Comparison and critical appraisal of dengue clinical guidelines and their use in Asia and Latin America


Department of Tropical Hygiene and Public Health, University Hospital Heidelberg, Germany, INF 324, 69120 Heidelberg, Germany

Instituto de Medicina Tropical Pedro Kouri, Autopista Novia del Mediodia, Km 6, PO Box 601, Marianao 13, Ciudad de la Habana, Cuba

Hospital Luis Vernaza, Calle Loja número 700 y Escobedo, Guayaquil, Ecuador

Faculty of Medicine, University of Malaya, Lembah Pantai, 50603 Kuala Lumpur, Malaysia

Departamento de Virología, Centro Nacional de Diagnóstico y Referencia CNDR-MINSA, Complejo de Salud Dra. Concepción Palacios, Colonia 1ro de Mayo, CP 2900, Managua, Nicaragua

San Lazaro Hospital, Quiricada Street, Sta Cruz, Manila 1003, Philippines

Núcleo Universitario Rafael Rangel, Universidad de los Andes, Avenida Medina Angelita, Sector Carmona, Trujillo, Venezuela

Special Programme for Research and Training in Tropical Diseases, TDR-World Health Organization, 20 Avenue Appia, CH-1211 Geneva 27, Switzerland

Universidade Federal de Ceará, Rua Carolina Sucupira, 770 ap. 202 Bairro Aldeota, Fortaleza, CE, CEP 60140-120, Brazil

Universidade Federal de Goiás, Rua 227 Q 68, Setor Leste Universitário Goiânia, GO, 74605080, Brazil

Section Clinical Tropical Medicine, University Hospital Heidelberg, INF 324, 69120 Heidelberg, Germany

Received 14 June 2009; received in revised form 2 July 2009; accepted 7 August 2009

KEYWORDS
Dengue; Dengue fever; Dengue haemorrhagic fever; Clinical guidelines; Case classification; Disease management

Summary The World Health Organization (WHO) dengue classification scheme for dengue fever (DF) and dengue haemorrhagic fever (DHF)/dengue shock syndrome (DSS) has been adopted as the standard for diagnosis, clinical management and reporting. In recent years, difficulties in applying the WHO case classification have been reported in several countries. A multicenter study was carried out in Asia and Latin America to analyze the variation and utility of dengue clinical guidelines (DCGs) taking as reference the WHO/PAHO guidelines (1994) and the WHO/SEARO guidelines (1998). A document analysis of 13 dengue guidelines was followed by a questionnaire and Focus Group discussions (FGDs) with 858 health care providers in seven countries. Differences in DCGs of the 13 countries were identified including...

∗ Corresponding author. Tel.: +49 15159114654.
E-mail address: santamariaruth@yahoo.com (R. Santamaria).
the concept of warning signs, case classification, use of treatment algorithms and grading into levels of severity. The questionnaires and FGDs revealed (1) inaccessibility of DCGs, (2) lack of training, (3) insufficient number of staff to correctly apply the DCGs at the frontline and (4) the unavailability of diagnostic tests. The differences of the DCGs and the inconsistency in their application suggest a need to re-evaluate and standardise DCGs. This applies especially to case classification and case management.

© 2009 Royal Society of Tropical Medicine and Hygiene. Published by Elsevier Ltd. All rights reserved.

1. Introduction

Dengue virus infections have spread to more than 100 countries worldwide with dramatic increases in incidence in the tropical regions of Latin America and the Caribbean and Asia. They are now a major challenge for public health.1–3

For more than three decades, the World Health Organization (WHO) scheme of dengue case classification, case definitions and clinical management guidelines have been adopted as the standard for diagnosis, clinical management and reporting of dengue cases in many dengue-endemic countries. This scheme separates symptomatic dengue infections into two main disease entities: dengue fever (DF), a non-specific viral illness, and dengue haemorrhagic fever (DHF), a syndrome characterised by increased vascular permeability and altered haemostasis that may progress to a potentially fatal hypovolaemic shock (DSS).4

However, as dengue spreads worldwide affecting people of various ages and ethnic groups, there is an emerging recognition that the DF/DHF/DSS classification scheme may not be universally applicable for clinical management.5,6 In recent years, difficulties in applying the WHO case classification scheme have resulted in a lack of consistency in the diagnosis, classification, treatment and epidemiological surveillance of dengue cases. Clinicians in several countries adapted the current classification (1997) to better reflect the patterns of disease seen locally; such adaptations include 'DF with bleeding'; 'DF with organ impairment' and 'DF with unusual manifestations'. As a result, surveillance data and case fatality rates are no longer comparable, also rendering clinical research studies and vaccine trials across countries and regions difficult.

Recognizing the importance of the consistency in implementing any dengue case classification and case management, we conducted a multicenter study in Asia and in Latin America (LA) on the variation and practical use of dengue clinical guidelines (DCGs) taking as reference the WHO/PAHO guidelines (1994)7 and the WHO/SEARO guidelines (1998)8 and looking at field application in clinical practice, acceptance by health care staff and user-friendliness in hospitals and primary care units.

2. Methods

This study was designed as a multi-dimensional evaluation research, which was conducted in two stages, the first being a comparative analysis of seven country DCGs from LA and six from Asia, followed by a qualitative/quantitative study conducted with health care staff from selected countries in Asia and LA (Figure 1).

2.1. Analysis of dengue clinical guidelines (DCGs)

A systematic document analysis of seven dengue guidelines currently used in LA9–14 and six in Asia15–20 was conducted. A purposive sample technique for selection of countries took into account the number of annually reported dengue cases, willingness of countries to participate, and use/availability of national dengue guidelines. Once the guidelines were identified, copies were obtained from different sources (WHO, Ministries of Health, consultants and researchers in the field). The WHO/PAHO guideline (1994) for LA and the WHO/SEARO guideline (1998) for Asia were used as standard documents for comparison.7,8

2.2. Field study among health staff

The second stage involved standardized interviews among doctors and nurses working in primary care units and hospitals of secondary and tertiary levels of care in five LA and two Asian countries (Figure 1). The choice for the study sites was based on the geographical distribution, number of annually reported dengue cases and the willingness of facilities to participate in the study. The field study was conducted between April and December 2008.

2.2.1. Questionnaires

A self-applied semi-structured 7-item questionnaire on the utility of DCGs (Figure 1) was pilot tested in two settings and then administered to health care staff working in health centres and hospitals (Table 1).

2.2.2. Focus group discussions (FGD)

A purposive sample technique was used to select the FGD participants among those in the questionnaire survey based on their experience in managing dengue patients. The average number of participants in each FGD was 10 and the average duration was 1 h. To ensure uniformity of the type of information to be obtained, a guide to the FGD was used in all study sites. The interview focused on the use and acceptance of DCGs in daily practice, suggestions for improvement in the current DCG and the management of dengue cases. The FGDs were conducted in the local language by trained interviewers, tape recorded and transcribed into text format for the analysis. Informed consent was obtained from
every participant. Content analysis was used to analyse the data. Systematic analysis between the information provided by doctors and nurses working in health care centres and in hospitals provided a powerful source of comparison for this study.

### 2.3. Data Analysis

The following categories were used to analyse the dengue guidelines: source of document, warning signs, case classification, other classifications, grades of severity of DHF, laboratory requirements, algorithm for dengue management and treatment. Inconsistencies among countries and between country guidelines and reference guidelines were highlighted. At least two of the authors checked the correct classification of different elements in the guidelines; in case of disagreement, agreement was reached by consensus. The responses to questions were tabulated in an Excel database (Microsoft Corp., Redmond, WA, USA) and analysed.

### 3. Results

#### 3.1. Analysis of dengue guidelines

All guidelines were based on the WHO case classification (1997). Target audience, warning signs, and laboratory requirements did not differ much from the reference guidelines (WHO/PAHO Supplementary Table 1; WHO/SEARO Supplementary Table 2) but considerable differences regarding the classification of DHF/DSS, the grading of severity and algorithms for dengue management were identified.

#### 3.2. Variation in warning signs

The WHO/PAHO guideline included specific warning signs to guide the process of dengue assessment (Supplementary Table 1) but country guidelines showed the following variation: Brazil was the only country that included all warning signs but included additionally ‘hepatomegaly’.

### Table 1  Self-applied questionnaires; sample distribution.

<table>
<thead>
<tr>
<th></th>
<th>Latin America (n = 627)</th>
<th>Asia (n = 231)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Level of care</td>
<td>Level of care</td>
</tr>
<tr>
<td></td>
<td>Primary (n = 14)</td>
<td>Primary (n = 18)</td>
</tr>
<tr>
<td></td>
<td>Secondary and tertiary (n = 14)</td>
<td>Secondary and tertiary (n = 6)</td>
</tr>
<tr>
<td>Doctors</td>
<td>71</td>
<td>33</td>
</tr>
<tr>
<td>Nurses</td>
<td>84</td>
<td>23</td>
</tr>
<tr>
<td>Laboratory technicians</td>
<td>20</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>175</td>
<td>57</td>
</tr>
</tbody>
</table>

*Mercosur countries include Argentina, Brazil, Chile, Paraguay and Uruguay*
The guidelines of Colombia, Cuba, Ecuador, El Salvador, Mexico and Mercosur did not include ‘cyanosis’ or ‘oliguria’. The WHO/SEARO guideline included eight warning signs (Supplementary Table 2). However, ‘severe vomiting’ was mentioned only in the guidelines of the Philippines, Thailand, Sri Lanka and Vietnam and not in the others. The Philippines included additionally ‘breathing difficulties, seizures, narrowing pulse pressure and other laboratory criteria’, while those of Thailand included ‘anorexia’ and ‘confusion’ and Sri Lanka ‘black coloured stools’.

3.2.1. Classification DF/DHF/DSS

The dengue case classification as recommended by WHO was not uniformly applied. In LA only Brazil, Cuba and Ecuador adopted the classification of dengue for DF and DHF in their national guidelines. Cuba and Ecuador included the exact WHO/PAHO criteria for classifying DSS while Colombia, El Salvador and Mexico included different criteria for defining DF/DHF/DSS. For example, the Colombian guideline included in the classification of DF ‘platelet count of \(\leq 180,000 \text{per mm}^3\)’ and stated, just as in Mexico, that for DHF only two out of the four criteria were sufficient for the classification as DHF. The Brazilian guideline included an additional category ‘complicated dengue’ to describe severe cases with neurological alterations, cardiopulmonary dysfunction, hepatic failure, thrombocytopenia (platelet count \(\leq 50,000/\text{mm}^3\)), and gastrointestinal haemorrhages that could not be classified as DF or DHF. In the Mercosur guideline ‘plasma leakage’ was not mentioned as a diagnostic criterion and ‘haemorrhage’ (without mentioning the tourniquet test) was the key element for diagnosing DHF. The El Salvador guideline classified dengue into four categories (Group A = mild dengue; B = moderate dengue; C = severe dengue; D = dengue shock). The Asian guidelines were more uniform. Only Malaysia and Sri Lanka included hepatomegaly and circulatory disturbances as additional criteria for DHF and Sri Lanka mentioned DHF with or without shock instead of DSS. Vietnam did not include a section on dengue classification but mentioned all criteria recommended by WHO/SEARO.

3.2.2. Grades of severity of DHF

The grading of severity of DHF into four levels (Supplementary Tables 1 and 2) was adopted only in the guidelines of three LA countries: Brazil, Ecuador, and Mexico but not in the others. All Asian guidelines used the grading system.

3.2.3. Algorithm for dengue management

The WHO/PAHO algorithm for dengue management was used only by Cuba and Mexico. Other countries like Colombia did not include some essential aspects such as warning signs, instructions for non-hospitalised patients or signs for urgent referral. In Ecuador, there were no recommendations on the level of care.

In Asia the WHO/SEARO algorithm for dengue management was inconsistently adopted. India, Malaysia and Thailand included criteria for diagnosing DHF, whereas the Philippines, Sri Lanka and Vietnam emphasized warning signs and admission criteria. All Asian countries mentioned criteria for hospital admission including several features of shock, but only the guideline of the Philippines listed other warning signs, such as persistent vomiting, difficulty of breathing, seizures, narrowing of pulse pressure, rise in haematocrit > 20%, platelet count \(\leq 100,000/\text{mm}^3\), prolonged bleeding time. India, the Philippines and Thailand defined criteria for identifying the patient for immediate treatment and the other Asian guidelines provided instruction about where the patient should be treated (triage mostly based on warning signs) and when to discharge inpatients (except for Vietnam).

3.3. Field study among health staff (health care provider study)

3.3.1. Questionnaire surveys

A survey with 858 self-applied questionnaires was conducted, 627 (73.1%) in LA and 231 (26.9%) in Asia (Table 1). The age range of the participants was 24 to 65 years in LA and 22 to 63 years in Asia. The male to female ratio of respondents was 1.5:1.

The most commonly used dengue guidelines came from Ministries of Health, followed by the different WHO guidelines (Figure 2). These guidelines have already been used for one year or more, as responded by 63.9% of the study participants in Latin America and 70.9% in Asia.

Less than one-third of respondents indicated that guidelines were kept within the facility (26.9% in LA and 31.1% in Asia) and about half of the participants reported that the guidelines were kept in places where they were difficult to access, such as the supervisor’s or epidemiologist’s office, or library. Wall posters were used only in Brazil 25.4%, Cuba 16.4%, and the Philippines 33.3% (Figure 3).

Training on clinical management as described in the locally used guideline was obtained either on the job, (35.1% in LA and 32.1% in Asia) or during in-service training in the current facility (30.9% in LA and 31.3% in Asia) but rarely at medical or nursing schools (11.1% in LA and 16.9% in Asia). Only 7% of respondents in LA and 6% in Asia had never received any training on clinical management of dengue.

Figure 4 shows the perceived barriers using DCGs. The most common complaints were poor training, lack of staff to apply the guideline, and unavailability of diagnostic tests. Less than 2% in both LA and Asia thought that the guidelines
were not useful while less than 1% disagreed with the guideline. Responses on the perceived barriers varied according to staff from hospitals and health centres (Table 2). ‘Poor training’ was mentioned by 23% in both groups. Other factors were the lack of time and lack of staff to follow guidelines. The unavailability of diagnostic tests (18.8%) contributed to the poor applicability of dengue guidelines in health centres.

### Table 2 Perceived barriers of usage of dengue guidelines by level of health care.

<table>
<thead>
<tr>
<th>Perceived barriers</th>
<th>Health centre</th>
<th>Hospital</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor training on use of guidelines</td>
<td>44 (23.0)</td>
<td>86 (23.1)</td>
</tr>
<tr>
<td>Diagnostic test are not available</td>
<td>36 (18.8)</td>
<td>22 (5.9)</td>
</tr>
<tr>
<td>Lack of staff to follow guidelines</td>
<td>21 (11.0)</td>
<td>68 (18.2)</td>
</tr>
<tr>
<td>Lack of time to follow guidelines</td>
<td>22 (11.5)</td>
<td>48 (12.9)</td>
</tr>
<tr>
<td>I do not agree with guidelines</td>
<td>15 (7.8)</td>
<td>3 (0.8)</td>
</tr>
<tr>
<td>Forms difficult to access</td>
<td>14 (7.3)</td>
<td>42 (11.3)</td>
</tr>
<tr>
<td>Appropriate treatments are not available</td>
<td>8 (4.2)</td>
<td>8 (2.1)</td>
</tr>
<tr>
<td>Guidelines/forms too complicated</td>
<td>4 (2.1)</td>
<td>12 (3.2)</td>
</tr>
<tr>
<td>Guidelines are not useful</td>
<td>1 (0.5)</td>
<td>6 (1.6)</td>
</tr>
<tr>
<td>Other</td>
<td>26 (13.6)</td>
<td>78 (20.9)</td>
</tr>
</tbody>
</table>

### 3.4. Focus group discussions

A total of 92 FGDs were conducted, with medical doctors and nurses in separate sessions. The participants represented various departments and functional roles within the health care setting.

Participants from Asia and LA in hospitals and health centres consistently described similar limitations and provided similar suggestions to improve dengue case management and clinical guidelines. The issues frequently mentioned and recommendations made were:

1. Increase accessibility of guidelines and provision of training:

Most participants proposed improved accessibility in terms of physical access to the document and improved knowledge sharing at all levels of care, including frontline clinics, provision and use of wall posters on dengue management and simplified guidelines that are specific to the level of care provided by the facility.

Training on disease recognition and clinical management particularly for front line doctors, junior doctors who run the primary health facilities and nurses who...
perform triage of patients was proposed in both Asia and LA. Training on dengue management should also be incorporated in undergraduate medical school curriculum.

(2) Improve case definition and dengue case classification:

Several participants mentioned that the WHO classification scheme is complex. They took it as a 'guide' to classify patients, but the criteria were not followed rigorously because in their view not all patients fulfilled all four criteria for DHF.

Three aspects were particularly stressed in the FGDs. (1) The terminology should better reflect plasma leakage, rather than haemorrhage; (2) the classification should emphasize signs and symptoms rather than laboratory findings and should include warning signs; and (3) 'unusual manifestations' such as organ failure should be included.

(3) Improve explanations about treatment:

In both Asia and LA it was proposed that the dengue case classification should help in triage and case management, including warning signs for severe disease and providing instructions for case management at each stage of the disease. Participants in Malaysia and the Philippines emphasized that the guideline should specify when to start the use of intravenous fluids and blood products, and the recognition of signs of improvement.

(4) Strengthen resource management:

A frequently mentioned problem was the lack of frontline doctors and nurses to perform triage and ambulatory management of dengue which involved time for explanation to the patient. For instance, in some urban health centres, each doctor might see about 150 patients per day leaving little time for explanations. Dengue cases were instead referred to hospitals, which became overcrowded with dengue patients, many of whom could have been managed at primary care levels. It was mentioned that the utilisation of available personnel resources be maximized, through identification of staff needs (training, equipment, etc.), staffing requirements, supervision of activities (use of dengue guidelines, etc.), and professional development. The lack of diagnostic resources in primary health facilities, especially at night and on weekends, was another issue which limited the possibility of classifying and managing patients. In the absence of on-site facilities, blood samples had to be sent to the nearest hospital, thus limiting the possibility of classifying and managing patients. In the absence of on-site facilities, blood samples had to be sent to the nearest hospital, thus increasing the waiting time and discouraging full blood counts from being done on a daily basis to establish patient’s status and evolution to more severe disease. It was also suggested that the number of hospital beds for dengue patients be increased and the triage procedures better defined in order to better apply the treatment instructions for severe cases.

4. Discussion

4.1. Guideline analysis

Considerable differences in the DCGs currently used by thirteen LA and Asian countries were identified. This was particularly evident with regards to ‘warning signs’. One explanation was that the concept of 'warning signs' was interpreted in different ways (Supplementary Tables 1 and 2). In some guidelines the signs and symptoms of shock were called 'warning signs', in others those of suspected dengue were mentioned and in yet others 'warning signs' for progression towards severe disease were included. The lack of consensus on warning signs in dengue management could lead to different interpretations in dengue guidelines across countries. Changes in dengue epidemiology with a shift towards the adult population may compound these differences. Likewise the large variation in the use of treatment algorithms both in LA and Asia suggested a lack of evidence and/or agreed standards of dengue case management. Nevertheless, there was an attempt in most countries to define criteria for ambulatory treatment and hospital admission and discharge, highlighting the importance of triage, particularly in dengue outbreaks. The WHO treatment recommendations implied that a patient had to be classified first and then treated accordingly. In clinical practice it was often the opposite: the patient had a clinical assessment first and was treated according to the clinical state, regardless of the classification which could be done at a later stage.

Regarding the classification of dengue into DF and DHF/DSS and the grading of severity for DHF, no major variation among Asian countries was observed, implying a tacit agreement with the WHO classification developed in Bangkok Childrens' Hospital in the early 1970. However, the LA guidelines adopted very different approaches in classifying dengue suggesting the dissatisfaction with the stringent DHF criteria, confusion with the terminology in DHF, the fact that many patients could not be classified and perhaps the epidemiological differences between LA and Asia. There was also an attempt to use a new classification into levels of severity, particularly in El Salvador, Brazil and others. Additionally terms such as 'complicated dengue' and 'DF with unusual haemorrhage' have been introduced to represent the observed patterns of disease.

4.2. Health care provider study

This study component focused on the experiences of health staff in using national dengue guidelines in their daily work. It became obvious that dengue guidelines were often not available in the work place which was a drawback to the improvement of dengue management. Other limitations were the lack of training in the use of guidelines which was described by Cabana et al. (1999). An important barrier to applying treatment guidelines correctly were the unfavourable doctor-to-patient ratio, especially at frontline clinics, leading to expedient hospitalisations of patients, who could have been managed at primary care level.

Additionally, medical staff had difficulties following strictly the dengue case classification promoted by WHO when discrepancies between the DF/DHF/DSS definitions and local disease patterns occurred. The difficulty of using laboratory information necessary for defining DHF was frequently mentioned. In practice, unless the patient had daily complete blood count (CBC) during the febrile, defervescence and critical phases, it would be difficult to
detect the rising haematocrit (HCT) and falling platelet count trends that heralded plasma leakage and thus classified the patient as DHF. Furthermore, 613 (81%) of 756 hospitalized dengue patients in a multi-country study sought medical attention within 48 h of illness onset, thus giving an opportunity to establish a baseline HCT in the early febrile phase. However, the doctor has to be aware that a seemingly normal full blood count in the first 1–2 days of fever does not discount the possibility of dengue, but serves as a baseline for comparison. Knowing the patient’s individual baseline HCT takes the guesswork out of determining if there is a rising HCT as the patient enters the critical phase in the next 24 to 72 h. Detection of a 20% rise in HCT above the baseline or population HCT to classify a patient as DHF might be unrealistic in an anaemic patient or a patient who had received intravenous fluid therapy. In these circumstances, thrombocytopenia plus a rising HCT as criteria for guidance for diagnosis of DHF might be more practical.

4.3. Suggestions for improvement of the dengue case classification and clinical management

This study underlines the difficulties with the current dengue case classification documented in the literature (as described above) and supports suggestions to work towards an evidence based, user-friendly and universally accepted case classification into levels of severity, which supports triage and case management according to the patients’ clinical status. Any new classification scheme for dengue should highlight the dynamic nature of dengue’s clinical, haematological and immunological profiles. Although the majority of dengue patients recover with or without treatment, the challenge to the clinician is to identify the minority that could progress to severe disease and may die without appropriate interventions after the first few days of fever. A modified case classification should:

1. be based on severity (severity of plasma leakage, severity of bleeding and severity of organ impairment)
2. be able to identify warning signs of plasma leakage prior to shock
3. draw the attention to haemodynamic assessment to detect shock from the early stages of compensated shock to late stages of hypotensive shock (AHA/PALS)
4. facilitate an algorithm of case management based on:
   - Presence or absence of warning signs prior to the development of severe disease
   - Compensated shock or hypotensive shock
   - Severity of bleeding

In general, plasma leakage precedes severe haemorrhage. How each of these phenomena could be identified and how both will impact on haematocrit values and the haemodynamic picture as the patient progresses through the illness, should form the core of dengue management guidelines.

Additionally, the current dengue case classification scheme has never been validated in clinical practice and with dengue spreading to new geographical areas, the necessity for validation becomes more important. Grol et al. (1998) found that recommendations based on evidence were followed more than those that were not based on scientific facts (71% vs. 57% respectively). Although evidence-base only does not ensure the correct use of dengue guidelines by all health providers, at least it could contribute to close the gap between dengue research and clinical practice.

Authors’ contributions: AK, OH, EM, LCSL and RS designed the study protocol; SK, AN, EV, HB, CS, EM, RS, IC, JBS, ED, LHT and LCSL carried out the field work; SK, AK and RS carried out the dengue guidelines analysis; AK, LCSL, SK and RS drafted the manuscript; TJ, SK and RS carried out the analysis and interpretation of the data. All authors read and approved the final manuscript. AK and LCSL are guarantors of the paper.

Acknowledgements: The study was supported by the Special Programme for Research and Training in Tropical Diseases (WHO/TDR) in Geneva. The authors wish to thank the WHO Regional Offices and colleagues from the DENC0 study group for their valuable help to get the country dengue guidelines and Dr Daniel Gonzalez (Pedro Kouri Institute, Havana-Cuba), Dr Arnaldo Izquierdo (Aballi Pediatric University Hospital, Havana-Cuba); Dr Edison Soria (IMEDIN - Institute for Children Medicine, Guayaquil-Ecuador), Dr Iris Villalobos (Hospital Central de Maracay) and Dr Silvia Runge-Ranzinger (Heidelberg University) to facilitate access to important documents and articles. The study would not have been possible without the active involvement of many colleagues in the participating countries.

Funding: The project has been funded by the Special Programme for Research and Training in Tropical Diseases (TDR/WHO).

Conflicts of interest: None declared

Ethical approval: Ethical approval was obtained in each study site from the Institutional Review Boards of the Department of Tropical Hygiene and Public Health, University Hospital Heidelberg, Germany; Instituto de Medicina Tropical Pedro Kouri, La Habana, Cuba; Hospital de Infectología José Rodriguez Maridueña, Guayaquil, Ecuador; University of Malaya, Kuala Lumpur, Malaysia; Departamento de Virologia, Centro Nacional de Diagnóstico y Referencia, Managua, Nicaragua; San Lazaro Hospital, Manila, Philippines; Universidad de los Andes, Nucleo Trujillo, Venezuela; Universidade Federal de Ceará, Fortaleza, Brazil; Universidade Federal de Goias, Goiania, Brazil; and also from the WHO Ethical Review Board (A70175). The study objectives were explained to the study participants (doctors and nurses) and written informed consent was received from them. All data collected were managed and stored in a confidential manner assuring that only key study personnel had access to the information; the information will be destroyed after five years. There were no known risks in this study.
Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.inhe.2009.08.006.

References