Cases of travel-acquired dengue fever in Denmark 2001–2009

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Abstract

Dengue fever (DF) remains one of the most important emerging infectious diseases. Whereas DF is well recognized in endemic countries, there are indications that the disease is underdiagnosed among travellers to endemic regions. Here, we present the first descriptive survey on cases of travel-acquired DF imported to Denmark diagnosed at the national reference laboratory for dengue virus diagnostics during a 9-year period. In our study, 16 – 46 travel-acquired dengue virus infections were diagnosed per year. DF is mainly imported by adults, mostly men, returning from Southeast Asian countries. The minimum incidence of dengue virus infection among Danish travellers is estimated to be 4.9 per 100 000 travellers. Our results confirm and expand studies from other European countries, and underline the importance of surveillance based on relevant diagnostic analyses.

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Introduction

Dengue viruses (DENVs) are single-stranded, non-segmented RNA viruses of the family *Flaviviridae*. Four distinct serotypes (DENV-1 to DENV-4) are recognized to be transmitted between humans by the day-active mosquitoes *Aedes aegypti* and *Aedes albopictus*, which breed in exposed stagnant water in urban settings [1]. The combination of high population density and day-active vectors contributes to the high transmission level of DENVs [2].

Dengue fever (DF) is usually a self-limiting febrile disease. However, in a small percentage of cases, DENV infection may cause life-threatening complications: dengue haemorrhagic fever (DHF) or dengue shock syndrome (DSS) [3]. Infection with one serotype provides life-long immunity against the homologous serotype, but no cross-protection against other serotypes. Partial immunity may increase the risk of severe subsequent DENV infections, including DHF or DSS [4].

Dengue transmission has expanded dramatically in recent decades, and DF continues to be one of the most important emerging diseases, being endemic in more than 100 countries. The WHO estimates that 50 000 000 people are infected per year, approximately 500 000 of whom develop severe disease and are hospitalized [4]. The risk of infection is higher for the local population in endemic regions than for the traveller. Nonetheless, many cases of DENV infection are imported to European countries [5–11], but the extent of imported DENV infections among travellers is poorly described and probably underestimated [3,12].

Until recently, only travel-acquired or imported cases have been reported in European countries. The autochthonous transmissions that were reported in France [13] and Croatia in 2010 [14] emphasize the need for increased attention to DENV surveillance. Establishment of *Ae. albopictus* in several European countries [15] and the continuous importation of DENV through travelling have increased the risk of such autochthonous infections.

Our objective was to conduct the first descriptive survey of diagnosed cases of travel-acquired DF imported to Denmark during a 9-year period. We have estimated the minimum incidence rate (IR) of DENV infection among Danish travellers, as travel-acquired DF is probably underdiagnosed.

Materials and Methods

Ethical considerations

The data for the survey were generated from the routine DENV diagnostics performed at the Department of Virology, Statens Serum Institut (SSI), Denmark. The study was approved by the Danish Data Protection Agency. According to Danish law (Sundhedsloven), no other ethical committee approval is required.

Diagnostic procedures

The Department of Virology, SSI, Copenhagen is the Danish national diagnostic reference laboratory for imported human viral diseases, and performs ≥80% of all national diagnostic tests for DENV. All samples received for diagnostic DENV serology and/or RT-PCR between 2001 and 2009 were included in the study.

Detection of DENV-specific IgM and IgG was performed on all samples included in the study. The detection of viral RNA (vRNA) by RT-PCR was introduced in the department on 1 September 2002, and performed on all samples thereafter. Samples were received from Danish hospitals and >200 general practitioners.

Serology

During the study period, diagnostic serology procedures (IgM and IgG) were performed, with different assays. From 2001 to 2006, DENV IgM was detected with the Dengue Duo IgM and IgG rapid strip test (R-DEN02D; PanBio, Queensland, Australia). In addition, an indirect immunofluorescence assay was used to detect and titrate DENV-specific IgG (at the SSI until 2005, and thereafter at Bernhard-Nocht-Intitut für Tropemedizin, Hamburg, Germany). Samples analysed during 2007 to 2008 were tested with the PanBio Dengue Duo cassette kit (R-DEN03D; PanBio). In this period, samples that were negative for vRNA and IgG but positive for IgM were confirmed serologically with the Dengue Fever Virus IgM Capture ELISA (Focus Diagnostics, Cypress, CA, USA), either at the SSI or at an independent laboratory (Bernhard-Nocht-Intitut für Tropemedizin, Hamburg, Germany). Since 2009, the Dengue Fever Virus ELISA IgG and Dengue Fever Virus IgM Capture ELISA have been used for primary diagnostic analysis (40-EL 1500M and 40-EL1500G; Focus Diagnostics).

RT-PCR

Between September 2002 and September 2004, modified and previously described serotype-specific primers [16,17] were used for the detection and typing of DENV vRNA. From September 2004 and onwards, DENV vRNA was detected by real-time RT-PCR, as described elsewhere [18]. Serotypes were determined from RT-PCR results or by sequence analysis, as described elsewhere [19].

Case definitions

The case definition followed the guidelines of the CDC; a clinically compatible case with laboratory results indicative of presumptive infection (probable case, e.g. DENV-specific IgM) or with confirmatory laboratory results (confirmed case, e.g. RT-PCR or antigen detection).

Results

During 2001–2009 we have investigated 3181 suspected cases of DENV infection. After introducing the PanBio Dengue Duo cassette kit in our routine diagnostic analysis, we experienced an increased number of samples that were positive only for DENV-specific IgM. Therefore, we decided to confirm these findings. A total of 76 'IgM-only' samples were retested, and only 17 (22.4%) were confirmed as being IgM-positive and therefore included in this study.

Following the case definition of the CDC, we diagnosed a total of 173 probable cases of DENV infection (13–36 cases per year) (Fig. 1a). In addition, from 2002 to 2009, we confirmed 114 cases of DENV infection (8–21 cases per year).

There was a trend towards an increase in the number of cases of DENV infection per year. However, the number of suspected DF cases (i.e. number of requested analyses) increased as well during this period, from 292 cases in 2001, to 469 in 2009. Therefore, the frequency of DF cases had not increased. The average proportion of confirmed or probable cases ranged between 6.5% and 13.8% (mean 9.9%) from 2002 to 2009.

Seasonal variation in suspected and diagnosed cases

During the study period, we found a relatively high proportion of confirmed or probable cases of DENV infection from April to August (11.2–13.2%), as compared with the other months (Fig. 1b). There was also a trend towards an increased number of returning travellers with suspected DENV infection in March and August. The frequency of positive samples in August was 11.9%, and this coincides with the peak of DENV transmission seen in Thailand (http://www.searo.who.int/en/Section10/Section332_9482.htm). A lower frequency was detected in March (8.6%). This may be related to the fact that the countries in which DENV activity peaks during March (e.g. Indonesia) are generally less visited by Danish travellers.

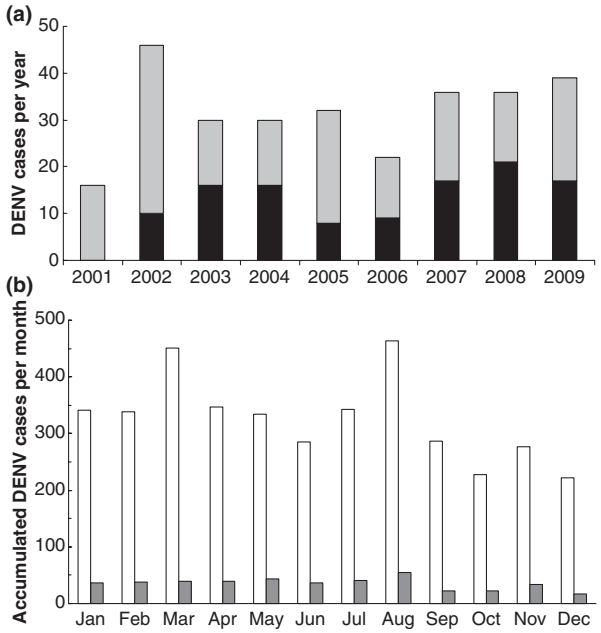

FIG. 1. (a) Number of probable (grey) or confirmed (black) dengue virus (DENV) infections per year. As RT-PCR analysis was introduced in 2002, only probable cases were found in 2001. (b) The seasonal distribution of suspected cases (white) and confirmed or probable cases (grey). Samples were collected in Denmark between 2001 and 2009.

Age and gender distribution

The age distribution of imported DENV cases is shown in Fig. 2. The mean ages (range) of males and females were 38 years (6–79 years) and 33 years (11–72 years), respectively (n = 365). The overall male/female (M/F) ratio was 1.18 : 1. Among 41–50-year-olds, the M/F ratio was significantly skewed (2.2 : 1, χ^2, p <0.05). Likewise, the calculated M/F ratio among the 31–50-year-olds was significantly skewed (1.82 : 1, p <0.05).

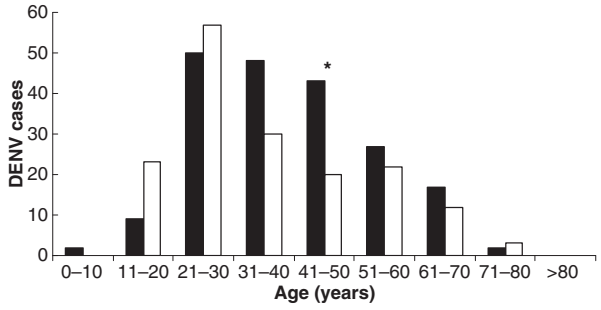

FIG. 2. Age distribution of probable or confirmed dengue virus (DENV) cases in Danish men (black) and women (white) between 2001 and 2009 (n = 365). The asterisk (*) indicates statistically more common DENV infections among men than among women (p >0.05).

Laboratory confirmation depends on the time of blood sampling

Information about onset of disease was available only from a limited number of patients registered between 2004 and 2009 (n = 21). Among this group of patients 75% were vRNA-positive within the first 7 days from onset of symptoms, and 50% were positive for IgM (data not shown). During the second week after onset of symptoms, all samples were IgM-positive, whereas 25% of samples were vRNA-positive.

Even though the time of onset of symptoms is uncertain, travel activity information is valuable for interpreting the results of the analysis. DENV vRNA was detected in the majority (83%) of the plasma samples (n = 88, 2004–2009) taken from day 0 to day 7 after return. All samples taken more than 11 days after return were negative for vRNA, implying that laboratory confirmation by RT-PCR should not be expected after this time (Fig. 3). Meanwhile, 62% of the samples taken during the first week after return were seronegative.

As expected, the frequency of IgG-positive samples increased with time from return. All DENV-positive samples taken ≥2 weeks after return were positive for DENV-specific IgG (titres ≥1 : 160).

Most cases are imported from Southeast Asia

Information on travel activity was collected from a total of 95 patients of 195 with probable or confirmed DENV infection during 2004–2009. The results show that DENV infections were imported from all endemic continents except Australia (Australasia) (Table 1). Most cases (67%) were imported from countries in Southeast Asia, particularly

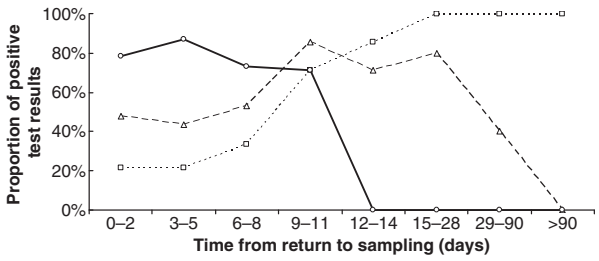

FIG. 3. Relationship between time of return from endemic region and sampling of plasma for dengue virus (DENV) diagnostic tests. The proportions of samples positive for viral RNA (○), IgM (Δ) or IgG (□) are shown.

TABLE 1. Travel activity of Danish patients with imported dengue virus infection (2004–2009)

Geographical region	Country	Number of imported cases
Southeast Asia	All	62
	Borneo[a]	2
	Indonesia, Bali	9
	Indonesia, other	2
	Malaysia	2
	Philippines	6
	Southeast Asia[a]	2
	Thailand	38
	Vietnam	1
South Asia	All	19
	Bangladesh	6
	India	10
	Maldives	1
	South Asia[a]	2
Central America	All	6
	Central America[a]	2
	Costa Rica	3
	Nicaragua	1
Africa	All	4
	Ghana	2
	Nigeria	1
	Somalia	1
Caribbean	All	2
	US Virgin Islands	2
South America	All	2
	Venezuela	2

[a]Country not specified.

Thailand, which are the most visited DENV-endemic destinations for Danish travellers.

Cases of all four serotypes are imported from Thailand

Between 2002 and 2009, the DENV serotype was determined for 71% of the confirmed cases (n = 87) (Fig. 4). Overall, DENV-2 was the most frequently detected, whereas DENV-4 was rarely detected, and not at all after 2004. However, country-specific differences were observed. In travellers returning from Indonesia, only DENV-2 was detected. In travellers returning from the Philippines, India and Bangladesh, we primarily found DENV-2 or DENV-3 infections. DENV-1 was more frequently found in cases imported from Thailand. Importantly, all four DENV serotypes were detected in travellers returning from Thailand. Our data also confirm that two to three different DENV serotypes co-circulated in Thailand and other countries during most of the study period.

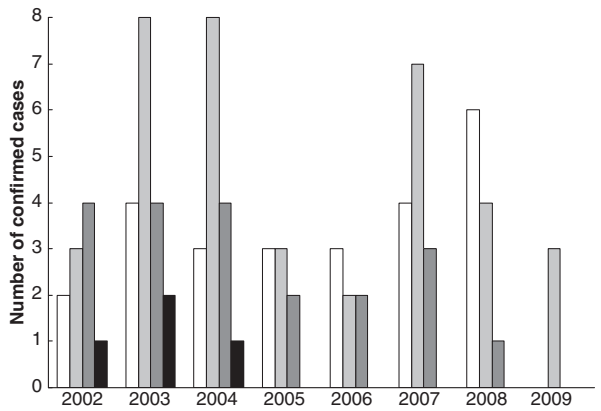

FIG. 4. Serotypes of imported dengue virus (DENV) cases are shown for each year. DENV-1 (white), DENV-2 (light grey), DENV-3 (dark grey), and DENV-4 (black).

There was one confirmed case of DENV infection per 20 400 Danish travellers to endemic regions

Between 2004 and 2008, the number of Danish travellers to DENV-endemic countries increased (e.g. from >87 000 to >151 000 per year for Thailand; http://www.e-unwto.org/content/v486k6/?v=search). The minimum proportion of travellers in which DENV infection was confirmed can be calculated. On the basis of accumulated numbers of travellers to countries from where most cases have been imported (Thailand, Indonesia, India, and Bangladesh), we can estimate an IR of 4.9 (95% CI 3.2–7.2) confirmed cases per 100 000 Danish travellers to endemic areas, or one confirmed DENV case per 20 400 (31 250–13 890) Danish travellers. The IR of confirmed or probable DENV infection in the Danish population is 0.57 (0.46–0.67) per 100 000 individuals.

Discussion

We have performed the first descriptive study on imported DENV infections among Danish travellers from 2001 to 2009. The Department of Virology, SSI, Copenhagen performs approximately ≥80% of all national diagnostic testing for DENV. Fewer than 150 suspected cases are tested serologically each year at major hospitals. In cases of positive serology, many of these samples are confirmed in our laboratory by RT-PCR. Therefore, the number of probable cases diagnosed in Denmark is likely to be only slightly higher than the numbers indicated here.

The increase in the number of DENV cases per year is probably a consequence of increased exposure resulting from travelling combined with greater awareness among patients and physicians, as reflected by the increasing numbers of suspected cases. The highest number of cases was found in 2002 (Fig. 1), which coincided with an outbreak in Thailand (http://www.searo.who.int/en/Section10/Section332_9482.htm).

The number of cases reported in a study from Sweden, where DENV infection is a notifiable disease, is approximately two-fold to three-fold higher than our results indicate [20]. In Denmark, DENV infection is not a notifiable disease. Although notification does not ensure that all cases are identified, it may increase awareness of the disease. Our results indicate that the number of cases of imported DENV infection in Denmark may be underestimated. With the recent

cases of autochthonous transmission in France and Croatia [13,14] and the high numbers of tourists visiting European countries with overwintering expanding populations of *Ae. albopictus* [15], we believe that a notification system, as in other EU countries, would be beneficial [8].

The overall M/F gender distribution of probable or confirmed cases in our study is very similar to that in other reports (Fig. 2) [8]. Between the ages of 31 and 50 years we found significantly more male cases, and this differs somewhat from the distribution in 20 other European countries combined [21]. The M/F ratio among all tested patients (positive or negative) was 1.21 : 1, and we found no indications of significant differences in age or gender over the years. Therefore, the age distribution is likely to represent the travel activity of the Danish population, rather than any gender differences in transmission or diagnosis.

The use of different commercial tests for the detection of DENV IgM showed some discrepancies. Our findings show that the number of probable cases may easily be overestimated if positive IgM test results are not confirmed or complemented by other diagnostic tests, as discussed earlier [11]. Evaluations of ELISA test kit performance conducted in our laboratory and by others show that false-positive IgM results are frequently obtained with the PanBio Dengue Duo cassette kit [22]. Although the reasons for false IgM-positive results are complex and not fully understood [11], this emphasizes the need for confirmatory RT-PCR or antigen testing. A number of studies have indicated that the proportion of DENV infection is 2–17% among febrile European travellers [9,23,24]. Consequently, given the high transmission level of DENV [2] and the volume of international travel, patients with acute fever of short duration should also be asked about recent foreign travel activity [3]. DENV infection should be considered in all patients with fever and myalgia returning from endemic areas, especially in the presence of maculopapular rash, thrombocytopenia, increased haematocrit, or elevation of liver enzymes. Likewise, information on onset of symptoms and return from endemic regions has important consequences for the diagnosis of travel-acquired DENV infection, as shown by our study and other studies [25]. DENV infection may be confirmed by detection of vRNA, which may be detected up to 11 days after return. Also, samples from most confirmed cases collected less than 1 week after return are often seronegative. In symptomatic patients, a negative IgM result does not exclude DENV infection [26]. We believe that RT-PCR-based diagnosis should be used together with IgM serology in suspected cases of acute DENV infection, as suggested by others [3,11,27,28]. Alternatively, NS1 antigen detection may provide confirmation.

Our results show that the majority of Danish imported DENV infections are acquired in Thailand and other Southeast Asian travel destinations (Table 1). Other studies support these findings [5,7,8,29]. We have demonstrated transmission of all four serotypes in Thailand, although transmission of DENV-1 and DENV-2 was more frequent. Genotype determination of DENV in European travellers indicates that DEN-1 has been transmitted in Thailand for a decade, whereas DENV-2 has been found mostly in recent years. DENV-3 and DENV-4 are also present, but less frequent [30]. Co-circulation of DENV serotypes increases the risk of severe disease in cases of repeated infections [4]. Obviously, this poses the greatest risk for the local population, but is also a risk for visiting travellers with past DENV infection.

We have estimated that there is one confirmed case of DENV infection per 20 400 Danish travellers to endemic regions (4.9 per 100 000 travellers), which is in good agreement with IRs reported from another study [31]. Also, the IR of the general population is in good agreement with those of neighbouring countries [21].

Studies of foreign residents and military personnel in endemic areas suggest that up to one per 1000 may become infected with DENV during several months of stay (reviewed in [25,32]). As the risk of infection increase with the length of stay [7,32], the risk is probably less for the average traveller. The average length of stay is approximately 15 days for Danish and German travellers in Thailand (http://www2.tat.or.th). In a study from Germany, where DENV infection is a notifiable disease, the IR of confirmed or probable cases of DENV infection was found to be 8.2–27.9 per 100 000 travellers [8]. Taking our findings together, the estimated IR in our study should be regarded as a minimum proportion. The true proportion is probably considerably higher, as there is probably under-reporting of mild cases, as discussed by Allwin *et al.* [12], and because DENV infection is not a notifiable disease in Denmark.

During the study period, we received no information about haemorrhagic manifestations, shock or death among Danish travellers with DENV infection. Reports of complications such as DHF, DSS and fatalities in travellers exist [5,10], but are generally rare [6,8,20,33]. In one study, haemorrhagic manifestations were common [7].

We have conducted a comprehensive survey of the travel-acquired cases of DENV infection in Denmark, based on data collected at the national reference laboratory for DENV over a period of 9 years. The results support findings from other European countries, and underline the importance of considering DENV infection in all febrile patients with recent travel activity, as well as the timing of sample collection in relation to the relevance and performance of available laboratory diagnostic tests.

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Transparency Declaration

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References

1. Lifson AR. Mosquitoes, models, and dengue. *Lancet* 1996; 347: 1201–1202.
2. Teixeira MG, Costa MC, Barreto F, Barreto ML. Dengue: twenty-five years since reemergence in Brazil. *Cad Saude Publica* 2009; 25 (suppl 1): S7–S18.
3. Bulugahapitiya U, Siyambalapitiya S, Seneviratne SL, Fernando DJ. Dengue fever in travellers: a challenge for European physicians. *Eur J Intern Med* 2007; 18: 185–192.
4. Halstead SB. Dengue. *Lancet* 2007; 370: 1644–1652.
5. Jelinek T. Trends in the epidemiology of dengue fever and their relevance for importation to Europe. *Euro Surveill* 2009; 14: 19250.
6. Wichmann O, Gascon J, Schunk M *et al.* Severe dengue virus infection in travelers: risk factors and laboratory indicators. *J Infect Dis* 2007; 195: 1089–1096.
7. Lindback H, Lindback J, Tegnell A, Janzon R, Vene S, Ekdahl K. Dengue fever in travelers to the tropics, 1998 and 1999. *Emerg Infect Dis* 2003; 9: 438–442.
8. Frank C, Schoneberg I, Krause G, Claus H, Ammon A, Stark K. Increase in imported dengue, Germany, 2001–2002. *Emerg Infect Dis* 2004; 10: 903–906.
9. Cobelens FG, Groen J, Osterhaus AD, Leentvaar-Kuipers A, Wertheim-van Dillen PM, Kager PA. Incidence and risk factors of probable dengue virus infection among Dutch travellers to Asia. *Trop Med Int Health* 2002; 7: 331–338.
10. Vainio K, Noraas S, Holmberg M *et al.* Fatal and mild primary dengue virus infections imported to Norway from Africa and South-East Asia, 2008–2010. *Euro Surveill* 2010; 15: 19666.
11. Domingo C, de Ory F, Sanz JC *et al.* Molecular and serologic markers of acute dengue infection in naive and flavivirus-vaccinated travelers. *Diagn Microbiol Infect Dis* 2009; 65: 42–48.
12. Allwinn R, Hofknecht N, Doerr HW. Dengue in travellers is still underestimated. *Intervirology* 2008; 51: 96–100.
13. Gould EA, Gallian P, De Lamballerie X, Charrel RN. First cases of autochthonous dengue fever and chikungunya fever in France: from bad dream to reality! *Clin Microbiol Infect* 2010; 16: 1702–1704.
14. Gjenero-Margan I, Aleraj B, Krajcar D *et al.* Autochthonous dengue fever in Croatia, August–September 2010. *Euro Surveill* 2011; 16: 19805.
15. Reiter P. Yellow fever and dengue: a threat to Europe? *Euro Surveill* 2010; 15: 19509.
16. Harris E, Roberts TG, Smith L *et al.* Typing of dengue viruses in clinical specimens and mosquitoes by single-tube multiplex reverse transcriptase PCR. *J Clin Microbiol* 1998; 36: 2634–2639.
17. Lanciotti RS, Calisher CH, Gubler DJ, Chang GJ, Vorndam AV. Rapid detection and typing of dengue viruses from clinical samples by using reverse transcriptase-polymerase chain reaction. *J Clin Microbiol* 1992; 30: 545–551.
18. Drosten C, Gottig S, Schilling S *et al.* Rapid detection and quantification of RNA of Ebola and Marburg viruses, Lassa virus, Crimean-Congo hemorrhagic fever virus, Rift Valley fever virus, dengue virus, and yellow fever virus by real-time reverse transcription-PCR. *J Clin Microbiol* 2002; 40: 2323–2330.
19. Domingo C, Palacios G, Niedrig M *et al.* A new tool for the diagnosis and molecular surveillance of dengue infections in clinical samples. *Dengue Bull* 2004; 28: 87–95.
20. Heddini A, Janzon R, Linde A. Increased number of dengue cases in Swedish travellers to Thailand. *Euro Surveill* 2009; 14: 19111.
21. European Centre for Disease Prevention and Control. *Annual Epidemiological Report on Communicable Diseases in Europe 2010*. Stockholm, ECDC, 2010.
22. Hunsperger EA, Yoksan S, Buchy P *et al.* Evaluation of commercially available anti-dengue virus immunoglobulin M tests. *Emerg Infect Dis* 2009; 15: 436–440.
23. O'Brien D, Tobin S, Brown GV, Torresi J. Fever in returned travelers: review of hospital admissions for a 3-year period. *Clin Infect Dis* 2001; 33: 603–609.
24. Askling HH, Lesko B, Vene S *et al.* Serologic analysis of returned travelers with fever, Sweden. *Emerg Infect Dis* 2009; 15: 1805–1808.
25. Rigau-Perez JG, Clark GG, Gubler DJ, Reiter P, Sanders EJ, Vorndam AV. Dengue and dengue haemorrhagic fever. *Lancet* 1998; 352: 971–977.
26. Schwartz E, Mileguir F, Grossman Z, Mendelson E. Evaluation of ELISA-based sero-diagnosis of dengue fever in travelers. *J Clin Virol* 2000; 19: 169–173.
27. Lindegren G, Vene S, Lundkvist A, Falk KI. Optimized diagnosis of acute dengue fever in Swedish travelers by a combination of reverse transcription-PCR and immunoglobulin M detection. *J Clin Microbiol* 2005; 43: 2850–2855.
28. Grobusch MP, Niedrig M, Gobels K, Klipstein-Grobusch K, Teichmann D. Evaluation of the use of RT-PCR for the early diagnosis of dengue fever. *Clin Microbiol Infect* 2006; 12: 395–397.
29. Potasman I, Srugo I, Schwartz E. Dengue seroconversion among Israeli travelers to tropical countries. *Emerg Infect Dis* 1999; 5: 824–827.
30. Domingo C, Niedrig M, Gascon J *et al.* Molecular surveillance of circulating dengue genotypes through European travellers. *J Travel Med* 2011; (in press).
31. Robert Koch Instute. Dengue-fieber. *Epidemiol Bull* 2006; 37: 322–323.
32. Jelinek T. Dengue fever in international travelers. *Clin Infect Dis* 2000; 31: 144–147.
33. Schwartz E, Weld LH, Wilder-Smith A *et al.* Seasonality, annual trends, and characteristics of dengue among ill returned travelers, 1997–2006. *Emerg Infect Dis* 2008; 14: 1081–1088.