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Surveillance of dengue fever in French Guiana by monitoring the results of negative malaria diagnoses

A. TALARMIN[1]*, C. PENEAU[2], P. DUSSART[1], F. PFAFF[1], M. COURCIER[3], B. DE ROCCA-SERRA[3] AND J. L. SARTHOU[1]

[1] *Institut Pasteur de la Guyane française, Cayenne, French Guiana*
[2] *Hôpital André Bouron, Saint-Laurent du Maroni, French Guiana*
[3] *Laboratoire d'Analyses Médicales, Kourou, French Guiana*

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SUMMARY

Surveillance of dengue fever is mainly based on specific laboratory tests. However non-specific systems, such as clinical surveillance, are also required. In French Guiana, we have tested a non-specific laboratory surveillance system where different biological examinations performed for other reasons than the diagnosis of dengue fever were analysed as methods for dengue fever surveillance. The number of negative malaria diagnoses in Cayenne and Kourou was found to be the best indicator of dengue fever infections in these towns. This surveillance system appears to be very simple and reliable, and a test which could serve as an indicator that is likely to be found everywhere.

INTRODUCTION

Dengue fever is a tropical mosquito-borne infectious disease caused by four serotypes of dengue virus. Dengue fever is a major public health problem which is responsible for millions of cases of illness and thousands of deaths in tropical countries every year [1]. It is transmitted to humans by mosquitoes of the *Aedes* genus, mainly *Aedes aegypti*. The principal symptoms of classical dengue fever are fever, headache, myalgia, and arthralgia with or without a rash. Dengue hemorrhagic fever includes these symptoms, haemorrhage, thrombocytopaenia and signs of increased capillary permeability [2]. Dengue fever is frequently associated with thrombocytopaenia and the serum level of transaminases is often high [2].

Dengue fever is also a major public health problem in French Guiana, an overseas French Department (administrative unit) located between Brazil and Surinam, in the Amazonian forest. An epidemic of dengue haemorrhagic fever caused by dengue virus type 2 was responsible for 40 cases and 6 deaths in 1991 and 1992 [3]. There have also been outbreaks in 1996, 1997 and 1998 in different cities of French Guiana (unpublished data).

Vector control is the only way to reduce the incidence of dengue fever. Mosquitoes can be controlled by the use of insecticides against larvae and adults, and continuous elimination of larval habitats has proved to be more effective in preventing the epidemics [1]. The contribution of the whole population to control, especially during interepidemic periods, is also a very important factor for success. Unfortunately, most people lose interest in mosquito control as soon as the level of dengue transmission is low, so that continuous routine control of larvae is difficult to maintain. When an epidemic has begun, the objective is to prevent spread to other areas.

* Author for correspondence: Centre National de Référence pour la Surveillance des Arboviroses, Institut Pasteur de la Guyane, 23 avenue Pasteur, BP 6010, 97306, Cayenne cedex, French Guiana.

Although very difficult, this is possible [4]. Various conditions are necessary to reduce efficiently the number of cases. One is a rapid-response emergency vector control programme immediately after the onset of the outbreak [1]. Consequently, an effective surveillance and an early warning system are necessary. Surveillance is usually based upon specific biological tests (e.g. serological or virological tests) and/or sentinel physicians who declare the number of clinically suspected cases to the local health authorities [5].

The only surveillance systems existing in French Guiana until December 1996 were the number of suspected cases and the number of probable and confirmed dengue fever cases. The terms suspected, probable and confirmed cases are used according to the definitions adopted by the Council of State and Territorial Epidemiologists/Centers for Disease Control and Prevention (Atlanta, USA). A suspected case is defined as an illness in a patient whose serum was sent to the Centre National de Référence pour la Surveillance des Arboviroses for the diagnosis of dengue fever. A probable case is an illness in a person that is clinically compatible with dengue combined with supportive serological test results (a single convalescent-phase serum specimen containing dengue virus IgM antibody, or a dengue virus IgG antibody titre of 1280). A confirmed case is an illness in a person that fulfils any of the following criteria for diagnosis; seroconversion from negative to positive, or a fourfold or greater increase in dengue virus IgG or IgM antibody titres to one or more dengue virus types in paired serum samples; or isolation of dengue virus from serum or autopsy specimens; or demonstration of dengue virus antigen in autopsy tissue samples by immunochemical analysis; or demonstration of a dengue virus cDNA fragment by amplification from a serum sample [6].

However, shortcomings in these surveillance and alert systems have been found during the last epidemics, and there have been excessive delays before epidemic declaration. This lead to the conclusion that our Department needed an improved dengue fever surveillance system to increase the efficacy of the mosquito control services after the onset of an epidemic.

We describe here a new surveillance system based on a non-specific biological test. We evaluated the results of tests performed for reasons other than dengue fever surveillance as indicator of dengue fever prevalence.

METHODS

Dengue fever laboratory assays

All tests were performed at the Institut Pasteur de la Guyane, National Reference Centre for Arboviruses. A dengue virus-specific IgM capture enzyme immunoassay (MAC-ELISA) was used as previously described [7]. For virus culture, acute phase serum samples from feverish patients (< day 4 after onset of fever) were diluted 10-fold in Leibowitz medium containing 3% foetal calf serum, and dilutions were used to inoculate subconfluent AP 61 cell cultures as previously described [3]. After 7 days of culture, cells were harvested, and dengue viruses were identified according to serotype by an indirect immunofluorescence assay using anti-dengue virus type-specific monoclonal antibodies obtained from the Center for Disease Control and Prevention, Fort Collins, CO, USA [8]. Dengue virus RNA was detected using reverse transcription–polymerase chain reaction (RT–PCR). Viral RNA was extracted from 10 μl of acute phase serum using silica as previously described [9]. The first run of RT–PCR and subsequent semi-nested PCR were performed following a previously described procedure [10].

Non-specific laboratory assays

The non-specific laboratory assays used for dengue fever surveillance were those most frequently requested by physicians to evaluate the severity of the illness ('haemogram' which consists of counting red and white blood cells, haematocrit, haemoglobin, and platelets; the serum transaminase assay and Giemsa-stained blood smears to exclude the presence of malaria infection). Malaria is also a major public health problem in French Guiana, but the areas of transmission are different for dengue fever and malaria. Malaria mainly occurs inland, along the Oyapock river which is the border with Brazil and along the Maroni river which is the border with Surinam. Dengue fever is limited to the coastal area, from Saint-Georges de l'Oyapock in the East to Saint-Laurent du Maroni in the West. Because of the menace of malaria in French Guiana, the blood smear technique for malaria diagnosis is frequently requested for patients presenting with symptoms of dengue fever, to distinguish it from malaria. Therefore we exploited this test for dengue surveillance.

To evaluate the value of non-specific tests for

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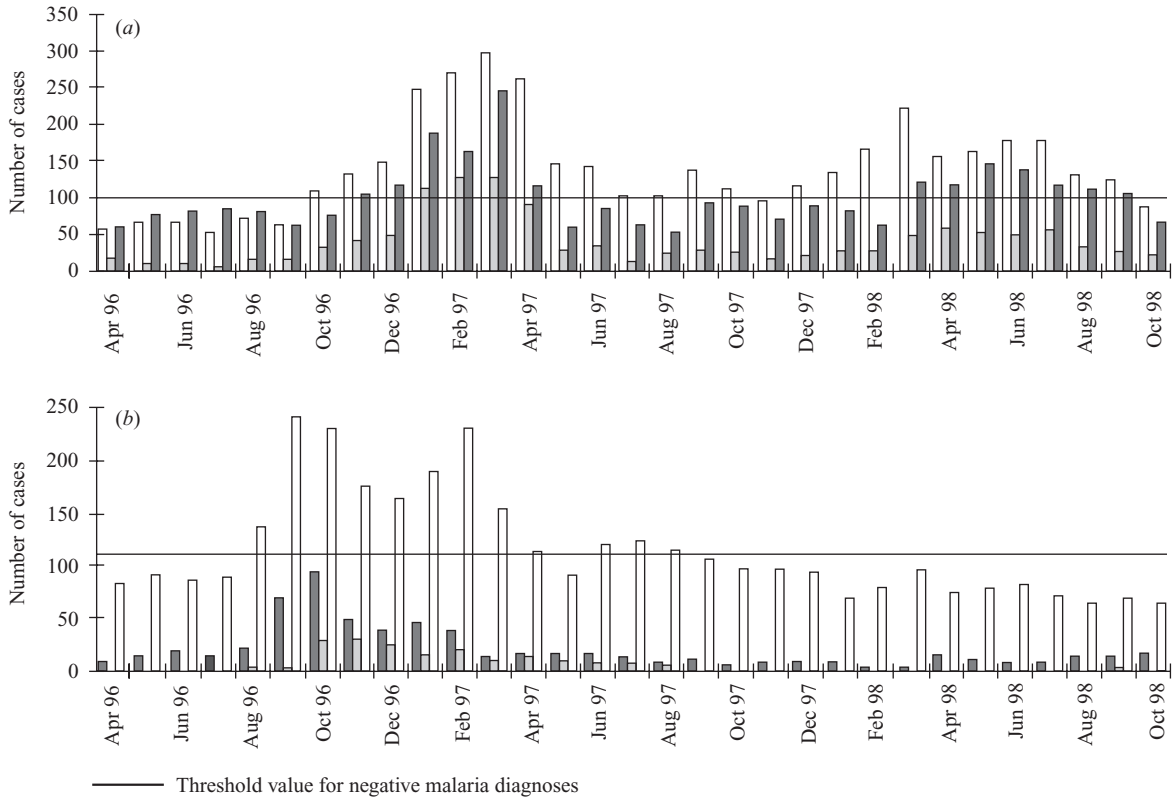

Fig. 1. Numbers of suspected cases (□), recent infections with a flavivirus (▨) and negative malaria diagnoses (▧) in Cayenne (A) and Kourou (B), French Guiana.

predicting the incidence of dengue fever, the monthly number of requests for these biological examinations was compared to the number of suspected dengue cases and to the number of probable and confirmed dengue cases. Two laboratories were included in this evaluation phase, one laboratory of the four in Cayenne, and the only laboratory in Kourou. The evaluation was performed retrospectively until December 1996, and prospectively after that date. The threshold value that should alert public health authorities was determined as the mean baseline during a non-epidemic period plus two standard deviations.

After the retrospective evaluation of the non-specific surveillance, a study was performed in the Cayenne laboratory to assess the value of the system in predicting dengue fever. After gaining informed consent, dengue fever diagnostic assays were performed for each patient with a malaria diagnosis, using the same methods. This evaluation was performed during an epidemic period (January, February and March 1997) and a period with a lower incidence of dengue fever (July to October 1998). Results of dengue fever diagnoses in samples from patients sent for malaria diagnosis were compared to those obtained in samples from patients also sent for dengue fever diagnosis. Almost all patients tested for dengue fever diagnosis were also tested for malaria.

Statistical analysis

To compare the specific and the non-specific surveillance systems, the correlation coefficients were calculated using Excel 5 software (Microsoft Corp., Redmond, WA) according to previously described methods [11]. The ξ^2 analysis was used to assess the value of negative blood smears for detecting dengue fever in comparison with the requests for dengue fever diagnoses.

RESULTS

All biological examinations were evaluated, but only results of the negative malaria diagnoses, which appeared to be the best indicator of dengue fever, are presented here. The number of confirmed cases correlated best with the number of negative malaria diagnoses ($r_{cay} = 0{\cdot}70$, $P < 0{\cdot}001$; $r_{kou} = 0{\cdot}69$, $P < 0{\cdot}001$) (Fig. 1).

The evaluation of the value of malaria diagnoses for

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Table 1. *Results of dengue fever diagnostic tests performed on samples sent to the Cayenne laboratory for malaria diagnosis and on samples sent to the same laboratory for the diagnosis of dengue fever*

Samples sent for	Tested *n*	Probable dengue fever cases *n* (%)	Confirmed dengue fever cases *n* (%)
Malaria diagnosis only			
Jan–Mar 1997	412	98 (23·8)	95 (23·1)
Jul–Oct 1998	304	35 (11·5)	74 (24·3)
Dengue fever and malaria diagnoses			
Jan–Mar 1997	550	138 (25·1)	132 (24·0)
Jul–Oct 1998	363	47 (12·9)	84 (23·1)

predicting dengue fever is presented in Table 1. Of 599 samples from patients sent for malaria diagnosis to the Cayenne laboratory from January to March 1997, 412 (68·8%) were tested for dengue fever diagnosis. From July to October 1998, 304 out of 401 (75·8%) of samples from patients sent for malaria diagnosis were tested for dengue fever diagnosis. The overall percentage of probable and confirmed dengue fever cases in patients whose samples were sent for malaria diagnosis was 46·8% during the epidemic period and 35·8% when the transmission of dengue fever decreased. The percentages of samples from patients sent for dengue fever diagnosis were 49·0% and 36·1% for the same periods respectively. Differences between the results of samples from patients sent for malaria diagnosis and those sent for dengue fever diagnosis were not significant.

DISCUSSION

To our knowledge, this is the first analysis of different non-specific biological assays as methods for dengue fever surveillance. Malaria is not frequent in Cayenne or Kourou and only imported cases (mostly from the two border rivers of the Department) are observed. Nevertheless there are some cases; therefore physicians, even if they are quite sure that the patient is infected with dengue fever, often request blood smears to exclude malaria in patients showing febrile illnesses without specific symptoms. The evaluation of the value of negative malaria diagnoses confirmed that this test is often prescribed in patients presenting with dengue fever, since percentages of probable and confirmed cases among patients addressed for malaria diagnosis only was similar to that observed among patients addressed for dengue fever and malaria diagnoses. Only 70–75% of the patients tested for malaria diagnosis were tested for dengue fever. However no sample bias existed and therefore the percentage of probable and confirmed dengue fever cases is accurate. As previously observed, the percentage of dengue fever cases increased during the epidemic period [3]. The percentages of probable and of confirmed cases were similar during the epidemic period, but the percentage of confirmed cases was higher than that of probable cases when the transmission of dengue fever decreased (Table 1). This is due to the fact that cell culture or RT–PCR were performed in almost all acute phase sera during the non-epidemic period, and only in some sera during the epidemic. In contrast with what is observed during dengue epidemics, no increase in the number of negative malaria diagnoses was observed during the influenza epidemics which occurred at the end of 1996 and at the beginning of 1998 in French Guiana (Fig. 1) [12]. Physicians probably do not think of malaria in patients presenting with respiratory symptoms.

Although potentially valuable, these tests are only an alternative to the clinical surveillance which does not exist in French Guiana. Both systems can only be accessory tools for dengue fever surveillance. An increase in the number of biological examinations requested, used for dengue fever surveillance, must always be compared with other data (e.g. the number of requests for dengue fever serological tests and/or the number of cases of thrombocytopaenia, the appreciation of local physicians, the number of confirmed cases and whether a new serotype of dengue virus is circulating). Only when all these indicators are in agreement should an epidemic be declared and the vector control services alerted. However non-specific surveillance (using sentinel physicians or laboratories) is useful since it can alert the Health Authorities and the laboratory in charge of the specific surveillance.

In conclusion, dengue fever surveillance using non-

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specific biological examinations is simple and appears to be reliable in the two different areas tested in our study. To alert the Health Authorities earlier, numbers of requested examinations have to be recorded weekly from sentinel laboratories. This will now be done in French Guiana and the French West Indies.

This system can be used as an indicator in all the regions where dengue fever and malaria exist together (South America, South East Asia). The need to associate negative malaria diagnosis with thrombocytopaenia may apply in some regions and should be determined after preliminary assays. We believe that a test which can serve as an indicator is likely to be found nearly everywhere that medical laboratories exist. In regions where malaria is not transmitted, the number of requests for platelet counts may be a very good indicator. It cannot be studied in our country since the platelet count is not requested alone but with the 'haemogram' which appeared to be a poor indicator because this test is requested for many pathologies. The number of patients with thrombocytopaenia could not be studied retrospectively but will be tested prospectively in French West Indies. Apart from simplicity and reliability, this surveillance system presents two advantages. Firstly, it can be performed even in places where the specific diagnosis of dengue fever is not possible (an increased number of these non-specific examinations should help the Health Authorities in their decision to request specific dengue fever diagnosis), and secondly the number of laboratories required for this surveillance is lower than the number of physicians necessary for the sentinel physicians system. Two limitations of this system are that to obtain a long-term compliance of the sentinel laboratories, this surveillance must be kept as simple as possible and the determination of the threshold value can be made only on a non-epidemic period and therefore it may be delayed in regions where an epidemic exists or when a retrospective study is not possible.

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