ORIGINAL ARTICLE

Epidemiology of Chikungunya in Malaysia: 2006-2009

K B Chua, M.Med., M.D., Ph.D., FRCPE, FRCPath

National Public Health Laboratory, Infectious Disease, Makmal Kesihatan Awam Kebangsaan, Kementerian Kesihatan, Lot 1853, Kg. Melayu, Sungai Buloh, Selangor 47000, MALAYSIA

SUMMARY

This is a retrospective cross-sectional study based on the database of clusters of patients with clinical diagnosis of chikungunya (CHIK) that were referred to the National Public Health Laboratory for diagnostic investigations from January 2006 to December 2009. Of the 13,759 referred patients, a total of 6314 (45.9%) patients were laboratory confirmed to have CHIK and 7445 (54.1) patients were considered as clinical cases of CHIK by epidemiological link. Epidemic curves plotted using date of onset of illness for all referred clusters of cases showed that there were three unrelated outbreaks of CHIK in Malaysia from 2006 to 2009. There were two small outbreaks that occurred within the state of Perak in 2006. The cluster of cases in 2008 and 2009 were of related outbreak which started in Johor state and subsequently spread to various parts of Malaysia.

The mean age of the patients was 37.0 years old and those patients in the laboratory confirmed group were significantly younger than those in the epidemiological linked group. The main presenting clinical features recorded in this study were fever, arthralgia, myalgia and rashes. Those patients in the laboratory confirmed group had a significant higher incidence of fever, arthralgia and rash than those in the epidemiological linked group.

KEY WORDS:
Malaysia, chikungunya, epidemiology

INTRODUCTION

Chikungunya (CHIK) virus is a small envelope positive sense RNA virus that belongs to the genus *Alphavirus* under the family *Togaviridae*. It is transmitted to human beings by infective female mosquitoes of the *Aedes* genus (especially *Aedes aegypti*), similar to the vectors of dengue viruses.[1-4] The symptoms of CHIK virus infection are characterized by fever, headache, severe back and joint pain, rash, and lymphadenitis. The incubation period varies but is usually between two to three days. In adults there is abrupt onset of fever, headache and severe joint pain without prodromal symptoms. The joint pains are the dominant complaint and affect mainly the small joints of the hands, wrists and feet. A maculopapular rash together with a generalized lymphadenopathy may appear later. Although the arthritis may resolve within a few weeks, pain, swelling and morning stiffness may continue for months and even a year after infection. Petechiae and bleeding from gums may occur, but there are no significant haemorrhagic manifestations. Clinical illness in children tends to be less specific and may manifest as non-specific febrile viral illness with rash, vomiting and abdominal pain.[1,2]

CHIK virus was first isolated from human and subsequently *Aedes aegypti* mosquitoes during an outbreak in Tanzania in 1953. Following which, CHIK virus has caused occasional outbreaks and some larger epidemics throughout most of sub-Sahara Africa and tropical Asia including India and the Western Pacific. Historical evidence suggests the spread of CHIK virus from Africa to Asia, where it has caused outbreaks in the Philippines, Thailand, Indonesia, India, Sri Lanka, Vietnam, Kampuchea and Myanmar since 1954.[5-8] The epidemiology of CHIK virus infection in Africa differs from that of Asia. In Africa, the most important animal host in maintaining the cycle of CHIK virus infection are the non-human primates such as baboons and Cercopithecus monkeys. Humans may be infected in African villages and rural areas, particularly where Aedes aegypti is present in large numbers. In contrast to the situation in Africa, transmission in Asia is primarily from human to human by Aedes aegypti in the urban areas .[3-8] However, Aedes albopictus has been implicated lately as the main mosquito vector in the human to human transmission of the virus in rural areas following genetic mutation of the virus that has enable the virus to adapt well in A. albopictus.[9]

The epidemiology of CHIK in Malaysia is rather interesting and unique in this region. Prior to the occurrence of CHIK in Malaysia, three alphaviruses (Getah, Bebaru and Sindbis) have been isolated in the Peninsular Malaysia, but were not known to be associated with any human clinical infections, except for a single case of mild fever attributed to Sindbis virus.10 A serological survey for alphaviruses, especially CHIK, conducted by Marchette et al (1978) in Peninsular Malaysia showed that anti-CHIK antibody was detected in persons older than 20 years and mainly in the northern states, such as Perlis, Kedah and Kelantan, bordering Thailand.[10] A second study by Marchette et al (1980) showed specific haemagglutination inhibition and neutralizing antibodies in a chicken in Kelantan and a pig in Kedah, further supporting CHIK activity along the Malaysia-Thailand border. The study found that Malays, who are mainly rural and aborigines, who are forest-dwellers, had higher frequencies of anti-CHIK antibody, and suggested that monkeys could serve as vertebrate hosts in Malaysia.[11] Further study conducted on Carey Island, situated in the state of Selangor (central western part of peninsular Malaysia), where monkeys were abundant in the mangrove and plantations, showed plantation workers had anti-CHIK antibody at fairly high frequency.[11] Despite the demonstration of the presence of anti-CHIK in human, there has been no report of clinical disease or outbreak due to this virus but the study anticipated

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Corresponding Author: Kaw Bing Chua, M.Med., M.D., Ph.D., FRCPE, FRCPath, Consultant Virologist, National Public Health Laboratory, Infectious Disease, Makmal Kesihatan Awam Kebangsaan, Kementerian Kesihatan, Lot 1853, Kg. Melayu, Sungai Buloh, Selangor 47000, MALAYSIA Email: chuakawbing@yahoo.com.sg

such an outbreak in peninsular Malaysia within the next two decades. As predicted by the previous study, Malaysia experienced the first outbreak of CHIK in late 1998 due to CHIK virus of Asian genotype, involving residents in the suburb of Klang, a coastal city within the state of Selangor in the central western part of peninsular Malaysia. Because of its first occurrence, the diagnosis was only confirmed in the laboratory at the end of January 1999.[12] This paper reports the epidemiology of subsequent outbreaks of CHIK in Malaysia from 2006 till 2009.

MATERIALS AND METHODS

Study population:
This is a retrospective cross-sectional study. The sample population was from the National Public Health Laboratory (NPHL), Ministry of Health databases of chickungunya laboratory diagnostic investigations, from January 2006 to December 2009. NPHL is the main laboratory in Malaysia that provides laboratory diagnosis of chikungunya from 2003. A substantial proportion of chikungunya laboratory diagnostic service is supported by the Institute for Medical Research, Ministry of Health Malaysia and a limited localized CHIK diagnostic service is provided by the Department of Medical Microbiology, University Malaya Medical Centre. Any suspected case of CHIK based on clinical diagnosis (fever, rash and/or joint pain) by doctors or public health specialists referred to NPHL for laboratory investigation are captured in the databases. The serum sample of any suspected case of CHIK is subjected to a combination of three types of laboratory tests (virus isolation, molecular detection of CHIK virus RNA, and assay of anti-CHIK specific IgM) for diagnostic confirmation

Laboratory Tests:
The serum samples from patients with a history of illness of 4 days or less were processed for virus isolation (routine) and molecular detection of CHIK virus RNA (only for cases of special request for urgent result). Serological assay of anti-CHIK IgM was routinely carried out on serum samples of patients with illness of more than 5 days and all three types of diagnostic tests were performed on serum samples collected from patients on the 5th day of illness.

Virus isolation was carried out using C6/36 cells (ATCC CRL-1660) and Vero cells (ATCC CCL-81) cultured in JM cell culture tubes. The presence of CHIK virus in infected C6/36 cells after 10 days of culture was identified using treated human convalescent serum known to contain high level of anti-CHIK specific IgG by indirect immunofluorescence assay. As for Vero cells, any inoculated cells that showed cytopathic effect (CPE) within 10 days of culture were harvested. The identification of CHIK virus was similarly carried out using same treated human convalescent serum by indirect immunofluorescence assay.

Molecular detection of CHIK virus genome was carried out by RT-PCR. Briefly, CHIK virus RNA was extracted from 200 µl of patient's serum sample using a viral RNA extraction kit (Roche Diagnostics, Germany). CHIK virus specific oligonucleotide primers used for the amplification of CHIK virus genomic sequence fragments was in accordance to Hasebe *et al*.[13] Reverse transcriptase polymerase chain reaction (RT-PCR) was performed in a single reaction tube using the Access RT-PCR Kit (Promega Corporation, USA). Each genomic fragment was amplified in a 50-µl reaction mix containing the respective CHIK virus forward and reverse primers of 20 pmol each and 2 µl of the extracted viral RNA as template. Each reaction mix was subjected to a 60-minute of reverse transcription at 420C, reverse transcriptase inactivation of 98 0C for 5 minutes, followed by 35 cycles of amplification at a denaturing temperature of 95 0C for 30 seconds, annealing temperature of 54 0C for 30 seconds and an extension temperature of 72 0C for 30 seconds per cycle. The amplified products were confirmed by electrophoresing 3 µl of each of the amplified products in a 1% agarose gel.

Qualitative serological assay for anti-CHIK virus specific IgM was performed by indirect immunofluorescent test using in-house prepared Vero cells infected with known CHIK virus that were seeded on wells of Teflon coated slide. The procedure for carrying the tests was as described previously except in this assay, the CHIK virus infected cells was used as antigen.[6]

Data management and analysis:
The demographic, epidemiological and clinical features of the patients together with laboratory data were tabulated in appropriate worksheets using the Microsoft Excel program. All data analyses were performed using both Microsoft excel 2007 and SPSS version 16.0. The distribution of the chikungunya records was analysed by age, sex, days of fever, presence of related symptoms and laboratory results. The patient was considered as a confirmed case CHIK if his or her serum sample was tested positive by any or a combination of the three types of laboratory tests performed in this study. If none of the laboratory test was positive or no laboratory result was available, it was considered as a clinical case of CHIK by epidemiological link to related laboratory confirmed case of the outbreak within the locality. The epidemic curve was plotted by using date of onset (according to respective epidemiology week) for each of the year 2006, 2008 and 2009 to describe the pattern of spread of the disease.

RESULTS

All cases referred for laboratory investigations were from clusters of patients with clinical diagnosis as suspected cases of chikungunya. A total of 13,759 patients with clinical diagnosis of chickungunya were referred to the National Public Health Laboratory for laboratory confirmation of the disease within the period of 2006 to 2009. The highest number of cases referred for investigation was in 2008 (8320 cases) while the lowest number was in 2006 (108 cases). The types of diagnostic laboratory tests performed on each of the patients' serum specimens received for laboratory confirmation of CHIK for the year 2006, 2008 and 2009 and their respective results are shown in Table I. Based on the case definition stated earlier, a total of 6314 (45.9%) patients were laboratory confirmed to have CHIK and 7445 (54.1) patients were considered as clinical cases of CHIK by epidemiological link. Overall, slightly less than 50% of the referred cases was laboratory confirmed to have CHIK and approximately the same percentage of patients were laboratory confirmed as having CHIK for the year 2006, 2008 and 2009.

From the number of clinical cases referred by each state for laboratory confirmation of CHIK, Perak state referred the highest number of cases (3709) for laboratory investigation for CHIK over the whole period and closely followed by Johor state (3497 cases). However, Johor referred the highest number of cases in the year 2008 (2964 cases) and Perak in the year 2009 (1369 cases) (Table II). As for the total number of

laboratory confirmed cases of CHIK, Johor ranked the highest with 1525 (24.2%) confirmed cases and Perak was the second highest (1515 cases, 24.0%). During the year 2006, there was no reported case from other states except the state of Perak. In that year, there were 108 cases reported from Perak state involving 3 district health offices (DHO) namely; DHO Larut Matang Lama, DHO Kinta and DHO Kerain. The only region within the state of Malaysia which did not refer any case for laboratory investigation of CHIK during the whole duration of 2006 to 2009 was the Federal Territory of Labuan.

Epidemic curves were plotted by using date of onset of illness (according to the respective epidemiology week) for all referred clusters of cases with clinical diagnosis of chikungunya to give a simple visual display of outbreak's magnitude and its time trend. The cluster of cases seen in 2006 was plotted separately from the clusters of cases in 2008 and 2009 as they appeared to be unrelated outbreaks with no case recorded in 2007. In 2006, the epidemic curve (Fig. 1) shows the epidemiology week (based on date of onset of illness) versus number of cases occurred during the outbreak in the Perak state. It started from epidemiology week 9 to week 17. There were no reported cases from epidemiology week 1 to 8 and week 12. The number of cases increased slowly from week 9 to 11, but there was a sudden upswing of cases which reached the peak at week 13 (41 cases) and declined gradually after week 14 but there was a second sudden upswing of cases seen in week 17 (Fig. 1).

The epidemic curve for the years from 2008 to 2009, shows a propagated or intermittent type whereby several peaks can be seen from the plot. Cases were reported from epidemiology week 2 of 2008 to week 52 of 2009. There were no cases being reporteded from week 3 to 16 of the year 2008 before it was seen again in week 17. Cases were noted to increase gradually in number from week 23 (about 25 cases), but surged in week 31 (almost 550 cases), and followed by a sudden drop to 200 cases in week 32. It peaked again at week 33 (380 cases). It was then followed by a declining trend to week 40 before it started to peak at week 49 and subsequently started to decline again at week 51(Figure 2). For the year 2009, the epidemic curve has the same pattern as in the year 2008 (propagated or intermittent type). Cases were first reported and peaked at epidemiology week 1(620 cases). Cases were noted to decrease gradually from week 2 to week 14 and started to increase and peaked at week 19. After the peak on week 19, it showed a declining trend until week 52 (Figure 10).

Table III summarizes the epidemiological and clinical features of the patients, both confirmed by laboratory tests and epidemiological linked cases. The mean age of the patients was 37.0 years old and those patients in the laboratory confirmed group were significantly younger than those in the epidemiological linked group. Though there were a slightly higher number of male patients in comparison to females, there was no significant gender difference. The main presenting clinical features recorded in this study were fever, arthralgia, myalgia and rashes. However, not all patients had all the presenting clinical features stated. Those patients in the laboratory confirmed group had a significant higher incidence of fever, arthralgia and rash than those in the epidemiological linked group.

DISCUSSION

Chikungumya is a re-emerging mosquito-borne viral infection. Malaysia experienced the first outbreak of CHIK in late 1998 involving residents staying in suburb of Klang, Selangor due to CHIK virus of Asian genotype.[12] Following a hiatus of 7 years, CHIK re-emerged in 2006 causing a localized outbreak in a north-western coastal town in the district of Larut Matang Lama within the state of Perak.[14] Based on the epidemic curve, the cluster of cases in Perak in 2006 appeared to be due to two unrelated outbreaks with no case reported in the epidemiology week 12. This was confirmed by molecular study of CHIK viruses isolated from cluster of CHIK cases from various districts. The CHIK virus isolated from patients in the district of Larut Matang Lama was of Asian genotype whereas the CHIK virus isolated from cases in Kinta district was of Central/East African genotype.[14,15] Detailed epidemiological investigation by Noridah et al showed that the cluster of cases from the district of Kerian recorded in epidemiology week 17 (Figure 1) was due to the spread from Kinta district.[15] This was further supported by molecular study of isolated viruses from both districts.[15] Molecular analysis also showed that the CHIK virus caused the outbreak in Kinta district was related to CHIK virus of Central/East African genotype which has spread from East African to Indian Ocean islands and India since 2004.[16,17] Study by Noridah et al showed the introduction of CHIK virus of Central/East African genotype into the Kinta district was due to movement of people between peninsular Malaysia and India.[15]

The clusters of CHIK cases seen in the years 2008 and 2009 appeared to be of related epidemic and not related to the outbreaks that occurred in Perak in 2006. The epidemiological data was supported by molecular analysis of CHIK viruses isolated from various states at various times of the period.[18] The latest epidemic appeared to begin in the state of Johor in early 2008, during which Singapore also experienced cluster of CHIK cases (over 200 notified).[19] The CHIK virus was a Central/East African genotype separately introduced into Malaysia apart from the strain seen in Kinta district earlier.[18] From the epicenter in Johor state, the epidemic has spread to various parts of the country. In 2008, besides Johor and Perak, the states with the most number of cases were Selangor, Melaka, Negeri Sembilan and Pahang. As the epidemic progressed into 2009, the number of cases peaked in Kelantan, Kedah, Terengganu and Perlis, the states in the north of peninsular Malaysia. Sarawak too recorded its peak in 2009. Sabah was largely spared in 2009, recording only three cases in 2008 but has a large surge of cases in early 2010 (unpublished data). The urban centres of Kuala Lumpur and Pualu Pinang recorded few cases throughout the whole epidemic even while the states surrounding them were affected. The outbreak was by far much larger than the previous ones that occurred and ended in Perak. The factors which could have contributed to the uncontrolled spread of CHIK in this epidemic were as suggested in Chem *et al*.[18]

The original plan was to perform a combination of laboratory tests for confirmation of CHIK. However, as the epidemic evolved, especially from mid-2008 onwards, the laboratory was overwhelmed by request for laboratory confirmation of CHIK. Thus, only a small number of serum samples were tested by RT-PCR in 2008 for urgent confirmation of CHIK outbreak and no molecular test (RT-PCR) was performed on serum samples received in 2009. Otherwise, the percentage of laboratory confirmed cases could be higher.

The presenting clinical features of patients with CHIK infection

Table I: The number of serum specimens received on each respective year and types and results of laboratory tests performed on each of the serum specimens.

Type of Test	Result by number (%)			
	2006	2008	2009	TOTAL
	n = 108	n = 8320	n = 5331	n = 13,759
IgM antibody:				
Detected	35 (32.4)	2008 (24.2)	1482 (27.8)	3525 (25.6)
Not Detected	39 (36.1)	2202 (26.5)	1539 (28.9)	3780 (27.5)
Borderline	8 (7.4)	1 (0)	0 (0)	9 (0.1)
Not process	12 (11.1)	4105 (49.3)	2310 (43.3)	6427 (46.7)
Missing value	14 (13.0)	4 (0.0)	0(0)	18 (0.1)
RT- PCR:				
Detected	13 (12.0)	115 (1.4)	-	128 (0.9)
Not Detected	55 (50.9)	86 (1.0)	-	141 (1.0)
Contamination	8 (7.4)	1 (0)	-	9 (0.1)
Not process	18 (16.7)	8118 (97.6)	5331	13469(97.9)
Missing value	14 (13.0)	0 (0)	-	14 (0.1)
Virus isolation:				
Virus isolated	13 (12.0)	1814 (21.8)	916 (17.2)	2743 (19.9)
No virus isolated	72 (66.7)	2262 (27.2)	1408 (26.4)	3742 (27.2)
Contamination	5 (4.6)	95 (1.1)	7 (0.1)	107 (0.8)
Not process	18 (16.7)	4148 (49.9)	3000 (56.3)	7166 (52.1)
Summary				
Laboratory confirmed	49 (45.4)	3870 (46.5)	2395 (44.9)	6314 (45.9)
Not confirmed	59 (54.6)	4450 (53.5)	2936 (55.1)	7445 (54.1)

Table II: Distribution of chikungunya cases by states and the year 2006, 2008 and 2009.

State	2006		2008		2009		Total	
	No. of cases	No. Confirmed (%)	No. of cases	No. Confirmed (%)	No. of cases	No. Confirmed (%)	No. of cases	No. Confirmed (%)
Johor	0	0	**2964**	1367 (46.1)	533	158 (29.6)	3497	**1525** (43.6)
Kedah	0	0	218	90 (41.3)	807	426 (52.8)	1025	516 (50.4)
Kelantan	0	0	247	136 (55.1)	1057	592 (56.0)	1304	728 (55.8)
Kuala Lumpur	0	0	21	9 (42.9)	10	1 (10.0)	31	10 (32.3)
Melaka	0	0	762	390 (54.1)	10	2 (20.0)	772	392 (50.8)
N. Sembilan	0	0	613	304 (49.6)	52	24 (46.2)	665	328 (49.3)
P.Pinang	0	0	1	0	39	16 (41.0)	40	16 (40.0)
Pahang	0	0	441	228 (51.7)	187	97 (51.9)	628	325 (51.8)
Perak	**108**	**49 (45.4)**	2232	917 (41.1)	**1369**	549 (40.1)	**3709**	1515 (40.8)
Perlis	0	0	0	0 (0.0)	20	11 (55.0)	20	11 (55.0)
W.P Putrajaya	0	0	34	18 (52.9)	27	10 (37.0)	61	28 (45.9)
Sarawak	0	0	3	1 (33.3)	348	121 (34.8)	351	122 (34.8)
Selangor	0	0	820	410 (50.0)	629	291 (46.3)	1449	701 (48.4)
Terengganu	0	0	2	0	243	97 (39.9)	245	97 (39.6)
Sabah	0	0	3	0	0	0	3	0
Total	**108**	**49 (45.4)**	**8320**	**3870 (46.5)**	**5331**	**2395(44.9)**	**13,759**	**6314 (45.9)**

Table III: Epidemiological and Clinical features of patients with chikungunya: laboratory confirmed cases verses epidemiological linked cases.

Variable	Laboratory confirmed (%) (n =6314)	Epidemiological linked cases (%) (n = 7445)	Total number of cases (%) (n = 13,759)	Statistic
Age	35.9 ± 18.1	38.0 ± 19.1	37.0 ± 18.6	p = 0.002
Gender:				χ^2 = 2.71
Female	3108 (49.2)	3557 (47.8)	6665 (48.4)	p = 0.0999
Male	3202 (50.7)	3877 (52.1)	7079 (51.5)	
Missing data	4 (0.1)	11 (0.1)	15 (0.1)	
Clinical Feature:				
Fever:				χ^2 = 21.55
Yes	5590 (88.5)	6386 (85.8)	11976 (87.0)	p < 0.0000
No	647 (10.3)	950 (12.8)	1597 (11.6)	
Missing data	77 (1.2)	109 (1.4)	186 (1.4)	
Duration of fever	4.28± 4.39[a]	4.81± 7.03[a]	4.55± 5.71[a]	p < 0.0000
Athralgia:				χ^2 = 79.53
Yes	2282 (36.1)	2156 (29.0)	4438 (32.2)	P < 0.0000
No	3953 (62.6)	5179 (69.5)	9132 (66.4)	
Missing data	79 (1.3)	110 (1.5)	189 (1.4)	
Myalgia:				χ^2 = 1.57
Yes	662 (10.5)	828 (11.1)	1490 (10.8)	p = 0.2107
No	5574 (88.3)	6506 (87.4)	12080 (87.8)	
Missing data	78 (1.2)	111 (1.5)	189 (1.4)	
Rashes:				χ^2 = 26.69
Yes	1119 (17.7)	1076 (14.4)	2195 (16.0)	p < 0.0000
No	5118 (81.1)	6261 (84.1)	11379 (82.7)	
Missing data	77 (1.2)	108 (1.5)	185 (1.3)	
Headache:				χ^2 = 1.13
Yes	256 (4.1)	275 (3.7)	531 (3.8)	p = 0.2871
No	5980 (94.7)	7059 (94.8)	13039 (94.8)	
Missing data	78 (1.2)	111 (1.5)	189 (1.4)	

Figure 1: Epidemic curve of chikungunya outbreak in Malaysia for the year 2006.

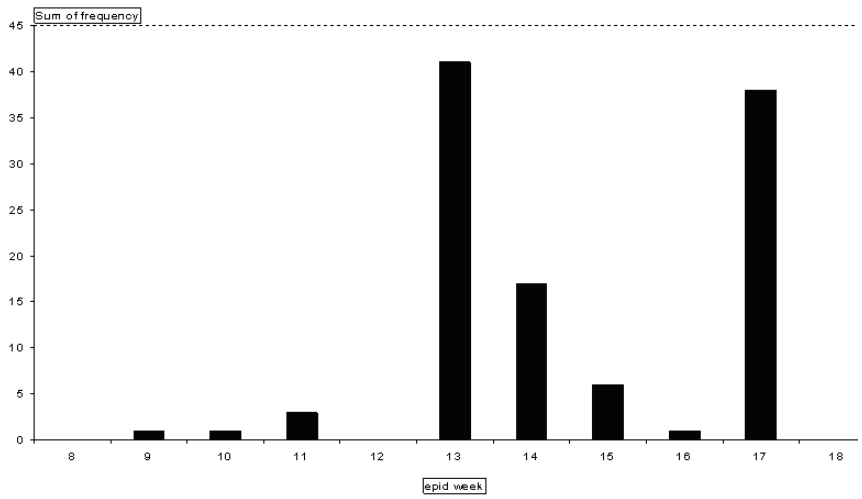
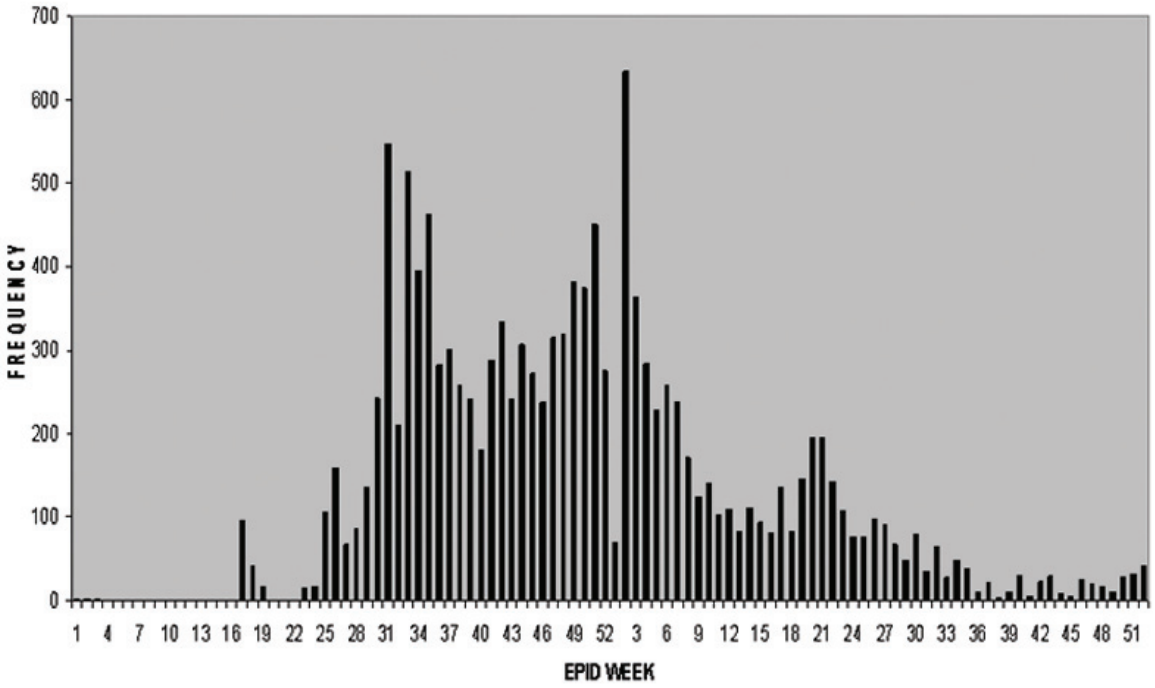


Figure 2: Epidemic curve of chikungunya outbreak in Malaysia for the years 2008 and 2009



were of no different from those reported in other studies.[16,17] There was no reported mortality due to CHIK for the years 2006, 2008 and 2009 except for a recent reported fatal case in Sarawak.[20]

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