THE 1993 DENGUE 2 EPIDEMIC IN NORTH QUEENSLAND: A SEROSURVEY AND COMPARISON OF HEMAGGLUTINATION INHIBITION WITH AN ELISA

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Abstract. An epidemic of dengue type 2 infection occurred in North Queensland during 1992 and 1993. A random serosurvey of 1,000 residents of a population that experienced this epidemic only during 1993 was conducted to determine the proportion of the population at risk for secondary infection in the event of another epidemic with a different serotype. The ability of an ELISA to detect prior exposure to the dengue virus was compared with the hemagglutination inhibition assay. Dengue 2 virus plaque-reduction neutralization assays were performed to evaluate the specificity of the antibody response. Antibodies to dengue virus, or closely related flaviviruses, were detected in 61.9%. Seroprevalence increased with age and correlated well with known previous epidemics in the region. The sensitivity and specificity of the ELISA was 99.2% and 96.2%, respectively. An estimated 26% of the population was infected during the 1993 epidemic.

During 1992 and 1993, North Queensland, in the eastern part of tropical Australia, was affected by a dengue 2 epidemic with 1,063 cases reported to public health authorities. The same area has previously been affected by epidemics in 1981 (dengue 1), 1954 (dengue 3), 1941–1943 (dengue 2) and 1925–1926 (possibly dengue 1). There were several earlier epidemics dating back to 1879.

A dengue epidemic with a serotype other than type 2 in the next few years would result in a number of cases of secondary dengue infections, a situation that has been shown to increase the likelihood of dengue hemorrhagic fever (DHF). It is of interest that the first clinical description of DHF was of cases that occurred in Charters Towers, North Queensland. This region, with a growing tourism industry, has an increasing risk for further introductions of the dengue virus; indeed, since 1993 imported cases of dengue 2 and 3 have occurred and one instance of local transmission of dengue 2 has been reported. Another epidemic caused by dengue 2 in 1996 and early 1997 affected the Torres Strait Islands, between Australia and Papua New Guinea, with several cases reported in the coastal city of Cairns. A dengue 3 epidemic began in Cairns in late 1997 and is currently ongoing.

The aim of the present study was to determine the proportion of a population that had been infected with the dengue 2 virus during the epidemic in 1993. A further aim of the study was to compare two methods for the determination of past exposure to the flavivirus group of viruses (which includes the dengue viruses, Japanese encephalitis virus, Murray Valley encephalitis virus, and several other viruses known to occur within Australia). The hemagglutination inhibition (HI) test is the existing gold standard for serologic surveys but is more difficult to perform than the ELISA, which has recently become available commercially.

MATERIALS AND METHODS

Study population. Charters Towers (20°5′S, 146°16′E), with a population of approximately 10,000 people, was chosen as the study site because the epidemic in this city had occurred only in 1993, unlike the other major center affected by the epidemic, Townsville, which recorded cases in both 1992 and 1993. The population of the city is predominantly middle class and there are no slum areas. The epidemic in Charters Towers was widespread throughout the town and was described by some as being of an explosive nature. In contrast, the epidemic in Townsville affected some suburbs more than others and the distribution varied during the course of the epidemic.

Research design. A random sampling of 1,000 people who had lived in Charters Towers during 1981 and 1993 was undertaken. Telephone contact was made using every tenth number in the local directory. The telephone provider company estimated that about 90% of the homes in Charters Towers had connections. Prospective participants had a study explained to them and signed consent was obtained. A questionnaire was administered and a blood specimen was taken. Where possible all eligible persons within the same household were enrolled. Children less than 14 years of age were excluded. The 1981 residential requirement was related to a proposed investigation of the clinical correlates of primary versus secondary infection. The study was approved by the Ethics Committee of the James Cook University of North Queensland.

Serum was separated from whole blood on the day of collection and sent daily by courier to Townsville where it was stored at −20°C.

Hemagglutination inhibition test. The HI test was performed using the method of Clarke and Casals. Gander cells in acid citrate dextrose were obtained from the Department of Primary Industries (Brisbane, Australia). Dengue hemagglutinin was prepared by polyethylene glycol precipitation of infected C6/36 cell culture supernatant fluid and was obtained from the State Health Laboratories (Brisbane, Australia). The dengue 3 hemagglutinin was used.

Enzyme-linked immunosorbent assay. A commercial assay for the detection of IgG antibodies to dengue viruses was used (Panbio, Brisbane, Australia). The test is an indirect IgG assay using all four virus types coated on the surface of polystyrene microwells in a strip format. The test was performed in accordance with the manufacturers’ instructions. Briefly, a 1:100 dilution of serum was added to each well, and incubated at 37°C for 20 min. The wells were washed with phosphate-buffered saline (PBS) and reacted with 100 μl of horseradish peroxidase–conjugated sheep anti-human IgG for 20 min at 37°C. After a final wash with...
PBS, tetramethylbenzidine/hydrogen peroxide was added. After incubation for 10 min at room temperature, 100 μl of 1 M phosphoric acid was added and the absorbance was measured at 450 nm. Negative and positive serum control samples and a duplicate cut-off calibrator serum control were included for each test run. The optical density (OD) of the specimen well was compared with the OD of the well containing a calibration cut-off (CO) serum sample provided with the kit. Those samples with OD:CO ratios ≥ 1 were recorded as positive.

**Plaque-reduction neutralization test (PRNT).** The method used for the PRNT was that of Morens and others. Baby hamster kidney (BHK)-21 (clone 15) cells were provided by J. Aaskov (Queensland University of Technology, Brisbane, Australia) and were maintained in RPMI 1640 media supplemented with 5% fetal bovine serum (FBS) at 37°C in 5% CO₂. Cells were separated from polystyrene flasks using trypsin-versene and subcultured at approximately 3 × 10⁶ cells/ml.

For the PRNT, cells were maintained in suspension in a sterile conical flask and 50 μl was added to each well of a 24-well tissue culture plate (Falcon; Becton Dickinson, Franklin Lakes, NJ) at a concentration of 1.5–3 × 10⁵ cells/ml.

Dengue 2 (New Guinea C strain) was grown in C6/36 cells and stored as viral culture supernatant fluid supplemented with 30% FBS, at −70°C. Plaque titrations were performed using BHK-21 cells.

Serum was diluted 1:30 in RPMI 1640 media and 100 μl was added to an equal volume of supernatant fluid estimated to contain approximately 80 plaque-forming virions. The serum plus virus mixture was incubated for 60 min at 37°C. Fifty microliters of the mixture was then added to each of three wells in the 24-well plates and the plates were incubated for 4 hr at 37°C. Each well was overlaid with 500 μl of 1.5% carboxymethyl cellulose (Sigma, St. Louis, MO) in RPMI 1640 media. Plates were incubated at 37°C for five days. Cells were stained with 250 μl of 0.5% crystal violet added directly to the media and left for 60 min. After washing three times with tap water, the plaques were counted. A 70% reduction in the number of plaques compared with negative control wells was used as the criteria for classifying results as positive or negative.

### RESULTS

Three thousand households were contacted to enroll 1,000 people from 801 households. Nonparticipation was due either to ineligibility or unwillingness to undergo venisection. Of the 1,000 people entered into the study, there were 367 males and 633 females. The distribution of the respondents’ ages is shown in Figure 1. The over representation of the older age groups reflects both their availability and willingness to participate in the study. Younger, more mobile people who had not lived in Charters Towers during 1981 were excluded. Males were both less available and, on the whole, less willing to agree to venisection.

A potential bias, that of over representation of people who had dengue fever in the recent past, was, we believe, avoided by stressing to potential participants the importance of their participation whether or not they thought they had dengue fever. This was again emphasized in local publicity associated with the study. There was no apparent bias in the volunteer group relating to the geographic distribution of households, socioeconomic level, or standard of housing.

Nine hundred ninety-nine serum samples were available for testing with the ELISA. There were two samples with insufficient volume for HI testing.

The overall seroprevalence for flavivirus antibodies was 60.4% as determined by HI and 61.4% by ELISA. Twenty serum samples gave discrepant results. Fifteen samples were positive by ELISA and negative by HI and five samples were positive by HI and negative by ELISA.

A comparison of HI and the ELISA (Table 1) showed that the sensitivity and specificity of the ELISA was 99.17% and 96.18%, respectively, when compared with HI results.

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<th>HI</th>
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<tr>
<td>+</td>
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<td>5</td>
<td>378</td>
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<td>Total</td>
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The OD:CO ratio in ELISA was close to the cut-off value for most of the discrepant serum compared with a mean OD: CO ratio of 2.84 where both HI and ELISA results were positive and 0.20 where both were negative.

Six hundred twenty-four samples, including all samples that were positive in either HI or the ELISA, were tested for dengue 2-specific antibodies by PRNT. Three hundred ninety-nine of these samples had neutralizing antibodies to dengue 2, or 39.9% of the total study population.

Sixty-one percent of the females and 63% of the males were seropositive by the ELISA ($P = 0.37; $ not significant).
The corresponding dengue 2 PRNT results were 41% and 38% ($P = 0.26$; not significant). The flavivirus seroprevalence, however, increased with age, as did the prevalence of antibodies to dengue 2 (Figure 2). The increased prevalence of flavivirus antibody positivity in people more than 40 years of age corresponds to a cohort of people who may have been present during the 1954 dengue 3 epidemic. People more than 50 years of age represent a cohort who may have been exposed to dengue 2 during the World War 2 epidemic, and this is reflected by an increase in both flavivirus and dengue 2 antibody frequencies in this age group. Twenty-six percent of those less than 50 years of age had dengue 2 antibodies. This group was no more likely to give a history of previous dengue infection away from Charters Towers ($P = 0.55$) or to have traveled to a country where dengue has occurred ($P = 0.41$) compared with dengue 2-seronegative people. There were 28 individuals less than 40 years of age (8.3%) whose serum was positive in the ELISA but they had no dengue 2 neutralizing antibody. Only three of these people had traveled to international locations where dengue has occurred. The other individuals may have been infected with dengue 1 during the small epidemic that occurred in 1981, or have been infected with another flavivirus.

The HI test is a reliable and sensitive method for the detection of dengue antibodies. The HI antibodies are thought to persist for more than 50 years, which has made this test popular for serologic surveys. Dengue 3 antigen was used in HI tests on the basis of advice that dengue 3 might be more broadly cross-reactive than the other dengue serotypes (Aaskov J, Queensland University of Technology, Brisbane, Australia, unpublished data). Certainly, many dengue 2 infections that occurred 50 years prior were detected in this study using this antigen. The ELISA was not as sensitive as the HI test in one study, but demonstrated high sensitivity and specificity in another. Up until recently, all ELISAs for the detection of dengue antibodies were in-house assays and the performance of an ELISA in one laboratory might not be reproduced in other laboratories. The release of a commercial dengue ELISA and the paucity of data supporting the use of an ELISA in retrospective serologic surveys prompted us to compare the two tests.

The results indicate that the ELISA is sensitive and specific in the detection of previous exposure to dengue infections. Given the history of dengue epidemics in Charters Towers, the test needed to be sensitive enough to detect antibody from 40 years ago (1954 dengue 3 epidemic), 50 years ago (1941–1943 dengue 2 epidemic), or even further in the past. An analysis of the PRNT results for the sera that yielded discrepant results suggested that the ELISA may be slightly more sensitive. The specificity of the ELISA was high in our study population. The simplicity and speed of the ELISA make it an ideal test for use in seroepidemiologic studies.

Dengue virus epidemics have been estimated to infect between 5.6% and 90% of the populations affected (Table 2). Factors related to the mosquito vector, the virus strain, climate, housing conditions, and previous immunologic exposure influence the intensity of an epidemic. The infection rate for an Australian community has only been determined for an outbreak on Thursday Island in 1981 in which 27% of the population was estimated to have been infected. Rowan reported that approximately 15,000 of Townsville’s then 40,000 inhabitants (37.5%) had been infected during the 1954 epidemic.

In Charters Towers, the prevalence of dengue 2 antibodies among those who could only have acquired antibodies during the 1992–1993 epidemic, i.e., those less than 50 years of age, was 26%. This is thought to be a reasonable estimate of the proportion of the Charters Towers population that was infected during the most recent epidemic. Of interest is that flavivirus, and not dengue 2, seroprevalence increased from 31% to 57% in the age categories representing people born before and after the 1954 dengue 3 epidemic (a difference of 24%). The dengue 2 seroprevalence increased from 29.5% to 53% in the age categories representing people born before...
and after the 1942–1943 epidemic (a difference of 23.5%). Without accurate records of residential histories it is difficult to draw firm conclusions. However, the data are consistent with each of the significant epidemics (1941–1943, 1954, and 1993) being associated with a seroconversion of approximately 25% of the population.

The different infection rates observed in the different studies may reflect some methodologic differences. Only a few of the studies set out to specifically look at the seroconversion rate during the respective epidemics. Different epidemics in the same communities have been observed to affect different proportions of the population, as occurred in the two Cuban epidemics.17 The variation in infection rates is therefore likely to be determined by a combination of ecologic and virologic attributes. There was an apparent difference in the size of the epidemics caused by dengue 1 in 1981 and the dengue 2 epidemic in 1993 in Charters Towers. During the 1981 epidemic, there were only two cases of dengue reported from Charters Towers, but it was possible that many more cases had occurred. In our sample, 23 people thought they had dengue fever in 1981 but only 16 had serum reactive in the ELISA or HI tests; 11 of these had dengue antibodies. Our data suggest that any dengue 1 epidemic was very limited in its extent and may not have become established in the town as did the epidemics in 1942–1943, 1954, and 1993. The epidemic on Thursday Island was caused by the same strain but was more extensive and occurred during the wet season. It is likely in this instance that ecologic rather than virologic factors acted to limit the extent of the 1981 epidemic in Charters Towers.

If the Charters Towers infection rate applies to Townsville (population = 130,000), which was similarly affected during the same epidemic, there is a sizable pool of children at risk should a different dengue serotype be introduced in the next few years.

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### References