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SUNNYDALE

Evolutionary studies have suggested an African origin for CHIKV. Since its identification, sporadic cases and outbreaks have been reported in several African countries, on the Indian subcontinent and in Southeast Asia. In the last decade, CHIKV has re-emerged, causing a series of large outbreaks, which started in Kenya in 2004 and ravaged the Comoros Islands, the Island of La Re Union and other Islands in the Southwest Indian Ocean in early

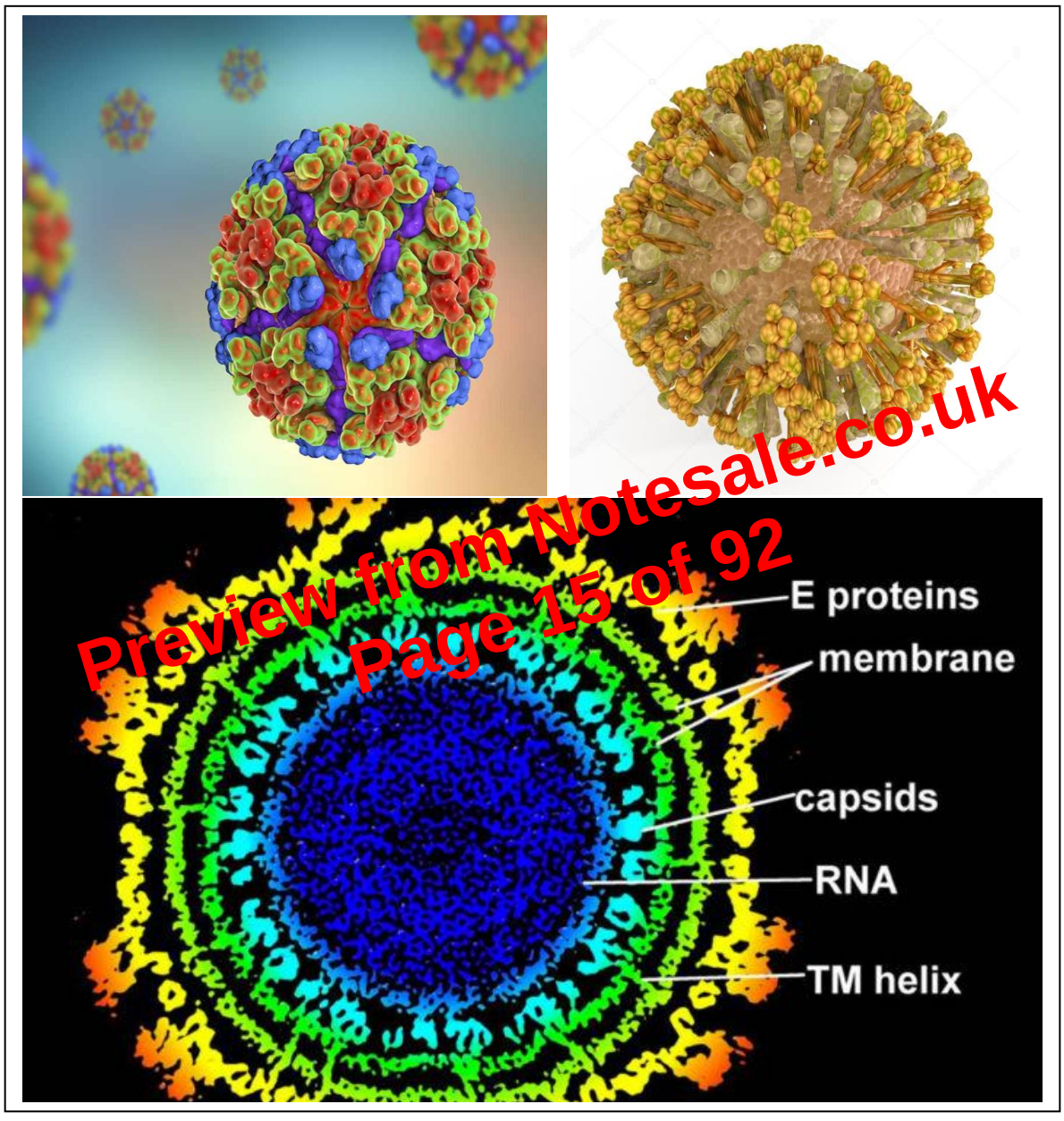

Fig 1.1: Chikungunya virus

2005, followed by an epidemic in the Indian subcontinent in 2005 – 2006.

Dengue is the most prevalent and dangerous of the emerging Arbovirus. According to World Health Organization (WHO), it is the most rapidly spreading arbovirus worldwide and is epidemic in every inhabited region of the world except for continental Europe. A study done in 2103 estimated that 96 million clinically significant cases occur annually, a dramatic increase from 50 million in 2009.

Dengue is a member of the Flavivirus genus, which also includes Yellow fever virus, West Nile virus and Zika virus. There are four distinct dengue virus serotypes, with type 2 considered to be the most virulent strain. Although the human-mosquito-human transmission cycle is the most prominent method of propagation, dengue can be transmitted vertically during pregnancy and via blood-borne transmission. Dengue is not transmitted via sexual contact or respiratory droplets.

Fig 1.2: Dengue Virus

Rationale

Dengue occurred sporadically in Bangladesh from 1964 until a large epidemic in 2000 made the virus well recognized to the people. However, our physicians at present, are experienced enough to handle this menace. But chikungunya infection is a newly emerging disease in our country and now a days, incidence of CHIKV disease is very much high in Dhaka city.

Chikungunya virus is most often spread to people by *Aedes aegypti* and *Aedes albopictus* mosquitoes. These same mosquitoes also transmit dengue virus. Signs and symptoms of both dengue and CHIKV disease are almost similar. Our physicians are familiar with dengue infection and they often misunderstood CHIKV infections as dengue fever. However, it is utmost important to properly investigate any viral disease in order to intervene in time to protect the individuals from severity and complications. However, observing the status of chikungunya by determining the antibodies against CHIKV in suspected dengue patients is crucial.

genotypes of the virus from the original site of first identification in East Africa. Recently, in additional site of origin has been identified in Asia.

The epidemiology of Chikungunya has been extensively evaluated from 2004 when the virus initiated its travel eastbound from the coast of Africa to the Indian Ocean. It is noteworthy that, this diffusion has been mainly sustained by Aedes albopictus, a new vector to which the virus become adapted due to the mutation in E1-Ala226Val gene. This mutation was also identified during the first, even small, outbreaks of Chikungunya related disease outside the tropics that occurred in Northern Italy in 2007 and in Southern France in 2010. Three years later, the virus appeared for the first time in the western hemisphere and since then, in less than 24 months spread to the North and South America.

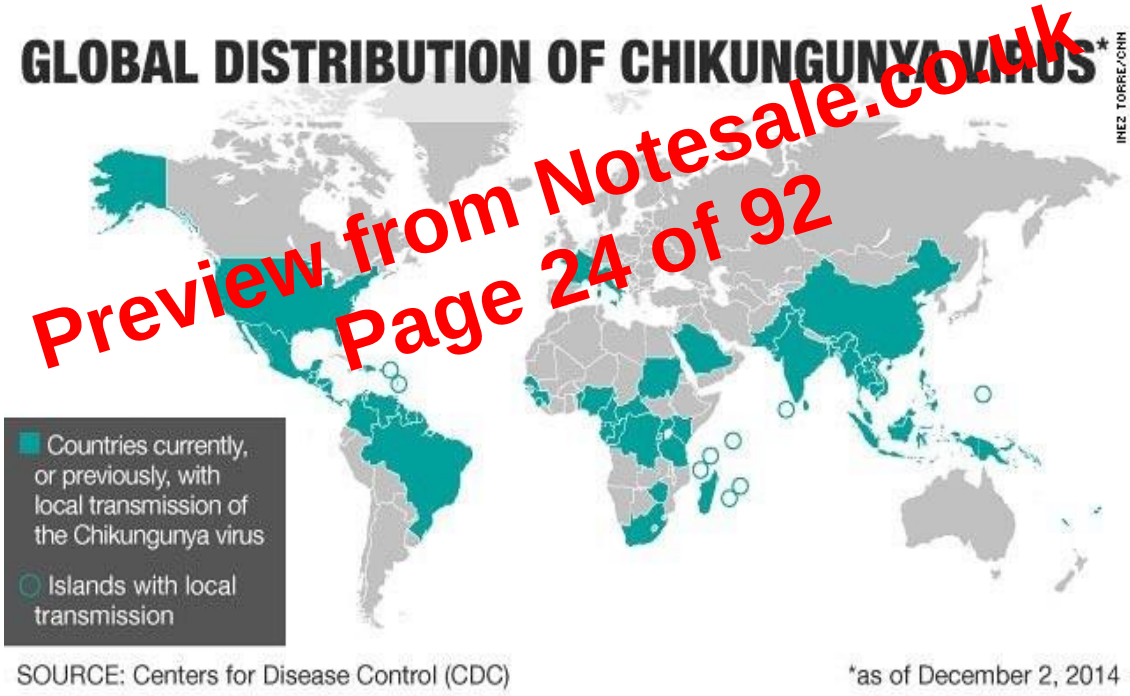

Fig 2.1: Epidemiology of Chikungunya Virus.

outbreaks that demonstrate early the ability of CHKV to cause atypical disease (Padbidri VS, Gnaneswar TT, 1979) as had been reported in outbreaks since 2005. A later 1973 outbreak in central India (Barsi) also involved large attack rates and resulted in over 37% of the population being infected (Sergon K et al, 2008). Even though these outbreaks in India were sufficient, there was no reported epidemic activity from the country for the next 30 years. This curious apparent lack of CHKV transmission does suggest the lack of establishment of an enzootic cycle in India after the cessation of epidemic activity.

During the 1950s to 1970s, a number of smaller scale outbreaks were recorded in Africa as well. Cases were reported in Zaire, Zambia, Senegal, Uganda, Zimbabwe, Nigeria, Angola, Central African Republic and South Africa (Halstead SB, 1996).

The epidemiological pattern was quite distinct from that seen in Asia during the same period, perhaps most significantly due to the association of enzootic maintenance of CHKV in Africa with a number of alternative vector species.

Re-Emergence of CHIKV

Perhaps not expected, due to the maintenance of CHIKV in zoonotic transmission in Africa, there was a re-emergence of epidemic CHIKV in 2004 in costal Kenya. During July, an unusual increase in the number of malaria-like illness was detected in the island community of Lamu. However, local physicians noted that the degree of joint pain associated with these cases was significantly more severe than that seen in standard malaria infections. In addition, 91% of the blood smears were negative for parasites; thus, the increase in cases could not be accommodated for by malaria. Additional testing for likely etiologies revealed positive CHKV – specific antibodies and nucleic acid results (Sergon K et al, 2007). The scope of this outbreak was quite large for Eastern Africa with an estimated 13,500 cases. Yet recognition of the outbreak on a global scale was minimal.

Prevention of Dengue

Dengue remain the leading arbovirus cause of morbidity in man. There are 3.6 billion people living in areas of dengue risk, with an estimated 390 million infections and 96 million symptomatic cases annually (Beatty, M.E. Letson, G.W. and Morgolis, H.S., 2009; Bhatt, S. Gething, P.W. Brady, O.J. Messina, J.P. Farlow, A.W. Moyes, C.L. Drake, J.M. Brownstein, J.S. Hoen, A.G. Sankoh, O. Meyers, M.F. George, D.B. Jaenisch, T. Wint, G.R.W., Simons, C.P. Scott, T.W. Farrar, J.J. and Hay, S.I., 2013).

Dengue is vectored by mosquitoes, with several members of the *Aedes stegomyia* subgenus serving as vectors. For example, Aedes albopictusis is an excellent vector of dengue in the laboratory and outbreaks in Hawii (Effler, P.V. Pang, L. Kitsutani, P. Vorndarm, V. Nakata, M. Ayers, T. Elm, J. Tom, T. Reiter, P. Rigau-Perez, J.G. Hayes, J.M. Mills, K. Napier, M. Clark, G.G. and Gubler, D.J., 2005) and Taiwan (Lambrechts, L. Scott, T. W. and Gubler, D.J., 2010) attest to their ability to vector the virus in the field. Differences in the ability for Aedes albopictus to develop disseminated infections of dengue viruses may explain its lower vector competence status relative to *Aedes aegypti* (Lambrechts, L. Scott, T. W. and Gubler, D.J., 2010). *Aedes scutellariscomplex* members, *Aedes polynesiensis* (Rosen, L. Rozeboom, L.E. Sweet, B.H. and Sabin, A.B., 1954), *Aedes katheriensis* (Leake, C.J., 1984) and *Aedes scutellaris* (Moore, P.R. Johnson, P.H. Smith, G.A. Ritchie, S.A. and Van Hurk, A.F., 2007) have been shown to be potential vectors of dengue virus in the laboratory. Aedes polynesiensis is suspected of vectoring outbreaks in French Polynesia [82] while Aedes hensilli (Savage, H.M. Fritz, C.L. Rutstein, D. Yolwa, A. Vorndam, V. and Gubler, D.J., 1998) and Aedes scutellaris (MacKerras, I.M., 1946) have been linked to dengue transmission in Yap and New Guinea, respectively. However, it is another transmission worldwide and is almost exclusively the vector in large, explosive urban epidemics of dengue (Gubler, D.J., 1998; Lambrechts, L. Scott, T.W. and guebler, D.J., 2010).

What is unique about Aedes aegypti that makes it such an effective vector for dengue? Aedes aegypti is arguably the most anthropophilic mosquito (Tabachnick W., 1991). Most of its behavior from immatures residing within male-made, water-holding containers to adult females living inside human domains where they feed almost exclusively on human blood is tightly liked to man. Its high domesticity truly makes Aedes aegypti the 'cockroach' of mosquitoes.

Association between Chikungunya and Dengue

To date, the number of diagnosed cases of DENV-CHIKV coinfections is surprisingly small and available information is often incomplete, making it difficult to establish epidemiological trends. However, it is noteworthy that, the number of reported cases has increased considerably during the past 10 years, indicating that the phenomenon is becoming a concern among the scientific community because of its potential impact on human health and economy. Indeed, although the first documented cases of DENV-CHIKV coinfections date back to the 1960s in Vellore, south India, when 14 cases were reported during a CHIKV epidemic outbreak and in Thailand, where nine cases were documented. It was not until 2006 that the diagnoses of concomitant infections experienced a real interest, possibly due to the burden of cases of chikungunya infection in Indian Ocean's island and Southeast Asia where DENV is an endemic.

In 2006, two cases of coinfection corresponding to two female patients were described in Malaysia, and 20 more were recorded during the CHIKV outbreak in La Reunion the same year. More cases of coinfection were reported in Madagascar and Sri Lanka in 2006-2007 and in Gabon, India, Nigeria and Singapore in 2007-2010, coinciding with the epidemics of CHIKV caused by IOL strains during this period in the area. The most recent cases were diagnosed in South America, India and Nigeria in 2013-2014. Of note, two of these cases

corresponded to infected travelers returning to Portugal and Germany after being infected in Angola and India respectively, raising concern about the possible spread of coinfection cases in Europe where *Aedes albopictus* is present.

Table VI: Duration of illness at the time of diagnosis:

Duration time	Frequency	Percent
2-5 days	58	48.33%
6-10 days	46	38.33%
11-15 days	9	7.5%
16-30 days	5	4.17%
More than 30 days	2	1.67%
Total	120	100%

About knowledge of respondents to the site of Incubation period where participated in this study and their duration of sign and symptoms like fever, joint pain etc. were categorized in the following five groups:

- **Group I:** 2-5 days of 58 (48.33%)
- **Group II:** 6-10 days of 46 (38.33%)
- **Group III:** 11-15 days of 9 (7.5%)
- **Group IV:** 16-30 days of 5 (4.17%)
- **Group V:** more than 30 days of 2 (1.67%)

CHAPTER 6

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APPENDIX IV

TEST PROCEDURES

Chikungunya test Principle

Standard Q chikungunya IgM/IgG test kit has “M”, “G” test lines and “C” control line. Monoclonal anti-human IgM and Monoclonal anti-human IgG are immobilized at two individual test lines respectively (M, G line) on the nitrocellulose membrane.

The IgM line in the result window is closer to the sample well and followed by IgG line. If human anti-chikungunya IgM or IgG exist in patient serum, the individual test line appear visible band respectively forming the complex with anti-human IgM/IgG, human IgM/IgG, inactivated chikungunya virus and anti-chikungunya EI-gold which means positive test result.

The violent line at the control region should always appear if the assay performed correctly.

Intended use of chikungunya test

Standard Q chikungunya IgM/IgG test is an Immunochromatographic assay for the detection of IgM/IgG antibodies against chikungunya virus in human serum, plasma or whole blood samples. The test kit is for in vitro only. This is intended for professional use, only for an initial screening test.

Capillary Whole Blood

1. Capillary whole blood should be collected aseptically by fingertip.
2. Clean the area to be lanced with an alcohol swab.
3. Squeeze the end of the fingertip and price with a sterile lancet.
4. Collect the capillary whole blood to the black line of the SD Ezi tube+ for the testing.
5. The capillary whole blood must be tested immediately after collection.

Venous Whole Blood

1. Collect the venous whole blood into the commercially available anti-coagulant tube such as heparin, EDTA or sodium citrate by venipuncture.
2. If venous whole blood in anti-coagulant tube is stored in a refrigerator at 2 – 8^0 C, the specimen can be used for testing within 1 – 2 days after collection.
3. Do not use hemolysed blood samples.

Test Procedure

Common check-up:

A. Read the instruction
B. Check the expiry date
C. Check the test device and silica gel pack in the foil pouch.

1. Using a SD Ezi tube+, collect the 10 ml of serum/plasma/whole blood to the black line of the SD Ezi tube+.
2. Add the collect serum plasma whole blood to the sample well of the test device.
3. Add 3 drops (90 ml) of assay diluent into the assay well of the test device.
4. Read the test result 15 minutes; the test result can be read up to 30 minutes.

Interpretation of the Test Results

1. Negative result: Only band (C control line) within the result window indicates a negative result.
2. IgM positive result: Two colored bands (C control line and M test line) within the result window indicate chikungunya IgM positive.
3. IgG positive result: Two colored bands (C control line and G test line) within the result window indicate chikungunya IgG positive.
4. IgM and IgG positive result: Three colored bands (C control line, M test line and G test line) within the result window indicate chikungunya IgM and IgG positive.
5. Invalid test: If the control band (C control line) is not visible within the result window, the result is considered invalid.

Limitation of the test:

1. The test procedure, precaution and interpretation of results for this test must be followed strictly when testing.
2. This test detects the presence of anti-chikungunya IgM and IgG and should not be used as the sole for criteria for the diagnosis of chikungunya virus infection.
3. The test result must be considered with other clinical data available to the physician.
4. Failure to follow test procedure and interpretation of test result may adversely affect test performance and/or products in valid results.

Dengue Test Principle