Dengue virus infection in travellers returning to Berlin, Germany: clinical, laboratory, and diagnostic aspects

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Received 25 June 2003; received in revised form 27 October 2003; accepted 6 November 2003

Abstract

Background: Dengue is a mosquito-borne viral infection endemic throughout the tropics and subtropics. The global prevalence of dengue has grown dramatically in recent years and it has been recognized as a potential hazard to tourists. Objective: In this study, we analyzed the epidemiology, clinical manifestations, laboratory features and serological/virological results in a series of German travellers returning to Berlin with acute dengue virus infection. Study design: Laboratory-confirmed dengue virus infections among German travellers returning to Berlin were studied retrospectively during the period of 1993–2001. Seventy-one patients tested positive for dengue fever and were included in this study. Results: The majority of patients (77.5%) contracted the disease in South Central and South East Asia. The most important clinical characteristics were fever and prostration (100%), headache, predominantly frontal or retroorbital (86%), arthralgia (79%), morbilliform rash (66%) and myalgia (48%). The most meaningful laboratory results were: marked leucopenia (72%), thrombocytopenia (70–89%), hyponatremia (41%) and increased hepatic enzymes ALAT (41%), ASAT (45%) and LDH (62%). Dengue virus infection was diagnosed by means of a matching clinico-epidemiological history and positivity of specific serology and/or virus isolation. Hemorrhagic phenomena appeared in 10 of the 71 patients (14%), out of which one was diagnosed with DHF according to WHO criteria. All patients recovered fully.

Conclusion: Pretravel advice should be given to all travellers to dengue-endemic areas. DF must be included in the differential diagnosis of patients returning febrile from tropical areas.

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Keywords: Viral diseases; Dengue; Dengue haemorrhagic fever; Arbovirus infections; Virus isolation

Abbreviations: ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase; CK, creatine kinase; CRP, C-reactive protein; DEN-1-4, Dengue virus serotypes 1–4; DF, dengue fever; DHF/DSS, dengue haemorrhagic fever/dengue shock syndrome; IFT, indirect immunofluorescence testing; IgM, immunoglobulin class M; IgG, immunoglobulin class G; LDH, lactate dehydrogenase; PTT, partial thromboplastin time; RNA, ribonucleic acid; RT–PCR, reverse transcriptase–polymerase chain reaction; WHO, World Health Organization

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doi:10.1016/j.actatropica.2003.11.004
1. Introduction

Dengue fever is an acute infectious disease in subtropical and tropical areas and one of the most important arthropod-borne viral diseases in terms of human morbidity and mortality. Dengue fever is caused by a single stranded ribonucleic acid (RNA) virus of the family of Flaviviridae, genus Flavivirus, transmitted by *Aedes* mosquitoes, the most important vectors being *Aedes aegypti* and *Aedes albopictus*. Four distinct serotypes, DEN-1, DEN-2, DEN-3, and DEN-4 are recognized. Infection with any of these viruses may be asymptomatic, may cause a self-limited febrile illness known as dengue fever (DF), or, in a small percentage of cases, may result in a life-threatening syndrome, the so-called dengue haemorrhagic fever/dengue shock syndrome (DHF/DSS) (Guzmán and Kouri, 2002; WHO, 2002a). Infection with one dengue serotype provides lifelong homologous immunity, but only transient cross-protection against other serotypes, thus allowing for sequential infection with possible progression towards DHF/DSS. The pathogenesis of DHF/DSS is only partially understood. Today, the majority view is that antibody-dependent viral infection enhancement is the main mechanism responsible for inducing DHF/DSS associated with secondary dengue virus infection (Cardosa, 2000; Guzmán and Kouri, 2002).

With approximately 50 million cases of dengue fever occurring annually and an estimated number of 500,000 hospitalized cases of DHF/DSS officially notified, dengue fever is a common disease in most tropical and subtropical regions (Cardosa, 2000). In endemic areas, the disease reaches epidemic proportions and constitutes a considerable threat for public health (Gubler, 2002). Among the factors that have been implicated in the current rise of dengue are unplanned and uncontrolled urbanization, overpopulation, crowding, poverty, a weakened public-health infrastructure and international journeys introducing new serotypes, genotypes and new strains to different parts of the world (Gubler, 2002).

Given the fact that dengue fever is spreading over a geographically expanding range and with growing emergence in the second half of the twentieth century, it has been recognized as a potential hazard to tourists and plays an important role in the differential diagnosis of fever of unknown origin in travellers returning from tropical areas (Rigau-Perez et al., 1994; Jelinek, 2000).

The epidemiology and the clinical manifestation of dengue fever in endemic countries have been extensively described, but few reports exist on the clinical and serological manifestations among the traveller population. In Europe the exact extent of travel-acquired imported dengue infections among travellers is unknown but of growing importance (Badiaga et al., 1999; Teichmann et al., 1999; Lindbäck et al., 2003). The objective of this study was to analyze the epidemiology, clinical manifestations, laboratory features and serological/virological results in a series of patients with imported acute dengue virus infection among travellers returning to Berlin, Germany.

2. Patients and methods

A retrospective study was performed on 71 patients with laboratory-confirmed dengue fever who presented as in- or outpatients at the Department of Infectious Diseases, Charité University Hospital in Berlin, Germany, during the period 1993–2001. The department is a referral clinic for patients with suspected infectious diseases.

Laboratory diagnosis of dengue fever was performed at the Robert Koch Institute, Berlin, Germany. Laboratory confirmation of dengue fever was defined as (i) the positive detection of dengue virus-specific IgM or an at least fourfold increase in DEN-specific IgG in indirect immunofluorescence tests, as described previously (Reinhard et al., 1998), and/or (ii) the positive isolation of dengue virus in cell culture on the insect cell line C6/36. DEN-1-4 was detected by indirect immunofluorescence using flavivirus and dengue type-specific monoclonal antibodies and/or reverse transcriptase-polymerase chain reaction (RT–PCR) as published previously (ter Meulen et al., 2000). The sensitivity of the immunofluorescence assay is comparable with standard diagnostic enzyme immunosays (Koraka et al., 2002). Whenever possible, at least two serum samples were tested from each patient, the first one obtained in the early symptomatic phase and the second one during convalescence. Virus isolation was only attempted out of the initial serum samples.

Patients with clinically suspected dengue fever but
Dengue virus type-1 (KeCa/Nicaragua; isolated in 1993 from the serum of a patient with dengue fever who had returned from a trip to Nicaragua) was used as a positive control and for the performance of immunofluorescence assays detecting dengue-specific IgM and IgG antibodies. The viruses were propagated in C6/36 cells grown at 28°C in Leibovitz L-15 medium. Infected tissue culture fluids were harvested on day 5 and stored at −70°C until use.

Dengue virus types 1–4 specific mouse monoclonal antibodies from two different sources were used. Antibodies from the hybridomas DE-1F1–3 Hawaii, DG-8A1–12 and 1H10–6–7H241 were kindly provided by Dr. D.J. Gubler of the Centers for Disease Control and Prevention, Atlanta, GA, USA. Monoclonal antibodies from hybridomas commercially available through the American Type Culture Collection, Rockville, MD, USA included anti-flavivirus group common antigen-specific (ATCC HB-114) and anti-dengue complex (ATCC HB114), DEN-1 (ATCC B47), DEN-2 (ATCC HB46), DEN-3 (ATCC HB49), and DEN-4 (ATCC HB48), and they were obtained after in vitro culturing of hybridomas in a tumbler chamber (Jaspert et al., 1995).

Dengue-specific IgM and IgG in serum samples was detected on DEN-1-infected cells on glass slides as described earlier (Reinhard et al., 1998). Briefly, one part of Dengue-1 virus (KeCa/Nicaragua)-infected Vero B4 cells and two parts of uninfected cells were mixed and grown for 1 day on glass slides with 12 marked wells (Menzel, Germany) at 37°C and 5% CO2, fixed with ice-cold acetone, dried and stored at −20°C. For specific IgM detection, the IgG was removed from the test sera by incubation with anti-human IgG antibody according to the manufacturer’s instructions (Mastsorb Diagnostica, Germany). Serum dilutions from 1:10 to 1:80 were analyzed for IgM and dilutions of up to 1:20,000 for IgG.

Fifty microlitres of patient sera was diluted with 1 ml Leibowitz-L15 medium and sterile filtered. After removing the supernatant media from confluent C6/36 monolayers in 25 cm² plastic tissue culture flasks, filtered serum dilution was inoculated on monolayers and incubated at room temperature for 1 h. After adding 5 ml of L-15 medium, cultures were incubated at 28°C and inspected daily for cytopathic effects. After 5–7 days, 1 ml supernatant was passaged onto fresh C6/36 monolayers. Adherent cells were harvested by scraping with a rubber policeman, pelleted by centrifugation, resuspended in L-15 medium and dropped on glass slides to be incubated for 1 day as described above. DEN types 1–4 were detected by indirect immunofluorescence using Flavivirus and dengue common antigen-specific monoclonal antibodies as well as two sets of monoclonal antibodies specific for DEN-1–DEN-4 and/or reverse transcriptase-polymerase chain reaction (RT-PCR).

The following data were recorded for each patient who was included in the protocol: age, sex, travel history (geographic area, length of stay) and reason for travel, chronic diseases, previous dengue virus infections, history of vaccination and malaria chemoprophylaxis details.

The appearance and duration of physical signs and symptoms were examined. In case of hospital admission, the duration of stay was documented. Laboratory results analyzed included complete blood count, C-reactive protein (CRP), electrolytes, liver enzymes aspartate and alanine aminotransferase (ASAT, ALAT) and lactate dehydrogenase (LDH), creatine kinase (CK), serum protein and albumin concentration and clotting tests (prothrombin level and partial thromboplastin time, PTT).

3. Results

3.1. Demography and epidemiology

A total of 71 patients had laboratory confirmed dengue fever during the study period. Seven patients were diagnosed in 1993, 7 in 1994, 13 in 1995, 7 in 1996, 7 in 1997, 8 in 1998, 6 in 1999, 9 in 2000, and 7 in 2001, respectively.

The mean age in the study population was 34 years (range 15–71 years), with 30 females (42.3%) and 41 males (57.7%). All individuals were German residents and had recently visited a dengue-endemic area. The length of travel ranged from 10 to 180 days (mean 29 days). Tourism was the main reason for travel (96%); only three patients were on a business trip.

Fifty-five patients (77.5%) contracted the disease in South Central and South East Asia (10 patients in India, 1 in Indonesia, 3 in the Philippines, 5 in Sri Lanka,
and 36 in Thailand). Fourteen patients (19.7%) got infected during their stay in South- and Central America and the Caribbean (three each in the Dominican Republic and Venezuela, two in Nicaragua, one each in Brazil, Columbia, El Salvador, French Guyana, Mexico, and Panama). One patient was infected during his stay on the Cook Islands, another while travelling to Ghana. Patients were infected throughout the year. A statistically significant seasonality in relation to time point and area of infection was not found (data not shown).

Chronic underlying diseases were present in five patients (7%). Three patients had arterial hypertension, one patient had a hypothyreosis, and one was HIV-infected. Two patients had a previous dengue virus infection with typical clinical manifestation in their past history. One was infected during childhood in Thailand; the other during a previous travel to India.

Fifty-nine patients (83%) took no malaria prophylaxis. Eleven patients received appropriate chemoprophylaxis and one patient was on stand-by medication. Five patients (7%) were previously vaccinated against yellow fever; none of the patients was vaccinated against Japanese encephalitis or tick borne encephalitis.

### 3.2. Clinical spectrum

In 20 patients (28.2%), the onset of fever was during the stay in the endemic area (median of 2 days prior to return, range 0–6 days). In 51 patients, symptoms started a median of 3 days after return (range 1–8 days). Fever was noted in all patients. The median duration of fever was 6 days (range 1–11 days) with a median maximal body temperature of 39.4 °C (range 38.5–40.6 °C). Eighteen of the 71 patients (25.4%) described a biphasic course with a nadir between two temperature peaks. Clinical signs and symptoms in our patients are summarized in Table 1. Fifty-eight patients required admission to hospital, and 13 received ambulatory care. The mean duration of hospitalization was 6 days (range 1–16 days).

<table>
<thead>
<tr>
<th>Sign/symptom</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td>100</td>
</tr>
<tr>
<td>Prostration</td>
<td>100</td>
</tr>
<tr>
<td>Headache</td>
<td>86</td>
</tr>
<tr>
<td>Bone pain/arthralgia</td>
<td>79</td>
</tr>
<tr>
<td>Rash</td>
<td>66</td>
</tr>
<tr>
<td>Myalgia</td>
<td>48</td>
</tr>
<tr>
<td>Lymphadenopathy</td>
<td>30</td>
</tr>
<tr>
<td>Enanthema</td>
<td>28</td>
</tr>
<tr>
<td>Pharyngitis</td>
<td>27</td>
</tr>
<tr>
<td>Conjunctivitis</td>
<td>16</td>
</tr>
<tr>
<td>Hemorrhagic manifestation</td>
<td>14</td>
</tr>
<tr>
<td>Nausea</td>
<td>14</td>
</tr>
<tr>
<td>Coughing</td>
<td>13</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>9</td>
</tr>
<tr>
<td>Vomiting</td>
<td>7</td>
</tr>
</tbody>
</table>

One of them fulfilled the WHO definition for DHF. The 15-year-old patient was admitted after returning from a 3-week trip to Thailand. He presented with an acute syndrome characterized with a sudden onset of high fever of 39.9 °C, general bone pain and arthralgia, severe headache and retroorbital pain, haemorrhagic pharyngitis, haemorrhagic maculopapular skin rash of the extremities, epistaxis, hypotension (90/70 mmHg), elevated liver enzymes, leukopenia (2.2 nl−1), severe thrombopenia (20 nl−1), hypoprotein- and albuminaemia (5.9 g/dl; 3.4 g/dl); prolonged PTT (102 s) and decreased level of prothrombin (60%). Haematocrit dropped to less than 20% of baseline (from 52 to 40 l/l) after volume-replacement treatment. After a monophasic fever of 7 days the patient recovered completely. Serology showed low titres of anti-dengue IgM and high titres of anti-dengue IgG in the initial sample (1:10/1:2560). The patient was born in Thailand and immigrated to Germany by the age of 8. At the age of 6 he was diagnosed of having DF in Thailand.

Unusual manifestations were present in five of our patients. Three patients suffered of generalized tenderness to the touch (dermal hyperaesthesia). One patient developed a pancreatitis, another developed meningitis. Cerebrospinal fluid of the patient revealed pleocytosis of 96 cell/μl (50% each lymphocytes and granulocytes). Protein, lactate and CSF to plasma glucose ratio were normal. None of the patients received...
transfusion of blood products. All patients made a full recovery.

3.3. Clinical laboratory results

Marked leucopenia was detected in 51 of the 71 patients (72%) early in the course of disease. Fifty patients (70%) initially had thrombocytopenia. In due course thrombocytopenia was noted in 63 patients (89%) with decreasing levels reaching their lowest value on day 6 after onset of illness. Eight patients had decreasing levels of thrombocytes with values still within the normal range.

Ten patients (14%) had a slightly reduced haemoglobin level. In contrast, haematocrit was within the normal range in all patients. Hyponatremia was evident in 29 patients (41%) noted at presentation. Elevated liver enzymes were frequently seen either on presentation or during the illness. ASA T was elevated in 32 patients (45%), ALA T in 29 patients (41%), and LDH in 44 patients (62%). In 10 patients (14%) hypoproteinaemia was noted, with hypoalbuminaemia in 4 patients (6%). An elevated CK was found in 15 patients (21%); less frequent than myalgia was reported (48% of the patients). Clotting tests were altered in 18 patients (25%) who showed a prolonged PTT. In addition, in five of those patients the prothrombin level was decreased. Level of CRP was on average only slightly elevated. The laboratory findings are summarized in Table 2.

3.4. Diagnostic aspects

Sixty-nine patients were determined to be primary infections and two patients to have a secondary infection. Ten patients (14%) presented early (between 24 and 96 h after onset of symptoms) and their serology was initially negative in both antibody classes. In all of these patients seroconversion was well documented, i.e. anti-dengue IgM and IgG antibodies were detected on follow-up. In another 18 patients (26%), the initial specimen contained detectable anti-dengue IgM antibodies but no IgG; IgG seroconversion was observed in convalescent sera. In 30 patients (42%), a repeated IgM positivity in the presence of an at least four-fold increase in DEN-specific IgG was observed.

Table 2

<table>
<thead>
<tr>
<th>Laboratory test</th>
<th>Number sampled</th>
<th>Mean ± S.D.</th>
<th>Range</th>
<th>Reference value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood count</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leucocytes</td>
<td>71</td>
<td>2.63 ± 0.7</td>
<td>1.1–6.8</td>
<td>(3.1–9.5) × 10^9 l^−1</td>
</tr>
<tr>
<td>Thrombocytes init.</td>
<td>71</td>
<td>116 ± 29</td>
<td>24–214</td>
<td>(130–340) × 10^9 l^−1</td>
</tr>
<tr>
<td>Thrombocytes min.</td>
<td>58</td>
<td>87 ± 32</td>
<td>12–177</td>
<td>(130–340) × 10^9 l^−1</td>
</tr>
<tr>
<td>Haematocrit</td>
<td>71</td>
<td>43 ± 3</td>
<td>36–52</td>
<td></td>
</tr>
<tr>
<td>Haemoglobin</td>
<td>71</td>
<td>14.4 ± 0.8</td>
<td>11.2–17.1</td>
<td>f 12–16/h. 14–18 g/dl</td>
</tr>
<tr>
<td>Biochemistry</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CK</td>
<td>71</td>
<td>109 ± 89</td>
<td>11–1890</td>
<td>&lt;80 U/l</td>
</tr>
<tr>
<td>LDH</td>
<td>71</td>
<td>315 ± 106</td>
<td>134–1160</td>
<td>&lt;240 U/l</td>
</tr>
<tr>
<td>ASAT initial</td>
<td>71</td>
<td>30 ± 18</td>
<td>8–168</td>
<td>&lt;21 U/l</td>
</tr>
<tr>
<td>ASAT maximum</td>
<td>58</td>
<td>45 ± 28</td>
<td>10–242</td>
<td>&lt;21 U/l</td>
</tr>
<tr>
<td>ALAT initial</td>
<td>71</td>
<td>36 ± 23</td>
<td>7–303</td>
<td>&lt;21 U/l</td>
</tr>
<tr>
<td>ALAT maximum</td>
<td>58</td>
<td>65 ± 50</td>
<td>8–472</td>
<td>&lt;21 U/l</td>
</tr>
<tr>
<td>Sodium</td>
<td>71</td>
<td>134 ± 2</td>
<td>126–140</td>
<td>134–145 mmoles/l</td>
</tr>
<tr>
<td>Potassium</td>
<td>71</td>
<td>4.0 ± 0.3</td>
<td>3.1–4.8</td>
<td>3.4–5.2 mmoles/l</td>
</tr>
<tr>
<td>Protein</td>
<td>71</td>
<td>7.0 ± 0.4</td>
<td>5.8–8.6</td>
<td>6.5–8.0 g/dl</td>
</tr>
<tr>
<td>Albumin</td>
<td>71</td>
<td>4.2 ± 0.3</td>
<td>3.5–5.2</td>
<td>3.6–5.0 g/dl</td>
</tr>
<tr>
<td>Prothrombin</td>
<td>71</td>
<td>94 ± 12</td>
<td>60–130</td>
<td>70–130%</td>
</tr>
<tr>
<td>CRP</td>
<td>71</td>
<td>2.2 ± 1.6</td>
<td>0.2–9.2</td>
<td>&lt;0.8 mg/dl</td>
</tr>
</tbody>
</table>

CK, creatine kinase; LDH, lactate dehydrogenase; ASAT, aspartate aminotransferase; ALAT, alanine aminotransferase; PTT, partial thromboplastin time; CRP, C-reactive protein; min, minimum values recorded; max, maximum values recorded; initial, values obtained from the initial blood sample.
including two patients with secondary dengue infection who showed high titres of anti-dengue IgG in the initial sample. In 13 outpatients (18%) IgM detection in the initial test was taken as evidence of dengue; for those a follow-up could not be performed as patients were not returning after clinical cure.

Twenty-six serum samples obtained on first presentation of the patients were used for virus isolation. Dengue virus was isolated from 11 (42%) out of those 26 acute serum samples. DEN-1 was the most frequent serotype isolated (nine times): from Nicaragua 1993, Thailand 1995, India 1996, Thailand 1997, two times each from Thailand 1998 and 1999, and Thailand 2001. DEN-2 was isolated in two patients (India 1996, Thailand 2001). When data were analyzed based on disease days, virus was isolated from 9 of 13 serum samples collected on disease day 5 or earlier and in 2 of 13 serum samples taken on disease day 6 or later (1 of 7 attempts on disease day 6; and 1 of 6 taken on disease day 7). With respect to the antibody response, 10 serum samples collected initially showed no dengue-specific IgM- or IgG-seroconverters, either in both or in one of the classes, respectively. Virus isolation was successful in six out of these serum samples.

4. Discussion

The geographical areas where dengue virus infection was acquired in the 71 cases presented here reflect on the one hand the global distribution of dengue and on the other hand travelling preferences of our patient cohort. The majority of patients had travelled to South Central and South East Asia; the second largest group had been to South- and Central America or the Caribbean. Reports of dengue in travellers from Africa or Oceania are by far less frequent (Jelinek et al., 1998); we had two among our dengue patients. In contrast to previous reports (Lindbäck et al., 2003; Witteşö et al., 1993) we could not detect a significant seasonality of infection in our patients.

In nearly a third of our patients the fever began during their stay in the endemic area. Because of the short incubation time of the disease it is conceivable that many infected travellers may experience dengue fever while still abroad, leading to an underestimation of the true incidence in the traveller population (Potasman et al., 1999). In our opinion the diagnosis should be considered second to malaria in travellers returning with fever. Clinical signs and symptoms in our patients were variable, with some classical clinical manifestations being absent in a considerable number of patients. For example, despite being considered as a typical feature of DF, only 25% of the patients described a biphasic fever course.

Dengue virus infection should be considered if clinical signs, travel history and a possible incubation period of 4–7 days (range 3–14) are suggestive. In general the following were the most important clinical characteristics: fever and prostration, headache mostly frontal or retroorbital, arthralgia, morbilliform rash and myalgia. The most helpful laboratory results were: marked leukopenia, thrombocytopenia, hyponatremia and increased hepatic enzymes. Most of the clinical manifestations and laboratory features presented in our study group are in line with previous reports (Badnaga et al., 1999; López-Vílez et al., 1996, Jelinek et al., 2002). Hyponatremia was a frequent laboratory feature in our study group. This sign was not mentioned in all of the previous reports. Although severe myalgia is one of the hallmarks of DF it was only present in every second patient. A laboratory correlation showing an elevation of the CK was even less frequently seen; it was present in every fifth patient.

In patients from nonendemic areas who acquire dengue abroad, the disease rarely takes a hemorrhagic course; this is in contrast to patients who live in areas endemic for dengue. In this series hemorrhagic phenomena like petechiae, epistaxis and hypermenorrhea appeared in 14% of the patients, including in two patients with secondary dengue infection. All of those patients had severe thrombocytopenia. In addition clotting tests were altered in 25% of the analyzed group, but only half of them showed hemorrhagic manifestations. In vitro findings suggest that coagulopathy plays an important role in the pathophysiology to develop DHF. However, a recent review concluded that an association between activation of coagulation and clinical outcome of dengue virus infections is conceivable but up to now inadequately assessed (Mairuhu et al., 2003).

According to the WHO case definition the observed hemorrhagic manifestations are less frequent observed but still within the normal clinical range of a DF (WHO, 1997a). Consequently, only one of those patients was diagnosed with DHF. According to
the current hypothesis, pre-existing partial immunity predisposes to development of DHF if the subsequent infection is caused by a different serotype. Based on clinical history and on serology results 2 of our patients were diagnosed with secondary dengue infection, amongst them—and not surprisingly—the one who developed DHF.

Laboratory diagnosis of dengue virus infection has mainly involved techniques such as viral RNA detection and virus isolation in clinical specimens in the acute phase of disease and determination of virus-specific antibodies in the later phase of disease (WHO, 1997b). Specific knowledge about the performance of different laboratory diagnostic methods is required. For example in 14% of our patients who presented early in the disease the initial serology was negative in both antibody classes. These patients subsequently underwent documented seroconversion, but the diagnosis could have been missed without a convalescent serum sample. The earliest observed IgM positivity in our study group was on the 3rd day of illness. As shown in recent external quality assurance for Dengue serology several diagnostic laboratories must improve their diagnostic procedures (Donoso Mantke et al., 2003). A successful virus isolation and/or positive RT-PCR very much depends on the time and the quality of samples taken. Regarding the quality of the RT-PCR of the respective diagnostic laboratories we also found a great difference in sensitivity and specificity as seen in a quality assurance evaluation.

In addition to the most widely accepted hypothesis of antibody-dependent immune enhancement for the pathogenesis of DHF/DSS in secondary infections, epidemiological and laboratory evidence suggests that virus strain and perhaps serotype may also be important as a risk factor to develop DHF (Gubler et al., 1978). Detection and typing of dengue virus in serum using RT-PCR is a fast technique but has two disadvantages: it cannot provide live virus for further biological characterization; and its sensitivity varies between serotypes (Sudiro et al., 1997). Virus isolation provides information concerning the dengue virus serotypes and also preserves the virus isolates derived from clinical manifestations for future virological and molecular epidemiological studies.

Virus isolation as performed here in 26 patients of the study group was successful in 11 patients. Attempts for virus isolation seem promising when patients are febrile. In typical dengue cases the fever persists for 5-6 days. Once fever has subsided, attempts for virus isolation are usually negative. Virus isolation is a reliable tool to diagnose acute dengue virus infections, particularly when antibody response of the initial serology shows no dengue-specific IgM or IgG reaction (Yamada et al., 2002). Studies of antibody kinetics have shown that, in primary infections, IgM to dengue viruses increases to high levels in nearly all patients within 2-3 days of defervescence and peaks within 2 weeks after onset of symptoms. In non-primary infections, the IgM response is variable, sometimes absent or lagging behind a dramatic increase of IgG antibodies (Vaughn et al., 1997). Both IgM and IgG anti-dengue antibodies neutralise dengue, thus virus making successful virus isolation unlikely. Isolation and typing of dengue virus have their limitations. Virus isolation may not be readily available for many clinicians, as it is often time-consuming and requires a well-equipped laboratory. Most importantly, the period during which dengue virus can be successfully detected in serum samples is brief; patients are thought to be viremic on average for 5 days (shortly before onset of fever and during the febrile period).

Without doubt, there is underreporting of dengue cases imported to Europe, either because the patient does not seek medical care or because the physicians fail to establish the correct diagnosis (Jelinek et al., 2002). In an era of pressure on cost effectiveness in medical care and with a diagnosis that requires in most cases no further treatment, it is difficult to persuade both patients and doctors to perform follow-up investigations. Therefore, some travellers with symptoms of an acute viral fever in whom malaria has been excluded might not come back after they have recovered.

In Germany, mandatory reporting of dengue fever cases imported to Europe, either because the patient does not seek medical care or because the physicians fail to establish the correct diagnosis (Jelinek et al., 2002). In an era of pressure on cost effectiveness in medical care and with a diagnosis that requires in most cases no further treatment, it is difficult to persuade both patients and doctors to perform follow-up investigations. Therefore, some travellers with symptoms of an acute viral fever in whom malaria has been excluded might not come back after they have recovered.
implemented an internet-based system (DengueNet) for the global epidemiological and laboratory surveillance of DF and DHF (WHO, 2002b).

In conclusion, acute dengue virus infection is common in patients presenting after a travel to an endemic area. While it is essential to rule out malaria infection as the commonest and life-threatening differential diagnosis requiring immediate treatment, dengue infection should be considered next and specific laboratory tests performed. The patients do not always present with the typical symptoms of dengue, but the combination of the clinical picture, laboratory features and the recent travel history should be helpful in the majority of patients to establish the correct diagnosis. The selection of laboratory methods applied for confirmation of DF must be adjusted to the knowledge of the specific antibody kinetics and course of disease. The outcome of imported dengue virus infections in non-immune travellers usually takes a favourable course, but global expansion of the dengue virus and increased international travel has intensified the possibility of sequential infection, thus the risk to develop DHF. Therefore, and under consideration that neither an effective vaccination nor an effective vaccine is available, protection measures like mosquito repellents and wearing clothes that keeps skin exposure to mosquitoes to a minimum are highly recommendable.

Acknowledgements

The authors thank Johanna Làge-Stehr for help and support for Dengue diagnostics and Jung-Won Sim-Brandenburg and Anette Teichmann for technical support performing virus isolation and virus typing.

References


