**Rapid Detection of Chikungunya virus**

**by Reverse Transcriptase – Polymerase Chain Reaction (RT-PCR)**


## ABSTRACT

*Chikungunya is an arthropod-borne viral disease, transmitted mainly by Aedes mosquitoes. The infection gives very disabilitating symptoms, which sometimes may mislead to other viral diseases. Therefore, diagnosis should be confirmed by virus isolation, detected by RT-PCR, as well as serologically (IgG and IgM antibodies), which will facilitate more accurate diagnosis.*



Keywords: Chikungunya, Aedes mosquitoes, RT-PCR


## INTRODUCTION

Chikungunya is an arthropod-borne viral disease commonly found in Africa and Southeast Asia. The viral agent is transmitted both in primate and human mainly by Aedes mosquitoes.

It is suggested that although human infection with this virus appears of low activity, it is widespread and the virus remains a potential menace and may be responsible for future epidemics (Matusop & Singh, 2000).

The infection gives a very disabilitating symptom due to the involvement of the joints. Even though chikungunya is considered as a self-limited disease, the symptoms it gives make the development of the

laboratory-based surveillance system more important in providing an early warning of chikungunya epidemics. Surveillance of mosquitoes infected with CHIKV provides an early warning sign for risk of transmission in an area. A reverse transcriptase-polymerase chain reaction (RT-PCR) was developed for the rapid detection of chikungunya virus in infected mosquitoes in epidemic areas.

## CHIKUNGUNYA VIRUS

### Historical Background

The word 'chikungunya' was first used by the indigenous people of Southern Province, Tanganyika Territory (Tanzania), in reference to a disease which afflicted them in epidemic form in 1952-1953 (Jupp & McIntosh, 1985). The word is Swahili meaning "that which bends up" and refers to the stooping posture adopted by patients because of the severity of the joint pains .The virus belongs to the genus Alphavirus of the family Togaviridae.

Chikungunya is systematically named in 1997, with alternative name 'Buggy Creek virus' and acronym CHIKV. It is a single RNA virus. The International Classification of Disease Codes (ICD) for chikungunya fever is A92.0 (www.cbwinfo.com/Biological/Pathogens/CHIK.html).

### Disease Distribution

CHIKV was first isolated by Ross in patients from Tanganyika (Tanzania) in 1957 (Powers, 2000). Reports of chikungunya epidemics have been described in Africa and India. The virus seems to be enzootic throughout tropical Africa, and it appears to have spread to other parts of

the world from Africa to cause pandemics in both the American and Asian tropics. Since 1953, CHIKV has caused numerous well-documented outbreaks and epidemics in both Africa and Southeast Asia, involving hundreds of thousands of people (Diallo, 1999; Powers, 2000). The disease has also been reported from Australia (Thaikruea, 1997).

In the Southeast Asia region, CHIKV infection has been reported in Burma, Philippines, Thailand and recently Malaysia (Thaikruea, 1997; Matusop & Singh, 2000; Lam, 2001).

In Thailand, CHIKV infection is classified as re-emerging disease when two outbreaks were reported in 1995. The first reported case of chikungunya diagnosed by serologically in Thailand was in 1960 and the last case was in 1991 (Thaikruea *et al.*, 1997).

In Malaysia, CHIKV was never reported until January 1999, confirmed by the WHO Collaborating Centre for Arbovirus, UM University Hospital and The Western Australian Centre for Pathology and Medical Research, Australia (Lam, 2001).

In Indonesia, a CHIKV epidemic was reported in April 1983, and reached the peak in December 1983 (Haksohusodo *et al.*, 1983). Re-emerging disease was identified throughout the country from September 2001 to March 2003 (Laras *et al.*, 2004).

## CLINICAL ASPECTS

### Clinical Features

The incubation period varies between 2-12 days, but usually two to four days, and the single most significant symptom is the arthralgia, presenting in 70% cases (Jupp & McIntosh, 1985).

In many patients, the onset of arthritis is followed in 1-10 days by a maculopapular rash, usually nonpruritic, affecting mainly the trunk and limbs. The rash resolves within 7-10 days, followed by a fine desquamation. Fever is sometimes absent. Cervical lymphadenopathy occurs frequently. Other signs and symptoms of chikungunya include paresthesias and tenderness of palms and soles, polyarthritis, minor haemorrhages and leucopenia.

Some studies showed that the joint pains, stiffness and swelling persisted for four months to three years (Matusop &Singh, 2000).

In contrast to the clinical presentation in adults, the most presenting symptom in children was vomiting and abdominal pain or anorexia. Arthritis and arthralgia appear to be less prominent features. The clinical examination showed that the most frequent sign was pharyngitis and facial flushing (Zuckerman *et al.*, 1994).

There has been no report of death due to chikungunya, except for one possible case during the epidemic in 1973 in India (Matusop & Singh, 2000). Chikungunya is generally considered as a non-fatal but very disabilitating disease.

**Diagnosis and Treatment**

Chikungunya can be provisionally diagnosed based on the presentation of the main signs and symptoms such as maculopapular rash following the onset of arthritis.

Although not listed as a haemorrhagic fever virus, illness caused by CHIKV can be confused with diseases such as dengue or yellow fever, based on the similarity of the symptoms. Therefore, diagnosis should be confirmed by virus isolation as well as serologically (IgG and IgM antibodies), which will facilitate more accurate diagnosis. A rise in titre from a second serology test or isolation in mice, mosquito or cell culture will give definitive results.

There is no specific treatment for the patients. This is mainly supportive and symptomatic. Rest is indicated during the acute illness, and non-steroidal anti-inflammatory drugs for the arthritis.

## CHIKUNGUNYA VECTORS

Various species of Aedes mosquitoes have been incriminated as potential vectors of chikungunya. In Thailand, *Aedes aegypti* and *Aedes albopictus* have been associated with chikungunya outbreaks in 1995 (Thaikruea *et al.*, 1997). In South Africa, *Aedes furcifer* and *Aedes cordellieri* are considered to be epidemic-epizootic vector during epidemics (Diallo *et al.*, 1999), while in West and Central Africa, *Aedes africanus* is the dominant vector (Matusop & Singh, 2000).

*Aedes aegypti*

Large epidemics occur in urban and semi-urban settings where the virus is transmitted by *Aedes aegypti*. This most prominent vector is widespread in tropical countries. The man-biting habits of domestic *Aedes aegypti* appear to vary in different countries where CHIKV epidemics have occurred.

It was suggested that the rapid spread of virus might have been due to the daytime biting habit of the local *Aedes aegypti*. During the day the human hosts are most likely to disturb the mosquitoes, thus interrupting their feeds, so that a mosquito moves to another host to finish feeding thus transmitting the virus mechanically. The present population growth in Africa with its associated urbanization could lead to large-scale future epidemics on the scale already experienced in Asia, with the possible maintenance of the virus in an *Aedes aegypti*-man cycle (Jupp & McIntosh, 1985).

**Vector Control**

CHIKV is strictly tropical in distribution, which is clear from its geographical distribution pattern in southern Africa, where the virus is absent from the temperate areas (Jupp & McIntosh, 1985). The transmission cycle of CHIKV is characterized by a periodicity of occurrence with silence intervals of 3-4 years (Diallo *et al.*, 1999). Most common epidemic occurs following the rainy season.

**Detection by RT-PCR**

RT-PCR (reverse transcriptase-polymerase chain reaction) is the most sensitive technique for mRNA detection and quantification currently available. Compared to the two other commonly used techniques for

quantifying mRNA levels, Northern blot analysis and RNase protection assay, RT-PCR can be used to quantify mRNA levels from much smaller samples. In fact, this technique is sensitive enough to enable quantification of RNA from a single cell (www.ambion.com/techlib/basics/rtpcr/).

CHIKV can be isolated by the intracerebral inoculation of suckling mice, or by viral culture in either mosquito or mammalian cell culture systems (Zuckerman, 1994).

There were several studies conducted using RT-PCR in detecting chikungunya virus. Pfeffer *et al.* (2002) used RT-PCR/Nested PCR combination and was successfully apply the technique to four CHIKV isolates from patient's serum samples from Asia and Africa. Hasebe *et al.* (2002) used culture fluid from *Aedes albopictus* (C6/36 cell line) to isolate the virus of Malaysian strain from the patients during epidemic 1998 in Malaysia.

Laras *et al.* (2005) extracted the RNA from all patient sera and mosquito samples by nested RT-PCR method during epidemic in Indonesia (September 2001 to March 2003). Overall, evidence of recent and acute CHIKV infections, based on serology and/or RT-PCR, was demonstrated in 47% of serum samples assayed. But the attempts failed to detect evidence of CHIKV by RT-PCR in the field infected mosquitoes.

Although chikungunya does not normally cause death, the disease may become a public health threat in the country if the virus is introduced due to the prevalence of the *Aedes* mosquito vectors. Early steps should be taken in order to prevent future outbreaks, especially in tropical areas,

where the vectors are available. RT-PCR can be useful as an early warning system to detect infected mosquitoes in epidemic areas.

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