Tracking the re-emergence of epidemic chikungunya virus in Indonesia

Kanti Larasa, Nono C. Sukria, Ria P. Larasatia, Michael J. Bangsa, Rizal Kosim, Djauzid, Tony Wandra, John Master, Herman Kosasih, Sri Hartati, Charmagne Beckett, Endang R. Sedyaningsih, H. James Beecham III, Andrew L. Corwin

a US Naval Medical Research Unit No. 2 (US NAMRU-2), Box 3, Unit 8132, Jakarta, Indonesia
b FPO AP 96520-8132, USA
c US NAMRU-2, Jl. Percetakan Negara No. 29, Kompleks P2M-PLP/LitBangKes, Jakarta Pusat 10560, Indonesia
d Centers for Communicable Diseases — Prevention and Environmental Health (P2M-PLP), Indonesian Ministry of Health, Jl. Percetakan Negara No. 29, Jakarta Pusat 10560, Indonesia
e National Institute of Health Research and Development (BalitBangKes), Indonesian Ministry of Health, Jl. Percetakan Negara No. 29, Jakarta Pusat 10560, Indonesia

Received 5 January 2004; received in revised form 15 March 2004; accepted 16 March 2004
Available online 28 October 2004

KEYWORDS
Chikungunya virus; Epidemic; Indonesia

Summary
Twenty-four distinct outbreaks of probable chikungunya (CHIK) etiology were identified throughout Indonesia from September 2001 to March 2003, after a near 20-year hiatus of epidemic CHIK activity in the country. Thirteen outbreak reports were based on clinical observations alone, and 11 confirmed by serological/virological methods. Detailed epidemiological profiles of two investigated outbreaks in Bogor and Bekasi are presented. Human sera were screened using an ELISA for IgM and IgG anti-CHIK antibodies. Additionally, reverse transcriptase PCR and virus isolation were attempted for virus identification. The mean age of cases was 37 ± 18 years in Bogor and 33 ± 20 years in Bekasi. There was no outstanding case-clustering, although outbreak-affected households were observed to be geographically grouped within villages. The attack rates in Bogor and Bekasi were 2.8/1000 and 6.7/1000 inhabitants respectively. Both outbreaks started in the rainy season following increased Aedes aegypti and A. albopictus densities.

© 2004 Royal Society of Tropical Medicine and Hygiene. Published by Elsevier Ltd. All rights reserved.

1. Introduction
Outbreaks of chikungunya (CHIK) have been reported in Africa and many parts of Asia. Epidemic
CHIK infections were reported in Bangkok until the mid-1970s (Burke et al., 1985), after which virus activity virtually disappeared. Sporadic clinical CHIK cases were again recognized in Bangkok in 1988 (Lam et al., 2001) with the last reported CHIK outbreak occurring in Thailand during the 1995 rainy season (Thaikruea et al., 1997). Sporadic and epidemic CHIK has also been reported from Cambodia (Chastel, 1963), Viet Nam (Dai and Thoas, 1967), Laos (Halstead and Udormakdi, 1966), Malaysia (Khai Ming et al., 1974), Indonesia (Siemons et al., 1984), and the Philippines (CDC, 1986). The first CHIK outbreak in Malaysia was documented in 1998—1999, although CHIK antibody had been found in human sera before then in the absence of disease (Lam et al., 2001). The most recently reported cases of CHIK in the Philippines were diagnosed from three US Peace Corps Volunteers in 1986, ending an apparent period of inactivity which began 18 years earlier in 1968 (CDC, 1986).

In Indonesia, CHIK antibody was first identified during a serosurvey conducted in 1972, suggesting that CHIK virus was widely distributed in the areas screened (Kanamitsu et al., 1979). Outbreaks of CHIK have historically been documented throughout Indonesia. The first outbreak, designated as a "knuckle fever," was inferred from Dutch anecdotal memoirs to have heralded from Batavia (present-day Jakarta) in 1779 (Carey, 1971). In 1973, a febrile disease outbreak of unknown origin was reported in Samarinda and Balikpapan, along the east coast of Kalimantan (Indonesian Borneo), presenting signs and symptoms compatible with CHIK (Kanamitsu et al., 1979). Ten years later (1983), a series of CHIK outbreaks occurred in the Special Area of Yogyakarta (central Java), with an estimated attack rate of 70—90% (Haksohusodo, 1990; Slemons et al., 1984). Additionally, eight other outbreaks of possible CHIK were reported between 1982 and 1985 throughout Indonesia (Mackenzie et al., 2001). There have been no published accounts of CHIK outbreaks in Indonesia since 1985, until the present report.

CHIK virus infection is notably characterized by abrupt clinical onset, involving fever and often severe arthralgia or arthritis in the extremities. This is followed by constitutional symptoms that include maculopapular rash on the trunk and limbs (Chin, 2000; Jupp and McIntosh, 1988), misdiagnosis and underreporting of this disease in dengue-endemic areas is considered common.

The objectives of this report are five-fold: (i) to confirm epidemic CHIK occurrence associated with febrile illness and arthralgia, (ii) to identify the causative etiology, (iii) to determine the "measure of impact" (attack rate) within the community, (iv) to identify possible demographic and/or environmental factors contributing to increased risk, and (v) to describe the temporal and spatial movement of epidemic CHIK eastward across the Indonesian archipelago, from 2001 to 2003.

2. Materials and methods

2.1. Outbreak occurrences

The 24 CHIK outbreak episodes were principally recognized from 2001 to 2003, through general disease reporting communicated through routine surveillance, namely weekly reports from community health centers (PusKesMas) to district and provincial health authorities, and ultimately, to the national level Centers for Communicable Diseases and Prevention—Environmental Health (CDC-EH). Additionally, outbreak information was communicated through anecdotal reporting from district and/or provincial sources, and public pronouncements in local newspaper accounts. Outbreaks were classified as either laboratory-confirmed or presumed on clinical presentation only. Laboratory-confirmed outbreaks were those in which specimens from patients were positive for CHIK by IgM/IgG ELISA and/or reverse transcriptase PCR (RT—PCR). Clinically recognized outbreaks were generally defined as clusters of persons ill with signs and symptoms compatible with CHIK etiology (fever with symptoms of rash and/or joint pain), but lacking laboratory test evidence. Specimens were made available for laboratory diagnostic evaluation from 11 of 24 clinically reported outbreaks.

2.2. Investigated outbreaks

Detailed epidemiological profiles are presented of two investigated CHIK outbreaks in Bogor and Bekasi, West Java Province, western Indonesia (Figure 1). The first outbreak investigated, lasting two months beginning in October 2001, was in Kedung Badak and Kebun Pedes kecamatan (subdistricts), Tanah Sareal District, on the periphery of the city of Bogor which is located approximately...
56 km south of Jakarta, the capital city of Indonesia. The two subdistricts are situated between 106°43′—106°51′E and 60°30′—60°40′S, at an elevation of 200–350 m asl. The second outbreak investigated, from February to June 2002, was in Kali Jaya village, Cikarang Barat district, Bekasi Regency, approximately 45 km east of Jakarta between 106°48′—107°27′E and 6°10′—6°30′S, at an elevation of 25 m asl.

2.3. Outbreak chronologies

In November 2001, national newspaper accounts referred to a ‘strange’ outbreak of disease near Bogor, reportedly involving approximately 100 cases with symptoms that included fever, rash, joint pain and loss of ability to walk in some cases. Anecdotal reports at the time suggested that the outbreak might have been associated with CHIK virus. An investigation team was assembled rapidly and deployed from 30 November to 7 December by the Indonesian Ministry of Health, with institutional representation from the Indonesian National Institute of Health Research and Development (NIHRD), CDC-EH, and US Naval Medical Research Unit No. 2 (US NAMRU-2) to support the local district health service’s response.

On 6 June 2002, the Bekasi Regency Health Authority Office contacted US NAMRU-2 reporting an outbreak of ‘unknown’ disease in Kali Jaya village. The outbreak was reported to have begun in February, in one part of the village, and had subsequently spread to two other areas. By early accounts, an estimated 163 people were believed to be afflicted. There was considerable confusion in identifying the cause of the outbreak, as earlier it had been presumed to be leptospirosis. However, testing on 12 specimens yielded only one reactive Leptospira sample by ELISA, whereas seven sera were ELISA-positive for IgM CHIK. An investigation team made up of the same institutions
mentioned in the outbreak response near Bogor from 17–22 June 2002, in support of the local district health service’s response.

2.4. Study subjects

Community-based, case–control investigations were conducted in both outbreak areas of Bogor and Bekasi. Patients presenting with fever, rash and/or joint pain were identified as suspected CHIK cases. Healthy family members of cases (those residing in the same household) were also screened to assess possible familial clustering. Controls were randomly selected from healthy individuals in the affected community, carefully matched by age and gender. Controls served to exclude possible remnant viral background infections with other outbreak etiologies. One village with no recent case reports suggestive of CHIK was chosen as a control village. For comparison, a cross-sectional study was carried out in the control village.

2.5. Specimen and data collection

After informed consent, one venous blood sample (5–7 ml) was obtained from each volunteer case and control subject using a Vacutainer® (Becton Dickinson, Franklin Lakes, NJ, USA) without additive. At the same time, day collections of indoor resting mosquito populations using a sweep net sampling device were made from suspected CHIK-infected households. Sera and mosquitoes were stored in liquid nitrogen and transferred to the US NAMRU-2 laboratory for further analysis. Demographic information and clinical signs and symptoms were collected from each participant using a study-specific questionnaire, administered by local health authorities. All investigative actions were predicated on formal request letters for participation and assistance through the NIHRD. Informed voluntary consent was obtained from all participating individuals before entering the study.

2.6. Laboratory methods

For detecting active, recent or past CHIK infections, tests were performed using ELISA for detecting IgM and IgG antibodies, a nested RT–PCR technique and virus isolation using cell culture. Clinically-suspected CHIK cases that presented with hemorrhagic manifestation that included bleeding of gums and/or epistaxis were additionally examined for IgM antibodies to dengue virus by ELISA using the Dengue Fever Virus IgM Assay (Focus Technologies, Cypress, CA, USA).

2.7. Detection of IgM and IgG antibodies against chikungunya virus

Serum samples were assayed for the presence of the IgM and IgG antibodies against CHIK using an antigen capture ELISA (Porter et al., 2004). The sample was considered as positive if the optical density (OD) value exceeded the mean plus three standard deviations from normal sera.

2.8. Detection of chikungunya viral RNA

Viral RNA was extracted from all sera and mosquito samples by the QiaAmp extraction method (Qiagen extraction method (Qiagen Viral RNA Mini Kit, Valencia, CA, USA), following the manufacturer’s protocols. Viral RNA was detected using a nested RT–PCR assay. Nested primer pairs were designed to amplify three structural gene regions, CapS (C), Envelope-2 (E2), and an area overlapping the 6K and E1 genes (6K/E1). All nested primer sets were also tested against all four dengue serotypes (DENV 1–4), Ross River virus and Japanese encephalitis virus and were found to be specific for CHIK. Viral RNA was reverse-transcribed to cDNA, which was concomitantly amplified in the same tube using the Promega Access RT/PCR Reagent Kit (Madison, WI, USA), utilizing a cocktail containing all outer primers (C: 5′ ATG GAG TTT ATC CCA ACC CA 3′, 1-20 and 5′ GCA GAC GCA GCG AGG GCC AG 3′, 1201–1220; E2: 5′ GCA GAC GCA GCG AGG GCC AG 3′, 1201–1220 and 5′ GTG GCT GCA AGG TAG TTC TC 3′, 1440–1460; 6K/E1: 5′ ATG CAA CAG ACC GGG CTA CA 3′, 2481–2500 and 5′ GCC TCA ATT GCG TAT TTT CA 3′, 2740–2761). Inner primer pairs (C: 5′ AAC TTT CTA CAA TAG GAG GT 3′, 21–40 and 5′ GTG GCT TTT TTG CCT TCT TA 3′, 220–220; E2: 5′ GCT ATT TGT AAG AAC GTC AG 3′, 1221–1240 and 5′ TAC GCT GCT GCG GTC GGG AA 3′, 1420–1440; 6K/E1: 5′ GCC CAA TGG TAC TGG AGA TG 3′, 2501–2520 and 5′ GTG TTC CAG AAG CAG TAG GC 3′, 2720–2701) were then used in separate nested-PCR reactions with separate reagents (PE Applied Biosystems, Foster City, CA, USA) for each gene. The predicted product size for the amplicon was 200 nucleotides for all three genes.

2.9. Chikungunya virus isolation

Serum was diluted 1:10 in PBS and inoculated onto confluent monolayers of C6/36 Aedes albopictus cells (ATCC Cell Repository Line 1660) in 24-well
culture plates. The plates were then incubated at 30°C for 14 days and observed daily for evidence of cytopathic effects (CPE). At the end of 14 days or upon recognition of CPE, cells were removed from the plates and evaluated for presence of CHIK virus by an immunofluorescence assay using anti-CHIK (arbovirus group A) hyperimmune mouse ascitic fluid (Pavri, 1973).

2.10. Mosquito collections

All mosquitoes collected from indoor resting sites were identified to species based on morphological characters. Mosquitoes were pooled by species and locality and tested for the presence of CHIK viral RNA using a nested RT–PCR.

3. Results

From September 2001 to March 2003, 24 suspected outbreaks of CHIK virus were reported throughout Indonesia (Figure 2). Most outbreaks (83%) occurred on the main island of Java, nearly half (46%) occurring in the densely populated province of Central Java. CHIK outbreak occurrences were
also reported from Aceh (1 episode), North Sumatra (1), North Sulawesi (1) and Lombok Island (2). These outbreaks occurred in urban (21%), semi-urban (neighborhood and village) (17%) and rural (62%) communities, mirroring roughly the distribution of the human population in Java. The first instance of suspected epidemic CHIK occurred in Bireun, Aceh province, located in northwesternmost Indonesia in September 2001, progressively moving eastward in the country, with transmission last seen in Pasuruan (East Java), Klaten (Central Java), Tangerang and Bekasi (West Java), in March 2003. Correspondingly, the number of epidemic CHIK foci increased with time, from just two in 2001, to eight in 2002 and 14 during the first three months of 2003. Seventy-five percent (18/24) of outbreak episodes were reported from November 2002 to March 2003 (Table 1). The total number of reported epidemic-associated cases during this time period was 5821 (mean 253 patients per outbreak event, range 37–1031 cases/event).

3.1. Laboratory confirmed outbreaks

From the 11 outbreak episodes subjected to laboratory follow-up examination, CHIK was incriminated as the etiology in all instances. Overall, evidence of recent and acute CHIK infections, based on serology and/or RT-PCR, was demonstrated in 47% (325/696) of serum samples assayed. Confirmation of ten outbreak episodes of CHIK was based on presence of IgM antibody and/or viral RNA, found in 25% to 100% of samples tested. CHIK was implicated in the Bolaang Mongondow (North Sulawesi) outbreak by presence of IgG antibody alone (Table 1).

3.2. Bogor outbreak

3.2.1. Background

Historical trends from community health center records dating from 1998 to January 2002

<table>
<thead>
<tr>
<th>No.</th>
<th>Regency/city</th>
<th>Province</th>
<th>Month, year</th>
<th>Cases</th>
<th>Laboratory confirmed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No. of case sera</td>
<td>Percentage positive (%)</td>
</tr>
<tr>
<td>1</td>
<td>Bireun, regency</td>
<td>Aceh</td>
<td>Sept–Nov 2001</td>
<td>420</td>
<td>NC</td>
</tr>
<tr>
<td>2</td>
<td>Bogor, city</td>
<td>West Java</td>
<td>Oct–Nov 2001</td>
<td>119</td>
<td>86</td>
</tr>
<tr>
<td>3</td>
<td>Klaten, regency</td>
<td>Central Java</td>
<td>Jan–Oct 2002</td>
<td>127</td>
<td>NC</td>
</tr>
<tr>
<td>4</td>
<td>Bekasi, regency</td>
<td>West Java</td>
<td>Feb–Jun 2002</td>
<td>169</td>
<td>93</td>
</tr>
<tr>
<td>5</td>
<td>Tegal, regency</td>
<td>Central Java</td>
<td>March 2002</td>
<td>78</td>
<td>NC</td>
</tr>
<tr>
<td>6</td>
<td>Purworejo, regency</td>
<td>Central Java</td>
<td>April–May 2002</td>
<td>371</td>
<td>NC</td>
</tr>
<tr>
<td>7</td>
<td>Boyolali, regency</td>
<td>Central Java</td>
<td>Nov–Dec 2002</td>
<td>73</td>
<td>NC</td>
</tr>
<tr>
<td>8</td>
<td>Kudus, regency</td>
<td>Central Java</td>
<td>Nov–Dec 2002</td>
<td>187</td>
<td>NC</td>
</tr>
<tr>
<td>9</td>
<td>Bolaang Mongondow, regency</td>
<td>North Sulawesi</td>
<td>Dec 2002–Feb 2003</td>
<td>803</td>
<td>222</td>
</tr>
<tr>
<td>10</td>
<td>Bandung, regency</td>
<td>West Java</td>
<td>Dec 2002–Feb 2003</td>
<td>246</td>
<td>11</td>
</tr>
<tr>
<td>11</td>
<td>Klaten, regency</td>
<td>Central Java</td>
<td>Jan–Feb 2003</td>
<td>185</td>
<td>NC</td>
</tr>
<tr>
<td>12</td>
<td>Tegal, regency</td>
<td>Central Java</td>
<td>Jan–Feb 2003</td>
<td>108</td>
<td>NC</td>
</tr>
<tr>
<td>13</td>
<td>Jember, regency</td>
<td>East Java</td>
<td>Jan–Feb 2003</td>
<td>202</td>
<td>141</td>
</tr>
<tr>
<td>14</td>
<td>Cirebon, regency</td>
<td>West Java</td>
<td>Jan–Feb 2003</td>
<td>107</td>
<td>NC</td>
</tr>
<tr>
<td>15</td>
<td>Lombok Barat, regency</td>
<td>West Nusa Tenggara</td>
<td>Jan–Feb 2003</td>
<td>120</td>
<td>NC</td>
</tr>
<tr>
<td>16</td>
<td>Lombok Tengah, regency</td>
<td>West Nusa Tenggara</td>
<td>Feb 2003</td>
<td>142</td>
<td>NC</td>
</tr>
<tr>
<td>17</td>
<td>Jepara, regency</td>
<td>Central Java</td>
<td>Feb 2003</td>
<td>37</td>
<td>NC</td>
</tr>
<tr>
<td>18</td>
<td>Bandung, regency</td>
<td>West Java</td>
<td>Feb 2003</td>
<td>38</td>
<td>23</td>
</tr>
<tr>
<td>19</td>
<td>Yogyakarta, city</td>
<td>Yogyakarta</td>
<td>Jan–Mar 2003</td>
<td>372</td>
<td>NC</td>
</tr>
<tr>
<td>20</td>
<td>Bantul, regency</td>
<td>Yogyakarta</td>
<td>Jan–Mar 2003</td>
<td>1031</td>
<td>23</td>
</tr>
<tr>
<td>21</td>
<td>Pasuruan, regency</td>
<td>East Java</td>
<td>Mar 2003</td>
<td>654</td>
<td>20</td>
</tr>
<tr>
<td>22</td>
<td>Tangerang, city</td>
<td>Banten</td>
<td>Mar 2003</td>
<td>47</td>
<td>17</td>
</tr>
<tr>
<td>23</td>
<td>Bekasi, regency</td>
<td>West Java</td>
<td>Mar 2003</td>
<td>NA</td>
<td>50</td>
</tr>
<tr>
<td>24</td>
<td>Klaten, regency</td>
<td>Central Java</td>
<td>Mar 2003</td>
<td>185</td>
<td>10</td>
</tr>
</tbody>
</table>

Data from Arbovirus Sub Directorate General, CDC, Indonesian Ministry of Health. NC: not laboratory confirmed; NA: data not available.

a Laboratory confirmed chikungunya outbreak.

b Positive by IgM ELISA and/or reverse transcriptase PCR.

c Based on an IgG anti-chikungunya response, because of the delay in specimen collection relative to acute disease phase.
show this outbreak episode to be the first instance of epidemic CHIK transmission in the affected area. Historical data on signs and symptoms compatible with CHIK found no evidence of prior outbreak occurrence in the previous three years. Monthly community health center records dating back to January 1998 presented signs and symptoms compatible with CHIK suggesting that it had first appeared in August 2001. Analysis of trend, with respect to fever of unknown origin (FUO) also shows a gradual increase of CHIK disease beginning in August 2001, with peak occurrence coinciding with outbreak recognition between August—November 2001 (Figure 3).

3.2.2. Clinical epidemiology
Clinically recognized CHIK cases (119) were identified during the outbreak period from the community health centers. No fatalities related to CHIK occurred in these two outbreak-affected villages. The mean age of recognized cases was 36.5 ± 18 years (range 3–87 years). The proportional distribution of cases in the age groups ≥ 40 years, 30–39 years, 20–29 years, 10–19 years and <10 years was 44, 19, 18, 14 and 5% respectively, and the age-specific attack rates (AR) in the affected villages were 4.6 cases per 1000 persons, 3.7/1000, 2.7/1000, 1.6/1000 and 0.5/1000 respectively. The male-to-female case ratio was 1:2. The most notable signs and symptoms among 108 cases
Interviewed were arthralgia (81%), fever (78%) and malaise (75%) (Figure 4). Duration of illness (morbidity) averaged 6.7 ± 5.05 days, with a range of 1–30 days.

3.2.3. Epidemic curve
The period of epidemic CHIK transmission (cumulative 16 weeks) began at week 35 (August 2001) and ended at week 50 (December 2001). There was temporal uniformity in the two affected villages (Figure 5). CHIK introduced itself slowly until week 40 (October 2001), when the first of three dramatic rises in cases were recorded. The epidemic curve reflected periods of intermittent or oscillating periods of activity, with repeated outbreak peaks occurring during weeks 44 and 48 (November 2001). At the close of the outbreak (week 50), only five suspected CHIK cases were recorded.

3.2.4. Case distribution
Clinically recognized CHIK cases were distributed in only two (Kedung Badak and Kebon Pedes) of the 11 villages comprising Tanah Sareal district. Out of 119 clinically defined cases, 69 were from Kebun Pedes village, where the outbreak was first recognized, and 50 from Kedung Badak village. The ARs based on community health center case records and extrapolated from census data, were 3.4 cases per 1000 persons in Kebun Pedes, and 2.3/1000 persons in Kedung Badak. CHIK cases were identified in 35% of 94 households surveyed in the outbreak-affected area. There was little evidence of familial case clustering in that 73% of 52 CHIK affected-households had just one family member with compatible signs and symptoms of CHIK. There was, however, geographical case clustering of affected households observed in both Kebon Pedes and Kedung Badak villages.
Table 2  Laboratory results from investigated chikungunya outbreaks in Bogor and Bekasi, West Java Province, Indonesia

<table>
<thead>
<tr>
<th>Village</th>
<th>Percentage of positive sera</th>
<th>IgM ELISA (%)</th>
<th>PCR (%)</th>
<th>Culture</th>
<th>IgG ELISA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Outbreak-affected village</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Bogor</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kebon Pedes</td>
<td>Cases (n = 50)</td>
<td>62</td>
<td>12</td>
<td>All negative</td>
<td>82</td>
</tr>
<tr>
<td></td>
<td>Controls (n = 18)</td>
<td>11</td>
<td>6</td>
<td>All negative</td>
<td>67</td>
</tr>
<tr>
<td>Kedung Badak</td>
<td>Cases (n = 36)</td>
<td>40</td>
<td>17</td>
<td>14</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>Controls (n = 27)</td>
<td>22</td>
<td>15</td>
<td>All negative</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>Family member of cases (n = 25)</td>
<td>48</td>
<td>All negative</td>
<td>4</td>
<td>52</td>
</tr>
<tr>
<td><strong>Bekasi</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kali Jaya</td>
<td>Cases (n = 93)</td>
<td>43</td>
<td>6</td>
<td>1</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td>Controls (n = 124)</td>
<td>10</td>
<td>0</td>
<td>Not done</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>Family member of cases (n = 21)</td>
<td>19</td>
<td>All negative</td>
<td>Not done</td>
<td>71</td>
</tr>
<tr>
<td><strong>Non outbreak-affected village</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kayu Manis (Bogor)</td>
<td>(n = 99)</td>
<td>All negative</td>
<td>9</td>
<td>All negative</td>
<td>13</td>
</tr>
<tr>
<td>Harja Mekar (Bekasi)</td>
<td>(n = 55)</td>
<td>All negative</td>
<td>All negative</td>
<td>Not done</td>
<td>6</td>
</tr>
</tbody>
</table>

3.2.5. Laboratory results

From 86 case sera, 63% showed laboratory evidence of recent CHIK infection by IgM ELISA (45) and/or RT–PCR (13) and/or viral isolation (5). Eleven of 45 control sera obtained from healthy individuals in the outbreak-affected villages, had evidence of recent CHIK infection: eight by IgM ELISA and five by RT–PCR. CHIK IgG was detected in 67% (58/86) of case sera and 49% (22/45) of the control sera. In 52% of sera obtained from 25 healthy family members from affected households (≥1 case), anti-CHIK IgM by ELISA was detected (12) or virus was isolated (1). Thirteen of 25 (52%) healthy family members also had anti-CHIK IgG indicating past exposure. Twelve had both IgM and IgG antibodies. In the control village of Kayu Manis, 9 and 13 (n = 99) specimens were positive by RT–PCR and IgG ELISA, respectively (Table 2).

Laboratory findings identified 63% of "symptomatic" cases and 11% of "asymptomatic" controls as confirmed CHIK infections. Symptomatic CHIK-positive cases comprised 50, 88, 64, 61, and 60% in the age groups <10, 10–19, 20–29, 30–39, and ≥40 years, respectively. Age-specific proportions as to asymptomatic representation among CHIK-positive cases was 0.6, 17, 38, and 15%, in the age groups <10, 10–19, 20–29, 30–39 and ≥40 years respectively. The mean age of laboratory confirmed, clinically recognized CHIK cases was 37.8 ± 18.3 years: 38.5 ± 18.9 by IgM ELISA, 33.2 ± 15.5 by RT–PCR, and 32.2 ± 16.6 by virus isolation. No significant difference (P = 0.199, analysis of variance [ANOVA]) was seen when these figures were compared with mean age of (i) laboratory negative, clinically recognized cases (40.7 ± 21.6 years); (ii) laboratory positive, village-affected controls (34.3 ± 17.4 years); and (iii) laboratory negative, village-affected controls (40.9 ± 17 years) (Table 3). There was little agreement between laboratory results involving 86 case specimens examined of which 54 were found positive from the two outbreak-affected villages. No specimen was "positive" by all three diagnostic methodologies, whereas 32 were found "negative" by all three tests.

3.2.6. Vector ecology

Daytime indoor resting collections for adult mosquitoes captured 120 mosquitoes of which 60% were identified as Culex quinquefasciatus Say, 39% A. aegypti (L.), and 1% A. albopictus Skuse.

3.2.7. Climatic influences

There was negligible variability in average monthly 24-hour maximum–minimum ambient temperatures from January 2000 to December 2001 (24–26.2 °C), however, the seasonal fluctuations in rainfall were pronounced (79–491 mm/month). There was an increase in rainfall from August 2001 corresponding to the leading edge of the outbreak and subsequent outbreak months of September to
Table 3: Age distribution of laboratory confirmed chikungunya infection in Bogor and Bekasi, West Java Province, Indonesia

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Laboratory confirmed a</th>
<th>Family members</th>
<th>Controls b</th>
<th>Laboratory confirmed a</th>
<th>Family members</th>
<th>Controls b</th>
</tr>
</thead>
<tbody>
<tr>
<td>1—9</td>
<td>2/4 (50%)</td>
<td>6/10 (60%)</td>
<td>0/0 (0%)</td>
<td>1/1 (100%)</td>
<td>2/3 (67%)</td>
<td>0/0 (0%)</td>
</tr>
<tr>
<td>10—19</td>
<td>7/8 (87.5%)</td>
<td>4/7 (57%)</td>
<td>3/5 (60%)</td>
<td>2/7 (14%)</td>
<td>1/7 (14%)</td>
<td>0/12 (0%)</td>
</tr>
<tr>
<td>20—29</td>
<td>9/14 (64%)</td>
<td>1/3 (33%)</td>
<td>1/6 (17%)</td>
<td>13/20 (65%)</td>
<td>1/4 (25%)</td>
<td>5/33 (15%)</td>
</tr>
<tr>
<td>30—39</td>
<td>11/18 (61%)</td>
<td>0/1 (0%)</td>
<td>3/8 (37.5%)</td>
<td>7/20 (35%)</td>
<td>0/1 (0%)</td>
<td>1/29 (3%)</td>
</tr>
<tr>
<td>≥40</td>
<td>24/42 (59.5%)</td>
<td>2/4 (50%)</td>
<td>4/26 (15%)</td>
<td>16/43 (37%)</td>
<td>0/6 (0%)</td>
<td>6/50 (12%)</td>
</tr>
</tbody>
</table>

Mean ± SD years (range) 37.8 ± 18.3 (3—87) 14.9 ± 8.7 (5—23)

3.3. Bekasi outbreak

3.3.1. Background

Historical trends over a four-year period (January 1998 to July 2002) from Kali Jaya community health center data showed no evidence of sporadic or epidemic CHIK, based on review of reported clinical signs and symptoms before the outbreak period. When data extraction from clinical records was restricted to FUO cases, possible CHIK was detected in September 2001, four months before the outbreak (Figure 3).

3.3.2. Clinical epidemiology

No deaths were attributed to CHIK infection among the 169 outbreak-related cases in Kali Jaya. The mean age of clinically recognized cases was 33.3 ± 19.9 years (range 2—85 years). The highest proportion (37%) of CHIK was in the age group ≥40 years, followed by the age groups 20—29 (28%), 30—39 (16%), 10—19 (12%), and <10 (7%) years. Extrapolation from Bekasi Regency population statistics (census data 2001), translated to the village (Kali Jaya) level, provided for an overall estimated AR of 6.7 cases per 1000 persons, with age-specific ARs of 11.4 cases/1000 persons (≥40 years of age), 8/1000 (30—39 years), 13/1000 (20—29 years), 4.0/1000 (10—19 years), and 1.5/1000 (<10 years). The male:female case ratio was 1:1.6. Principal clinical signs and symptoms included arthralgia (89%), malaise (83%), and fever (76%) (Figure 4), while the duration of illness (morbidity) ranged from 1 to 60 days (mean 7.9 ± 8.1 days).

3.3.3. Epidemic curve

The outbreak period lasted 23 weeks, starting in week 5 (January 2002) and ending week 27 (July 2002). The epidemic curve (Figure 5) showed three dramatic increases in cases beginning week 7 (February), week 18 (May) and week 24 (June), followed by an abrupt decline.

3.3.4. Case distribution

All suspected and confirmed outbreak cases were detected from only one village, Kali Jaya, located in Cikarang Barat district, which comprises 11 villages. The outbreak cases were restricted to only one of seven Rukun Wargas (RW, areas within a village normally consisting of between 300 and 750
households each), and further distributed within only two of three Rukun Tetangga (RT, smaller unit areas within an RW). Seventy-nine percent of suspected CHIK cases were residents of RT 1, and 21% from RT 2. Cases were identified in 51% (91/180) of households surveyed, 24% of which had more than two family members with symptoms compatible with CHIK. There was some evidence of familial case clustering because 47% of 91 households had more than two cases per family unit.

3.3.5. Laboratory results
Overall, 46% of 93 case sera examined had evidence of recent CHIK infection, compared with only 10% of 124 asymptomatic control sera from the outbreak-affected village, 19% of 21 sera from healthy family members of cases, and none of the 53 sera from the control village. Forty-three percent of case sera were positive by IgM ELISA, 6% by RT–PCR, and 1% by virus isolation. Detection of IgG anti-CHIK among (i) cases, (ii) village-affected controls, (iii) healthy family members of cases, and (iv) matched controls from the non-outbreak village, was 73, 40, 71, and 6% respectively (Table 2). Laboratory findings revealed confirmed infection in 46% 'symptomatic' and 10% 'asymptomatic' controls from Kali Jaya. Age-specific proportions of confirmed infection relative to clinical symptomatic group status were 100, 29, 65, 35, and 37% in the <10, 10–19, 20–29, 30–39, and ≥40 year age groups respectively. In the control groups, no asymptomatic infections were found among the <20 year age group, whereas 15% of those 20–29 years, 3% of those 30–39 years, and 12% of those ≥40 years of age were considered to be asymptomatic infections.

There were significant differences (P = 0.03, ANOVA) between mean ages in laboratory diagnosed CHIK cases based on confirmatory method: 33.5 ± 13.5 years by IgM ELISA; 13.8 ± 13.1 years by RT–PCR; and 70 years by virus isolation. The mean age of laboratory confirmed CHIK cases (34.5 ± 14.2 years), regardless of the testing methodology used, did not vary significantly (P = 0.2, ANOVA) by mean age from: (i) laboratory negative, clinically defined cases (40.6 ± 16.9 years), (ii) laboratory positive, village-affected asymptomatic controls (32.8 ± 9.4 years), and (iii) laboratory negative, village-affected controls (36.7 ± 15.1 years) (Table 3). There was poor test agreement (specificity and sensitivity) between the three assay methods; only one serum (out of 43 confirmed cases) was positive by all three applied laboratory methods. In contrast, 50 case sera from 93 examined were found 'negative' by the three diagnostic assays.

3.3.6. Vector ecology
Daytime indoor resting collections for adult mosquitoes captured 224 mosquitoes in Kali Jaya from which 91% were identified as C. quinquefasciatus Say, 6% as A. albopictus and 3% as A. aegypti. Residual insecticide fogging just prior to the investigation, however, likely nullified any meaningful data collection. All attempts to detect evidence of CHIK virus by RT–PCR failed.

3.3.7. Climatic influences
There was little variability in the average 24-hour maximum–minimum ambient temperatures from January 2001 to August 2002, ranging from 26.2 to 29.6 °C. Rainfall increased dramatically during the period leading up to the beginning (January and February 2002) of the outbreak. Cumulative rainfall for January (109 mm) and February (837 mm) 2002 greatly exceeded the mean cumulative monthly rainfall (177 mm, range 1–565) for pre-outbreak months of January to December 2001. The heavy rains resulted in significant flooding in Bekasi Regency. There was a precipitous drop in recorded rainfall of only 132 mm in March 2002 and it remained below 150 mm/month from April to July followed by a rapid decline of cases. The monthly rainfall (mm) and temperature (°C) data were obtained from Bekasi Meteorology and Geophysics office.

4. Discussion
The re-emergence of epidemic CHIK across Indonesia after almost 20 years of quiescence is similar to that previously experienced in India (Pavri, 1973), Myanmar (Khai Ming et al., 1974), Sri Lanka (Wasinjak-Hirjan et al., 1949) and Thailand (Burke et al., 1985; Thaikrua et al., 1997). In Myanmar, a large outbreak of CHIK was reported in 1984, 12 years after the last reported instance of epidemic occurrence (Thaung et al., 1975; Thein et al., 1992). This apparent silence of virus and disease activity is an epidemiological feature that distinguishes epidemic CHIK from most other vector-borne viral diseases that share similar vectors and transmission dynamics, principally dengue viruses. It has generally been recognized that re-emergent and epidemic CHIK occurs in cycles of seven to eight years, although intervals of two and three decades have been reported in countries like Uganda (Lanciotti et al., 1998; Pavri, 1986).

Some of the outlying (outside of Java Island) purported CHIK outbreak episodes lacking in laboratory confirmation may have in fact been attributed to
Re-emergence of epidemic chikungunya virus in Indonesia

...chikungunya virus, commonly associated with epidemics of febrile disease throughout Indonesia (Corwin et al., 2001; Sukri et al., 2003). Nevertheless, it is critical in viewing the relatively few instances in which laboratory confirmation was possible against the background of archipelago-wide epidemic occurrence.

Most (62%) of the outbreak episodes during the recent Indonesian epidemic occurred in densely populated rural localities on Java, although most cases were recognized from urban and semi-urban areas. Epidemic CHIK has been predominantly an urban feature in Asia, whereas in Africa, outbreaks have been characterized as being smaller in scale and affecting principally rural populations (Jupp and McIntosh, 1988). In Indonesia, as in many other dengue-endemic countries in Southeast Asia, sporadic occurrences and outbreaks of CHIK are likely to be underreported as the disease is not commonly included in clinical diagnostic algorithms used in the region. A coincident rise in FUOs months before the suspicion that CHIK was involved in the outbreaks in Bogor and Bekasi attests to the general unfamiliarity of the disease for most health care professionals, while negligible FUO cases were recorded from control villages. Epidemic curves from the two investigated outbreaks revealed similar trends and progression; a gradually increasing case load early on, followed by three distinctive peaks in disease incidence. CHIK outbreaks in Thailand (Thaikruea et al., 1997) and Malaysia (Lam et al., 2001) exhibited the same pattern, reflected in epidemic curves described in west Java.

Age-specific attack rates associated with epidemic CHIK increased with age in both investigated outbreaks. Similar demographic disease profiles have been recognized in other outbreaks, including that described during the Malaysian epidemic in 1998. The two-fold greater female representation among cases seen in both Indonesian outbreaks was comparable to that reported in Malaysia (Lam et al., 2001). This outbreak feature may reflect a greater occupational exposure associated with women’s daytime activities in and around the home.

Hemorrhagic presentation has occasionally been associated with acute CHIK infections (Jupp and McIntosh, 1988; Nimmannitya and Mansuwan, 1986). In general, hemorrhagic presentation related to CHIK in outbreak settings has not been documented from the region, including recent outbreaks in Malaysia and Thailand (Lam et al., 2001; Thaikruea et al., 1997). In both outbreaks described in Java, 15 of 179 (8.3%) suspected CHIK cases were described as having hemorrhagic presentation during illness; 73% (11/15) of which were CHIK-positive by ELISA IgM and/or RT-PCR. Anti-dengue IgM was identified from only one case, in the absence of IgM anti-CHIK. Hemorrhagic manifestations associated with recent CHIK infection has been reported from Thailand, previously, where 8% of hemorrhagic fever cases seen in Bangkok were attributed to CHIK (Burke et al., 1985). A hospital-based study in Myanmar revealed similar rates of hemorrhage, such as epistaxis (9%), petechiae (8%), and melena (1%), associated with sporadic clinical CHIK (Thein et al., 1992). The mosquito vectors associated with the two outbreaks remain speculative, in so far as field collections were restricted to species discrimination only. In Bekasi, extensive insecticide applications (fogging) against adult mosquitoes immediately following outbreak recognition complicated proper assessment. The relative contribution of *A. aegypti* and *A. albopictus* is not known, although it is presumed that *A. aegypti* was the primary vector responsible for transmission in all outbreaks in Indonesia. Although the most abundant indoor resting mosquito was *C. quinquefasciatus*, this ubiquitous species is refractory to infection with CHIK virus and thus is not considered a vector (Jupp and McIntosh, 1988).

The CHIK outbreaks in Bogor and Bekasi began during the start of rainy seasons, during which there was a rapid transition from low to high precipitation. Additionally, the first outbreak peak in cases occurred five (Bogor) and four (Bekasi) weeks following the start of the 2001 and 2002 rainy seasons respectively. The association with climatic events has also been described in other CHIK outbreak settings (Jupp and McIntosh, 1988). In urban outbreaks where *A. aegypti* is the vector, possibly supplemented by *A. albopictus* in Asian countries, the seasonal distribution of CHIK transmission has shown a relationship with rainfall patterns in several countries, generally increasing in response to increased precipitation. At least two separate outbreak episodes occurred at the beginning of the rainy season (June–August 1995) in Thailand, with increases in case recognition a month into the wet season (Thaikruea et al., 1997). Exceptions exist in other areas of South and Southeast Asia where rainfall is not markedly seasonal and cases of CHIK may occur throughout the year (Halstead, 1966). A reasonable assertion would be that the increased abundance of rainwater-filled containers provides greater success rates for mosquito oviposition and larval development, resulting in higher densities of adult vectors (Jupp and McIntosh, 1988), thus increasing risk of virus transmission.

A distinguishable, albeit non-linear trend was observed in the eastward progression of epidemic
CHIK occurrence across the Indonesian archipelago, resulting in 24 recognized outbreak episodes over a 19-month period in 2001–2002. It is likely that many more outbreaks occurred in the country that were not reported. The long duration between epidemic disease occurrences in Indonesia likely contributed to the general underreporting and poor recognition of CHIK during sporadic episodes and long intervals of inter-epidemic activity. The apparent temporal distribution of virus has been attributed to the agency of human movement from different localities and islands, a very common activity in Indonesia. This extensive series of outbreaks beginning in Sumatra and extending to Lombok and Sulawesi were considered all the more remarkable given that there has been a notable absence of recognized CHIK disease reported clinically in Indonesia for nearly two decades. To our knowledge, CHIK did not spread to islands further east in the country, remaining confined to the Oriental zoogeographical region (Mackenzie et al., 1994). The ferocity with which this epidemic presented itself after years of apparent inactivity caught health authorities across the archipelago nation off-guard. Countries neighboring Indonesia, yet to witness a resurgence or an emergence of epidemic CHIK, would find it useful to learn from Indonesia’s recent experience.

5. Disclaimer

The opinions and assertions contained herein are of the authors’ own choosing and are not to be construed as official or as reflecting the views of the US Navy or the Department of Defense.

Conflicts of interest statement

The authors have no conflicts of interest concerning the work reported in this paper.

Acknowledgments

The authors gratefully acknowledge the cooperation from the respective health authority offices in Bogor and Bekasi, West Java and all government community health centers and other institutions for assisting in the investigations. Special thanks to Nursyarofah, Ratna P. Kusumaningrum, Yayu Fathiyah, Rohani Simanjuntak, Erlin Listyaningsih, and Chairin Ma’roef and other laboratory technicians in the US NAMRU-2 Virology Program for specimen/data processing and laboratory assistance. Primers used for the RT–PCR were designed by James McArdle. These investigations could not have been conducted without the full support of Umar F. Achmadi and Soemarjati Arjoso from the Centers for Communicable Diseases — Prevention and Environmental Health (P2M-PLP) and the National Institute of Health Research and Development (LitBangKes), Indonesian Ministry of Health, Jakarta, respectively. This work received financial support from the US Department of Defense and the Global Emerging Infections System (GEIS).

References


K. Laras et al.
Re-emergence of epidemic chikungunya virus in Indonesia


