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Short Report: Dengue-3 Outbreak in Paraguay: Investigations Using Capillary Blood Samples on Filter Paper

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Abstract. During a dengue-3 outbreak in Paraguay at the beginning of 2007, capillary blood samples absorbed onto filter papers were collected from 44 suspected cases. These samples were subjected to three molecular and serologic tests, and 31 of the 44 samples gave a positive result by at least one of the techniques used. Molecular analyses detected the dengue-3 serotype in 22 patients and additionally the dengue-2 serotype in two patients. Therefore two different serotypes were co-circulating during this outbreak. Overall, this study validates the use of dried-blood samples for field screening investigations. Indeed, all types of laboratory studies of dengue were possible with samples consisting of a few drops of dried blood from finger pricks.

Dengue fever (DF) is a common viral disease that is a major public health problem in most tropical countries. There are currently about 100 million dengue virus infections annually, with more than 500,000 cases of the severe forms of the disease, dengue hemorrhagic fever (DHF) or dengue shock syndrome.[1] Several factors have contributed to the spread of this arbovirus in South America since the 1970s. In particular, the suspension of *Aedes aegypti* vector eradication programs, and the increase of human population movements within and between endemic regions have led to the resurgence of epidemic DF and the emergence of DHF in South America.[2–4] Four distinct serotypes of dengue virus (DENV-1 to DENV-4) currently co-circulate in this sub-continent and dengue virus outbreaks are frequent.[5] The persistence of dengue outbreaks during recent years has become a public health problem in Paraguay, a country adjacent to Argentina, Brazil, and Bolivia. In early 2007, the Ministry of Public Health and Social Welfare of Paraguay declared a nationwide epidemiologic alert after the confirmation of 390 cases of dengue infection during the first week of January. This outbreak has been the worst in the country's history in terms of the number of cases and percentage mortality. By March 2007, 19,000 suspected dengue cases, including 46 DHF and 10 deaths, had been reported. The DENV-3 serotype was identified as the serotype responsible.[6,7] To help in managing this outbreak and to evaluate the epidemiologic situation, the Institut Pasteur de la Guyane set up a scientific expedition in March 2007. We recently proposed that capillary blood samples absorbed on filter papers could be an alternative to venous blood samples for the diagnosis of dengue virus infection.[8] This expedition was an opportunity to validate the usefulness of collecting capillary blood samples onto filter papers for field screening studies, in particular in an area where the storage and transport conditions were incompatible with the molecular diagnosis of dengue infection from venous blood samples. Blood samples dried onto filter paper have the advantage of simplicity of collection (from the finger), and allow the identification of the viral serotype by molecular methods and the detection of dengue virus–specific immunoglobulin (IgM) antibodies and antigens.[8]

Capillary blood samples were collected, in four hospitals in Asuncion, between March 3 and 13, 2007, from 44 patients suspected of dengue virus infection and who agreed to participate in the study.[9] For each patient, three drops of about 20 μL of capillary blood were obtained from a finger and absorbed onto filter papers (Schleicher & Schuell, Germany) for subsequent reverse transcriptase-polymerase chain reaction (RT-PCR), and antigen (NS1) and IgM detection. All the samples were stored at room temperature until arrival at the Centre National de Référence des Arbovirus, Institut Pasteur de la Guyane, on March 17, 2007, when they were transferred to −80°C until studied. All capillary blood samples were first analyzed by RT-PCR: filter papers were cut into strips, placed into sterile tubes, and total RNA was extracted as previously described.[8] Dengue virus genome detection and typing was then done according to Lanciotti and others.[10] Although NS1 antigen detection is still not considered by the World Health Organization (WHO) to be a reference test for confirming dengue infection, it is widely used for diagnosis. We therefore decided to use it as a confirmatory test for the diagnosis of dengue virus infection. The Platelia dengue NS1 antigen capture ELISA kit (Bio-Rad Laboratories, Marnes la Coquette, France) was used to detect the presence of the NS1 antigen secreted by dengue virus during infection in capillary blood samples from all acute cases and from those convalescent cases for which sufficient biologic material was available.[11] For each patient, one drop of capillary blood absorbed onto filter paper was cut and placed in a sterile tube containing 150 μL of the dilution buffer provided in the kit. In addition to the controls provided in the kit, we also included two NS1-positive and -negative sera as internal controls: 10 μL of these sera were absorbed onto filter paper and thereafter processed in the same way as the capillary blood samples. After 30 minutes of incubation at room temperature, 100 μL of the capillary blood samples or controls were incubated with 100 μL of diluted conjugate for 90 minutes. The subsequent steps were performed according to the manufacturer's recommendations.[12,13] Serum samples were tested for IgM antibodies to

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dengue using an IgM antibody-capture enzyme-linked immunoabsorbent assay (MAC-ELISA).[14] All capillary blood samples obtained from cases in the convalescent phase of infection were tested (n = 18), whereas only those acute phase samples with sufficient biologic material were tested (n = 3). Briefly, each dried drop was cut into strips and incubated in 600 μL of phosphate buffered saline (PBS) 1X containing 0.5% Tween, 20% and 5% non-fat dried milk for 2 hours at room temperature, and 100 μL aliquots of the eluate were used as serum in the MAC-ELISA technique. Thereafter, the test was performed as described by Talarmin and others.[14]

The first day of fever was defined as day 1 of the disease. According to information provided by the patients, 26 of the 44 samples were collected during the acute phase of infection (day 1 to day 4) and 18 during the convalescent phase (≥ day 5) (Table 1). Dengue infection was confirmed for 26 cases (59%; 22 acute phase samples and 4 convalescent phase samples) by RT-PCR and/or NS1 antigen detection using capillary blood samples (Table 1). Of the 22 positive acute phase samples, 19 were scored positive by both tests, one was positive only by RT-PCR, and two were positive only by NS1 antigen detection. The three cases of discordance between RT-PCR and NS1 antigen detection may have been the result of blood spots not being uniform between papers or the viral load being below the threshold of sensitivity of the RT-PCR (the two NS1-positive RT-PCR-negative samples were collected on day 4 of the disease). We reported similar observations in our previous studies.[12,13] All four positive samples obtained during the convalescent phase of the disease were positive by RT-PCR, but only two also scored positive by NS1 antigen detection (Table 2). The NS1 antigen detection approach is a specific test for confirmation of acute phase dengue, so it is possible that these patient samples were collected during the acute phase of infection; this possibility is reinforced by the absence of IgM.[12,13] The other two RT-PCR positive samples collected during convalescent phase of infection (day 6 of the disease), although positive for dengue IgM, were negative for NS1. These results confirm previous observations that it is possible to detect viral particles in capillary blood samples late in the course of infection.[8] Thus, on the 26 RT-PCR- and/or NS1 detection-positive samples, 21 were positive by both approaches (80% concordance), 23 were positive by NS1, and 24 by RT-PCR. One of the three acute phase patients tested and four of the 18 convalescent patient samples gave a positive result only by IgM serology. Therefore, on the 44 samples tested, 31/44 (70.5%) scored positive by RT-PCR, NS1 antigen detection, or IgM serology (Table 1). Although presenting clinical signs compatible with dengue infection, biologic diagnosis indicated that the 13 other samples were negative. Molecular typing showed that 22 (19 acute and 3 convalescent) samples were infected by a DENV-3 serotype and two (one acute and one convalescent) by a DENV-2 serotype. Although these results confirm circulation of DENV-3 serotype during this outbreak, the identification of two DENV-2 infected patients indicates the co-circulation of another serotype. These results were confirmed by sequencing five DENV-3 C/prM (GenBank accession nos. EU076555 to EU076559) and one DENV-2 C/prM (GenBank accession no. EU076554) partial genes sequences.

TABLE 1

Overall virologic and serologic results from the 44 capillary blood samples absorbed onto filter papers from suspected dengue cases

	Acute phase (day 1 to day 4)	Convalescent phase (≥ day 5)	Total
Suspected cases	**26**	**18**	**44**
Confirmed DEN cases (RT-PCR and/or NS1 detection)	22	4	26
Probable DEN cases (IgM serology only)	1	4	5
Positive DEN patients	**23**	**8**	**31**

RT-PCR = reverse transcriptase-polymerase chain reaction.

TABLE 2

Results from the four capillary samples collected during the convalescent phase of the disease that were positive by RT-PCR

	Day post infection	RT-PCR	NS1 detection	IgM serology
Patient 1	Day 5	DENV-3	Positive	Negative
Patient 2	Day 5	DENV-3	Positive	Negative
Patient 3	Day 6	DENV-3	Negative	Positive
Patient 4	Day 6	DENV-2	Negative	Positive

RT-PCR = reverse transcriptase-polymerase chain reaction.

This study confirms the value of this type of clinical sampling for virologic and serologic diagnosis of dengue infection for field screening studies. Indeed, because of the ease of collection, storage, and transport, dried-blood samples on filter paper offer many practical advantages over conventional methods of serum collection and storage. Moreover, we showed here for the first time that the Platelia dengue NS1 Ag kit can be used with capillary blood samples absorbed onto filter papers to confirm a dengue infection. This shows that this type of biologic specimen is suitable for diverse types of laboratory studies of dengue. Finally, although this study involved only a small number of samples, the obtained results tend to confirm previous observations suggesting that viral particles might persist longer in capillary blood than in peripheral blood.[8] It would be valuable to confirm this finding by following viremia in a larger series of sequential capillary blood samples from dengue-infected patients.

The study protocol was reviewed by the Paraguayan Ministry of Public Health and determined to be an outbreak study and public health response that did not require further human subject review. Written informed consent was obtained from each patient or from his or her legal representative. No undesirable event associated with the blood collection technique was observed. All information concerning the patients, their identity, date of birth, the day of the beginning of the clinical symptoms, the date of blood collection, and the diagnosis results obtained have been recorded in a secure computerized system.

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REFERENCES

1. Rigau-Perez JG, Clark GG, Gubler DJ, Reiter P, Sanders EJ, Vorndam AV, 1998. Dengue and dengue haemorrhagic fever. *Lancet 352:* 971–977.
2. Gubler DJ, 1998. Dengue and dengue hemorrhagic fever. *Clin Microbiol Rev 11:* 480–496.
3. Gubler DJ, 2002. Epidemic dengue/dengue hemorrhagic fever as a public health, social and economic problem in the 21st century. *Trends Microbiol 10:* 100–103.
4. Gubler DJ, 2002. The global emergence/resurgence of arboviral diseases as public health problems. *Arch Med Res 33:* 330–342.
5. Guzman MG, Kouri G, 1996. Advances in dengue diagnosis. *Clin Diagn Lab Immunol 3:* 621–627.
6. Emerging, Infectious, Disease, Updates, 29 January 2007. *Emerging and Reemerging Infections Diseases, Region of the Americas: Dengue Outbreak in Paraguay*. Washington, DC: The Pan American Health Organization. Available at: http://www.paho.org/English/ad/dpc/cd/eid-eer-2007-01-29.htm.
7. Emerging, Infectious, Disease, Updates, 15 March 2007. *Emerging and Reemerging Infectious Diseases, Region of the Americas: Dengue Outbreak in Paraguay*. Washington, DC: The Pan American Health Organization. Available at: http://www.paho.org/English/ad/dpc/cd/eid-eer-2007-03-15.htm.
8. Matheus S, Meynard JB, Lacoste V, Morvan J, Deparis X, 2007. Use of capillary blood samples as a new approach for diagnosis of dengue virus infection. *J Clin Microbiol 45:* 887–890.
9. World Health Organization, 1997. *Dengue Haemorrhagic Fever: Diagnosis, Treatment, Prevention and Control*. Geneva: World Health Organization.
10. Lanciotti RS, Calisher CH, Gubler DJ, Chang GJ, Vorndam AV, 1992. Rapid detection and typing of dengue viruses from clinical samples by using reverse transcriptase-polymerase chain reaction. *J Clin Microbiol 30:* 545–551.
11. Alcon S, Talarmin A, Debruyne M, Falconar A, Deubel V, Flamand M, 2002. Enzyme-linked immunosorbent assay specific to dengue virus type 1 nonstructural protein NS1 reveals circulation of the antigen in the blood during the acute phase of disease in patients experiencing primary or secondary infections. *J Clin Microbiol 40:* 376–381.
12. Dussart P, Labeau B, Lagathu G, Louis P, Nunes MR, Rodrigues SG, Storck-Herrmann C, Cesaire R, Morvan J, Flamand M, Baril L, 2006. Evaluation of an enzyme immunoassay for detection of dengue virus NS1 antigen in human serum. *Clin Vaccine Immunol 13:* 1185–1189.
13. Kumarasamy V, Wahab AH, Chua SK, Hassan Z, Chem YK, Mohamad M, Chua KB, 2007. Evaluation of a commercial dengue NS1 antigen-capture ELISA for laboratory diagnosis of acute dengue virus infection. *J Virol Methods 140:* 75–79.
14. Talarmin A, Labeau B, Lelarge J, Sarthou JL, 1998. Immunoglobulin A-specific capture enzyme-linked immunosorbent assay for diagnosis of dengue fever. *J Clin Microbiol 36:* 1189–1192.