A PRACTICAL COMMUNITY-BASED APPROACH TO THE DIAGNOSIS OF DENGUE VIRUS INFECTIONS

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ABSTRACT

A study is described in which private physicians participated in the surveillance and diagnosis for dengue disease within a community. Using simple, economical methods it was shown that a significant proportion of specimens submitted were positive for dengue infection. Of 610 fingerprick blood specimens collected on filter paper strips, 7% to 33% were positive by serology. Of these specimens, 423 (69%) were single specimens and 92 (22%) of the single specimens were positive by serology. Of 153 fingerprick blood specimens collected in heparinized, glass capillary tubes, 15% to 50% yielded a dengue virus isolate. When a positive result was based on serology and/or virus isolation, an overall positive rate of 39% was obtained for one of the participating clinics. Inclusion of thrombocytopenia and leukopenia in selection of cases significantly improved the positive rate. It is argued that this simple and practical community-based approach will result in more accurate surveillance of dengue transmission within an endemic area and thus aid in the development of long-term strategies for control of disease.

Keywords: dengue, surveillance, laboratory diagnosis.

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INTRODUCTION

The effective control of transmissible diseases depends to a large extent on accurate epidemiological data reflecting the extent and level of transmission and endemicity of an infectious agent. A reliable epidemiological profile is essential in the planning and implementation of long-term strategies to contain and control disease (1). The syndromes caused by the dengue viruses, dengue fever (DF) and dengue haemorrhagic fever/dengue shock syndrome (DHF/DSS), still pose important public health problems in many parts of the tropical world (1). The precise prediction of dengue outbreaks still remains an elusive goal exacerbated, in part, by inaccurate, incomplete and infrequent monitoring of virus transmission within a particular community. Typically, transmission levels are estimated on the basis of the number of dengue cases reported by physicians, as diagnosed on the basis of clinical criteria (2). This is often supplemented by occasional monitoring of the relevant mosquito vectors. In a large majority of cases, these reports are not followed up and substantiated by the diagnostic laboratory and data on the actual number of confirmed cases and virus serotypes circulating are inadequate in most areas where dengue is endemic. It is also important to note that an accurate, overall picture of disease activity would require the effective participation of private clinics, in addition to the government health centres and hospitals. In an effort to improve this unsatisfactory situation, a simple, economical and practical diagnostic approach involving private practising physicians within a community was tested in Kuala Lumpur, Malaysia, a dengue-endemic area. This approach was based on an easily performed fingerprick blood collection from suspected dengue cases using filter paper strips and/or glass capillary tubes. The success of this approach would suggest that its more widespread use within a community will produce a more accurate epidemiological profile of virus prevalence and transmission and, ultimately, result in the more effective epidemiological surveillance necessary in attempts to prevent and control this important human disease.

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MATERIALS AND METHODS

Patients and case selection

610 selected patients attending two private, general practice clinics in the Kuala Lumpur area over a 30 month period were screened for a potential dengue infection. For both participating clinics, these patients were essentially those who presented with symptoms of pyrexia of unknown origin. In addition, clinic 1 (see Table I) also selected cases utilizing some of the diagnostic criteria for dengue fever syndrome outlined in a World Health Organization guide (2). This included: fever of more than 2-3 days duration, leukopenia (<10,000/mm3) and thrombocytopenia (<100,000/mm^3).

Table I
DIAGNOSIS OF DENGUE INFECTIONS ON SPECIMENS SENT BY PRIVATE CLINICS

Clinic	Diagnostic criteria	No. positive for dengue infection by Serology*	Virus isolation
1	PUO/THR/LKP+	176/525 (33%)	28/56 (50%)
2	PUO	6/85 (7%)	15/97 (15%)

* Positive result based on single specimen or paired sera tested by haemagglutination inhibition and interpreted according to a World Health Organization guide (2); also see Materials and Methods.

+ PUO = pyrexia of unknown origin, THR = thrombocytopenia, LKP = leukopenia.

Blood specimens

Fingerprick blood specimens were collected using Nobuto's filter paper (Strip type, Type I, Toyo Roshi Kaisha Ltd., Tokyo, Japan). Both sides of the filter paper strip were saturated with blood and the strips then allowed to dry at room temperature. In some cases a simultaneous sample was collected into heparinized, glass capillary tubes (75mm length, 0.5-0.9 mm diameter, Erma Optical Works Ltd, Tokyo, Japan) and the end of the tubes sealed with plasticine. These tubes were then kept at 4°C until they were transported to the laboratory, usually within 24 hours.

Serological methods

Specimens on filter paper strips were eluted by soaking the strips (after cutting into 3 pieces to facilitate and optimize extraction) in 0.8 ml of a 12.5% kaolin (in borate-saline) solution for a minimum of 3 hours at 4°C. Specimens were centrifuged at 1,500 rpm for 10 minutes and then absorbed by adding 50 ul of a 50% goose erythrocyte suspension for 30 minutes at 4°C. After centrifugation at 1,500 rpm for 10 minutes specimens were used in the standard haemagglutination-inhibition test to detect flavivirus antibodies (3). In accordance with established criteria (2), a specimen was regarded as positive when a titre of ≥1:2560 was obtained with a single specimen or at least a four-fold increase in titres detected with paired sera.

Virus isolation

Approximately 50 ul of serum removed from glass capillary tubes was diluted with 50 ul of diluent solution (phosphate-buffered saline containing 0.5% gelatin and 5% fetal calf serum). Virus isolation was then attempted by inoculation of the diluted serum intracerebrally into *Toxorhynchites* mosquito larvae as described previously (4). Identification of isolated dengue serotypes was carried out by immunofluorescence using type-specific monoclonal antibodies (5).

RESULTS

The present study showed that, based on serological criteria, 7% to 33% of the blood specimens collected on filter paper strips were positive for dengue infection (Table I). Of the specimens submitted for serology, 69% were single specimens and 4% to 26% of these were positive (Table II). With regard to virus isolation, 15% to 50% of the specimens collected in glass capillary tubes yielded a dengue virus isolate (Table I). Of the 44 virus strains isolated, 18 (41%) were dengue-1, 3 (7%) were dengue-2, 5 (11%) were dengue-3, 3 (7%) were dengue-4 and 15 (34%) were untyped. Further analysis of specimens from clinic 1 showed that 39% of the specimens were positive by serology and/or virus isolation. It may also be seen that positive rates were significantly higher when other diagnostic criteria, such as thrombocytopenia and leukopenia, were used in case selection (Table I). From the clinical findings, all the patients in the present study were cases of dengue fever and none had dengue haemorrhagic fever/dengue shock syndrome.

DISCUSSION

The present study investigated the workability and usefulness of involving private physicians working within a community in the overall attempt to accurately monitor dengue disease activity. It is concluded that such an effort was workable and generated useful information. The overall rates of serological positives (7% to 33%) and isolation positives (15% to 50%) compares favourably with the rates obtained from cases diagnosed in a hospital setting within the same study area (6). Most importantly, the study showed that a significant percentage of single specimens submitted were positive by serology; 92 of the 182 serological positives (50%) being obtained with single specimens

Table II
PROPORTION OF SINGLE SPECIMENS IN STUDY SENT FOR SEROLOGY

Clinic	Total No. of specimens sent	No. of single specimens	No. of positive single specimens
1	525	341 (65%)	89/341 (26%)
2	85	82 (96%)	3/82 (4%)
Total	610	423 (69%)	92/423 (22%)

only. As has been noted previously (7), and confirmed in the present study, a majority (70-80%) of the specimens submitted for laboratory diagnosis are single specimens. The methods employed (i.e. fingerpricks, filter paper strips and glass capillary tubes) are also simple, economical, practical and more acceptable. The present approach can also be extended to rural health centres and the smaller district hospitals. The results of the present study have several important implications.

Most importantly, by extending coverage into the community it would improve the accuracy and extent of surveillance of dengue transmission. This is particularly important in the light of the association of certain dengue serotypes (e.g., dengue-2) with more severe disease. Under actual field conditions in a community, it would be reasonable to assume that the overall incidence of dengue disease in many tropical areas are being underestimated. Several factors may contribute to this situation. For example, some physicians may not be fully aware of the clinical signs and symptoms of dengue disease which often present with non-specific features, especially the milder dengue fever. The inconvenience of dealing with the government bureaucratic process when notifying a case makes many medical practitioners in the private sector reluctant to notify cases. The proposed scheme of involving private physicians may thus help to solve the problem of underestimation of cases and thus give a more accurate picture of dengue activity within a community. Furthermore, the present study has also confirmed the fact that all four dengue serotypes are endemic in Malaysia and that, for the duration of the study in Kuala Lumpur, dengue-1 appeared to be the major serotype. The majority of these isolates were obtained from acute-phase blood specimens with low antibody titres.

Such a scheme may also result in better management of individual cases. With the more rapid diagnostic methods available recently, such as IgM detection (7) rapid virus isolation in mosquito larvae (4) and the promise of hybridization probes (8), the scheme becomes more attractive in that physicians will obtain rapid feedback from the laboratory and this may prevent, for example, unnecessary antibiotic therapy. The overall cost of heath care may thus be reduced. The feedback obtained may also serve to improve the doctors' clinical acumen in recognizing and diagnosing cases of dengue disease thus improving the diagnostic capabilities of the medical community as a whole. This, in turn, may be invaluable in providing early warning of potential outbreaks of dengue in various parts of the country. With regard to case selection, the study also showed that the inclusion of certain diagnostic criteria, namely thrombocytopenia and leukopenia, significantly improved the positive rate. These two criteria have been shown to be present in 26-50% and 76-100% of dengue fever cases respectively (2).

The collection of dengue virus isolates from the present study is also extremely valuable for basic research into the phenomenon of genetic variation among the dengue viruses. It has been proposed previously that variation may, in fact, be the basis of virus virulence (9). In relation to this, the molecular analysis of dengue virus isolates from all over the globe is being intensively pursued at the present moment (10,11). Such data on the types of viruses circulating are also valuable in epidemiological terms in tracing virus movement over time between endemic areas which are closely linked geographically, as is found in the Southeast Asia region.

In conclusion, it would seem that the widespread application of the methods outlined in the present study in endemic areas would contribute significantly to improving surveillance for dengue virus transmission and thus form the basis for more rational approaches in the prevention and control of dengue disease.

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