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Retrospective seroepidemiological study of chikungunya infection in South Asia, Southeast Asia and the Pacific region

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SUMMARY

Chikungunya virus (CHIKV) and Ross River virus (RRV) of the genus *Alphavirus*, family Togaviridae are mainly transmitted by *Aedes* mosquitoes and the symptoms they cause in patients are similar to dengue. A chikungunya (CHIK) outbreak re-emerged in several Asian countries during 2005–2006. This study aimed to clarify the prevalence of CHIKV infection in suspected dengue patients in six countries in South Asia and Southeast Asia. Seven hundred forty-eight serum samples were from dengue-suspected patients in South Asia and Southeast Asia, and 52 were from patients in Fiji. The samples were analysed by CHIKV IgM capture ELISA, CHIKV IgG indirect ELISA and focus reduction neutralization test against CHIKV or RRV. CHIK-confirmed cases in South Asia, particularly Myanmar and Sri Lanka, were 4·6%, and 6·1%, respectively; and in Southeast Asia, particularly Indonesia, the Philippines and Vietnam, were 27·4%, 26·8% and 25·0%, respectively. It suggests that CHIK was widely spread in these five countries in Asia. In Fiji, no CHIK cases were confirmed; however, RRV-confirmed cases represented 53·6% of suspected dengue cases. It suggests that RRV is being maintained or occasionally entering from neighbouring countries and should be considered when determining a causative agent for dengue-like illness in Fiji.

Key words: Asia, chikungunya virus, Fiji, Ross River virus, seroepidemiology.

INTRODUCTION

Chikungunya virus (CHIKV; genus *Alphavirus*, family Togaviridae) is transmitted to humans by the bite of infected mosquitoes, particularly *Aedes aegypti* and *A. albopictus* [1]. CHIKV, which shares the same antigenic complex with Ross River (RRV), O'nyong-nyong, Semliki Forest, Getah, Sagiyama and Mayaro viruses [2], and the dengue viruses (DENV; genus *Flavivirus*, family Flaviviridae) are transmitted by the same mosquito vectors. CHIKV and DENV are often found co-circulating [3, 4] and

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cause similar clinical symptoms, including fever, headache, rash, and in a few cases, haemorrhage. Because of the similarity of symptoms, misdiagnosis and underreporting of chikungunya (CHIK) in dengue-endemic areas are common [4]. CHIK is endemic in the tropical areas of Africa and Asia [5] and generally manifests as mild disease, but outbreaks in the Indian Ocean region showed that it could lead to severe and life-threatening complications [6]. CHIK fever re-emerged as an explosive epidemic in India during 2005–2006 and it is believed to have originated from Kenya in 2004. The recent epidemic in the Indian Ocean spread to several Asian countries, Europe and United States during 2005–2010 [5]. The first reported outbreaks of CHIK occurred in South Asian countries such as Myanmar in 1973 [5] and Sri Lanka in 2006 [7], and in Southeast Asian countries such as Indonesia in 1973 [8], the Philippines in 1985 [5] and Vietnam in 1965 [5]. In the Pacific region, Fiji, American Samoa, Wallis and the Cook Islands, no CHIKV outbreak was reported in 2006 or the preceding years. However, the first reported outbreak of RRV occurred in 1979. Some of the serum samples collected during the RRV outbreak were found to be cross-reactive with both CHIKV and RRV [9].

This study aimed to clarify the prevalence of CHIKV infection that occurred in and before 2006 in the six different countries in South Asia, Southeast Asia and the Pacific region by measuring the titres of anti-CHIKV IgM, IgG and neutralizing antibodies from suspected dengue patients in each country. Titres of RRV neutralizing antibodies from suspected dengue patients in the Pacific region were also measured.

METHODS

Viruses and cell lines

The CHIKV strain S-27 African prototype was used as assay antigen for CHIKV IgM capture ELISA, CHIKV IgG indirect ELISA and neutralization tests. RRV strain (T48) Australian isolate was used for the neutralization tests. C6/36 mosquito cells were used for virus propagation, and Vero (African Green monkey kidney epithelial cells ATCC, CCL81) cells were used for virus titration and neutralization tests.

Study population

For the detection of CHIKV infection, 748 serum samples were collected in different years from febrile patients clinically suspected of dengue in South Asia: Myanmar (2004–2006), Sri Lanka (2005–2006), and in Southeast Asia: Indonesia (2004–2005), the Philippines (2001) and Vietnam (2006). For the detection of CHIKV as well as RRV infection, 52 samples were collected from patients suspected of dengue in Fiji in 2005. The study protocol was approved by the Institutional Ethical Review Committee of the Institute of Tropical Medicine, Nagasaki University (approval no.: 150 917 143).

Laboratory methods

Detection of anti-CHIKV IgM

Presence of anti-CHIKV IgM in serum samples was determined by an in-house IgM capture ELISA (IgM ELISA), which was performed following the protocol described previously [10, 11] with minor modifications. All wells with the exception of the blank in 96-well microplates (Maxisorp, Nalge Nunc International, Denmark) were coated with 100 μl (5·5 μg/100 μl) of anti-human IgM goat IgG (Cappel ICN Pharmaceuticals, USA) with ELISA coating buffer [0·05 m carbonate-bicarbonate buffer (pH 9·6), containing 0·02% sodium azide] as the diluent. The plate was kept at 37 °C for 1 h or at 4 °C overnight. All wells except the blank were blocked with 100 μl of the original concentration of Block Ace (UK-B 80, Yukijirushi, Japan), and were incubated at room temperature (RT) for 1 h. The reagent was removed from all of the wells and washed three times with phosphate-buffered saline containing 0·05% Tween 20 (PBS-T). The test sera, as well as the positive and negative control sera, were diluted at 1:100 in PBS-T and 100 μl aliquots were distributed into duplicate wells. The plate was incubated at 37 °C for 1 h and the serum was removed by washing as described above. One hundred microlitres of CHIKV antigen (128 ELISA units) was added as an assay antigen and incubated at 37 °C for 1 h. After washing as described above, the plate was allowed to react with horseradish peroxidase (HRP)-conjugated anti-CHIKV rabbit polyclonal antibody at a dilution of 1:150, at 37 °C for 1 h. After washing, the colour was developed by adding to each well 100 μl substrate solution, 5 mg *o*-phenylenediamine dihydrochloride (OPD; Sigma Chemical Co., USA) with 0·03% hydrogen peroxide in 0·05 M citrate phosphate buffer (pH 5·0). The plates were kept in the dark at RT for 1 h. To terminate the reaction, 100 μl of 1 N sulphuric

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acid was added per well, and the optical density (OD) was read at 492 nm using a Multiscan JX (model no. 353, Thermolabsystem, Japan). A P/N [positive control (or sample) OD_{492}/negative control OD_{492}] ratio $\geqslant 2{\cdot}0$ was considered positive.

Detection of anti-CHIKV IgG

To detect the presence of anti-CHIKV IgG in serum samples, the in-house IgG ELISA was performed and purified CHIKV was used as the assay antigen [12]. In the procedure, all wells of the 96-well microplates except the blank were coated with 100 μl antigen (250 ng/100 μl per well) diluted with ELISA coating buffer. The plate was incubated at 37 °C for 1 h or at 4 °C overnight. The wells except the blank were blocked with 100 μl of the original concentration of Block Ace and were incubated at RT for 1 h. The plate was washed three times with PBS-T and then 100 μl of each test serum sample diluted at 1:1000 in PBS-T+10% Block Ace was added in duplicate to the plates. A control serum sample known to contain the antibody to the test antigen was run on each plate as a positive control. After incubation at 37 °C for 1 h, the plate was washed and 1:30 000 diluted HRP-conjugated anti-human IgG goat IgG (American Qualex, USA) in PBS-T+10% Block Ace was added at 100 μl/well. The plate was incubated at 37 °C for 1 h, followed by washing and initiation of the peroxidase reaction by the addition of OPD substrate solution (described above) at 100 μl/well. The plate was incubated at RT for 15–30 min in the dark and the reaction was stopped by the addition of 1 N sulphuric acid at 100 μl/well. A standard curve was prepared by using the OD_{492} values of the CHIKV infection high-titre-positive control serum starting with a 1000-fold dilution, followed by serial twofold dilutions up to $1{:}2^{12}$ in PBS-T+10% Block Ace. Next, the IgG titres of patients' serum samples were determined from the positive standard curve. A sample titre $\geqslant$1:3000 was considered to be positive [12].

Fifty percent focus reduction neutralization test ($FRNT_{50}$)

To confirm the status of CHIKV and RRV infection in the patients, their serum samples were checked for the ability to neutralize CHKV or RRV by FRNT with a cut-off point titre of $\geqslant$1:20 as positive result. Serum samples, heat-treated at 56° C for 30 min, were diluted serially and 150 μl of each diluted sample was mixed with an equal volume of 60 focus-forming units of CHIKV or RRV, followed by incubation at 37 °C for 1 h for a virus-antibody neutralization reaction. One hundred microlitres of virus and serum mixture was then used to inoculate a Vero cell monolayer in a 96-well plate. After incubation at 37 °C for 1 h, the infected cells were overlaid with 150 μl of 1·25% methylcellulose 4000 in 2% FCS MEM. The plate was then incubated at 37 °C for 30 h. After removing the methylcellulose, the plates were fixed with 4% paraformaldehyde (Wako, Japan) for 30 min at RT, rinsed with phosphate-buffered solution (PBS), and permeabilized with 200 μl of 1% NP-40 solution in PBS per well for 30 min at RT. The plates were washed three times with with PBS followed by blocking with an original concentration of Block Ace for 30 min at RT. Focus immunostaining was performed after blocking. One hundred microlitres of in-house anti-CHIKV rabbit IgG (diluted 1:3000) was added per well and incubated at 37 °C, for 1 h, followed by three washes with PBS. Subsequently, 1:1000 diluted HRP-conjugated goat anti-rabbit IgG (American Qualex) was added to the plate at 100 μl/well followed by incubation at 37 °C for 1 h. The staining was visualized by the addition of 0·5 mg/ml solution of substrate 3,3′-diaminobenzidine tetrahydrochloride (DAB; Wako, Japan) in PBS with 0·03% hydrogen peroxide at 100 μl/well at RT for 10 min, and the staining reaction was allowed to proceed. After washing the stained cells with distilled water and air-drying the plates, the number of foci per well were counted using a stereomicroscope. The reciprocal of the end-point serum dilution that provided a $\geqslant$50% reduction in the mean number of foci relative to the control wells that contained no serum was considered to be the $FRNT_{50}$ titre.

RESULTS

The overall CHIKV IgM-positive rate was 2·7% (20/748), and the positive rate in each country was 1·8% (4/225) in Myanmar, 1·7% (2/121) in Sri Lanka, 3·5% (7/198) in Indonesia, 4·4% (7/160) in the Philippines and 0·0% (0/44) in Vietnam (Table 1). The overall CHIKV IgG-positive rate was 36·8% (276/748). The IgG-positive rate for each country was: 12·9% (29/225) in Myanmar, 38·0% (46/121) in Sri Lanka, 43·9% (87/198) in Indonesia, 57·5% (92/160) in the Philippines and 50·0% (22/44) in Vietnam (Table 1). The positive rate of CHIKV IgM and/or IgG ELISA (A) was 14·2% (32/225) in Myanmar, 39·7% (48/121) in Sri Lanka, 44·4% (88/

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Table 1. *Positive rates of CHIKV infection in South Asia and Southeast Asia regions*

Country	Total no. of samples	CHIKV IgM and/or IgG ELISA positives				CHIKV FRNT tested samples			CHIKV FRNT positives in ELISA positives					CHIKV neutralization-confirmed cases (A × B)
		IgM	IgG	Total*	Positive rate (A)	IgM	IgG	Total*	IgM	IgG	Total*	Positive rate (B)	$FRNT_{50}$ GMT	
Myanmar	225	4	29	32 (1)	14·2%	4	25	28 (1)	2	8	9 (1)	32·1%	34	4·6%
Sri Lanka	121	2	46	48	39·7%	2	37	39	1	5	6	15·4%	25	6·1%
Indonesia	198	7	87	88 (6)	44·4%	7	80	81 (6)	7	49	50 (6)	61·7%	205	27·4%
Philippines	160	7	92	98 (1)	61·3%	7	83	89 (1)	5	35	39 (1)	43·8%	87	26·8%
Vietnam	44	0	22	22	50·0%	0	22	22	0	11	11	50·0%	90	25·0%
Total	748	20	276	288 (8)	38·5%	20	247	259 (8)	15	108	115 (8)	44·4%		17·1%

CHIKV, Chikungunya virus; $FRNT_{50}$, 50% focus reduction neutralization test; GMT, geometric mean titre.
* Both IgM- and IgG-positive cases.

198) in Indonesia, 61·3% (98/160) in the Philippines and 50·0% (22/44) in Vietnam (Table 1).

Of the CHIKV IgM and/or IgG ELISA positives, CHIKV $FRNT_{50}$ was performed on those serum samples with sufficient volume. The overall CHIKV $FRNT_{50}$ positive rate (A × B) was 17·0% (Table 1). Of these samples, the positive rates of CHIKV $FRNT_{50}$ (B) were 32·1% (9/28) in Myanmar, 15·4% (6/39) in Sri Lanka, 61·7% (50/81) in Indonesia, 43·8% (39/89) in the Philippines and 50·0% (11/22) in Vietnam (Table 1). The geometric mean titres (GMT) of CHIKV $FRNT_{50}$ in Myanmar, Sri Lanka, Indonesia, the Philippines and Vietnam, were 34, 25, 205, 87 and 90, respectively (Table 1). Of the total samples, CHIKV neutralization confirmed cases were 4·6% in Myanmar, 6·1% in Sri Lanka, 27·4% in Indonesia, 26·8% in the Philippines and 25·0% in Vietnam (Table 1).

Out of 52 samples from Fiji, CHIKV IgM and IgG ELISA positives were 7·7% (4/52) and 50% (26/52), respectively (Table 2). For determination of $FRNT_{50}$, only 25 samples had enough volume for analysis in IgM and/or IgG positives. The positive rate of RRV $FRNT_{50}$ was 96·0% (23/25) and for CHIKV $FRNT_{50}$ was 64·0% (16/25). The GMTs of $FRNT_{50}$ against RRV and CHIKV were 329 and 32, respectively (Table 2). As previously reported in $FRNT_{50}$, cross-reactivity of serum samples against RRV and CHIKV occurs [10]. We compared the neutralization titres against these two viruses (Fig. 1). The neutralization titre against RRV was significantly higher than against CHIKV in all samples examined, thus patients having these samples were concluded as having RRV infections. The RRV neutralization-confirmed cases were 53·6% in the Fiji samples (Table 2).

DISCUSSION

Results of this study provided data that extend our knowledge on the distribution of CHIKV infection in South Asia and Southeast Asia. In the present study, we found 4·6–27·4% CHIKV neutralization-confirmed cases in the five countries, i.e. Myanmar and Sri Lanka in South Asia, and Indonesia, the Philippines and Vietnam in Southeast Asia. In Myanmar, among the suspected dengue patients from 2004 to 2006, 4·6% could neutralize CHIKV. There have been only a few serological studies reported in this country on the presence of CHIKV-specific antibodies in patients and these antibodies

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Table 2. *Positive rates of CHIKV and RRV infection in Fiji*

Total no. of samples	CHIKV IgM and/or IgG ELISA positives				CHIKV/RRV FRNT tested samples			CHIKV FRNT positives in ELISA positives						RRV FRNT positives in ELISA positives						RRV neutralization confirmed cases (A x B)
	IgM	IgG	Total*	Positive rate (A)	IgM	IgG	Total*	IgM	IgG	Total	Positive rate	$FRNT_{50}$ GMT		IgM	IgG	Total*	Positive rate (B)	$FRNT_{50}$ GMT		
52	4	26	29 (1)	55·8%	1	25	25 (1)	0	16	16	64·0%	32		1	24	24 (1)	96·0%	329		53·6%

CHIKV, Chikungunya virus; RRV, Ross river virus; $FRNT_{50}$, 50% focus reduction neutralization test.
* Both IgM- and IgG-positive cases.

were seen in 24·7%, 30·4% and 19·0% in suspected dengue patients between 1970–1972 [13], 1973–1974 [14] and in 1984 [15], respectively. In Sri Lanka, although the first outbreak was reported in 2006 [16], our results (samples collected in 2005–2006) indicated 6·1% positivity for CHIKV infection even before the recognition of the outbreak [16]. In this study, detection of anti-CHIKV IgM is a proof of recent CHIKV infection during the collection period and the detection of anti-CHIKV IgG further confirmed by neutralization test indicate the circulating situation of CHIKV in Myanmar, Sri Lanka, Indonesia, Philippines and Vietnam. Of the CHIKV IgM(+) and/or IgG(+) cases confirmed by ELISA, the percentage of positives by CHIKV FRNT were very much different in the five countries in South Asia and Southeast Asia and this might be due to the presence of different *Alphavirus* infections apart from CHIKV. Of the CHIKV ELISA-positive cases, we attempted to detect neutralizing antibodies against RRV from the Sri Lanka samples by using RRV FRNT; however, we were unable to detect them (data not shown), but we detected CHIKV neutralizing antibodies.

In the present study, relatively high prevalence of CHIKV infection was found in Indonesia, the Philippines and Vietnam. In Indonesia, other studies describing the occurrence of CHIKV infections were 9·7%, 14·3% and 50·7% in 1960–1973 [8], 1972 [17] and 1998–1999 [18], respectively. In 2001–2003, 24 distinct outbreaks most likely due to CHIKV based on clinical or laboratory methods were identified throughout Indonesia and where laboratory results were available, 8·0–94·0% confirmed CHIK-positive cases were determined [19]. In the present study, the high percentage (27·4%) of CHIKV infection in Indonesia based on the samples collected in 2004–2005, might be related to the 2001–2003 CHIKV outbreak in Indonesia.

In the Philippines, the percentages or reported number of cases of CHIKV infection in patients were 15·9% in 1966 [20], 20·7% in 1968–1974 [17], three cases in 1986 [21], 73% in 1996 [22], and 18·5% in 1999 [23]. Our results corroborate previous reports in the literature on the high incidence of CHKV infection found in the Philippines.

In Southern Vietnam, a previous study showed 24·0% of serum samples collected in 1972 were positive for CHIKV-specific antibody [17]. In our study, the high incidence of CHIKV neutralizing antibodies was observed in CHIKV IgM(-) and IgG(+) ELISA

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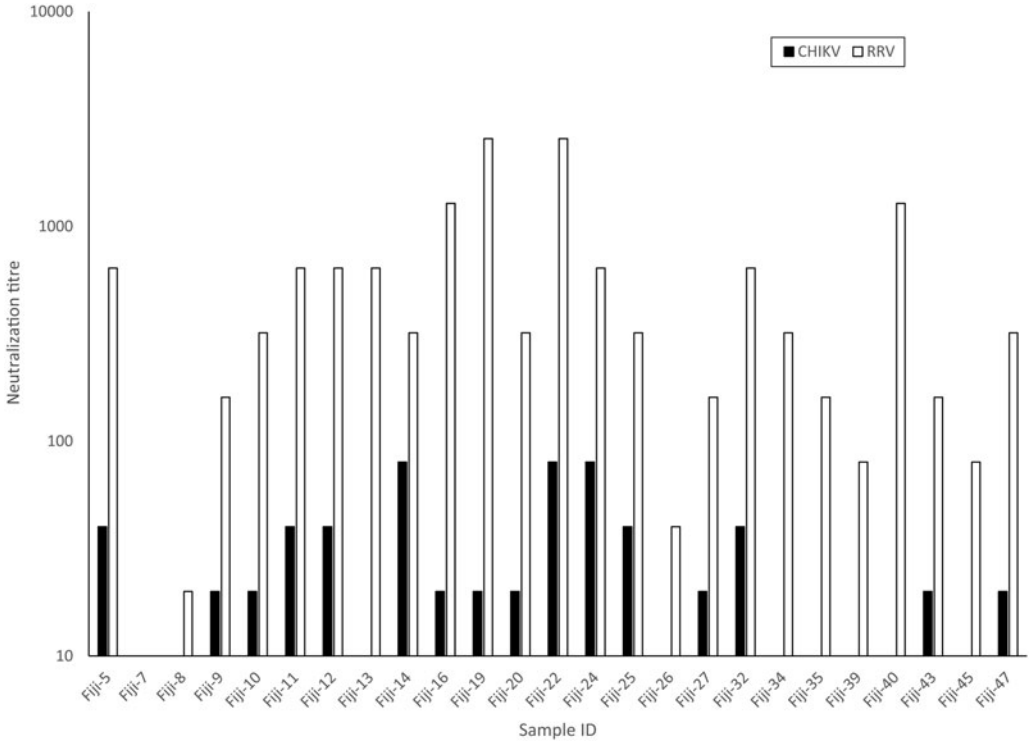

Fig. 1. Comparison of chikungunya virus (CHIKV) and Ross River virus (RRV) 50% focus neutralization test ($FRNT_{50}$) titres from 25 individual patients in Fiji. □, RRV $FRNT_{50}$ titre; ■, CHIKV $FRNT_{50}$ titre.

samples collected in 2006 in Vietnam. It appears that CHIKV infection had already occurred in Vietnam even prior to 2006 because of the positive detection of IgG in the absence of CHIKV IgM antibodies, which are associated with recent infection.

It was noted in this study that some of the IgM- and IgG-positive samples had antibodies against both DENV and CHIKV (data not shown). Since DENV and CHIKV are both transmitted by *A. aegypti* mosquitoes, it is possible that sequential infection of these two viruses during a short period of time or concurrent infection occurred in the affected patients. The difference in the results between CHIKV IgM/IgG ELISA and FRNT for positive detection of CHIKV infection indicates that it could be due to cross-reactivity of the serum samples with other alphaviruses [24].

In our study, the high RRV neutralization titre found in the Fiji samples could be due to the outbreak of RRV disease in 2003–2004 [25]. One sample (Fiji-7, Fig. 1) was CHIKV ELISA IgG positive; however, it did not show any neutralizing antibodies against CHIKV and RRV. This might be due to infection of the patient by other alphaviruses. Other studies provided evidence on the activity of other alphaviruses such as chikungunya, Getah and Sinbis viruses in Fiji; however, they did not give definite conclusions because of the insufficient volume of serum samples for further study [9, 17]. Our study suggests that RRV is being maintained in a limited number of the population in Fiji or occasionally imported from Australia.

In conclusion, our retrospective serological data suggest that CHIKV infection is widely spread in these five countries in South Asia and Southeast Asia, especially in Indonesia, the Philippines and Vietnam. However, the positive rates of CHIKV neutralization-confirmed cases in Myanmar and Sri Lanka were relatively low in the five countries and it may be due to different alphaviruses apart from CHIKV. CHIKV infection has been regionally reported in many parts of South Asian and Southeast Asian countries, and our data together with other data helped us to understand the situation of CHIK before the 2006 CHIKV outbreak.

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DECLARATION OF INTEREST

None.

REFERENCES

1. **Schwartz O, Albert ML.** Biology and pathogenesis of chikungunya virus. *Nature Reviews Microbiology* 2010; **8**: 491–500.
2. **Pfeffer M, *et al.*** Genus-specific detection of alphaviruses by a semi-nested reverse transcription-polymerase chain reaction. *American Journal of Tropical Medicine and Hygiene* 1997; **57**: 709–718.
3. **Caron M, *et al.*** Recent introduction and rapid dissemination of Chikungunya virus and Dengue virus serotype 2 associated with human and mosquito coinfections in Gabon, central Africa. *Clinical Infectious Diseases* 2012; **55**: e45–53.
4. **Chahar HS, *et al.*** Co-infections with chikungunya virus and dengue virus in Delhi, India. *Emerging Infectious Diseases* 2009; **15**: 1077–1080.
5. **Powers AM, Logue CH.** Changing patterns of chikungunya virus: re-emergence of a zoonotic arbovirus. *Journal of General Virology* 2007; **88**: 2363–2377.
6. **Economopoulou A, *et al.*** Atypical Chikungunya virus infections: clinical manifestations, mortality and risk factors for severe disease during the 2005–2006 outbreak on Reunion. *Epidemiology and Infection* 2009; **137**: 534–541.
7. **Kularatne SA, *et al.*** Epidemiology, clinical manifestations, and long-term outcomes of a major outbreak of chikungunya in a hamlet in Sri Lanka, in 2007: a longitudinal cohort study. *Journal of Tropical Medicine* 2012; **2012**: 639178.
8. **Porter KR, *et al.*** A serological study of Chikungunya virus transmission in Yogyakarta, Indonesia: evidence for the first outbreak since 1982. *Southeast Asian Journal of Tropical Medicine and Public Health* 2004; **35**: 408–415.
9. **Aaskov JG, *et al.*** An epidemic of Ross River virus infection in Fiji, 1979. *American Journal of Tropical Medicine and Hygiene* 1981; **30**: 1053–1059.
10. **Bundo K, Igarashi A.** Antibody-capture ELISA for detection of immunoglobulin M antibodies in sera from Japanese encephalitis and dengue hemorrhagic fever patients. *Journal of Virological Methods* 1985; **11**: 15–22.
11. **Ngwe Tun MM, *et al.*** Serological characterization of dengue virus infections observed among dengue hemorrhagic fever/dengue shock syndrome cases in upper Myanmar. *Journal of Medical Virology* 2013; **85**: 1258–1266.
12. **Inoue S, *et al.*** Evaluation of a dengue IgG indirect enzyme-linked immunosorbent assay and a Japanese encephalitis IgG indirect enzyme-linked immunosorbent assay for diagnosis of secondary dengue virus infection. *Vector Borne and Zoonotic Diseases* 2010; **10**: 143–150.
13. **Thaung U, *et al.*** Epidemiological features of dengue and chikungunya infections in Burma. *Southeast Asian Journal of Tropical Medicine and Public Health* 1975; **6**: 276–283.
14. **Khai Ming C, *et al.*** Clinical and laboratory studies on haemorrhagic fever in Burma, 1970–72. *Bulletin of the World Health Organization* 1974; **51**: 227–235.
15. **Thein S, *et al.*** Development of a simple indirect enzyme-linked immunosorbent assay for the detection of immunoglobulin M antibody in serum from patients following an outbreak of chikungunya virus infection in Yangon, Myanmar. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 1992; **86**: 438–442.
16. **Panning M, *et al.*** No evidence of chikungunya virus and antibodies shortly before the outbreak on Sri Lanka. *Medical Microbiology and Immunology* 2009; **198**: 103–106.
17. **Tesh RB, *et al.*** The distribution and prevalence of group A arbovirus neutralizing antibodies among human populations in Southeast Asia and the Pacific islands. *American Journal of Tropical Medicine and Hygiene* 1975; **24**: 664–675.
18. **Kanamitsu M, *et al.*** Geographic distribution of arbovirus antibodies in indigenous human populations in the Indo-Australian archipelago. *American Journal of Tropical Medicine and Hygiene* 1979; **28**: 351–363.
19. **Laras K, *et al.*** Tracking the re-emergence of epidemic chikungunya virus in Indonesia. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 2005; **99**: 128–141.
20. **Basaca-Sevilla V, Halstead SB.** Laboratory detection of arthropod-borne virus infection in the Philippines. *Journal of the Philippine Medical Association* 1966; **42**: 211–224.
21. **Centers for Disease Control (CDC).** Chikungunya fever among U.S. Peace Corps volunteers – Republic of the Philippines. *Morbidity and Mortality Weekly Report* 1986; **35**: 573–574.
22. **Retuya TJA, *et al.*** Chikungunya Fever Outbreak in an Agricultural Village in Indang, Cavite, Philippines. *Philippine Journal of Microbiology and Infectious Disease* 1998; **27**: 93–97.
23. **Inoue S, *et al.*** Detection of chikungunya virus from sera of dengue-suspected patients in the Phillippines. *Transactions of the National Academy of Science & Technology* 2000; **22**: 157–166.

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24. **Calisher CH, *et al.*** Complex-specific immunoglobulin M antibody patterns in humans infected with alphaviruses. *Journal of Clinical Microbiology* 1986; **23**: 155–159.

25. **Klapsing P, *et al.*** Ross River virus disease reemergence, Fiji, 2003–2004. *Emerging Infectious Diseases* 2005; **11**: 613–615.

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