

# Chikungunya fever in two German tourists returning from the Maldives, September, 2009

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This report describes the first isolation and molecular characterisation of a chikungunya virus from two German tourists who became ill after a visit to the Maldives in September 2009. The virus contained the E1 A226V mutation, shown to be responsible for an adaptation to the Asian tiger mosquito *Aedes albopictus*. The E1 coding sequence was identical to chikungunya virus isolates from Sri Lanka and showed three nt-mismatches to the only available E1 nt sequence from the Maldives.

## Introduction

Since the start of the current chikungunya fever pandemic on the east coast of Africa in 2005, many cases have been reported in countries in Asia and south-east Asia [1,2]. These cases were attributed to a particular chikungunya virus (CHIKV) strain that has adapted to very efficient transmission to humans via the Asian tiger mosquito (*Aedes albopictus*) due to a A226V mutation in the E1 envelope protein [3,4]. The Maldives were first hit by the chikungunya virus pandemic in late 2006 after the wet season which usually lasts until September. Based on almost 12,000 suspected cases of chikungunya fever the disease was reported on 121 of the 197 inhabited islands with incidence rates between 82 and 722 per 1,000 population [5]. A small set of blood samples from febrile patients with symptoms meeting the chikungunya fever case definition at that time confirmed CHIKV as causative agent in 64 of 67 cases by reverse-transcription PCR (RT-PCR) [5]. However, no further characterisation of the virus strain responsible for the 2006-7 outbreak was performed. One case of a traveller returning to Singapore in January 2007 was confirmed by RT-PCR and the nt sequence of the E1 gene was determined [6]. In early 2009, an outbreak of a viral fever with symptoms including myalgia or arthralgia and rash occurred on several islands of the Laamu Atoll about 400 km south of Malé [7], but no further virological investigation was carried out to determine whether this was due to dengue or chikungunya fever.

## Case report and laboratory findings

Between 1 and 10 September 2009, a German couple visited the Dhiffushi Holiday Island resort at the southern tip of the Ari Atoll, the Maldives (Figure 1), together with their seven year-old son. They flew directly from Munich to Malé with a stopover in Dubai, United Arab Emirates.

Two and three days respectively after the family had returned to Munich, the son and the 35 year-old father developed symptoms compatible with either dengue or chikungunya fever (Table) while the wife stayed healthy. A test for dengue virus showed neither virus RNA nor anti-dengue virus (DENV) IgM for both patients, but the father had IgG antibodies reactive against DENV indicating an earlier anamnestic dengue fever or a cross-reaction with an earlier flavivirus vaccination. CHIKV-specific real-time RT-PCR yielded ct-values of 23 (son) and 22.5 (father) in the respective acute serum samples obtained on 14 September, indicating high-level viremia [8,9]. Chikungunya virus was isolated in Vero B4 cells from both sera and the entire nucleotide sequence of the isolate from the father was determined. The viral genome was 11,811 nucleotides in length and showed high levels of identity with the pandemic CHIKV that is circulating in many parts of the Indian subcontinent and other parts of Asia since 2006. Most interestingly the CHIKV isolate from the Maldives contained the A226V change in the E1 glycoprotein which has been shown to be responsible for shorter extrinsic incubation periods in *Aedes albopictus* mosquitoes [4]. While the son made an uneventful recovery after one week of symptoms, the father developed persisting arthralgias with limited mobility in the affected extremities and still requires analgesic treatment (Table).

## Discussion

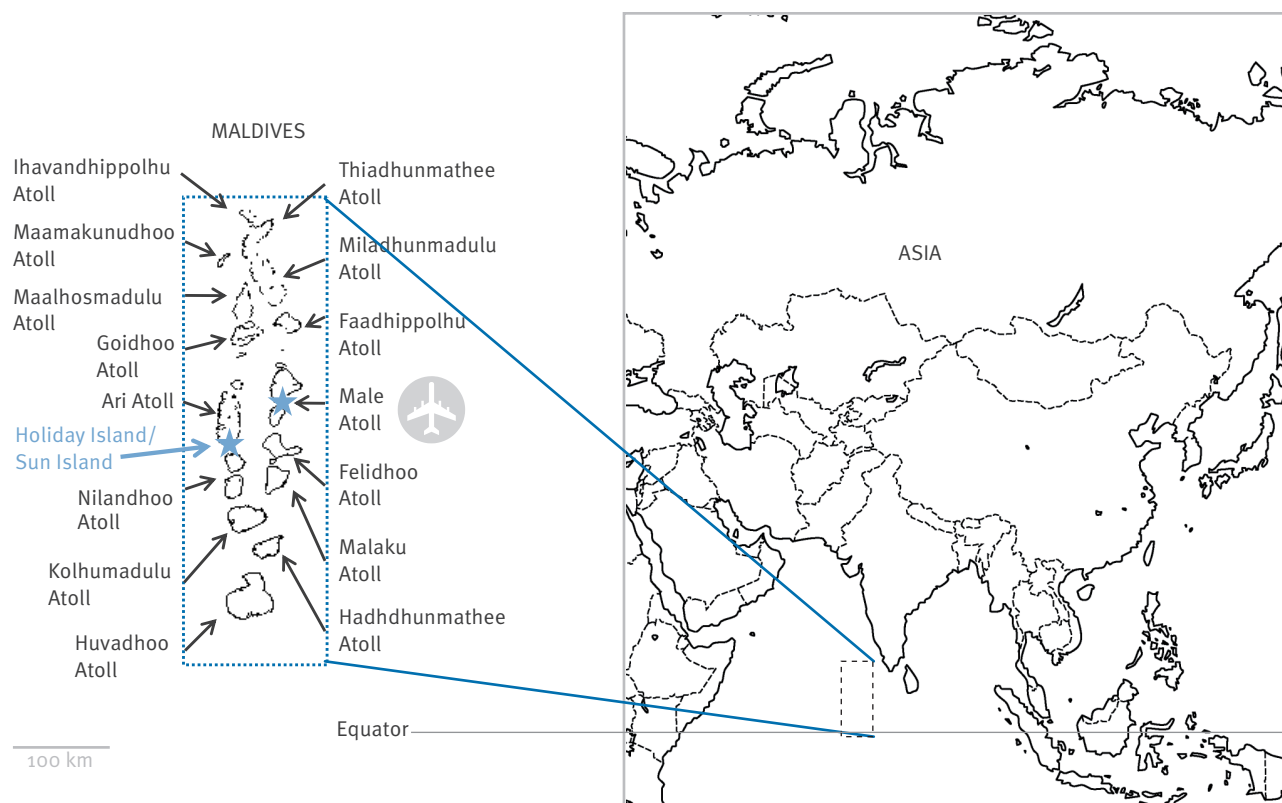
Together with a very recent report on chikungunya fever in a French traveller returning from the northern part of Malé Island, Maldives, in October 2009 [10], our findings suggest a continuous circulation of CHIKV also in other parts of the Maldives. The family stayed

on Dhiffushi Holiday Island throughout their holidays with a daytrip to the neighboring Sun Island. Malé with its international airport was only visited for the inter-continental flight connection, leaving not much time to

become exposed to mosquito bites. We cannot rule out that both infections were acquired while waiting at the airport, because this would fit well with both the incubation period of the disease and with the previous case

**FIGURE 1**

Location of Holiday and Sun Islands on the southernmost rim of the south Ari Atoll, about 100 km away from Male International Airport



**TABLE**

Clinical and laboratory data of patients diagnosed with chikungunya fever, Germany, September 2009

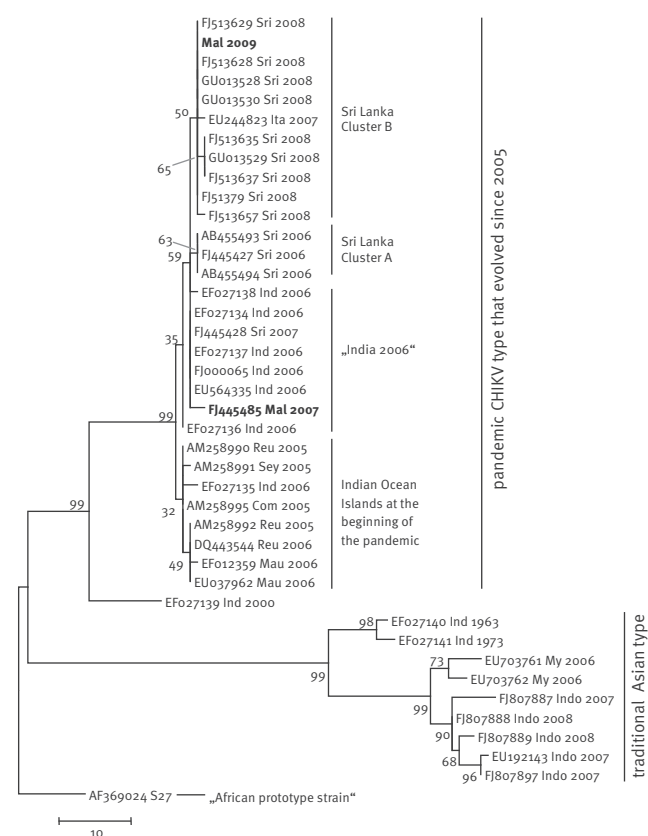
Patient	Son (7 years)	Father (35 years)
Travel schedule	Munich-Dubai-Male and back on 1-10 September, 2009	Munich-Dubai-Male and back on 1-10 September, 2009
Onset of disease	12 September 2009	13 September 2009
Clinical presentation	Fever 39.5°C	Fever 39.0°C
	Headache	Ague, retrobulbar pressure and pain, arthritis of both wrists and ankles
	Macular and partially confluent exanthema (mainly on face and torso)	Erythema, macular and partially confluent exanthema (mainly on torso and arms)
Laboratory findings	Leucocytes 2,700/μl CRP 2.5 mg/dl	Leucocytes 5,300/μl CRP 13 mg/dl Creatinine 1.4 mg/dl
	CHIKV RT-PCR positive	CHIKV RT-PCR positive
	DENV RT-PCR negative	DENV-PCR negative; anti-DENV IgG 15E
Therapy	Paracetamol, Ibuprofen	Paracetamol, Ibuprofen
Further course	Since 16 September fever-free, exanthema gone on 17 September, no further complications since then	Since 16 September fever-free and creatinin back to normal (1.1 mg/dl), exanthema gone, but arthralgias of ankles, wrists, and digital joints persist for more than six months including limited mobility and requiring NSAID treatment

CRP: C-reactive protein; CHIKV: Chikungunya virus; DENV: Dengue virus; RT-PCR: reverse transcription-polymerase chain reaction; NSAID: non steroidal anti-inflammatory drugs

report of the French traveller, who became infected while staying at the Malé Atoll. However, given the high incidence rates of 65.2 per 1,000 population previously reported for the Ari Atoll [5], both infections could likewise have been acquired on Holiday Island. Further, a considerable number of people travel constantly between India and Sri Lanka and the tourist resorts on the Atolls' islands of the Maldives where they are employed. This frequent exchange may argue for a repeated and renewed introduction of CHIKV from India or Sri Lanka via viraemic workers or tourists and limited local transmission through aedine mosquitoes at the respective islands. Analyses of the E1 gene revealed three nt-mismatches when compared to the 2007 case that was analysed in Singapore [6], but identical nt sequences to a series of CHIKV strains from Sri Lanka (Figure 2) [6,11].

## FIGURE 2

Phylogenetic relationship generated by Maximum Parsimony method as implemented in MEGA4 based on the complete E1 protein coding sequence (1314 nt) of a set of CHIKV of different geographic origin



Sequence data are provided with their accession number, country and year. The term Cluster A and B for sequence data from Sri Lanka is adapted from [10]. Please note that the only available CHIKV E1 sequence from the Maldives from 2007 clusters together with CHIKV from India from 2006, while the CHIKV reported here is part of the Cluster B from Sri Lanka in 2008. Sri = Sri Lanka, Mal = Maldives, Ita = Italy (imported from India in 2007), Ind = India, Reu = Reunion, Sey = Seychelles, Com = Comores, Mau = Mauritius, My = Malaysia, Indo = Indonesia. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (100 replicates) are shown next to the branches. The branch lengths are informative (bar length corresponds to 10 nt- differences)

It will be seen in the near future whether more cases of chikungunya fever will be reported for the Maldives, but we feel that this is already an issue in travel medicine although the German Robert Koch Institute reported only three chikungunya fever cases in returning travellers from the Maldives in 2009 (two of which we describe here). A crucial question concerning the current global situation on chikungunya fever is the adaptation of the pandemic CHIKV strain to *Ae. albopictus*. *Aedes aegypti* has been long known to occur on several islands of the Maldives and seems to be the predominant vector on Malé itself while *Ae. albopictus* has established foci on other islands where it seems to be the main mosquito vector species [5]. We do not know which *Aedes* species has infected the German tourists, but we do know that the A226V mutation is suggestive for *Ae. albopictus* as the vector. This particular mosquito is present in many areas around the Mediterranean Sea and was responsible for a CHIKV outbreak in Italy in 2007 resulting in more than 300 cases [12,13]. With a continuing circulation of CHIKV in major tourist destinations in Asia and Africa, imported cases of chikungunya fever will also be seen in Europe and North America. In countries where *Ae. albopictus* is abundant, returning viraemic tourists could cause smaller outbreaks.

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