

# A SEROLOGICAL STUDY OF CHIKUNGUNYA VIRUS TRANSMISSION IN YOGYAKARTA, INDONESIA: EVIDENCE FOR THE FIRST OUTBREAK SINCE 1982

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**Abstract.** A study of epidemic transmission of Chikungunya virus (CHIK) was initiated in April 1999 in Yogyakarta, Indonesia. Three hundred seventeen volunteers from three kelurahans (sub-districts) were recruited. Anti-CHIK IgG antibodies were detected in 68% to 74% of cases and 28% to 32% of controls. In the kelurahan with no reported CHIK illness, 29% of cases and 28% of controls had anti-CHIK IgG antibodies. None of these cases demonstrated anti-CHIK IgM antibodies. In the two kelurahans with disease activity, anti-CHIK IgM antibodies were detected in 3% to 36% of cases, with the highest percentage from the kelurahan with recently reported cases. Ten percent of controls from Gowok had anti-CHIK IgM detected in their serum. Twelve acutely ill volunteers were later included from the kelurahan Pilahan for virus identification. Samples from two volunteers were culture- and RT-PCR-positive for CHIK. This is the first documentation of epidemic transmission of CHIK in Indonesia since 1982.

## INTRODUCTION

Chikungunya virus (CHIK) is an RNA virus that belongs to the *Alphavirus* genus of the family *Togaviridae*. The virus is transmitted primarily by the urban vector *Aedes aegypti* and has been responsible for epidemics in cities of Africa and Asia (Lumsden, 1955; Robinson, 1955; Carey *et al*, 1969). The illness caused by CHIK is associated with distinct clinical manifestations that include a rather abrupt onset of fever, rash, crippling arthralgia, and occasional frank arthritis (Robinson, 1955; Carey *et al*, 1969). The incubation

period is usually two to ten days, with the constitutional symptoms lasting one to seven days. The articular symptoms usually resolve within days to a few weeks, but in severe cases, these symptoms may last for months.

In Southeast Asia, CHIK activity was documented in Thailand (Halstead *et al*, 1969), Vietnam (Tesh *et al*, 1975), Philippines (Campos *et al*, 1969), and Myanmar (Thaung *et al*, 1975). In Indonesia, an outbreak of CHIK was first recognized in June 1982 (Wuryadi, 1986), in a small district located in Jambi Province, South Sumatra. The illness rapidly moved north to involve Palembang, Padang, Medan, Ache, Nias, and other cities and islands. Later in the same year, the islands of Java and West Kalimantan were affected. Between 1983 and 1985, the disease spread to involve South, East and Central Kalimantan, South and North Sulawesi, East Timor, East Nusa Tenggara, the Mollucca Islands, and Papua (formerly Irian Jaya). The attack rates ranged from 40% to 85% and the most common clinical symptoms were fever, skin rash, and myalgia/arthralgia (Wuryadi, 1986).

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In the province of Yogyakarta, Indonesia, the outbreak was recognized in April 1983 and continued until August 1984 (Haksohusodo, 1986). Nearly all of the districts in the province were involved, with an estimated attack rate of 70% to 90%. The apparent peak of the epidemic was in December 1983.

In December 1998, local clinics in the kelurahan (sub-district) of Suryatmajan, located in the kecamatan (district) of Danurejan, region of Yogyakarta, Yogyakarta Province, began reporting clusters of a febrile illness associated with arthralgia and rash. Cases continued to be reported until February 1999, when vector control by fogging was instituted. To confirm CHIK as the etiological agent of disease and to investigate the extent of CHIK transmission in this area, representatives from the National Institutes of Health Research and Development, Indonesian Ministry of Health, and the Naval Medical Research Unit #2, Jakarta, along with physicians from Gadjah Mada University, Yogyakarta, Indonesia, initiated community-based serological surveys in areas of reported cases. This report documents the findings of this investigation.

## MATERIALS AND METHODS

### Study population and sampling

Local health officials in the kelurahan of Suryatmajan provided a list of individuals who had reported to the community health center (Puskesmas) between December 1998 and February 1999 with symptoms consistent with CHIK disease. Invitations were sent to these individuals requesting voluntary participation in the study. Volunteers were both male and female, age 4 years and older. For control cases, asymptomatic volunteers from the same kelurahan were also invited to participate. Volunteers from the kelurahan Bausasran, where no disease consistent with CHIK was reported, were also recruited to investigate prevalence rates of infection prior to the recognition of the outbreak. The kelurahan Gowok, in the kecamatan Catur Tunggal, was included in the survey because more recent CHIK cases were being reported at the time of the study. Samples were collected between 14 May 1999 and 20 May 1999, approximately three months after the number of cases was reported to begin declining. Twelve acutely ill volunteers from the

kelurahan Pilahan were included after May 1999 to try to directly identify CHIK as the infecting virus by either RT-PCR or virus isolation.

Approximately 3 to 7 mls of blood were obtained from each volunteer. The clotted blood samples were centrifuged, the serum removed and stored at -20°C until assayed. Serum samples taken from volunteers suffering from acute illness were stored at -70°C. These samples were used for attempted virus isolation in C6/36 mosquito cell line and viral RNA detection by RT-PCR. Prior to obtaining the samples, participants were asked to complete a questionnaire that included clinical and demographic information.

All samples were obtained after informed consent and under US NAMRU-2 and National Institute of Health Research and Development-approved human use protocols.

Based on answers to the questionnaire, volunteers were assigned as either cases or controls. A CHIK case was defined as an individual experiencing fever, and joint or bone pain, with or without other constitutional symptoms within the last six months. Control cases were those who were without these symptoms within the same time period.

### Serology for CHIK

Serum samples were assayed for the presence of IgG and IgM antibodies against CHIK using a microtiter plate ELISA. For detection of anti-CHIK IgM antibodies, an antigen capture technique was used. Ninety-six well microtiter plates (Immulon 2, Dynex Technologies, Chantilly, VA, USA) were coated overnight at 4°C with anti-human IgM antibodies (Kirkegard and Perry, Gaithersburg, Maryland) diluted 1:500 in phosphate-buffered saline (PBS), pH 7.4. Serum for testing was diluted 1:100 in serum dilution buffer (PBS, 0.1% Tween-20 and 5% Bacto skim milk) and 100 µl added to the anti-human IgM coated plates. A panel of normal sera, negative for CHIK-specific antibodies, was used to determine the cut-off value. Following one-hour incubation at 37°C, the plates were washed with PBS/Tween and CHIK antigen lysate, diluted 1:25, was added. The antigen was prepared from CHIK-infected BHK21 clone 15 cells and inactivated with beta propiolactone. Uninfected BHK21 clone 15 cell lysate was used as control antigen. Normal human serum diluted 1:50 was added to each ly-

sate preparation. After one-hour incubation at 37°C, the plates were washed and anti-CHIK hyperimmune mouse ascitic fluid (diluted 1:1000) was added. The plates were then incubated for an additional hour. Bound anti-CHIK mouse antibody was detected using horseradish peroxidase-conjugated anti-mouse IgG and ABTS substrate. The substrate was allowed to react for one hour and the plates read at 415 nm. The adjusted optical density value (OD) for each sample was determined by subtracting the OD obtained with the control antigen from the OD obtained using the CHIK antigen. A sample was considered positive if its OD value exceeded the mean plus three standard deviations of the normal control sera.

For detection of CHIK-specific IgG antibodies, the CHIK and control antigen lysates were prepared from CHIK-infected and uninfected Vero cell lysates, respectively. Ninety-six well plates were coated directly with antigen and diluted 1:1,500 in PBS. The plates were coated overnight at 4°C, washed three times and reacted with test sera, diluted 1:100. Following one-hour incubation at 37°C, the plates were washed three times and reacted with horseradish peroxidase conjugated mouse anti-human IgG Fc (Kirkegard and Perry). Bound antibody was detected as indicated above, using anti-human IgG conjugate and ABTS substrate.

The endpoint ELISA IgM and IgG titers were determined by re-testing ELISA-positive samples at serial two-fold dilutions beginning at 1:100. The largest dilution giving a positive result was considered the endpoint titer.

### Serology for Ross River virus

To evaluate the possibility of Ross River virus (RRv) as the etiology of the illness, samples that were positive by CHIK serology were tested for anti-RRv IgM and IgG antibodies by antibody capture and indirect ELISA, respectively. These ELISA procedures were similar to the CHIK assays except that RRv antigen and TMB substrate were used instead of CHIK antigen and ABTS substrate, respectively.

### Virus detection by RT-PCR

RNA from acutely ill patients was purified from serum samples using the QIAamp Viral RNA Isolation Kit (Research Biolabs, Singapore). The purified RNA was then used in a nested RT-PCR assay. Twenty microliters of the purified

RNA suspension were combined with 20 pmole each of oligonucleotide primers JM1 (5' GCAGACGCAGAGAGGGCCAG3'; bp 1201 to 1220) and JM2 (5' GCTGCTGCAAGGTAGTT CTC3'; bp 1440 to 1421), 10 µl 5x RT-PCR buffer, 0.2 mM of deoxynucleotide triphosphates, 5 units of reverse-transcriptase enzyme, and 5 units of *Thermus flavius* polymerase. The primers were designed to amplify a 240 base pair fragment of the E2 gene region. Primer coordinates are based on a published CHIK RNA structural gene sequence (Genbank accession no. L37661). The mixture was incubated at 48°C for 45 minutes for reverse transcriptase reaction and subsequently subjected to 40 cycles of PCR, consisting of denaturation at 94°C for 38 seconds, primer annealing at 60°C for 1 minute, and primer extension at 68°C for 2 minutes. A second nested PCR reaction was done using the product from the first reaction. For this reaction, 20 pmoles each of the internal primers JM3 (5' GCTATTTGTAAGAA CGTCAG3'; bp 1221 to 1240) and JM4 (5' TACCGTGCTGCGGTCGGGAA3'; bp 1420 to 1401) were combined with 5 µl of 10x PCR Buffer II (Perkin Elmer, Foster City CA), five units of Taq Gold (Perkin Elmer), and water to a total volume of 50 µl. The mixture was then incubated at 95°C for 10 minutes and subjected to 40 cycles of amplification, with each cycle consisting of 95°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds. Following the last cycle, the mixtures were held at 72°C for 7 minutes. Amplified PCR products were resolved by electrophoresis on 2% agarose gel and ethidium bromide stain. Positive samples resulted in a 200 base-pair fragment.

The CHIK RT-PCR assay was performed using purified RNA isolated from RRv and dengue virus serotypes 1, 2, 3, and 4. This was done to evaluate the specificity of the assay. The RRv isolate was from Australia and the dengue virus isolates were from Indonesia. The results showed that the CHIK primer pairs only produced a PCR-product with the CHIK virus RNA (data not shown).

### Virus isolation

Serum samples were diluted 1:10 in PBS and applied to confluent monolayers of C6/36 cells in 24-well culture plates (Corning, New York). The plates were centrifuged at 400g for 45 minutes and then 1 ml of medium (MEM) added. 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 the presence of virus by standard immunofluorescence assay using anti-CHIK hyperimmune mouse ascitic fluid. RNA from virus isolates was also subjected to RT-PCR with CHIK-specific oligonucleotide primers as another method of virus identification.

### Antibody avidity assays

The method of Gassmann and Bauer (1997) was used, with some modifications, to evaluate the avidity of CHIK IgG antibody to determine whether the detected IgG antibody response was the result of a recent, versus remote, infection. To determine the appropriate urea concentrations to use in the test, high avidity and low avidity positive control sera, diluted 1:100, were tested at different urea concentrations that ranged from 6M to 9M in 0.5 M increments. Using the best urea concentration, 6.5 M, the samples were tested in duplicate for IgG as indicated above. After the initial sample incubation, one duplicate plate was incubated for 3 minutes with 6.5 M urea in PBS and then washed three times. The plates were further processed according to the usual procedure. For samples giving an OD value > 2.0, the assay was repeated using two-fold serial dilutions starting at a 1:100 dilution. For samples giving an OD less than 0.6, samples were re-tested at two-fold dilutions, starting at 1:10.

For samples tested at a single 1:100 dilution, the avidity index was calculated by dividing the OD from the urea-treated sample by the OD of the untreated control. For samples requiring serial dilutions, fine determinations of the avidity indexes were calculated by dividing the dilution of the urea-treated curve necessary for a defined OD by the respective dilution of the control curve at the same OD (Gassmann and Bauer, 1997). The defined OD was selected in the range 0.25-0.6 fold of the maximal OD.

### Statistical analysis

The number of anti-CHIK antibody positive volunteers in the case and control groups for each sub-district were compared using the chi-square test. The Fisher exact test was used when the number of positives in either the case or control groups was five or less. The frequency of symptoms be-

tween CHIK antibody-positive volunteers and antibody-negative volunteers was compared using the chi-square test. The mean avidity indexes for cases and controls in each sub-district were compared using analysis of variance and Dunnett's test for multiple comparisons.

## RESULTS

A total of 317 volunteers were enrolled in the study. Seventy were from Bausasran, 110 from Gowok, and 137 from Suryatmajan. Two volunteers in the Gowok group were actually from the neighboring kelurahan Ambarukmo, but for the purposes of this study, were kept in this group.

The mean ages of the participants from Suryatmajan, Gowok, and Bausasran were 32, 41, and 24, respectively. The male-to-female ratios were 0.76:1, 0.49:1, and 2.33:1, respectively. For Suryatmajan, the mean ages for cases and controls were 28 and 34 years, respectively, and for Gowok, 42 and 40 years, respectively. The mean age for both cases and controls in Bausasran was 24. As indicated, volunteers from Bausasran tended to be male and younger compared to volunteers from the other two kelurahans.

Table 1 summarizes the IgM and IgG ELISA results for all volunteers. In Suryatmajan, 74% of the cases were positive for IgG antibodies against CHIK, but only 3% of cases still possessed CHIK IgM antibodies indicating a recent infection. The median reciprocal titer was 150 for the IgM-positive cases. Among the controls, anti-CHIK IgM was detected in 4% (median reciprocal titer 100) and 49% were positive for IgG antibodies. Overall, a significantly higher number of cases was positive for anti-CHIK IgG compared to controls. In contrast, there was no significant difference between the number of cases positive for anti-CHIK IgM and the number of anti-CHIK IgM positive controls.

A larger number of cases from Gowok, where acute cases were being reported at the time of the serosurvey, possessed anti-CHIK IgM antibodies (36%) at a median reciprocal titer of 1600. The higher median IgM antibody titer compared to the titer in the Suryatmajan IgM-positive volunteers is consistent with more recent CHIK disease activity. Eighty-one percent of all cases from Gowok were positive for anti-CHIK IgG antibodies. Seven controls from Gowok dem-

Table 1  
Results of serological screening for anti-CHIK IgM and IgG antibodies<sup>a</sup>.

Sub-district	No. volunteers	IgM and IgG Pos (%) / Median titer	IgG Pos (%) / Median titer	Total (%)
Suryatmajan (n=137)				
Cases	61	2 (3) / 150	43 (70) / 6400	45 (74) <sup>b</sup>
Controls	76	3 (4) / 100	34 (45) / 3200	37 (49)
Gowok (n=110)				
Cases	42	15 (36) <sup>b</sup> / 1600	19 (45) / 3200	34 (81) <sup>b</sup>
Controls	68	7 (10) / 1600	18 (26) / 1600	25 (37)
Bausasran (n=70)				
Cases	15	0	5 (33) / 3200	5 (33)
Control	55	0	15 (27) / 3200	15 (27)

<sup>a</sup>Anti-CHIK IgG was always found together with anti-CHIK IgM antibodies.

<sup>b</sup>The number of positives among the cases was significantly greater than controls ( $p < 0.001$ ).

onstrated CHIK IgM antibodies. Twenty-five controls had IgG antibodies, including the seven IgM-positive controls. In no instance was anti-CHIK IgM detected without the presence of anti-CHIK IgG. Like Suryatmajan, the number of anti-CHIK IgG cases was significantly higher compared to the controls. However, as a reflection of the ongoing CHIK disease activity in Gowok, a significantly higher number of cases was positive for anti-CHIK IgM antibodies compared with the control group.

In the kelurahan Bausasran, an area of little to no reported CHIK disease activity, 33% of cases were found to have IgG antibodies to CHIK (median reciprocal titer, 3200) and there were no cases positive for anti-CHIK IgM antibodies. Of 55 control cases, 15 (27%) had anti-CHIK IgG antibodies. The difference in the number of volunteers positive for anti-CHIK IgG, between the case and control groups, was not significantly different.

To assess whether the IgG antibodies found in the volunteers living in Gowok were due to recent infection rather than remote infection, IgG antibody avidity assays were performed using the IgG positive samples. Fig 1 shows the mean avidity index plus the standard deviation for the case and control samples from each kelurahan. When compared with the mean avidity indexes for the samples from Bausasran, where no disease activity was reported, and Suryatmajan, the mean indexes for the Gowok control and case samples were significantly lower ( $p < 0.001$ ). In contrast,

Table 2  
Symptoms exhibited by volunteers with and without serological evidence of CHIK infection.

Symptom	Other (%) n=156	CHIK (%) n=161
Bone/joint pain <sup>a</sup>	63 (40)	122 (76)
Headache	99 (63)	114 (71)
Fever <sup>a</sup>	53 (34)	96 (60)
Myalgia <sup>a</sup>	52 (33)	83 (52)
Chills <sup>a</sup>	18 (12)	54 (34)
Cough	73 (47)	55 (34)
Joint swelling <sup>a</sup>	11 (7)	33 (20)
Rash <sup>a</sup>	12 (8)	49 (30)
Bleeding gums	11 (7)	8 (5)

<sup>a</sup>Differences are statistically significant by Pearson chi-square test.

there was no significant difference between the mean avidity indexes for the Suryatmajan samples and Bausasran samples. No significant difference was noted between the avidity indexes for the Gowok cases and controls. This provided laboratory confirmation of more recent CHIK disease activity in Gowok.

Table 2 shows the type and frequency of symptoms experienced by those individuals with and without serological evidence of CHIK infection. Bone and joint pain, fever, chills, and muscle pain were significantly more common in the CHIK infection group. Joint swelling, suggestive

of frank arthritis, was reported by 20% and skin rash by 30%. Although not encountered frequently, significantly more individuals positive for anti-CHIK antibody reported these symptoms.

The majority of anti-CHIK IgM and IgG ELISA-positive cases occurred in individuals 15 years old or greater (Fig 2). Most of the anti-CHIK IgG positive cases were in the 31-to 45-year old age group. The same number of IgM-positive cases was seen in the 15-to-30 and 31-to-45 year-old age groups, with a slightly higher number of cases detected in the >45 year-old group. In the age group 4 to 14 years, one anti-CHIK IgM case and 20 IgG cases were detected. The presence of anti-CHIK IgG antibodies in this age group supports the occurrence of relatively recent CHIK disease activity, since they were not born during the 1983 epidemic.

Following the community-based serological survey, 12 acutely ill individuals who met our case definition were later identified by a public health clinic. These individuals were residents of the kelurahan Pilahan, located approximately 10 km southwest of Bausasran and approximately 15 km southwest of Gowok. Of the 12, six were positive for IgM and eight for IgG. All IgM-positive cases also had anti-CHIK IgG. No cases were positive for anti-CHIK IgM alone. Two of these samples were positive for virus by culture and RT-PCR detection of CHIK RNA. RNA from viruses isolated from culture was also subjected to RT-PCR and produced RT-PCR products of the appropriate size. This provided further identification of the isolated viruses as CHIK.

CHIK antibody-positive samples were evaluated for anti-RRv ELISA antibodies. Eighty-two (50%) of the 163 CHIK-positive samples were positive for anti-RRv IgG immunoreactivity by indirect ELISA. No samples revealed anti-RRv IgM immunoreactivity (data not shown). The anti-RRv antibody titers were lower than the anti-CHIK antibody titers in all instances, with RRv ELISA values that were 16 to 128-fold lower. This suggested that the RRv immunoreactivity was more likely due to cross-reactivity between the anti-CHIK antibodies and RRv proteins.

DISCUSSION

This article documents the first outbreak of CHIK in Indonesia since the 1983/84 epidemic

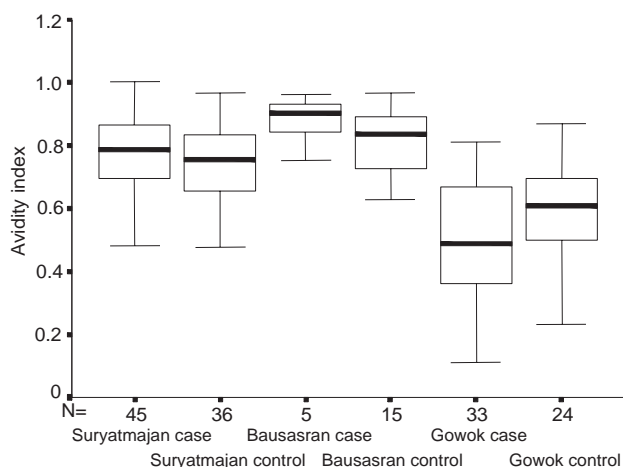


Fig 1—Mean IgG avidity indices for samples obtained from volunteers living in the three indicated kelurahans (sub-districts) of Yogyakarta, Indonesia. The center line in the box represents the mean avidity index. The boxed area on either side of the mean indicates 95% confidence intervals. The bars represent the minimum and maximum avidity indices. Samples from three anti-CHIK antibody-positive volunteers had insufficient volume and were not included in avidity testing.

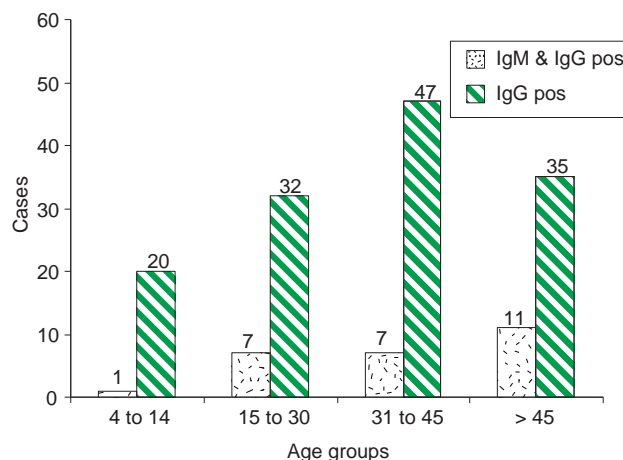


Fig 2—Age distribution of volunteers who showed serological evidence of CHIK infection. The numbers above the bars represent the number of volunteers with the indicated serological status.

in Yogyakarta. Prior to 1983, there was very little documented evidence of CHIK transmission. In other countries of Southeast Asia, CHIK transmission was well documented prior to this date.

In Thailand, the first reported case was diagnosed in 1960 (Thaikruea *et al*, 1997) and since then outbreaks were reported from the provinces of Khon Kaen (1991), Nakhon Si Thammarat (1995), and Nong Khai (1995). All three of these outbreaks occurred during the rainy season (June to August) and were associated with clinical manifestations that included fever, severe arthralgia, and maculopapular rash. Cases were identified by the presence of anti-CHIK IgM antibody and virus isolation. The IgM antibody declined within 3 months (Thaikruea *et al*, 1997).

The outbreak described in this article involved two sub-districts (kelurahans), with the outbreak in Suryatmajan apparently beginning in December 1998. According to health center clinics in that area, peak activity occurred in February with a sudden decline due to the institution of mosquito control by fogging. The reports of acute disease occurring in the neighboring kelurahan of Gowok at the time of the initial serological survey suggested spread of disease activity from Suryatmajan. The antibody avidity assays support this conclusion, showing that the anti-CHIK antibodies in the sera of volunteers from Suryatmajan and Bausasran were significantly more avid compared with the anti-CHIK antibodies from the Gowok volunteers. Gowok is located approximately 5 km northeast of Suryatmajan. The occurrence of acute cases in Pilahan (10 km southwest), where viral isolates were obtained, also suggests spread from Suryatmajan. The antibody patterns obtained from the volunteers also supports the initial occurrence of CHIK transmission in Suryatmajan. Only 3% of cases from Suryatmajan demonstrated anti-CHIK IgM antibodies, compared with 36% of cases from Gowok. In addition, the median IgM antibody titer was about ten-fold lower in the Suryatmajan samples (150) compared with the Gowok samples (1600), indicating earlier infections in Suryatmajan. There was no statistically significant difference in the number of anti-CHIK IgM-positive volunteers, between cases and controls, in Suryatmajan. This is consistent with waning IgM antibodies. The significantly greater number of anti-CHIK IgM-positive cases compared to controls in Gowok provides additional evidence of more recent disease activity.

Although there can be variability between patients in the time course of antibody maturation, antibody avidity can increase significantly within

one month after infection, as in the case of tick-borne encephalitis virus (TBE) infection (Gassmann and Bauer, 1997). By six months' post-infection with TBE, nearly all patients possess high avidity antibodies (Gassmann and Bauer, 1997). The overall finding of high avidity anti-CHIK antibodies in volunteers from Suryatmajan, who were infected in December 1998, approximately four months prior to the serosurvey, is consistent with the projected spread of the epidemic from Suryatmajan to Gowok. The lack of any significant difference in mean antibody avidity, between the Suryatmajan and Bausasran samples, indicates that anti-CHIK antibody-positive individuals in Bausasran were infected due to sporadic, low-level transmission, either during or before the December 1998 outbreak.

The clinical manifestations of CHIK infection in Yogyakarta were similar to those reported from other epidemics. Fever and arthralgia occurred in the majority of cases, but rashes were only reported in 19%. Because much of the clinical information obtained relied on volunteer recall, the appearance of a rash may have been overlooked in some instances.

The symptoms experienced by the volunteers could also have been due to RRv, another alphavirus associated with fever and severe arthralgia. Earlier serological studies indicated that RRv only circulated in West New Guinea and the Moluccas and was not present in Yogyakarta or Java (Tesh *et al*, 1975). Nevertheless, to rule out this agent as a possible etiology of the outbreak in Yogyakarta, samples positive for anti-CHIK antibodies were also tested for anti-RRv antibodies. Half of the samples were immunoreactive by RRv ELISA, but this reactivity was attributed to the anti-CHIK antibodies cross-reacting with RRv antigens. Although some cross-reactivity exists between alphavirus antigens and heterologous alphavirus antibodies, prior work showed that in most, if not all, cases, immunoreactivity against the homologous antigen consistently produces higher ELISA antibody titers (Roehrig, 1982; Xiao *et al*, 1986). The fact that CHIK antibody titers were several-fold higher, compared to anti-RRv antibody titers, supports our conclusion that CHIK was the infecting virus.

The ratio of symptomatic CHIK-positive cases to asymptomatic CHIK-positive volunteers ranged from 1.3:1 in Gowok to 1.2:1 in Suryatmajan. This suggests that most cases were

symptomatic, although more than 40% experienced, no or very mild, illnesses. The fact that 26% to 49% of the controls demonstrated serological evidence of CHIK infection indicates high levels of transmission prior to the recognition of the outbreak. To obtain more accurate measures of inter-epidemic transmission, prospective cohort studies are needed.

The data presented in this article provide evidence that the 1998/1999 outbreak in Yogyakarta of a febrile disease associated with arthralgia and occasional rash was due to CHIK. That cases were still being reported in kelurahan Gowok suggests that transmission of CHIK continues to occur in the province. Continued transmission was further supported by the occurrence of acute virus isolation-positive cases in Pilahan. Both of the virus isolation cases were positive by RT-PCR. The gene fragment targeted by the RT-PCR primers lies within the E2 region. This region is the target of anti-alphavirus neutralizing antibody responses. Because of the selection pressure elicited by these responses, the E2 region is the most variable between the different alphaviruses. The production of a RT-PCR product from serum samples, as well as tissue culture supernatants, confirms that the infecting virus was CHIK, given the lack of cross-reactivity between the RT-PCR primers and RRv RNA.

The data presented in this article indicate that a CHIK outbreak began in December 1998 and spread to multiple areas of Yogyakarta. Further studies are needed to define the full extent of this epidemic and to determine whether endemic transmission of this virus is occurring.

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