TO STUDY THE EVALUATION OF CONVENTIONAL CULTURE METHODS AND SERODIAGNOSTIC METHODS FOR CLINICALLY DIAGNOSED AS MENINGOENCEPHALITIS IN PATIENTS ADMITTED IN THANJAVUR MEDICAL COLLEGE HOSPITAL WITH SPECIAL REFERENCE TO JAPANESE ENCEPHALITIS.

Dissertation submitted to

THE TAMILNADU Dr. M. G. R. MEDICAL UNIVERSITY, CHENNAI.

In partial fulfillment of the regulations

For the award of degree of

M.D (MICROBIOLOGY)

BRANCH-IV

THANJAVUR MEDICAL COLLEGE

THANJAVUR 613004.

THE TAMILNADU Dr. M .G. R. MEDICAL UNIVERSITY

CHENNAI-600032

APRIL 2017

CERTIFICATE

I hereby certify that the dissertation entitled **"TO STUDY THE EVALUATION OF CONVENTIONAL CULTURE METHODS AND SERODIAGNOSTIC METHODS FOR CLINICALLY DIAGNOSED AS MENINGOENCEPHALITIS IN PATIENTS ADMITTED IN THANJAVUR MEDICAL COLLEGE HOSPITAL WITH SPECIAL REFERENCE TO JAPANESE ENCEPHALITIS"** submitted to THE TAMILNADU Dr. M .G. R. MEDICAL UNIVERSITY, In partial fulfillment of the regulations required for the award of M.D Degree in Microbiology is a record of original research done by **DR.R.GOPINATHAN**, carried out in the Department of Microbiology, Thanjavur Medical College, Thanjavur during the period from June 2015 to May 2016 under my guidance and supervision and the conclusions reached in this study are his own

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submitted by Dr. R. GOPINATHAN **of**

Dept. of MICROBIOLOGY **Thanjavur Medical College, Thanjavur**

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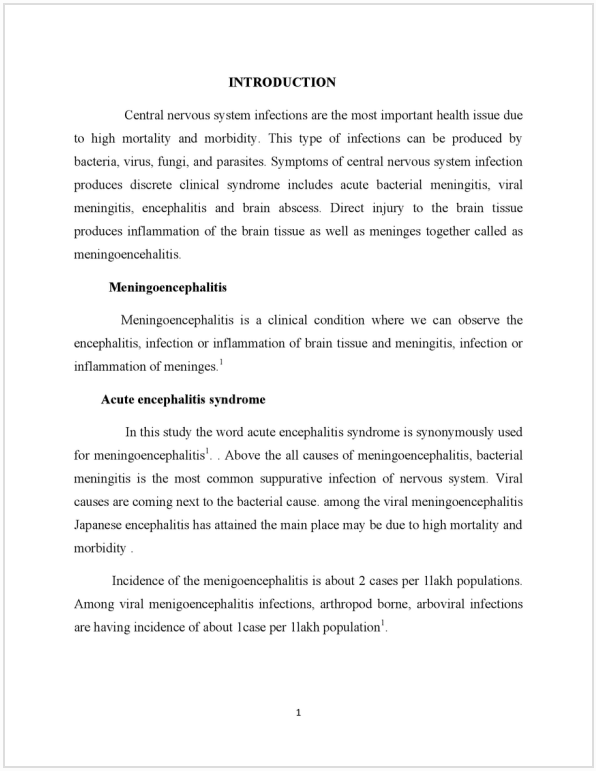

INTRODUCTION

Central nervous system infections are the most important health issue due to high mortality and morbidity. This type of infections can be produced by bacteria, virus, fungi, and parasites. Symptoms of central nervous system infection produces discrete clinical syndrome includes acute bacterial meningitis, viral meningitis, encephalitis and brain abscess. Direct injury to the brain tissue produces inflammation of the brain tissue as well as meninges together called as meningoencehalitis.

Meningoencephalitis

Meningoencephalitis is a clinical condition where we can observe the encephalitis, infection or inflammation of brain tissue and meningitis, infection or inflammation of meninges.[1]

Acute encephalitis syndrome

In this study the word acute encephalitis syndrome is synonymously used for meningoencephalitis[1]. . Above the all causes of meningoencephalitis, bacterial meningitis is the most common suppurative infection of nervous system. Viral causes are coming next to the bacterial cause. among the viral meningoencephalitis Japanese encephalitis has attained the main place may be due to high mortality and morbidity .

Incidence of the menigoencephalitis is about 2 cases per 1lakh populations. Among viral menigoencephalitis infections, arthropod borne, arboviral infections are having incidence of about 1case per 1lakh population[1].

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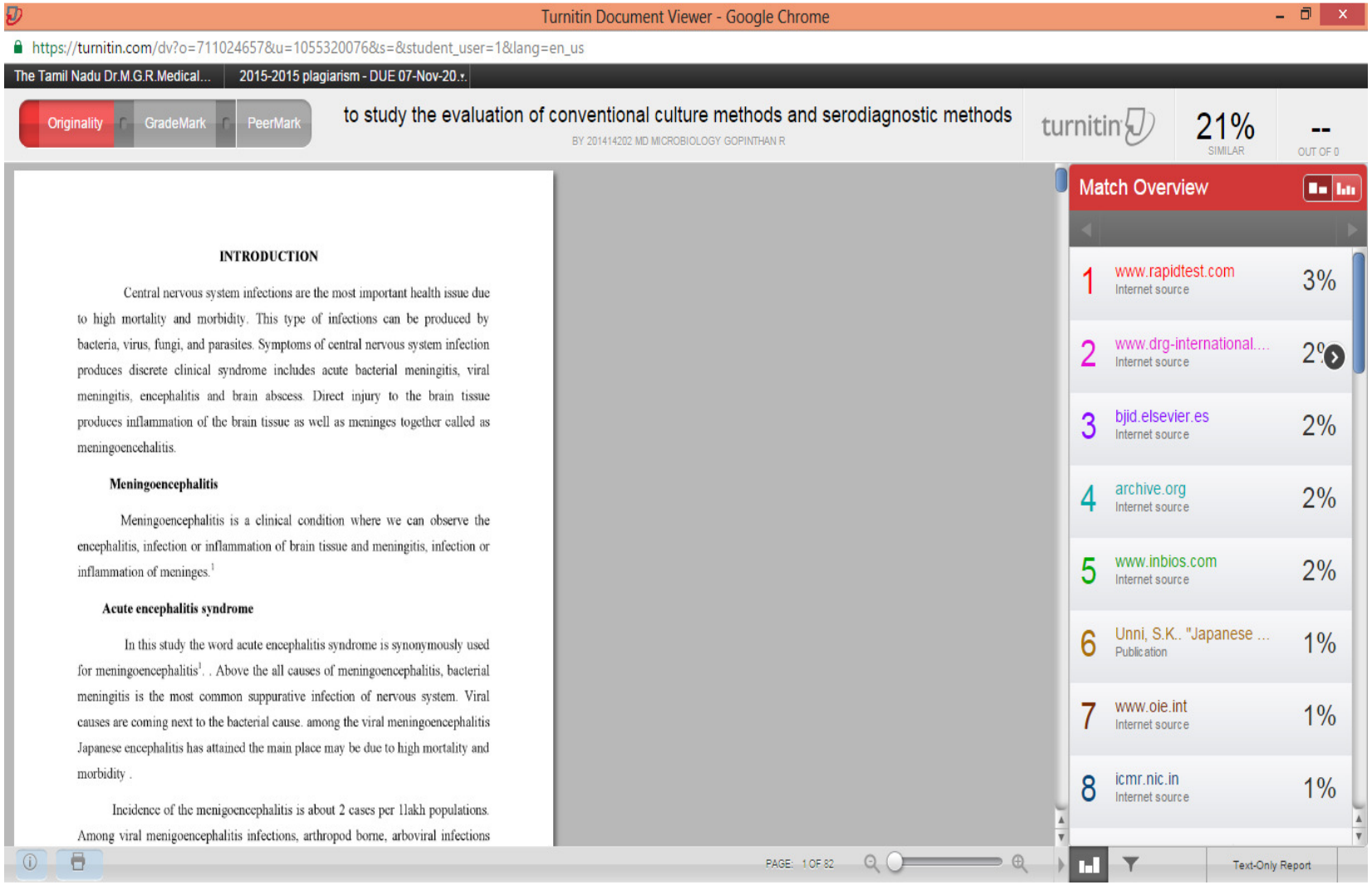
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DECLARATION

I **DR.R.GOPINATHAN,** solemnly declare that this dissertation "**TO STUDY THE EVALUATION OF CONVENTIONAL CULTURE METHODS AND SERODIAGNOSTIC METHODS FOR CLINICALLY DIAGNOSED AS MENINGOENCEPHALITIS IN PATIENTS ADMITTED IN THANJAVUR MEDICAL COLLEGE HOSPITAL WITH SPECIAL REFERENCE TO JAPANESE ENCEPHALITIS"** is a bonafide record of work done by me in the Department of Microbiology, Thanjavur Medical College, Thanjavur under the Guidance and Supervision of my Professor **Dr.EUNICE SWARNA JACOB,M.D.,**The Head of the Department, Department of Microbiology, Thanjavur Medical College, Thanjavur between May 2015 and April 2016.

This dissertation is submitted to The Tamilnadu Dr. M.G.R Medical University , Chennai in partial fulfillment of University regulations for the award of M.D Degree (Branch – IV) in Microbiology to be held in April 2017.

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Date:

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Postgraduate Student,
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LIST OF ABBREVIATION

JEV- Japanese encephalitis virus

PCR- Polymerase chain reaction

CSF- Cerebrospinal fluid

AES- Acute encephalitis syndrome

CNS- Central nervous system

HSV- Herpes simplex virus

RER- Rough endoplasmic reticulum

BBB-Blood brain barrier

RT-PCR-Reverse transcription polymerase chain reaction

ELISA- Enzyme linked Immunosorbant assay

CF- Complement fixation

VN- Virus neutralization

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LIST OF TABLES

LIST OF FIGURES

CONTENTS

INTRODUCTION

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Incidence of the menigoencephalitis is about 2 cases per 1lakh populations. Among viral menigoencephalitis infections, arthropod borne, arboviral infections are having incidence of about 1case per 1lakh population[1].

Japanese encephalitis

Japanese encephalitis is an acute viral infection caused by mosquito borne Japanese encephalitis virus. JE virus is a virus from flaviviridae. Domestic pigs and wild birds are reservoirs of the virus, the most important vectors of the disease are the culex mosquitos .Japanese encephalitis virus causes most serious clinical disease among the flavivirus group.

Infection with Japanese encephalitis virus may cause febrile illness, meningitis, or encephalitis and meningoencephalitis, clinically indistinguishable from other cause of acute encephalitis syndrome Approximately 3 billion people live in endemic regions. Incidence of 55,000 human cases and 11,000 deaths have been noted annually in world wide[3]. Roughly 26% patients of encephalitis die due to the infection of JEV.[1, 2]

50% of the patients who are all the survivors will develop permanent neurological symptoms. Mortality being high in this disease, identification of viral infection and early diagnosis in infection prone area has the crucial role in the management. In India, epidemics of JE were reported from many parts of the country, and it was considered a major health issue.

The first recognition of JE was based on serological surveys which were conducted in 1955, in Tamil Nadu .Totally 66 cases were reported between the period of 1956 and 1966 in Southern India. Successive surveys were carried out by that indicates approximately half of the population in South India has neutralizing antibodies to the virus. A major outbreak was recorded in 1973 in west Bengal resulting in a 42.6% fatality rate[4]. Consequently, the disease spread to other states, caused a series of outbreaks in various parts of the country. Cases were reported from 21 states and union territories in 1978. In Uttar Pradesh, the first major JE epidemic occurred in Gorakhpur in 1978, with 1,102 cases and 297 deaths reported. In 2005 epidemic surpassed all previous reported

outbreaks in the country. In that year, Uttar Pradesh faced a demoralizing outbreak of JEV, mostly restricted to Gorakhpur, with 6,051 cases and 1,510 deaths. Subsequently another outbreak occurred in 2006 where with 2,320 cases and 528 deaths. Likewise, JE cases in Uttar Pradesh were cramped predominantly to Gorakhpur during 2007, with 3,534cases and 756 deaths, and then onwards till 2007 there have been 103,874 reported cases in India, and 33,729 deaths[5]. Roughly 597,657,000 people in India live in JE-endemic regions, and 1,600 to 4,500 cases are reported every year.[4]

A relative study exhibit the total number of cases and deaths in India from 2005 to 2010 shows higher incidence of cases in Northern India than in Tamil nadu[4]; but the actual enormity of JE may be higher due to lack in reporting and expensive diagnostic methods.

In India, Serum and CSF samples, are taken for IgM by ELISA test, is widely used for the diagnosis of JE. The time of diagnosis with CSF sample is earlier (1-2days) compared to the serum (5-6days). The other diagnostic methods are PCR and Cell culture. IgM ELISA test is readily available and economical when compared to PCR and Cell culture. Hence I choose this study to compare the reliability of IgM ELISA and PCR along with cytological analysis of cerebrospinal fluid.

AIMS AND OBJECTIVES

1. To study the incidence of JE in patients admitted with meningoencephalitis.

2. To study the cytological and chemical analysis of all CSF samples in suspected meningoencephalitis patients admitted in Thanjavur Medical College hospital

3. To study the serodiagnosis of JE by IgM capture ELISA in all CSF samples of patients admitted with suspected meningoencephalitis in a tertiary care hospital Thanjavur .

4. To study the molecular characterization of JE virus by polymerase chain reaction (RT-PCR)

5. To study the comparison of IgM ELISA and polymerase chain reaction (RT-PCR) in association with cytological and bio chemical analysis of CSF.

REVIEW OF LITERATURE

Meningitis is an acute purulent infection with in the subarachnoid space. This may be due to bacterial, viral, fungal, and parasites. This type of infections may lead to central nervous system inflammation. Subsequently it will produce fever, head ache, seizure, hemiparesis, neck rigidity, elevated intra cranial pressure, loss of consciousness and stroke.

Bacterial causes

1. Hemophilus influenza type b
2. Neisseria meningitides
3. Listeria monocytogen
4. Mycobacterium Tuberculosis
5. Leptospira

Viral causes

1. Japanese encephalitis virus
2. Herpes simplex virus
3. Enterovirus

Protozoal and fungus

1. Toxoplasma
2. Cryptococcus.

Epidemiological characters

Bacterial causes are the commonest type of central nervous system infection, with a yearly incidence of 2 cases/1lakh populations[5].

The most frequently responsible organisms of bacterial meningitis are pneumococcus (-49%), Neisseria (-26%), group B streptococci(-16%), and Listeria (-12%). Haemophilus type b records for less than 10% of cases among bacterial meningitis. N. meningitidis is the most common organism causing epidemics of meningitis among 8 to 12 years of age group[5].

Causative organisms

- **Streptococcus pneumonia**

Streptococcus pneumonia is the most common organism producing purulent infections in adults. There are number of conditions that increase the risk ofpneumococcal meningitis.

The morbidity and mortality is high even in antimicrobial treatment. Rate of mortality is up to 20% in untreated cases.[5]

The cases of meningitis have been declined due to Neisseria infection, due to regular and routine immunization programs. The vaccine contains serogroup A[6].sero group B is not present in the vaccine .This is the reason for the present cases.

- Gram negative bacilli are one of the cause for bacterial meningitis among diabetics and immune compromised.
- **Streptococcus, agalactiae,** which was previously responsible for meningitis in infants but now the cases in neonates have declined when compared to geriatric age group.
- **L. monocytogenes** is a progressively more important cause of meningitis in pregnancy, neonatal period and individuals more than 60 years old and immune compromised of all ages.
- **H.influenzae type B** meningitis in pediatric age group has decreased due to the introduction of HIB vaccination.
- **Staphylococcus aureus** and coagulase negative staphylococci (CONS) are the significant cause of meningitis after invasive surgeries.
- **Coagulase negative staphylococci (** Cons) ,the normal skin flora produces the meningitis in patients who are all underwent neurosurgical procedures**.**

Pathophysiology

Commonest bacteria that cause meningitis are S. pneumonia and Neisseria which forms colonization over the pharynx by attaching to the epithelial cells of nasopharynx[7]. Bacterial organisms are elated across epithelial cells in membrane bound vacuoles to the intravascular space. If the organisms escapes from the blood stream from phagocytosis, it can easily reach the intraventricular choroid plexus and get in to the CSF. Normally CSF contains some white blood cells and small amount of complement proteins and immunoglobulins. Phagocytosis ,the procedure of engulfment to destroy the bacteria is impaired by the fluid nature of CSF. Fluid nature of CSF contributing less in phagocytosis when compared to solid tissue [7].

Clinical features

Meningitis can present as an acute illness that progress rapidly in a few hours or as a sub acute infection that progressively worsens over several days. The classical pictures of meningitis are temperature, vomiting, head ache and neck rigidity. A decreased level of consciousness occurs in more than 75% of patients[8]. Nausea, vomiting and photophobia are also common complaints of patients of acute encephalitis syndrome. Seizures are mostly seen among children. It occurs as a part of initial presentation of bacterial meningitis. Elevated intracranial pressure is an expected complication and a dangerous presentation of bacterial meningitis. More than 80% of patients will have

pressure of more than 180 mmH2O in the CSF. The worst thing in raising ICP is coning .The incidence of coning among patients with suspected bacterial meningitis is reported from the range of 1% to 7%[8].

Diagnosis

Blood cultures should be obtained immediately when bacterial meningitis is suspected. Diagnosis is made by the examination of CSF. The classic CSF abnormalities in bacterial meningitis are PMN increased white cell count, decreased glucose level, increased protein level, increased pressure of CSF. bacterial cultures are positive in more than 70% of patients[7]

CSF gram stain demonstrates more than 50% of patients. CSF glucose concentrations are abnormal in patients of meningitis. Serum/CSF, glucose ratio should be monitored for getting the diagnosis[9]. A 16s rRNA sequence broad based bacterial PCR can identify small numbers of viable and non viable organisms in CSF and is expected to be useful for making a diagnosis of bacterial meningitis in patients who have been admitted and treated with antibiotics and where gram stain and CSF culture are negative.[9]

TABLE 1: CEREBROSPINAL FLUID ABNORMALITIES

Pressure	>180 mmH2O
White blood cells	10μL to 10000 Ml
Red blood cells	Absent
Glucose	<40mg /DL
CSF/serum glucose	<0.4
Protein	>45 mg /DL
Gram stain	Positive >60%
Culture	Positive >80%
PCR	Detects bacterial DNA
Limulus lysate	Positive in gram negative meningitis
Latex agglutination	Positive in S.pneumoniae,N. meningitides

Viral meningitis

Viral meningitis are caused by the infection or the inflammation produced by the viruses. This can be classified in to acute meningitis, sub acute meningitis and chronic meningitis.

Mostly viral meningitis are in acute stage with the complaints of head ache, temperature, vomiting, and decreased level of consciousness.

In Immunocompetent patients having viral meningitis normally presents with head ache, fever and signs of meningeal irritation together with an inflammatory CSF profile.

Head ache is the most common symptom almost invariably present in all cases which is often characterized as frontal or retro-orbital and is often associated with photophobia and pain when moving the eyes to the edges.

Neck rigidity is present in most of cases but may be mild and present only near the limit of neck extension movements.

Causative organisms are

1. Japanese encephalitis virus

2. Herpes simplex virus

3. Entero virus

By using different diagnostic methods, including CSF PCR, culture, and serology, a precise viral cause can be found in most of the viral meningitis cases. In viral meningitis 75-90% of cases can easily diagnosed by cell culture, PCR, and serology[9].

The most important organisms producing meningitis are (HSV type-2) and arboviruses. CSF cultures results shows positive only in 30-70% of patients. Isolation of virus is mainly depends on the specific viral organisms and viral load. In the region of one-third of culture negative cases are classified as “aseptic” meningitis.

Epidemiological features

Viral meningitis is not a nationally notifiable disease; on the other hand it has been estimated that the yearly incidence of cases in world wide is about - 75000 per year/1 lakh populations[5].

There is a substantial increase of cases during the summer reflecting which reflects the seasonal predominance of enetrovirus and arthropod-bone virus infections .

Laboratory diagnosis

Examination of cerebrospinal fiuid

- **On macroscopic appearance[10]**

  CSF appears clear in viral meningitis. The terminology aseptic meningitis most widely used to identify the viral meningitis.

- **On microscopic appearance**

  Minimal white blood cells normally present in CSF.

Cell count analysis

The typical profile is an elevated count of lymphocytes (5 to500cells/µl) and increased level of white cell count.

Biochemical analysis

A normal or slightly raised protein level (10-100 mg/dl).

A normal glucose concentration or slightly decreased, and

A normal or mildly elevated opening pressure (100 to 400mm H_2O).

JAPANESE ENCEPHALITIS

Family of flaviviridae of viral pathogens are classified into three groups. It includes pestivirus, flavivirus, hepacyvirus. Flaviviruses are recognized by causing diseases among humans and animals. Commonest flaviviral infections are dengue viral infection, West nile virus infection, Yellow fever virus infection, Japanese encephalitis virus infection

.Flaviviral infections are mainly transmitted through vectors. Mosquitoes are the main vector in transmitting the infections, ticks are also playing role in transmitting the infection.

Zoonotic disease

Japanese encephalitis, yellow fever disease, dengue fever disease, together called as zoonotic diseases .This type of infections can be classified into two categories 1.enzoonotic 2.epizoonotic

This zoonotic cycle needs pigs as the key amplifier, birds, ticks, mosquitoes are used as a carriers for the transmission of infection.

Japanese encephalitis

- 25% of patients with JEV infection may die due to encephalitis complication.

- 50% of patients will develop neurological complications like memory impairment, paralysis, behavioral changes, seizures and impaired cognition.

Humans are considered as dead end host due to low level of viremia in the blood circulation. Low level of viremia is insufficient to infect the feeding mosquitoes.

Vectors

Culex

- It belongs to culex vishnui subgroup,which acts as a main vector for the transmission of Japanese encephalitis infection.
- Anopheles barbirostris van der Walp and Anopheles subpictus have also been identified from India for harboring JEV

Incidence

- Incidence is about 45000 cases and 10000 deaths annually in world wide.
- In 1871 the first case was identified in Japan afterwards tropical countries like south East Asia and north Australia[6].
- An epidemic occurs during summer period in north Asian region.
- Peak infections can be seen during rainy season in south Asian region.

CLASSIFICATION OF FLAVIVIRUS

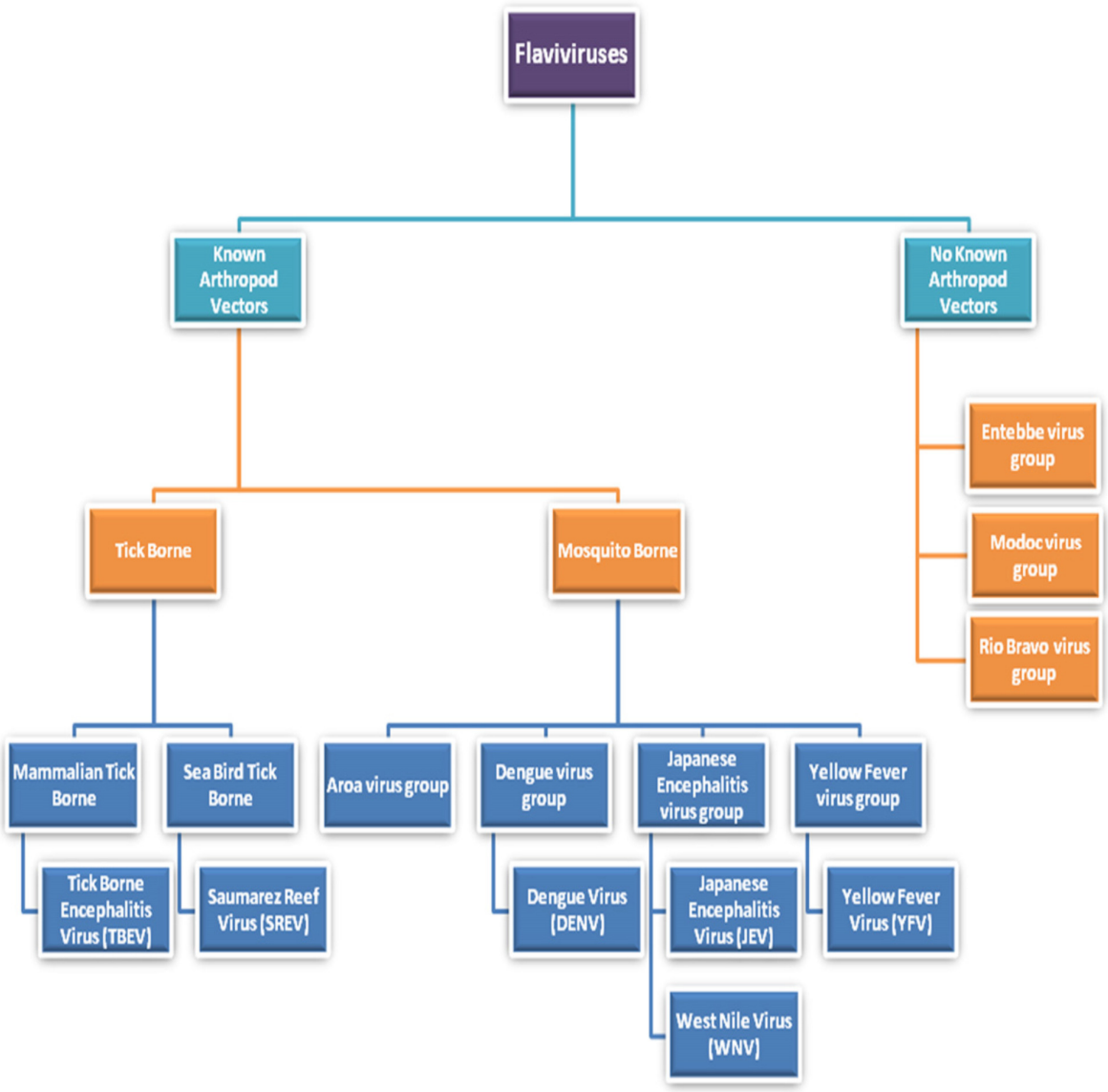


FIG: 1 FLOW CHART OF CLASSIFICATION OF FLAVIVIRUS

JEV GENOME

- Japanese encephalitis virus is an enveloped virus of 55nm in diameter size.
- It has a single standard RNA virus with positive sense.
- The RNA viral genome is 11kb in length.
- It contains three structural proteins

1.Precursor to membrane (prM)

2. Envelope (E)

3. Capsid (C).

- .Non structural proteins

NS1, NS2, NS3, NS4, NS5

- 5 genotypes have been identified such as genotype I, II, III, IV and V
- Hemagglutination test monoclonal antibody test and polyconal antibody test can be used to make a difference between the isolates within the same gene

Precursor to membrane (prM)

- Precursor to membrane protein is a Glycoprotein in nature having two trans membrane helices.
- It serves as a chaperone to form E protein and for assembly of protein.
- Maturation is taken place in Golgi apparatus

C protein

- It has 120 amino acids and it forms the homodimers in shape.
- This protein is involved in packaging of the viral genome and
- It also involved in the formation of the neucleo capsid prM and E .

E protein

- Main intention of E protein is to form neutralizing antibodies, which contains receptor binding site for NS1**.**
- It has the skill to induce the protective antibody secretion in the host

NS-2B protein

- NS-2B protein ruins as a heterodimer material along with NS-3 protein and helps in anchoring of this heterodimeric complex to the endoplasmicreticulam membrane.

NS3 protein

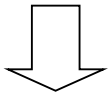
- NS-3protein is participating in viral replication and viral assembly by its RNA helicase and NTPase activity.

NS5 protein

- This protein is the biggest among all the proteins of JEV.
- It has methyl transferase activity on its N terminal end and RNA-dependent RNA polymerase activity on c-terminal end.

Viral replication

- Viral replication starts with entry of JE virus



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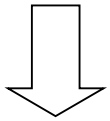
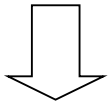
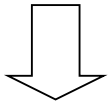
- It enters in to the host cell by the mechanism of receptor endocytosis.



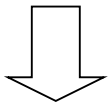
- A viral replication complex is formed by NS3 and NS5.



- By using replicating complex double standard replicative form is formed.



- Replication force starts with the formation of RNA strands which is complementary to the parent strand..



- Cyclization of viral genome confirms that replicated viral RNA is in full length.

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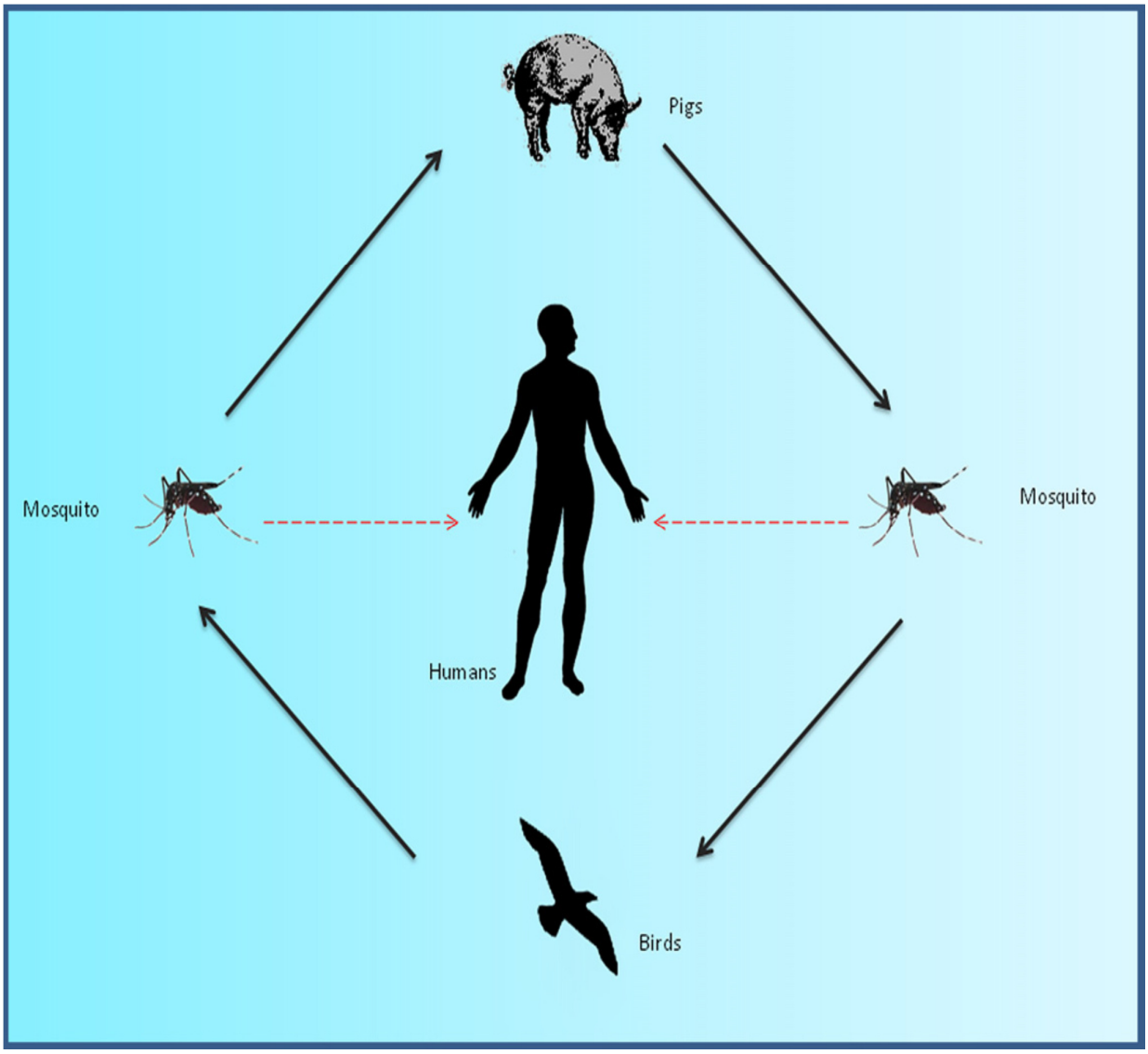

FIG 2: LIFE CYCLE OF JAPANESE ENCEPHALITIS VIRUS

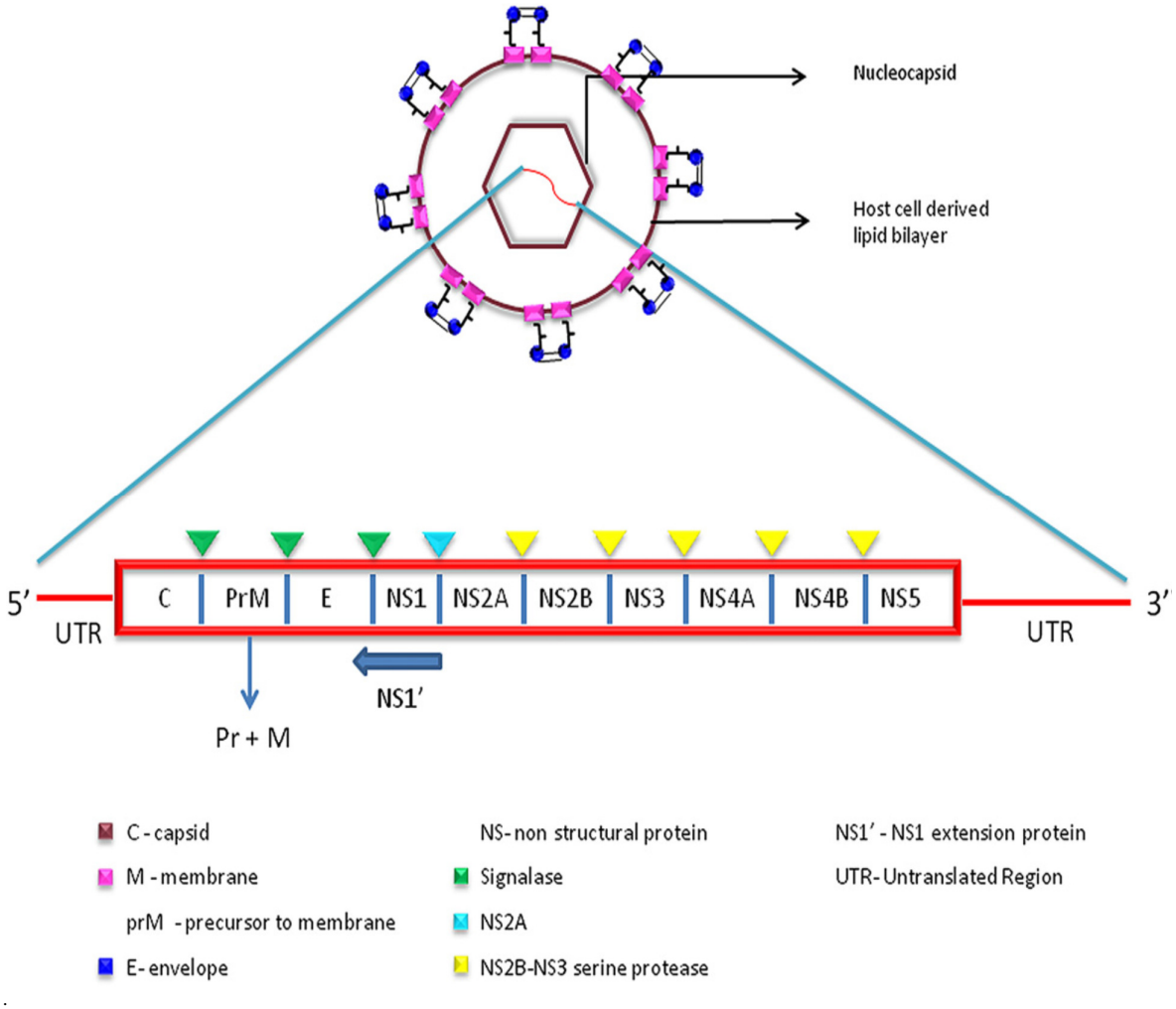

FIG3: GENOME OF THE JAPANESE ENCEPHALITIS VIRUS

PATHOGENESIS

- Data available for this viral encephalitis due to JEV has been derived from animal models and in vitro.

- This JEV encephalitis starts with bite of infected mosquitoes. It has a peripheral replication cycle that we can see in vitro studies

- Japanese encephalitis viral infection produces impairment of hot immune system which leads to functional reduction of differential count.

- Differential count population is altered after JEV infection

- Japanese encephalitis viral infection transmitted to central nervous system takes place mainly through antipodal transport.

- Rough endoplasmic reticulum is the site of replication in neurons.

- Infection of neuronal stem cells leads to loss of neurons in subvendricular zone.

- Microglial cells and astrocytes acts as a scavenger cells in the site of neuronal injury.

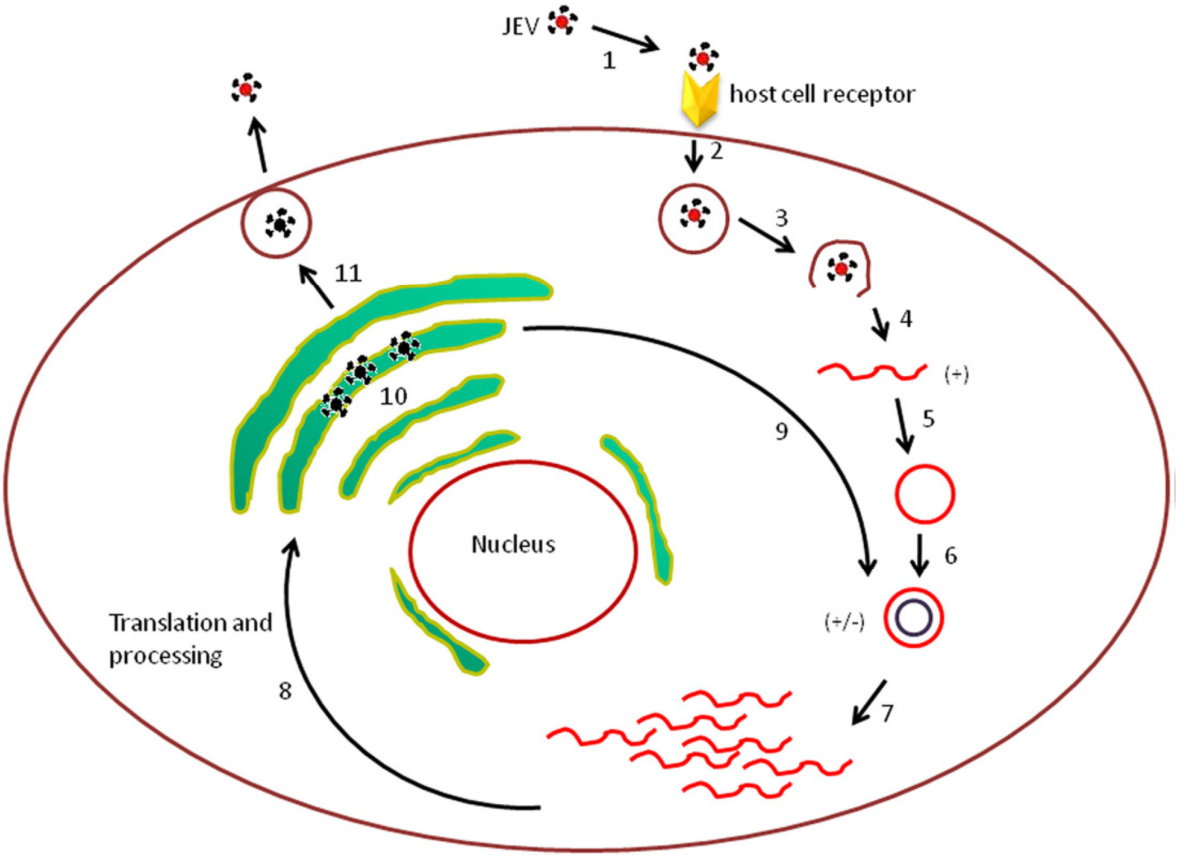

FIG4: REPLICATION OF JAPANESE ENCEPHALITIS VIRUS

CLINICAL FEATURES

- Most of the Japanese encephalitis infections are asymptomatic. Symptoms are developed only with less than1% of the populations.
- Infection principally affects children who are all age group of less than 15 years[2]
- Incubation time is 5-15 days in Japanese encephalitis infection.

- **Symptoms**

  Fever

  Head ache

  Vomiting

  Myalgia

  Altered mental status

  Seizure

  Paralysis

  Impaired cognition

  Gastrointestinal symptoms of abdominal pain in children.

- Most of the symptoms are due to cerebral edema and congested lepto meninges of brain tissue.

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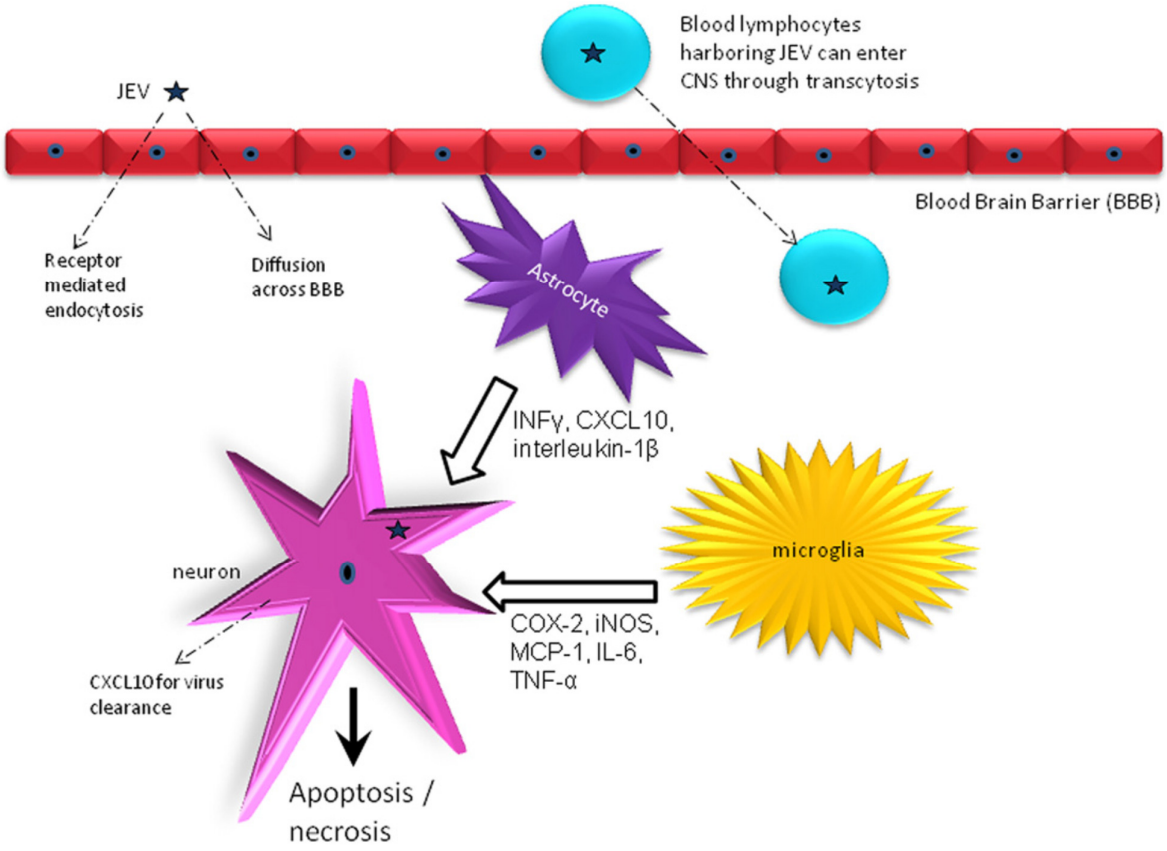

FIG: 5 NEUROPATHOGENESIS OF JAPANESE ENCEPHALITIS VIRUS

RESPONSE TO JEV INFECTION

IMMUNITY

Humoral and cell mediated immunity

- Immunity has a major role in Japanese encephalitis infection.
- Antiviral antibodies have been reported as an imperative to deal with the arbovirus induced encephalitis.
- Neutralization occurs though antibodies which will usually clear the cell free viruses. Phagocytosis is taken place to clear the virion.
- If there is any neuronal infection, antibodies are acted over the surface of infected neuron cells to alter intracellular replication of viruses with the aim of follow the non cytolytic mechanism.
- Development of IgM antibodies after infection plays major role in the identification of Japanese encephalitis infection.
- IgM antibodies can be identified in both serum and cerebrospinal fluid.
- IgM antibodies can be detected in CSF on day 1.
- In case of serum it will take 5 to 7 days.
- Identification of IgG helps to cross check the other flaviviral infection.

STRAIN VARIABILITY

JEV occurs as a single serotype, though considerable antigenic variation is observed. More than 50 strains being isolated in Japan alone. Five JEV genotypes have been described (GI–GV)[22] , however genotype relationship to phenotype or virus fitness is still unclear. The basis for genotyping is phylogenetic analysis of the viral envelope *E* gene, vitally important in viral attachment and entry into host cells. A potential link does exist between JEV genotype and climate. GIa and GII are significantly associated with tropical climates, GIb and GIII with temperate climates, and GIV is confined geographically within Indonesia. Despite its current isolation, GIV is believed to be ancestral to all other circulating genotypes and shows the largest antigenic and phylogenetic difference of all the genotypes. GV has been found in both tropical and temperate climates and recently reemerged in Tibet and South Korea after remaining undetected for nearly 60 years. Since its reemergence, GV has shown a high capacity for dispersal in a short time period and also warrants continued monitoring given its potential for high virulence. At present GIb is believed to be the dominant JEV genotype throughout Asia[23].

CLEANING AND DISINFECTION

SURVIVAL

The thermal inactivation point of JEV is 40˚C, and the virus can be easily destroyed completely by heating for 30 minutes at 56 ˚C. JEV is labile, sensitive to ultraviolet light and gamma radiation, and does not survive well in the environment. The virus is stable in alkaline environments (pH 7–9) and inactive in acidic environments.

Seasonal precipitation, humidity, and temperature changes are thought to influence JEV transmission by affecting human agricultural practices and the life cycle of mosquito vectors. Higher humidity is a key factor, known to

influence longevity, mating, dispersal, and feeding behavior of mosquitoes. Environmental temperatures in the range of 22–34˚C have been shown to increase mosquito density,10 decrease larval development time, and reduce the extrinsic incubation period of the virus in mosquito vectors, affecting potential JEV transmission.

In endemic areas, cases occur sporadically throughout the year with occasional increases during the rainy season. JEV express more epidemic viral activity in northern temperate Asian climates, with disease outbreaks in summer months. Subtropical regions, such as Thailand and Vietnam, may see a combination of epidemic and endemic disease characteristics[21]. Year-round maintenance of JEV in temperate climates is thought to occur by overwintering mosquitoes, poikilothermic vertebrates, hibernating bats, and/or by vertical transmission from female mosquitoes to offspring. Annual re introduction into temperate climates by migrating birds, bats, or wind-borne mosquitoes is also a possibility, though phylogeny studies of JEV genotypes suggest more local rather than widely dispersed cycles of transmission. Genotypes in tropical climates are maintained year-round by traditional vector-host transmission cycles.

Disinfection

Disinfection can be achieved with organic and lipid solvents, common detergents, iodine, phenol iodophors, 70% ethanol, 2% glutaraldehyde, 3–8% formaldehyde, or 1% sodium hypochlorite[25].

EPIDEMIOLOGY

SPECIES AFFECTED

Pigs are the main amplifying host of JEV, exhibiting high and prolonged viremia and a natural infection rate of 98–100%[26]. Reproductive failure is a trademark of the clinical disease in pigs, and total losses can reach 50% or

more.JEV infection in humans, predominantly children and travelers from non-endemic areas who have not developed immunity to the virus. Sporadic encephalitis is observed in horses, and the virus is manifested either as severe encephalitis or more commonly as subclinical infection. In horses, the mortality rate is not about 5% [25].Neither humans nor are horses believed to contribute to virus transmission due to their relatively low titers and short-term viremia. Subclinical infection has been reported in cattle, sheep, goats, dogs, cats, chickens, ducks, wild mammals, reptiles and amphibians, but these species are not known to contribute to spread of the disease.

ZOONOTIC POTENTIAL

JEV is a zoonotic disease persisting in nature through a cycle of transmission primarily between mosquitoes, some domestic and wild birds, domestic and pigs, and humans. Humans can become infected with the virus, sometimes fatally, but are considered dead-end hosts. Amplification of JEV in swine often precedes human epidemics. A recent multi-criteria decision analysis (MCDA) conducted in Australia found JEV, along with rabies, Nipah virus, and Eastern equine encephalitis, to be the highest priority diseases in the swine industry when considering zoonotic criteria alone. In regions where the virus exists, several key characteristics influence human infection risk: the density, size, and spatial organization of rice paddies, swine farms, and human communities.

GEOGRAPHIC DISTRIBUTION

JEV is endemic in much of Asia and the Pacific, encompassing regions occupied by nearly half of the human population[1]. The greatest population density in endemic areas can be found in India and China, though the disease has been reported as far west as Pakistan, as far south as northern Australia and Papua New Guinea, as far north as maritime Siberia, and as far east as the

island of Saipan.The potential for JEV to spread quickly upon introduction into new areas, similar to closely related WNV in 1999, is a public health concern[27]. Recent isolation of genetic material of JEV in birds and mosquitoes in Italy further substantiates the need for continued vigilance.

MORBIDITY AND MORTALITY

Rates of JEV infection are higher in animals than humans, and detrimental biological and economic consequences in swine production arise primarily from reproductive failure and high piglet mortality. The mortality rate in infected piglets with no immunity to JEV is near 100%, while mortality in infected adult swine is close to zero. Reproductive losses can reach 50–70%. In humans, JEV is the greatest known cause of epidemic viral encephalitis worldwide and more than one-third of the world's population is at risk of infection. The virus primarily affects children; on the other hand less than 1% of JEV infections in humans actually progress to encephalitis[22]. An estimated 50,000 documented human cases of JEV occur annually, resulting in at least 15,000 deaths[22]. Due to the lack of surveillance and data collection in many areas, the actual incidence may be much higher. Up to half of those infected can suffer permanent neurologic damage[33, 45].

TRANSMISSION

JEV relies on vector-borne transmission. Wading water birds (such as herons and egrets) are the primary natural reservoirs of the virus, while pigs act as amplifying hosts. Over 90 bird species are known to be amplifying and reservoir hosts of JEV[32], capable of wide virus distribution and introduction into new areas. Additional proposed amplifying hosts include orangutans and bats, dogs, and flying foxes have also exhibited seropositivity for anti-JEV antibodies. Known vectors include Culex mosquito species, primarily Cx. tritaeniorhynchus, as well as Cx. fuscocephala, Cx. annulirostris,Cx. annulus,

Cx. sitiens, and potentially Cx. quinquefasciatus. Other important regional vectors include Cx. vishnuiin India and *Cx. gelidus*in Indonesia. In addition to the preferred *Culex*species, *Anopheles sinensis*and *Aedesalbopictus*are known vectors, along with several *Armigeres*and *Mansonia*species. Potential natural JEV vectors, *Cx. pipiens, Cx. Quinquefasciatus*[34].

Epizootics involving swine appear to be cyclical, consisting of two separate amplification cycles. During the first cycle, roughly 19% of pigs will become infected and develop antibodies within ten days[30]. This is followed by a second cycle, one to two weeks later, in which mosquitoes transmit the virus to remaining naïve pigs effectively raising the rate of sero conversion to almost 100%. Clinical cases in humans typically occur following this cycle of amplification in swine. As each extrinsic incubation period in mosquitoes ranges from 5–15 days, it can take up to 30 days for the virus to complete its infection process in humans. In addition to natural transmission cycles, attention is also given to artificial insemination practices in modern swine production.

JEV is known to cause inflammation of the genital tract in boars, which can lead to subsequent shedding of the virus in semen and possible transmission to breeding sows. Laboratory-acquired JEV infection has been reported in humans, and work with the virus is restricted to Biosafety Level 3 (BSL-3) facilities and practices. Transmission can occur through needle sticks and potentially at mucosal surfaces if exposed to high concentrations of aerosolized virus.

INFECTION IN SWINE/PATHOGENESIS

CLINICAL SIGNS

Reproductive failure is the most common clinical manifestation in swine. Sows may give birth to stillborn, mummified, or darkened fetuses, and boars may experience reduced number and motility of sperm. Testicular edema and temporary infertility in boars has also been observed.

POSTMORTEM LESIONS

Gross lesions of affected piglets include hydrocephalus, cerebellar hypoplasia, subcutaneous edema, and spinal hypo myelinogenesis. Multifocal hepatic and splenic necrosis may also be seen grossly, while histological lesions are generally restricted to the central nervous system. Serosal petecchiae, bicavitary effusions, neuronophagia, glial nodules, and perivascular cuffing in the brain and spinal cord have also been observed.[35]

DIAGNOSIS

CLINICAL HISTORY

Reproductive failure is the most common manifestation of the disease, and there are no characteristic gross lesions of infected sows. JEV infection prior to 50–70 days of gestation can cause abortion, fetal mummification or stillbirth, and encephalitis in young animals[35]. In endemic areas, the breeding impact may actually be seen more in boars than sows. Some sows may be able to develop protective immunity prior to reaching sexual maturity, whereas earlier maturing boars are at risk of infertility if they have not yet developed protective immunity.

TESTS TO DETECT NUCLEIC ACIDS, VIRUS, OR ANTIGENS

Virus isolation, though time consuming, is the gold standard for a definitive diagnosis. To isolate the virus in laboratory animals, tissue homogenates from infected animals are first inoculated intra cerebrally into two to four day old mice. If the mice show neurological signs, followed by death within 14 days, their brain tissue is collected for a second passage in mice. At this stage, if viral antigen collected from the brain tissue supernatant of infected mice is able to agglutinate red blood cells of one-day-old geese or chickens, it is then available for use in hemagglutination inhibition (HI) testing with JEV antiserum. Primary cultures can be used, inoculated either with tissue directly from infected swine or brain suspension from inoculated mice. A variety of cell lines are suitable; the Aedesalbopictus mosquito cell line C6/36 is useful, as are chicken embryo, African green monkey kidney (Vero) cells, baby hamster kidney (BHK) cells, and porcine kidney (PSEK) cells. Cytopathic effect may not be observed in C6/36 cells, requiring further culture or other diagnostics (such as detection of antigen or RNA). Following culture, indirect immunofluorescence can be used to identify the viral antigen using monoclonal antibodies to JEV specifically or flaviviruses in general. Isolated virus can also be identified by reverse transcription polymerase chain reaction (RT-PCR) or serological methods.

RT-PCR is routinely used in diagnostic laboratories, and both conventional and real-time assays are available. Several multiplex PCR (multiplex PCR) assays have been developed for simultaneous detection of six common diseases of swine—pseudo rabies virus (PRV), porcine parvovirus (PPV), porcine circo virus (PCV) type 2, porcine reproductive and respiratory syndrome virus (PRRSV), classical swine fever virus (CSFV), and JEV—in a single reaction system. Additional reduction of cost and overall assay time is

achieved with multiplex real-time PCR. Sensitivity remains higher with single PCR; however multiplex tests have high specificity for each individual virus.

An additional testing method of interest is multiplex ligase detection–polymerase chain reaction and microarray (MLPM). MLPM has been described for the simultaneous detection of PCV, PRRSV, CSFV, PPV, PRV, and JEV. This test shows a high degree of specificity, more rapid diagnostic time, and enhanced sensitivity compared to real time RT-PCR, as well as modification capacity to include additional pathogens[41]. A nucleic acid detection method, the reverse transcriptase-loop-mediated isothermal amplification (RT-LAMP) assay, has been described for detection of viral RNA, though veterinary application is still limited. One-step real time RT-LAMP assays are experimentally proven to have sensitivity equal to real time RT-PCR and greater than conventional RT-PCR in targeting the *NS1* gene of JEV. Real time RT-LAMP also demonstrates greater sensitivity than conventional RT-PCR in targeting the highly conserved *NS3* gene. Additional targets in RT-LAMP assays are the *E* genes of JEV genotypes I and III. This method demonstrates a ten-fold increase in sensitivity compared to conventional RT-PCR.

Genotypes I and III can be differentiated with RT-LAMP by targeting a single nucleotide polymorphism located in the capsid *C* gene, and RT-LAMP coupled with a lateral flow dipstick (LFD) is able to detect multiple strains of JEV and eliminate the need for potentially unstable fluorescent dyes.

A high degree of specificity can be achieved with RT-LAMP assays, showing no cross-reactivity with other clinically and/or serologically related swine diseases. Given the efficacy, simplicity, and speed of these assays, they are a potentially desirable option for field-level testing and accommodation of a large number of samples.Flavivirus-specific monoclonal antibodies (MAb) can detect JEV antigen in serum, and immunohistochemistry can be used to detect JEV antigen in fetal tissues.

Antigen capture enzyme-linked immunosorbent assay (ELISA) has been developed for simple and rapid detection of JEV infection, as an alternative to virus isolation and RT-PCR. M Ab against the E protein is used for its high specificity to JEV, followed with a polyclonal antibody (PcAb) conjugate to enhance sensitivity and detection of multiple strains. It is important to note that sensitivity is slightly higher with RT-PCR, and antigen capture ELISA may be unable to detect very low viral loads.[44]

TESTS TO DETECT ANTIBODY

A variety of serological tests are available to establish the prevalence of infection in a population or to make a diagnosis in a diseased individual. Methods include immunofluorescent antibody (IFA), virus neutralization (VN), hemagglutination inhibition (HI), complement fixation (CF), and ELISA. The VN test is specific, enabling the greatest differentiation between JEV and other flaviviruses. JEV, typically the Nakayama or JaGAr-01 strain, is propagated in cell culture using chicken embryo, Vero or BHK cells for the preparation of aliquots of virus-containing supernatant.

Hemagglutination inhibition is widely used, despite its cross-reactivity with other flaviviruses[45]. A four-fold difference in titer between serum samples from acute and convalescent phases is sufficient for diagnosis of flavivirus infection, though not necessarily JEV .A faster more efficient potential alternative to HI is a newly developed immunochromatographic test for the detection of anti-JEV IgG in pig serum.

For this test, domain III of the JEV E protein, known to be bound by anti-JEV antibodies, is expressed in soluble form in a bacterial expression system. This eliminates the need for erythrocytes and mouse brain-derived JEV antigen. Immunochromatographic assays have already been used for the detection of avian influenza, PRRSV, and PCV, as well as contagious diseases in humans.

Complement fixation is rarely used for diagnosis by combining test serum, antigen extracted from inoculated mouse brains with acetone, and pooled fresh guinea pig serum (complement). The highest test serum dilution where no hemolysis occurs is the titer for this particular test, and a four-fold change in titer is considered to be significant for diagnosis. The IgM capture-ELISA (MAC-ELISA) is commonly used[11]. IgM can be detected within two to three days and for up to three weeks post-infection in pigs. To distinguish between antibodies from natural infection and antibodies to inactivated vaccines, ELISA detection of antibody to the nonstructural NS1 protein of the virus is utilized.

The interpretation of serological results, especially in older pigs, must consider vaccination history and age; maternal antibody can persist for up to eight weeks. It is also important to note that there is some level of cross reactivity with other flaviviruses for all of the serological tests.

SAMPLES

REJECTION CRITERIA

1. Insufficient volume.
2. Specimens in leaky containers
3. Possibility of contamination

Infected tissues are preferred for virus isolation, as isolation from blood and cerebrospinal fluid is occasionally achieved[21]. Due to the uneven spread of viral pathogens among fetuses *in utero*, it is important to sample tissue from an adequate number of fetuses.

In general, four to six should be sampled to avoid missing a diagnosis.

The OIE recommends brain or spinal cord be sampled, though spleen, liver, or placental tissues from stillborns, neonates, or fetuses may also be acceptable RT-PCR can identify the virus in cerebrospinal fluid, sera, and tissue culture supernatants.

Recent multiplex PCR tests for detection of multiple diseases of swine have used tissue homogenates from lung, spleen, kidney, and lymph nodes of aborted fetuses or pigs displaying clinical signs of disease. The ability to detect low levels of the virus in blood samples of newly infected swine can be achieved with the real time RT-LAMP assay.

Antigen capture ELISA is able to detect JEV in cerebrospinal fluid, brain tissue, and mosquito homogenate, and detection of anti-JEV antibodies in serum, thoracic, or abdominal fluid of stillborn piglets is also considered diagnostic[32].

Classically, any serological tests should include paired samples to demonstrate a rise in titer from acute to convalescent phases of disease. The use of oral fluids as a diagnostic specimen has not been evaluated for JEV.

IMMUNITY

POST-EXPOSURE

Natural infection contributes to long lasting immunity in adult pigs and surviving piglets. The host immune system primarily targets the E protein, a viral envelope glycoprotein consisting of three structural domains.

VACCINES

Currently, complete control and eradication of JEV by vaccination is implausible. Vaccination of swine can decrease amplification of the virus; though, regular vaccination of piglets can be cost prohibitive and maternal antibodies can decrease effectiveness of the existing live-attenuated vaccine.

Existing live-attenuated swine and human vaccines are GIII-derived and elicit greater GIII strain-specific neutralizing antibodies.

The E protein of JEV is critical in neutralizing antibody responses and is also the basis for separation of JEV into different genotypes.

Some level of protection using current vaccines is still observed against GI strains, possibly aided by a T cell response induced by vaccination.

There is still no specific treatment for Japanese encephalitis, and alternative vaccination methods are being developed. In spite of their limitations, vaccines can help to prevent JEV infection in humans and animals.

Efforts have been made to develop bivalent and multivalent vaccines as a time and cost-saving measure.

An experimental recombinant pseudorabies virus (PRV) vaccine expressing the NS1 protein of JEV has been shown to induce long lasting antibody against JEV as well as cell-mediated immunity in piglets.

Pigs receiving simultaneous intradermal and oronasal vaccination with an experimental chimeric classical swine fever (CSF)-JEV virus replicon particle (VRP) exhibit more rapid anti-JEV IgG and neutralizing antibody responses than those vaccinated subcutaneously with a commercial inactivated vaccine.

Viremia is also undetectable or transient in challenge studies with the VRP vaccinated pigs. In its place is a gene expressing domain III of the antigenic JEV E protein.

Inactivated and live-attenuated vaccines are available in some countries for pregnant sows to increase chance of live births and for breeding boars to protect against reduction or deformity of sperm.

The live-attenuated vaccine has shown greater efficacy than the killed vaccine in both experimental and naturally occurring infection and an obtainable trivalent vaccine offers combined protection against JEV, PPV, and Getah virus (GETV).

Attributable to the genotypic shift from predominantly GIII to GI in large parts of Asia in the last decade, efficacy of some existing vaccines may need to be reevaluated.

In a study of weaned piglets receiving live-attenuated JEV vaccine protective against GIII, only limited cross-reactive protection against GI was observed. It is possible to increase the immunogenicity of existing vaccines with the use of novel adjuvants.

Specifically, an inactivated JEV GI vaccine containing the cytokine recombinant porcine granulocyte-monocyte colony stimulating factor (rGM-CSF) has been shown to augment humoral immunity in pigs.

Live-attenuated vaccines can be difficult and costly to produce and carry associated bio safety concerns.

Efforts are happening to develop stable cell lines capable of continuously expressing JEV virus-like particle (VLP) antigens that can effectively induce neutralizing antibodies to JEV.

One such cell line, BJ-ME, produced JEV VLPs able to induce high titers of neutralizing antibodies and complete protection in mice exposed to lethal JEV challenge.

Experimental DNA vaccines have also shown some ability to induce high antibody titers and have potential to be advantageous in swine production.

Proteins expressed from plasmids encoding the JEV *E* genes associate to form particles, similar to VLPs, effective in eliciting neutralizing antibody and cytotoxic T lymphocyte (CTL) responses in mice.

These same plasmids also elicit a higher neutralizing antibody response than the inactivated vaccine in swine, with antibodies persisting for up to eight months post-vaccination.

CROSS-PROTECTION

Cross-protective antibody responses have been induced experimentally in pigs exposed to closely related flaviviruses, suggesting the potential for prevention or inhibition of JEV infection in areas where multiple flaviviruses are endemic.

Though, the coexistence of JEV with MVEV in Papua New Guinea and WNV in Pakistan suggests that adequate numbers of susceptible hosts may exist to accommodate multiple viruses.

Of the three domains of the immunogenic E protein component of the JEV viral envelope, flavivirus cross-reactive epitopes are found predominantly in domain II.

Until now, neutralizing epitopes have been mapped in domain III, showing a high level of conservation between multiple strains of JEV and WNV, suggesting some level of cross-protection[43].

PREVENTION AND CONTROL

Successful decline of JEV in endemic areas has been attributed to large scale human immunization, immunization of pigs, separation of pigs from human communities, alterations in agricultural practices to reduce the presence of mosquitoes, and better overall standards of living.

Other examples of preventative measures include removal of stagnant water around pig enclosures, insecticides, insect screens, and use of fans inside buildings where pigs are housed to disrupt mosquito activity.

In pigs that do become infected, alpha interferon (IFN-α) shows promise as an antiviral treatment method able to experimentally increase expression of interferon-stimulated genes with strong anti-JEV effect.

There are recommendations for importation of horses from countries or zones infected with JEV, but no recommendations for importation of swine.

TABLE 2: INCIDENCE OF CASES AND DEATHS IN INDIA

s.no	states	2004 C/D	2005 C/D	2006 C/D	2007 C/D	2008 C/D	2009(P) C/D	2010(P) C/D
1	Andra	7/4	34/1	11/1	22/1	6/1	13/1	131/1
2	Assam	235/45	145/50	392/88	424/99	319/99	462/92	274/97
3	Bihar	85/25	190/62	21/3	336/164	204/45	325/95	19/0
4	Haryana	37/25	46/39	2/2	32/18	13/2	12/11	0/0
5	Karnataka	182/6	122/10	73/2	32/1	3/0	247/8	-
6	Kerala	9/1	0/0	3/3	1/0	2/0	3/0	19/0
7	Maharashtra	22/0	51/0	1/0	0/0	24/1	1/0	0/0
8	Manipur	0/0	1/1	0/0	66/1	4/0	6/0	111/6
9	Nagaland	0/0	1/1	1/1	7/0	0/0	9/2	11/5
10	Punjab	0/1/1	1/0	0/0	1/0	0/0	0/0	-
11	Uttarkanad	0/1	1/1	1/2	2/2	12/1	0/0	-
12	**Tamilnadu**	**88/9**	**51/11**	**18/11**	**37/1**	**144/1**	**266/7**	**242/3**

MATERIALS AND METHODS

APPROVAL OF THE ETHICAL COMMITTEE

This study was approved by the ethical committee of our institute and informed consent was obtained from all patients who are included in this study

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PERIOD OF STUDY

This study was conducted over a period of one year from May 2015 to April 2016.

PLACE OF STUDY

It was carried out in the Department of Microbiology, Thanjavur Medical College.

COLLABORATING DEPARTMENTS

This study was conducted in collaboration with Department of Medicine, Thanjavur Medical College Thanjavur and Department of Pediatrics, Government Raja Mirasudar Hospital Thanjavur.

DESIGN OF STUDY

It was cross sectional and comparative study.

MATERIALS

This study was included 100 patients. A total of 100 patients who were admitted Department of Medicine, Thanjavur Medical College and Department of Pediatrics, Government Raja Mirasudar Hospital Thanjavur were included in this study during the period from May 2015 to April 2016. CSF sample was collected from each patient with all age groups. Furthermore basic demographic characteristics such as age, sex, socio economic status and place were also obtained

METHODOLOGY:

This is cross sectional and comparative study that aims to analyze 100 cases of clinically diagnosed meningoencephalitis, admitted in thanjavur medical college hospital. CSF samples for cytological, biochemical analysis, antibody detection by IgM capture ELISA method, and molecular characterization of JE virus to be done by RT-PCR in serologically positive cases. .

INCLUSION CRITERIA:

Patients clinically diagnosed as acute encephalitis syndrome suspected Japanese encephalitis.

- Fever
- Headache
- seizure
- Vomiting
- Neck rigidity
- Hemiparesis

EXCLUSION CRITERIA:

- Already diagnosed positive JE cases
- Clinically diagnosed as non-infectious causes.
- TB Meningitis.
- Fungal meningitis
- Parasitic meningitis

CSF

SPECIMEN COLLECTION

LUMBAR PUNCTURE

To disinfect the puncture site, antiseptic solution and alcohol is used to clean the site. Gloves and mask was used when doing the procedure.

A needle with stylet is inserted at the L3-L4, inter space. While the subarachnoid space is reached, stylet is removed; spinal fluid appeared in the needle hub.

Measurement of the hydrostatic pressure with a manometer is taken. 2ml of CSF is collected into 3 sterile labeled tubes. Collected samples were immediately to the laboratory and processed.

VENTRICULAR SHUNT FLUID

Site is cleaned with antiseptic solution prior to removal of fluid and to prevent the introduction of infection. Cerebrospinal fluid is aspirated from the ventricular drain or shunt.

CSF is collected into a minimum of 3 sterile tubes. An initial CSF sample is collected before the administration of antibiotic therapy for diagnostic sensitivity.

Consequent CSF samples are collected every 2 to 3 days after antimicrobial therapy is started to monitor for resolution of the infection.

SPECIMEN TRANSPORT

Collected samples are labeled properly. Then labeled samples are transported to the laboratory immediately along with proper requestion.one sample is not refrigerated for bacterial culture processing and other two samples are refrigerated for cytological, biochemical analysis, antibody detection by serological method and for molecular study..

CSF PROCESSING

3 containers to be used

- One sample is used for bacterial identification, not to be refrigerated.
- One sample is used for viral identification, to be refrigerated**.**
- One sample is used for biochemical analysis and cell count analysis**.**

PERFORMANCE SPECIFICATIONS:

Neonates less than 28 days: E.coli, Group B streptococci

Less than two months: Group B streptococci .

Less than10 yrs: H. influenzae, pneumococci

More than 10yrs: *S.* pneumoniae,Neisseriameningitidis

Immunocompromised : Cryptococcus neoformans

CNS shunt infection : (CONS)

ON DAY I

APPEARANCE OF CSF

Clarity of CSF –noted.

Noted for blood.

Noted for clot.

CENTRIFUGE CSF

Centrifugation done for samples collected less than 1ml-vortex 30 seconds

Centrifugation done for samples collected more than 2ml-3000rpm for 20 min.

WET MOUNT EXAMINATION

Examined for pus cells, RBCs, bacteria and yeast cells.

India ink stain examination done to identify the capsulated organisms

GRAMS STAIN

1-2 drops of CSF is used for the smear preparation.

Dried the slide at free air

Fixed the smear with methanol or heat

Noted for pus cells, bacteria and yeast cell

CULTURE

Chocolate agar (CHOC),

Blood agar,

MacConkey agar

RCM broth were used .

Incubated at 37ºC for 48 hours; examined daily.

ON DAY II

CULTURE EXAMINATION:

Examined all plates and Medias for macroscopic evidence of growth at 24hours.

JEV DETECT IgM ANTIBODY CAPTURE ELISA (MAC_ELISA)

SUMMARY AND EXPLANATION OF THE TEST

Exposure to JEV causes a disease with a number of symptoms Including meningitis and encephalitis. JE Detect IgM Antibody Capture ELISA(MAC-ELISA) employs a recombinant antigen called JERA, which can be used as a rapid serological marker for JEV infection. The JERA protein is a recombinant antigen, which consists of a stretch of peptides from different parts of the JEV.

PRINCIPLE OF THE TEST

The JE Detect IgM Antibody Capture ELISA(MAC-ELISA) consists of Detect IgM Positive Control (represents reactive serum),and unknown serum samples are diluted with Sample Dilution Buffer, then incubated in micro titration wells which have been coated with anti-human IgM antibodies. This is followed by incubation with both JEV- derived recombinant antigen (JERA) AND Normal Cell Antigen(NCA) discretely. After incubation and washing ,the wells are treated with a JERA-specific antibody labeled with the enzyme horseradish peroxidase .After a third incubation and washing step, the wells are incubated with the(TMB)substrate. An acidic stopping solution is then added and the degree of enzymatic turnover of the substrate is determined by absorbance measurement at 450 nanometers. Above a certain threshold, the ratio of the absorbencies of the JERA and the control wells accurately determines whether antibodies to JEV are present.

PROCEDURE 1.Positive and Negative controls should be assayed in duplicate for both JERA and NCA portions of assay. Unknown serum samples to be tested can be assayed singly or in duplicate but must be

assayed for both JERA and NCA portions of assay. Up to forty-four specimens can be tested on one 96-well plate.

2. Mark the micro titration strips to be used.

3.Diluted test sera and controls to 1/100 using the provided Sample Dilution Buffer. Used small polypropylene tubes for these dilutions and atleast 4µL of sera and positive and negative control.

4.Applied the 50µL/well of 1/100 diluted test sera, JE Detect Negative Control, and JE Detect Negative Control to the plate by single or multi-channel pipettor as appropriate. An perfect arrangement is shown below. Cover the plate with parafilm or similar cover just on the well opening surface, so the bottom of the plates is not covered.

5.Incubated the plate at 37 C for 1 hour in an incubator.

Do not stack plates on top of each other. They should be spread out as a single layer. This is very important for even temperature distribution. Do not use CO2 or other gases. Do not place plates in contact with any wet substances such as wet paper towel .

CORRECT METHOD

6.After the incubation ,wash the plate 6 times with an automatic washer using Wash buffer. Use 300 µL per well in each wash cycle.

7.Added 50 µL /well of JERA into row A-D and 50µL/well of NCA into row E-H by multi-channel pipettor. A correct application for JERA and NCA shown below.

8.Covered the plate with the parafilm or imilar cover just on the well opening surface, so the bottom of the plate is not covered.

9.Incubated the plate at 37ºC for 1 hour in an incubator.

10.After the incubation, washed the plate 6 times with an automatic plate washer using 1LWash buffer. Used 300 µL per well in each wash cycle.

11.Added 50µL/well of ready to use Enzyme-HRP conjugate into all wells by multi channel pipettor.

12.Covered the plate with the parafilm or similar cover just on the well opening surface ,so the bottom of the plate should not be covered.

13.Incubated the plate at 37ºC for 1 hour in an incubator in darkness.

14.After the incubation, washed the plate 6 times with an automatic plate washer using 1µWash buffer. Use 300µL per well in each wash cycle.

15. Added 150 µL/well of EnWash into all wells by multichannel pipettor.

16.Incubated the plate at room temperature for 5 minutes without any cover on the plate.

17.After the incubation, washed the plate 6 times with an automatic plate washer using 1 Wash buffer. Use 300 L per well in each wash cycle.

18.Added 75 µL/well of Liquid TMB substrate into all wells by multichannel pipettor.

19.Incubated the plate at room temperature in a dark place (or container) for 10 minutes without any cover on the plate.

20.After the incubation, added 50µL/ well of Stop solution into all wells by multi channel pipettor.

22.Within 5 minutes, read the RAW OD 450 value with a Microplate reader.

CSF SAMPLE:

Undiluted CSF samples were used. CSF sample optimization was done to get the proper dilution factor. The rest of the process was the same as described for serum.

Necessary validation of CSF to be followed in laboratory before processing the unknown samples.

QUALITY CONTROL

Each kit contains positive and negative control sera to ensure assay performance. The negative and positive controls are intended to monitor for substantial reagent failure. The positive control will not ensure précising at the assay cut-off. The test is invalid and must be repeated if the ISR values of either of the controls do not meet the specifications. Acceptable Immune Status Ratio(ISR) values for these controls are found on the specification table below. If the test is invalid, patient results cannot be reported.

CALCULATIONS FOR UNKNOWN SAMPLE ANALYSIS

CALCULATION OF THE IMMUNE STATUS RATIO(ISR):

Calculated the average JERA and NCA OD values for each of the controls. Test samples ran in singlet and obtained the individual JERA and NCA values.

Determined the Immune Status Ratio(ISR) value for the controls and all test sera by dividing the JERA OD /NCA OD .

The assay performance is deemed valid when the ISR for the positive control is greater than6.0, and the ISR for the negative control is less than 2.8.

SELECTION OF THE CUT-OFF:

The cut-off was selected using values from a small set of field data and is an estimate only.

Negative control JERA/NCA ratio more than 2.8 shows the test procedure is invalid

Positive control JERA/NCA ratio less than 5.0 shows the test procedure is invalid

TABLE: 3 ELISA INTERPRETATIONS

ISR	RESULTS	INTERPRETATION
Less than 4	Negative	No detectable antibody by the test
4-6	Equivocal	Need confirmatory test
Above6	Positive	Indicates the presence of detectable antibodies

REAL-TIME PCR DETECTION OF JEV

MATERIAL & METHODS:

PureFast® Viral nucleic acid mini spin purification kit [Kit contains Proteinase-K, Lysis
buffer, Wash Buffer-1, Wash Buffer-2, Spin columns with collection tube and elution buffer.

HELINI JEV Real-time PCR kit is from HELINI Biomolecules, Chennai, India.

REAL-TIME PCR MODEL: AGILENT MX3000P, USA

HELINI Dengue virus [Universal] Real-time PCR kit components:

RT-PCR Probe PCR Master Mix

RT-Enzyme mix

JEV Primer Probe Mix

Internal control Primer Probe Mix

Internal control template

Positive control

Instruction manual

VIRAL RNA PURIFICATION

1. 200µl of Plasma/serum transferred into fresh 1.5ml centrifuge tube.
2. 200µl Viral lysis buffer added.
3. Mixed well by vortex
4. Add 20µl of Proteinase K and 5µl of internal control template, Mixed well by inverting several times.
5. Incubate at 56ºC for 15min.
6. Added 220µl of Ethanol and mixed well.
7. Transferred entire sample into the Purefast® spin column. Centrifuged at 10000rpm for 1min. Discard the flow-through and place the column back into the same collection tube.

8. Added 500µl Wash buffer-1 to the Purefast® spin column. Centrifuge at 10000rpm for 1min and discard the flow-through. Place the column back into the same collection tube.
9. Added 500µl Wash buffer-2 to the Purefast® spin column. Centrifuge a 10000rpm for 1min and discard the flow-through. Place the column back into the same collection tube.
10. Discard the flow-through and centrifuge for an additional 1 min. This step is essential to avoid residual ethanol.
11. Transferred the PureFast® spin column into a fresh 1.5 ml micro-centrifuge tube.
12. Added 60µl of Elution Buffer to the center of Purefast® spin column membrane.
13. Incubate for 1 min at room temperature and centrifuge for 2 min.
14. Discard the column and store the purified DNA at -20°C. 10µl of elute used in real-time

PCR PROCEDURE:

DETECTION MIX

COMPONENTS VOLUME

Components	**volume**
RT Probe PCR mastermix	8µl
RT enzyme mix	2µl
JEV Primer Probe Mix	2.5µl
Internal control Primer Probe Mix [IC PP Mix	2.5µl
Purified Viral RNA sample	10µl
Total reaction volume	25µl

Centrifuged PCR vials briefly before placing into thermal cycler.

NEGATIVE CONTROL SETUP

Included 10µl of nuclease free.

POSITIVE CONTROL SETUP

Included 10µl of Positive control.

TABLE: 4 AMPLIFICATION PROTOCOL

	Step	Time	Temp
45 cycles	Reverse transcriptase	30sec	42° c
	Taq enzyme activation	15sec	95° c
	Denaturation	20sec	95°c
	Annealing/Data collection	20sec	56°c
	Extension	20sec	72°c

JEV = FAM channel **Internal Control** = JOE/HEX/VIC/Cy3 Channel

ELISA RESULTS

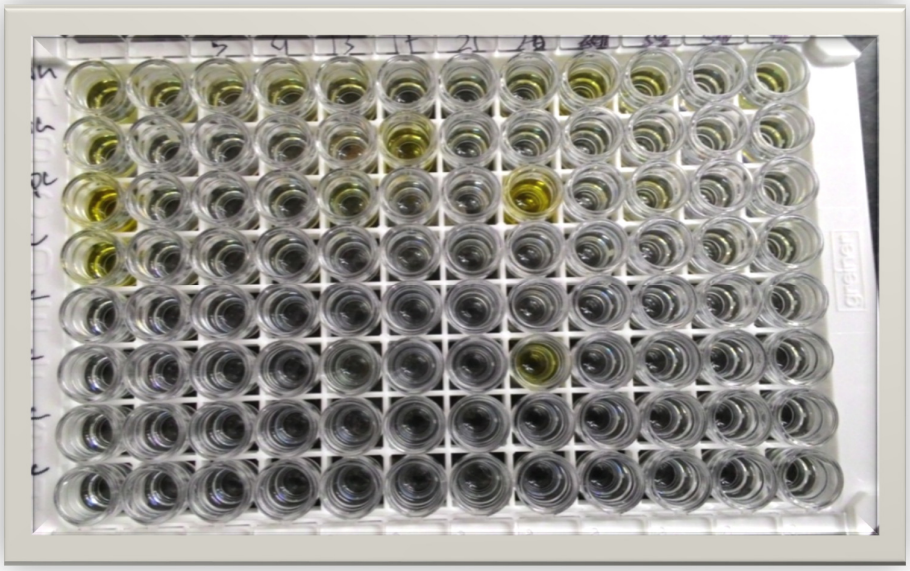
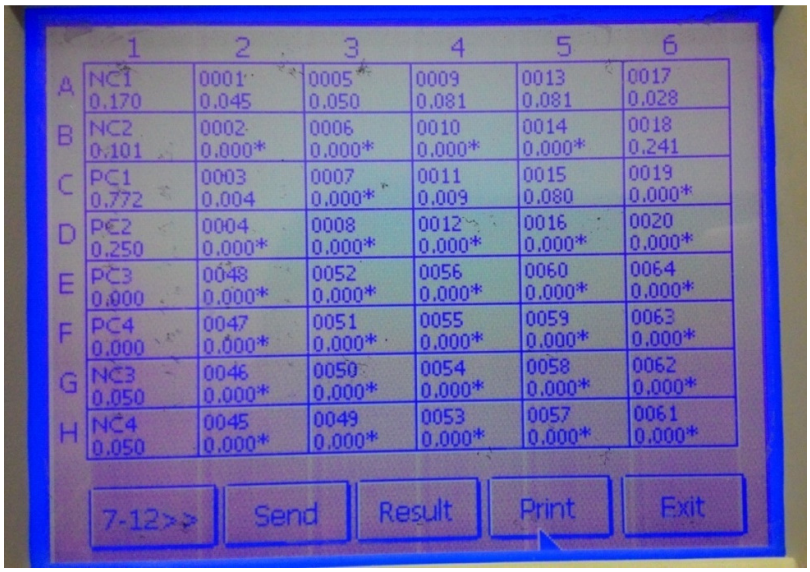


FIG: 6 ELISA KIT



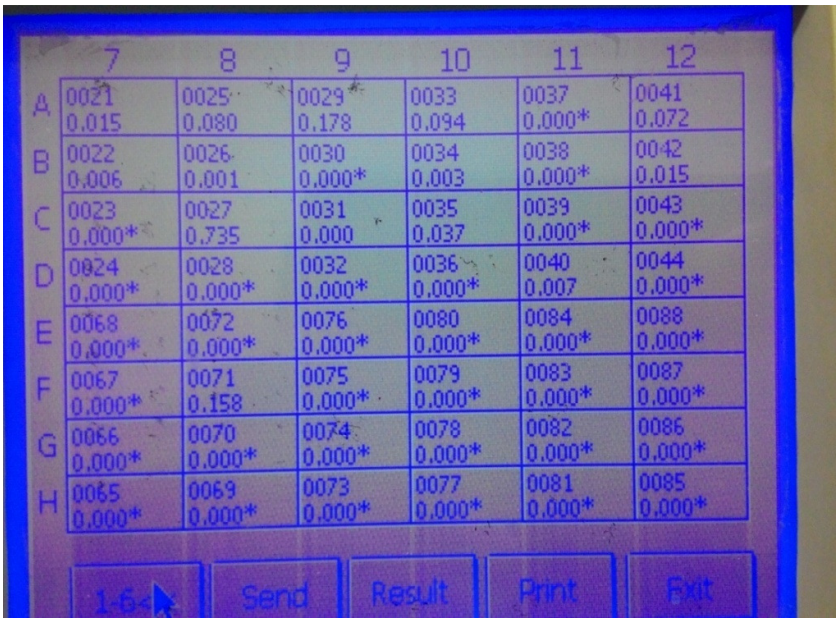


FIG 7: ELISA INTERPRETATION

CALCULATION OF ISR VALUE

NEGATIVE CONTROL

ANTIGEN	JERA	NCA
NO 1	0.170	0.50
NO2	0.101	0.50
TOTAL	0.271	0.100

AVERAGES FOR JERA = 0.271/2 =0.135

AVERAGE FOR NCA=0.100/2=0.50

JERA/NCA RATIO=0.135/0.50=0.27

CALCULATION OF ISR VALUE

POSITIVE CONTROL

ANTIGEN	JERA	NCA
NO 1	0.772	0.100
NO2	0.350	01.00
TOTAL	1.222	0.200

AVERAGES FOR JERA = 1.222/2 =0661

AVERAGE FOR NCA=0.200/2=0.100

JERA/NCA RATIO=0.661/0.100=6.61

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PCR RESULTS

MxPro - Mx3000P

Multiplex Quantitative PCR Systems
Quantitative PCR - Consolidated Report
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Filter gain factors: CY5 x1 ROX x1 HEX-JOE x1 FAM x8
Run date: September 19, 2016

Replicates: Treated individually (since no replicates in selection)

Plate Setup

	1	2	3	4	5	6	7	8	9	10	11	12
A			JEV NTC FAM		Dengue NT FAM							
B			1 FAM		1 FAM							
C			2 FAM		3 FAM							
D			3 FAM		5 FAM							
E			4 FAM		7 FAM							
F			5 FAM		9 FAM							
G			Positive co FAM		Positive co FAM							
H												

MxPro - Mx3000P

Multiplex Quantitative PCR Systems
Quantitative PCR - Consolidated Report
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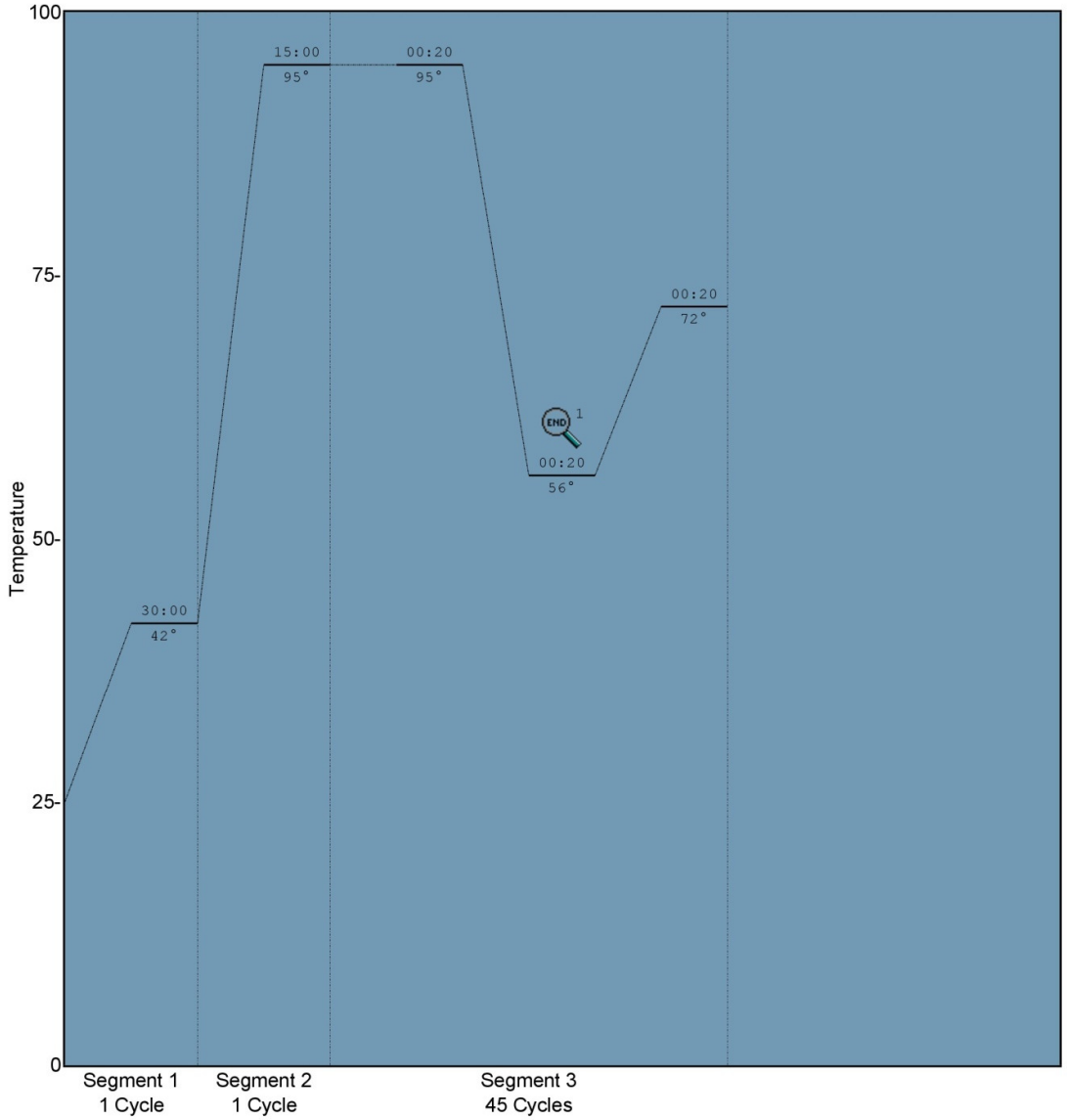
Filter gain factors: CY5 x1 ROX x1 HEX-JOE x1 FAM x8
Run date: September 19, 2016

Thermal Profile
(Estimated Run Time: 02:09:32)



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MxPro - Mx3000P

Multiplex Quantitative PCR Systems
Quantitative PCR - Consolidated Report
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Filter gain factors: CY5 x1 ROX x1 HEX-JOE x1 FAM x8
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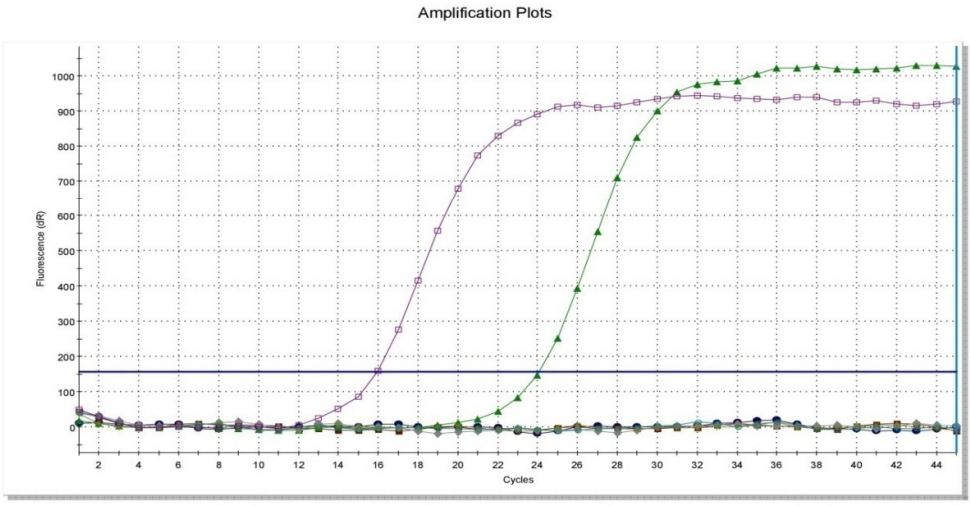


Amplification plot of RT PCR genotyping of JEV. Cycle threshold for 1 sample were detected as sample-1 belongs to JEV genotype – 3.

MxPro - Mx3000P

Multiplex Quantitative PCR Systems
Quantitative PCR - Text report
C:\HELIN\\2016\Customs service\JEV-Dengue-Madurai and Thanjavur\19-09-2016.mxp
Filter gain factors: CY5 x1 ROX x1 HEX-JOE x1 FAM x8
Run date: September 19, 2016

Thermal Profile Summary

Segment	Cycles	Plateau	Temp. (degrees)	Temp. Inc. (deg/sec)	Duration (min:sec)	Time Inc. (min:sec)	Collect
1	1	Plateau 1	42.0	0.0	30:00	00:00	<none>
2	1	Plateau 1	95.0	0.0	15:00	00:00	<none>
3	45	Plateau 1	95.0	0.0	00:20	00:00	<none>
3	45	Plateau 2	56.0	0.0	00:20	00:00	1 Endpoints
3	45	Plateau 3	72.0	0.0	00:20	00:00	<none>

Replicates: Treated individually (since no replicates in selection)
* Fluorescence term used: dR

Text Report

Well	Well Name	Dye	Well Type	Threshold*	Ct*
A3	JEV NTC	FAM	NTC	157.771	No Ct
B3	1	FAM	Unknown	157.771	No Ct
C3	2	FAM	Unknown	157.771	24.12
D3	3	FAM	Unknown	157.771	No Ct
E3	4	FAM	Unknown	157.771	No Ct
F3	5	FAM	Unknown	157.771	No Ct
G3	Positive control-QS1	FAM	Unknown	157.771	15.99

Interpretation

Sample 1-JEV genotype 3 identified in FAM channel

RESULTS AND INTERPRETATION

During the study period from June 2015-June 2016 a total of 100 CSF samples were collected and tested for JE IgM antibody at central service diagnostic laboratory, Departmentment of microbiology, Tertiary care hospital, Thanjavur medical college, Thanjavur.

TABLE: 5 AGE WISE DISTRIBUTION

AGE(years)	NO.OF CASES	PERCENTAGE (%)
<20 yrs	42	42%
21-30 yrs	5	5%
31-40yrs	8	8%
41-50 yrs	12	12%
51-60 yrs	18	18%
61-70 yrs	9	9%
71 &ABOVE	6	6%
TOTAL	100	100%

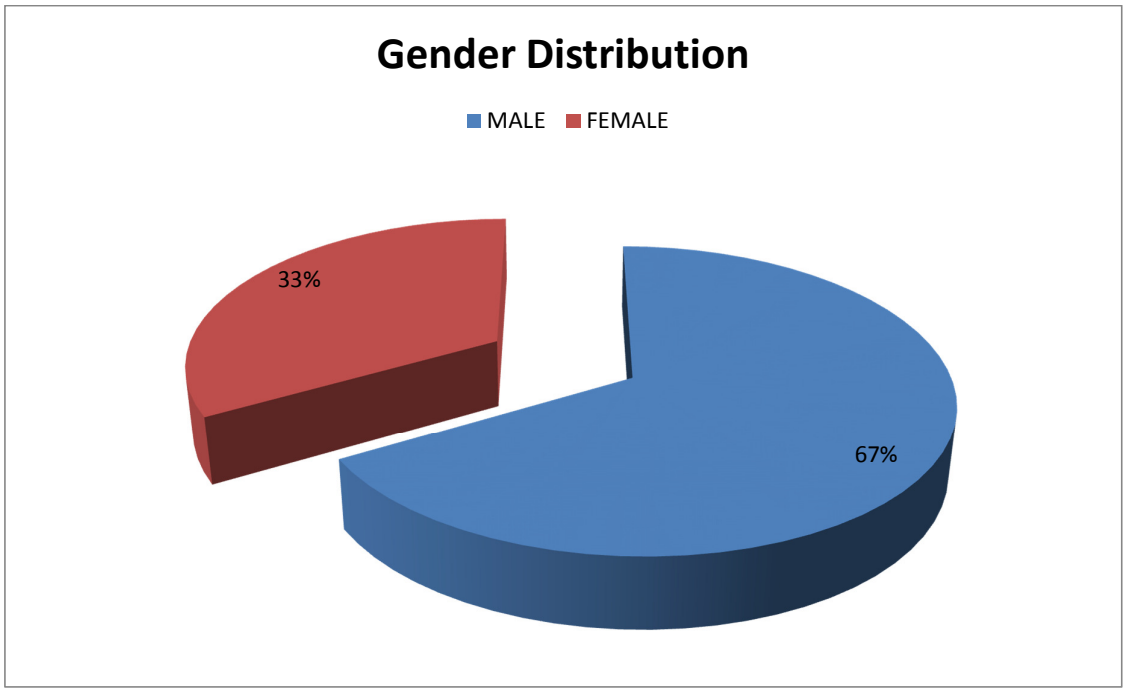
Table 5 shows the age wise distribution of cases. A total of 100 CSF samples were tested for JE IgM.

TABLE 6: GENDER DISTRIBUTION OF SUSPECTED CASES

GENDER	< 20	>20	Total	Percentage
MALE	4	50	**54**	**72 %**
FEMALE	2	19	**21**	**28 %**

Out of 100 samples 54 samples collected from male patients and 21 samples collected from female patients.

In this study male population contributes 72%, female population contributes 28%.



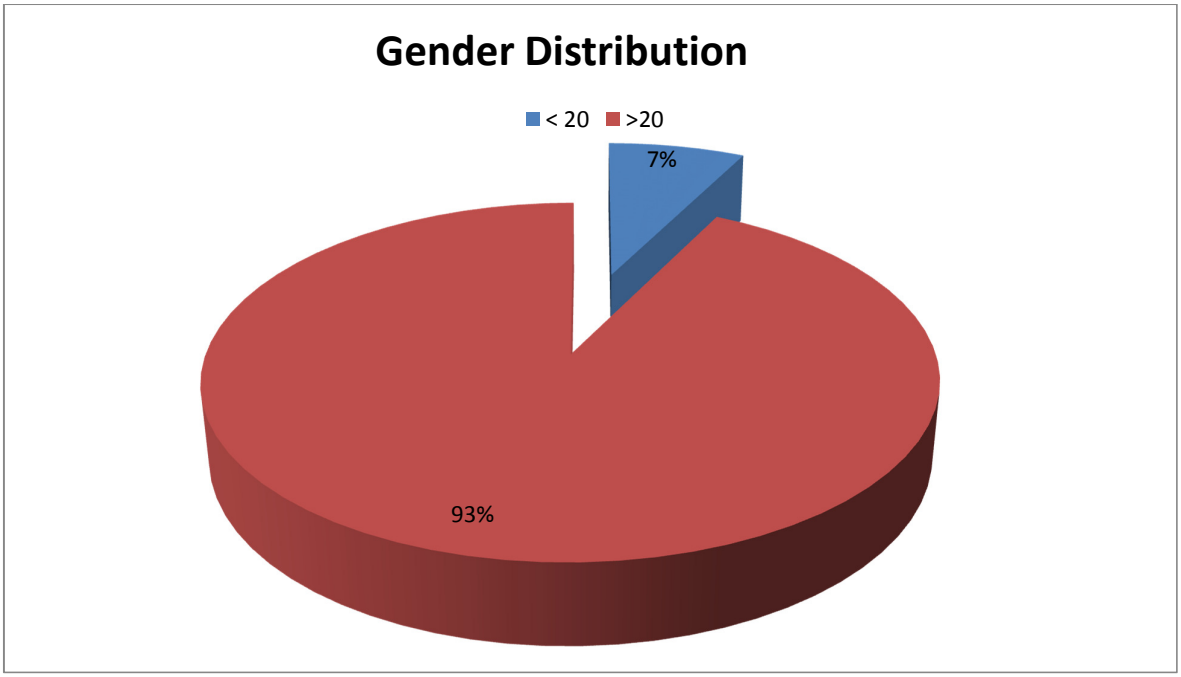


FIG 8: GENDER DISTRIBUTION

TABLE 7: MALE AND FEMALE CHILD DISTRIBUTION

GENDER	<2	>2	Total
MALE CHILD	11	4	15
FEMALE CHILD	5	5	10

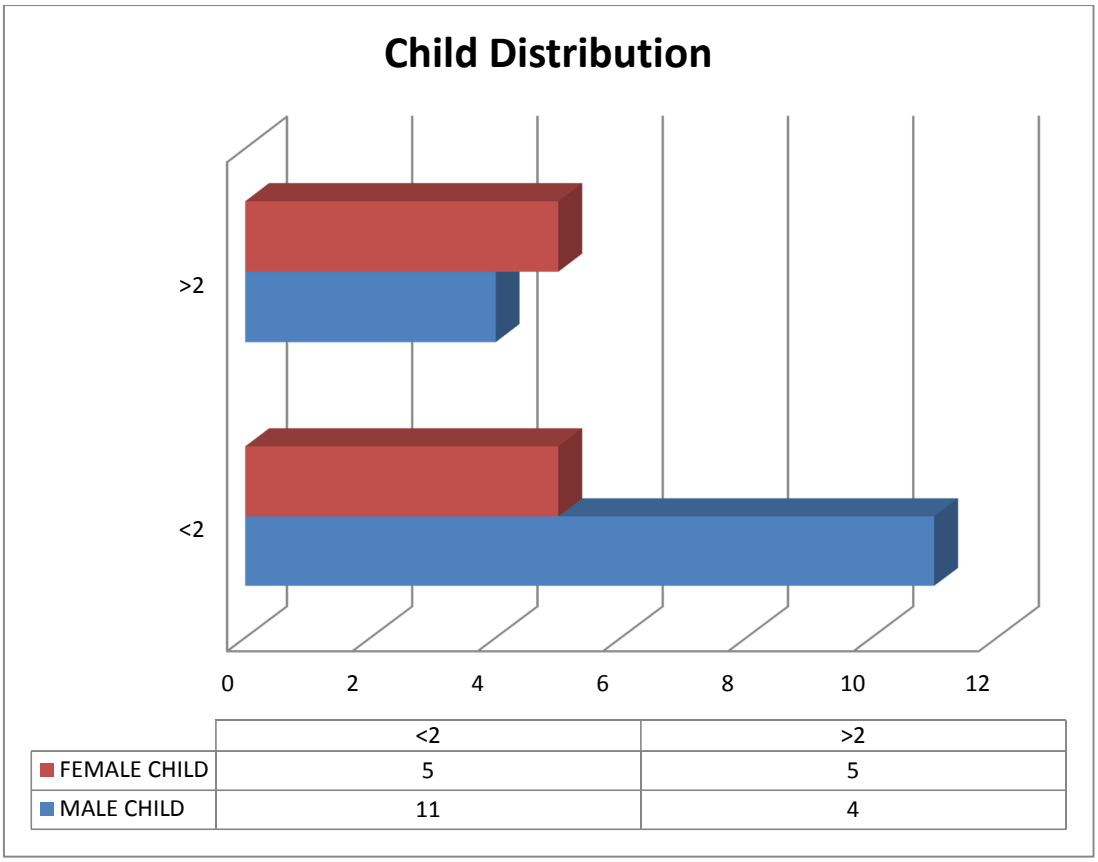


FIG 9: CHILD DISTRIBUTION

TABLE 8: JEV IGM POSITIVE CASES BY ELISA

RESULT	TOTAL CASES	PERCENTAGE (%)
POSITIVE	1	1%
NEGATIVE	99	99%

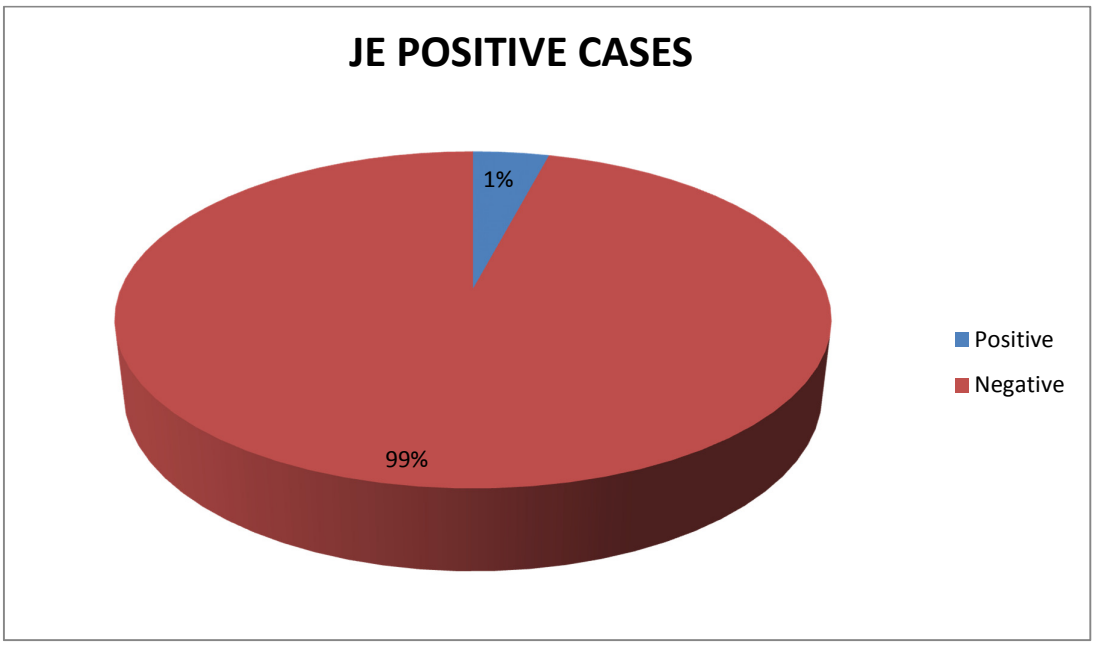
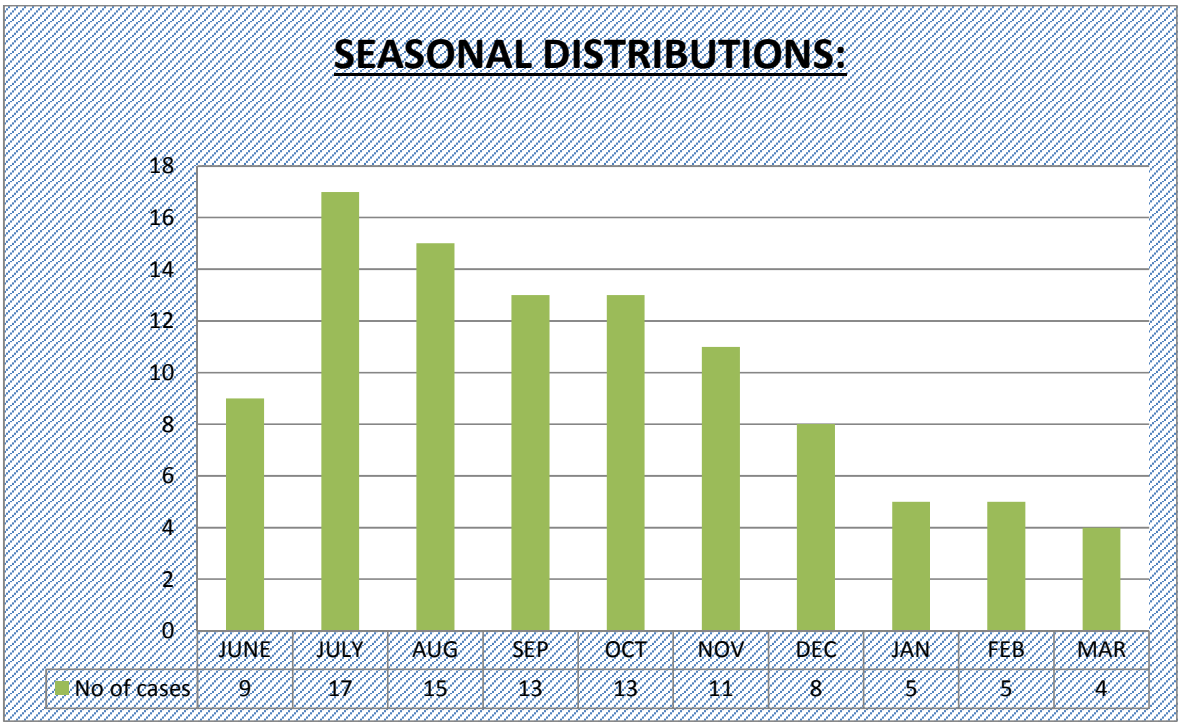


FIG: 10 JEV POSITIVE AND NEGATIVE PERCENTAGE

TABLE 9: SEASONAL DISTRIBUTIONS

MONTHS	NO OF CASES	PERCENTAGE
JUNE	9	9 %
JULY	17	17 %
AUGUST	15	15 %
SEPTEMBER	13	13 %
OCTOBER	13	13 %
NOVEMBER	11	11 %
DECEMBER	8	8 %
JANUARY	5	5 %
FEBRUARY	5	5 %
MARCH	4	4%
Total	**100**	**100**

In this study out of 100 samples maximum number of sampled were collected on July and lowest samples were collected from March.



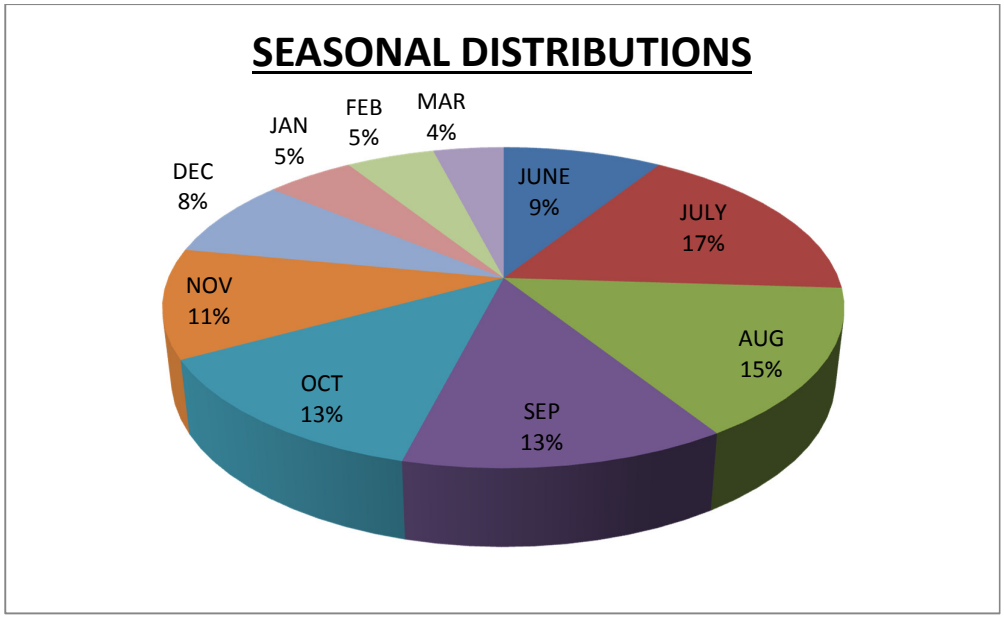


FIG 11: SEASONAL DISSTRIBUTION

TABLE 10: CLINICAL FEATURES STUDIES

Disease	No of Cases	Percentage
Fever	71	71 %
Headache	12	12 %
Seizure	8	8 %
Vomiting	4	4 %
Neck Rigidity	4	4 %
Hemiparesis Fever	1	1 %
Total	**100**	**100**

In this study out of 100 patients, 71 patients came with fever, 12 patients with head ache, 8 patient with seizure, 4 patients with vomiting, 4patients with neck rigidity and 1patient with hemiparesis .

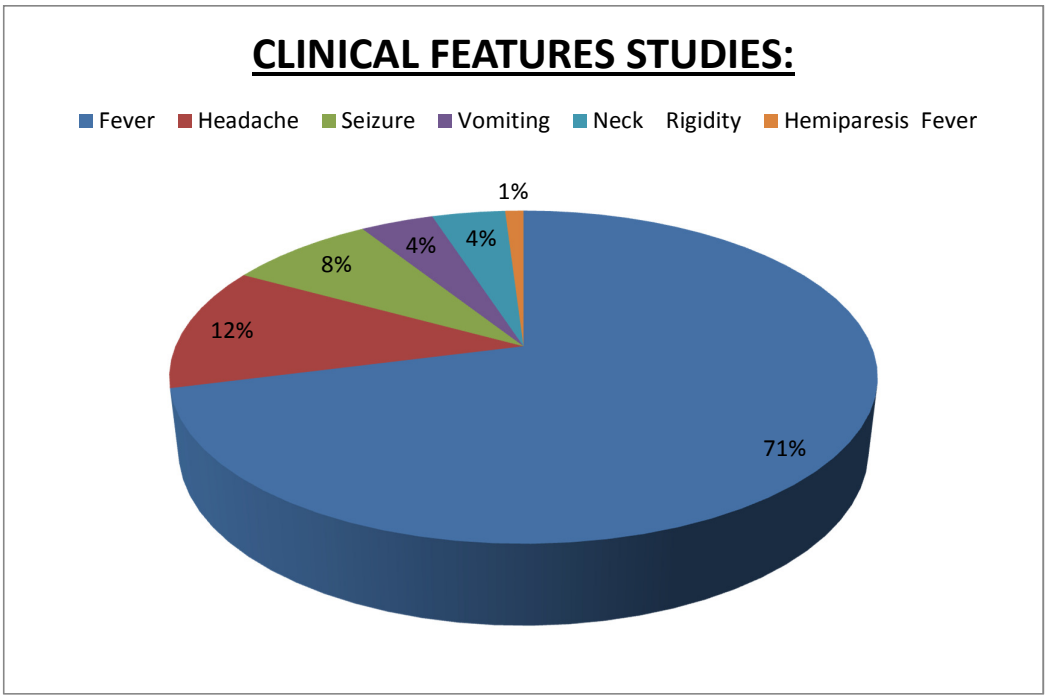


FIG 12: CLINICAL FEATURE DISTRIBUTION

TABLE10: CLINICAL FEATURES WITH AGE

Disease	< 20	>20
Fever	**33**	**38**
Yes	28	30
No	5	8
Headache	**1**	**11**
Yes	**1**	6
No	0	5
Seizure	**8**	**0**
Yes	**7**	0
No	1	0
Vomiting	**0**	**4**
Yes	**0**	2
No	0	2
Neck Rigidity	**0**	**4**
Yes	**0**	3
No	0	1
Hemiparesis Fever	**0**	**1**
Yes	**0**	1
No	0	0
Total	**100**	

In this study age is compared with clinical features

Less than 20 age groups and more than 20 age groups shows fever the main clinical symptom**.**

TABLE 11: REAL TIME PCR AND ELISA

METHOD	POSITIVE	NEGATIVE	TOTAL
ELISA	1	99	100
Real-time PCR	1	99	100

Table11 shows that out of 100 samples,1 sample (1%) was positive for JEV RNA and 99 samples were negative (99%) for JEV RNA by Real- time PCR assay. Out of 100 samples, 1(1%) sample was positive both by ELISA and Real- time PCR assay.

DISCUSION

The thriving supervision of patients suffering from illness depends upon the identification of the type of organisms that cause the diseases. Consequently data offered in this study could endow with information of immediate public health importance to physicians in Thanjavur Medical College hospital for the management of patients suffering from meningoencephalitis. Laboratory investigation of CSF or serum specimens in suspected cases of meningoencephalitis are important for prompt diagnosis and management of patients

100 CSF samples collected from patients suspected meningoencephalitis processed for CSF biochemical analysis, cell count analysis, IgM capture ELISA for JEV and molecular characterization by RT-PCR for JEV ELISA positive sample

- **Greenlee et al**[1] explained about the CSF changes in the meningitis as well as in encephalitis that elevation of protein level in CSF correlates with this study.

- Biochemical analysis shows increased protein levels in 7 samples. Sugar level was elevated in 2 samples.

- Out of 100 samples 7 sample shows abnormal results which is 7%, among the 7 samples 5 samples shows increased protein levels and other two samples shows increased sugar level.

- Out of 7 samples 1 sample exhibit bacterial culture positive remaining 6 shows negative, so there is definite correlation between the CSF sugar and protein elevation and bacterial culture positivity
    - Cell count analysis was observed.
    - 6 samples shows abnormal results which accounts for 6%.
    - **Kida S, et al[4]** .explains the CSF cell count analysis.

In this study among the 6 samples which shown abnormal cell count, out of this 6 samples

- 2 to 3 neutrophils were observed in 2 samples.
- 4 to 5 lymphocytes were noted in 4 samples.
- Single neutrophil in the CSF sample is pathogenic.
- In this study, age wise distribution exhibit most number of suspected cases from the age group of less than 20 which accounts for 43%.5% from the group of age 20 to 30,7% from the group of age 30 to 40,12% from the group of age 40 to 50,18% from the group age 50 to 60,9%from the group of age 60 to 70,6% from above 70.
- Male female distribution exhibits the male dominance which is 72% when compared to female which is 28%.
- Fever is the most common presentation of suspected cases which accounts for 71%, headache accounts for 12% and seizure for 8%.
- Seasonal distribution exhibit most number of suspected cases come from July then October and November.
- Out of 100 suspected cases, positive from march month which is contrary to the previous statement.

- Negative samples may be due to other infections like bacterial, fungal, and other viral meningoencephalitis.
- Diagana M[11], Preux PM et al studied about the sero positivity of test IgM ELISA.
- IgM capture ELISA was done for 100 CSF samples, out of 100 samples 1 sample exhibit positive which accounts for 1%.
- This sample is compared with cell count and sugar protein level of CSF,
- 9 lymphocytes were observed in this sample as well as raised protein level, sugar level observed in normal limits.
- This explains about the infection of the central nervous system strongly associated with the elevated levels of protein and the cell count.
- **Gourie-Devi M, Ravi V et al[18]**, explains the demographic profile as well as the seasonal distribution of cases shows the positive IgM ELISA test with cytological profile**.**
- CSF positive for JEV in ELISA sample is processed for RT-PCR, for genome identification as well as to access the reliability of the IgM capture ELISA
- IgM positive sample shows positivity in PCR also, and the identified gene is E.
- There are 5 genotypes have been identified till now, genotype 1 and 3 were more common in India.
- In this study genotype 3 is identified which is more commonly identified in India previously.
- In our country like india, cost effective diagnostic method is necessary.
- Standard test like PCR and cell culture methods can not be done for all suspected cases.

- Cell culture method is time consuming and costly for our setup.
- PCR is costly, well trained staffs are needed to initiate the procedure.
- So we are in need of cost effective diagnostic method and it should be very reliable when compared to the standard tests like PCR and cell culture.
- In this study, 100 samples are tested for IgM ELISA , out of 100 samples one sample shows positive which is one percentage.
- This positive sample was sent to the PCR study where they identified the E gene which is more specific for Japanese encephalitis. In my study results of IgM ELISA is concurrent with PCR.
- In our setup, IgM ELISA can be used for the diagnosis of JEV in place of PCR .
- IgM ELISA along with biochemical analysis and cell count can be used in place of PCR for earlier diagnosis and management.

SUMMARY

- The study was conducted at Thanjavur Medical College Hospital. Thanjavur over a period of June 2015 to June 2016.
- The aim of the study is to determine the seroprevalence Japanese encephalitis virus in patients attending tertiary care hospital and molecular characterization for JEV.
- CSF samples were collected from 100 suspected meningoencephalitis patients.
- CSF is examined macroscopically for clarity and microscopically for pus cells, bacteria and yeast cell .
- Then all the samples were analyzed for cell count and biochemical parameters.
- Then samples were tested for IgM antibodies by capture ELISA method. Finally IgM positive samples were processed to identify the molecular characterization by RT-PCR method..
- Real time PCR was done for 6 samples, including both one positive and 5 negative by IgM ELISA for JEV.
- Out of 6 samples, 1 sample shown positive by RT-PCR, which was already noted positive by ELISA.
- Prevalence of JEV in thanjavur 1%.
- High prevalence was seen among children in the age group 5-10 years.
- Real –time PCR for JEV was positive for one sample (100%) which is shown positive by Elisa.
- IgM antibody measurement in CSF sample by ELISA along with cytological and biochemical analysis is as good as RT-PCR in the early diagnosis

CONCLUSION

This study estimates the seroprevalence of Japanese encephalitis in a tertiary care hospital Thanjavur. The seroprevalence of Japanese encephalitis was estimated as 1% .The prevalence of JEV was high among children in the age group of 5 to 15.In this study the observed prevalence of 1% is coincided with the actual prevalence in thanjavur.This study estimates the magnitude and dynamics of disease transmission .Isolation of virus by cell culture method and Real –time PCR are the standard diagnostic methods. RT-PCR estimates the viral load and can identify the genotyping, because both viral load and genotyping plays an important role in management strategy. Moreover knowing about the genotypes in the community helps in the development of future vaccine. Further studies of genotype distribution will helps in the development, adaptation and prevention strategies RT-PCR and isolation is very difficult because of its cost. At this fact IgM antibody measurement in CSF sample by ELISA along with cytological and biochemical analysis is as good as RT-PCR in the early diagnosis. Early diagnosis prevents the disease progression and further complications.

BIBLIOGRAPHY

1. Greenlee, BURKE D.S., HISALAK A. & USSERY M.A. (1982). Japanese encephalitis. *In:* Proceedings of International Seminar on Viral Diseases in SE Asia and the Western Pacific, Mackenzie J.S., ed. Academic Press, Sydney, Australia, 537–540.
2. CHUNG Y.J., NAM J.H., BAN S.J. & CHO H.W. (1996). Antigenic and genetic analysis of Japanese encephalitis viruses isolated from Korea. *Am. J. Trop. Me. Hyg.,* **55**, 91–97.
3. CLARKE D.H. & CASALS I. (1958). Techniques for haemagglutination with arthropod-borne viruses. *Am. J. Trop. Med. Hyg.,* **7**, 561–573.
4. Kida S FENNER F.J., GIBBS E.P.J., MURPHY F.A., ROTT R., STUDDERT M.J. & WHITE D.O. (1992). Flaviviridae. *In:* Veterinary Virology, Second Edition. Academic Press, New York, USA, 441–455.
5. HALE J. H. & LEE L.H. (1954). A serological investigation of six encephalitis viruses isolated in Malaya. *Br. J. Exp.Pathol.,* **35**, 426–433.
6. HASEGAWA H., YOSHIDA M., FUJITA S. & KOBAYASHI Y. (1994). Comparison of structural proteins among antigenically different Japanese encephalitis virus strains. *Vaccine,* **12**, 841–844.
7. HOKE C.H. JR & GINGRICH J.B. (1994). Japanese encephalitis. *In:* Handbook of Zoonoses, Second Edition, Beran G.W., ed. CRC Press, Boca Raton, Florida, USA, 59–69.
8. HORI H., MORITA K. & IGARASHI A. (1986). Oligonucleotide fingerprint analysis on Japanese encephalitis virus strains isolated in Japan and Thailand. *Acta Virol.,* **30**, 353–359.
9. JAN L.R., YUEH Y.Y., WU Y.C., HORNG C.B., & WANG G.R. (2000). Genetic variation of Japanese encephalitis virus in Taiwan. *Am. J. Trop. Me. Hyg.,* **62**, 446–452.
10. KIMURA-KURODA J. & YASUI K. (1986). Antigenic comparison of envelop protein E between Japanese encephalitis virus and some other flaviviruses using monoclonal antibodies. *J. Gen. Virol.*, **67**, 2663–2672.
11. KONISHI E., SHODA M, AJIRO N & KONDO T. (2004). Development and evaluation of an enzyme-linked immunosorbent assay for quantifying antibodies to Japanese

encephalitis virus nonstructural 1 protein to detect subclinical infections in vaccinated horses. *J. Clin. Microbiol*., **42**, 5087–5093.

12. LIAN W.C., LIAU M.Y. & MAO C.L. (2002). Diagnosis and genetic analysis of Japanese encephalitis virus infected in horses. *J. Vet. Med. B Infect. Dis. Vet. Public Health*, **49**, 361–365.
13. MACKENZIE J.S. (2005). Emerging zoonotic encephalitis viruses: lessons from Southeast Asia and Australia. *J. Neurovirol.*, **11**, 434–440.
14. PARIDA M. M., SANTHOSH S. R., DASH P. K., TRIPATHI N. K., SAXENA P., AMBUJ K. SAHNI A. K., LAKSHMANA RAO P. V. & MORITA K. (2006). Development and evaluation of reverse transcription loop-mediated isothermal amplification assay for rapid and real-time detection of Japanese encephalitis virus. *J. Clin. MIcrobiol*., 4172–4178.
15. SOLOMON T., NI H., BEASLEY D.W.C., EKKELENKAMP M., CARDOSA M.J. & Barrett A.D.T. (2003). Origin and evolution of Japanese encephalitis virus in Southeast Asia. *J. Virol.,* **77**, 3091–3098.
16. TANAKA M. (1993). Rapid identification of flavivirus using the polymerase chain reaction. *J. Virol. Methods,* 311–322. Phylogenetic analysis of Japanese encephalitis virus: envelope gene based analysis reveals a fifth genotype, geographic clustering, and multiple introductions of the virus into the Indian subcontinent. *Am. J. Trop. Med. Hyg*. 242–251.
17. WILLIAMS D.T., WANG L.F. DANIELS P.D. & MACKENZIE J.S. (2000). Molecular characterization of the first Australian isolate of Japanese encephalitis virus, the FU strain. *J. Gen. Virol.,* **65**, 2471–2480.
18. Gourie-Devi M, Ravi V XINGLIN J., HUANCHUN C., QIGAI H., XIANG W., BIN W., DEXIN Q. & LIURONG F. (2002) The development and application of the latex agglutination test to detect serum antibodies against Japanese encephalitis virus. *Vet.* [1] A.F. van den Hurk, S.A. Ritchie, J.S. Mackenzie, Ecology and geographical expansion of Japanese encephalitis virus, Annu. Rev. Entomol. 54 (2009) 17e35.
19. S.H. Karunaratne, J. Hemingway, Insecticide resistance spectra and resistance mechanisms in populations of Japanese encephalitis vector mosquitoes, Culex tritaeniorhynchus and Cx. gelidus, in Sri Lanka, Med. Vet. Entomol. 14 (2000) 430e436.

20. V. Thenmozhi, R. Rajendran, K. Ayanar, R. Manavalan, B.K. Tyagi, Long-term study of Japanese encephalitis virus infection in Anopheles subpictus in Cuddalore district, Tamil Nadu, South India, Trop. Med. Int. Health 11 (2006) 288e293.
21. T. Solomon, N.M. Dung, R. Kneen, M. Gainsborough, D.W. Vaughn, V. T. Khanh, Japanese encephalitis, J. Neurol. Neurosurg. Psychiatry 68 (2000) 405e415.
22. T. Solomon, H. Ni, D.W. Beasley, M. Ekkelenkamp, M.J. Cardosa, A.D. Barrett, Origin and evolution of Japanese encephalitis virus in southeast Asia, J. Virol. 77 (2003) 3091e3098.
23. N. Nitatpattana, A. Dubot-Peres, M.A. Gouilh, M. Souris, P. Barbazan, S. Yoksan, X. de Lamballerie, J.P. Gonzalez, Change in Japanese encephalitis virus distribution, Thailand, Emerg. Infect. Dis. 14 (2008) 1762e1765.
24. P.D. Uchil, V. Satchidanandam, Phylogenetic analysis of Japanese encephalitis virus: envelope gene based analysis reveals a fifth genotype, geographic clustering, and multiple introductions of the virus into the Indian subcontinent, Am. J. Trop. Med. Hyg. 65 (2001) 242e251.
25. H. Hasegawa, M. Yoshida, Y. Kobayashi, S. Fujita, Antigenic analysis of Japanese encephalitis viruses in Asia by using monoclonal antibodies, Vaccine 13 (1995) 1713e1721.
26. M. Saito, K. Taira, K. Itokazu, N. Mori, Recent change of the antigenicity and genotype of Japanese encephalitis viruses distributed on Okinawa Island, Japan, Am. J. Trop. Med. Hyg. 77 (2007) 737e746.
27. S. Mukhopadhyay, R.J. Kuhn, M.G. Rossmann, A structural perspective of the flavivirus life cycle, Nat. Rev. Microbiol. 3 (2005) 13e22.
28. D. Ding, P.E. Kilgore, J.D. Clemens, L. Wei, X. Zhi-Yi, Cost-effectiveness of routine immunization to control Japanese encephalitis in Shanghai, China, Bull. World Health Organ 81 (2003) 334e342.
29. C.L. Liao, Y.L. Lin, S.C. Shen, J.Y. Shen, H.L. Su, Y.L. Huang, S.H. Ma, Y.C. Sun, K.P. Chen, L.K. Chen, Antiapoptotic but not antiviral function of human bcl-2 assists establishment of Japanese encephalitis virus persistence in cultured cells, J. Virol. 72 (1998) 9844e9854.
30. S.K. Unni et al. / Microbes and Infection 13 (2011) 312e321 319

31. P.W.Mason, Maturation of Japanese encephalitis virus glycoproteinsproduced by infected mammalian and mosquito cells, Virology 169 (1989) 354e364.
32. J.Y. Leung, G.P. Pijlman, N. Kondratieva, J. Hyde, J.M. Mackenzie, A.A. Khromykh, Role of nonstructural protein NS2A in flavivirus assembly, J. Virol. 82 (2008) 4731e4741.
33. S.A. Shiryaev, B.I. Ratnikov, A.E. Aleshin, I.A. Kozlov, N.A. Nelson, M. Lebl, J.W. Smith, R.C. Liddington, A.Y. Strongin, Switching the substrate specificity of the two-component NS2B-NS3 flavivirus proteinase by structure-based mutagenesis, J. Virol. 81 (2007) 4501e4509.
34. A.K. Bera, R.J. Kuhn, J.L. Smith, Functional characterization of cis and trans activity of the Flavivirus NS2BeNS3 protease, J. Biol. Chem. 282 (2007) 12883e12892.
35. A. Utama, H. Shimizu, S. Morikawa, F. Hasebe, K. Morita, A. Igarashi, M. Hatsu, K. Takamizawa, T. Miyamura, Identification and characterization of the RNA helicase activity of Japanese encephalitis virus NS3 protein, FEBS Lett. 465 (2000) 74e78.
36. D. Luo, T. Xu, R.P. Watson, D. Scherer-Becker, A. Sampath, W. Jahnke, S.S. Yeong, C.H.Wang, S.P. Lim, A. Strongin, S.G. Vasudevan, J. Lescar, Insights into RNA unwinding and ATP hydrolysis by the flavivirus NS3 protein, EMBO J. 27 (2008) 3209e3219.
37. C.W. Lin, C.W. Cheng, T.C. Yang, S.W. Li, M.H. Cheng, L. Wan, Y.J. Lin, C.H. Lai, W.Y. Lin, M.C. Kao, Interferon antagonist function of Japanese encephalitis virus NS4A and its interaction with DEAD-box RNA helicase DDX42, Virus Res. 137 (2008) 49e55.
38. R.J. Lin, B.L. Chang, H.P. Yu, C.L. Liao, Y.L. Lin, Blocking of interferon- induced Jak-Stat signaling by Japanese encephalitis virus NS5 through a protein tyrosine phosphatase-mediated mechanism, J. Virol. 80 (2006) 5908e5918.
39. M. Nawa, T. Takasaki, K. Yamada, I. Kurane, T. Akatsuka, Interference in Japanese encephalitis virus infection of Vero cells by a cationic amphiphilic drug, chlorpromazine, J. Gen. Virol. 84 (2003) 1737e1741.
40. C.J. Lee, H.R. Lin, C.L. Liao, Y.L. Lin, Cholesterol effectively blocks entry of flavivirus, J. Virol. 82 (2008) 6470e6480.

41. Y.J. Chien, W.J. Chen, W.L. Hsu, S.S. Chiou, Bovine lactoferrin inhibits Japanese encephalitis virus by binding to heparan sulfate and receptor for low density lipoprotein, Virology 379 (2008) 143e151.
42. J. Ren, T. Ding, W. Zhang, J. Song, W. Ma, Does Japanese encephalitis virus share the same cellular receptor with other mosquito-borne flaviviruses on the C6/36 mosquito cells? Virol. J. 4 (2007) 83.
43. C.J. Chen, M.D. Kuo, L.J. Chien, S.L. Hsu, Y.M. Wang, J.H. Lin, RNAprotein interactions: involvement of NS3, NS5, and 30 noncoding regions of Japanese encephalitis virus genomic RNA, J. Virol. 71 (1997) 3466e3473.
44. C.S. Hahn, Y.S. Hahn, C.M. Rice, E. Lee, L. Dalgarno, E.G. Strauss, J. H. Strauss, Conserved elements in the 30 untranslated region of flavivirus RNAs and potential cyclization sequences, J. Mol. Biol. 198 (1987) 33e41.
45. P.D. Uchil, V. Satchidanandam, Characterization of RNA synthesis, replication mechanism, and in vitro RNA-dependent RNA polymerase activity of Japanese encephalitis virus, Virology 307 (2003) 358e371.
46. P.D. Uchil, A.V. Kumar, V. Satchidanandam, Nuclear localization of flavivirus RNA synthesis in infected cells, J. Virol. 80 (2006) 5451e5464.
47. P. Kumar, P.D. Uchil, P. Sulochana, G. Nirmala, R. Chandrashekar, M Haridattatreya, V. Satchidanandam, Screening for T cell-eliciting proteins of Japanese encephalitis virus in a healthy JE-endemic human cohort using recombinant baculovirus-infected insect cell preparations, Arch. Virol. 148 (2003) 1569e1591.
48. S.I. Yun, Y.J. Choi, B.H. Song, Y.M. Lee, 30 cis-acting elements that contribute to the competence and efficiency of Japanese encephalitis virus genome replication: functional importance of sequence duplications, deletions, and substitutions, J. Virol. 83 (2009) 7909e7930.
49. G.N. Sapkal, N.S. Wairagkar, V.M. Ayachit, V.P. Bondre, M.M. Gore, Detection and isolation of Japanese encephalitis virus from blood clots Am. J. Trop. Med. Hyg.
50. A.G. Aleyas, J.A. George, Y.W. Han, M.M. Rahman, S.J. Kim, S.B. Han, B.S. Kim, K. Kim, S.K. Eo, Functional modulation of dendritic cells and macrophages by Japanese encephalitis virus through MyD88 adaptor J. Immunol. 183 (2009) G. Aleyas, Y.W.

Han, J.A. George, B. Kim, K. Kim, C.K. Lee, S.K. Eo, J. Immunol. 185 (2010) 1429e1441.

51. Tunkel A, Initial therapy and prognosis of bacterial meningitis in adults.UpToDate. 2013 Feb.

52. Schuurman T, Boer R, et al. Prospective Study of Use of PCR Amplification and Sequencing of 16S Ribosomal DNA from Cerebrospinal Fluid for Diagnosis of Bacterial Meningitis in a Clinical Setting. JournalofClinical Microbiology. 2004

53. Shin S, Kwon K, et al. Evaluation of the Seeplex® Meningitis ACE Detection Kit for the Detection of 12 Common Bacterial and ViralPathogens

54. Saravolatz L D, Manzor O, et al. Broad-Range Bacterial Polymerase Chain Reaction for Early Detection of Bacterial Meningitis. Clinical Infectious Diseases 2003 January;36:40–5

55. Mackenzie JS, Williams DT, Smith DW. Japanese Encephalitis Virus: The Geographic Distribution, Incidence, and Spread of a Virus with a Propensity to Emerge in New Areas. In: Tabor E, ed. *Emerging Viruses in Human Populations.* Vol 16. Amsterdam: Elsevier Science Bv; 2007:201-268.

56. Tajima S, Kotaki A, Yagasaki K, Taniwaki T, Moi ML, Nakayama E, Saijo M, Kurane I, Takasaki T. Identification and amplification of Japanese encephalitis virus and Getah virus propagated from a single porcine serum sample: A case of coinfection. *Arch Virol.* 2014;159(11):2969-2975.

57. Le Flohic G, Porphyre V, Barbazan P, Gonzalez JP. Review of climate, landscape, and viral genetics as drivers of the Japanese encephalitis virus ecology. *PLoS Negl Trop Dis.* 2013;7(9):e2208.

58. Schuh AJ, Ward MJ, Brown AJ, Barrett AD. Phylogeography of Japanese encephalitis virus: genotype is associated with climate. *PLoS Negl Trop Dis.* 2013;7(8):e2411.

59. Gao X, Liu H, Li M, Fu S, Liang G. Insights into the evolutionary history of Japanese encephalitis virus (JEV) based on whole-genome sequences comprising the five genotypes. *Virol J.* 2015;12(1):43.

60. Schuh AJ, Ward MJ, Leigh Brown AJ, Barrett ADT. Dynamics of the emergence and establishment of a newly dominant genotype of Japanese encephalitis virus throughout Asia. *J Virol.* 2014;88(8):4522-4532.
61. Wang L, Hu W, Magalhaes RJS, Bi P, Ding F, Sun H, Li S, Yin W, Wei L, Liu Q, Haque U, Sun Y, Huang L, Tong S, Clements AC, Zhang W, Li. The role of environmental factors in the spatial distribution of Japanese encephalitis in mainland China. *Environ Int.* 2014;73:1-9.
62. Brookes VJ, Hernandez-Jover M, Cowled B, Holyoake PK, Ward MP. Building a picture: Prioritisation of exotic diseases for the pig industry in Australia using multi-criteria decision analysis. *Prev Vet Med.* 2014;113(1):103-117.

LIMITATIONS

1. Faulty biochemical reports due to
    - Improper procedure of lumbar puncture
    - Antibiotic treatment prior to lumbar puncture.
    - Delay in transport of specimen
    - Improper storage.
    - Autolytic enzymes in CSF.
    - Fastidious nature of pathogen.

2. Sample size is small

3. One year comparative study

PROFORMA

Name:	Serial No:
Age:	Lab No:
Sex:	Op/Ip No:
Address:	Date of Sample Collection:
Occupation:	Specimen:
Income:	Test:

Chief Complaints:

1. Fever
2. Altered Sensorium
3. Neck Stiffness
4. Vomiting
5. Convulsions

Past History:

Clinical Diagnosis:

CONSENT FORM

I _____hereby give consent to participate in the study conducted by **Dr.R.GOPINATHAN**, post graduate in the department of microbiology, thanjavur medical college thanjavur-613004 and to use my personel clinical data and result of investigation for the purpose of analysis and to study the nature of disease .I also give consent for further investigations

Place: Signature of participant

Date:

INFORMATION SHEET

- We are conducting cross sectional and comparative study on

- **To study the evaluation of conventional culture methods and serodiagnostic methods for clinically diagnosed as meningoencephalitis in patients admitted in Thanjavur medical college hospital with special reference to Japanese encephalitis in** the department of microbiology thanjavur medical college Thanjavur.

- At the time of announcing the results and suggestions ,name and the identy of the patients will be confidential.

- Taking part in the study is voluntary . You are free to decide wheather to participate in this study or to withdraw at any one time ;your decision will not result in any loss of benefits to which you are otherwise entitiled.

- The results of the study may be intimated to you at the end of the study period or during the study if anything is found abnormal which may aid in the management or treatment.

Signature of Investigator **Signature of Participant**

Date

MASTER CHART

S.NO	BIOCHEMICAL ANALYSIS Sugar/Protein	CELL COUNT	CULTURE RESULTS	JE ELISA	PCR
1	100 / 50	Nil	No Growth	Negative	Negative
2	48 / 40	Nil	No Growth	Negative	Negative
3	55 / 50	Nil	No Growth	Negative	Negative
4	20 / 40	Nil	No Growth	Negative	Negative
5	49 / 50	Nil	No Growth	Negative	Negative
6	83 /40	Nil	No Growth	Negative	Negative
7	71 / 50	Nil	No Growth	Negative	Negative
8	112 / 70	Nil	No Growth	Negative	Negative
9	63 / 40	Nil	No Growth	Negative	Negative
10	64 / 50	Nil	No Growth	Negative	Negative
11	30 / 50	Nil	No Growth	Negative	Negative
12	40 / 70	Nil	No Growth	Negative	Negative
13	50 / 60	Nil	No Growth	Negative	Negative
14	40 / 60	Nil	No Growth	Negative	Negative
15	46 / 63	Nil	No Growth	Negative	Negative
16	52 / 476	N1/L3	No Growth	Negative	Negative
17	42 / 472	N3/L9	No Growth	Negative	Negative
18	51 / 63	Nil	No Growth	Negative	Negative
19	47 / 59	Nil	No Growth	Negative	Negative
20	36 / 58	Nil	No Growth	Negative	Negative
21	49 / 62	Nil	No Growth	Negative	Negative
22	39/48	Nil	No Growth	Negative	Negative
23	52/61	Nil	No Growth	Negative	Negative
24	43/59	Nil	No Growth	Negative	Negative
25	36/47	Nil	No Growth	Negative	Negative
26	51/597	L3	No Growth	Negative	Negative
27	55/489	L5	No Growth	Negative	Negative
28	42/61	Nil	No Growth	Negative	Negative
29	49/71	Nil	No Growth	Negative	Negative
30	39/62	Nil	No Growth	Negative	Negative
31	43/62	Nil	No Growth	Negative	Negative
32	44/67	Nil	No Growth	Negative	Negative
33	48/71	Nil	No Growth	Negative	Negative
34	55/65	Nil	No Growth	Negative	Negative
35	52/67	Nil	No Growth	Negative	Negative
36	41/59	Nil	No Growth	Negative	Negative

S.NO	BIOCHEMICAL ANALYSIS Sugar/Protein	CELL COUNT	CULTURE RESULTS	JE ELISA	PCR
37	44/64	Nil	No Growth	Negative	Negative
38	45/72	Nil	No Growth	Negative	Negative
39	51/465	N1/L5	No Growth	Negative	Negative
40	45/72	Nil	No Growth	Negative	Negative
41	46/61	Nil	No Growth	Negative	Negative
42	49/69	Nil	No Growth	Negative	Negative
43	57/72	Nil	No Growth	Negative	Negative
44	54/68	Nil	No Growth	Negative	Negative
45	55/65	Nil	No Growth	Negative	Negative
46	56/62	Nil	No Growth	Negative	Negative
47	35/60	Nil	No Growth	Negative	Negative
48	51/70	Nil	No Growth	Negative	Negative
49	61/71	Nil	No Growth	Negative	Negative
50	58/72	Nil	No Growth	Negative	Negative
51	42/63	Nil	No Growth	Negative	Negative
52	44/75	Nil	No Growth	Negative	Negative
53	38/60	Nil	No Growth	Negative	Negative
54	42/61	Nil	No Growth	Negative	Negative
55	55/71	Nil	No Growth	Negative	Negative
56	42/71	Nil	No Growth	Negative	Negative
57	52/63	Nil	No Growth	Negative	Negative
58	32/51	Nil	No Growth	Negative	Negative
59	51/62	Nil	No Growth	Negative	Negative
60	42/52	Nil	No Growth	Negative	Negative
61	61/73	Nil	No Growth	Negative	Negative
62	57/65	Nil	No Growth	Negative	Negative
63	49/58	Nil	No Growth	Negative	Negative
64	60/70	Nil	No Growth	Negative	Negative
65	51/59	Nil	No Growth	Negative	Negative
66	61/75	Nil	No Growth	Negative	Negative
67	55/78	Nil	No Growth	Negative	Negative
68	52/71	Nil	No Growth	Negative	Negative
69	62/80	Nil	No Growth	Negative	Negative
70	55/59	Nil	No Growth	Negative	Negative
71	62/71	Nil	No Growth	Negative	Negative
72	36/71	Nil	No Growth	Negative	Negative
73	45/71	Nil	No Growth	Negative	Negative
74	55/65	Nil	No Growth	Negative	Negative
75	42/59	Nil	No Growth	Negative	Negative

S.NO	BIOCHEMICAL ANALYSIS Sugar/Protein	CELL COUNT	CULTURE RESULTS	JE ELISA	PCR
76	55/76	Nil	No Growth	Negative	Negative
77	49/76	Nil	No Growth	Negative	Negative
78	51/73	Nil	No Growth	Negative	Negative
79	50/60	Nil	No Growth	Negative	Negative
80	55/72	Nil	No Growth	Negative	Negative
81	39/58	Nil	No Growth	Negative	Negative
82	59/78	Nil	No Growth	Negative	Negative
83	61/82	Nil	No Growth	Negative	Negative
84	47/49	Nil	No Growth	Negative	Negative
85	51/72	Nil	No Growth	Negative	Negative
86	52/71	Nil	No Growth	Negative	Negative
87	44/52	Nil	No Growth	Negative	Negative
88	52/455	N1/L3	klebsella pneumoniae	Negative	Negative
89	36/62	Nil	No Growth	Negative	Negative
90	42/49	Nil	No Growth	Negative	Negative
91	32/54	Nil	No Growth	Negative	Negative
92	54/68	Nil	No Growth	Negative	Negative
93	57/81	Nil	No Growth	Negative	Negative
94	43/61	Nil	No Growth	Negative	Negative
95	51/75	Nil	No Growth	Negative	Negative
96	53/74	Nil	No Growth	Negative	Negative
97	59/78	Nil	No Growth	Negative	Negative
98	51/76	Nil	No Growth	Negative	Negative
99	**55/423**	**N2/L5**	**No Growth**	**Positive**	**positive**
100	45/75	Nil	No Growth	Negative	Negative