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## A review on Zika infection: From strain identification to Guillain-Barré syndrome complication

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## ABSTRACT

Zika infection, caused by Zika virus, is one of the life-threatening viral diseases of the 21st century. Though this virus came into our focus about 50 years ago, our understanding regarding this has not been improved till then. The probable reason lies within its characteristics of not showing any physical or physiological symptoms until the virus is spread to our next generation, new-born babies, who become mostly victims of microcephaly. The prime focus of this review was to demonstrate two focusing etiological features and complications related to Zika infection along with their findings. In the first study, the purpose was to isolate the virus strain and to find out the reason for not showing any physiological symptoms. The next study was about how Guillain-Barré syndrome can complicate the Zika infection in vector body. In short, a partial depiction of infection by Zika virus was tried to be focused.

## 1. Introduction

Zika virus (genus *Flavivirus*, family *Flaviviridae*) is a type of pathogen for which mosquito acts as the vector. Initially, this virus was discovered in 1947 from sentinel rhesus monkeys located in Zika forest, Uganda. The identification of the virus in human beings was done in 1952[1]. Fever, arthralgia, rash, neurological abnormalities like microcephaly are the characteristic symptoms of this viral disease[2]. Though the number of incidents was rather negligible at the beginning, a recent devastating outbreak in Brazil in 2015 caused about 4000 people to be suspected of the infection, which drove World Health Organization to declare the level of alarm as 'extremely high'[3]. The most challenging part of the Zika is that there is no developed vaccine or treatment to cure the patients especially the pregnant women. So if a pregnant woman is affected by Zika, then the future of the new-born baby will be in a question mark[4]. Here, in this article, we have reviewed two studies related to the Zika infection.

## 2. Isolation of a new strain of Zika virus

Isolation and the after effects of inoculation of two new strains of Zika virus from *Aedes (Stegomyia) africanus* (*A. africanus*) mosquito species of Lunyo forest, Uganda was reported for the first time. The history of the previous isolations of Zika virus strains dated back to 1947 followed by the discovery of some other strains in 1948 and 1954[5]. The use of infant mice was uncommon in the laboratory when the original isolations were made. The mosquito-catching program was carried on at that side of the forest which was close to a coffee plantation. The time between 9th May and 27th August in 1956 was fixed to carry on the mosquito-catching program. The investigation of the activity of Zika virus in *A. africanus* of this area was also carried out. A total of 1355 of the captured *A. africanus* were categorized in 11 groups and other insects caught were discarded. Usually, 2-hour sessions were taken to carry out the catching at ground level, depending upon the availability of the catching team. So, on some days, no catching was made while two sessions might have been done on some days, but as a rule, the minimum of one session was to be done on any single day except Saturdays or Sundays when no catching was carried out.

Eleven catches were conducted dated between 9th May and 27th August in 1956. Among them, Lunyo catch No. III was used to isolate single Rift Valley fever virus strain whereas the two groups of mosquitoes from which the isolations related to the discussion were made and marked as Lunyo V and Lunyo VI[5].

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### 2.1. Lunyo V

Three groups of mice were selected in which infant mice containing two groups who were inoculated with Lunyo V isolations, were labeled as Lunyo VA and Lunyo VB and the adults as Lunyo VC. In Lunyo VC, one dead mouse was discarded as it showed no illness prior to its death on the 16th day. In box of Lunyo VA, one “non-specific” death took place on Day 5. On day 9, the brains of the rest of the four mice were kept in glycerol saline after being killed when they were on the verge of dying. In box of Lunyo VB, one infant was dead while one became sick on Day 6 and the other three were sacrificed when they were sick on Day 7[3,6,7]. The brains removed from the sick or dead mice on day 6 were utilized to make a suspension and were inoculated in two forms as unchanged and as filtrate following Seitz filtration. Inoculation caused sickness and death of the inoculated infant mice. A histological study of tissues of these dead mice revealed skeletal myositis, encephalitis of viral type and myocarditis. Then, successful passages from brain and heart of the sick mice were done after inoculating them with the Seitz-filtered brain suspensions. These passages were mainly attempted to investigate and compare the characteristic of progeny viruses with their progenitor ones.

### 2.2. Lunyo VI

Three groups of mice were selected and inoculated just like Lunyo V. In the adult group, one mouse was removed on the 27th day due to death following an illness for 3 days. In case of two groups of infant mice, one dead mouse was found in each on the 9th post-inoculation day; the remaining eight were killed due to moribundity. The brains of the killed mice were kept at  $-20^{\circ}\text{C}$  overnight. In the next day, a suspension with and without Seitz filtration was prepared for passage. Generally, the incubation period was kept constant at 5 days, but sometimes it may be dropped to 4 days. When the first batch of mice became sick, thawing of the original suspension was done, followed by inoculation into four three-day-old infant mice. After observing them for the following 30 days, all of them remained well.

During the first passage obtained from Lunyo VIA and B, unfiltered suspension was inoculated into the five adult mice. Among the five mice, one became dead on Day 9, one died on Day 15, one remained sick for thirteen days and the other two remained well. From this result, we can assume that the adult mice have the adaptation capability to the agent. So, based upon this supposition, a suspension was prepared from 13th-passage infant mouse brain and was then applied to commence a series of passages in ten 35-day-old mice. After applying this brain suspension, all mice from the first adult group became ill and then dead on the 9th day. After administering intracerebrally in these 35-day-old mice, the agent gave rise to a titer of  $10^{5.5}$  LD<sub>50</sub> per 0.03 mL within this time frame. A rhesus monkey, coded M.R. 1063, showed no reaction initially after receiving inoculation of Lunyo VI mosquito suspension, but it died unfortunately after 3 weeks due to retroperitoneal abscess[5].

Stocks of both Lunyo V and VI were prepared, as well as the homologous antiserum. In case of Lunyo V, the serum of M.R. 1059, a monkey, proved sufficient and for Lunyo VI, two rabbits, marked as R. 34 and R. 41, became hyper immunized.

These agents did not belong to the Coxsackie group as it was susceptible to show sensitivity following exposure to diethyl ether and they were also found to be remarkably stable. When keeping a  $10^{-1}$  suspension in bovine plasma albumin at  $4^{\circ}\text{C}$  for 24 h, it caused a decrease of only 0.2 logs, i.e. from  $10^{7.0}$  to  $10^{6.8}$ . Heating this suspension for 1 h at  $37^{\circ}\text{C}$  caused a drop to  $10^{6.5}$ .

### 3. Association between Zika infection and Guillain-Barré syndrome (GBS)

In November 2013, a Polynesian woman in her early 40s was hospitalized for neurological problems in Polynesian institution having only past medical history of acute articular rheumatism. From the Emergency Department, diagnosis report confirmed paraesthesia of the distant four limbs a day before and she was discharged. In Day 1, she was admitted to the Department of Neurology because paraesthesia, for which she had been diagnosed, had caused weakness of ascendant muscles which was a distinguishing feature of GBS. At Day 3, she was affected by tetraparesis mainly in the lower limbs, along with paraesthesia of the extremities, diffuse myalgia and a bilateral but asymmetric peripheral facial palsy and absence of deep tendon reflexes. No respiratory or deglutition disorders were present. The patient developed dysautonomia due to chest pain, prolonged ventricular tachycardia and orthostatic hypotension. The echocardiography appeared normal. Diffuse demyelinating disorder, associated with elongated F-wave, raised distal motor latency, conduction hindrance and acute loss of nerve supply without axonal abnormalities were confirmed by electromyogram. After the administration of intravenous polyvalent immunoglobulin at a dose of 0.4 g/kg/day for 5 days, she recovered a lot. At day 13, the patient was discharged home. Paraparesis caused the patient to walk with the help of a walking frame even after the end of hospitalization and the facial palsy slowly disappeared. At Day 40, the condition improved and she was able to walk without assistance along with a muscular strength score of 85/100 which is satisfactory. Retrospective data showed that she was affected from influenza-like syndrome, along with myalgia, febricula, cutaneous rash, and conjunctivitis at Day 7. The patient, presenting these symptoms, was suspected of Zika fever as Zika virus infection was epidemic[8].

Laboratory analysis revealed that the blood count was normal and there was no inflammation. Transaminase enzyme level was increased by two fold; cerebrospinal fluid analysis showed an albuminocytological dissociation of 1.66 g/L proteins (normal: 0.28–0.52) and 7 white cells/ml (normal < 10); glycorrachia was within normal level of 0.60 g/L. Blood testing for HIV, hepatitis B and C, *Campylobacter jejuni* and *Leptospira* were proved negative, all of which were usual aetiologies of GBS whereas serological tests for cytomegalovirus, Epstein-Barr virus, and type 1 and 2 herpes simplex virus were positive which confirmed resolute infections.

Before administering immunoglobulin intravenously, the blood samples were investigated with direct detection of dengue virus (DENV) by non-structural protein 1 antigen and RT-PCR and only RT-PCR for Zika eight days after commencing influenza-like symptoms (corresponding to day 1). All of them proved negative[9,10]. At the 8th and 28th days after the appearance of the influenza-like syndrome, the presence of Zika-specific immunoglobulin M (IgM) and Zika- and DENV-specific immunoglobulin G (IgG) in blood samples were

confirmed by in-house enzyme-linked immune sorbent assays (in-house IgM antibody capture-ELISA and indirect IgG ELISA using inactivated antigen). The presence of specific antibody in serum sample collected 28 days after the onset of influenza-like syndrome was determined by plaque reduction neutralization test against serotype 1 to 4 DENV (DENV1–4) and Zika. A 90% neutralization titer > 1/320 for DENV1, 1/80 for DENV2, > 1/320 for DENV3, 1/20 for DENV4 and > 1/320 for Zika was found which confirmed the presence of antibodies against the four DENV serotypes and Zika in the serum of the patient. These serological tests confirmed recent infection by Zika and resolute infections by DENV1-4.

#### 4. Discussion

After inoculation with Lunyo V and VI, the resemblance in behavioral characteristics of the mice and the histological picture in both the cases led us to believe that they are the two strains of Zika virus. Besides, some new findings about Zika virus came into light which was not previously recorded. Firstly, in infant mice, skeletal myositis and myocarditis was found along with pulmonary edema in Zika infection. Secondly, the virus containing material extracted from sources except the central nervous system can be transferred to infant mice intra-cerebrally or by a peripheral route. Thirdly, Zika virus can easily and rapidly adapt to become more pathogenic for adult mice as compared to the original isolation. The reason for this rapid adaptability may be attributed to the fact that the passage series was initiated with virus which had already experienced several transfers in infant mice. Finally, this virus can resist itself from deterioration by preservation as a 10% suspension in bovine plasma albumin at 4 °C.

Again, in an attempt to enquire the association of Zika infection and GBS, Zika infection was differentiated from cross-reactions due to DENV based on IgM/IgG serological results and progressive neural resolution therapy. Again, the correlation between types 1 and 3 dengue fever and GBS during Zika fever was revealed by DENV and Zika specific blood tests in the patient as DENV infection had also been related to GBS[11-13]. Based upon this, a hypothesis was implicated regarding sequential arboviral immune stimulation and its relation with unusual occurrence of GBS cases during concurrent Zika infection and two dengue serotypes. The underlying pathological mechanism of Zika-related GBS is yet to find out but an assumption of its immunological origin can be drawn[14]. The risk of GBS is related to precise sequence of DENV and Zika infections. So, diffusing demyelinating disorder may occur in case of Zika infection for which the clinician must be aware.

#### 5. Conclusion

From the above study it can be stated that Zika virus infection has started to change its character with time while expanding its geographical range. The change ranges from a native, mosquito driven infection causing mild illness to large outbreaks associated with neurological disorders like GBS and microcephaly. The lack of effective treatment strategy has aggravated the complication of Zika which guided the US experts from the National Institute of Health to commence the trials of Zika vaccine.

#### Conflict of interest statement

We declare that we have no conflict of interest.

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