Prevalence of arthropod borne viral infections in a tertiary care hospital, with special reference to the 2012 dengue fever outbreak in Thanjavur and Thiruvarur districts.

MOHAMED ALI
Department of Microbiology, THANJAVUR MEDICAL COLLEGE

Abstract:
Aim: To study the prevalence of Japanese encephalitis, Chikungunya and dengue fever, to appreciate the seasonal changes, age and sex distribution of the diseases, to find out the serotype and reasons for rapid increase in number of dengue cases in 2012.

Materials and Methods:
The study was conducted at a tertiary care hospital in suspected patients of dengue, Chikungunya, Japanese encephalitis from January to November 2012. Number of samples received for Japanese Encephalitis, Chikungunya, dengue were 209, 234, 12,519 respectively. The patients' samples were tested using IgM capture ELISA kits for dengue, Japanese Encephalitis, Chikungunya diseases. For early detection of dengue, NS1Ag MICROLISA and for Rapid test, Dengue IgG IgM WB kit, Dengue Day 1 Test kits were used. Serotyping was done for ten NS1 antigen positive samples by RT-PCR.

Results:
There is almost absence of Japanese Encephalitis and Chikungunya infections. Dengue fever out of 12,519 samples, 2774 samples were positive. Maximum number of positive samples (96) was received between September, October, and November. Males are affected more than females. The most affected age group is below 20 years (50). The use of NS1 ELISA has helped to detect 60 of the cases in early stage. Dengue serotype 3 was the predominant serotype. The water stored in open containers in areas of water scarcity in Nagapattinam favored Aedes mosquito growth and spread of dengue fever. Conclusion: Physicians should consider arboviral infections in fever, encephalitis patients. Vaccination should be done in Japanese Encephalitis endemic area. Effective vector control measures should be taken prior to the monsoon season. Creating awareness regarding usage of mosquito nets, repellants should be done. The study emphasizes the need for continuous seroepidemiological surveillance for the timely implementation of effective Japanese Encephalitis, Chikungunya, Dengue control programmes.

Keywords: Japanese Encephalitis, Chikungunya, Dengue, IgM capture ELISA, Serotype.

Introduction:
The arboviruses are arthropod borne virus which produces clinical illness in humans. More than 385 virus are classified as arboviruses. Epidemics occur frequently. The fever is biphasic and is associated with congestion of conjunctiva, retro orbital myalgia, joint pain. Haemorrhagic manifestations occur in dengue.

Japanese encephalitis is characterized by headache, fever, vomiting, altered sensorium, convulsions and weakness. Chikungunya infection is characterized usually by fever, rash and joint pains.

Japanese Encephalitis:
Nearly 3 billion people live in Japanese encephalitis endemic area. It causes 35,000 cases of encephalitis and 10,000 deaths annually (1). The disease was first observed in Vellore district of Tamil Nadu (2). Japanese Encephalitis epidemics occurred in Bihar, West Bengal, Uttar Pradesh, Karnataka, Andhra Pradesh, Manipur (3). Culex tritaeniorhynchus is the most important vector followed by Culex vishnui, Culex pseudovishnui, Culex bitaeniorhynchus (4). These vectors breed in paddy fields. Amplifying host are pigs. Human infections are more in October-November.

Chikungunya Fever:
Chikungunya fever outbreak was first reported in Tanzania in 1952 (5). Chikungunya belongs to the genus Alpha virus and Family Togaviridae (6). In India, Chikungunya infection was first reported in June 2006. In India, Chikungunya infection was first reported in Andhra Pradesh, Karnataka and involved 8 states in India affecting 1.3 million people (8). Both Aedes aegypticus and Aedes albopictus are vectors.

Dengue Fever:
Nearly 2.5 billion people live in dengue endemic area and 50 million people are being infected annually (9). Death due to dengue fever is estimated to be 12,500 to 25,000 annually. It is endemic in Africa, south East Asia, America. In India, the dengue epidemics has affected Calcutta (1963), Vellore (1964) Nagpur (1965), Kanpur (1968), Delhi (1969) and in 1999, there was a dengue outbreak in Madras. Aedes aegypti is the vector and it breeds well in urban environment.
Materials and methods:
The study was conducted at the department of microbiology, in a tertiary care hospital. All cases with clinical features suggestive of dengue, Chikungunya, Japanese encephalitis were included in this study. The informed consent was obtained from the patients and the ethical committee clearance was obtained. The study was conducted from January 2012 to November 2012. From the patients 2 – 5 ml of blood was obtained using syringe after aseptic precautions. Serum was separated by centrifuging the whole blood sample. The serum sample was stored in sterile aliquots after proper labeling and preserved in refrigerator at 4°C. Proper registers were maintained separately for dengue, Japanese encephalitis, Chikungunya infection. Number of suspected samples received for Japanese Encephalitis is 209, Chikungunya fever is 234, and Dengue fever is 12,519 in 2012. The patients sample were tested using dengue- IgM capture ELISA kit, Japanese Encephalitis- IgM capture ELISA kit, Chikungunya- IgM capture ELISA kits supplied by National institute of virology Pune (Maharashtra).

For early detection of dengue, NS1Ag MICROLISA is used as a screening test for the suspected cases of dengue. The kit detects all four dengue serotypes: DEN1, DEN2, DEN3 & DEN4. Rapid card Test was done by using Dengue IgG/IgM WB kits, supplied by SD BIO STANDARD DIAGNOSTICS PVT LTD, Gurgaon, Haryana and Dengue DAY 1 test kits. DENGUE NS1Ag MICROLISA and Dengue DAY 1 test kits were supplied by J. mitra & co.pvt.ltd. New Delhi 110020 – India. In all the ELISA procedures, the manufacturer’s instructions were strictly followed and results were obtained.

Ten NS1 antigen positive samples were sent to Trirunelveli medical college for serotyping. Viral RNA was isolated from each of these samples and was subjected to the RT-PCR medical college for serotyping. Viral RNA was isolated from each of these samples and was subjected to the RT-PCR.

RT-PCR: Of these 10 NS1 positive samples, six samples were positive; four samples were negative by RT-PCR assay. All the 6 PCR positive samples were subjected to Molecular Typing of dengue virus namely D1, D2, D3, and D4. Out of the six samples, two samples were positive for Dengue virus serotype 1 and four samples were positive for Dengue virus serotype 3.

Discussion:
JAPANESE ENCEPHALITIS: In 2012, four and half year old child was positive indicating Japanese encephalitis is more common in children which correlates with the other study (11).The incidence of Japanese encephalitis in Tamil Nadu as stated by Ministry of health and family welfare, 51 cases in 2005, and 18 cases in 2006, and 37 cases in 2007 indicates there is significant under reporting of true cases (12).The high pig to pig ratio accounts for low Japanese encephalitis incidence in Thanjavur (13). CHIKUNGUNYA FEVER:

In 2012, all the Chikungunya samples are negative, which correlates with the near absence of Chikungunya cases at AIIMS locality (14). Chikungunya outbreaks are separated by periods of greater than 10years of absence of apparent infections. There is a prolonged gap of 32 years between 1973 Chikungunya outbreak and 2006 Chikungunya outbreak in Andhra Pradesh (15). Similarly in Indonesia, 1983 Chikungunya outbreak is followed by Chikungunya outbreak in 2001 after a long interval. DENGUE FEVER: In 2012, Dengue infection is more in males than in females which correlates with the other study (17). Number of samples received post monsoon was more in September, October, and November which correlates with the other study (10). The most affected age group is below 20years which correlates with the other study (16). In 2012, the Dengue outbreak started in Nambiar Nagar of Nagapattinam and spread to Thiruvarur , Thanjavur .In Nagapattinam, due to water scarcity, water is stored in open containers for domestic purposes .Numerous Aedes mosquito larvae were identified in house to house surveys .The movement of travelers to festival at Velankani may be a reason for rapid spread of infection throughout Tamil Nadu which correlates with the other study (10). The use of NSI ELISA has helped to detect 60% of the cases that cannot be detected in early stage of infection with IgM ELISA alone. The predominance of dengue serotype 3 correlates with the other study (10). In 2011, the serotype present was serotype 1 and serotype 4 in Thanjavur. The emergence of new Dengue serotype 3 has lead to a massive outbreak in Thanjavur which correlates with the other study (19). Conclusion: Japanese encephalitis should be ruled out first, in a case of encephalitis before considering any other viral etiology .Vaccination with mouse brain derived inactivated vaccine should be done in endemic areas .Physicians while treating viral fever, should keep dengue, Chikungunya fever in differential diagnosis and if necessary, serological testing should done to reduce under reporting of true cases. As all this infections are mosquito borne, effective vector control measures should be taken prior to the monsoon season.

Residents in endemic area should take personal protection measures to reduce mosquito bites by using mosquito nets, repellants .Stagnation of water, accumulation of disposable plates around residential area has to be avoided. Clean environment will certainly decrease arboviral infections in the future.

The study emphasizes the need for continuous seroepidemiological surveillance.
for the timely implementation of effective Japanese Encephalitis, Chikungunya, Dengue control programmes.

References
10. CSE DRAFT DOSSIER: HEALTH AND ENVIRONMENT>>
15. Centers for Disease Control and Prevention, Emerging Infectious Diseases ISSN: 1080-6059, Chikungunya Outbreak, South India, 2006.
17. Study of seroprevalence of Dengue Fever in Central India PM ukey, sa bondade, PV Paunipager, RM Power, and SL Akulwar
18. The changing epidemiology of dengue in Delhi India. Ekta Gupta, Lalit Dar, Geetanjali Kapoor and Shobha Broor.

An Initiative of The Tamil Nadu Dr. M.G.R. Medical University
University Journal of Pre and Para Clinical Sciences