Dengue in Brazil II: laboratory aspects and perspectives

Summary

The establishment of Public Health Laboratory Network for dengue diagnosis as well as their activities and problems in the last 10 years are described. Research laboratorial activities in dengue by different groups in the country are presented and briefly discussed. Some perspectives and objectives for dengue research in the country are summarized as a contribution to a closer collaboration of the laboratories involved in flavivirus studies in Brazil.

Dengue-like fever has been clinically described in the state of Rio de Janeiro in the last century between 1846 and 1847; by the same time dengue cases were reported in the states of Bahia, Pernambuco and some other areas in the north of the country (1). Dengue outbreak was also described in Rio Grande do Sul in 1916 probably related to early epidemics (1905 and 1911) in the Argentinian cities close to the border (1). By 1923, a splendid report of a dengue outbreak at Niterói city (Rio de Janeiro state) allowed comparative evaluations of the clinical picture observed in the same area 53 years later (2,3).

In 1981 a dengue outbreak was reported at Boa Vista city, Roraima state, in the Amazon region, when dengue virus type 1 (DEN-1) and 4 (DEN-4) were isolated by the Instituto Evandro Chagas (IEC), Belém (4). This episode could be controlled by local measures of vector elimination and did not start epidemics in other parts of the country. However, by April 1986, an outbreak caused by DEN-1 virus was described and confirmed by the Department of Virology / Instituto Oswaldo Cruz (IOC) in a densely populated area close to Rio de Janeiro city (5,6,7). A rapid spread of the disease to the city and later, in the same year, along the coast, was impossible to control specially due to the high infestation rates of the vector Aedes aegypti in many country’s areas. Lack of political decision and resources to face the new disease contributed to the establishment of outbreaks in Alagoas and Ceará by June and September 1986, respectively.

Before dengue outbreak in Rio de Janeiro just two laboratories (Instituto Adolfo Lutz, São Paulo and Instituto Evandro Chagas, Belém)
were responsible for arthropod-transmitted viruses diagnosis in Brazil, both covering the Southeast and mostly the Amazon area, respectively. The low circulation of arthropod transmitted viruses in Rio de Janeiro demonstrated by a seroepidemiological survey carried out in school children in 1977 (8) lead us to decide invest no efforts in this virus group, facing other state’s public health priorities. However, the isolation of the first dengue strains in Rio de Janeiro changed completely this situation and, in short time, many virus laboratories had to start their diagnosis activities of the new disease. Recently, by March 1986, we attended an International Course for Dengue Diagnosis that took place in Venezuela sponsored by the Pan American Health Organization (PAHO). One month later IgM capture immunoassay (Mac-Elisa) and dengue virus isolation system were settled in our laboratory, when we confirmed by DEN-1 virus isolation 100% of the first 9 sera samples collected from suspected cases.

Soon became clear that efforts should be made to organize training courses for the State Public Health Laboratories in order to implement virus isolation system in Aedes albopictus clone C6/36, dengue virus identification by using monoclonal antibodies and serological assay (Mac-Elisa). Supported by the National Health Foundation (FNS), PAHO and IEC/Belém, the Department of Virology/IOC organized three National Training Courses that took place at FIOCRUZ, Rio de Janeiro and at FUSAM, Recife (Table 1). Ae. albopictus cell line and reagents were distributed for those laboratories and soon dengue infections by DEN-1 virus were confirmed in different parts of the country.

By 1990, exactly 4 years after the isolation of the first strains of DEN-1 virus, an active virological surveillance detected dengue virus type 2 (DEN-2) circulation in Rio de Janeiro before the establishment of an extensive epidemic that followed the same distribution of DEN-1 in the country (9,10). In the following years numerous dengue epidemics could be described with laboratory support, showing the dengue dissemination in the country, along the routes of vector infestation (11,12,13,14,15,16,17). Nowadays the Public Health Laboratory Network for Dengue Diagnosis is composed by seven Reference Laboratories (IEC/PA - National Reference, IOC/RJ, IAL/SP, FUNED/MG, ISDF/Brasília, LACEN/PE, LACEN/CE) and six Central Laboratories (RJ, BA, GO, MS, PR, MT).

The network response for dengue diagnosis laboratories in the country was very efficient, although local problems often had brought difficulties for the services continuous maintenance provided by the laboratories. One difficult point in some states, was the very large number of sera samples collected during epidemics, provenient from areas already known to have virus circulating. Considering the time consuming for handling samples and the lack of personnel at the laboratories, a close collaborative contact with the epidemiological services in the state was considered essential to keep an overview of the dengue situation in the area, avoiding the shortcomings that may arise from non-testing rapidly all serum samples received in the laboratory.

Other aspect to be considered is the limited source of the reagents, basically dengue virus antigen availability. The task of antigen preparation was taken over mainly by the IEC, besides the IAL and IOC; the participation of Yale University/USA in a collaborative project with IEC for antigen preparation has been an
important support for our dengue diagnosis activities.

The establishment of an active surveillance system in dengue showed to be very important and two examples could illustrate this point. First, the city of Niteroi, was able to establish in the late 80’s, a good virological and epidemiological surveillance system through a close collaboration between laboratory, epidemiological services and health centers in areas heavily infested with *Aedes aegypti*. As a result our laboratory could isolate DEN-2 virus in the community before the recognizing of a large epidemic in the state (9,10). This allow the establishment of local vector control measures and a more accurate evaluation of new cases, which certainly contributed to reduce the impact of the introduction of the new serotype in the Rio de Janeiro.

Another example of successful virological surveillance system occurred in the state of Bahia, 1996. Based on clinical observation that dengue-like infections was occurring for the second time in the same patients, our group, in cooperation with LACEN/BA, using a reverse transcriptase-polymerase chain reaction (RT-PCR) could show that DEN-1 was already circulating in that state where only DEN-2 had been detected. DEN-1 was later confirmed by tissue culture isolation (18). The speed of the RT-PCR assay for detecting and typing dengue virus is an important tool in a virological surveillance. Then, training courses should be implemented soon, in order to make this technology available to the main dengue laboratories in the country.

The possibility of the introduction of dengue virus types 3 and 4 in Brazil, coming from other countries of the American region, make the establishment of effective epidemiological and virological surveillance an essential measure to the early detection of new serotypes in the country.

The implementation in the country of good quality kits for dengue diagnosis would join the private laboratories in the system of dengue virus surveillance, specially during interepidemic periods, when the public sector very often do not follow carefully the presence of dengue virus circulation. One example for that application occurred in Fortaleza, Ceará where a private laboratory stored negative sera collected from rubella suspected cases. A retrospective study in those samples carried out in our laboratory showed that dengue virus was circulating in Fortaleza since December 1993, peaking to a large epidemic caused by DEN-2 with 48.000 notified cases in May/June 1994 (19). The introduction of commercial kits would also allow the expansion of the network of dengue diagnosis laboratories to smaller municipalities, which would be able to detect dengue virus IgM antibodies, as an early virus circulation warning in the community. This point is essential for the planned Program of Eradication of *Aedes aegypti* in the country, which will require the early diagnosis of every dengue suspected case, in cooperation with the Vector Surveillance System, to be also established next year.

Other essential information for the Program of Eradication is the role of *Aedes albopictus* in dengue transmission. In Brazil the presence of *Aedes albopictus* was determined by first time in 1986 at the campus of the Federal Rural University, about 50 km outside Rio de Janeiro city and soon could be detected in other states like São Paulo, Minas Gerais and Espírito Santo (20).

In 1993, our laboratory isolated and demonstrated by RT-PCR DEN-1 virus from a pool of *Aedes albopictus* mosquitoes collected at the state of Minas Gerais (21). Dengue
transmission by this vector however, could not be demonstrated in that area.

During the last ten years our laboratory confirmed a total of 33.6% (6435/19175) of dengue studied cases basically from 3 epidemics occurred in the state of Rio de Janeiro and epidemics occurred in the states of Bahia and Espirito Santo, besides cases provenient from other states and even countries. In the state of Rio de Janeiro we observed a high rate of virus isolation during the first years of dengue epidemic (virgin soil phenomenon) (7). The decline of this rate were observed in the following years (Table 2). Mac-Elisa was choosen as a serological method for epidemiological surveillance because of its positivity as early as day 2 after the onset of disease and persistence of IgM for about three months (22).

The isolation of this strains allowed us to study the molecular epidemiology of dengue virus in the country. With the cooperation of CDC/Puerto Rico and Institute Pasteur/Paris we have been able to characterize Brazilian strains as Caribbean (DEN-1) and Jamaica (DEN-2) genotypes by using restriction enzymes (23) and virus sequencing (24). More efforts should be applied in collaborative projects in order to follow dengue virus evolution patterns in Brazil.

The possibility of recovery of flavivirus from complementary DNA, opened new perspectives to better understanding the molecular background of many biological properties including virulence and attenuation. Studies with cDNA obtained from yellow fever vaccine strains may conduct to new live flavivirus vaccines, since cDNA for other virus of the group, like dengue and Japanese encephalitis are already available (25).

Studies on dengue virus replication, using in situ hybridization and immuno-labelled specific antibodies in Aedes albopictus cell cultures clone C6/36, showed that the replication is processed inside the rough endoplasmic reticulum, using smooth membrane structures for virus particle synthesis. Virus particles leave the infected cells through the Golgi system and exocitosis when the last cleavage of the M protein occurs. The majority of the virus particles remain inside the vesicles derived from the rough endoplasmic reticulum, even after the lysis of the infected cells (26,27,28).

Studies on dengue immunology have been established by the analysis of the tumor necrosis factor-alfa serum level in Brazilian patients with dengue-2, showing that this factor can be detected in dengue disease, including more severe forms. Very high level could be demonstrated in one fatal case (29).

For the biological control of Aedes aegypti and Aedes albopictus, a micro crustacean Mesocyclops longisetus, has been used with excellent results. The predator survive in hot climates and in experimental conditions may destroy 98% of the Aedes albopictus larvae (30).

The bacterial toxins of the genus Bacillus have a strong effect on Culicidae larvae and one product, already prepared in pre-industrial scale presents a LC50 of 0.00071 ± 0.00048 mg/l and a LC90 of 0.021 ± 0.0046 mg/l and is being applied in fields studies, aiming its use, as a complementary tool, in vector control (31).

All these data, show a broad spectrum of activities in dengue viruses and related fields, since the reintroduction of the virus in the Rio de Janeiro area in 1986.

In conclusion, we recommend as basic approaches for dengue research and public health activities the following points:
- PCR technology set up in the main laboratories for dengue diagnosis
- Introduction of kits for dengue laboratory diagnosis.
- Evaluation of the role of Aedes albopictus in dengue transmission in Brazil.
- Characterization of dengue viruses genome strains circulating in Brazil.
- National and international research support on:
  i. molecular and cellular dengue biology, ii. immune response, iii. the role of Aedes albopictus in dengue transmission
- International cooperative projects, considering the magnitude of the problem for the tropical world.
References


