rainy season. YF is endemic in many parts of West, Central and East Africa, principally between latitudes 15°N and 15os, extending northwards into Ethiopia and Sudan Continuing activity has been encounter in Nigeria with occasional outbreaks in other parts of West There are relatively few outbreaks in East Africa. (1)

1.4-Epidemic:

Epidemiology There are two distinct cycles of yellow fever mosquito, urban the reservoir of the virus is humans and the vector the Aedes aegypti. tree-dwelling monkeys and the vector sylvatic or jungle the reservoir is various species of forest mosquito.

America; disease occurrence: tropical regions of Central Africa and Latin the Aedes eradication has re-emerged in South America, since the 1970s when of urban programme was relaxed, with the ever present risk of re-establishment cycles of yellow fever.

During 1995 there were at least 490 cases in Peru, representing the largest epidemic in South America in 40 years.

The last major outbreak was in Kenya in 1993 with 54 cases and a case fatality rate of 50%. Ae.(3)

2- Classification of the virus :

Family: Flaviviridea
Genus: flavivirus
Virus:Yellow fever virus (4)

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3- Structure and Genome:

Shape	ocazaheadraly
Size	40 – 60nm
Envelop	enveloped
Nucleic acid	(+ssRNA)

3.1-Shape: yellow fever virus has ocazaheadraly shape (cubic).(2)

3.2- Size: slightly small.(1)

The total diameter(40-60)nm.(2)

3.3- Enveloped or not: enveloped

3.4- Nucleic acid: flaviviruses have positive-strand RNA genome (+ssRNA). That is 11-12 000 nucleotides in length The RNA does not have a polyadenylate sequence.(1)

3.5- Molecule weight: $(4.2 – 4.4)x10^6$. (2)

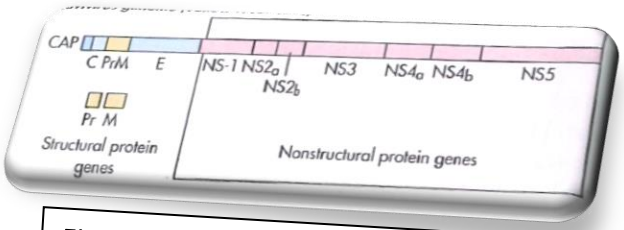

Figure1: Flavivirus genome (yellow fiver

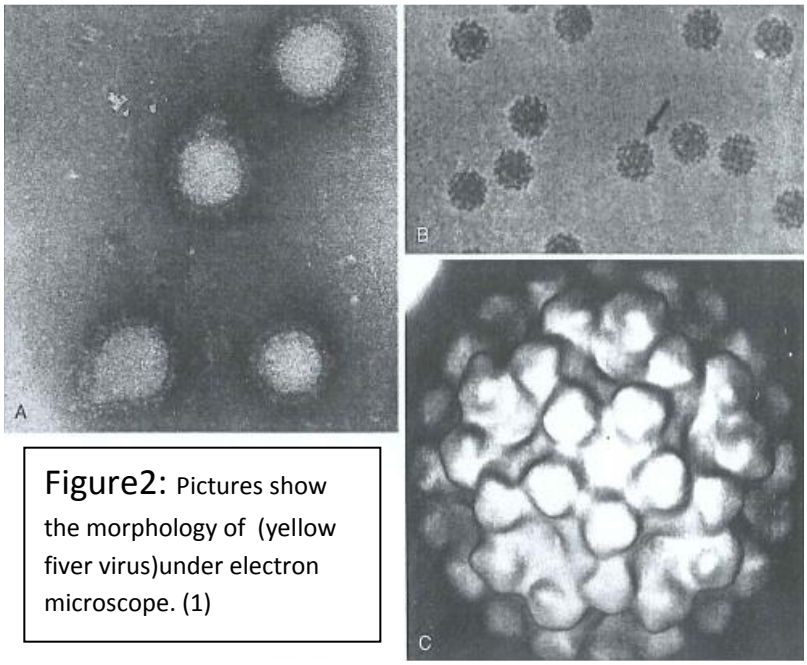

Figure2: Pictures show the morphology of (yellow fiver virus)under electron microscope. (1)

4- Protein

flavivirus viruses have three structural proteins and other seven non-structural proteins.

4.1- Structural proteins and their function.

One-third of the flavivirus genome encodes the three structural proteins genes, which are small core protein encapsidated the viral RNA genome(C) and there are two other structural proteins, termed the membrane (M) and envelope (E), on the outside of virus particles.The E protein is the major protein of the virus It is normally glycosylated, has haemagglutination activity and is the target of neutralizing antibodies.(1)

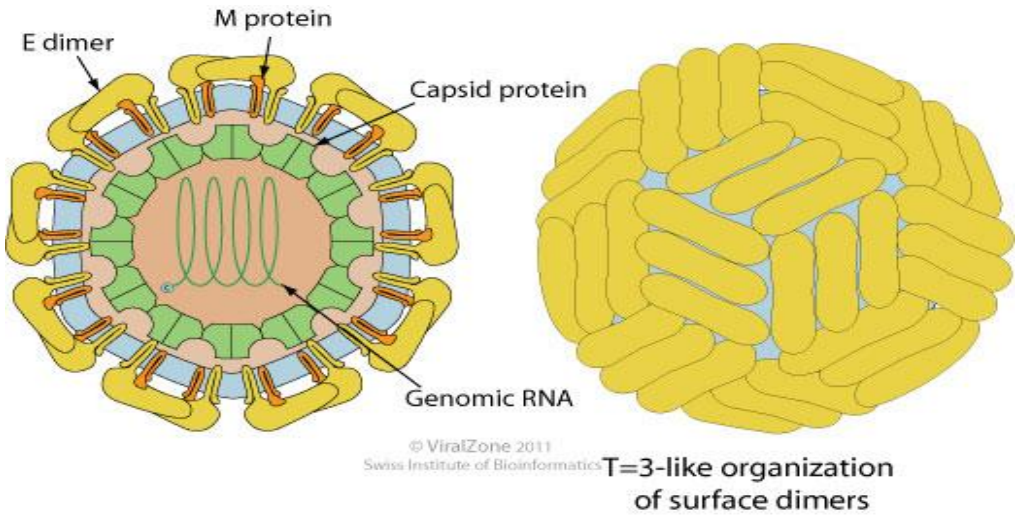

Figure3: Yellow fiver virus (structural

4.2- Non- Structural proteins.

while the remaining tow-third of the genome encodes the other seven non-structural proteins genes(NS1, NS2A, NS2B, NS3, NS4A,NS4B and NS5).

NS3 protein contains the majority of T cell epitopes. Also the amino terminus of NS3 and NS2B function together as a serine protease, while NS5 encodes the RNA-dependent RNA polymerase of the virus.(1)

5- Transmission:

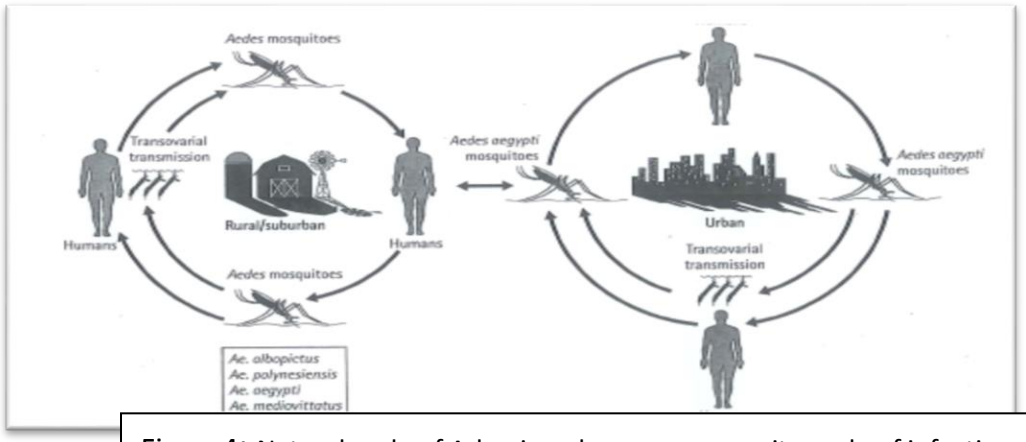

Figure4: Natural cycle of Arbovirus: human – mosquito cycle of infection .(2)

Arboviruses are maintained in natural transmission cycles involving reservoir hosts and arthropod vectors, usually mosquitoes.(1)

The virus is maintained by Aedes mosquitoes in a sylvatic or jungle cycle, in which monkeys are natural host, and also in an urban cycle, in the which humans host.(2)

Transmission may then occur upon a subsequent blood meal, when mosquitoes deposit saliva in extravascular tissues while probing to locate a venule. Infection of the vertebrate then leads to viraemia and the opportunity for infection of additional vectors.

Examples of natural cycles. Human-mosquito cycle as in dengue and urban yellow fever . Urban vectors of dengue viruses are typically the highly peridomestic and anthrophilic Aedes aegypti, but peridomestic Ae. albopictus has also been implicated; humans are the vertebrate reservoirs for rural and urban dengue.(1)

Patterns of flavivirus transmission. The cycle of arbovirus transmission maintains and amplifies the virus in the environment.(2)

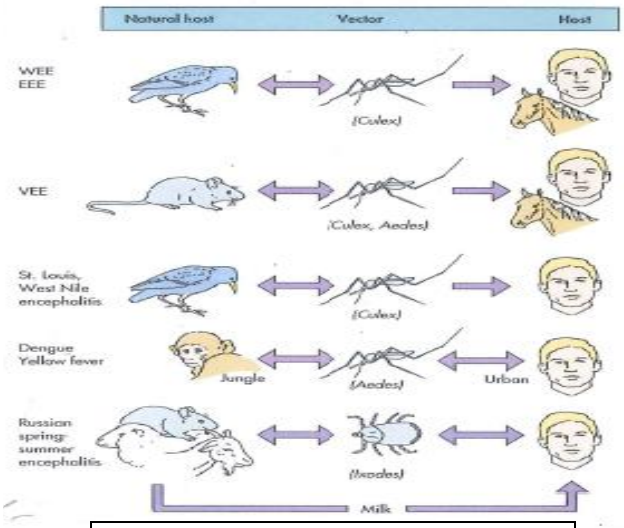

Figure5: Virus transmission.(2)

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6- Penetration And The Target Organ :

The yellow fever virus can attack almost any body cell in all of the body's organ systems. The ability of the virus to bind to certain types of proteins on the surface of cells results in the range of hosts and the specificity of body cells. Cells of different organisms have slightly dissimilar proteins. This is also true for proteins on the surfaces of different cells in the body. Apparently, the yellow fever virus can bind to a variety of protein types on the surfaces of body cells. (6)

So This virus multiplies on the skin. then, It spreads to the local lymph nodes, liver, spleen, kidney, bone marrow, and myocardium, where it may persist for days. It is present in the blood early during infection. (7)

The attachment of any virus to the cell surface is a critical and determinant characteristic. In the case of Flaviviruses they have only a three structural proteins to work with when creating more viruses. As a result, the surface of the finished viral particle is a simple mix of nucleocapsid (C), membrane proteins (prM/M), and cell surface recognition proteins (E). Although it might not seem like much, these proteins are used to their full potential, and many perform multiple functions throughout the virus life-cycle.

The most determinant protein which affects a flavivirus's tropism is the E glycoprotein, which acts as a binding factor and initiates membrane fusion. Phagocytic cells which express the immunoglobulin gamma (IgG) receptors on their cell-surface—specifically, the Fc-gamma-receptor—have a greater affinity for binding most flaviviruses due to opsonization with GpE and the complement IgG.

The flaviviruses enter the cell via receptor-mediated endocytosis, and that membrane fusion only occurs between the E protein and the endosomal membrane, *not the cell membrane*. Below is a figure1 which–rather artfully–shows the internalization of Dengue Virus into cancerous, human liver cells (Huh7 cell line). This figure simplifies and provides a visual basis for the attachment and entry of flaviviruses.

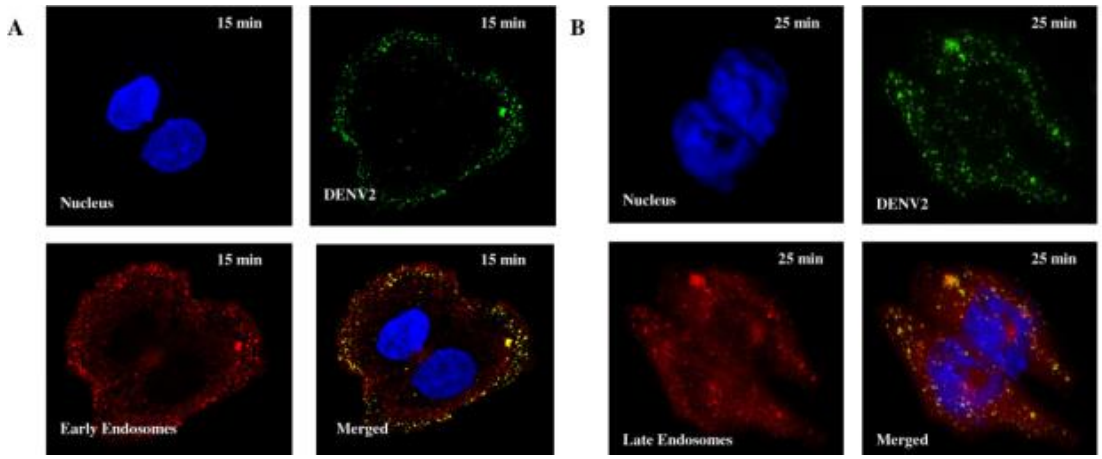

Figure6. Internalization of Dengue Virus by the endosome

So, in general, the E protein holds two specific functions: Binding to the cell-surface (attachment), and inducing fusion with the endosomal membrane (release).How the E protein is signaled to fuse with the membrane is still unknown, though it is widely accepted that the low pH of the endosome plays the main role in this phenomenon.

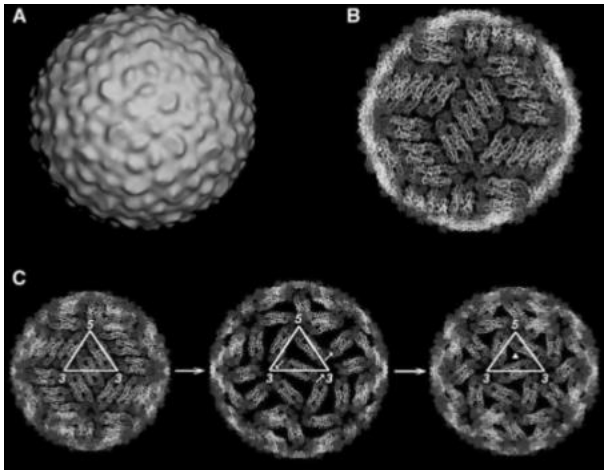

Figure7. The conformational change (C) that the E protein undergoes when in an acidic environment.

This conformational change is critical to the fusion activity between the viral proteins and the endosomal membrane, which allows the nucleocapsid to be released into the cytoplasm.

Following the fusion of the membrane, a flavivirus will then have its nucleocapsid released into the cytoplasm where it is ready for the next stage of the life-cycle, replication and biosynthesis. (8).

7- Replication Cycle (The Main Site):

Since the flaviviruses have a (+)sense RNA genome it will replicate in the cytoplasm and bud at internal membrane of the host cell. (2)

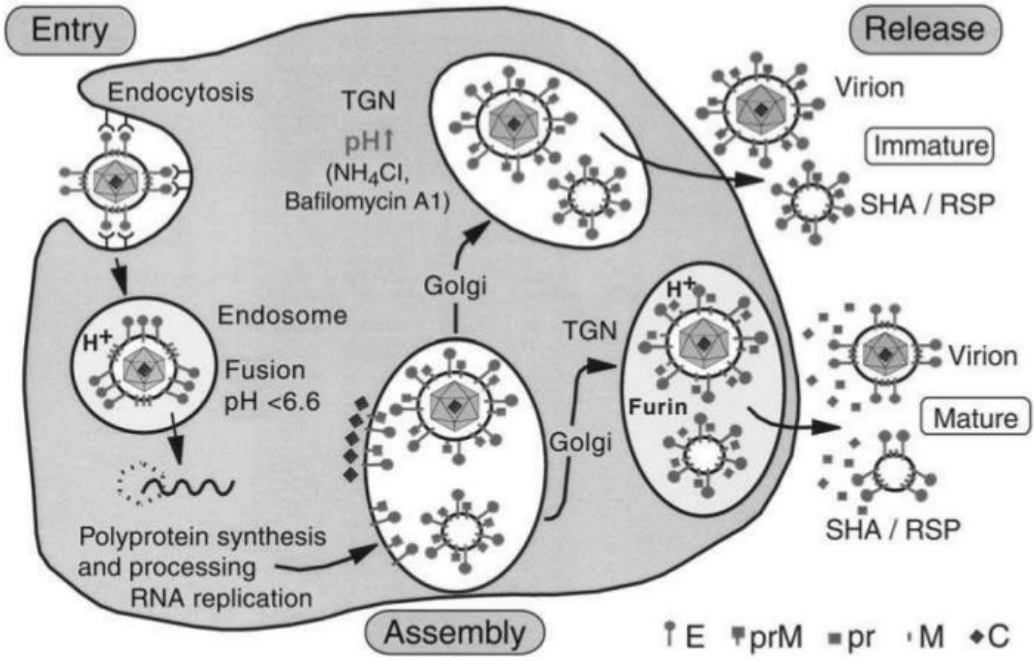

Figure8. The flaviviruses replication.(8)

Once the virus fuses with the endosome, the genome is released into the cytoplasm, where it functions as an mRNA. At the rough ER, translation of the open reading frame occurs, and makes one large polyprotien. This is polyprotein is cleaved into separate, mature proteins after translation including, NS5 – the viral RdRP1. After translation occurs, synthesis of viral RNA takes place in viral complexes, with the production of a (-)-strand of RNA serving as the template strand. After the template strand is made, many more (+) strand RNA's are made, which make up the genome of what will soon be the progeny of the flavivirus that came into the cell and began replicating! An interesting finding is that is takes about 15 minutes for progeny RNA synthesis to be made.(9)

8- Assembly And Egression :

Ultrastructural studies indicate that virion morphogenesis occurs in association with intracellular membranes. Electron microscopic studies of flavivirus-infected cells have consistently observed fully formed virions within the lumen of a compartment believed to be the ER . In many studies, virions appear to accumulate within disorderly arrays of membrane-bound vesicles . Budding

intermediates and clearly distinguishable cytoplasmic nucleocapsids have not been frequently observed, suggesting that the process of assembly is rapid. Nascent virions appear to be transported by bulk flow through the secretory pathway and released at the cell surface . Budding at the plasma membrane has been occasionally observed, but does not appear to be a major mechanism for virion formation. These ultrastructural observations, together with studies on structural protein biosynthesis, oligomer formation, and the properties of intracellular and released virions, suggest the following model for virion assembly and maturation. which has been reviewed by others . proper folding of E requires cosynthesis with prM , and both proteins remain associated as detergent-stable heterodimers that are retained in the ER via sequences in their C-terminal transmembrane anchors . The highly basic C protein interacts with the viral genome RNA in the cytoplasm to form nucleocapsid precursor that acquires an envelope by budding into the ER lumen. Recent studies suggest that the basic building block of the flavivirus nucleocapsid is likely to be a C protein dimer.

Later stages in virion maturation include glycan modification of E (for some viruses) and prM by trimming and terminal addition , implying that virions move through an exocytosis pathway similar to that used for synthesis of host plasma membrane glycoproteins. Although differences in the efficiency of prM cleavage have been noted, this cleavage generally distinguishes released virus from intracellular virus particles . Intracellular M-containing virions have not been detected, suggesting that prM cleavage occurs just before release of mature virions. This cleavage can be inhibited by elevating the pH in intracellular compartments or by introducing mutations at the basic pr/M cleavage site . The cleavage site specificity and biochemical data indicate that furin is the enzyme responsible for prM cleavage.Although inhibiting prM cleavage does not impair virus release, studies on prM-containing particles suggest that furin cleavage or a major structural alteration in prM is required to generate highly infectious virus. Also, the current hypothesis is that uncleaved prM prevents E fromundergoing an acid-catalyzed conformational change during transit of immature virions through an acidic intracellular compartment. Cleavage of prM by furin and release renders the mature virion ready for acid-catalyzed rearrangements required for productive entry. Morecomplex interactions are also involved in flavivirus assembly and egress. As mentioned, assembly is coupled to RNA replication and emerging evidence links determinants in NS2A and NS3 to the assembly process, independent of their roles in proteolytic processing and RNA replication . An RNA packaging signal in the flavivirus genome has yet to be identified but the ability of

subgenomic replicons lacking the structural region to be packaged in trans suggests that an obligate packaging signal does not reside in this region of the genome. Further studies are needed to define the roles of this and other cellular pathways in flavivirus morphogenesis and release. (10)

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9.Symptoms

Yellow fever is caused by a mosquito-borne flavivirus that is found in tropical South America and Africa. the disease is characterized by the sudden onset of headache and fever with temperatures exceeding 39°C, and is accompanied by generalized myalgia, nausea and vomiting, after an incubation period of 3-6 days. Jaundice may appear by the third day of illness, but frequently this is mild or absent. Hematemesis and melena may occur from bleeding into the gastro-intestinal tract and epistaxis and bleeding gums may also be noted. Albuminuria and oliguria may also begin suddenly during the first week of illness. In severe cases, death may occur from 3 to 6 days after the onset of illness, and midzonal necrosis is observed in the liver. The case fatality rate is estimated at 20% but may be as high as 50%. Mild cases may develop fever, headache and myalgia only, without gastro-intestinal upsets, jaundice or albuminuria. (1)

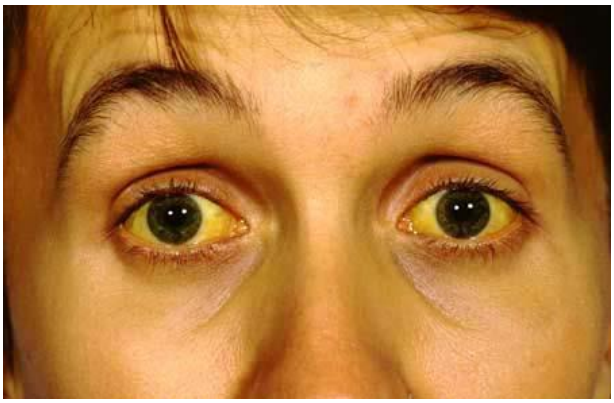

Figure9.Jaundice. (11)

10.Laboratory Diagnosis

Diagnosis of arbovirus infections depends on:

- the isolation of virus from blood, Cerebrospinal fluid (CSF) or tissues

- detection of arbovirus-specific RNA in, blood, CSF or tissue
- serology.

Although virus isolation is an important aspect of diagnosis, it should be noted that this is normally a very slow process and is unlikely to generate results until after an n acute virus infection. Detection of virus-specific RNA by reverse transcriptase polymerase chain reaction (RTPCR) is a rapid approach to diagnosis but is dependent on the availability of oligonucleotide primers that will specifically amplify a region of the genome of a given virus. Given the large number of arboviruses, there has to be significant differential diagnosis prior to selection of appropriate primers. Similarly, enzyme-linked immunosorbent assays (ELISAs) can be used to detect serum antibodies from patients. However, these assays are dependent on availability of virus and/or antigens for use in ELISAs. One procedure that is commonly used to identify arbovirus infections is indirect immunofluorescence as this can give a result in a few hours.

10.1. Virus isolation

The causative virus can be isolated from blood collected during the initial 3-day febrile illness induced by viruses such as dengue, Ross River and yellow fever, when viremia titers are at a maximum. Some arboviruses can also be isolated from CSF or brain biopsy, or from brain at autopsy of fatal encephalitis cases. Liver may yield virus isolation from fatal cases of yellow fever.

10.2. Arbovirus-specific RNA detection

viral RNA is extracted from serum or suspensions of tissues from patients, or from tissue culture cells or mosquito homogenate. This is amplified by RTPCR and the products analyzed by restriction digestion and/or determination of the nucleotide sequence of the PCR W product and comparison with nucleotide sequences databases.

10.3. Serology

Serological tests frequently offer the only available means of laboratory diagnosis of encephalitis. The detection of a four-fold or greater rise of antibody titer by hemagglutination inhibition (HI) or ELISA tests on paired sera collected during the initial week and several days later after defervescence provides good, but not definitive, evidence of concurrent infection. Antibodies detected by Cstic fibrosis (CF) first appear 2 or more weeks after onset and become undetectable

by 3 years. Note that HI and CF result are usually not as specific as those obtained by neutralization. Virus-specific IgM antibody may be detected within 1 day of onset of clinical symptoms using an IgM capture ELISA test. IgM antibody wanes 6 weeks after onset and is replaced by IgG antibodies. IgM antibodies are indicative of a recent infection. (1)

11.Control

Strategies for prevention of arbovirus infections depend on either control or active immunization with Vaccine.

11.1Vector control

This is possible for mosquito-borne viruses in urban and suburban localities: suppression of populations of vector mosquito species can halt virus transmission during epidemics. This can be achieved by:

-The use of insecticides to kill adult mosquitoes. aerial sprays of malathion, although this may kill many other insect species.
-The elimination of breeding sites of domestic Aedes aegypti by removal of objects such as tin cans and motor tyres that could contain rainwater, both near human habitations and in public parks and drainage systems, has prevented occurrence of dengue in Singapore and urban yellow fever in metropolitan areas in Caribbean countries.
-The chemical control of larvae, termed "larviciding, by use of temephos granules or malathion in oil for even coverage of small breeding sites, has reduced mosquito vector populations substantially in irrigated localities in California and elsewhere.
-The biological control of larvae is attempted by microbiological agents such as Bacillus thuringiensis israeliensis, larvivorous fish, flatworms or mermithid nematodes, or insect growth regulators such as the juvenile hormone mimic methoprene
-Personal protection against bites by mosquitoes involves a combination of wearing protective clothing, preferably impregnated with permethrin, screening of dwellings to prevent entry of mosquitoes and frequent application of mosquito repellants such as diethyl toluamide to exposed skin areas. For tick- borne viruses,

protective clothing should be worn outdoors, followed by rigorous inspection to remove attached ticks from skin. (1)

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12- Treatment:

No treatments exist for arbovirus disease other than supportive care, when Walter Reed and his colleagues discovered that yellow fever was spread by A. aegypti, the number of cases was reduced from 1400 to none within 2 years.

Patrick R. murray, ken S. Rosenthal, George S. kobayashi, Michael A. pfaller, (2002) medical microbiology. United states of America : mosby.

12.1-Vaccines:

A live vaccine against yellow fever virus. This vaccine is meant for people working with the virus or at risk for contact. The yellow fever vaccine is prepared from the 17D strain isolated from a patient in 1927 and grown for long periods in monkeys, mosquitoes, embryonic tissue culture, and embryonated eggs. The vaccine is administered intradermally and elicits lifelong immunity to yellow fever and, possibly, other cross-reacting flaviviruses.

Patrick R. murray, ken S. Rosenthal, George S. kobayashi, Michael A. pfaller, (2002) medical microbiology. United states of America : mosby.

12.2- Medication:

There is no specific treatment for yellow fever, and patients can benefit from supportive care only able to treat dehydration and fever and respiratory failure device. (12)

13- Host Immune Defense:

Immune Response Both humoral immunity and cellular immunity are elicited and are important to the control of primary infection and the prevention of future infections with the flaviviruses. Replication of the flaviviruses promunity to one flavivirus can provide some protection against infection with other flaviviruses through recognition of the type-common antigens expressed on all viruses in the family. Cell-mediated immunity is also important in controlling the primary infection. Immunity to these viruses is a double-edged sword. A non-neutralizing antibody can enhance the uptake of flaviviruses into macrophages and other cells that express Fc receptors. Such an antibody can be generated to a related strain of virus in which the neutralizing epitope is not expressed or is different. Inflammation resulting from the cell-mediated immune response can destroy tissues and significantly contribute to the pathogenesis of encephalitis. Hypersensitivity reactions, such as delayed-type hypersensitivity, the formation of immune complexes with virions and viral antigens, and the activation of complement, can also occur. They can weaken the vasculature and cause it to rupture, leading to hemorrhagic symptoms. Immune responses to a related strain of dengue virus that do not prevent infection can promote immunopathogenesis, leading to dengue hemorrhagic fever or dengue shock syndrome.(2)

14- Genetics (Gene Mutation):

yellow fever virus as a result of the T380R mutation in the EDIII of Asibi strain following extensive in vitro passage in mice and chicken embryos was found to contribute to the more rapid clearance in mice challenged with 17D. However, viral infectivity and dissemination in mosquitoes had not been evaluated for this mutant.(13)

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