Using routine diagnostic data as a method of surveillance of arboviral infection in travellers: A comparative analysis with a focus on dengue

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**KEYWORDS**

Dengue; Diagnostics; Travellers; Sentinels; Surveillance

**Summary**  
**Background:** In a large part of the developing world, limited infectious disease surveillance is performed. In laboratory information management systems data on diagnostic requests is available and may be amenable to trend analyses. We explored this potential, using DENV diagnostic requests as a model.  
**Method:** Test results and anonymised information provided by clinicians were received for 8942 patients from diagnostic centres in the Netherlands from January 2000 to May 2011. The data were evaluated for completeness of a predefined minimal dataset and trends in DENV positive results by travel destination. Population travel data were obtained from a commercial registry, and dengue case notification data by country from WHO DengueNet.  
**Results:** Vaccination history was rarely reported (0.4%); travel destination was completed for 42% of requests; trends in diagnostic requests and IgM positive tests for this subset correlated to the WHO DENV notifications for the three main travel destinations, with some discrepancies. Additionally, this approach may provide information on disease outbreaks with other...
Introduction

New diseases continue to emerge across the world, due to a complex array of factors relating to demographics, increasing demand for animal protein, deforestation, and a steep increase in international travel and trade [1,2]. Vector-borne diseases, with the exception of West Nile and Dengue virus, are considered neglected tropical diseases and no or little surveillance is performed in a large part of the developing world. Systematic evaluation of health complaints through travel clinics has shown a high incidence of health complaints (8–10%) [3,4]. As a consequence, international travellers can be seen as sentinels and sources of introduction for infectious diseases occurring worldwide [5–8]. Developing diagnostic tools and approaches to monitor health complaints in returning travellers could provide an interesting addition to traditional surveillance [8]. Geosentinel clinics form an international network that collects data on diseases in travellers. However, the number of clinics participating is limited and the reporting system depends on the extent to which clinicians actively upload information. A large amount of diagnostic data is therefore available in diagnostic laboratories but remains unused for surveillance purposes. This had led us to explore the use of routine laboratory submission data and results for additional monitoring of trends of illness through international travellers. Here, we set out to explore this potential by using dengue virus as an example.

Dengue virus (DENV) is considered one of the most important arboviruses globally, with 2.5 billion people at risk of infection according to the World Health Organization (WHO) [9]. It is a well-recognized disease in travellers to tropical and subtropical regions and therefore diagnostic tests are requested frequently [10–12]. Surveillance is done in many countries that are popular travel destinations, and DENV probable and confirmed cases are notified on voluntary basis to the WHO. This currently provides the information on public health threats by possible introduction of viremic patients and trends in local disease activity. We use DENV as an example since WHO surveillance information is available for some countries as comparison and complementary data.

Methods

Diagnostic data

During a consensus meeting between the diagnostic laboratories and responsible researchers, a minimal dataset needed for data analysis was proposed and discussed based on the question what minimal information was essential for use in surveillance of disease in travellers and the countries they visit. The defined minimum dataset was age, sex, travel date and destination, description of clinical symptoms, vaccination history, diagnostic results and test(s) used. Age and sex were considered to be of importance in order to identify risk groups and make results compatible between diagnostic centres. Information of travel history and dates were needed to correlate demographic distribution of infections in Dutch travellers to current known and unknown outbreaks. This information was also needed for interpreting results as new or old infections and possible cross-reactions with co-circulating cross-reactive arboviral infections [16–19]. Records of clinical symptoms were needed to evaluate the usefulness of the clinical data for syndromic surveillance [20]. Finally, vaccination history was considered essential for the data analysis since a number of flavivirus vaccinations (Tick-borne encephalitis virus, Japanese encephalitis virus, Yellow fever virus) are known to cross-react causing false positive IgG diagnostic results [16,19]. Data containing the diagnostic results, interpretation, and the information provided by clinicians with the requests for DENV diagnostics were retrospectively extracted from the laboratory information management systems (LIMS) from the three main arboviral diagnostic labs in the Netherlands from 2000 to 2011. This represents the vast majority of all DENV diagnostic requests in the Netherlands. The information was provided in excel format as raw data. As there is no standardized testing for DENV infections, each laboratory provided the interpretations of the results. Diagnosis was based on determination of IgM and IgG antibodies by rapid lateral flow Immunochromatographic Test (ICT) by Panbio (Brisbane, Australia) (one laboratory), immunofluorescence assay (IFA) by Progen (Heidelberg, Germany) and Scimedix (Denville, New Jersey, USA) (one laboratory) and Enzyme-linked immunoassays (ELISA) from Focus (Cypress, CA, USA) (two laboratories). Each laboratory provided a cut-off for defining if a diagnostic result was considered positive or negative.
Labs using IFA and ELISA tests used a higher but comparable cut-off than recommended by the manufacturer to increase specificity. These cut-offs were defined by validation prior to implementation of the assays in the diagnostic routine. In compliance with the medical ethical guidelines, no personal identifiers were included.

Data analysis

A database was created in Microsoft Excel (edition 2007). The raw data was organized and imported into ‘R’ (version 2.14.0) for further analysis. The data was evaluated for completeness of the minimal defined dataset and for trends in DENV positive results by travel destination. These trends were compared with those based on data collected by WHO DengueNet 2001 to 2011 that are published by the WHO regions online (South-East Asia only published data until 2010).

Travel data

Data were obtained from a commercial research registry ‘ContinuVakantieOnderzoek’ (CVO) that follows travel trends by interviewing, every three months, 10,000 out of a panel of 150,000 Dutch homes about their travel behaviour [21]. The research has been conducted since the 1980’s and is used for monitoring Dutch travel behaviour in the tourist and travel industry. Basic information is requested on number and length of vacations within and outside the country (if any), location and lodging, activities, method of transport and booking. Information is analysed in condensed form for reports and raw data is available in an online database HolidayTracker [21].

Estimation of the proportion of DENV infections in travellers, 2010

As travellers may be seen as a source of introduction of arboviral infections, we tried to use the available data to provide an estimate range for the possible proportion of travellers infected with Dengue during one year. For this, the lower limit estimate was made by:

\[
E_{\text{low}} = \left( P \times 4 \right) / N
\]

Where \( E_{\text{low}} \) is the lower estimate of DENV infections, \( P \) is the number of DENV IgM positive patients, 4 is the multiplication factor assuming 25% of all cases are symptomatic [22], and \( N \) is the number of travellers to DENV endemic countries.

The higher estimate was made by:

\[
E_{\text{high}} = \left( \left( N + 0.1 \right) \times \left( P/T \right) \right) / N \times 4
\]

Where 4, \( N \) and \( P \) are as indicated above, 0.1 is the estimated proportion of travellers returning with febrile illness [3,4,23,24], and \( T \) is the number of samples tested.

Statistical analysis

Proportions were tested with Pearson’s chi-squared test for equality of proportions. Trend lines were tested with F-test and \( R^2 \) and correlations were calculated using Spearman’s rank correlation coefficient for describing independence between variables (\( r_s \)).

Results

Completeness of diagnostic data

In total, data were received on 10,540 diagnostic samples from 8942 patients from the three diagnostic centres in the Netherlands from January 2000 until May 2011. Each laboratory provided diagnostic result and interpretation per patient. The completeness of data ranged from 0.5% for vaccination history to 98% and 99% for sex and birth date. Some basic clinical information was provided with 75% of requests, but only 42% of the requests mentioned information on travel history (Table 1). Only 0.2% of diagnostic requests contained all parameters.

Trends in diagnostic requests and results

Between 2000 and 2011 RT-PCR for detection of DENV viral RNA was performed on 275 samples (3%) of DENV diagnostic requests. More than 50% of the RT-PCR requests have occurred in the last three years and the percentage of positive samples has dropped from 30% to 13% (data not shown). RNA was more often detected in IgM positive (23%) than in IgM negative patients (9%).

The annual number of serological diagnostic requests for DENV has increased significantly over time (Fig. 1). In correspondence, the proportion of travellers with diagnostic requests has also increased (Fig. 1). Correlation between the number of requests, and IgG and IgM positive samples per year was very high \( (r_s = 0.82 \text{ to } 0.99 \text{ and } P < 0.01) \). The proportion of patient samples testing positive for DENV IgG or IgM antibodies fluctuated, with peaks in 2005 and in 2009/2010. IgG and IgM proportions show a reasonably strong correlation \( (r_s = 0.70 \text{ and } P < 0.01) \). When studying trends in more detail, the increase relative to the previous year of IgG positives compared to IgM was higher in 2005 than in 2009, which indicates some independence between IgG and IgM proportions. A third peak was observed in 2002 but this is less clear as the total number of diagnostic requests was too low to draw conclusions based on this.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Information provided with diagnostic requests per patient population for 8942 patients.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameter</td>
<td>Percentage available</td>
</tr>
<tr>
<td>Sex</td>
<td>98%</td>
</tr>
<tr>
<td>Birth date</td>
<td>99.9%</td>
</tr>
<tr>
<td>Clinical history</td>
<td>75%</td>
</tr>
<tr>
<td>Travel history</td>
<td>42%</td>
</tr>
<tr>
<td>Sex, Age, Clinical, Travel history</td>
<td>38%</td>
</tr>
<tr>
<td>Vaccination history</td>
<td>0.5%</td>
</tr>
<tr>
<td>Clinical, Travel, Sex, Age, Vaccination</td>
<td>0.2%</td>
</tr>
<tr>
<td>Total patients</td>
<td>8942</td>
</tr>
</tbody>
</table>

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Trends in travel history by region

The 3751 (42%) patients that had information on travel history provided with their diagnostic requests were subdivided into the geographical regions Africa, Americas, Europe and Asia-Oceania. For 263 patients, travel history was stated as 'Tropics' (228), 'No' (12) or 'Yes' (23) and these could not be assigned into a geographical category. Patients (n = 219) that had travelled to more than one region were included multiple times in the subdivision. Most patients traveling outside of Europe had travelled to Asia-Oceania followed by the Americas and then Africa (Table 2). The prevalence of DENV IgM and IgG positive patients differed significantly for travellers from different regions (Table 2, P < 0.01). Only 29% (79) of the samples tested with PCR were accompanied by travel information (Table 2).

Trends in travel history by country

In total 131 different countries were listed in the travel history of patients. The top five travel destinations were Indonesia, Thailand, Surinam, India and the combination of the former and current Dutch Caribbean Islands, i.e. Bonaire, St Eustatius and Saba (BES islands), St Maarten, Aruba and Curacao (2000 patients, 53%). Travellers from Surinam were most frequently DENV IgG positive (Fig. 2 and Supplement Fig. 3(A)). The Dutch Caribbean islands were the travel destination associated with the highest number of patients with DENV IgM positive tests (129 patients). Results are plotted for all countries that had been listed for at least 50 persons (Supplement Fig. 3(A)) or 20 persons (Supplement Fig. 3(B)) as travel destination. The proportion of DENV positive patients differed greatly between travel destinations. Large differences were observed in percentage IgM positives for patients returning from different countries. This is partly explained by the low number of diagnostic requests for travellers to these countries (Supplement Fig. 3(A)&(B)). Per top five travel destinations only one or two patients were PCR positive, but did show a higher than average number of positive samples compared to other destinations (20%).

Trends in DENV IgM positive results by country compared to WHO DENV notifications

Data representing the number of Dutch patients with DENV positive tests was combined with data representing the WHO reported DENV cases per country for the pooled data from patients returning from the top five travel destinations.

WHO notifications preceded the trends in DENV diagnoses from the pooled dataset by one year (data not shown). When analysing trends by country, however, clear differences were observed. For the Dutch Caribbean islands, Suriname and Thailand, the diagnostic data and the number of WHO reported cases show a high correlation, although the timing differed. The data from Thailand corresponded almost perfectly with the WHO

Table 2

<table>
<thead>
<tr>
<th>Travel destination</th>
<th>Number of patients</th>
<th>% Requests</th>
<th>% IgG</th>
<th>% IgM</th>
<th>% IgG and IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Africa</td>
<td>643</td>
<td>17</td>
<td>11</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Americas</td>
<td>1283</td>
<td>34</td>
<td>34</td>
<td>20</td>
<td>15</td>
</tr>
<tr>
<td>Asia-Oceania</td>
<td>1744</td>
<td>46</td>
<td>21</td>
<td>16</td>
<td>10</td>
</tr>
<tr>
<td>Europe</td>
<td>37</td>
<td>1</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>P-value</td>
<td></td>
<td></td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>
data. For the Caribbean islands, the diagnostic trends preceded the increase seen in WHO reporting by one to two years. For the data from travellers to Surinam, the increase in the diagnostic trend line from 2008 onwards was not seen in WHO notifications (Fig. 2). For Indonesia, the diagnostic IgM trend showed some dependency with the WHO data but seemed to lag behind by one year in 2004–2005. Finally, the proportion of IgM positive samples for India did not show any correlation with the WHO reported cases or the proportion of travellers with diagnostic requests. However, the proportion of requests did show a correlation with the WHO data. All these observed correlations ($r = 0.7–0.9$) were significant ($P < 0.01$).

**Estimation of the proportion of DENV cases in travellers, 2007 and 2010**

As travellers may be seen as a source of introduction of arboviral infections, we tried to use the available data to provide an estimate range for the possible number of dengue cases during one year, as described in the methods section. By this approach, the lower estimate, assuming that all clinical DENV imported cases would be detected, was 0.02% for 2007 and 0.06% for 2010 of travellers to tropical or subtropical regions. The higher estimate, made based on the assumption that the current diagnostic requests reflect a fraction of all febrile travellers, was 3.7% for 2007 and 7.6% for 2010 of travellers to tropical or subtropical regions. We did not perform further calculations using the number of viremic travellers because of the low number of travellers tested by RT-PCR.

**Discussion**

**Completeness of diagnostic data**

We explored the potential use of diagnostic request data for systematic monitoring of trends in arboviral disease in Dutch travellers around the world, using DENV diagnostic data as a model [15]. We received information on 10,540 diagnostic samples from 8942 patients. Only 42% of the diagnostic requests provided information on travel history thereby limiting the usable amount of data to a large extent. Because of such a large amount of requests this loss of usable data seemed to be partially compensated as...
analysis still provided results. The literally complete lack of vaccination background data is of concern, as this information is essential for interpretation of serological diagnostic results of patients. The envelope protein of flavivirus is the main target for immune response [25–27]. Many common epitopes on the envelope protein cause cross-reactivity between flaviviruses in diagnostic tests [18,28,29]. Recent vaccination could cause cross-reactive antibody titres against other flaviviruses, mainly for IgG antibodies [16,19,30]. Although this factor has been taken into account when validating the cut-offs per laboratory for travellers to countries where YFV vaccination is compulsory (parts of South America) or highly recommended (Central Africa), this possible confounding factor could not be excluded when evaluating the IgG test results [16]. We tried to reduce the influence of this possible confounder by focusing further analysis on IgM data. This lack of information should however be further investigated to identify probable causes along the diagnostic request chain, for example (1) shortage of time by physicians to complete request forms, (2) the lack in understanding of how vital this information is for interpretation or (3) the lack in knowledge of the usability of this data of surveillance. The use of digital request forms with compulsory fields might prove beneficial for improving completeness of diagnostic data. Lack of additional information on travel times of individual patients could be compensated by extending the national information used on travel destinations into seasons and link these to the of diagnostic trends per month. This approach can only be used when sufficient diagnostic requests are available, reflecting travel behaviour of the patient population that is subject to national preferences. Potentially combining information on an international level might provide a better spread in data per country and strengthen the data per visited country. This data could provide laboratory and background parameters for surveillance supplementary to what is already done through the Geosentinel system [6,7].

**Trends in diagnostic requests and results**

The low number of requests for PCR is also of concern, especially because of the high percentage of PCR positive samples in both IgM positive and negative samples. In the Netherlands diagnostic samples may only be tested based on what the physician specifically requests. The lack in PCR requests is therefore probably based on the assumption that travellers are frequently not viremic anymore on time diagnosis. The number of PCR positive samples is therefore so low (one or two per destination) that no surveillance on travel destination is possible, but show that potentially a large number of viremic travellers return to the Netherlands annually.

There was a clear increase in the DENV requests and IgM positive patients over recent years (Fig. 1), similar to what was observed in other countries across the EU like Germany and the United Kingdom [31–33]. The proportion of travellers with diagnostic requests has increased (Fig. 1). This may reflect true increase in DENV prevalence or more awareness of DENV risk among Dutch physicians.

**Trends in travel history per country**

Although most diagnostic requests involved patients returning from Asia, the most positive samples came from the Americas, possibly for the reasons given above, whereas DENV was rarely diagnosed in travellers returning from Africa (Table 1). This is interesting as DENV is endemic in a large part of Africa. Possibly physician awareness might play a role in requesting dengue testing in travellers to Africa.

**WHO in DENV IgM positive results by country compared to WHO DENV notifications**

WHO notification data on the Caribbean, Central America and the northern part of South America indicate large DENV outbreaks in 2007, 2009, 2010 and high DENV activity in 2008 [34]. This corresponds to our diagnostic findings for Surinam from 2007 onwards, but does not correspond to the WHO dengue data reported on Surinam itself. It seems implausible that all Surinam surrounding countries would experience outbreaks and Surinam would remain unaffected. Possibly, reporting of DENV cases to the WHO was affected for some reason during this period. Monitoring based on diagnostic results with WHO notification therefore has a clear added value.

The diagnostic results for travellers from Indonesia and India show less correlation. Both these destinations are less popular than the previously mentioned three. Dutch travellers focus their travels mainly on Borneo, Bali and Java islands making them less representative for all Indonesia. Focusing outbreak information on only these three islands may prove to be more accurate in relation to travellers.

Occasionally, a deviation from the trend indicated presence of high numbers of patients with dengue-like
surveillance of arboviral infection in travellers

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[33] Her Z, Kam YW, Lin RTP, Ng LFP. Chikungunya: a bending re-


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