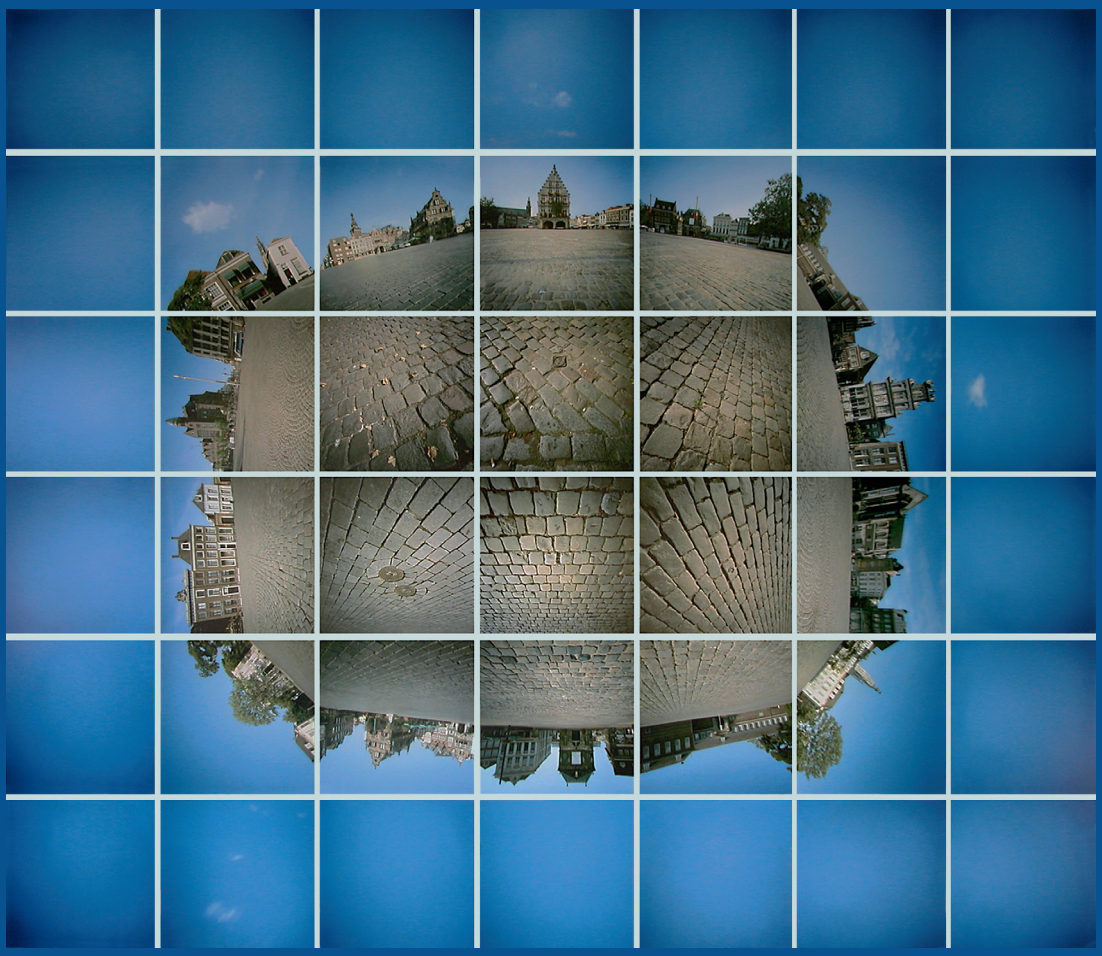

Dutch travelers in the global village

The rise and fall of travel-related infectious diseases

Femke Wendy Overbosch

Dutch travelers in the global village:
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Dutch travelers in the global village:
the rise and fall of travel-related infectious diseases

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Promotor:	prof. dr. M. Prins	AMC-UvA
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Faculteit der Geneeskunde

Voor Henriëtte en Evert, mijn allerliefste ouders

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1

Introduction

1. GENERAL INTRODUCTION

"One of us suggested performing the following experiment to prove that the population of the Earth is closer together now than they have ever been before. We should select any person from the 1.5 billion inhabitants of the Earth – anyone, anywhere at all. He bet us that, using no more than five individuals, one of whom is a personal acquaintance, he could contact theselected individual using nothing except the network of personal acquaintances."

Frigyes Karinthy. Chain-Links, 1929. Translated from Hungarian and annotated by Adam Makkai and Enikö Jankó (1).

In 1929, the Hungarian author Frigyes Karinthy hypothesized that social distance between people had become smaller due to technological advances in communication and travel (1). Though his assumption of the world population was probably not fully accurate at the time (United Nations (UN) estimations of 1930: 2 billion inhabitants) (2), his ideas have inspired for both scientific experiments and entertainment for decades, such as 'The Small World Problem' in 1967 by Jeffrey Travers and Stanley Milgram, the play 'Six degrees of separation' written by John Guare in 1990 and the parlor game 'Six degrees of Kevin Bacon' (3-5). All these, speculate how few 'friendship' or 'handshake'-links are needed to connect any person with a random other person in the world.

1.1 Global village

Where these projects mostly concern social aspects, other factors including population growth, urbanization, migration, transportation of goods, technological developments electronic communication and the internet, international travel, reduction of poverty and prosperity contribute to interconnectedness as well. Especially in South America and Asia, large progress has been made in the reduction of extreme poverty, but all other factors have increased tremendously since the publication of Karinthy (6). The global population currently includes 8.0 billion people (November 2022), of which more than half live in urban areas (figure 1) (7-9).

In 1962, the Canadian philosopher Marshall McLuhan introduced the term 'global village' (10). His interpretation of 'global village' mostly concerned global media and communication. However, looking at other developments such as urbanization, transportation and travel, the world has become an interconnected global village in many other aspects. This global interconnectedness, and in particular travel – of humans, animals, and goods-, has large effects on the epidemiology and spread of infectious diseases.

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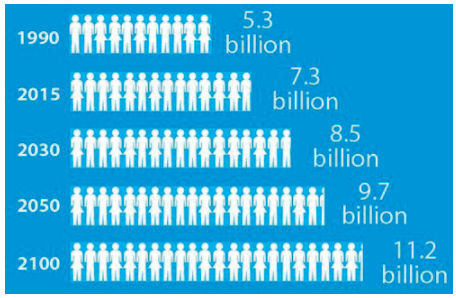

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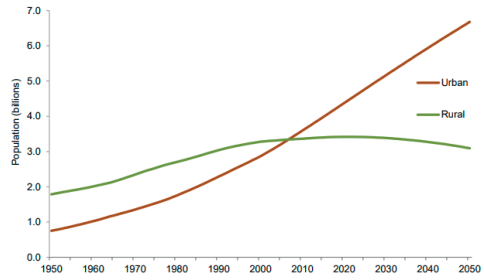


Figure 1 a. World population prospects until 2100 (estimation of 2015). b. Urban and rural population prospects until 2050 (estimations of 2018).
Source: from *World Population Prospects: The 2015 Revision* by the United Nations Department of Economic and Social Affairs, © (2015) United Nations, and from *World urbanization prospects 2018 highlights*, by the United Nations Department of Economic and Social Affairs, © (2019) United Nations. Reprinted with the permission of the United Nations.

1.2 International travel

People travel for different reasons, such as holiday, business, migration (e.g., work, family reunification) or to flee (e.g., for war, other safety reasons). All categories of travelers have increased tremendously in recent decades.

The numbers of international tourist arrivals have increased from 25 million in 1950, to 1.46 billion in 2019 (figure 2) (11). The number of migrants in 2017 was estimated at 258 million globally in 2017, an increase of 11% compared to 2013 (12). The UN Refugee Agency estimated that more than 84 million people were forcibly displaced worldwide in 2021. Of these, 20.8 million persons were regarded to be refugees, an increase of 20% since 2016 (13).

Irrespective of the reason for travel, travelers can carry pathogens –such as bacteria, viruses, fungi, and helminths- to other areas in the world. Pathogens can cause infectious diseases; illnesses which can be transmitted to a susceptible host by infected humans, animals or by contaminated objects. Travelers can contract (new) pathogens in the destination area, that they have not previously been exposed to or vaccinated against (14-16). Travelers can also carry (new) pathogens back home (17). Introduction of new pathogens in susceptible populations under certain circumstances such as bad hygiene or crowding can be conducive for infectious disease outbreaks (18-20).

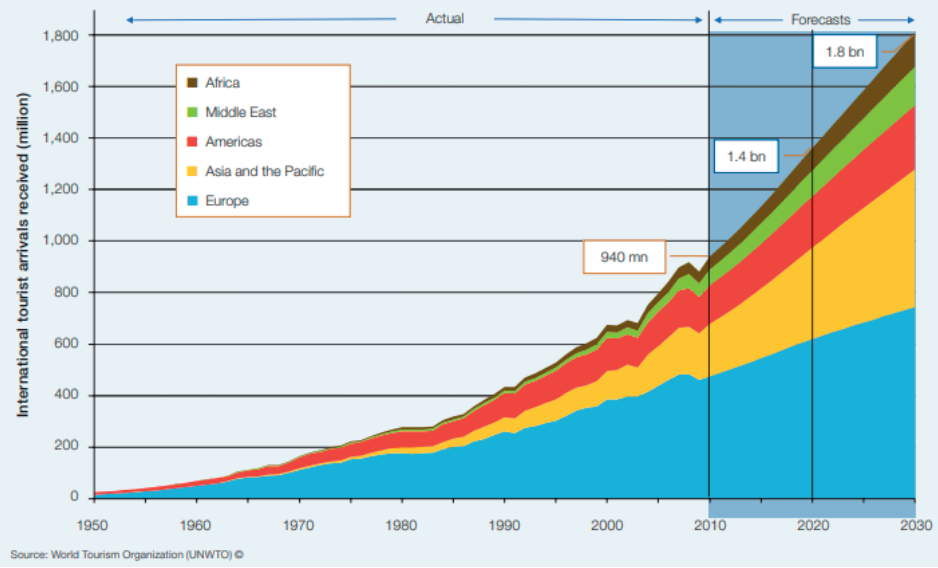


Figure 2 Trends and forecast of international tourist travelers until 2030 (estimations of 2010).
Source: World Tourism Organization (2017). UNWTO Tourism Highlights: 2017 Edition, UNWTO, Madrid, p. 14, DOI: https://doi.org/10.18111/9789284419029. © UNWTO

2. HISTORY OF TRAVEL-RELATED INFECTIONS

2.1 International travel-related outbreaks in the past

Travelers already contributed to the spread of pathogens and vectors when the global village was still in its infancy, often alongside existing and newly developed trade routes (21). It was centuries before mass (air) travel existed, that large worldwide outbreaks of influenza, smallpox and cholera were already described. Most notorious were the pandemics of the plague (caused by *Yersinia pestis* bacteria) -also known as the black death- in 1347-1353 (22). In an effort to protect the city of Venice, Italy, in the 14th century against plague outbreaks, ships had to drop their anchor for 'quaranta giorni' –40 days in Italian-, before they were allowed to unload. Columbus likely contributed to the introduction of infectious pathogens in the Americas in the 15th century such as smallpox, measles, bubonic plague, and yellow fever (table 1), killing thousands of Native Americans (23, 24). Before Coronavirus Disease 2019 (COVID-19, table 1), the Spanish flu in 1918-1920 was the most notorious recent worldwide pandemic with a very high proportion of deaths among diagnosed people (i.e. the case fatality rate) of 2-3% and an estimated 50 million deaths (25). The exact origin of the Spanish flu –a H1N1 influenza – still remains unknown (26). It is generally assumed that this pandemic has had three waves; in spring, autumn and winter 2018, and that World War I contributed to the high death toll by spreading the disease by migrating armies, but also by the hygiene and sanitation circumstances worsened by the war (26).

With a growing population and circumstances in which (long distance) traveling becomes increasingly feasible, outbreaks of infectious diseases continue to occur in the progressively globalized world. A more recent example of a widespread outbreak is the Severe Acute Respiratory Syndrome (SARS, table 1) epidemic in 2003, with eventually 8098 reported cases globally and a case fatality rate of 9.6% (27, 28). The first cases of SARS were traced back to November 2002 in the Guangdong Province of China, but the first report of an outbreak with 305 cases from the Chinese Ministry of Health to the WHO was not before February 2003 (27). Subsequently, other countries reported SARS outbreaks; amongst others, Canada reported 375 cases and 43 deaths after a viremic traveler returned home from Hong Kong and introduced the disease in Toronto (29). Two months of close international collaboration of 13 laboratories resulted in the identification of the new SARS coronavirus on 16 April 2003 (30). As the WHO virologist Dr Klaus Stöhr spoke in 2003 *"In this globalized world, such collaboration is the only way forward in tackling emerging disease. (30)".*

Goods, products, animals, and vectors

Not just people can carry (new) pathogens to other areas in the world, but also goods and products. The world merchandise trade volume (of major products) increased between 1950 and 2009 with an average of 6 percent per year; a development which contributes to the risk of spread of unwanted vectors and pathogens (31, 32).

Furthermore, animals can transfer pathogens across the world. Both the transportation of livestock for trade purposes -with more than 1.6 billion live animals across and outside the EU in 2019- as well as the growing livestock population in the EU to 146 million pigs, 76 million bovine animals and 75 million sheep and goats in 2020, pose risks (33-35). For example, during an unprecedented Q fever outbreak in the Netherlands in 2007-2009, in which 3523 human cases and many cases among goats were identified (36, 37). Potential introductions of pathogens by migrating birds are also well known, and surveillance systems for avian influenza (table 1) are currently in place worldwide (38, 39). The first West Nile Virus (table 1) outbreak in the Americans in New York City in 1999, which was preceded by an epizootic among birds with a high case fatality rate, was likely associated with migrating birds (40).

Insects such as ticks and mosquitoes should not be neglected as carriers of pathogens either, especially now global climate change including a rise in temperature favors multiplication and survival of certain mosquitos. The introduction of invasive mosquitoes to new areas is often associated with the transportation of used tires or importation of lucky bamboo. Along its trade routes, the spread of these vectors cause an increase of

the areas where outbreaks of infectious diseases such as dengue, Zika and chikungunya can occur (41-43).

2.2 International response to (emerging) infectious diseases: from past to present

The first steps towards an international approach to control infectious disease took place in 1851, when the first International Sanitary Conference was held after several cholera (table 1) epidemics hit European regions between 1830 and 1847 (44, 45). The aim of the conference was to reach international agreement toward standardization of quarantine to prevent import of cholera, plague and yellow fever (45). In 1951, the WHO member states adopted the International Sanitary Regulations, which were replaced by the International Health Regulations (IHR) in 1969 (44). The latter regulation intended to monitor and control three notifiable diseases: cholera, plague, and yellow fever.

The SARS epidemic in 2003 caused an urgent call to action. Because of the increasing threat of pandemic outbreaks, the IHR were regarded too restricted, and in 2005 the IHR were revised. The adapted aim was to prevent, control and respond to the international spread of (infectious) diseases, whilst unnecessary interference with global traffic and trade are to be avoided. State parties are now obliged to notify all events which could constitute a public health emergency of international concern (PHEIC) to the WHO. In case a PHEIC is declared, temporary recommendations can be advised and include, amongst others, implementation of source and contact tracing, implementation of quarantine of suspected and isolation of affected persons, and mandatory vaccination or prophylaxis (46).

As foreseen, new large-scale outbreaks occurred, such as the introduction of chikungunya virus in the Americas in 2013, a pathogen which previously circulated in Africa and Asia only (47). This outbreak however did not meet the criteria for a PHEIC as the case fatality rate of chikungunya was very low, in stark contrast to the SARS outbreak in 2003, for example. Since the introduction of the revised IHR2005, the WHO declared seven outbreaks PHEICs: the swine flu (H1N1) pandemic (2009), the resurgence of wild poliovirus in areas in central Asia, Middle East and Central Africa (2014), the Ebola virus outbreak in west Africa (2014), the Zika virus outbreak in the Americas (2016), the Ebola virus outbreak in the Democratic Republic of Congo (2019), the COVID-19 pandemic (2020) and the global Mpox-outbreak (2022) (48, 49).

2.3 Achievements in the global prevention and control of infectious diseases

Besides the intensified internationally organized outbreak responses as described in the IHR, internationally coordinated efforts to prevent outbreaks from either new or endemic pathogens have become increasingly important. Potential effectivity of programs depends on the reservoir of the pathogens, the mode of transmission (e.g. airborne, droplet, direct contact, vehicle or vector-borne transmission) and the availability of preventive measures including vaccines and medication (50, 51).

Safe sanitation, for example, contributes to a reduction of diarrhea, soil-transmitted diseases and vector proliferation, and large improvements have been made towards 'clean water and proper sanitation', one of the seventeen UN Sustainable Development Goals in the past century (52, 53). Where the three leading causes of death in 1900 in the USA consisted of infectious diseases: pneumonia, tuberculosis and diarrhea/enteritis; in 1997 the top 3 was replaced by non-communicable diseases: heart disease, cancer and stroke (54). Infectious diseases remain a threat though: in 2020, the COVID-19 pandemic instantly replaced the third rank (55).

The availability of effective vaccines and medication also contribute to population-based prevention and control of infectious diseases. Examples of very successful internationally organized control programs are the Expanded Programme on Immunization (EPI, since 1974), and the Global Polio Eradication Initiative (GPEI, since 1988), which set as respective goals to make vaccines against diphtheria, pertussis, tetanus, poliomyelitis, measles and tuberculosis available to every child globally by 1990, and to eliminate polio (table 1) worldwide (56-58). The most successful vaccination program so far has probably been the smallpox (table 1) eradication program, as the World Health Assembly declared smallpox as fully eradicated in 1980 (59).

Internationally organized mass drug administration campaigns, utilized in combating the 'neglected tropical diseases' lymphatic filariasis, onchocerciasis, dracunculiasis, schistosomiasis and trachoma, are an example of campaigns that use medication to effectively control and reduce infectious diseases. Neglected Tropical Diseases' (NTD) cover 20 infectious diseases that prevail in tropical and subtropical areas and thrive in areas with limited or no access to water, sanitation and hygiene (WASH), and include -amongst others– the more commonly known diseases dengue, chikungunya, filariasis and schistosomiasis. In 2019 -for the fourth consecutive year- more than a billion people in Africa, Asia and Latin America were treated as a result of international efforts of the WHO and partners, and donation of medication by several pharmaceutical companies (60-62).

2.4 Global control of infectious diseases in the context of present travel

Population-based control measures for infectious diseases in low-income countries are intended for local populations, but travelers from high- and middle-income countries also benefit from reduced local infections risks (14, 63, 64). Despite these programs, the burden of disease for local populations are generally still much higher than for travelers to these countries. This is explained by disparities in exposure to pathogens, living and hygienic conditions and access to health care. However, with ever expanding volumes of travel in the global village, the risks for travelers of contracting and subsequently spreading of infectious diseases remains considerable (65). Therefore, in the past two decades, the scale, frequency and speed of spread of emerging diseases has increased substantially (66). Recently, the multiple local outbreaks of dengue in France, Spain and Italy -which likely started with returning viremic travelers- have shown the potential consequences for the general population when new pathogens are introduced (67, 68). Also, the risk for worldwide spread of Ebola became reality during the 2014-2016 outbreak in urban Western Africa (69). The Ebola outbreak, with eventually 28,600 infected patients and 11,325 deaths, made clear how easily Ebola can spread and accelerate after introduction in urban areas (70). The extensive travel between affected and unaffected areas, including travelers who attended funerals of deceased relatives with traditional burial methods and unprotected touching, probably contributed considerably to transmission and spread, which eventually occurred to seven other countries outside the outbreak areas (69, 71).

Prevention and control measures to prevent spread and large outbreaks after introduction of a new pathogen, can include a wide range of measures such as isolation and quarantine, use of facemasks, social distancing, curfew, (development of) medications and vaccines. Internationally developed and aligned preparedness and prevention guidelines would be beneficial for populations worldwide (72): uniformity of guidelines increase the compliance with preventive measures. Including specific measures for visiting and departing travelers in these guidelines would further contribute to the prevention of spread of disease across countries.

Table 1 Characteristics of well-known examples of infectious diseases causing high disease burden throughout the global village in the ancient and recent history.

Source		*Transmission*	*Host*	*Public health impact*	
Disease	**Causal agent**	**Main route of infection**	**General symptoms**	**Estimated case fatality rate**	**Treatment or vaccine available in case of an outbreak?**
Avian influenza (bird flu) (73)	Avian influenza type A viruses	Direct contact with infected birds	Mild to severe flu like symptoms	Unknown (depending on variant)	In case of in infection, and prophylactic antiviral treatment available
Cholera (74)	Vibrio cholerae (bacteria)	Contaminated water and food	Watery diarrhea and vomiting	2% (to 40% in case of limited access to health care)	In case of infection antibiotics, and prophylactic vaccine available
COVID-19 (75, 76)	SARS-coronavirus-2	Respiratory droplets	Fever, cough, dyspnea, dysgeusia, fatigue	Depending on variant, health, and access to health care. ~0.7-11%	Prophylactic vaccine available
Ebola (77, 78)	Ebolavirus	Direct contact with blood or body fluids	Malaise with fever followed by unexplained hemorrhaging	20-90%	Prophylactic vaccine against species Zaire ebolovirus available
(Seasonal) Influenza (79, 80)	Influenzavirus	Respiratory droplets	Flu like symptoms	Depending on variant: 0.53-3.1% Spanish flu:2-3%	(Annual seasonal) prophylactic vaccine available
Measles (81, 82)	Morbillivirus	Airborne spread and respiratory droplets	Malaise including fever and 'koplik spots' followed by rash	2.2% (worldwide), <0.01% (The Netherlands)	Prophylactic vaccine available
Plague (83, 84)	Yersinia pestis (bacteria)	Bite of rodent flea or respiratory droplets (pneumonic plague)	Malaise with bubonic (swollen lymph nodes), septicemic or pneumonic symptoms	30-60% (bubonic type)	In case of infection antibiotics available
Polio – WPV* 2 & 3: eradicated (85-87)	Wild polio and circulating vaccine-derived poliovirus type 1,2,3	Contaminated water or food, and respiratory droplets	Flu like symptoms, with(out) neurological symptoms	0.25-0.5%	Prophylactic vaccine available

Table 1 Characteristics of well-known examples of infectious diseases causing high disease burden throughout the global village in the ancient and recent history. (*continued*)

Source		*Transmission*	*Host*	*Public health impact*	
Disease	**Causal agent**	**Main route of infection**	**General symptoms**	**Estimated case fatality rate**	**Treatment or vaccine available in case of an outbreak?**
SARS[#] (27, 88)	SARS-coronavirus	Respiratory droplets	Malaise with high fever and pneumonia	9.6%	Potential treatment available in case of infection
Smallpox – eradicated (89-91)	Variolavirus	Respiratory droplets and sores	Malaise including fever followed by pustular rash	Variola major: 1%, variola minor 30%	Treatment in case of infection, and prophylactic vaccine available
West-Nile virus (92)	West-Nile virus	Bite of mosquito	Malaise with(out) neurological symptoms	<1-50% in case of severe disease (severe disease occurs in 1%)	no
Yellow fever (93)	Yellow fever virus	Bite of mosquito	Malaise including fever, with(out) organ failure, shock, bleeding	20-57% in case of symptomatic illness	Vaccine available

[*] WPV: Wild *polio* virus

[#] SARS: Severe Acute Respiratory Syndrome

3. TRAVEL MEDICINE

The practice and organization of travel medicine and tropical medicine differs between countries. Most commonly, pre-travel medicine -'travel medicine'- focuses on the primary prevention (prevention of disease before it occurs) and management of travel-related health problems during travel, while tropical medicine focuses on diagnostics and treatment of ill returned travelers from subtropical and tropical countries (94). Both travel and tropical medicine require up-to-date knowledge of travel-related diseases and its global epidemiology. An individual traveler who wants to prepare for travel to tropical or subtropical areas can consult a 'travel medicine clinic' or 'travel health clinic' for travel health advice.

3.1 Tailoring of travel health advice

A pre-travel health consultation can include advice about hygiene measures, use of insect repellents, (international mandatory) vaccinations, anti-malaria prophylaxis, antibiotic use for standby self-treatment and/or prophylactic use against altitude illness (94). Travel medicine recommendations –such as the American 'Yellow book' guidelines from the Centers of Disease Control and Prevention (CDC) or the Dutch guidelines from the National Coordination Centre for Travelers Health Advice (Landelijk Coördinatie centrum Reizigersadvisering, LCR)- are generic recommendations that have to be tailored to each individual traveler (95, 96). Tailoring is the expertise of the travel health specialist, taking travelers' behavior and underlying conditions, characteristics of itinerary, and experience and capabilities into account (97-123). Tailored advice aims to mitigate individual risks, to enhance adherence and it considers costs versus risk of infection. It also aims to prevent overtreatment such as administration of vaccinations that are not indicated or prescribing stand-by antibiotics for traveler's diarrhea to healthy travelers (124-126). Preventing such overtreatment reduces unnecessary side-effects, or the risk of importation and spread of antibiotic resistant bacterial strains (127, 128). The latter poses a public health risk, and is particularly of concern when introduced in hospitals.

Development of evidence based pre-travel health guidelines usually rely on epidemiological information from local settings at destination countries (129-132), ill returned travelers visiting tropical medicine clinics (including sentinel studies), and retrospective research among (ill) travelers (114, 117, 120, 133-138). These sources offer valuable information especially about the incidence of local cases, severity of illness of local patients, and ill returned travelers who sought healthcare. However, information about the actual infection risk is lacking, because denominators (i.e. the number of people at risk during travel) are missing, as well as data on travelers with mild or asymptomatic disease who did not seek healthcare. Therefore, estimates of true infection risk is often

not available. Especially in case the majority of cases experience a self-limiting infectious disease, prospective studies following travelers closely from pre-travel onwards until after their return enables us to obtain knowledge of the actual infection risk during travel and in general the epidemiology of infections in travelers, including determinants for infection and trends in risk over time. Findings of prospective research are essential for tailoring individual travel-health advice and to minimize individual risks. Professor Steffen introduced and regularly updates an overview of risks of travel vaccine preventable disease relative to each other, providing insightful risks for travelers (figure 3) (139). In addition to 'sentinel' studies that report on travelers who seek health care, which give insight into the occurrence of symptomatic infectious disease, these prospective studies also provide important data on the occurrence and spread of asymptomatic or mild infectious diseases and the risk of infection in the global village (140).

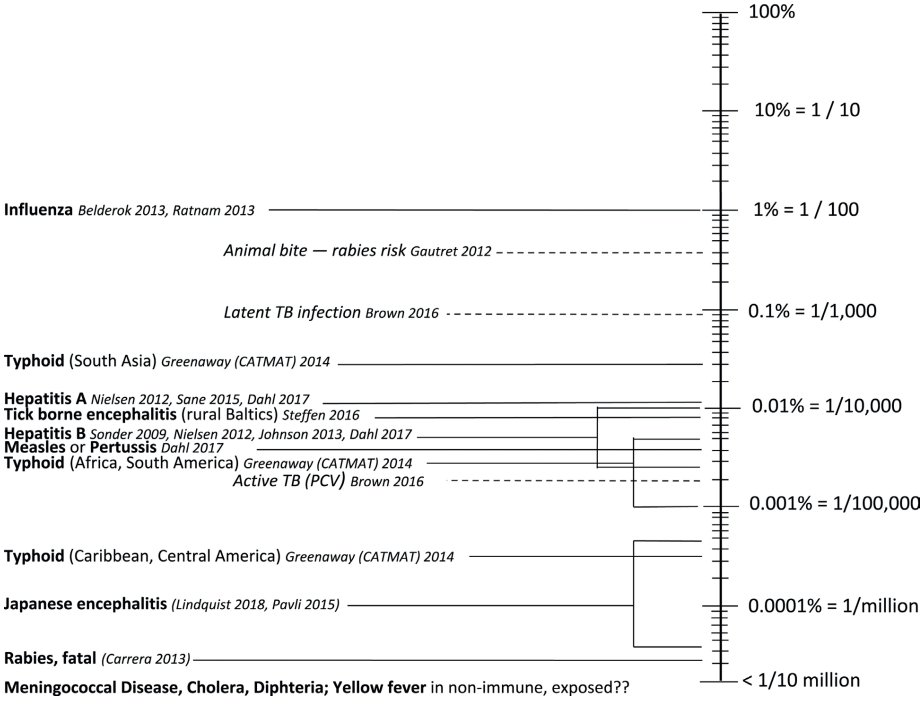


Figure 3 Professor Steffen's logarithmic scale of vaccine preventable disease travel health risks of travelers from high-income countries: estimated incidence per month in lower income countries among non-immunes (update 2018) (139). Source: R Steffen, *Travel vaccine preventable diseases – updated logarithmic scale with monthly incidence rates*, Journal of travel Medicine:25(1) 2018, by permission of Oxford University Press.

Groups of travelers

Travelers can be classified on certain characteristics that are related to specific risks, as described by UN classification as well as American Centers for Disease Control and Prevention (CDC) Yellow book (figure 4) (95, 141).

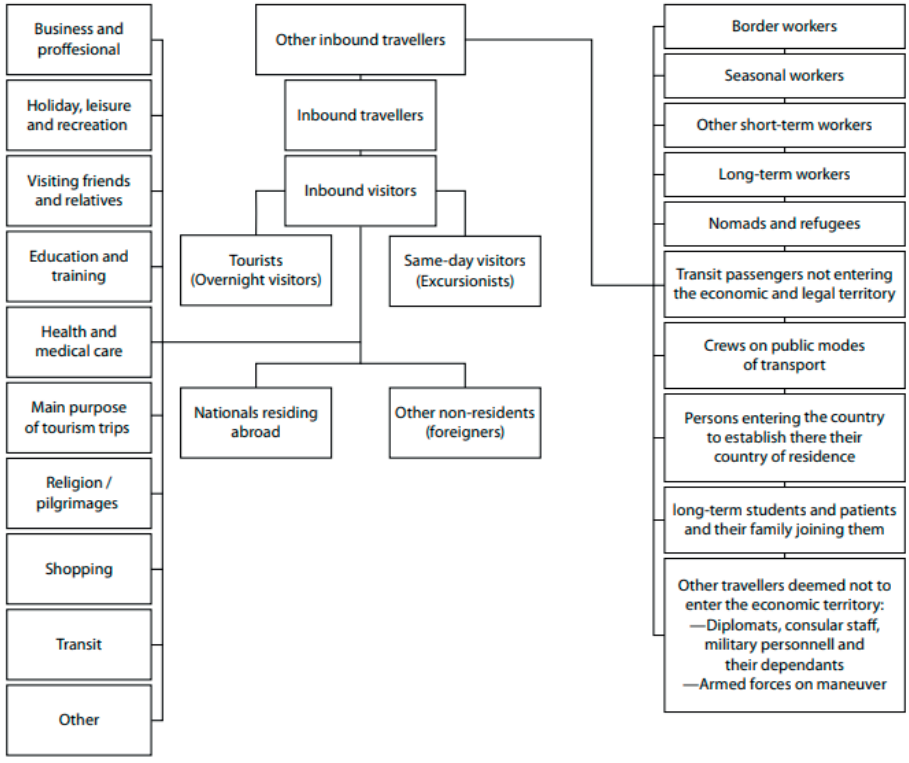


Figure 4 Classification of travelers by the United Nations.
Source: from *International Recommendations for Tourism Statistics 2008* by the United Nations Department of Economic and Social Affairs, © (2010) United Nations. Reprinted with the permission of the United Nations.

Most travelers visiting travel health clinics belong to one of the following four groups: business and study travelers, tourists, visiting friends and relatives (VFR), and long-term travelers (11, 142). Business-related travelers are often at less risk for infectious diseases compared to the other groups of travelers, because of their short travel duration, their residence in more luxury accommodations and less contact with the local population, though better adherence of malaria chemoprophylaxis in this group could be encouraged (114, 142). Travelers for leisure, 'the tourist travelers' are often short-term travelers, the majority traveling to well-known touristic areas, usually participating in local touristic activities (11, 143, 144). This thesis predominantly focuses on the last two groups: the VFR travelers and the long-term travelers.

The term 'VFR traveler' is usually used for migrants from lower-income countries who now live in higher-income countries and travel to their country of origin to visit friends and relatives. High-income countries generally inhabit specific groups of immigrants, often depending on historical (ex-colonies) or economic ties, making comparisons of VFR studies from different areas challenging. Also definitions of VFR travelers differ between studies and have changed over time, as travel patterns have changed in the interconnected world. Broader VFR definitions including all travelers whose primary purpose of travel is visiting friends or relatives, have been proposed in recent years (145). Nevertheless, previous studies as to VFR travelers found similarities in travel-related risk factors in different VFR groups, such as high frequencies of travel to countries of origin and longer travel duration, but also lower uptake of pre-travel advice, malarial chemoprophylaxis or adherence to other preventive measures compared to tourist travelers (146-149).

The risk of acquiring infections among long-term travelers is generally higher than among short-term tourist travelers due to cumulative risks caused by longer travel duration, waning adherence to preventive measures such as use of anti-malarial chemoprophylaxis, higher number of casual sexual contacts and engagement in non-travel related (medical) activities (e.g. dentist visits) (135, 150, 151). Also, long-term travelers are more likely to have chronic diarrhea, giardiasis, malaria (all species), fatigue >1 month, eosinophilia, cutaneous leishmaniasis, and schistosomiasis compared to short-term (<1 month) travelers (135). However, not all studies found that long-term travelers are at increased risk. For example, Wieten et al (2015) demonstrated that long-term traveling was not associated with animal associated incidents that potentially require post-exposition treatment due to risk of rabies (152). Also (first) episodes of travel diarrhea among travelers are often experienced in the first weeks or months of travel, and less often thereafter (153-155).

3.2 Travel medicine in the Netherlands

In the Netherlands, the LCR, established in 1996, develops guidelines for pre-travel medicine (96). To improve the uptake and compliance of travel health advice, the LCR has a two-fold aim: to promote uniformity in travelers' health advice and to improve the quality of pre-travel clinics. In the Netherlands, pre-travel health advice is provided by travel clinics of Public Health Services (PHS) and private travel clinics. In addition, a number of general practitioners, occupational health services and hospitals provide pre-travel advice, especially hospitals with tropical diseases departments (96). LCR publishes quality criteria, and national guidelines for travel medicine specialists (156). Updates of guidelines must be approved by the LCR consensus group before distribution. This group comprises experts representing different fields within travel health (e.g., public health medicine, occupational health, academic medical centers with tropical

disease departments, Ministry of External Affairs) (96). Updates of guidelines incorporate most recent information available from the WHO (including *International Travel and Health,* pre-COVID-19 published annually), the CDC (including the biennial published *Yellow Book),* and new insights from scientific literature (95, 157). Also, notification data of (imported) infectious diseases contribute to up-to-date information, including evaluation and monitoring of notifiable diseases among travelers (14, 63, 64, 158-160).

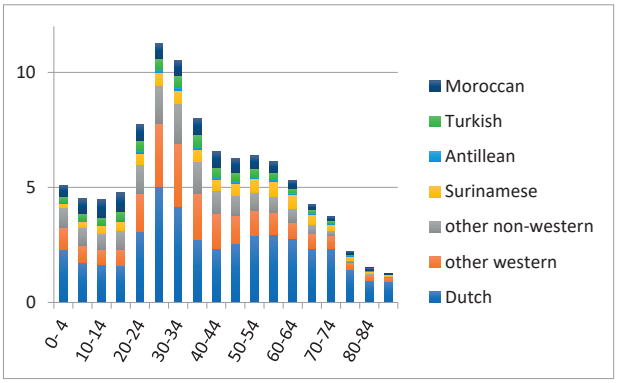
Where travel health consultations aim to improve the health of individual travelers who seek voluntary advice, IHR international regulations aim to prevent and control the spread of infectious diseases for public health reasons. In the IHR context, immunizations or other public health measures can be made mandatory (157). Some measures serve both individual and public health purposes. LCR Members -such as travel health services- receive rapid alerts. Rapid alert reports comprise urgent individual recommendations for travelers in response to emerging health risks as well as newly published IHR mandatory recommendations that urge prompt and uniform implementation. In the global village, quick implementation of IHR recommendations is increasingly important to comply with the international public health response and to control the spread of travel-related infectious infections.

3.3 Travelers from Amsterdam

Amsterdam, the capital of The Netherlands, has a population of 872,497 people (2021), and is an ethnically diverse (56% non-Dutch ethnic origin in 2021) city with inhabitants with a migration background from 208 different countries (161). With Schiphol Airport nearby – the third largest airport in Europe, handling nearly 72 million passengers annually before the COVID-19 pandemic, Amsterdam welcomes travelers from all over the world: in 2019 22.4 million tourists visited the capital (162-164). First generation migrants (residents born abroad) and second generation migrants (residents with one or both parents born abroad) from Suriname, Morocco and Turkey form the largest ethnic groups (7, 14 and 5% of total population, 2021) (figure 5) (161). A population-based cohort (HELIUS) study among the largest ethnic groups living in Amsterdam revealed that of those with a migration background and who traveled in the previous year (28.7-51.4%), 57.4 to 94.8% undertook a VFR-trip. Most of these VFR travelers (69.6-96.4%) were first generation migrants. Uptake of pre-travel health advice varied from <6% (Turkish and Moroccan VFRs) to 59% (Ghanaian VFRs) (manuscript in progress). The infection risks of travelers visiting friends and relatives in Suriname, and of those of tourist travelers to Suriname, have been studied in this thesis (**chapter 6-7**).

Another group of travelers from Amsterdam studied in this thesis are long-term travelers to tropical and subtropical countries who sought pre-travel health advice at the PHS of

Amsterdam (**chapter 2-5**). The majority of these long-term travelers were young adults. Like in other university-oriented cities, young and higher educated adults represent a relatively large group of the population of Amsterdam: 30% of Amsterdam' population belongs to the 20-34 year age group (161). Not yet being attached to permanent jobs, children, or a mortgage, many of these young adults take advantage of this opportunity to take a 'gap year' and go on a long-term trip abroad (165, 166).



F**igure 5** Demographics of Amsterdam (2021): percentage of total population of Amsterdam by age group and ethnicity (161).

Travel Health Clinic of the Public Health Service of Amsterdam

In the city center of Amsterdam, the PHS of Amsterdam houses a Travelers's advice and vaccination clinic. The participants for the studies included in this thesis were recruited among its clients. In 2018, 32,500 persons attended this clinic for tailored pre-travel health advice and/or vaccinations by specialized nurses and physicians (167). The top 10 destinations among travelers visiting the PHS clinic in Amsterdam in 2018 were Indonesia, Thailand, Vietnam, India, Colombia, Sri Lanka, Ghana, Suriname, South Africa and Brazil (167). As The PHS of Amsterdam also houses a public health laboratory and research department, it offers a suitable setting for travel-related studies.

4. AIM AND OUTLINE OF THIS THESIS

4.1 Aim

In the global village, individual travel medicine and public health has become more and more intermingled. An integrated approach of (international) obligatory public health measures as required by the IHR and voluntary individual-based measures are becoming increasingly important. This thesis focuses mainly on individual risks for travelers from the Netherlands. The overall aim of this thesis is to increase knowledge

of the epidemiology of travel-related infectious diseases among individual travelers in the context of a fast-changing, interconnected world. This knowledge provides input for evidence-based guidelines for primary and secondary (identification of disease in an early stage) preventive strategies for travelers, particularly for travelers at increased risk. It will contribute to the knowledge of potential diagnoses in ill returned travelers, and provides an evidence-based baseline which is necessary for public health policy.

4.2 Study population and outcomes

Two important groups of travelers in Amsterdam were studied: a cohort of long-term travelers of Dutch origin to tropical and subtropical areas and a cohort of tourist and VFR travelers to Suriname (table 2). We also studied two imported cases of Lassa fever, an infectious disease known for a high case fatality rate and therefore belonging to the notifiable diseases group A -the class with the most urgent pace of notification in which public health measures are taken under the command of the Dutch Minister of Health, Welfare and Sport-.

In our studies, we focused on a selection of infectious diseases that are currently not or low endemic in the temperate climate of the Netherlands and for most a vaccine or treatment is lacking. These include the arthropod-borne viral diseases dengue, Zika and chikungunya, the rodent-borne viral disease Lassa fever, fecal-orally transmitted hepatitis E, and the helminth infections schistosomiasis, filariasis, strongyloidiasis and toxocariasis (table 3).

4.3 Outline

This thesis is divided into two parts. Part one focuses on studies estimating the risk of infection during long-term travel (part 1a) and travel to Suriname (part 1b). Part two describes cases of imported Lassa infection, including the assessment and management of the risks and the public health control measures taken upon the diagnosis after travel to the Netherlands -a non-endemic country for Lassa virus-.

Part 1a: travel-acquired infections among long-term travelers to (sub)tropical areas

Here, we focus on the prevalence, attack rate, incidence, and determinants of specific travel-related diseases among a prospective cohort of long-term travelers, predominantly born in high income countries. These immune competent participants traveled from the Netherlands to tropical and subtropical areas between 2008 to 2011. The studies were part of a large cohort study on travel-related diseases among long-term travelers (Supplementary table 1).

In **chapter 2**, we focused on four helminth infections -schistosomiasis, strongyloidiasis, filariasis and toxocariasis- among this cohort of long-term travelers. We estimated the pre-travel prevalence, attack and incidence rate and aimed to identify determinants for these travel-acquired infections. We also measured the symptoms reported by participants who contracted an infection during travel, assessed reported use of anti-helminth medication and evaluated the predictive value of post-travel eosinophilia.

In **chapter 3**, we assessed the pre-travel prevalence, attack- and incidence rate of travel-acquired hepatitis E among our cohort of long-term travelers and examined determinants for infection.

In **chapter 4** we assessed the pre-travel prevalence, attack- and incidence rate of travel-acquired chikungunya infections in our cohort of long-term travelers -including the travelers to Latin America-, before introduction of the virus in the Americas was first reported.

In **chapter 5**, we estimated the pre-travel prevalence, attack- and incidence rate of travel-acquired dengue virus infection and studied determinants - including potential cross-reaction between (vaccination-induced) flavivirus antibodies - associated with infection. We also measured dengue-related symptoms reported by all participants during travel and reported health seeking behavior among participants with a travel-acquire DENV infection.

Part 1b: travel-acquired infections among VFR and tourist travelers to Suriname

In this part, we focused on the prevalence, attack- and incidence rate and determinants of dengue during travel, Zika and chikungunya virus infections among (VFR) travelers to Suriname, using data from a cohort of first-generation migrants from Suriname, and data from a prospective cohort of travelers to Suriname. All participants were recruited at the travel health clinic of the PHS of Amsterdam.

In **chapter 6**, we assessed the seroprevalence of previous dengue virus infections among a cohort of first-generation Surinamese migrants living in the Netherlands, using stored serum from participants who had previously been tested for immunity against hepatitis A or hepatitis B.

In **chapter 7**, we estimated the pre-travel prevalence, attack rate and incidence of travel-acquired dengue virus infections, among Dutch tourist and VFR travelers to Suriname and identified determinants for travel-acquired infections. As chikungunya and Zika virus were introduced in Suriname during the study period, pre-travel prevalence, attack rate

and incidence of travel-acquired infections with these two viruses were also estimated. To gain more insight in potential cross-reacting antibodies against other flavivirus and used DENV or ZIKV ELISA tests, a selection of samples of serological confirmed travel-acquired dengue and Zika virus infection were retested using virus neutralization tests.

Part 2: Public health measures to prevent spread of imported hemorrhagic fever
This part presents two cases of a travel-related illness (Lassa fever) in order to prevent introduction or spread of the disease in the Netherlands.

In **chapter 8**, we described two imported cases of Lassa virus infection contracted in Sierra Leone in two Dutch health care workers, and the public health measures taken in the Netherlands upon their diagnosis to prevent further transmission.

In **chapter 9**, the main findings of the studies are summarized and discussed and related to the current epidemiology in the areas studied and the most recent literature. Furthermore, we provide recommendations for preventing acquisition and transmission of travel-related diseases for both travel medicine and public health guidelines and for future research.

An overview of the data sources used in this thesis is presented in table 2, an overview of disease characteristics of the travel-related infections studied is provided in table 3.

Table 2 Overview of data sources used in this thesis.

Data source	Study design/type	Study population	Period of data collection	Chapter
Long-term travelers' study*	Prospective cohort study	Adults born in high income countries, and traveling to tropical or subtropical areas for 3 to 12 months (n=600-604).	2008-2011	2-5
Serumbank study*	Cross-sectional study	First generation migrants from Suriname (n=400)	2008-2011	6
Travelers to Suriname study*	Prospective cohort study	Adult VFR$ travelers born in Suriname and adult tourist travelers (born in the Netherlands) to Suriname (n=481)	2014-2017	7
RIVM#	Case report	Imported cases of Lassa fever in the Netherlands in Dutch healthcare workers rom Sierra Leone (n=2)	2019^	8

* participants recruited at the travel health clinic of the Public Health Service of Amsterdam.
$ VFR: Visiting friends and relatives
# RIVM: National Institute for Public Health and the Environment (Rijksinstituut voor Volksgezondheid en Milieu)
^ notification date: 20 November 2019

Table 3 Overview of characteristics of the travel-related infectious diseases studied in this thesis.

		Source			*Transmission*	
	Infectious agent	**Causal agent**		**Endemic regions**	**Reservoir or vector**	**Route of infection**
Helminth infections	Schistosomiasis (168-170)	Intestinal	*S. mansoni* *S. japonicum* *S. mekongi* *S. guineensis* *S. intercalatum*	Africa, Middle East, parts of South America, parts of Asia, Corsica (France)	Aquatic snails	Contact with contaminated fresh water sources
		Urogenital	*S. haematobium*			
	Strongyloidiasis (170-172)		*S. stercoralis* *S. fülleborni*	mainly (sub) tropical regions, but also in temperate climates	Humans, dogs, monkeys	Contact with contaminated soil
	Filariasis (170, 173-176)	Lymphatic	*W. bancrofti,* *B. malayi,* *B. timori*	Mainly regions in Africa and Asia	Mosquitoes: ao *Culex, Anopheles, Aedes spp*	Bites of vector
		Onchocerciasis	*O. volvulus*		Blackflies: *Simulium* spp	
		Loiasis	*Loa loa*		Deerflies: *Chrysops*	
		Mansonellosis	*M. perstans,* *M. ozzardi,* *M. strepocerca*		Midges: *Culicoides* spp. Blackflies: *Simulium* spp	
	Toxocariasis (170, 177, 178)		*T.canis* *T.cati*	Worldwide	Dogs Cats	Ingestion of eggs on soil/plants contaminated by dog/cat feces

	Host			*Public health*	
	Port of entry	**Incubation period**	**General symptoms**	**Estimated numbers infected/ at risk worldwide**	**Treatment or vaccine available?**
	Skin	2-6 weeks	Abdominal pain, diarrhea, bloody stool, hepato-/ splenomegaly	241.3 million at risk	Treatment in case of infection available (praziquantel)
			Hematuria, fibrosis of bladder/ureter, kidney damage		
	Skin	14-30 days	Abdominal pain, intermittent/ persistent diarrhea, respiratory systems, pruritis, urticaria	613.9 million infected	Treatment in case of infection available (ivermectin or albendazole)
	Skin	5–18 months (range 1 month-2 years)	Lymphoedema, elephantiasis, hydrocele	863 million at risk	Treatment in case of infection available (doxycycline + ivermectin)
			Severe itching, disfiguring skin conditions, (severe) visual impairments	220 million at risk	Treatment in case of infection available (doxycycline + ivermectin)
			Itchy swellings, eye worm	> 29 million at risk	Treatment in case of infection available (diethylcarba-mazine [DEC]/ prednisone or albendazole)
			Ao angioedema, pruritis, fever, headache, neurological symptoms	114–580 million at risk in Africa (m. perstans)	Treatment in case of infection available (doxycycline)
	Gastro-intestinal	1 week-2 years	Systemic, abdominal, respiratory and (sometimes) dermatological symptoms	~1.41 billion infected	Treatment in case of infection available (albendazole)

Table 3 Overview of characteristics of the travel-related infectious diseases studied in this thesis. (*continued*)

		Source		*Transmission*	
	Infectious agent	**Causal agent**	**Endemic regions**	**Reservoir or vector**	**Route of infection**
Virus infections	Hepatitis E (179-181)	Genotype 1	Asia and Africa	Humans	Ingestion of fecal contaminated drinking water
		Genotype 2	Asia, Africa, previously in Mexico		
		Genotype 3	Europe, USA, New Zealand, Japan	Animals, including pigs	Ingestion of undercooked meat (particularly pork)
		Genotype 4	Southeast Asia		
	Chikungunya*[C] [§] (182, 183)		Americas, Africa, Asia	Mosquitoes: *A.Aegypti* *A.Albopictus*	Bites of vector
	Dengue*[C] [§] (184)	Serotypes 1-4	Americas, Africa, Asia	Mosquitoes: *A.Aegypti* *A.Albopictus*	Bites of vector
	Zika*[C] (185)		Americas, Africa, Asia	Mosquitoes: *A.Aegypti* *A.Albopictus*	Bites of vector, or sexually transmitted
	Lassa fever*[A] (186)		Parts of west Africa	Rodents (*M. natalensis*)	Direct contact or exposure to rodent excreta, or exposure to blood, tissue, secretions or excretions of an infected person

*[A/C] notifiable diseases in the Netherlands: the Dutch Public health act requires that Dutch physicians and head of laboratories notify a human case to the local Public Health Service if one of the determined pathogens is suspected (group A) or confirmed (group B-C). As described in the Dutch Public Health act, notifiable diseases are grouped according to the legal measures that may be impose (187). The Public Health Service notifies the case anonymous to the National Institute of Public Health and the Environment (RIVM). In case of an infectious disease which requires notification under the International Health Regulation (IHR), the RIVM -the appointed National Focal Point– will notify the case to World Health Organization within 24 hours.

§ only notifiable in the Caribbean Netherlands

	Host			*Public health*	
	Port of entry	**Incubation period**	**General symptoms**	**Estimated numbers infected/ at risk worldwide**	**Treatment or vaccine available?**
	Gastro-intestinal	5-6 weeks (range 2-10 weeks)	Fever, nausea, vomiting, abdominal pain, itch, rash jaundice, hepatomegaly	20 million infections per year	Vaccine (Hecolin®) available in limited number of countries
	Skin	3-7 days	Fever, arthralgia, myalgia, headache, rash	Unknown	No
	Skin	4-7 days	Fever, retro-orbital pain, arthralgia, myalgia, headache, rash, (severe) bleeding and/or shock	400 million infections, 3 billion at risk	Vaccine (Dengvaxia®, Qdenga®) available in limited number of countries
	Skin, or anogenital tract including oral mucosa	3-14 days	Fever, arthralgia, myalgia, conjunctivitis, headache, rash During pregnancy: fetal abnormalities (ao microcephaly)	3.6 billion at risk	No
	Respiratory inhalation of aerosolized excreta, or direct contact	2-21 days	Fever, malaise, myalgia, headache, gastro-intestinal and respiratory symptoms, shock, bleeding, hearing loss	Estimates range from 100,000-500,000 to 900,000 infections per year	No vaccine available, limited experience with potential effective treatment

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SUPPORTING INFORMATION

Supplementary table 1 Overview of the studies of the large cohort study on travel-related diseases among long-term travelers of the Travel Health Clinic, Public Health Service of Amsterdam, the Netherlands, 2008-2011.

Infectious diseases under study	Title, authors and journal
Sexual transmitted disease	*Unprotected casual sex equally common with local and Western partners among long-term Dutch travelers to (sub)tropical countries.* J Whelan, SM Belderok, Anneke vd Hoek, GJB Sonder. Sex Transm Dis. 2013. Oct;40(10):797-800. doi: 10.1097/OLQ.0000000000000013.
Tuberculosis	*Screening travellers to high-endemic countries for infection with Mycobacterium tuberculosis using interferon gamma release assay; a prospective study.* F Elfrink, A vd Hoek, ME Mensen, GJB Sonder. BMC Infect Dis. 2014 Sep 23;14:515. doi: 10.1186/1471-2334-14-515.
Travelers diarrhea	*Diarrhea among long-term travelers: high attack rates and highest incidence in the beginning of travel.* SM Belderok, A vd Hoek, J Whelan, GJB Sonder. Traveling hosts and pathogens – epidemiology of travel-related infections: Thesis SM Belderok (2014), chapter 5. https://hdl.handle.net/11245/1.432035
Influenza	*Influence in long-term Dutch travelers in the tropics: symptoms and infections.* J Whelan, GF Rimmelzwaan, A vd Hoek, SM Belderok GJB Sonder. BMC Infect Dis. 2016 Apr 16;16:158. doi: 10.1186/s12879-016-1502-6.
Dengue (**chapter 5**)	*Dengue virus infections among long-term travelers from the Netherlands: a prospective study, 2008-2011.* FW Overbosch, J Schinkel, IG Stolte, M Prins, GJB sonder. PLoS One. 2018 Feb 7;13(2):e0192193. doi: 10.1371/journal.pone.0192193. eCollection 2018.
Helminth infections (**chapter 2**)	*Low incidence of helminth infections (schistosomiasis, strongyloidiasis, filariasis, toxocariasis) among Dutch long-term travelers: a prospective study, 2008-2011.* FW Overbosch, T van Gool, A Matser, GJB Sonder. PLoS One. 2018 May 30;13(5):e0197770. doi: 10.1371/journal.pone.0197770. eCollection 2018.
Hepatitis E (**chapter 3**)	*Hepatitis E in long-term travelers from the Netherlands to subtropical and tropical countries, 2008-2011.* F Elfrink, FW Overbosch, J Schinkel, G Koen, GJB Sonder. Emerg Infect Dis. 2018 Jun;24(6):1055-1060. doi: 10.3201/eid2406.171513.
Chikungunya (**chapter 4**)	*No chikungunya virus infections among Dutch long-term travellers to (sub)tropical countries: a prospective study 2008-2011.* FW Overbosch, F Elfrink, J schinkel, GJB Sonder. BMC Infect Dis. 2019 Feb 26;19(1):196. doi: 10.1186/s12879-019-3819-4.
Malaria	*Malaria in long-term travelers: infections risks and adherence to preventive measures – a prospective cohort study.* FST Suryapranata, FW Overbosch, A Matser, MP Grobusch, MBB McCall, GGC van Rijckevorsel, M Prins, GJB Sonder. Travel Med Infect Dis. 2022 Sep-Oct;49:102406. doi: 10.1016/j.tmaid.2022.102406.

Part 1a

Travel-acquired infections among long-term travelers to (sub)tropical areas

2

Low incidence of helminth infections (schistosomiasis, strongyloidiasis, filariasis, toxocariasis) among Dutch long-term travelers, a prospective study, 2008-2011

Femke W Overbosch, Tom van Gool, Amy Matser, Gerard JB Sonder

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ABSTRACT

Background

Despite the considerable burden of helminth infections in developing countries and increasing international travel, little is known about the risks of infection for travelers.

Objective

We studied the attack and incidence rate of serology confirmed strongyloidiasis, filariasis, and toxocariasis among long-term travelers and associated factors. A second objective was to evaluate eosinophilia as a positive/negative predictive value (PPV/NPV) for a recent helminth infection.

Methods

From 2008 to 2011, clients of the Public Health Service travel clinic planning travel to (sub)tropical countries for 12-52 weeks were invited to participate in a prospective study. Participants kept a weekly diary, recording itinerary, symptoms, and physician visits during travel and completed a post-travel questionnaire. Pre- and post-travel blood samples were serologically tested for the presence of IgG antibodies against *Schistosoma* species*, Strongyloides stercoralis,* filarial species, and *Toxacara* species and were used for a blood cell count. Factors associated with recent infection were analyzed using Poisson regression. Differences among groups of travelers were studied using chi square tests.

Results

For the 604 participants, median age was 25 years (interquartile range [IQR]: 23-29), 36% were male, median travel duration was 20 weeks (IQR: 15-25), and travel purpose was predominantly tourism (62%). Destinations were Asia (45%), Africa (18%), and the Americas (37%).

Evidence of previous infection was found in 13/604 participants: antibodies against *Schistosoma* spp. in 5 (0.8%), against *S.stercoralis* in 3 (0.5%), against filarial species in 4 (0.7%), and against *Toxocara* spp. in 1 (0.2%). Ten recent infections were found in 9 participants (3,1, 6, 0 cases, in the above order), making the attack rates 0.61, 0.17, 1.1 and 0, and the incidence rates per 1000 person-months 1.5, 0.34, 2.6 and 0. The overall PPV and NPV of eosinophila for recent infection were 0 and 98%, respectively.

Conclusions

The risk of the helminth infections under study in this cohort of long-term travelers was low. Routine screening for eosinophilia appeared not to be of diagnostic value.

INTRODUCTION

Being among the most widespread infectious agents in human populations, helminths (i.e., roundworm and flatworm parasites) are an enormous burden for many low-income countries (1, 2). Millions of people in developing countries are chronically infected with at least one helminth species (1). Infection can produce a wide range of illnesses, depending on the involved species. The World Health Organization was requested by its World Health Assemby in 1974 to intensify research into the major tropical parasitic diseases (3). Since then, several programs regarding helminths have been launched, like the Onchocerciasis Elimination Program for the Americas (OEPA, 1993), African Programme for Onchocerciasis Control (APOC, 1995), Global Programme to Eliminate Lymphatic Filariasis (GPELF, 2000), and Schistosomiasis Control Initiative (SCI, 2002) (4-8). Several such programs include mass drug administration (MDA) which often can prevent and alleviate symptoms of disease and reduce infection prevalence to levels that mitigate transmission and new infections (9). MDA proved to be an effective global public health control measure that could by-pass the cost of screening diagnostics and use drugs donated by pharmaceutical companies (1, 10). However, while important progress was made, the global burden of schistosomiasis, for example, is still estimated at 3.5 million disease-adjusted life-years (DALYs) and for lymphatic filariasis, it is more than 2 million DALYs (2). Although complete elimination of helminth infections will depend amongst others on mosquito-control, improvement of sanitation, and access to clean water, fundamental research is still needed to develop alternative treatment or medication targeting various stages of the parasites (1, 8, 9).

Travelers to helminth-endemic countries may be at risk for contracting helminth infections, for example, when they are exposed to vectors and/or engage in risk behavior such as walking bare-foot. International travel has increased tremendously in recent years, with >1 billion tourist arrivals worldwide since 2012. As this increase includes developing countries, research into helminth infections among travelers seems justified, especially as asymptomatic infection with helminths can cause morbidity long after the primary infection (8, 11-13). However, research into prevalence (P), attack rates (AR) and incidence rates (IR) of helminth infections among travelers is scarce. A previous prospective study showed a low risk among short-term travelers (AR: 0.08-0.51%, and IR: 1.1-6.4 per 1000 person-months) (14). In 2008 though, among 6,957 ill travelers returning to Europe, 156 (2%) were diagnosed with a helminth infection: strongyloidiasis in 54/156 (35%) and loiasis in 10/156 (6%). Schistosomiasis was reported separately, and found in 129/6957 (2%) cases (15). Data collected within the Geosentinel study among 43,722 ill returning travelers from 1997 to 2004 revealed 271 (0.62%) filarial infections (16). Africa contributed by far the most to the contracted helminth infections among travelers (16,

17). Eosinohilia (i.e., >450 eosinophils per µl of blood) is often used as marker pointing to a helminth infection, though reports of eosinophilia coinciding with helminth infections vary considerably (14, 18-22).

Due to the extended period of exposure, one would expect higher attack rates in long-term travelers compared to short-term travelers. In addition, other factors may differ and be of influence on risks, such as compliance with preventive measures and behavioural factors such as as closer contacts with local populations (23-28). To gain more insight into helminth infections among this specific group of long-term travelers, we focused on four frequently diagnosed helminth infections among ill returning travelers which also have been previously studied among short-term travelers: schistosomiasis, strongyloidiasis, filariasis, and toxocariasis (14-16). Their primary species, vectors, regions involved, symptoms and numbers infected worldwide are summarized in Table 1. Our aim was to estimate their attack rate and incidence rate among long-term travelers and to investigate factors associated with infection. A second objective was to evaluate the diagnostic relevance of eosinophilia as a predictor for helminth infection.

METHODS

Study population

This study was conducted as part of a prospective mono-center study of Dutch travelers at the Public Health Service travel clinic in Amsterdam from December 2008 through September 2011. All clients aged ≥ 18 years planning to travel to any subtropical or tropical country for ≥12 and ≤52 weeks were invited to participate. All participants were seen by a medical doctor or nurse who specialized in travel medicine. They were advised according to Dutch National Guidelines on Travelers' Health Advice, receiving oral and written information about how to avoid mosquito-borne infections (36). The study protocol was approved by the Medical Ethics Committee of the Academic Medical Center of Amsterdam (MEC 08/064). Participants were included after obtaining their written informed consent.

Survey methods

At enrollment, a standardized questionnaire in Dutch or English was used to collect pre-departure data on socio-demographics, travel history, vaccination status, and purpose of travel: tourism, work/education, visits to friends and/or relatives (VFR). Participants donated a pre-travel blood sample for serologic testing and were given a digital thermometer (Huikeshoven Medical, Tiel, the Netherlands) and asked to take their temperature if they felt feverish. They were asked to keep a structured, weekly

Table 1 Key characteristics of four helminth infections: schistosomiasis, strongyloidiasis, filariasis, and toxocariasis.

infection		causal agent	reservoir or vector	route of infection	incubation period	general symptoms	regions	estimations of numbers infected/at risk
Schistosomiasis (10, 12, 29)	intestinal	*S. mansoni* *S. japonicum S. mekongi* *S. guineensis* *S. intercalatum*	aquatic snails	Contact with contaminated freshwater sources	2-6 weeks	abdominal pain, diarrhea, blood in stool, hepatomegaly, splenomegaly	Africa, Middle East, Caribbean, parts of South-America, parts of Asia, Corsica (France)	218 million at risk
	urogenital	*S. haematobium*				hematuria, fibrosis of bladder/ureter, kidney damage		
Strongyloidiasis (11, 29)		*S. stercoralis S. fülleborni*	Human, dog, monkey	contact with contaminated soil; direct penetration of human skin by infective larvae	14-30 days	abdominal pain, intermittent/persistent diarrhea, cough, wheezing, chronic bronchitis, pruritis, urticaria	mainly (sub) tropical regions, but also in temperate climates	30-100 million infections
Filariasis (8, 29-33)	lymphatic	*W. bancrofti, B. malayi, B. timori*	mosquitoes	bites of vector	5-18 months (range 1 month-2 years)	lymphoedema, elephantiasis, hydrocele	mainly regions in Africa and Asia	120 million infections
	onchocerciasis	*O. volvulus*	blackflies			severe itching, disfiguring skin conditions, visual impairment including permanent blindness		120 million at risk
	loiasis	*Loa loa*	deerflies			itchy swellings (Calabar swellings), eye worm		> 29 million at risk
	mansonellosis	*M. perstans, M. ozzardi, M. strepocerca*	midges blackflies			amongst others angioedema, pruritis, fever, headache, neurological symptoms		114-580 million at risk in Africa (*m.perstans*)

Table 1 Key characteristics of four helminth infections: schistosomiasis, strongyloidiasis, filariasis, and toxocariasis. (*continued*)

infection	causal agent	reservoir or vector	route of infection	incubation period	general symptoms	regions	estimations of numbers infected/at risk
Toxocariasis (21, 29, 34, 35)	*T.canis*	dogs	ingestion of eggs on soil/ plants contaminated by dog/cat feces (less frequently by eating undercooked meat containing larvae)	1 week-2 years	systemic, abdominal or respiratory symptoms, sometimes dermatological symptoms as well	worldwide	unknown (estimates for just USA already tens of millions)
	T.cati	cats					

travel diary during travel and two weeks after return, recording their itinerary, signs of disease, physician visits, diagnoses and possible self-treatment. Two to six weeks after return, they completed a short post-travel questionnaire including questions about potential helminth exposure (swimming in a fresh- water source, drinking of unboiled water, walking bare-foot, and having had wounds on feet), and donated a second blood sample for serologic testing.

Laboratory methods

All blood samples were immediately stored at 6°C. The total leukocyte count and the eosinophil count of both pre-travel and post-travel samples were determined within 24 hours by automated analyzer (Sysmex, Kobe, Japan). Blood samples for serologic testing were centrifuged and frozen at -80°C within 24 hours, to be tested after all participants had returned. Serodiagnosis of *Schistosoma mansoni*, *haematobium*, and *japonicum* was performed using indirect hemagglutination assay (IHA) with adult *S. mansoni* worm antigens (Fumouze Laboratories, Levallois-Perret, France) and an enzyme-linked immunosorbent assay (ELISA) with *S. mansoni* soluble egg antigens (37). For *S. stercoralis*, an in-house ELISA based on an antigen of *S. stercoralis* was used (38). For filariasis, a commercially available ELISA on microtitration wells sensitized with *Acanthocheilonema viteae* somatic antigens was used (Bordier Affinity Products, Crissier, Switzerland). For toxocariasis, a commercially available ELISA on microtitration wells sensitized with *T. canis* E/S larval antigens was used (Bordier Affinity Products, Crissier, Switzerland). Sensitivity and specificity in clinical settings were 100% and 93% for the combined IHA ELISA for schistosomiasis; 93% and 95% for the ELISA for strongyloides; 95% and 98% for the ELISA for filariasis; and 91% and 86% for the ELISA for toxocariasis (37-40).

For participants whose post-travel sample yielded positive test results, corresponding pre-travel samples were also tested. The presence of antibodies in both pre- and post-travel sample was considered suggestive for a previous infection. The presence of antibodies in the post-travel sample together with the absence of antibodies in the pre-travel sample was considered suggestive for an incident infection acquired during travel (i.e., a recent infection). If antibodies were detected in the post-travel samples together with a weak positive test result from the corresponding pre-travel sample, previous infection was assumed.

Data & statistical analysis

Participants were considered at risk for infection if they were susceptible to the disease (i.e., having a seronegative pre-travel sample) and visited at least one helminth-endemic country. Endemicity was based on information from The Global Infectious diseases and Epidemiology Online Network (29). Visited countries were analysed at continent-level

due to small numbers of visiting participants per country. If a traveler visited more than one continent, the continent in which the traveler spent most time was designated as the visited continent. Attack rates, incidence rates, sensitivity, specificity, positive predictive value, and negative predictive value were calculated as previously described (14). Eosinophilia was defined as an eosinophil count of ≥ 450 per mm^3.

The prevalence of previous infection with *Schistosoma* spp.*, S.stercoralis,* filarial species and/or *Toxocara* spp. and the corresponding 95% confidence intervals were calculated. Logistic regression analysis was used to examine the association between previous infection and the following variables: sex, age, country of birth, and total length of stay at previous travel destinations. Variables with a p-value <0.1 in univariable analysis were included in the multivariable model.

Incidence rates and 95% confidence intervals of infection with *Schistosoma* spp.*, S.stercoralis,* filarial species, and/or *Toxocara* spp. were calculated. Univariable logistic regression analysis for recent infection was performed using a Generalized Estimating Equation (GEE) model. This model takes account of clustered data, as participants could be at risk for 1 to 4 helminth infections, depending on previous immunity and visited endemic countries. To investigate factors associated with incident infection, we selected the variables sex, age, country of birth, purpose of travel, travel duration, visited continents, number of visited countries, and high-risk behavior. Participants were considered positive for high-risk behavior if they had been swimming in a fresh-water source (schistosomiasis) and/or had been walking bare-foot (strongyloidiasis and toxocariasis). Explorative analysis as to participants' using anti-helminth medication was performed using the chi-square test for categorical data. Anti-helminth medication against schistosomiasis, strongyloiadiasis, filariasis, and/or toxocariasis include albendazole, mebendazole, praziquantel, ivermectine, and diethylcarbamazine (DEC). A p value <0.05 was considered statistically significant.

Only the time spent in helminth-endemic countries was used as denominator to calculate incidence rates for *Schistosoma* and filarial infection. For those who became infected while traveling, the moment of infection was estimated as the midpoint between their arrival and departure dates in endemic countries. All analyses were conducted using STATA Intercooled version 13 (College Station, TX, USA).

RESULTS

Study population

Overall, 685 travelers intended to participate. Of these, 42 (6%) were excluded based on changed travel arrangements, 38 (6%) due to loss to follow-up, and 1 due to a missing post-travel blood sample.

For the remaining 604 participants, the median age was 25 years (interquartile range [IQR]: 23-29), 36% were male, the purpose of travel was predominantly tourism (62%), and the median travel duration was 20 weeks (IQR: 15-25). Seven participants traveled less or more than the intended period of 12-52 weeks. The median interval between return from travel and post-travel blood donation was 25 days (IQR 21-33).

One participant visited Tonga (Oceania) exclusively and was counted as visitor to Asia for simplicity purposes. Of all participants, 494 (82%) traveled to one or more countries endemic for schistosomiasis and 566 (94%) to countries endemic for filariasis.

Serologic results of previous infection

Thirteen participants (95% CI: 0.68-2.8%) had a previous infection, as they tested both pre- and post-travel-positive for the same helminth infections. In 5 (0.8%) participants, antibodies against *Schistosoma* spp. were found, in 3 (0.5%) antibodies against *S.stercoralis,* in 4 (0.7%) antibodies against filarial species, and in 1 (0.2%) antibodies against *Toxocara* spp. (Table 2). Three of the 13 individuals (23%) with a previous helminth infection (one *S.stercoralis* infection and two infections with filarial species) did not report being born in a developing country nor previous travel to one. In univariable logistic regression analysis, a previous infection was associated with older age, but in the multivariable model none of factors remained significant.

Serologic results of recent infection

Nine participants acquired 10 recent helminth infections as indicated by a negative pre-travel test and a corresponding positive post-travel test. In 2 participants antibodies against *Schistosoma* spp. were found, in 1 antibodies against *S.stercoralis*, in 5 antibodies against filarial species, and in 1 participant antibodies against both filarial and *Schistosoma* spp. were found. Characteristics of the 9 recently infected participants are shown in Table 3. In total, 603 subjects were at risk for toxocariasis; none of them acquired an infection during travel. The attack rate (AR) for *Schistosoma* spp. was 0.61 (3/494) and the incidence rate (IR) was 1.5/1,000 person-months (pm); for *S.stercoralis* the AR was 0.17 (6/601) and the IR 0.33/1,000 pm; for filarial species the AR was 1.1 (6/566) and the IR 2.6/1,000 pm (Table 4).

Table 2 Characterstics of 604 long-term travelers attending a Dutch travel health clinic, including prevalence of suggested previous infection with *Schistosoma* spp., *S.stercoralis*, filarial species, and/or *Toxocara* spp. from December 2008 to September 2011.

Characteristic	Total		no.*	P (%)	univariable				multivariable			
	no.	%			OR	95% CI lower	95% CI upper	p	OR	95% CI lower	95% CI upper	p
No. participants	604		13									
Sex												
female	389	64	9 (4,2,2,1)	2	1			0.710				
male	215	36	4 (1,1,2,0)	2	0.80	0.24	2.6					
Median age, y (IQR)	25 (23-29)											
Age, y												
< 24	203	34	4 (1,1,2,0)	2	2.6	0.47	14.4	0.027	2.6	0.47	14.5	0.060
24-29	262	43	2 (1,1,0,0,)	1	1				1			
≥ 30	139	23	7 (3,1,2,1)	5	6.8	1.4	33.6		5.7	1.2	28.8	
Country of birth												
Netherlands	563	93	12 (4,3,4,1)	2	1			0.596				
Other European country/US	26	4	1 (1,0,0,0)	4	1.83	0.23	14.7					
Other	15	2	0 (0,0,0,0)	0	na							
Previous travel destinations												
not Asia	299	50	6 (1,2,3,0)	2	1			0.807				
Asia	305	51	7 (4,1,1,1)	2	1.1	0.38	3.5					
not Africa	379	63	5 (0,1,3,1)	1	1			0.073	1			0.203
Africa	225	37	8 (5,2,1,0)	4	2.8	0.89	8.5		2.1	0.66	6.8	
not Latin America	362	60	6 (3,1,2,0)	2	1			0.311				
Latin America	242	40	7 (2,2,2,1)	3	1.8	0.59	5.3					
Total duration at previous travel destinations												
< 1 months	264	44	5 (0,2,3,0)	2	1			0.791				
1-3 months	116	19	2 (1,0,0,1)	2	0.91	0.17	4.7					
> 3 months	224	37	6 (4,1,1,0)	3	1.4	0.43	4.7					

* In parentheses, number of cases of *Schistosoma* spp., *S.stercoralis*, filarial species, and *Toxocara* spp., in that order.

Table 3 Characteristics, eosinophil counts, and risk behavior of participants with serologic evidence for infection with *Schistosoma* spp., *S.stercoralis*, filarial species, and/or *Toxocara* spp.

	serological conversion for*	sex	age in years	country of birth	destinations**	travel duration in weeks	eosinophil count per mm^3 (proportion of leukocytes)		purpose of travel	number of times swimming in lakes, rivers or streams	drinking of unboiled water from natural sources	walking bare-foot	wounds on feet
							pre-travel	post-travel					
1	strong	M	30	Germany	Argentina, Paraguay, Bolivia, Peru, Colombia, Panama	23	350 (5.3%)	290 (6%)	tourism	>10	yes	no	no
2	schis	F	25	Netherlands	**Uganda**, Tanzania	17	300 (3.3%)	120 (2.9%)	work/education	2-5 (bilharzia +)	yes	don't know	no
3	schis	M	31	Germany	Indonesia, Malaysia, Thailand, Vietnam, Cambodia	13	60 (1%)	70 (0.8%)	tourism	2-5	no	yes (rarely)	don't know
4	schis/fil	F	27	Netherlands	Thailand, Malaysia, Singapore, Cambodia, Laos	26	110 (0%)	100 (1.3%)	other	2-5 (Cambodia)	no	yes (rarely)	no
5	fil	F	39	Netherlands	Kenya, **Sudan**	40	60 (0.9%)	220 (4.7%)	work/education	0	no	no	no
6	fil	F	22	Netherlands	**Argentina**, Brazil, Bolivia, Peru	20	150 (3.4%)	190 (1%)	work/education	0	yes	yes (rarely)	no
7	fil	F	24	Netherlands	**Mexico**	43	560 (6.7%)	220	other	1	yes	yes (rarely)	yes
8	fil	M	31	Netherlands	**Congo D.R.**	15	130 (2.9)	210 (3.7%)	work/education	2-5	don't know	no	no
9	fil	F	28	Netherlands	**Indonesia**	23	150 (2.3%)	100 (1.7%)	work/education	>10	don't know	no	no

* strong=strongyloidiasis, schis=schistosomiasis, fil=filariasis

** country of primary destination in bold

Table 4 Attack and incidence rates of infection with *Schistosoma* spp., *S.stercoralis*, filarial species, and/*or Toxocara* spp. among long-term travelers with evidence of seroconversion during travel.

helminth	region	number of seroconversions	susceptibles at risk	person-months of travel	attack rate, % (95% CI, %)		incidence rate per 1000 person-months (95% CI)	
Schistosoma spp.	All regions	3	494	1938	0.61	0.13-1.8	1.5	0.50-4.8
	Asia	2	264	1117	0.76	0.092-2.7	1.8	0.44-7.2
	Africa	1	105	513	0.95	0.024-5.2	1.9	0.27-13.8
	Latin America	0	125	307	0	NA	0	NA
S.stercoralis	All regions	1	601	2958	0.17	0.0042-0.92	0.34	0.048-2.4
	Asia	0	269	1267	0	NA	0	NA
	Africa	0	106	528	0	NA	0	NA
	Latin America	1	226	1163	0.44	0.011-2.4	0.86	0.12-6.1
Filaria spp.	All regions	6	566	2308	1.1	0.39-2.3	2.6	1.16-5.8
	Asia	2	266	1203	0.75	0.091-2.7	1.7	0.42-6.6
	Africa	2	107	520	1.9	0.23-6.6	3.8	0.96-15.4
	Latin America	2	193	585	1.0	0.13-3.7	3.4	0.86-13.7
T. canis	All regions	0	603	2968	0	0-0.61^	0	NA
	Asia	0	271	1274	0	NA	0	NA
	Africa	0	107	533	0	NA	0	NA
	Latin America	0	225	1160	0	NA	0	NA

^ one-sided, 97.5% confidence interval

Overall, 454/601 (76%) participants reported that they had been swimming in fresh water, 228/601 (38%) had been drinking unboiled water from natural sources, and 375/601 (62%) had been walking bare-foot on warm humid soil. If only those participants who had visited schistosomiasis-endemic countries and who reported swimming in fresh water were considered at risk, the AR and IR for schistosomiasis rose slightly (AR 0.81, 95%CI: 0.002-0.024, IR 2.1, 95%CI: 0.43-6.1)

In univariable analysis, an association was found between recent helminth infection and the purpose of travel. Compared to tourists, participants traveling for work/education and VFR/other seroconverted more often for the four studied infections (OR 5.5, 95%CI: 1.1-28.2 and OR 10.5, 95%CI: 1.7-63.3 respectively, p=0.033).

Use of anti-helminth medication

Overall, 18 (3%) participants reported having used anti-helminth medication, predominantly albendazole. These 18 participants more often traveled for work/education or to visit friends and relatives than for tourism (5.8% and 3.6% vs 1.6%, p=0.027). Their travel duration was longer compared to non-users of helminth medication (7.6% (≥26 weeks) vs 0.6% (<16 weeks), 1.9% (16-20 weeks) or 2.7% (21-25 weeks), p=0.003) and they traveled more often to Africa or Asia than to Latin America (6.5% and 3.3% vs 0.88%, p=0.016). A doctor was visited by 12 of the 18 participants. Praziquantel was prescribed once post-travel to an asymptomatic traveler whose pre-travel blood sample showed anti-schistosomiasis antibodies. This traveler did however seroconvert for a filariasis (patient number 5, Table 3). The other 8 seroconverters did not report using one of abovementioned anti-helminth medications.

Eosinophilia

The median pre-travel eosinophil count among the 604 participants was 150 per mm3 (IQR: 90-240), and 28/604 subjects (5%) had a pre-travel eosinophilia. Among the 13 participants with a previous infection, the median pre-travel eosinophil count was 130 per mm3 (IQR: 100-230), and none of them had a pre-travel eosinophilia.

Post-travel, the median eosinophil count was 170 per mm3 (IQR: 100-260), and 38 (6%) had a post-travel eosinophilia. Among the 9 participants with a recent infection, the median post-travel eosinophil count was 190 per mm3 (IQR: 100-220), yet none of these 9 had a post-travel eosinophilia. The sensitivity, specificity, PPV and NPV of eosinophilia in pre-travel samples for previous infection and in post-travel samples for recent infection are described in Table 5.

Table 5 Post-travel eosinophilia among long-term travelers during travel, including participants with evidence of infection with *Schistosoma* spp., *S.stercoralis* and/or filarial species.

	Total, n	eosinophils median / mm^3 (IQR)	eosinophil count > 450/ mm^3	≥ 8% eosinophils / total leukocyte count	≥ 10% eosinophils / total leukocyte count
all participants	604	170 (100-260)	38 (6%)	n=31 (5%)	16 (3%)
recent helminth infection	9	190 (100-220)	n=0 (0%)	n=1 (11%)	n=1 (11%)
sensitivity			0%	11%	11%
specificity			94%	95%	97%
positive predictive value			0%	3%	6%
negative predictive value			98%	99%	99%

DISCUSSION

In this prospective study of long-term travelers to subtropical and tropical countries, the risk of acquiring one of the studied helminth infections during travel was very low, though quite a lot of travelers used anti-helminth medication. Post-travel eosinophilia was not a good marker for seroconversion during travel.

The found incidence rates and attack rates are in line with two previous prospective studies, although the IRs and ARs in our long-term study are even lower than the previously studied short-term travelers (14, 41). One of the reasons for the low IRs and ARs is probably the decrease of helminth prevalence due to control and elimination programs in endemic countries, which likely also have led to a lower risk of infection in travelers (2). In addition, a sizeable number of participants used anti-helminth medication. Although some used the medication against other parasitic infection and not specifically against one of the studied infections, some participants used the medication without reporting any symptoms, suggesting preventive treatment against a suspected helminth infection. Remarkably, the travelers who used anti-helminth medication were predominantly VFRs and study- and work-related travelers, as were the participants in our study who appeared most prone to a helminth infection. Anti-helminth medication might have had a preventive effect, which might have resulted in a slight underestimation of the risk of helminth infection. Considering the possible association of travel purpose and helminth infections, it seems sensible that VFRs and study- and work-related travelers should be specifically informed during pre-travel consultation to avoid high-risk behavior and risks of helminth infections during pre-travel health consultation.

Eosinophilia is frequently associated with helminth infections, and laboratory screening of patients at tropical medicine clinics often include eosinophil counts (16, 19, 21, 22). The eosinophil count found in symptomatic helminth infections is often very high; in a

recent German study they found a median eosinophil count of 981 cells/µl among 71 confirmed infections (range 508-15,100 cells/µl) (22). However, eosinophilia can arise from other medical conditions, including allergic disorders, and in several studies the diagnostic relevance of eosinophilia as predictor or screening for parasitic infections seemed of limited value (14, 18, 20). In our study, the PPV of eosinophilia for the four helminth infections under study was very low. We found only 3 travelers with an eosinophil count higher than 981 cells/µl in the pre-travel screening as well as in the post-travel screening, all of these being different participants. Our study should be compared with great caution to retrospective and cross-sectional studies that include eosinophilic screening of symptomatic returning travelers; although it might be valuable in those travelers, our study confirms that screening of western asymptomatic travelers seems of limited value.

Our study has some limitations. First, due to the low number of infections during travel, the sample size is too small to calculate incidences for all four studied helminth infections and for possible risk factors.

Second, some factors could have led to an underestimation of the number of helminth infections during travel. For example, incubation periods can vary widely and could have exceeded the time between travel return and blood donation, leading to an underestimation. However, the median time between return and blood donation was 25 days. We therefore assume that the possible number of infections still in window phase would be negligible. Also, the 13 participants with evidence of a previous infection could have been re-infected with the same helminth during the study period. As the serologic tests we used cannot discriminate between primary and secondary infections, we may have underestimated the number of infections during travel. Furthermore, the used laboratorium kits were not designed for detection of all possible species per helminth. Selection bias may also have occurred, as all participants were seeking pre-travel health advice when recruited. Their health awareness was perhaps higher than average, particularly after receiving oral and written advice about protection against mosquitoes, learning about the study, and agreeing to participate.

Third, cross-reaction with antibodies against other helminth infections cannot be excluded (38, 42), especially as two of previous filarial infections were found among travelers who reported no tropical or subtropical travel abroad before participating in our study. However, this finding could have been an error, as data about previous travels was self-reported. In the absence of a gold standard, additional laboratory diagnostics like stool microscopy could have yielded valuable information, but were not part of the study protocol (18).

Finally, our study did not collect data about nature of travel (adventurous, backpacking, high quality hotels). This could have been of influence on the found incidence rates per continent.

CONCLUSIONS

As far as we know, this is the first prospective study of serology confirmed schistosomiasis, strongyloidiasis, filariasis, and toxocariasis in long-term travelers. We showed that the risk of acquiring one of these infections during travel was very low for long-term travelers. Routine screening of eosinophilia among asymptomatic western travelers appeared to have no value for the four helminths under study.

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SUPPORTING INFORMATION

SI 1 Travel diary (in Dutch).

	Week nr.	Week nr.	Week nr.	Week nr.
Algemeen				
Datum				
Land(en)				
Preventieve maatregelen				
Verblijft u in malariagebied? (volgens GGD, landenkaart)				
Aanbevolen malariatabletten te slikken?				
Heeft u tabletten geslikt volgens schema? Indien ja, welke?				
DEET gebruikt?				
Klamboe gebruikt?				
Gedurende de gehele nacht met airco+afgesloten ruimte geslapen?				
Klachten				
Koorts gehad? (hoger dan 38˚C) Hoogst gemeten temperatuur? Hoe? (oraal, onder de oksel of rectaal)				
Hoofdpijn?				
Pijn achter de ogen?				
Spierpijn? (behalve de spierpijn na inspanning)				
Gewrichtspijn? Één gewricht? Meerdere gewrichten?				
Overgeven?				
Diarree? Alleen aakruisen bij minimaal 3x diarree per dag! Indien ja: bloed/slijm?				
Huiduitslag? Zo ja, waar? Wat?				
Hoesten langer dan een week?				
Overige klachten Indien ja, welke?				
Behandeling				
ORS (Orale rehydratie solutie)				
Antidiarree middel? Welk middel?				
Andere medicatie dan u gewend bent gebruikt? Welk middel?				
Arts bezocht? Indien ja, waarom?				
Diagnose? Behandeling gestart? Indien ja, welke?				

SI 2 Travel diary (in English).

	Week nr.	Week nr.	Week nr.	Week nr.
General questions				
Date				
Country/Countries on itinerary				
Preventive measures				
Risk area for malaria? (see malaria map, LCR)				
Is malaria prophylaxis recommended?				
Did you take malaria prophylaxis? Which kind?				
Did you use an insect repellent containing DEET?				
Did you sleep under a bed-net?				
Did you sleep in an air-conditioned room during the entire night, with the windows closed?				
Symptoms				
Fever? (above 38°C) Highest measured temperature? How? (oral, armpit, rectal?				
Headache?				
Pain behind the eye(s)?				
Muscle ache? (unrelated to physical activity)				
Joint pain? (unrelated to physical activity) One or more joints?				
Did you vomit?				
Diarrhea? Containing blood/ mucus?				
Skin rash? Where?				
Other symptoms? Which?				
Treatment				
Did you use ORS? (Oral Rehydration Solution)				
Did you use anti-diarrhoea drugs? Which medication?				
Did you take other medication? (other than your routine medication) Which medication?				
Did you consult a doctor If so, why?				
What was the diagnosis? Did the doctor start treatment? What treatment?				

SI 3 Post-travel questionnaire (in Dutch).

Vragen na de reis:

Heeft u in meren, rivieren of stromen gezwommen?

- ja — Zo ja: hoe vaak?(of zoals volg. Vraag): 1 keer / 2-5 keer / >10 keer
- nee

Heeft u ongekookt water uit een natuurlijk bron gedronken?

- ja
- nee
- weet niet

Heeft u blootsvoets buiten gelopen op warm, vochtige grond?

- ja (altijd, bijna altijd, helft, zelden, nooit, weet niet)
- nee
- weet niet

Had u wondjes onder de voeten?

- ja
- nee
- weet niet

SI 4 Post-travel questionnaire (in English).

Questions after travel:

Did you swim in lakes, rivers or streams?

- yes — If yes: how often? (or like next question): 1 time / 2-5 times / >10 times
- no

Did you drink unboiled water from natural sources?

- yes
- no
- don't know

Have you been walking bare-foot outside on warm, humid soil?

- yes (always, almost always, half of time, rarely, never don't know)
- no
- don't know

Did you have wounds on your feet?

- yes
- no
- don't know

SI 1 Dataset Dataset long-term travelers (de-identified).
Available at: https://doi.org/10.1371/journal.pone.0197770.s005

3

Hepatitis E among in long-term travelers from the Netherlands to subtropical and tropical countries, 2008-2011

Floor Elfrink, Femke W Overbosch, Janke Schinkel, Gerrit Koen, Gerard JB Sonder

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ABSTRACT

Hepatitis E virus (HEV) is a common cause of acute viral hepatitis. Virus genotypes 1 and 2 infect humans in developing countries by the fecal–oral route. To assess attack rates and disease incidence for travelers, we prospectively studied 604 long-term travelers to subtropical and tropical countries. Participants donated blood samples pretravel and posttravel and kept a diary. A total of 89/604 (15%) pretravel samples were positive for HEV IgG by ELISA, suggesting previous HEV infection. Seroconversion for HEV was found for 19/515 travelers (attack rate 3.7%, incidence 1.8 cases/1,000 person-weeks). We believe there is a substantial risk for acquiring HEV infection among long-term travelers. Although HEV infection does not seem to be a major problem in this healthy cohort, hygienic measures should be stressed in all pretravel health advice, particularly for pregnant women and immunocompromised travelers who are at risk for severe disease.

INTRODUCTION

Hepatitis E virus (HEV) is a common cause of acute viral hepatitis worldwide (1). There are 4 genotypes of HEV. Genotypes 1 and 2 infect humans in developing countries in areas with poor sanitation; transmission occurs through the fecal–oral route, causing occasional large outbreaks or frequent sporadic cases. Genotype 1 is found in Asia and Africa, and genotype 2 is found in Mexico and Africa. Genotypes 3 and 4 are transmitted zoonotically from animal reservoirs in industrialized and developing countries, mainly through consumption of uncooked or undercooked meat, and are responsible for sporadic cases of disease (1). In the Netherlands, genotype 3 is endemic in pigs and responsible for cases in humans.

The mean incubation period for hepatitis E is 40 days (range 15–60 days). Symptoms range from subclinical to fulminant and include fever, fatigue, loss of appetite, nausea, vomiting, abdominal pain, jaundice, joint pain, and hepatomegaly (1).

Hepatitis E is usually a self-limiting disease. The mortality rate for fulminant hepatitis is 0.5%–4%. Pregnant women, immunosuppressed persons, and persons with preexisting liver disease are at risk for severe hepatitis E. However, fulminant liver disease in immunocompetent persons has also been reported (2). Mortality rates for hepatitis E caused by genotype 1 for pregnant women are 20%–25% (3,4).

A previous HEV infection is characterized by the presence of specific IgG and is assumed to protect against reinfection. There is cross-neutralization among all genotypes (5).

Travelers from industrialized countries to developing countries are assumed to be at risk for acquiring an HEV infection through the fecal–oral route (genotypes 1 and 2). A vaccine against HEV is available only in China (4).

A study that included data in the GeoSentinel surveillance network for returned travelers with infectious gastrointestinal diseases during 1996–2005 reported a proportionate HEV illness rate of 1.2 cases/1,000 ill returned travelers (6). A recent case report identified a nonpregnant immunocompetent traveler who returned to Canada from India and was given a diagnosis of HEV infection, in whom fulminant liver failure developed (2). A study in Israel of 4,970 ill returning travelers during 1997–2012 reported 49 (1%) with acute hepatitis (32 cases were enterically transmitted): 19 travelers were given a diagnosis of hepatitis E, of whom 16 contracted their cases on the Indian subcontinent (7). The estimated risk for acquiring HEV for this study was 3.2 cases/100,000 travelers.

A prospective study of 1,206 short-term travelers from the Netherlands to subtropical and tropical countries (8), a prospective study of 105 long-term backpackers in Israel (9), and a prospective study of American missionaries (10) showed no seroconversions for HEV. Another prospective study of 356 short-term travelers from the United States reported 4 (1.7%) seroconversions (11). However, because seroconversions were found only for samples obtained 6 months after return of travelers and not in samples obtained 6 weeks posttravel, HEV might have been contracted after their return.

To our knowledge, no recent prospective studies of long-term travelers have been conducted. Because the risk for hepatitis E in subtropical and tropical countries might have increased, and the sensitivity of ELISAs for diagnosing HEV infection has improved over the past decade (3), we determined the incidence and risk factors of acquiring hepatitis E among long-term travelers (12–52 weeks) from the Netherlands to subtropical and tropical countries.

METHODS

Study Population and Design

This study was conducted as part of a larger, prospective, monocenter study of immunocompetent travelers >18 years of age who visited the Public Health Service travel clinic in Amsterdam, the Netherlands, during December 2008–September 2011. All clients planning to travel to subtropical or tropical countries for 12–52 weeks were invited to participate. Subtropical and tropical countries were defined as those with moderate-to-high risk for hepatitis A according to the World Health Organization (12). All participants consulted a nurse or physician specialized in travel medicine, and oral and written information was provided about how to avoid travel-related diseases. The study was approved by the Medical Ethics Committee of the Academic Medical Center in Amsterdam.

At their return, travelers were asked additional questions regarding behavior during travel, including drinking unboiled tap water or water from natural sources. Pretravel written informed consent was obtained, and travelers were interviewed by a nurse or physician about travel purpose, travel duration, planned destination(s), and demographic details.

Participants were given a digital thermometer (Huikeshoven Medical, Tiel, the Netherlands) and asked to record their temperatures if they felt feverish while traveling. Travelers kept a structured, weekly travel diary until 2 weeks after return and recorded their itinerary, symptoms, and physician visits while ill. Predefined symptoms that could be related to hepatitis E were fever and vomiting. It was also possible to report other

complaints. Diaries were completed on paper or digitally. Travelers received a weekly email reminder and were seen 2–6 weeks after return. Blood samples were obtained before and after travel. The pretravel sample was tested only if the posttravel sample tested was positive for antibodies against HEV.

Primary regions visited were grouped into regions according to the classification of the United Nations Country Classification with some modifications. For our study, Oceania included only Melanesia, Micronesia, and Polynesia and was merged with Southeast Asia because New Zealand and Australia matched exclusion criteria for participating. Latin America was divided into South America and the Caribbean/Central America.

Laboratory Methods

Blood samples were immediately stored at 6°C, centrifuged, and frozen at −80°C. We tested serum samples for HEV IgG by using an ELISA (Wantai Biologic Pharmacy Enterprise, Beijing, China), according to the manufacturer's instructions. This assay had a reported sensitivity of 98% but does not discriminate between different virus genotypes. If HEV IgG was detected in a posttravel sample, pretreatment samples were also tested. Presence of HEV IgG in a pretravel sample was regarded as evidence of previous HEV infection. A recent infection was defined as a positive posttravel sample and a negative pretravel sample.

Data Analysis

We calculated risk factors for previous HEV infection by using SPSS version 19.0 (IBM, Armonk, NY, USA) to obtain prevalence, univariable and multivariable prevalence ratios (PRs), and 95% CIs by means of logistic regression modeling. A p value <0.05 was considered statistically significant. All variables with p values <0.10 by univariable analysis were included in a multivariable model.

We calculated the attack rate of a recent HEV infection by dividing the number of seroconversions by the total number of participants still at risk for infection (i.e., all travelers who did not have HEV antibodies pretravel). We also calculated incidence rates by dividing the number of seroconversions by the total number of travel weeks of travelers still at risk. Person-time denominators for seroconversion were divided in half, assuming that the infection occurred halfway through travel.

We used univariable Poisson regression models to examine the effect of covariates (sex, age, travel purpose, primary destination, hospital admission) on seroconversion. Variables with p values <0.10 in univariable analysis were included in multivariable analysis. Outcomes were expressed as incidence rate ratios with 95% CIs. A p value <0.05 was considered statistically significant.

RESULTS

Study Population

During December 2008–September 2011, a total of 685 persons who intended to travel to subtropical and tropical countries for 12–52 weeks provided informed consent. Of these persons, 81 (12%) were excluded after completion of the study: 42 had their travel arrangements changed and no longer met the study criteria, 38 were lost to follow up, and 1 did not provide a posttravel blood sample. The remaining 604 persons formed the study population.

Median age of the study population was 25 years (interquartile range [IQR] 23–29 years), ≈66.6% were female, and 20% had never been to subtropical or tropical regions. Tourism was the main purpose for traveling (62.9%). Median interval between the first sample and departure was 38 days (IQR 20–55 days). Median interval between return and the second blood sample was 25 days (IQR 21–33 days).

Previous HEV Infection

A total of 89 of 604 persons were positive for HEV pretravel and posttravel, which indicated previous HEV infection, for a pretravel seroprevalence rate of 14.7% (Table 1). Univariate analysis indicated that previous HEV infection showed a positive correlation with older age, a nonwestern origin, and a history of travel to subtropical or tropical regions. Multivariate analysis showed that age, travel history, and nonwestern origin remained major predictors for previous HEV infection.

Table 1 Characteristics of 604 travelers who visited a travel clinic for pretravel advice and prevalence of previous HEV infection, the Netherlands, December 2008–September 2011*

Characteristic	Travelers	HEV IgG positive pretravel	Univariable analysis		Multivariable analysis	
			PR (95% CI)	p value	PR (95% CI)	p value
Total	604	89 (14.7)				
Median age, y (IQR)	25 (23-29)	26 (23–30)	1.0 (1.01–1.07)	**0.002**	1.0 (1.01–1.06)	**0.01**
Sex						
F	389 (64.4)	54 (13.9)	1.0	0.43		
M	215 (35.6)	35 (16.3)	1.2 (0.76–1.92)			
Region of birth						
Western (the Netherlands), n=563	590 (97.7)	84 (14.2)	1.0	**0.034**	1.0	**0.03**
Nonwestern	14 (2.3)	5 (35.7)	3.4 (1.10–10.23)		3.6 (1.5–11.28)	
Previous travel to subtropical region						
No	122 (20.2)	7 (5.7)	1.0	**0.003**	1.0	**0.01**
Yes	482 (79.8)	82 (17.0)	3.4 (1.5–7.5)		2.9 (1.27–6.45)	

*Values are no. (%) except as indicated. Bold indicates statistical significance. HEV, hepatitis E virus; IQR, interquartile range; PR, prevalence ratio.

HEV Infection Acquired during Current Travel

IgG seroconversion was found for 19/515 travelers, resulting in an attack rate of 3.7% and an incidence of 1.8 (95% CI 1.1–2.8) per 1,000 person-weeks. We obtained characteristics, attack rates, and incidence for recent HEV infections (seroconversions) (Table 2). At return, 32% (163/510) of participants reported they had used unboiled or untreated tap water for consumption, 19 did not remember, and 5 had missing results. The remaining 328 travelers did not consume unboiled or untreated water. Six persons who showed seroconversion reported having drunk unboiled or untreated tap water; 1 person had a missing result. Logistic regression of characteristics tested did not identify any major risk factors for acquiring HEV infection during travel.

Signs and Symptoms in Travelers Showing Seroconversion

A total of 215 (42%) of 515 travelers reported vomiting during their trip, and 35% (180/515) reported fever at least once. Nine of 19 travelers showing seroconversion reported >1 nonspecific symptoms possibly associated with HEV infection: 2 participants reported fever, 3 reported vomiting, 1 reported vomiting and fatigue, 2 reported vomiting and fever, and 1 reported abdominal pain and nausea.

A total of 31 (6%) of 515 participants were admitted to a hospital while abroad, of whom 1 person who showed seroconversion, was admitted because of symptoms of fever and dehydration caused by diarrhea. Jaundice, dark-colored urine, and light-colored stool were not reported as other complaints in the diary.

DISCUSSION

In this prospective study of long-term travelers from the Netherlands to subtropical and tropical countries, we found a substantial hepatitis E attack rate of 3.7% and an incidence of 1.8 cases/1,000 person-weeks. Results were obtained by using an HEV IgG ELISA and were higher than those in the 4 previous prospective studies of travelers (8–11).

The relatively high HEV seroconversion rate we found compared with those for previous prospective studies could be explained by an increase of hepatitis E incidence in developing countries over time. However, our results probably reflect improved sensitivity of currently available tests compared with those used in these previous studies. A combination of these factors is possible. Therefore, comparison of results of previous prospective and seroprevalence studies with those of our study should be interpreted with caution. We also found no major risk factors for acquiring HEV infection during travel.

Table 2 Attack rates and incidence of seroconversions in HEV antibody levels for 515 long-term travelers to subtropical and tropical countries, the Netherlands, December 2008–September 2011*

Characteristic	Travelers at risk	HEV seroconversions	Attack rate, % (95% CI)	Person-weeks of travel	Incidence/1,000 person-weeks (95% CI)	Univariable analysis	
						IRR (95% CI)	p value
Total	515	19	3.7 (2.4-5.7)	10,715	1.8 (1.1-2.8)		
Median age, y(IQR)	25 (23-29)	26 (22-31)					
Sex							
F	335 (65)	9	2.7 (1.4-5.0)	6,795	1.3 (0.7-2.5)	1.0	0.16
M	180 (35)	10	5.6 (3.0-9.9)	3,920	2.6 (1.4-4.7)	1.9 (0.8-4.7)	
Region of birth							
Western	506 (98.3)	19	3.8 (2.4-5.8)				
Nonwestern	9 (1.7)	0	NA				
Purpose of travel							
Holiday	324 (62.9)	10	3.1 (1.7-5.6)	6,556	1.5 (0.8-2.8)	1.0	0.54
Work or study	184 (35.7)	8	4.3 (2.2-8.3)	3,970	2.0 (1.0-4.0)	1.3 (0.5-3.3)	
VFR	7 (1.4)	1	14.3 (2.6-51.3)	188.5	5.3 (0.9-29.4)	3.5 (0.4-27.0)	
Primary region of travel							
Southeast Asia and Oceania	172 (33.4)	5	2.9 (1.2-6.6)	3,543	1.4 (0.6-3.3)	1.0	0.57
South America	148 (28.7)	5	3.4 (1.5-7.7)	3,211	1.6 (0.7-3.6)	1.1 (0.3-3.8)	
Sub-Saharan Africa	94 (18.3)	2	2.1 (0.6-7.4)	1,972	1.0 (0.3-3.7)	0.7 (0.1-3.7)	
Southern Asia	47 (9.1)	3	6.4 (2.2-17.2)	898	3.3 (1.1-9.8)	2.4 (0.6-9.9)	
Central America and Caribbean	37 (7.2)	3	8.1 (2.8-21.3)	754	4.0 (1.4-11.6)	2.8 (0.7-11.8)	
Asia, other	17 (3.3)	1	5.9 (1.0-27.0)	336	3.0 (0.5-16.7)	2.1 (0.2-18.1)	
Travel duration, wk							
12-16	189 (36.7)	6	3.2 (1.5-6.8)	NA			
17-24	190 (36.9)	7	3.7 (1.8-7.4)	NA			
25-52	136 (26.4)	6	4.4 (2.0-9.3)	NA			
Hospital admission							
no	484 (94)	18	3.7 (2.4-5.8)	10,032.5	1.8 (1.1-2.8)	1.0	0.84
yes	31 (6)	1	3.2 (0.6-16.2)	682.5	1.5 (0.3-8.2)	0.8 (0.11-6.11)	

*Values are no. (%) except as indicated. HEV, hepatitis E virus; IQR, interquartile range; IRR, incidence rate ratio; NA, not applicable; VFR, visiting friends and relatives.

Two nonprospective studies reported that hepatitis E is associated with travel to southern Asia (7,13). We found higher attack rates and incidences for southern Asia, other regions of Asia, and Central America than for Africa, Southeast Asia, and South America, but this finding did show a major difference.

In 2 other studies, travelers visiting friends and relatives were found to be at greater risk than tourist travelers for infectious diseases such as typhoid fever (6) and hepatitis E (14). Our study showed a higher attack rate (14%) and incidence (5.3 cases/1,000 person-weeks) for travelers visiting friends and relatives than for persons traveling for tourism or work/study, but this association was not strong. This finding could be caused by the small numbers of travelers visiting friends and relatives.

The pretravel seroprevalence of 15% we found was higher than the 2% found in the study of travelers from the Netherlands conducted during 2006–2007 (8) and the 6% found in the study of Boston, Massachusetts, USA, area travelers conducted during 2009–2010 (15). However, seroprevalence in this study was lower than the 27% found in the study of blood donors from the Netherlands conducted during 2011 (16) and in the population of Amsterdam during 2004 (17). Although these differences should also be interpreted with caution, there are several possible explanations for the differences in prevalence between studies.

The major difference in sensitivity between different assays could be an explanation for the higher prevalence we found than the prevalence of 2% found in the previous prospective study among travelers from the Netherlands. The test we used in our study was the same test used in the study of blood donors from the Netherlands (16) and for the population of Amsterdam (17). However, because HEV immunity increases with age and depends on ethnicity, the difference in characteristics between the different study groups could explain why we found a prevalence of 15% rather than 27%. Our study population was composed of mostly young persons of western origin.

Independent risk factors for previous HEV infection were being born in a nonwestern country and previous travel to subtropical and tropical regions, which can be explained by higher endemicity in nonwestern countries. Also, older age was a major risk factor for previous HEV infection, as observed by Sadik et al. (17).

A total of 9 of the 19 persons who showed seroconversion reported nonspecific symptoms possibly related to HEV infection. Only 1 of 31 hospitalized travelers showed seroconversion for antibodies against HEV, but hospitalization was probably not related to HEV infection (self-reported diagnosis was dehydration caused by diarrhea). None of the

persons who showed seroconversion were given a diagnosis of HEV infection during the study. Because many cases of hepatitis E are subclinical in otherwise healthy persons and only immunocompetent travelers were included in our study, it is not surprising that so many persons who showed seroconversion did not report specific symptoms.

The strength of our study is that it is a prospective study in which blood samples before and after travel and diaries kept during travel were available for all 604 long-term travelers. However, our study also had limitations. Because this study was part of a larger study, the travel diary contained general clinical symptoms instead of hepatitis E–specific symptoms. Thus, we could have missed signs of a mild clinical HEV infection. Also, the median interval between obtaining a posttravel blood sample and return from travel was only 25 days. Because the incubation period for hepatitis E is 15–60 days, this period could have led to an underestimation of cases. However, because this study involved long-term travelers who traveled for 12–52 weeks, it is unlikely that many infections were contracted in the last weeks of travel. Therefore, we assume the short interval between return from travel and obtaining a blood sample had limited consequences for the final results. In addition, the median interval between obtaining the first blood sample and travel departure was 38 days. Therefore, persons who showed seroconversion might have contracted the virus before travel, which could have led to overestimation of travel-related attack rates and incidences, but we assume this had limited effect on the final results.

We assumed that persons with HEV IgG were protected against reinfection and did not include them in additional analyses. However, reinfection is possible, even in immunocompetent persons (1,4). We compared pretravel and posttravel sample titers and found only 1 person with a high posttravel titer and a much lower but still above the positive threshold pretravel titer. This result could have been a reinfection, but a low false-positive value for the pretravel sample is also possible. We considered this traveler immune in our additional analysis; this person also did not report any symptoms of HEV infection. A 4-fold increase in titer between pretravel and posttravel samples was not found for other persons.

Our study could have had a selection bias because all participants sought pretravel health advice in which advice on personal hygiene was stressed. Because genotypes 1 and 2 of HEV are contracted through the fecal–oral route, this finding could have led to an underestimation of HEV incidence. However, most (82%) travelers in our cohort experienced travelers' diarrhea, which could also be contracted through the same route. Therefore, we believe that this selection bias had limited consequences on the outcome.

Finally, seroprevalence research most often lacks a diagnostic standard because it resembles a postinfection status in which conformatory tests using PCR are not feasible. In previous studies, the HEV IgG ELISA appeared to be one of the most sensitive tests available (18–21). However, in the absence of World Health Organization HEV-negative reference material, studies investigating the specificity of the test are scarce. Although results from a study in France were promising (specificity 97.8%) (21), possible false-positive test results cannot be excluded.

Using the HEV IgG ELISA, we found an attack rate for HEV infection of 3.7% and an incidence of 1.8 cases/1,000 person-weeks, which are higher than values from previous prospective studies. This finding could be a reflection of an increasing risk for travelers, but it could also (partially) reflect improved sensitivity of the available test. Almost half of persons who showed seroconversion had mild, nonspecific clinical symptoms possibly associated with HEV infection. Therefore, HEV infection does not seem to be a major problem in healthy immunocompetent travelers. However, rare fulminant liver failure in immunocompetent travelers has been reported (2), and in pregnant women and immunocompromised travelers, the risk for severe or fatal disease is much higher. Because travel has increased during the past few decades, at-risk groups also travel more (22). Good sanitation and clean drinking water should be discussed in all travel health advice. If an HEV vaccine were approved and found to be safe and effective for pregnant women and immunocompromised travelers, these vulnerable travelers could especially benefit from its protection.

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4

No chikungunya virus infection among Dutch long-term travellers to (sub)tropical countries, a prospective 2008-2011

Femke W Overbosch, Floor Elfrink, Janke Schinkel, Gerard JB Sonder

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ABSTRACT

Background

Chikungunya is an arthropod-borne viral disease now identified in over 60 countries in Asia, Africa, Europe, and the Americas. Chikungunya virus (CHIKV) has spread in the last 15 years to many countries, causing large local outbreaks. CHIKV infection can be clinically misdiagnosed in areas where dengue and/or Zika infections occur. Prospective studies are necessary to calculate the true incidence rate of CHIKV infection in travellers. The aim of this study was to obtain the attack and incidence rates of CHIKV infection among long-term travellers and identify associated risk factors.

Methods

A previously collected prospective cohort of Dutch long-term travellers (12-52 weeks) to subtropical and tropical countries was tested. From December 2008 to September 2011, participants were recruited at the travel clinic of the Public Health Service Amsterdam. A weekly diary was kept during travel in which participants recorded their itinerary, symptoms, and physician visits. On return, their pre- and post-travel blood samples were tested for the presence of IgG antibodies to CHIKV antigen. Seroconversions were confirmed by an in-house CHIKV neutralisation test.

Results

The median age of 603 participants was 25 years (interquartile range [IQR]: 23-29); 35.7% were male; median travel duration was 20 weeks (IQR: 15-25), and purpose of travel was predominantly tourism (62%).

The presence of anti-CHIKV IgG in the pre-travel sample, suggestive of previous CHIKV infection, was found for 3/603 participants (0.5%); all three had been previously travelling in either Africa or Asia. In one traveler who visited Latin America, a seroconversion was found (0.2 %) but the CHIKV neutralisation test was negative, making the incidence rate 0.

Conclusion

No chikungunya virus infections were found in this 2008-2011 prospective cohort of long-term travellers. We recommend the research be repeated, particularly as the sample size of our cohort might have been too small. Also, extensive spread of chikungunya virus has likely increased incidence rates among travellers since 2013.

BACKGROUND

Chikungunya is a mosquito-borne viral disease whose agent belongs to the alphavirus genus of the family Togaviridae, and the mosquitoes most commonly involved as vector are the daytime-active *Aedes aegypti* and *Aedes albopictus*. Symptoms of CHIKV infection can be mild and unrecognized or confused with the similar symptoms of dengue and Zika virus infection [1-3] . However, chikungunya's characteristic symptoms are high fever accompanied by severe arthralgia, which can be debilitating [1]. There is no specific treatment for chikungunya, and no vaccine is available [1].

The first recorded chikungunya epidemic was reported in Tanzania in 1952. The disease was subsequently reported in other parts of Africa, Asia, and the Indian subcontinent [1]. In 2007, the first local transmission was reported in Europe, and after the first autochthonous case was reported at the Caribbean island of St Martin in 2013, over a million cases were reported in the Americas within 9 months [1, 4].

Due to the emerging CHIKV endemicity and recent reports of CHIKV infections among travellers in endemic countries [3, 5-13], we were interested in the CHIKV incidence rate in Dutch travellers. Therefore, we tested a previously collected prospective cohort of Dutch long-term travellers (12-52 weeks) to subtropical and tropical regions [14]. Our primary aim was to estimate the attack rate and incidence rate of CHIKV infections during travel in Africa and Asia and to identify the associated risk factors. As we were also curious as to possible exposure in countries with no evidence of autochthonous CHIKV transmission at the time of our study, we also tested samples of all cohort travellers to Latin America [15, 16].

METHODS

Study population and study procedure

The study design and sample collection methods of the long-term travellers study have been described in detail previously [14]. In brief, this study was conducted as part of a prospective mono-centre study of long-term travellers aged ≥ 18 years to subtropical and tropical countries who were recruited at the Public Health Service travel clinic in Amsterdam from December 2008 through September 2011. Long-term travel was defined as travel for ≥12 and ≤52 weeks.

A standardised questionnaire in Dutch or English was used to collect data before departure on individual socio-demographics, travel history, and purpose of travel. Travellers

were asked to keep a structured, weekly travel diary. Travellers gave a blood sample once during their pre-travel visit and once 2-6 weeks after return.

Laboratory methods

After all study participants had returned, all post-travel serum samples were tested for immunoglobulin (Ig) G antibodies to CHIKV antigen by using an anti-CHIKV enzyme-linked immonosorbent assay (ELISA) IgG test (Euroimmun, Lübeck, Germany), according to manufacturer's instructions. For participants whose post-travel sample yielded a positive test result for anti-CHIKV IgG, their pre-travel sample was also tested for anti-CHIKV IgG.

Travel-acquired infection was considered the primary interest of this study. The presence of anti-CHIKV IgG in both the pre- and post-travel sample was considered suggestive of a previous CHIKV infection. Participants with a previous CHIKV infection were considered no longer at risk for a travel-acquired CHIKV infection. The presence of anti-CHIKV IgG in the post-travel sample, together with the absence of anti-CHIKV IgG test in the pre-travel sample, was considered a seroconversion. It was considered as evidence for a CHIKV infection if confirmed by a positive in-house CHIKV neutralisation test (Erasmus University Medical Center, Rotterdam, the Netherlands).

Statistical analysis

Analysis of destinations was performed at continent-level. The use of DEET (N, N-diethyl-meta-toluamide) was quantified by dividing the number of reported weeks of DEET usage, by the total number of travel-weeks.

Possible symptoms of a travel-acquired CHIKV infection were described using data reported in the travel diaries. Fever with arthralgia in ≥2 joints were considered the most characteristic symptoms of CHIKV infection. Myalgia, headache, skin rash, or vomiting reported simultaneously with fever and arthralgia in ≥2 joints were considered as frequently accompanying symptoms of CHIKV infection. The combination of fever and arthralgia in ≥2 joints followed by symptoms of arthralgia in ≥2 joints in consecutive week(s) was considered suggestive of persisting arthralgia after CHIKV infection.

To identify potential risk factors, following variables were selected to study: sex, age, purpose of travel, visited continent, and use of DEET. The prevalence of previous CHIKV infection and the corresponding 95% confidence interval (CI) were calculated. A p-value <0.05 was considered significant (STATA).

RESULTS

Characteristics of the study population

The prospective cohort consisted of 603 participants which formed the study population. The median age was 25 years (interquartile range [IQR]: 23-29), 35.7% were male, and the median interval between return from travel and post-travel blood donation was 25 days (IQR 21-33).

Results suggestive of previous CHIKV infection were found in both pre- and post-travel samples for 3/603 participants (0.5%; 95% CI -0.066-1.1%) (table 1). All three had been either in Africa or Asia before.

Table 1 Characteristics of 603 Dutch long-term travellers including the prevalence of previous chikungunya virus infection.

Characteristic	Total, no.	%	previous CHIKV*	
			no.	%
No. participants	603	100	3	0.5
sex				
Female	388	64	2	0.5
Male	215	36	1	0.5
Median age, years (IQR†)	25 (23-30)			
Age, years				
< 24	203	34	1	0.5
24-29	261	43	0	0
≥ 30	139	23	2	1.4
Total duration of(sub)tropical travel prior to study, in months				
< 1	263	44	1	0.4
1-3	116	19	1	0.9
> 3	224	37	1	0.5

The participants attended and were recruited at a Dutch travel health clinic between December 2008 and September 2011.
*CHIKV= chikungunya virus, †IQR= interquartile range

Travel-acquired CHIKV infection

The median travel duration was 20 weeks (IQR: 15-25); purpose of travel was predominantly tourism (62%), and the three most-visited countries were Thailand (175/600), Indonesia (137/600) and Argentina (130/600) (table 2). Only one CHIKV seroconversion was found in the 600 participants at risk for CHIKV infection. This participant had travelled in 2011 for 7.5 months through Argentina, Bolivia, Chile, and Peru, and reported no fever nor physical symptoms except coughing for three consecutive weeks. Moreover, the CHIKV neutralisation test was negative. Therefore we found no evidence of travel-acquired CHIKV infection in this cohort of travellers.

The characteristic symptoms of possible chikungunya (fever and in ≥2 joints) were reported by 40/600 (6.7%) participants. Frequently accompanying symptoms were: headache (85%, 34/40), myalgia (90%, 36/40), skin rash (23%, 9/40) and/or vomiting (38%, 15/40).

One of these 40 participants was diagnosed with chikungunya during travel whilst having joint pain and fever. This participant was also the only one who persisted in reporting pain in ≥2 joints in the 12 following weeks until the study ended. The participant had travelled predominantly in India in 2010, but the travel diary did not include information on how the diagnosis was made. Seroconversion for CHIKV was not found in this traveller.

Table 2 Travel-related characteristics of 600 Dutch long-term travellers at risk for CHIKV infection.

Characteristic	Total, no.	%
No. participants	600	100
Median duration of travel, weeks (IQR)	20 (15-25)	
Duration of travel, weeks		
<16	167	28
16-20	156	26
21-25	146	24
≥26	131	22
Purpose of travel		
tourism	371	62
work/education	173	29
VFR^/other	56	9
Visited continents		
Asia	269	45
Africa	107	18
Latin America	224	37
Use of DEET*, % of total travel duration		
< 25	175	29
25-50	134	22
51-75	102	17
≥ 75	189	32

The participants attended and were recruited at a Dutch travel health clinic between December 2008-September 2011.
†IQR= interquartile range, ^ VFR= visiting friends & relatives, * DEET= N,N-diethyl-meta-toluamide

DISCUSSION

The results of this 2008-2011 study of long-term travellers indicate a negligible risk for Dutch travellers to contract a CHIKV infection, since none of the 600 at-risk participants seroconverted. The results are in line with the available data that CHIKV was not yet introduced in the Americas at the time of the study period. The lack of seroconversion in Asia and Africa was rather unexpected, however, as 40/600 participants reported symptoms which could be characteristic of CHIKV infection. Large outbreaks of chikungunya were described in Asia preceding the study period [1, 17]. During the study period, the virus continued to spread in Southeast Asia, where large outbreaks were reported from popular tourist destinations in Indonesia and Thailand [18, 19]. As a substantial number of our cohort visited these two countries, exposure to CHIKV would have been likely. Concurrent to our study, the EuroTravNet study, investigated the proportion of chikungunya and indeed found some CHIKV infections (0.2% of 6,957 and 0.4% of 7,408 febrile returning travellers in 2008 and 2010, respectively); however it confirmed that the proportion of travellers with chikungunya was substantially lower than the proportion with dengue. In 2010; 357 of 7,408 persons (5%) contracted dengue [7].

Misdiagnosis seemed likely in our one participant who reported characteristic symptoms and chikungunya diagnosis during travel, but showed no CHIKV seroconversion in the post-travel sample. Surprisingly, this participant was not one of the travellers who seroconverted for dengue virus [14], and thus another pathogen probably caused all the symptoms.

Since we found no seroconversions, we could not calculate incidence rate ratios nor perform regression analysis to identify possible risk factors for travel-acquired CHIKV infection. Mosquito-borne infections depend often on seasonality including the wet seasons, as higher temperatures and heavy rainfall influences breeding sites. Possible explanations of our finding no CHIKV infection could be that travellers avoided wet-season-related outbreak areas or that anti-mosquito measures, like fumigation or spraying of insectides, were more extensively implemented in tourist areas than elsewhere. On the other hand, as 39 travellers of our cohort (6.5%) contracted dengue, local anti-mosquito measures cannot be the only reason why no one contracted chikungunya [14].

Our study has some limitations. First, selection bias may have occurred, as all participants were seeking pre-travel health advice when recruited and thus perhaps had a higher health awareness. Second, we did not collect information about specific areas that participants visited in the countries we studied. Therefore, we do not know if participants specifically avoided wet-season-related outbreak areas which might have

influenced our incidence findings. Third, the diagnostic test we used probably does not have a 100% sensitivity and could therefore underestimate the true incidence. Fourth, self-reported diaries introduce some bias, though they might be more accurate than recall influenced post-travel questionnaires. Finally, to our knowledge, prospective estimates of the incidence of chikungunya among travellers have not been published before. Probably, the incidence of chikungunya was much lower than for dengue at the time of our study. Therefore, the sample size of our prospective cohort might have been too small to reflect a solid incidence.

CONCLUSION

No CHIKV infections were found in this 2008-2011 prospective study among long-term Dutch travellers. Due to the extensive spread of the virus in the Americas since 2013, incidence rates among travellers have likely increased. We therefore recommend the study be repeated, preferably in a larger cohort of travellers. Travellers should be well informed about emerging arthropod-borne infectious diseases and urged to take appropriate anti-mosquito measures.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study protocol was approved by the Medical Ethics Committee of the Academic Medical Center of Amsterdam (MEC 08/064). Participants were included after providing informed and written consent.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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5

Dengue virus infection among long-term travelers from the Netherlands, a prospective study, 2008-2011

Femke W Overbosch, Janke Schinkel, Ineke Stolte, Maria Prins, Gerard JB Sonder

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ABSTRACT

Background

Dengue is increasing rapidly in endemic regions. Data on incidence among travelers to these areas are limited. Five prospective studies have been performed thus far, mainly among short-term travelers.

Objective

To obtain the attack and incidence rate (AR, IR) of dengue virus (DENV) infection among long-term travelers and identify associated risk factors.

Methods

A prospective study was performed among long-term travelers (12-52 weeks) attending the Public Health Service in Amsterdam. Clients planning to travel to (sub)tropical countries were invited to participate. Participants kept a travel diary, recording itinerary, symptoms, and physician visits. Pre- and post-travel blood samples were serologically tested for the presence of Anti-DENV IgG antibodies. Seroconversion was considered suggestive of a primary DENV infection. Anti-DENV IgG present in both corresponding samples in combination with a post-/pre-travel ratio of ≥4:1 was suggestive of a secondary infection. Risk factors for a DENV infection were studied using poisson regression.

Results

In total, 600 participants were included; median age was 25 years (IQR: 23-29), 35.5% were male, and median travel duration was 20 weeks (IQR: 15-25).

In 39 of 600 participants (AR: 6.5%; 95% CI 4.5-8.5%) anti-DENV IgG test results were suggestive of a recent infection, yielding an IR of 13.9 per 1,000 person-months traveling (95%CI: 9.9-19.1). No secondary infections were found. IR for Asia, Africa, and America were comparable and 13.5, 15.8, and 13.6 per 1,000 person-months respectively. Of participants with a recent DENV infection, 51% did not report dengue-like illness (DLI) or fever, but 10% were hospitalized.

In multivariable analysis, travelers who seroconverted were significantly more likely to be vaccinated with ≥2 flavivirus vaccines for the current trip or to have reported DLI in >1 consecutive weeks.

Conclusion(s)

Long-term travelers are at substantial risk of DENV infection. Half of those with a DENV infection reported no symptoms, but 10% were hospitalized, demonstrating the importance of advising anti-mosquito measures during travel.

INTRODUCTION

Dengue is an arthropod-borne viral disease found in (sub)tropical regions and is endemic in more than 100 countries in Africa, the Americas, the Eastern Mediterranean, South East Asia, and the Western Pacific. The disease is transmitted by Aedes mosquitoes, and caused by four serotypes of dengue virus (DENV-1, -2, -3, and -4) (1). An estimated 3.9 billion people worldwide are at risk (1). Infection with a particular serotype provides lifelong immunity against that serotype after recovery, but cross-immunity to the other serotypes is only partial and temporary (1). Infection is often asymptomatic or subclinical, but can produce a wide range of illness in which symptoms vary from a mild febrile self-limiting illness to a severe disease (2). An estimated 390 million infections occur per year (3). Individual risk factors determine the severity of disease and include age (such as infants with a primary infection born to dengue-immune mothers or children with a secondary dengue infection), ethnicity (white individuals), certain chronic diseases, and secondary DENV infection with a different serotype (2). No specific curative treatment is available and although DENV vaccines have been developed, they are not yet available for travelers (4).

Transmission in endemic areas has increased and the proportionate morbidity of DENV among travelers returning ill has risen (5). In endemic areas, predominantly in (semi-) urban settings, the increase is related to demographic and societal changes, such as the unprecedented population growth and uncontrolled urbanization of the past 50-60 years (2). The worldwide incidence has increased 30-fold, though it is not clear if this is related to the increase among travelers.

Clinical surveillance studies are not suitable to calculate the risk of DENV infections among travelers, as they disregard asymptomatic and subclinical DENV infections, and do not include data regarding numbers of travelers (6). Only five prospective studies performed in the last two decades calculated incidence rates of all DENV infections in cohorts of travelers to endemic countries, of which four predominantly focused on short-term travelers (7-10) and one on participants who traveled 3-6 months (11).

To gain more insight into the risk of DENV infection for long-term travelers, we prospectively studied Dutch long-term travelers who traveled ≥12 and ≤52 weeks to (sub) tropical countries. We estimated the prevalence (P) before traveling and the attack rate (AR) and incidence rate (IR) of DENV infections during travel. In addition, we identified risk factors associated with DENV infection.

METHODS

Study population

A prospective mono-center study of immunocompetent Dutch travelers was conducted at the Public Health Service travel clinic in Amsterdam from December 2008 to September 2011. All clients aged ≥18 years planning to travel to any (sub)tropical country in Sub-Saharan Africa, Central America, the Caribbean, South America, or Asia for ≥12 and ≤52 weeks were invited to participate. All participants were seen by a medical doctor or nurse specialized in travel medicine and were advised according to Dutch National Guidelines on Traveler's Health Advice (12), including oral and written information about how to avoid mosquito-borne infections.

Ethics statement

The study protocol was approved by the Medical Ethics Committee of the Academic Medical Center of Amsterdam (MEC 08/064). Participants were included after obtaining written informed consent.

Study procedures

A standardized questionnaire in Dutch or English was used to collect data before departure on socio-demographics, travel history, vaccination status, and purpose of travel (tourism, work/education, visits to friends and/or relatives). Participants were given a digital thermometer (Huikeshoven Medical, Tiel, the Netherlands) and asked to take their temperature if they felt feverish. They were also asked to keep a structured, weekly travel diary, recording their itinerary, signs of disease, use of insect repellent containing N,N-diethyl-meta-toluamide (DEET), physician visits, diagnoses, and possible (self) treatment. To encompass incubation periods of DENV infection, participants made weekly diary entries from the first week of travel to two weeks after their return. Diaries were filled out on paper or digitally, and travelers received a weekly email as reminder. Blood samples for serologic testing were taken once during their pre-travel advice and once during a visit 2-6 weeks after return.

Dengue-endemic countries

All (sub)tropical countries between the ten degrees January and July isotherm were considered dengue-endemic (13). Although Spain, Italy, Turkey, United States, Japan, New Zealand, and Australia are countries (partially) within the ten degrees isotherms, these countries have good surveillance and therefore we know that there is no dengue risk or that the risk is limited to areas not frequently visited by travelers. Therefore, we considered these countries as "non-dengue-endemic country". All countries outside the 10 degrees isotherms were also designated as "non-dengue-endemic country".

Laboratory methods

Blood samples were immediately stored at 6°C, then centrifuged and frozen at -80°C within 24 hours after collection. After all study participants had returned, all post-travel serum samples were thawed and tested for IgG antibodies to DENV antigen serotypes 1, 2, 3, and 4 by using an indirect ELISA (Panbio Diagnostics, Brisbane, Queensland, Australia) according to manufacturer's instructions. For participants whose post-travel sample yielded positive test results, pre-travel samples were also tested for anti-DENV IgG.

The ELISA for anti-DENV IgG has a sensitivity of 90-100% and a specificity of 90-98% (14-16). These test characteristics concern the use of paired serum samples. The presence of anti-DENV IgG in the pre-travel sample was considered suggestive of a previous DENV infection. The presence of anti-DENV IgG in the post-travel sample together with no anti-DENV IgG in the pre-travel sample was considered suggestive of acquiring a primary DENV infection during travel in the study period. We assumed that participants with a previous infection were still at risk for a secondary infection. Therefore, anti-DENV IgG in both the post- and pre-travel sample, but with a post-travel-to-pre-travel ratio of ≥4:1, was considered suggestive of a recent secondary DENV infection.

Statistical analysis

To study the association between possible symptoms of a DENV infection and the presence of anti-DENV IgG, dengue-like illness (DLI) was defined as fever (temperature ≥38°C) with one of the following symptoms: myalgia, arthralgia, headache, retro-orbital pain, or skin rash (7, 8). We defined 'Extended DLI' as DLI reported in ≥2 consecutive weeks. To study possible cross-reactivity of antibodies resulting from previous flavivirus vaccinations, we analyzed whether these vaccinations were predictive of the presence of anti-DENV IgG. For this purpose, we defined 'vaccination status' at the pre-travel visit as the total number of previously received yellow fever (YF), Japanese encephalitis (JE), and tick-borne encephalitis (TBE) vaccines. Vaccination status at the post-travel visit consisted of all additional YF/JE/TBE vaccinations received for the current travel. Due to small numbers of visiting participants per country, the visited countries were analyzed at continent level. If a traveler visited more than one continent, the continent in which the traveler spent most time was designated as the visited continent (17). The use of DEET was quantified by dividing the number of weeks that the use of DEET was reported, by the number of weeks spent in dengue-endemic areas.

The following variables were examined as possible determinants of previous DENV infection at the pre-travel visit: sex, age, country of birth, total duration at previous travel destinations, and vaccination status (previously administered flavivirus vaccines). In the analysis of recent DENV infection, the following possible risk factors were examined: sex,

age, country of birth, vaccination status (flavivirus vaccines administered before current travel), purpose of travel, (extended) DLI, and use of DEET.

Prevalence of previous DENV infection and the corresponding 95% confidence interval were calculated. We examined whether DENV prevalence differed by characteristics using univariable logistic regression. Variables with a p value <0.1 in univariable analysis were included in the multivariable model. The AR of recent DENV infection was calculated by dividing the number of study participants with a recent DENV infection by the total number of participants at risk. Incidence rate, incidence rate ratios (IRR), and 95% confidence intervals of recent DENV infections by potential risk factors were analyzed using poisson regression. Variables with a p value <0.1 in univariable analysis were included in the multivariable model. A p value <0.05 was considered statistically significant. As participants could have visited both dengue- and non-dengue-endemic countries, only the time spent in dengue-endemic countries was used as denominator to calculate DENV incidence rates. For those who became DENV infected while traveling, the moment of infection was estimated as the midpoint between the arrival and departure date in dengue-endemic countries, and therefore person-time denominators were divided in half. All analyses were conducted using STATA Intercooled version 13 (College Station, TX, USA).

RESULTS

Characteristics of the study population

Between December 2008 and September 2011, 685 subjects who intended to travel to (sub)tropical countries for 13-52 weeks provided informed consent. Of these, 80 (12%) were excluded upon completion of the study: 42 had their travel arrangements changed, and 38 were lost to follow-up. We excluded another 4 individuals due to a missing pre- or post-travel blood sample, and one individual whose subtropical itinerary appeared not to include any dengue-endemic areas. We did not exclude three participants who traveled a few days less than 12 weeks, nor 5 participants who had traveled a few days over 52 weeks.

The median age of the 600 participants included in the present study was 25 years (interquartile range [IQR]: 23-29), 35.5% were male, 97.5% were born in a non-dengue-endemic country, median travel duration was 20 weeks (IQR: 15-25), and purpose of travel was predominantly tourism (62.2%) (Table 1). The median interval between return from travel and post-travel blood donation was 25 days (IQR 21-33). Fifty-five participants (9.2%) traveled to countries in ≥2 continents, of whom 24 participants were allocated as visitors to Asia, 24 to Latin America, and 7 to Africa. One participant visited Tonga (Oceania) exclusively and was counted as a visitor to Asia for simplicity purposes.

Table 1 Characteristics of 600 long-term travelers attending a Dutch travel health clinic for pre-travel advice including prevalence and determinants of previous dengue infection, December 2008 – September 2011.

Characteristic	Total, no	%	Previous DENV*		Univariable analysis				Multivariable analysis			
			No.	P %	OR	95% CI		p value	OR	95% CI		p value
						lower	upper			lower	upper	
No. Participants	600	100	19	3.2								
Sex								0.901				
Female	387	64.5	12	3.1	1							
Male	213	35.5	7	3.3	1.06	0.41	2.7					
Median age, y (IQR)	25 (23-29)				1.1	1.0	1.1	0.017				
Age, y								0.083				0.383
< 24	203	33.8	3	1.5	1				1			
24-29	261	43.5	8	3.1	2.1	0.55	8.0		1.8	0.47	7.1	
≥ 30	136	22.7	8	5.9	4.2	1.1	16.0		2.6	0.63	10.9	
Country of birth								0.491				
Non-dengue-endemic country	585	97.5	18	3.1	1							
Dengue-endemic country	15	2.5	1	6.7	2.3	0.28	18.1					
Total duration at previous travel destinations, mth								0.006				0.025
< 1	263	43.8	2	0.76	1				1			
1-3	114	19.0	6	5.3	7.3	1.4	36.5		6.6	1.3	33.6	
> 3	223	37.2	11	4.9	6.8	1.5	30.9		5.0	1.0	24.6	
Vaccination status: total of pre-travel YF/JE/TBE** vaccinations								0.221				
0	377	62.8	9	2.4	1							
1	202	33.7	8	4.0	1.7	0.64	4.4					
≥ 2	21	3.5	2	9.5	4.3	0.87	21.3					

* DENV= Dengue virus. ** YF= yellow fever, JE= Japanese encephalitis, TBE= tick-borne encephalitis

Vaccination status

Before inclusion, 223 participants (37%) had received one or more flavivirus vaccinations: 217 travelers (36%) had received ≥1 prior yellow fever vaccination, 8 (1%) had ≥1 Japanese encephalitis vaccination, and 4 (1%) ≥1 tick-borne encephalitis vaccination. For the current trip, 177(30%) received ≥1 YF/JE and/or TBE vaccinations, including 18 previously vaccinated individuals. Of these 177, 8 participants received ≥2 vaccinations: all 2 or 3 JE vaccinations for a trip to Asia.

Previous DENV infection

Anti-DENV IgG in the pre-travel sample suggestive of previous DENV infection were found in 19/600 participants (3.2%; 95% CI 17.6-45.7) (Table 1). In univariable logistic regression analysis, previous DENV infection was associated with older age and longer duration of previous travels to (sub)tropical regions, but not with vaccination status, gender, and country of birth (yes/no dengue-endemic country). In multivariable analysis, only previous duration of travel remained significantly associated with a previous DENV infection.

Recent DENV infection

In 39 of 600 participants the anti-DENV IgG test results were suggestive of recent DENV infection (AR = 6.5%; 95% CI 4.5-8.5); the IR was 13.7 per 1,000 person-months (95% CI 9.8-18.8) (Table 2). The attack rate was 4.2% (7/166) for those traveling 12-15 weeks, 5.7% (9/158) for those traveling 16-20 weeks, 8.9% (13/146) for those traveling 21-25 weeks and 7.7% (10/130) for those traveling ≥26 weeks.

The median age of the 39 participants with recent DENV infection was 27 years (IQR: 23-35), 17 (44%) subjects were male, and median total travel duration was 22 weeks (IQR: 17-26). All 39 participants were born in a non-endemic country. One participant with a recent DENV infection traveled in both Latin America and Asia. As he spent most days in dengue-endemic areas in Latin America, he was categorized as a traveler to Latin America, making the incidence rate of recent DENV infection 13.5 (95% CI: 8.3-22.0), 15.8 (95% CI: 7.9-31.7), and 13.6 (95% CI: 8.2-22.5) for Asia, Africa, and Latin America respectively (Fig 1). None of the 39 travelers with recent DENV infection had evidence of anti-dengue antibodies in the pre-travel sample suggesting they all had a primary DENV infection.

In total, DLI was reported in 262 weeks by 178/600 (30%) participants. Among the 39 participants with recent DENV infection, DLI was reported by 16 (41%) travelers (29 weeks in total) and included 5 participants with a period of extended DLI. Three travelers with recent DENV infection (8%) reported fever during travel but did not meet the DLI criteria,

and the other 20 travelers with a recent DENV infection (51%) did not report any episodes of fever at all. Of all travelers, 26/600 (4.3%) participants were hospitalized, including 4 of the 39 travelers (10%) with recent DENV infection. All four were hospitalized during a period of extended DLI, but none of them reported signs or symptoms of a hemorrhagic fever. Whereas DLI was not predictive of the presence of anti-DENV IgG in the post-travel sample in the univariable analysis, the effect of extended DLI was significantly increased (IRR 3.1, 95% CI 1.2-7.8) compared to participants without extended DLI.

Vaccination status (all additional YF/JE/TBE vaccinations received for the current trip) was also predictive of recent DENV infection; the IRR was significantly higher for participants who received 2 or more vaccinations compared to those who did not receive additional vaccinations (IRR 8.2, 95% CI 2.5-27.4).

The effect of sex, age, purpose of travel, visited continents, and use of DEET was not significant. In the multivariable model, both extended DLI and the vaccination status remained significantly related to a recent DENV infection.

Our analysis suggested that the 3 recent DENV infections among participants who had received 2 or more JE-vaccinations for a trip to Asia, could be false-positive due to cross reaction caused by these vaccinations. Therefore, we recalculated the attack and incidence rate without these cases. The AR was now 6.0% (36/597), the IR was 12.9/1,000 person-months (95% CI 9.1-17.9), and the IR_{Asia} decreased to 11.0/1,000 person-months (95% CI 6.4-18.9). Vaccination status (all additional YF/JE/TBE vaccinations received for the current trip) was now no longer predictive of a recent DENV infection in univariable analysis; the IRR was comparable for participants who received 1 or more vaccinations compared to those who did not receive additional vaccinations (IRR 1.2, 95% CI 0.59-2.3, p=0.648). Only extended DLI remained significantly related to a recent DENV infection.

DISCUSSION

In this prospective study of long-term travelers to (sub)tropical countries we found a substantial AR and IR for DENV infection during travel. Our estimated AR ranged from 6.0% to 6.5% and is higher than found in previous prospective studies among short-term travelers in which ARs ranged between 1% and 2.9% (7-10). The only other prospective study among long-term travelers (3-6 months) found a comparable AR of 6.7% (11). As expected, the duration of travel influences the AR, while it does not influence the incidence rate. Hence, unlike the AR, the DENV incidence observed in our study was comparable to those reported in other prospective studies, which varied from 6.7 to 30

Figure 1 World map: Countries visited by the participants with evidence suggestive of recent dengue virus infection.
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Table 2 Characteristics of 600 long-term travelers to dengue-endemic areas attending a Dutch travel health clinic for pre-travel advice including their incidence rates and risk factors of suggestive recent dengue virus infection, December 2008 – September 2011.

Characteristics	Total			Recent DENV*		Univariable analysis				Multivariable analysis			
	No.	%	Person-months	No.	IR/1000 pm	IRR	95% CI		p value	IRR	95% CI		p value
							lower	upper			lower	upper	
No. participants	600	100	2796.0	39	13.7								
Sex									0.412				
Female	387	64.5	1756.6	22	12.5	1							
Male	213	35.5	1039.5	17	16.4	1.3	0.69	2.5					
Age, y									0.285				
< 24	203	33.8	883.7	10	11.3	1							
24-29	261	43.5	1225.0	15	12.2	1.1	0.49	2.4					
≥ 30	136	22.7	687.4	14	20.4	1.8	0.80	4.1					
Purpose of travel													
Tourism	373	62.2	1656.3	27	16.3	1			0.333				
Work/education	172	28.7	849.6	10	11.8	0.72	0.35	1.5					
VFR/other	55	9.2	290.1	2	6.9	0.42	0.10	1.8					
Visited continents									0.926				
Asia	268	44.7	1187.2	16	13.5	1							
Africa	107	17.8	505.2	8	15.8	1.2	0.50	2.7					
Latin America	225	37.5	1103.7	15	13.6	1.0	0.50	2.0					
Vaccination status: all additional YF/JE/TBE** vaccinations for current trip†									0.027				0.020
0	423	70.5	1967.0	24	12.2	1				1			
1	169	28.2	799.2	12	15.0	1.2	0.62	2.4		1.3	0.64	2.6	
≥ 2	8	1.3	29.8	3	100.6	8.2	2.5	27.4		9.3	2.8	31.1	

Table 2 Characteristics of 600 long-term travelers to dengue-endemic areas attending a Dutch travel health clinic for pre-travel advice including their incidence rates and risk factors of suggestive recent dengue virus infection, December 2008 – September 2011. (*continued*)

Characteristics	Total			Recent DENV*		Univariable analysis				Multivariable analysis			
	No.	%	Person-months	No.	IR/1000 pm	IRR	95% CI		p value	IRR	95% CI		p value
							lower	upper			lower	upper	
Use of DEET***, percentage of total travel duration									0.932				
< 25	173	28.8	869.9	12	13.8	1							
25-49	124	20.7	559.8	9	16.1	1.2	0.49	2.8					
50-74	111	18.5	525.5	6	11.4	0.83	0.31	2.2					
≥ 75	192	32.0	840.8	12	14.3	1.0	0.46	2.3					
Dengue-like illness^									0.196				
No	422	70.3	1923.9	23	12.0	1							
Yes	178	29.7	872.1	16	18.3	1.5	0.81	2.9					
Extended dengue-like illness^^									0.041				0.028
No	574	95.7	2668.1	34	12.7	1				1			
Yes	26	4.3	127.9	5	39.1	3.1	1.2	7.8		3.4	1.3	8.8	

* DENV= Dengue virus. ** YF= yellow fever, JE= Japanese encephalitis, TBE= tick-borne encephalitis.

*** DEET= N,N-diethyl-meta-toluamide. The use of DEET was quantified by dividing the number of weeks that the use of DEET was reported, by the number of weeks spent in dengue-endemic areas. † Vaccination status in this table includes all additional flavivirus vaccines received from pre-travel until post-travel visit.

^ DLI was defined as fever (temperature ≥38°C) with one of the following symptoms: myalgia, arthralgia, headache, retro-orbital pain or skin rash. In this table, DLI was considered positive if a traveler reported DLI in ≥ 1 week. ^^ 'Extended DLI' includes all travelers who reported at least DLI in ≥2 consecutive weeks.

per 1000 person-months among the predominantly short-term travelers, and was 11 per 1000 person-months among the long-term travelers (7-11). Comparison of results between different prospective studies should, however, be interpreted with caution. The risk for travel-related DENV infection depends not only on endemicity, but also on outbreaks in a particular country during a particular time of travel. As the journeys of the participants were scattered over countries and over time, the influence of possible rain seasons or outbreaks in this cohort of long-term travelers could not be studied. Also, comparison of prospective studies with studies based on surveillance data of travelers returning ill should be done with greatest caution: increased awareness of dengue in hospitals and improvements in diagnostic procedures could also have influenced the increase of diagnosed DENV infections in surveillance data (5).

Similar to previous prospective studies, we did not find differences between IRs among travelers to Asia, Latin America, and Africa (7, 9, 11). Therefore, it is remarkable that dengue is still diagnosed relatively less often in febrile returning travelers from Africa compared to Asia and Latin America (5). DENV diagnosis may be missed in patients returning from Africa, as it is not considered in the differential diagnosis.

In our study, 51% of the participants with a recent DENV infection did not report any episode of fever suggesting an asymptomatic infection. On the other hand, 16/39 of the travelers with test results suggestive of a DENV infection during their trip reported DLI at least once, 5 of whom reported extended DLI. Although DLI was not related to a recent DENV infection, participants who reported extended DLI were more likely to have a recent DENV infection. Possibly because DLI includes common and aspecific symptoms like headaches, brief episodes of DLI are mostly caused by other infections with fever, whereas extended and more severe symptoms of DLI are more specific for a recent DENV infection. The fact that all four hospitalizations among the travelers with a recent DENV infection occurred in the 5 participants with an extended DLI episode supports this hypothesis. Considering the possible severity of the disease, it is still important that travelers are advised pre-travel to take proper anti-mosquito measures, especially as no dengue vaccine is yet available for travelers.

Selection bias may have influenced risk estimates found in studies among cohorts of travelers, including ours. First, travelers often choose to avoid areas if an outbreak is reported, in contrast to people living in endemic regions. This could partly explain why incidence rates of DENV virus infection among travelers seems to have remained rather similar in the past two decades, despite an increase in reported cases among populations in endemic countries. Furthermore; participants who seek pre-travel health advice may have higher than average health awareness, particularly once they learn about the

study, agree to participate, and keep a diary during travel. This could have led to an underestimation of the true incidence of DENV infections.

Although travelers who had received one flavivirus vaccination for the current trip were not more likely to acquire a recent DENV infection than travelers who did not receive any additional flavivirus vaccines, the 8 travelers who received 2 or more flavivirus vaccines were significantly more likely to acquire a recent DENV infection. As all of these eight participants had received JE vaccines (vs. 2 out of 169 participants who had 1 additional JE vaccine), cross-reaction between JE and anti-DENV IgG cannot be excluded. However, when the potential false-positive cases were excluded, the AR and IR only decreased slightly. Therefore, at most, it could be considered of limited influence on the outcome of this study. Although not significantly, Asia's IR became notably lower than both the IRs of Africa and Latin America after excluding the potential false positives. This was somewhat surprising as a previous review article concluded that most cases of dengue infections in febrile travelers were diagnosed among travelers from Asia (18). It reinforces our conclusions that some of these cases were possibly true infections and other unmeasured confounders (e.g., the kind of travel (low budget/adventurous) or infection with yet another flavivirus) could also have been of influence, as all participants with ≥2 JE vaccines traveled to Asia.

Thus far no true standard exists to serologically confirm or rule out dengue after an infection. We considered testing anti-DENV IgM as well as anti-DENV IgG in our cohort, but argued this would have been of limited added value for our group, as all participants had been traveling for a substantial period and most of them donated their post-travel blood sample at least two weeks after return. We therefore assumed it to be highly unlikely for travelers to contract DENV in their last days of travel. As a consequence, positive post-travel IgM results without any detectable IgG-levels would have been unlikely, and waning IgM-levels could have led to negative post-travel results. The use of a plaque-reduction neutralization test, which is considered the laboratory standard, was not part of the study protocol, but could also have cross-reacted with other flavivirus antibodies (19). However, the study was performed before the large Zika virus outbreaks in the Americas; therefore cross-reaction with Zika is probably of little influence. Furthermore, we found no significant relation between flavivirus vaccines and the presence of anti-DENV antibodies in previous serology-based studies (7, 20).

Finally, some caution is required in the interpretation of our data, as data collection happened through self-reported weekly diaries. Participants could have interpreted the weekly questions differently. For example, some may have ticked 'used of DEET' if they used DEET at least once, others may have ticked itif they used DEET every day

that specific week. This might have influenced the results regarding 'use of DEET' as a predictor for DENV infection. However, as diaries decrease the effect of recall bias, we do not consider this limitation to have significantly affected other findings.

CONCLUSION

This is the second prospective study investigating DENV infection among long-term travelers. It confirms that the incidence rate of DENV infection among long-term travelers is substantial. As expected, the attack rate was higher among long-term than among previously investigated short-term travelers. Half of the travelers with a recent DENV infection reported no symptoms of dengue-like illness, suggesting they had asymptomatic infections, but almost all DENV-infected travelers who reported DLI symptoms in >1 consecutive weeks were hospitalized.

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SUPPORTING INFORMATION

SI 1 file Permission form for Fig 1
Available at: https://doi.org/10.1371/journal.pone.0192193.s001

S1 Dataset. Dataset long-term travelers (de-identified).
Available at: https://doi.org/10.1371/journal.pone.0192193.s002

Part 1b

Travel-acquired infections among VFR and tourist travelers to Suriname

6

High prevalence of previous dengue virus infection among first-generation Surinamese immigrants in the Netherlands

Femke W Overbosch, Anneke van den Hoek, Janke Schinkel, Gerard JB Sonder

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ABSTRACT

Background

A substantial portion of Dutch travellers is comprised of immigrants returning to their country of origin to visit friends and relatives (VFRs), including VFRs returning to dengue-endemic areas such as Suriname. Limited attention has been focused on dengue among immigrants, therefore it is unknown whether immigration has effect on the epidemiology of (severe) dengue among VFRs.

To get more insight in the seroprevalence of dengue among Surinamese immigrants, we conducted a seroprevalence study on a convenience sample of first-generation Surinamese immigrants living in the Netherlands.

Methods

Blood samples were tested for IgG antibodies to DENV antigen serotypes (1, 2, 3 and 4). Gender, age, years lived in Suriname before immigration, history of yellow fever vaccination, and time between yellow fever vaccination and blood sample collection were examined as possible predictors for previous infection.

Results

Of the studied 400 Surinamese travellers with a mean age of 52 years (range 18–89), 37% were male. Serology suggestive of past DENV infection was found in 325 individuals (81.3%; 95% CI: 77-85%). The time lived in Suriname before immigration was the only significant predictor for previous DENV infection.

Conclusions

Most first-generation Surinamese immigrants have evidence of past DENV infection, probably comparable to Surinamese inhabitants. Whether this influences the number of cases of (severe) dengue when travelling requires more study.

BACKGROUND

Dengue is a mosquito-borne infection found in tropical and sub-tropical regions. The spectrum of clinical manifestations of dengue varies from a mild febrile self-limiting illness to a severe, potentially fatal disease. Substantial gaps remain in the basic understanding of the pathogenesis. Known is that there are four distinct, but closely related, serotypes of the virus that cause dengue (DENV-1, -2, -3 and -4). Recovery from infection by one serotype provides lifelong immunity against that particular type (1). Hypothesized and strengthened by epidemiologic studies (2,3) is that subsequent infection by other serotypes increases the risk of developing "severe dengue" also known as Dengue Haemorrhagic Fever.

In recent years, transmission in endemic areas has increased, predominantly in urban and semi-urban settings, and has become a major international public health concern. The disease is now endemic in more than 100 countries in Africa, the Americas, the Eastern Mediterranean, South East Asia and the Western Pacific, the latter two being the most seriously affected. Over 2.5 billion people (which is over 40% of the world's population) are at risk (1). The WHO estimates there may be 50–100 million dengue virus (DENV) infections worldwide every year. An estimated 500,000 people with severe dengue require hospitalisation each year, a large proportion of whom are children. About 2.5% of those affected die (1).

The Netherlands is not a dengue-endemic area; therefore Dutch citizens are not at risk of contracting a DENV infection in their home country. On the other hand, Dutch travellers are at substantial risk for DENV infection when travelling to endemic areas. A Dutch prospective study among short-term travellers conducted in 2006–2007 showed a serology-based attack rate of 1.2% and an incidence rate of 14.6 per 1000 person-months (4).

A substantial portion of Dutch travellers is comprised of immigrants returning to their country of origin to visit friends and relatives (VFRs), including VFRs returning to dengue-endemic areas such as Suriname, a former Dutch colony in the Caribbean (population 492,000 people) (5). In 2010, 101,578 travellers from the Netherlands arrived in Suriname (6).

Although previous reports investigated the seroprevalence of dengue among people living in dengue endemic areas, limited attention has been focused on dengue among immigrants. Immigration to a non dengue endemic area causes deviation of exposure to DENV among immigrants compared to inhabitants of dengue endemic areas. Continuous exposure to DENV shifts to sporadic exposure during visits to the country of origin,

which probably has consequences for the moment of encounter with a secondary, and potentially more severe, DENV serotype among immigrants. As far as we know, no research has been performed on dengue seroprevalence rates among Surinamese immigrants, nor among Surinamese nationals in their home country. Taking into account that different serotypes have been introduced in the Americas in past decades (7) and that predominant DENV serotypes can vary by year (8), immigration could influence the epidemiology of (severe) dengue among Surinamese immigrants. To get more insight in the seroprevelance among this group of travellers, we conducted a seroprevalence study among first-generation Surinamese immigrants living in the Netherlands who sought travel health advice at the Public Health Service's Travel Clinic in Amsterdam.

METHODS

Study population and design

A serum bank was used for this study, which consisted of blood samples of Surinamese first-generation immigrants who attended the Public Health Service's Travel Clinic in Amsterdam from February 2008 to December 2011. These participants had been tested for immunity against hepatitis A or hepatitis B. Inclusion criteria for the hepatitis A and B immunity project were to have been born before 1970 or 1989, respectively. Data were collected concerning date of birth, gender, vaccination record, country of origin of both the participant and parents, and duration lived in country of origin. All participants had provided written informed consent to use the remains of the blood sample for anonymous scientific research on other infectious diseases. Following the rules stated in the Medical Research Involving Human Subjects Act (in Dutch: Wet Medisch-wetenschappelijk Onderzoek [WMO]), the informed consent letters for the hepatitis A and hepatitis B study were reviewed by the Medical Ethical Committee of the Academic Center of Amsterdam. The Medical Ethical Committee of the Academic Medical Center of Amsterdam approved all documents of the hepatitis A study (MEC 07/270), and reviewed all documents of the hepatitis B study and judged that evaluation of the hepatitis B study was not required (MEC 09/014). Therefore, we did not seek further waiver or approval for this specific dengue virus study.

Laboratory

Blood samples were immediately stored at 6°C, then centrifuged and frozen at −80°C within 24 hours after collection. After being thawed they were tested for total immunoglobulin (IgG) antibodies to DENV antigen serotypes 1, 2, 3 and 4 by using an indirect ELISA (Panbio Diagnostics, Brisbane, Queensland, Australia) according to the manufacturer's instructions. Test outcomes for DENV IgG antibodies were expressed as signal-to-

cutoff ratios (s/co) and were interpreted according to the manufacturer's instructions: ratios <0.9 were considered negative and thus as no evidence for past DENV infection, ratios ≥ 1.2 were considered reactive and thus as evidence for past DENV infection, and ratios 0.9-1.1 were considered boundary values.

Statistical analysis

Test outcomes for DENV IgG antibodies which were considered boundary values according to manufacturer's instructions, were allocated as negative for simplicity in statistical analysis.

SPSS for windows version 19.0 was used to obtain prevalence, prevalence ratios and 95% confidence intervals, by means of Poisson regression analysis with robust standard errors (9). All variables with an overall p-value < 0.1 in univariate analysis were included in a multivariate analysis.

RESULTS

The serum bank included 400 unique samples of first-generation Surinamese immigrants living in the Netherlands, who intended to travel to (sub-)tropical countries. Eleven participants (2.8%) did not meet the age inclusion criteria for the original study; 3 participants from the hepatitis A project were born after 1970, and 8 participants from the hepatitis B project were born after 1989. We did not exclude these participants in our analysis. The mean age was 52 years (range 18–89) and 37% were male.

In 325 participants, DENV IgG was present, suggesting past dengue virus infection, making the serologic results suggestive of previous dengue virus infection in 325/400 (81.3%; 95% CI: 77-85%) participants. Six participants (1.5%) had DENV IgG levels at boundary values and therefore considered as negatives. Table 1 shows the prevalence (Ps) and prevalence ratios (PRs) with univariate and multivariate 95% confidence intervals. In univariate analysis, the prevalence in Surinamese > 60 years was significantly higher than in Surinamese aged 40 years or younger and positively related to the duration participants had lived in Suriname before immigration. In multivariate analysis, only duration of living in Suriname before immigration remained a significant predictor for previous DENV infection. The seroprevalence was not related to history of yellow fever vaccination or time between yellow fever vaccination and blood sample collection.

Table 1 Characteristics of 400 first-generation Surinamese immigrants living in the Netherlands and their prevalence suggestive of previous dengue virus infection, February 2008 - December 2011

	Total	DENV IgG		Univariable analysis* PR (95% CI)	Multivariable analysis PR (95% CI)
		Positive	P%		
	n = 400	n = 325	81.3		
Sex					
male	148	119	80.4	1	
female	252	206	81.7	1.0 (0.92-1.1)	
Age (in years)					1
≤ 40	63	46	73.0	1	
41-50	112	90	80.4	1.1 (0.92-1.2)	1.5 (0.79-3.0)
51-60	117	94	80.3	1.1 (0.92-1.3)	1.6 (0.84-3.2)
≥ 61	108	95	88.0	1.2 (1.0-1.4)	1.4 (0.74-2.9)
Duration lived in Suriname (in years)					
≤ 15	79	43	54.4	1	1
16-20	112	88	78.6	1.4 (1.2-1.8)	1.4 (0.96-2.1)
21-25	90	82	91.1	1.7 (1.4-2.1)	1.6 (1.1-2.3)
≥ 26	115	108	93.9	1.7 (1.4-2.1)	1.7 (1.1-2.5)
History of yellow fever vaccination					
no	258	211	81.8	1	
yes (1 or 2)	142	114	80.3	0.98 (0.89-1.1)	
Time between (most recent) yellow fever vaccination and blood sample collection (in years)					
≤ 5	41	29	70.7	1	1
> 5-10	41	35	85.4	1.2 (0.96-1.5)	1.2 (0.94-1.4)
> 10-15	41	36	87.8	1.2 (0.99-1.5)	1.2 (0.94-1.4)
> 15	10	6	60.0	0.85 (0.49-1.5)	0.79 (0.47-1.3)

Participants attended the Public Health Service's Travel Clinic in Amsterdam for pre-travel advice.
P = prevalence.
PR = prevalence ratio.
* Variables that were significant at $p < 0.1$ were selected for inclusion in the multivariable model.

DISCUSSION

In this study the prevalence of dengue virus IgG antibodies among first-generation Surinamese immigrants was 81%. Although seroprevalence studies among Surinamese inhabitants are not available, this is comparable with the results from seroprevalence studies among populations in Latin America (10-13). It seems like immigration to a non dengue endemic country causes little difference in dengue seroprevalence between the Surinamese immigrants and the majority of populations living in dengue-endemic areas of the Americas and the Caribbean get infected with the dengue virus.

As expected, the seroprevalence of previous DENV infection in our study was positively related to the duration participants had lived in Suriname before immigration. This is in line with studies performed among persons living in dengue-endemic countries in Latin America where they found higher seroprevalence rates of DENV antibodies by increasing age, which can be seen as a marker for duration of exposure (13,14).

A limitation of this study is that the used ELISA was not DENV serotype specific, making the number of participants at risk for a secondary infection with a different serotype still unidentified. If we however hypothetically assume that most of the DENV IgG positive participants had only one previous DENV serotype infection, a high number of Surinamese VFRs would be at risk for a secondary, potentially more severe dengue. Especially, as before 1963 only DENV-2 American genotype was reported in the Americas, but since then the region has been subject to repeated importation of new dengue serotypes and strains (7). Over the last three decades a 4.6-fold increase in reported cases was observed in the Americas and even an 8.3-fold increase in DHF (15). In the past ten years, all 4 dengue serotypes have been circulating in Suriname (16,17).

The number of reported dengue cases or cases of severe dengue among Surinamese VFRs in Suriname or after return to the Netherlands, however, does not seem to be as high as one would expect. In 2010, the Pan American Health Organization (PAHO) reported only 113 clinical cases of dengue in Suriname (all lab confirmed) of which 20 cases were severe dengue including one death (16). It is likely, though, that a substantial number of under- or misdiagnosis occurs in Suriname as only lab-confirmed dengue virus infections have been reported. Also, the number of dengue cases or severe dengue infections in the Netherlands is not clear as dengue is not a notifiable disease in this country. However, severe dengue is extremely rare among Surinamese travelers returning to the Netherlands who attend the Academic Medical Center in Amsterdam, which serves an important portion of Surinamese immigrants in the Netherlands (in 2008, 338,000 Surinamese lived in the Netherlands of whom 20% lived in Amsterdam) (18). This low incidence of severe dengue among Surinamese could possibly be explained by host factors. Guzman's review summarises host factors that may reduce the risk of severe disease during a second dengue virus infection, which include race, second- or third-degree malnutrition, and polymorphisms in the Fcγ receptor and vitamin D receptor genes (19). Perhaps one or more of these factors can be applied to the Surinamese population.

Our study has some other limitations. Cross-reactivity between the dengue virus and other flaviviruses cannot be excluded. However, in our study, cross-reactivity with yellow fever vaccination is not likely since no relation was found with either previous yellow

fever vaccinations or time between yellow fever vaccination and sample collection. This is in agreement with the results of a study on the incidence of dengue virus infection among Dutch short-term travellers (4).

Second, the data used for this study is extracted from a serum bank of travellers visiting the Public Health Service's Travel Clinic in Amsterdam. The serum bank population may not be representative for the population of first-generation Surinamese living in the Netherlands and travelling to Suriname.

Third, data about duration lived in Suriname was self-reported by the participants. In part, this was also the case for history of yellow fever vaccination. Available data concerning these variables could thus deviate from the actual data, however, we do not consider these limitations to have significantly affected our findings.

Lastly, frequency and duration of stays in endemic areas after immigration was not incorporated as variables as these data was missing in the serum bank. This could have been of influence to the seroprevalence we found.

CONCLUSIONS

Most first-generation Surinamese immigrants living in the Netherlands display evidence of past dengue virus infection, probably comparable to Surinamese inhabitants. Surinamese immigrants have possibly been infected with fewer DENV serotypes, but whether this influences the number of cases of (severe) dengue is unknown. Incidence rates of severe dengue among Surinamese, a seroprevalence study among Surinamese inhabitants and serological tests which can discern the different DENV serotypes should be performed to uncover immigrants as a potential risk group for (severe) dengue. This could be of great importance for the development of specific dengue preventive policies.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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7

Dengue, chikungunya and Zika virus infections among Dutch travellers to Suriname: a prospective study during the introduction of chikungunya and Zika virus, 2014 to 2017

Femke W Overbosch, Janke Schinkel, Amy Matser, Gerrit Koen, Irene Prange, Maria Prins, Gerard JB Sonder

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ABSTRACT

Background

Suriname, a country endemic for dengue virus (DENV), is a popular destination for Dutch travellers visiting friends and relatives and tourist travellers. Chikungunya and Zika virus (CHIKV, ZIKV) were introduced in 2014 and 2015, respectively. Data on infection risks among travellers are limited.

Aim

We aimed to prospectively study incidence rate (IR) and determinants for DENV, ZIKV and CHIKV infection in adult travellers to Suriname from 2014 through 2017.

Methods

Participants kept a travel diary and were tested for anti-DENV, anti-ZIKV and anti-CHIKV IgG antibodies (Euroimmun). Selected samples were subjected to an in-house DENV and ZIKV PRNT50. The IR (infections/1,000 person-months of travel) and IR ratio and determinants for infection were calculated.

Results

Travel-acquired infections were found in 21 of 481 participants: 18 DENV, four ZIKV and two CHIKV, yielding an IR_{DENV} of 47.0 (95% CI: 29.6–74.6), IR_{ZIKV} of 11.6 (95% CI: 4.4–31.0) and IR_{CHIKV} of 5.6 (95% CI: 1.4–22.2)/1,000 person-months. In nine DENV and three ZIKV infected participants, infections were PRNT50-confirmed, yielding a lower IR_{DENV} of 23.3 (95% CI: 12.1–44.8) and an IR_{ZIKV} of 8.4 (95% CI: 2.7–26.1) per 1,000 person-months. Tourist travel was associated with DENV infection. ZIKV and CHIKV infections occurred soon after their reported introductions.

Conclusions

Despite an overestimation of serologically confirmed infections, Dutch travellers to Suriname, especially tourists, are at substantial risk of DENV infection. As expected, the risk of contracting ZIKV and CHIKV was highest during outbreaks. Cross-reaction and potential cross-protection of anti-DENV and -ZIKV antibodies should be further explored.

KEY PUBLIC HEALTH MESSAGE

What did you want to address in this study?

Travellers to Suriname can acquire dengue, and since 2014/2015 Zika and chikungunya as well. We wished to know the risks and risk factors of these three infections among travellers visiting friends and relatives and tourist travellers from the Netherlands.

What have we learnt from this study?

Dutch travellers run a substantial risk of dengue, especially the tourist travellers. We also found that participants who travelled during outbreak periods had a considerable risk to contract Zika or chikungunya.

What are the implications of your findings for public health?

The European areas where the mosquitoes live that can transmit dengue, Zika and chikungunya virus have expanded and are at risk for virus introduction by ill travellers, especially in summer months. Knowledge about the risk of dengue, Zika and chikungunya among travellers to popular endemic destinations informs targeted travel health advice to people most at risk, and can therefore reduce the risk of virus introduction in Europe.

INTRODUCTION

Several countries in Europe have close ties with specific tropical or subtropical countries, and travellers from connected European areas visit these (sub)tropical countries frequently. Suriname for example, a dengue-endemic country in South America with 575,763 inhabitants (2016) and a former colony of the Netherlands that gained its independence in 1975, is one of the most popular destinations among Dutch travellers to tropical and subtropical countries [1-3].

Dengue is a mosquito-borne viral disease with a clinical spectrum ranging from asymptomatic or mild influenza-like to severe disease, including death. Identification of risk groups for infection with dengue virus (DENV) during travel – both for primary and secondary infections – is important, as risk groups will benefit most from tailored prevention strategies including future vaccines. This is challenging, however, as retrospective research on travellers returning with illness underestimates incidence rates because it overlooks asymptomatic and mild infections. Also, clinical misdiagnosis can occur in countries endemic for Zika and chikungunya viruses (ZIKV and CHIKV), as the clinical spectrum of these mosquito-borne viruses may resemble dengue [4-5]. Finally, cross-reactivity of antibodies against flaviviruses such as DENV, ZIKV, yellow fever virus (YFV), tick-borne encephalitis virus (TBEV) and Japanese encephalitis virus (JEV) can occur and complicate interpretation of serological results [6,7].

So far, no cure exists, nor is a vaccine for travellers available [8]. Avoiding mosquito bites is currently the most appropriate prevention strategy. In addition, secondary DENV infection with a heterotypic serotype ($DENV_{1, 2, 3 \text{ or } 4}$), can be more severe, but tertiary DENV infections are rarely seen [6]. Presence of pre-existing DENV antibodies is therefore not conclusive for immunity, nor for severe disease. A previous study found pre-existing DENV antibodies among 81% of Surinamese migrants in Amsterdam [9]. Migrant travellers visiting friends and relatives (VFR) are known to be at increased risk of travel-related diseases in their country of origin, such as malaria, hepatitis A and typhoid fever, and might similarly be at increased risk for secondary DENV infection [10-12]. Identifying determinants for DENV infections among European travellers to tropical or subtropical countries such as Suriname will therefore be important.

To estimate the risk and determinants of travel-acquired DENV infection, we conducted a prospective study among VFR and tourist travellers to Suriname. As ZIKV and CHIKV were introduced on the American continent during the study period, we decided to expand the study with ZIKV and CHIKV infections.

METHODS

The aim was to prospectively study the attack rate (AR), incidence rate (IR) and determinants of travel-acquired DENV, ZIKV and CHIKV infections among Dutch travellers visiting friends and relatives and tourist travellers to Suriname.

Study population

Travellers to Suriname seeking pre-travel services at the Public Health Service (GGD) of Amsterdam from March 2014 through October 2017 were eligible to participate if born in Suriname (defined as VFR) or the Netherlands (defined as tourist). Inclusion criteria were age ≥ 18 years and intended travel duration of ≤ 3 months. All participants were seen by a medical doctor or nurse specialising in travel medicine and were advised according to Dutch National Guidelines on Traveller's Health Advice [13], receiving oral and written information about avoiding mosquito bites.

Study procedures

Before departure, a standardised questionnaire in Dutch was used to collect each traveller's socio-demographic data and vaccination history. Participants were given a digital thermometer (Domotherm Rapid 30 s, UEBE Medical GmbH, Wertheim, Germany) and asked to take their temperature if they felt feverish and also to keep a structured, daily paper travel diary until 2 weeks after return, recording their itinerary (in Suriname, the capital Paramaribo, coastal areas or inland; the Netherlands; other countries), their use of N,N-diethyl-meta-toluamide (DEET), presence of symptoms (fever if ≥ 38 °C, myalgia, arthralgia, headache, retro-orbital pain, diarrhoea, rash or other) and physician visits. An English translation of the full questionnaire is provided in the Supplement (part 1). After their return, the travellers were to present to the health service once more and their diaries were checked for clarity and completeness and, if necessary, complemented by a specialised nurse or physician together with the participant. The diaries were subsequently entered in a digital database using single data entry, in which unticked boxes – indicating absence of symptoms or treatment – were considered the default setting. Participants donated a blood sample pre-travel and during their return visit 2–4 weeks after return.

Laboratory methods

All blood samples were centrifuged, and serum samples were frozen at −80 °C within 24 h after blood donation. After all participants had returned, post-travel samples were serologically tested for immunoglobulin (Ig)G antibodies against DENV antigen serotypes 1, 2, 3 and 4. If the participants had consented, post-travel samples were also tested for anti-ZIKV IgG and anti-CHIKV IgG. Pre-travel samples were only tested in participants with a positive or borderline post-travel test result (Figure 1). Anti-DENV enzyme-linked

immunosorbent assay (ELISA) IgG, anti-ZIKV ELISA IgG and anti-CHIKV ELISA IgG tests were used according to manufacturer's instructions (Euroimmun, Lübeck, Germany). Reported sensitivity and specificity were, respectively, 98–100% and 100% for DENV, 100% and 97% for ZIKV, and 88% and 95% for CHIKV [14-16]. Paired sera were tested in participants with a positive or borderline post-travel test result (Figure 1). The presence of anti-DENV, anti-ZIKV or anti-CHIKV IgG in the pre-travel sample was considered suggestive of a previous infection. Seroconversion between paired sera was considered suggestive of a travel-acquired infection. An at least fourfold post- to pre-travel ratio of anti-DENV IgG was considered suggestive of a secondary travel-acquired DENV infection.

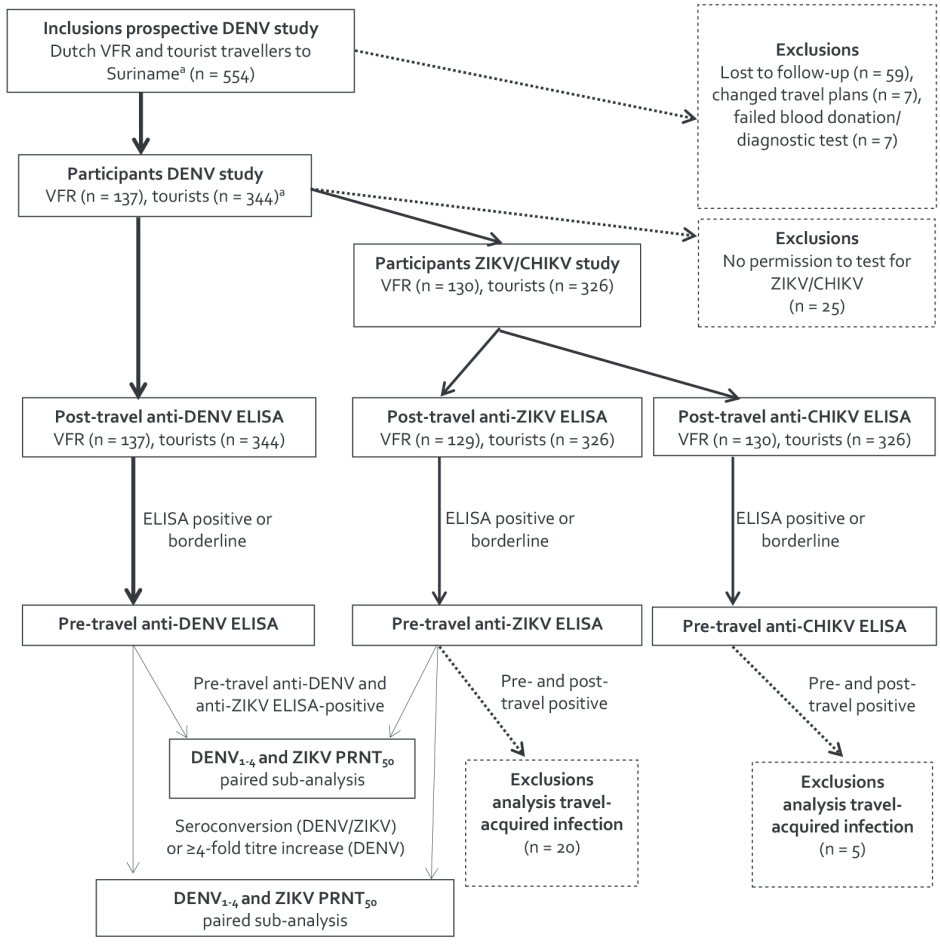

Figure 1 Prospective study among Dutch travellers to Suriname on travel-acquired dengue, Zika and chikungunya virus infections, the Netherlands, 2014–2017 (n = 554)

CHIKV: chikungunya virus; DENV: dengue virus; ELISA: enzyme-linked immunosorbent assay; PRNT: plaque-reduction neutralisation test; VFR: travellers visiting friends and relatives; ZIKV: Zika virus.

[a] 14 participants who donated their post-travel sample < 14 days after travel (range 9–13 days) were not excluded, as we assumed very limited bias by including them. Tourist travellers include 65 second-generation migrants.

To examine for possible cross-reactivity in the ELISAs, for budgetary reasons, we subjected a selection of samples to an in-house (Amsterdam University Medical Centers) 50% plaque-reduction neutralisation test (PRNT50) against DENV serotypes 1–4 and ZIKV [17]. The PRNT is a serological test in which antibodies neutralise virus by preventing the virus to form plaques in a cell monolayer. We compared plaque formation after addition of a participant sample (in which the antibodies of interest might be present) vs a control without an added sample. The PRNT50 laboratory procedures are described in the Supplement (part 2). Samples were selected for PRNT50 if (i) the pre-travel anti-DENV and anti-ZIKV ELISA yielded a positive test result or (ii) a travel-acquired DENV and/or ZIKV was found in paired ELISA results. The PRNT50 was considered (i) a confirmation of seropositivity for DENV and/or ZIKV if the pre-travel serum inhibited plaque formation by ≥ 50% compared with sample-free controls and (ii) a confirmation of seroconversion if in paired sera the PRNT50 result converted from < 50% to ≥ 50%, or a confirmed secondary DENV infection if the pre-travel PRNT50 result was ≥ 50% for one serotype combined with a PRNT50 result conversion in paired sera from < 50% to ≥ 50% for at least one other serotype.

Definitions

To examine a potential association due to cross-reaction between flavivirus vaccines and serological test results, we defined 'pre-travel vaccination status' as the total number of all flavivirus vaccinations against yellow fever (YF), Japanese encephalitis (JE) and tick-borne encephalitis (TBE) received before pre-travel blood donation (for example for previous travel), registered as in the participants' international certificate of vaccination and/or the public health service patient file system and/or self-reported by the participant. As pre-travel blood donation was incorporated in the pre-travel health consultation for a trip to Suriname, which potentially included vaccination against YF, the 'post-travel vaccination status' was defined as the total number of all additional YF, JE and TBE vaccinations received during or after the pre-travel blood donation. Use of DEET was quantified by dividing the reported number of days of DEET usage by the number of travel days.

Statistical analysis

Travel-acquired infections

We used Poisson regression analysis to examine the association between selected variables and travel-acquired infections. Participants having anti-ZIKV or anti-CHIKV IgG in the pre-travel sample were considered immune and excluded from the analysis of travel-acquired ZIKV or CHIKV infection. Participants having anti-DENV IgG in the pre-travel sample were considered at risk for secondary DENV infections and therefore included in the analysis of travel-acquired DENV infections. For individuals infected dur-

ing travel, the moment of infection was estimated as the midpoint between their arrival and departure dates.

We calculated attack rates (AR, the number of participants with a travel-acquired infection divided by total number of participants, expressed as percentage), incidence rates (IR, expressed as the number of infections acquired during 1,000 person-months (pm) of travel), and incidence rate ratios (IRR) of infections based on serology suggestive of travel-acquired infection for the entire cohort, independent of when ZIKV and CHIKV were introduced into Suriname. For each disease under study, we assessed the association with sex, age group, country of birth, vaccination status, usage of DEET and calendar year of travel. Variables with a p value < 0.1 in univariable analysis were included in the multivariable model and retrained in the final model using a backwards stepwise approach.

In a sensitivity analysis, we repeated the Poisson analysis for the PRNT50-confirmed travel-acquired DENV and ZIKV infections. Finally, we estimated IR for travel-acquired ZIKV and CHIKV infections based on serology and on PRNT50 during the outbreak periods, limiting the exposure time to the estimated duration of the outbreaks. For ZIKV, duration was 1 September 2015 through 25 April 2016 (first case reported on 2 October 2015); for CHIKV, duration was 1 June 2014 (also date of first case reported in Suriname) through 9 March 2015 [4,5,18,19].

Pre-travel infections

To determine previous DENV exposure among the participants and immunity against ZIKV or CHIKV – because ZIKV/CHIKV-immunity was an exclusion criterion for analysis of the ZIKV/CHIKV travel-acquired infections – we calculated the prevalence of previous DENV, ZIKV or CHIKV infection and the corresponding 95% confidence intervals (CI). Logistic regression analysis was used to examine determinants of previous infection. Variables with a p value < 0.1 in univariable analysis were included in the multivariable model and retrained in the final model using a backwards stepwise approach. A p value < 0.05 was considered statistically significant. Data were analysed using STATA versions 15.1 (StataCorp LLC, Texas, United States).

RESULTS

Study population

Overall, 554 Dutch travellers, of whom 169 were VFR travellers, intended to participate. Of the total, 73 (13%) were excluded (Figure 1). Included in the Dutch tourist travellers

were eight participants born in non-endemic countries other than the Netherlands. The resulting 481 participants consisted of 137 VFR and 344 tourist travellers. The overall median age was 48 years (interquartile range (IQR): 31–59), 177 (37%) were male, and the median travel duration was 22 days (IQR: 15–29). More than half of the participants (273, 57%) had no record of previous flavivirus vaccinations; 112 (23%) had received one and 96 (20%) had received two or more previous flavivirus vaccinations. The median interval between travel return and post-travel blood donation was 19 days (IQR: 16–23). The median age of the VFR travellers was 57 years (IQR: 49–62), 41 (30%) were male, the median travel duration was 29 days (IQR: 22–39), 51 (37% migrated before 1975 to the Netherlands and 64 (47%) lived 15–24 years in Suriname before migrating. the median age of the tourist travellers was 39 years (IQR: 27–55), 136 (40%) were male and the median travel duration was 19 days (IQR: 15–24).

Travel characteristics

Of the 481 participants, 192 (40%) kept their travel diary daily, 125 (26%) retrospectively, and 148 (31%) kept it during travel but not in the 2 weeks post travel, for which information was obtained retrospectively. For 16 (3%) participants, this post-travel information was missing.

Overall, 275 pm were spent in Paramaribo, 64 pm in the coastal areas, 55 pm in inland areas and 8 pm in other countries (predominantly in neighbouring Guyana or French Guyana, or in the Dutch Antilles).

In total, 171 of 481 participants reported one or more symptoms which are frequently reported with dengue at least once. Headache was reported most often by these 171 participants (n = 341), followed by rash (n = 324), diarrhoea ≥ 3 times/day (n = 215), arthralgia (n = 182), muscle ache (n = 154), fever (n = 76), retro-orbital pain (n = 61) and vomiting (n = 54).

Travel-acquired infections

During travel, four VFR travellers acquired four infections: two had a DENV infection (one primary and one secondary, attack rate $(AR)_{DENV}$: 1.5), one seroconverted for ZIKV (AR_{ZIKV}: 0.9) and one for CHIKV (AR_{CHIKV}: 0.8). In total, 20 infections were detected in 17 tourists: 16 with DENV (15 primary and one secondary, AR_{DENV}: 4.7), three with ZIKV (AR_{ZIKV}: 0.9) and one with CHIKV (AR_{CHIKV}: 0.3). Of these tourists, three seroconverted for both DENV and ZIKV (Figure 2). In the Supplement (part 3), we provide additional information about the occurrence of these travel-acquired infections on a timeline.

The overall IR were IR_{DENV} = 47.0 (95% CI: 29.6–74.6), IR_{ZIKV} = 11.6 (95% CI: 4.4–31.0) and IR_{CHIKV} = 5.6 (95% CI: 1.4–22.2) per 1,000 pm of travel. The IR for VFR travellers were IR_{DENV} = 13.6 (95% CI: 3.4–54.5), IR_{ZIKV} = 8.5 (95% CI: 1.2–60.5) and IR_{CHIKV} = 7.6 (95% CI: 1.1–54.1), and those for tourist travellers were IR_{DENV} = 67.8 (95% CI: 41.5–110.7), IR_{ZIKV} = 13.3 (95% CI: 4.3–41.1) and IR_{CHIKV} = 4.4 (95% CI: 0.6–31.0) per 1,000 pm of travel (Table 1, Figure 2).

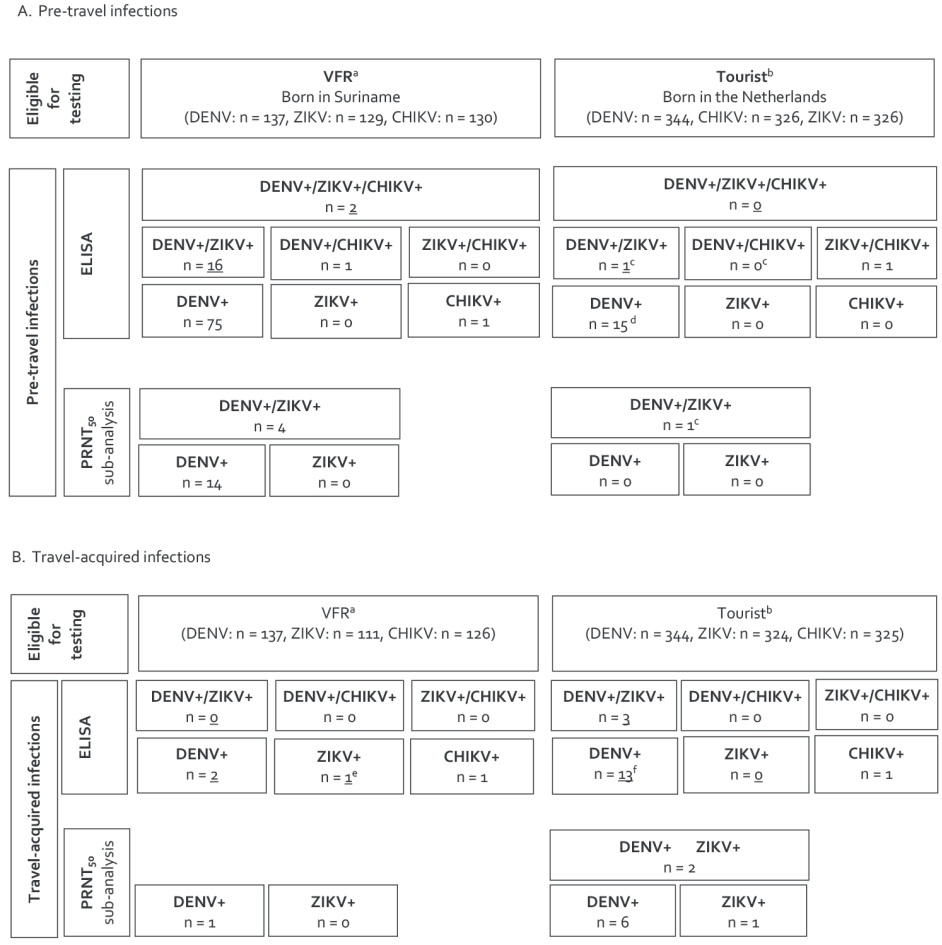

Figure 2 Serological results of pre-travel and travel-acquired dengue, Zika and chikungunya virus infections among Dutch travellers to Suriname, prospective study, the Netherlands, 2014–2017 (n = 481)

+: positive test result; CHIKV: chikungunya virus; DENV: dengue virus; ELISA: enzyme-linked immunosorbent assay; PRNT50: plaque-reduction neutralisation test (≥ 50% neutralisation); VFR: visiting friends and relatives; ZIKV: Zika virus.

[a] Dutch travellers visiting friends and relatives in Suriname.

[b] Dutch tourist travellers who visited Suriname (including 65 second-generation migrants).

[c] Including one second-generation migrant.

[d] Including seven second-generation migrants.

[e] One travel-acquired infection was non-confirmed due to ZIKV-PRNT50 activity in pre-travel sample.

[f] Two travel-acquired infections were non-confirmed due to DENV-PRNT50 activity in pre-travel sample.

The figure includes sub-analysis presenting DENV/ZIKV-PRNT50 results for selected participants. Underlined numbers indicate participants who were eligible for PRNT50 sub-analysis. As various numbers and combinations of pre-travel or travel-acquired infections were found, the results are grouped according to positive test results.

Table 1 Serologically determined travel-acquired dengue virus infection among Dutch VFR and tourist travellers to Suriname, prospective study during the primary introduction in Suriname of CHIKV and ZIKV, the Netherlands, 2014–2017 (n = 481)

Characteristics	Total			ELISA-determined travel-acquired DENV infections			$PRNT_{50}$-confirmed travel-acquired DENV infections		Univariable analysis of $PRNT_{50}$-confirmed DENV infections		
	n	%	pm	n	IR/1,000 pm	pm	n	IR/1,000 pm	IRR	95% CI	p value
Number of participants	481	100	383	18	47.0	386	9	23.3		NA	
Sex											
Male	177	37	141	3	21.3	141	2	14.2	1	Reference	0.352
Female	304	63	242	15	62.1	245	7	28.6	2.0	0.4–9.7	
Age in years											
≤ 35	160	33	115	7	61.0	116	4	34.4	1	Reference	
36–55	161	33	125	5	40.1	126	2	15.9	0.5	0.09–2.5	0.634
≥ 56	160	33	143	6	41.8	144	3	20.8	0.6	0.1–2.7	
Type of traveller											
Tourist (born in the Netherlands)	344	72	236	16	67.8	239	8	33.5	1	Reference	0.068
VFR (born in Suriname)	137	28	147	2	13.6	147	1	6.8	0.2	0.03–1.6	
Additional flavivirus vaccinations[a]											
0	229	48	197	7	35.6	198	3	15.1	1	Reference	0.277
1	252	52	186	11	59.1	188	6	31.9	2.1	0.5–8.4	
Visited areas											
Paramaribo only	67	14	62	2	32.2	63	1	16.0	1	Reference	0.665
Paramaribo and/or other areas	414	86	321	16	49.9	324	8	24.7	1.5	0.2–12.4	
Usage of DEET, % of total travel time											
< 25	113	23	118	3	25.4	118	3	25.4	1	Reference	0.182
25–49	55	11	39	1	25.8	40	0	0		NA	
50–74	58	12	49	1	20.5	49	0	0		NA	
≥ 75	255	53	177	13	73.5	179	6	33.5	1.3	0.3–5.3	
Year of (midpoint of) travel											
2014	142	30	119	1	8.4	119	1	8.4	1	Reference	0.222
2015	120	25	90	5	55.8	90	2	22.1	2.6	0.2–29	
2016	128	27	95	11	115.6	98	5	51.1	6.1	0.7–52	
2017	91	19	79	1	12.7	79	1	12.7	1.5	0.1–24	

CI: confidence interval; DEET: N,N-diethyl-meta-toluamide; DENV: dengue virus; ELISA: enzyme-linked immunosorbent assay; IR: incidence rate; IRR: incidence rate ratio; pm: person-months; NA: not applicable; PRNT50: plaque-reduction neutralisation test (≥ 50% neutralisation); VFR: visiting friends and relatives.

[a] Flavivirus vaccinations include yellow fever, tick-borne encephalitis and Japanese encephalitis vaccinations received during or after the pre-travel blood donation.

Table 2 Characteristics of Dutch VFR and tourist travellers to Suriname with a serologically determined travel-acquired dengue, chikungunya and/or Zika virus infection, including PRNT50 confirmation results, the Netherlands, 2014–2017 (n = 21)

Age group	Sex	Type of traveller	Flavivirus vaccination[a]		Dengue virus						Zika virus			
					ELISA (RU/mL)				$PRNT_{50}$		ELISA (RU/mL)			
			Pre-travel	Additional travel-related	Pre-travel IgG	Previous infection	Post-travel IgG	Travel-acquired infection	Previous infection	Travel-acquired infection	Pre-travel IgG	Previous infection	Post-travel IgG	Travel-acquired infection
40–49	F	Tourist	0	1	36	**Yes**	177	**Yes**	**Yes**	**Yes**		No	4	No
30–39	M	Tourist	0	1	ND	No	4	No	ND		ND	No	1	No
50–59	F	VFR	0	0	ND	No	7	No	ND		ND	No	1	No
60–69	F	Tourist	2	0	6	No	27	**Yes**	No	No	ND	No	<2	No
18–29	F	Tourist	0	1	5	No	87	**Yes**	No	**Yes**	5	No	69	**Yes**
60–69	F	Tourist	0	1	2	No	23	**Yes**	No	No	ND	No	15	No
50–59	F	Tourist	1	1	<2	No	65	**Yes**	No	No	ND	No	7	No
60–69	F	Tourist	2	0	<2	No	114	**Yes**	No	**Yes**	<2	No	129	**Yes**
18–29	F	Tourist	0	1	3	No	132	**Yes**	No	No	2	No	106	**Yes**
50–59	F	VFR	1	0	14	No	51	**Yes**	No	No	ND	No	2	No
18–29	F	Tourist	0	1	20	No	164	**Yes**	No	**Yes**	ND	No	<2	No
18–29	M	Tourist	2	0	8	No	34	**Yes**	No	No	ND	No	3	No
50–59	F	Tourist	2	0	13	No	87	**Yes**	No	**Yes**	ND	No	6	No
18–29	F	Tourist	0	1	8	No	50	**Yes**	Brd	No	ND	No	2	No
30–39	M	VFR	0	1	153	**Yes**	179	No	**Yes**	No	15	No	39	**Yes**
18–29	F	VFR	0	1	31	**Yes**	185	**Yes**	Yes	**Yes**	ND	No	6	No
30–39	F	Tourist	0	1	7	No	39	**Yes**	No	**Yes**	ND	No	2	No
50–59	F	Tourist	0	1	6	No	45	**Yes**	**Yes**	No	ND	No	2	No
50–59	F	Tourist	2	0	14	No	29	**Yes**	**Yes**	No	ND	No	7	No
60–69	M	Tourist	0	1	<2	No	44	**Yes**	No	**Yes**	ND	No	3	No
70–79	M	Tourist	1	0	9	No	180	**Yes**	No	**Yes**	ND	No	5	No

Brd: borderline; ELISA: enzyme-linked immunosorbent assay; F: female; M: male; IgG: immunoglobulin G; ND: not done; P'bo: Paramaribo; PRNT50: plaque-reduction neutralisation test (≥ 50% neutralisation); RU: relative units; VFR: traveller visiting friends and relatives.

[a] Yellow fever, tick-borne encephalitis and Japanese encephalitis.

DENV and ZIKV PRNT50 was performed in participants with an ELISA-confirmed travel-acquired dengue and/or Zika virus infection.

Zika virus		Chikungunya virus		Year of departure	Travel time (days)	Destinations	Reported symptoms
$PRNT_{50}$		ELISA (RU/mL)					
Previous infection	Travel-acquired infection	Previous infection	Travel-acquired infection				
No	No	No	No	2014	15	P'bo	Muscle ache
ND		No	**Yes**	2014	18	P'bo, coast, inland	Fever, headache, muscle ache, arthralgia, vomiting, rash
ND		No	**Yes**	2014	32	P'bo, coast, inland	Headache, arthralgia, rash
No	No	No	No	2015	15	P'bo, coast, inland	None
No	**Yes**	No	No	2015	89	P'bo	Arthralgia, rash
No	No	No	No	2015	17	P'bo, coast, inland	Fever, headache, muscle ache, nose bleeding
No	No	No	No	2015	17	P'bo, coast, inland	Fever
No	**Yes**	No	No	2015	31	P'bo, coast, inland	Fever, headache, arthralgia, rash
No	**Yes**	No	No	2015	19	P'bo, inland	None
No	No	No	No	2016	33	P'bo, coast	Arthralgia
No	No	No	No	2016	20	P'bo, coast, inland	None
No	No	No	No	2016	24	P'bo, coast, inland	None
No	No	No	No	2016	19	P'bo, inland, Dutch Antilles (10 days)	None
No	No	No	No	2016	53	P'bo, coast, inland	Headache, diarrhoea, rash
Yes	No	No	No	2016	21	P'bo, inland	None
No	No	No	No	2016	30	P'bo, coast	None
No	No	No	No	2016	12	P'bo, inland	None
No	No	No	No	2016	24	P'bo, Guyana (3 days)	None
Yes	No	No	No	2016	15	P'bo, inland	None
No	No	No	No	2016	10	P'bo, coast, inland	None
No	No	No	No	2017	22	P'bo, coast	None

Of all 21 participants (four VFR and 17 tourists) with travel-acquired infections, 16 were female, the median age was 50 years (range: 19–70 years) and the median travel duration was 20 days (range: 10–89 days). Of the 19 participants with a DENV and/or ZIKV infection, seven reported at least one symptom during the study period, of whom only three reported fever (Table 2). Both participants with a CHIKV infection reported multiple symptoms including arthralgia (Table 2). Poisson regression analysis for serological determined travel-acquired DENV is described in part 4 of the Supplement. Regression analyses were not performed for ZIKV and CHIKV due to the small numbers. Sensitivity analyses, restricting the exposure period to the outbreak period, yielded an IR_{ZIKV} of 85.8 (95% CI: 27.7–266.1; n = 60) and an IR_{CHIKV} of 18.7 (95% CI: 4.7–74.6; n = 134) per 1,000 pm.

PRNT50-confirmed travel-acquired infections

In VFR travellers, one of two DENV (AR_{DENV}: 0.7) serology-based travel-acquired infections were PRNT50-confirmed; the one ZIKV (AR_{ZIKV}: 0) was not PRNT50-confirmed (Figure 2, Table 2). In tourist travellers, eight of 16 DENV (AR_{DENV}: 2.3) and all three ZIKV travel-acquired infections were PRNT50-confirmed (Figure 2, Table 2). Of the three tourist travellers who were serologically positive for a travel-acquired infection with both DENV and ZIKV, two were PRNT50-confirmed for both and the third was confirmed for travel-acquired ZIKV infection only (Figure 2).

Based on PRNT50-confirmed travel-acquired infections, IR_{DENV} was 23.3 (95% CI: 12.1–44.8) and IR_{ZIKV} 8.4 (95% CI: 2.7–26.1) per 1,000 pm of travel. The IR for VFR travellers were IR_{DENV} 6.8 (95% CI: 1.0–48.2) and IR_{ZIKV} 0 (95% CI: 0–0.024) and for tourist travellers, IR_{DENV} 33.5 (95% CI: 16.8–67.0) and IR_{ZIKV} 13.2 (95% CI: 4.3–41.0) per 1,000 pm of travel. In univariable Poisson regression analysis, tourist travel was associated with PRNT50-confirmed travel-acquired DENV infection (IRR = 4.9; 95% CI: 0.6–39.4). Although the effect was not statistically significant, 2016 was the calendar year with the highest overall (VFR and tourist) IR_{DENV} of 51.1 (95% CI: 21.3–122.8) per 1,000 pm (IRR = 6.1; 95% CI: 0.7–52.2), compared with 2014, 2015 and 2017.

Sensitivity analysis based on the PRNT50 test results yielded an overall IR_{ZIKV} of 83 (95% CI: 26.9–258.3; n = 61) per 1,000 pm during the outbreak period for travel-acquired ZIKV infection.

Pre-travel infections

Of the VFR travellers, 95 had serological evidence of previous infection: 94 of 137 (69%; 95% CI: 60–76) DENV, 18 of 129 (14%; 95% CI: 8.5–21) ZIKV, and four of 130 (3%; 95% CI: 0.9–7.7) CHIKV. In contrast, 17 tourist travellers had serological evidence of a previous infection: 16 of 344 (5%; 95% CI: 2.7–7.4) DENV, two of 326 (0.6%; 95% CI: 0.1–2.1) ZIKV

Table 3 Characteristics and serological evidence of previous infection in Dutch VFR and tourist travellers to Suriname attending a Dutch travel health clinic for pre-travel advice and participating in a prospective study of travel-acquired DENV, ZIKV and CHIKV infections, the Netherlands, March 2014-October 2017 (n = 481)

Characteristics	Total	%	Previous DENV infection		Univariable analysis		
			n	%[c]	OR	95% CI	p value
Participants	**481**	**100**	**110**	**23**			
Sex							
Male	177	37	35	20	1	Reference	0.214
Female	304	63	75	25	1.3	0.8–2.1	
Age in years							
≤ 35	160	33	10	6	1	Reference	
36–55	161	33	40	25	5.0	2.4–10.3	< 0.001
≥ 56	160	33	60	38	9.0	4.4–18.4	
Type of traveller							
Tourist (born in the Netherlands)	344	72	16	5	1	Reference	< 0.001
VFR (born in Suriname)	137	28	94	69	45	24.2–83.1	
Total of pre-travel flavivirus vaccinations[a]							
0	273	57	53	19	1	Reference	0.039
≥ 1	208	43	57	27	1.6	1.0–2.4	
Calendar year of migration (VFR travellers only)[b]							
≤ 1974	51	37	29	57	1	Reference	
1975–1981	37	27	23	62	1.2	0.5–3.0	0.010
≥ 1982	44	32	37	84	4.0	1.5–10.7	
Data missing	5	4	NA			NA	
Years lived in Suriname before migration (VFR travellers only)[b]							
≤ 15	34	25	6	18	1	Reference	
15–24	64	47	54	84	25	8.3–76.5	< 0.001
≥ 25	34	25	29	85	27	7.4–98.9	
Data missing	5	4	NA			NA	

CHIKV: chikungunya virus; CI: confidence interval; DENV: dengue virus; NA: not applicable; OR: odds ratio; VFR: visiting friends and relatives; ZIKV: Zika virus.

[a] Flavivirus vaccinations include yellow fever, tick-borne encephalitis and Japanese encephalitis vaccinations.

[b] Subgroup analysis: not applicable for multivariable analysis.

[c] These numbers reflect the percentages of previous infections among the total number of participants of the corresponding category.

and one of 326 (0.3%; 95% CI: 0.1–1.7) CHIKV (Table 3 and Figure 2). Nineteen participants (18 VFR, one tourist) tested positive for both a previous DENV and ZIKV infection, of whom two (both VFR) also tested positive for previous CHIKV infection. One VFR tested positive for both previous DENV and CHIKV and one tourist for both ZIKV and CHIKV infections (Figure 2). Of the 460 participants for whom this information was available, 13 of 460 reported having had a previous DENV infection, of whom nine were serologically

confirmed. Of the 19 participants (18 VFR, one tourist) who tested positive for DENV as well as ZIKV before travel, only five were PRNT50-confirmed for a previous infection with both DENV and ZIKV. The remaining 14 (all VFR) were confirmed only for a previous DENV infection (Figure 2). Characteristics including ELISA and PRNT test results of these 19 participants care provided in part 5 of the Supplement.

In univariable logistic regression analysis of the serology-based results, previous infections were associated with VFR travelling (DENV, ZIKV, CHIKV), older age (DENV, ZIKV) and a history of at least one flavivirus vaccination (DENV) (Table 3); the logistic regression analysis to previous ZIKV and CHIKV are appended in the Supplement, part 6. In the multivariable model, only VFR travelling remained significantly associated with a previous DENV infection (OR: 38; 95% CI: 20–76).

DISCUSSION

This prospective study among Dutch tourist and VFR travellers to Suriname found a considerable incidence of travel-acquired DENV infections which was five times higher in tourist than in VFR travellers, although the 95% CI overlapped. This finding is in contrast with studies of other travel-related illnesses which found a higher incidence of travel-acquired infections in VFR travellers [10-12]. Although not statistically significant, travelling in 2016 was also associated with travel-acquired DENV infection.

The overall IR of travel-acquired DENV infection based on 47.0 per 1,000 pm is within the range of previous reported serology-based prospective studies among travellers (6.7–58.7 per 1,000 pm) [7,20-25]. Of these studies, only one used PRNT50 in a selection of participants; as in our study, it could not confirm all serology-based DENV infections. This suggests that serology-based DENV infection rates are likely to overestimate the true incidence.

For DENV, we found the highest overall IR in 2016. Peaks of DENV infections occur every 3 to 4 years in dengue-endemic areas in South America [26]. From 2001 through 2012, peaks in DENV infections in Suriname were recorded in 2009 and 2012, and our finding of a peak in 2016 appears to correspond with this cycle [27]. Surprisingly, the Pan American Health Organisation reported only six DENV cases in Suriname in 2016, and this total was lower than in the preceding 2 years [28]. Although under-reporting is possible, an unexpectedly low number of DENV cases was likewise reported in other American countries following the large ZIKV epidemics in 2015 and 2016, possibly due to temporary cross-protection by recent ZIKV infections [29,30].

For ZIKV and CHIKV, the incidence was highest during our estimated outbreak periods in Suriname. No CHIKV infections were found outside the outbreak period (see the arrows on the timeline in Supplement part 3), an observation comparable to those of a previous study [31]. We identified only one other small prospective study which estimated ZIKV infection incidence among travellers; it reported a somewhat higher incidence of 17% per pm (170 per 1,000 pm) among 49 Belgian travellers to South America in 2016 [32].

Many European countries have close ties with populations from former colonies, or large groups of migrants from other sub-tropical countries that are endemic for DENV and other arboviruses. With increasing travel, these arboviral infections can cause a large and increasing disease burden in returning travellers. The arthropod vector for DENV, ZIKV and CHIKV is expanding rapidly in continental Europe and in the past 15 years, multiple local dengue and chikungunya outbreaks have been reported after virus introduction by a viraemic traveller [33-41]. More insight in travel risk groups and determinants for disease is needed, both for travel health consultations and for arbovirus preparedness and control in Europe.

Our study was designed to investigate the risks and predictors of DENV infection based on serology. After the introduction of ZIKV and CHIKV in Suriname, however, we expanded the study to include these two viruses because of potential serological cross-reactions between DENV and ZIKV viruses and our interest in the risks of these two additional mosquito-borne infections for travellers. To study potential cross-reactions of DENV and ZIKV antibodies, we performed additional DENV and ZIKV PRNT50 confirmation tests, using selected samples due to budget limitations.

Cross-reaction probably contributed to a substantial overestimation of the prevalence of anti-ZIKV antibodies in VFR travellers, as most pre-existing ZIKV antibodies in VFR with both anti-ZIKV and anti-DENV antibodies were not confirmed in PRNT50. All non-confirmed ZIKV cases in this group had very high titres of anti-DENV antibodies, which probably caused cross-reactions in the anti-ZIKV IgG ELISA test (according to the test results appended in the Supplement, part 5). The confirmation of false positivity in these participants was also expected because it was unlikely that they had travelled since the ZIKV introduction in Suriname in 2015. The PRNT50 results of the five participants with confirmed pre-existing DENV and ZIKV should therefore also be interpreted with care. Another study also found cross-neutralising antibody responses in focus reduction neutralisation tests (FRNT50), however cross-reacting ZIKV antibody responses showed lower neutralisation activity compared with the antibodies against the infecting DENV serotype [42]. Perhaps a stricter cut-off, such as PRNT90, would be more accurate in participants who demonstrate both DENV and ZIKV antibodies in serological tests.

The incidence of travel-acquired DENV infections may have been overestimated, but possibly for other reasons than cross-reactivity, as eight of nine travellers with a non-confirmed DENV infection in PRNT50 had no ZIKV co-infection, and four of nine did not receive any additional flavivirus-vaccines. Although PRNT50 is still considered the most reliable test to confirm seroconversion, PCR in the viraemic phase of disease remains the gold standard. Further research is necessary to gain more insight into the associations between clinical symptoms, serological test results and PCR-confirmed DENV and ZIKV infections.

After the Zika epidemic in the Americas, a remarkable temporary decline in DENV infections was seen across the Americas, for which temporarily cross-protecting ZIKV antibodies have been hypothesised as a possible reason [29]. While cross-reactions of antibodies can overestimate incidences based on serology, cross-protection of anti-DENV and anti-ZIKV antibodies can lead to underestimations of infection risks in non-immune persons. Cross-protection against congenital ZIKA syndrome due to DENV antibodies was found in north-eastern Brazil [43,44]. Although (intermediate antibody levels from) prior infections have also been associated with more severe disease, cross-protection could perhaps be a reason why none of our travellers with high titres of DENV antibodies contracted a travel-acquired ZIKV infection and why none with a travel-acquired DENV infection had evidence of pre-existing anti-ZIKV antibodies [45].

A further argument for the cross-protection hypothesis is that all 19 participants with pre-existing antibodies to both DENV and ZIKV had high serological anti-DENV titres (DENV IgG titres ≥ 125 relative units/mL), and the 17 with complete PRNT50 results all had neutralising activity of ≥ 90% against at least two DENV serotypes in PRNT50 (see the extra material in part 5 of the Supplement). It is likely that these 19 travellers, all but one VFR, had a past secondary DENV infection and therefore not at risk for following DENV infections anymore. These VFR travellers, and probably other participants with high pre-existing anti-DENV antibody titres that we could not retest, contributed to the low incidence of DENV infections among VFR travellers.

Finally, it is noteworthy that four of five previous CHIKV infections were among VFR travellers, although CHIKV was introduced only recently. Other alphaviruses such as Mayaro virus may have cross-reacted with the CHIKV test and caused false-positive results [46,47].

Our study has some limitations. Firstly, as participants were recruited at a travel vaccination clinic, they may have had a higher health awareness than those not seeking pre-travel health advice; thus, our findings may not be generalisable to all travellers to

Suriname. Secondly, as risk of infection can differ between first- and second-generation migrants, these two groups are preferably analysed separately, but this analysis was not part of our study. Thirdly, some data such as pre-study travel history were incomplete and could therefore not be used in our analyses. Fourthly, no risk factors could be identified for ZIKV or CHIKV infection due to our small numbers. Finally, the default answers regarding symptoms and use of DEET in the diary were 'no' or 'not used', respectively. Therefore, symptoms or usage of DEET could have been under-reported if participants forgot to tick the box in the diary, making potential associations more difficult to demonstrate.

CONCLUSIONS

Dutch tourist and VFR travellers to Suriname, especially tourist travellers, run a substantial risk of contracting DENV, and since the introduction of ZIKV and CHIKV, they run a considerable risk of ZIKV and CHIKV infections during outbreaks as well. Cross-reacting anti-DENV antibodies probably contributed to an overestimation of the pre-travel ZIKV prevalence and cross-reaction may also have contributed to overestimation of the travel-acquired incidence of DENV infections. Conversely, high titres of pre-travel DENV antibodies potentially protect against other flavivirus infections. As the habitat of the vectors for DENV, ZIKV and CHIKV has expanded in recent years, expanded epidemiological knowledge of these arboviruses will be necessary both for travel health advice and for arbovirus control in Europe. Future studies into the incidence of flavivirus infections should consider the use of diagnostic tools during travel such as dried blood spots (travellers collecting their own blood sample using a finger prick) that allow for PCR testing after travel. This will offer additional information about the infecting virus and serotype and lead to more reliable incidence estimates in travellers.

ETHICAL STATEMENT

The study protocol was approved by the Medical Ethics Committee of the Amsterdam University Medical Center (METC 2013_323). Participants were included after providing written informed consent. Testing for infectious diseases other than DENV was part of the consent procedure.

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CONFLICT OF INTEREST

None declared.

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SUPPORTING INFORMATION

Supplementary files (1-6)

This supplementary material is hosted by Eurosurveillance as supporting information alongside the article Incidence of dengue, chikungunya and Zika virus infections among Dutch travellers to Suriname: a prospective study among tourists and travellers visiting friends and relatives during the recent introduction of chikungunya and Zika virus, on behalf of the authors, who remain responsible for the accuracy and appropriateness of the content. The same standards for ethics, copyright, attributions and permissions as for the article apply. Supplements are not edited by Eurosurveillance and the journal is not responsible for the maintenance of any links or email addresses provided therein.

Supplementary 1 The questionnaire participants filled out daily in their travel diary (original travel diary in Dutch). The data was collected for each day of travel, including 14 days post-travel.

Week no. ….	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Date							
General information	Tick all boxes and/or answer		the questions which apply to you				
Stay in Suriname:							
- Paramaribo	❑	❑	❑	❑	❑	❑	❑
- coastal area	❑	❑	❑	❑	❑	❑	❑
- inlands	❑	❑	❑	❑	❑	❑	❑
Stay in the Netherlands:	❑	❑	❑	❑	❑	❑	❑
Stay in other country, namely:							
Used DEET?	❑	❑	❑	❑	❑	❑	❑
Symptoms	Tick all boxes and/or answer		the questions which apply to you				
Fever	❑	❑	❑	❑	❑	❑	❑
If yes, body temperature:	°C	°C	°C	°C	°C	°C	°C
How did you measure? (rectally/axillary/orally)							
Headache	❑	❑	❑	❑	❑	❑	❑
Retro-orbital pain	❑	❑	❑	❑	❑	❑	❑
Muscle ache (unrelated to physical activity)	❑	❑	❑	❑	❑	❑	❑
Arthralgia	❑	❑	❑	❑	❑	❑	❑
Vomiting	❑	❑	❑	❑	❑	❑	❑
Diarrhoea ≥ 3x/day	❑	❑	❑	❑	❑	❑	❑
Rash	❑	❑	❑	❑	❑	❑	❑

Other symptoms, namely:*									
Needed treatment?	Tick all boxes and/or answer		the questions which apply to you						
Measures taken by itself?	❑	❑	❑	❑	❑	❑	❑		
- Medication:*									
- Other, namely:*									
Did you consult a physician?	❑	❑	❑	❑	❑	❑	❑		
- If yes, why?*									
Blood donation collected?	❑	❑	❑	❑	❑	❑	❑		
Diagnosis:*									
Treatment initiated by the physician?	❑	❑	❑	❑	❑	❑	❑		
- Medication:*									
- Hospitalised	❑	❑	❑	❑	❑	❑	❑		
- other, namely:*									

* = if necessary, continue answer under notes

Notes:

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Supplementary 2 Procedures used for the in-house (Amsterdam University Medical Centers) Plague Reduction Neutralization Test50 against Dengue virus serotypes 1-4 and Zika virus.

An in-house Plaque Reduction Neutralization Test (PRNT50) was performed to determine the virus type-specific neutralizing antibody response against dengue virus (DENV) serotypes 1-4 and Zika virus (ZIKV). Samples of selected participants were tested in pair. Participants were selected for PRNT50 if: 1) the pre-travel anti-DENV and anti-ZIKV Enzyme Linked ImmunoSorbent Assay (ELISA) Immunoglobulin (Ig)G yielded a positive test result (n=19), or 2) a travel-acquired DENV and/or ZIKV infection was determined based on the pre- and post-travel anti-DENV and anti-ZIKV ELISA IgG test results (n=19). The serum was heat-inactivated at 56˚C in a water bath for 30 minutes and 1:10 diluted. Next, 200 µL serum and a 200 µL virus control (Eagle's Minimal Essential Medium 2% Fetal Calf Serum) was mixed with an equal volume of each reference virus (DENV serotype 1-4 or ZIKV). The virus-serum mixture was then incubated at 37°C in a 5% CO2 incubator for 1 hour. The virus-serum mixture was inoculated on VERO cell line in wells of a 6-well plate and incubated at 37°C in a 5% CO2 incubator for 1 hour.

After removal of excess virus-serum mixture, 1ml overlay medium was added to each well, and this was allowed to solidify for 15 minutes at room temperature. Plates were incubated at 37°C in a 5% CO2 incubator for 5 days. For fixation after incubation 10% formaldehyde was added for 20 minutes at room temperature.

After removal of the overlayer, plates were washed 3 times. One ml Cristall Violett was added for incubation of 30 minutes at room temperature. At last, the plates were washed with water 5 times and air-dried at room temperature. The plaques were now ready for counting.

A positive control (virus without sera) were included for each assay. In addition, positive serum controls with high-titer convalescent sera against four dengue serotypes or Zika virus were included as well.

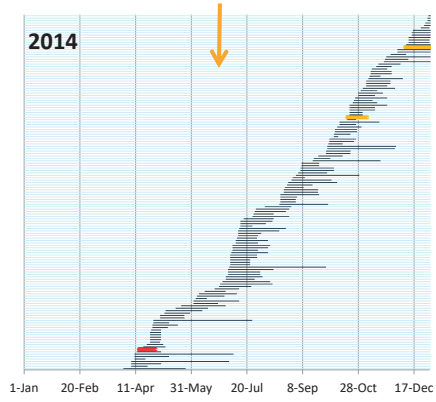
The percentage of plaques counted in test sera was compared with the number of plaques from the virus control (without sera):
<50% inhibition compare to the positive control was considered negative
50% inhibition compare to the positive control was considered borderline
>50% inhibition compare to the positive control was considered positive

Supplementary 3 Time-line of our prospective study from 2014-2017 (a-d) among Dutch VFR and tourist travellers to Suriname, representing all participants. Travellers who contracted a serologically determined travel-acquired DENV, CHIKV and/or ZIKV infection are marked.

Each line represents the travel period of a participant. Dark blue line: no travel-acquired infection, red: travel-acquired DENV infection, orange: travel-acquired CHIKV infection, purple: travel-acquired ZIKV infection, brown: travel-acquired DENV and ZIKV infections

Orange arrow: first reported chikungunya case in Suriname*. Purple arrow: first reported Zika case in Suriname^.

a..



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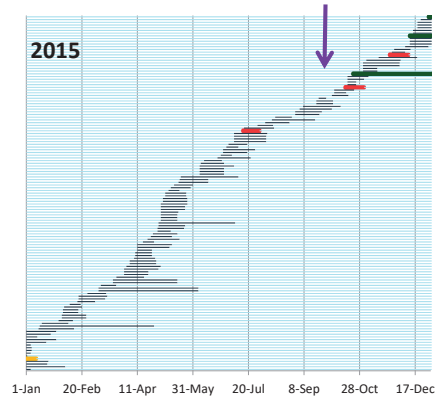

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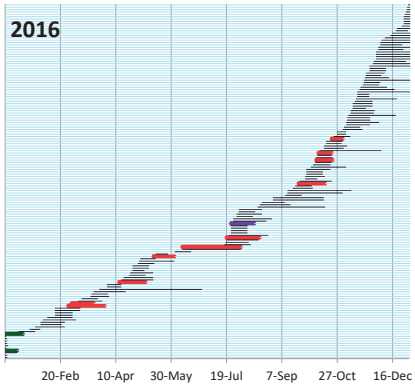
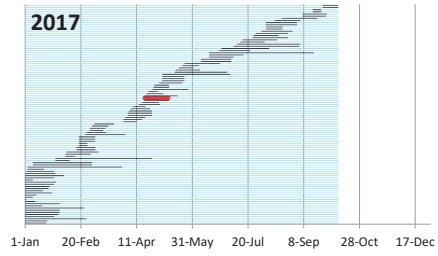


d.



* Sensitivity analysis starting from the first reported CHIKV infection in Suriname onwards yielded a serology-based overall IR_{CHIKV} of 5.8 (96% CI: 1.5-23.3 per 1,000 pm of travel (n= 435 participants).

^ Sensitivity analysis starting from the first reported ZIKV infection in Suriname onwards yielded a serology-based overall IR_{ZIKV} of 23.4 (95% CI: 8.8-62.4) per 1,000 pm of travel (n=226 participants), and an overall IR_{ZIKV} of 16.8 (95% CI: 5.4-52.0) per 1,000 pm of travel based on PRNT50 test results (n=234).

Supplementary 4 Serologically determined travel-acquired DENV infection among Dutch VFR and tourist travellers to Suriname: a prospective study of travel-acquired DENV, ZIKV and CHIKV infections during the primary introduction of CHIKV and ZIKV in Suriname, 2014-2017 (n=481).

Characteristics	Total			travel-acquired DENV infections		univariable analysis				multivariable analysis			
	No.	%	person-months	No.	IR/1000 pm	IRR	95% CI lower	95% CI upper	p value	IRR	95% CI lower	95% CI upper	p value
no. Participants	481	100%	383	18	47.0								
Gender									0.059				0.037
Male	177	37%	141	3	21.3	1				1			
female	304	63%	242	15	62.1	2.9	0.8	10.1		3.2	0.9	11.2	
Age, y									0.720				
≤35	160	33%	115	7	61.0	1							
36-55	161	33%	125	5	40.1	0.7	0.2	2.1					
≥56	160	33%	143	6	41.8	0.7	0.2	2.0					
Type of traveler									0.009				0.005
Tourist (born in the Netherlands)	344	72%	236	16	67.8	1				1			
VFR (born in Suriname)	137	28%	147	2	13.6	0.2	0.04	0.9		0.2	0.04	0.8	
Additional flavivirus vaccinations^									0.286				
0	229	48%	197	7	35.6	1							
1	252	52%	186	11	59.1	1.7	0.6	4.3					
Visited areas									0.536				
Paramaribo only	67	14%	62	2	32.2	1							
Paramaribo and/or other areas	414	86%	321	16	49.9	1.6	0.4	6.7					

Supplementary 4 Serologically determined travel-acquired DENV infection among Dutch VFR and tourist travellers to Suriname: a prospective study of travel-acquired DENV, ZIKV and CHIKV infections during the primary introduction of CHIKV and ZIKV in Suriname, 2014-2017 (n=481). (*continued*)

Characteristics	Total			travel-acquired DENV infections		univariable analysis				multivariable analysis			
	No.	%	person-months	No.	IR/1000 pm	IRR	95% CI		p value	IRR	95% CI		p value
							lower	upper			lower	upper	
Usage of DEET, % of total travel time									0.168				
<25	113	23%	118	3	25.4	1							
25-49	55	11%	39	1	25.8	1.0	0.1	9.8					
50-74	58	12%	49	1	20.5	0.8	0.08	7.8					
≥75	255	53%	177	13	73.5	2.9	0.8	10.2					
Year of (midpoint of) travel									0.002				0.001
2014	142	30%	119	1	8.4	1				1			
2015	120	25%	90	5	55.8	6.7	0.8	57		7.2	0.8	62	
2016	128	27%	95	11	115.6	13.8	1.8	107		14.6	1.9	113	
2017	91	19%	79	1	12.7	1.5	0.1	24		1.7	0.1	27	

DENV= dengue virus

^=Flavivirus vaccinations include yellow fever, tick-borne encephalitis and Japanese encephalitis vaccinations received during or after the pre-travel blood donation.

Supplementary 5 Characteristics of Dutch VFR and tourist travellers to Suriname with serologically determined previous DENV and ZIKV infections, including PRNT50 confirmation results, 2014-2017 (n=19).

	agegroup	gender	type of traveler	years in Suriname before migration	total pre-travel FV vaccinations	pre-travel anti-DENV IgG RU/ml (ELISA)	result previous DENV inf (ELISA)	result previous DENV inf ($PRNT_{50}$)	% neutralization DENV1-PRNT50)	% neutralization DENV2-PRNT50)	% neutralization DENV3-PRNT50)	% neutralization DENV4-PRNT50)	pre-travel anti-ZIKV IgG RU/ml (ELISA)	result previous ZIKV inf (ELISA)	result prev ZIKV inf ($PRNT_{50}$)	% neutralization (ZIKV-PRNT50)	% neutralization (repeated ZIKV-PRNT50)	year of departure
1	50-59	F	VFR	>=25	1	143	P	P	76	100	96	84	42	P	N			2014
2	50-59	F	VFR	>=25	2	129	P	P	100	100	99	100	184	P	N			2014
3	60-69	M	VFR	15-24	0	133	P	P	100	100	91	76	41	P	N			2014
4	**40-49**	**M**	**VFR**	>=25	**1**	**150**	**P**	**P**	100	100	96	73	**73**	**P**	**P**	54		**2014**
5	**50-59**	**M**	**VFR**	15-24	**2**	**> 200**	**HP**	**P**	100	100	97	96	**52**	**P**	**P**	58		**2015**
6	50-59	M	VFR	15-24	2	156	P	P	100	100	97	96	44	P	N			2015
7	50-59	F	VFR	>=25	0	154	P	P	100	100	100	69	116	P	N			2015
8	60-69	F	VFR	>=25	2	125	P	P	100	100	96	63	46	P	N			2015
9	40-49	M	VFR	>=25	0	194	P	P	100	100	97	97	156	P	N			2015
10	80-89	F	VFR	15-24	1	154	P	P	100	97	99	100	74	P	N			2016
11	70-79	M	VFR	>=25	2	152	P	P	100	100	97	97	40	P	N			2016
12	60-69	F	VFR	15-24	2	140	P	P	na*	na*	87	na*	81	P	N			2016
13	60-69	F	VFR	>=25	0	177	P	P	91	100	88	86	49	P	N			2016
14	40-49	F	VFR	m	1	180	P	P	96	100	93	97	32	P	N			2016
15	60-69	M	VFR	>=25	0	189	P	P	100	100	96	83	49	P	N			2016
16	**40-49**	**F**	**tourist**	na	**0**	**> 200**	**HP**	**P**	100	100	99	94	**> 200**	**HP**	**P**	100		**2016**
17	60-69	F	VFR	15-24	1	161	P	P	na*	na*	96	na*	114	P	N			2017
18	**60-69**	**F**	**VFR**	15-24	**0**	**170**	**P**	**P**	100	100	100	97	**34**	**P**	**P**	H	100	**2017**
19	**50-59**	**F**	**VFR**	>=25	**1**	**190**	**P**	**P**	100	100	95	100	**80**	**P**	**P**	52	-32	**2017**

FV=flavivirus (yellow fever, tick-borne encephalitis and Japanese encephalitis) vaccinations

M=missing, N=negative, NA=not applicable, na*=% neutralization in post-travel sample: 100%,

P=positive (ELISA: ≥22 relative units/ml; PRNT50: >50%), HP=high positive (ELISA: >200 relative units/ml)

VFR=travelers visiting friends and relatives

In bold: participants with serologically and PRNT50-confirmed DENV and ZIKV infections.

prev DENV inf (ELISA)= serologically confirmed previous DENV infections using an anti-DENV IgG test (enzyme-linked immunosorbent assay)

prev DENV inf ($PRNT_{50}$)= $PRNT_{50}$-confirmed previous DENV infections among participants with a serologically confirmed previous DENV and ZIKV infections.

prev ZIKV inf (ELISA)= serologically confirmed previous ZIKV infections using an anti-ZIKV IgG test (enzyme-linked immunosorbent assay)

prev ZIKV inf ($PRNT_{50}$)= $PRNT_{50}$-(non)-confirmed previous ZIKV infections among participants with a serologically confirmed previous DENV and ZIKV infections.

Supplementary 6

a. Determinants of serologically determined previous ZIKV infection among Dutch VFR and tourist travellers to Suriname who attended a Dutch travel health clinic for pre-travel advice and participated in a prospective study of travel-acquired DENV, ZIKV and CHIKV infections, 2014-2017 (n=455).

Characteristics	Total, no	%	previous ZIKV inf		univariable analysis			
					OR	95% CI		p value
			No.	%		upper	lower	
no. Participants	455	100	20	4.4%				
Gender								0.871
male	167	37	7	4.2%	1			
female	288	63	13	4.5%	1.1	0.4	2.8	
Age, y								0.013
≤55	303	67	8	2.6%	1			
≥56	152	33	12	7.9%	3.2	1.3	7.9	
Type of traveller								<0.001
Tourist (born in the Netherlands)	326	72	2	0.6%	1			
VFR (born in Suriname)	129	28	18	14%	26	6.0	115	
Total of pre-travel flavivirus vaccinations^								0.106
0	262	58	8	3.1%	1			
≥1	193	42	12	6.2%	2.1	0.8	5.3	
Year of migration (VFRs only) $								0.460
≤1974	48	37	5	10%	1			
1975-1981	35	27	4	11%	1.1	0.3	4.5	
≥1982	42	33	8	19%	2.0	0.6	6.7	
Data missing	4	3						
Years lived in Suriname pre-migration (VFRs only) $								
≤15	31	24	0	0	na			
15-24	60	47	7	12	na			
≥25	34	26	10	29	na			
Data missing	4	3						

VFR= visiting friends and relatives, ZIKV= Zika virus,

^=Flavivirus vaccinations include yellow fever, tick-borne encephalitis and Japanese encephalitis vaccinations.

$=subgroup analysis: not applicable for multivariable analysis

b. Determinants of serologically determined previous CHIKV infection among Dutch VFR and tourist travellers to Suriname who attended a Dutch travel health clinic for pre-travel advice and participated in a prospective study of travel-acquired DENV, ZIKV and CHIKV infections, 2014-2017 (n=456).

Characteristics	Total, no	%	previous CHIKV inf		univariable analysis			
					OR	95% CI		p value
			No.	%		upper	lower	
no. Participants	456	100	5	1.1				
Gender								0.417
male	167	37	1	0.60	ref			
female	289	63	4	1.4	2.3	0.26	21.0	
Age, y								0.221
≤55	304	67	2	0.66	ref			
≥56	152	33	3	2.0	3.0	0.50	18.4	
Type of traveller								0.016
Tourist (born in the Netherlands)	326	71	1	0.31	ref			
VFR (born in Suriname)	130	29	4	3.1	10.3	1.1	93.2	
Year of migration (VFRs only)								
≤1974	48	37	3	6.3	na			
1975-1981	36	28	1	2.8	na			
≥1982	42	32	0	0	na			
missing	4	3						
Years lived in Suriname before migration (VFRs only)								
≤15	32	25	1	3.1	na			
15-24	60	46	3	5.0	na			
≥25	34	26	0	0	na			
missing	4	3						

VFR= visiting friends and relatives, CHIKV= chikungunya virus,
^=Flavivirus vaccinations include yellow fever, tick-borne encephalitis and Japanese encephalitis vaccinations.
$=subgroup analysis: not applicable for multivariable analysis

Part 2

Public health measures to prevent spread of imported hemorrhagic fever

8

Public health response to two imported, epidemiologically related cases of Lassa fever in the Netherlands (ex Sierra Leone), November 2019

Femke W Overbosch, M de Boer, KE Veldkamp, P Ellerbroek, CP Bleeker-Rovers, B Goorhuis, M v Vugt, A vd Eijk, T Leenstra, M Khargi, J Ros, D Brandwagt, M Haverkate, C Swaan, C Reusken, A Timen, M Koopmans, J v Dissel, Lassa fever response team of The Netherlands

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ABSTRACT

On 20 November 2019, Lassa fever was diagnosed in a physician repatriated from Sierra Leone to the Netherlands. A second physician with suspected Lassa fever, repatriated a few days later from the same healthcare facility, was confirmed infected with Lassa virus on 21 November. Comprehensive contact monitoring involving high- and low-risk contacts proved to be feasible and follow-up of the contacts did not reveal any case of secondary transmission in the Netherlands.

Two patients with Lassa haemorrhagic fever were diagnosed following nosocomial exposure in a hospital in the district Tonkolili in Sierra Leone. We aimed to reconstruct the measures undertaken regarding the patients and their contacts, identify the lessons learned and formulate recommendations for future cases of importation of patients with haemorrhagic fever.

EPIDEMIOLOGICAL DESCRIPTION OF THE CASE PATIENTS

Both cases – Case 1 (C1) and Case 2 (C2) - had been working in a rural hospital in the Tonkolili district in Sierra Leone. On 4 November 2019, two Dutch healthcare workers (C1 and C2) and one local (C3) participated in obstetric surgical procedures in two local patients who were later presumed to be the source of Lassa virus (LASV) infection. One patient died on the day of surgery after resuscitation during which C1 was not wearing optimal personal protective equipment (PPE). The other patient died 2 weeks later. Haemorrhage was reported in both patients.

On 11 November, while attending an international course in Freetown, C1 started to develop non-specific symptoms (headache, muscle ache, arthralgia, fever, diarrhoea, vomiting and cough). C1 was treated locally for the most probable tropical diseases (such as malaria and typhoid fever).

After 8 days of persisting symptoms, C1 was medically evacuated to the Netherlands on 19 November on a commercially run private plane with a German flight crew, with a transit in Morocco. No specific infection precautions were taken on the flight. C1 was initially transferred by ambulance (Ambulance 1) to the Amsterdam University Medical Center (location AMC, Hospital 1), also without specific infection precautions. Staff in the hospital used MRSA (meticillin-resistant *Staphylococcus aureus*) airborne strict isolation measures and PPE (gloves, FFP2 masks and gowns). Upon suspicion of Lassa fever, C1 was relocated in a dedicated ambulance (Ambulance 2) to the Leiden University Medical Center (LUMC, Hospital 2) dedicated facility for treatment of highly contagious infections. The diagnosis of Lassa fever was confirmed on 20 November by RT-PCR and genome sequencing performed at Erasmus Medical Center (EMC) in Rotterdam. After rapid clinical deterioration, the patient died on 23 November. Stringent hygienic precautions were taken for management of the corpse.

C2 also started to develop non-specific symptoms (fever, vomiting and anorexia) on 11 November and was unsuccessfully treated in Sierra Leone for the most probable tropical diseases. RT-PCR on plasma samples of C2, sent to the EMC, tested positive for LASV on

21 November at and the decision was made for medical evacuation to the Netherlands. In a clinically stable condition, C2 was airlifted on 23 November under strict isolation measures by a French flight crew of Airlec Medical. C2 was transported in a dedicated ambulance (Ambulance 3) to the Major Incident Hospital at the University Medical Centre Utrecht (UMCU, Hospital 3), and admitted to a facility for highly contagious infections. C2 was discharged on 12 December, after two negative results within an interval of 48 h in serum tests for presence of LASV RNA. The patient was discharged into home isolation; as LASV RNA remained positive in the urine, strict instructions regarding hygiene were enforced until urine tested negative after 12 days.

C3 was a local healthcare worker who was confirmed with Lassa fever infection by the authorities in Sierra Leone. The case history and contact tracing around this case are not part of this report.

CONTACT TRACING

Contact tracing was initiated upon confirmation of the diagnosis in C1 as viral haemorrhagic fevers are mandatorily notifiable according to Dutch law (1). Immediately, a response team convened at the Centre for Communicable Diseases (CIb), consisting of representatives of the hospitals, the reference laboratory (EMC), involved public health services (PHS 1–5), ambulance services and experts from the CIb. The response team provided scientific advice on the risk assessment, risk classification and control measures regarding contacts and coordinated the risk communication (2,3).

The contacts of C1 and C2 (including all transportation and hospital staff) were interviewed to assess the intensity of exposure to the cases. All Dutch healthcare workers repatriated from Sierra Leone and the ones who were contacts of the presumed source patients were listed. Contacts were classified into three risk groups according to the nature of their exposure (Table). The control measures were targeted to each risk level, a procedure validated in a previous case (4,5).

CONTACT MONITORING

In total, 164 contacts who (temporarily) resided in the Netherlands were identified for follow-up. Nineteen were classified as high-risk contacts (6) (Figure). Monitoring of high- and low-risk contacts, respectively, ended on 15 December and 2 January 2020, 21 days after the last exposure (Table). Post-exposure prophylaxis (PEP) was not prescribed

Table Dutch risk classification of contacts exposed to healthcare workers with RT-PCR-confirmed Lassa fever contracted in Sierra Leone, including numbers of contacts inventoried in the Netherlands, December 2019.

Type of contact	Risk	Mandatory measures until 21 days post exposure	Number of contacts in the Netherlands
High-risk contacts[a]	Contact with patient or body fluids without appropriate PPE	- Temperature check 2×/day - Daily contact with public health service or hospital staff - Prohibition to travel abroad - Work restrictions - Safe sex (condom use)	19 (Hospital 1, Ambulance 1, friends SL, colleagues SL, family)
Low-risk contacts[a,b]	Contact with patient or body fluids with use of appropriate PPE	Temperature check 2x/day	31 (Hospital 1-4, ambulance 2-3, family)
Sporadic contacts	Presence in same room without direct contact	No risk, no measures	14 (Hospital 1)

PPE: personal protective equipment, SL: Sierra Leone.

[a] In case of fever ≥ 38 °C measured twice,12 h apart, contacts are instructed to consult their assigned healthcare worker (municipal health service or hospital staff) and to avoid new contacts.

[b] Including contacts without direct contact, but who have been working in and around the hospital in Sierra Leone.

to contacts in the Netherlands. Two contacts (one high- and one low-risk) developed a fever, but an acute LASV infection was excluded in EDTA-plasma with RT-PCR. All high-risk contacts were considered as non-infected as paired serum samples taken at the beginning and at the end of the tracing of high risk contacts revealed no seroconversion for LASV-specific IgM and IgG by both immunofluorescence assay and ELISA (Bernhard Nocht Institute, Hamburg, Germany).

Large grey spheres: assumed source patients with Lassa fever in the local hospital, Sierra Leone. Large white spheres: confirmed secondary cases (C1, C2 and another healthcare worker (C3) involved in the surgical procedures of the assumed source patients). Small spheres: contacts. Red line: high-risk contact, blue line: low-risk contact, no line: contact without direct contact, but followed up as low-risk as they have been working in and around the hospital in Sierra Leone. Red outer line of sphere: contact returned from Sierra Leone, black outer line of sphere: contact in the Netherlands.

Dutch hospitals involved: AUMC location AMC (Hosp 1), LUMC (Hosp 2), UMCU (Hosp 3) and EMC (Hosp 4). Dutch Public Health Services involved: Public Health Service Hollands Midden (PHS 1), Amsterdam (PHS 2), Rotterdam-Rijnmond (PHS 3), Kennemerland (PHS 4), and region Utrecht (PHS 5).

The figure displays the confirmed cases (C1, C2, C3), the presumed source patients and those contacts that were followed up in the Netherlands. The figure does therefore not display: the 14 sporadic cases identified in the Netherlands, the high- and low-risk contacts identified elsewhere in the European Union, the German and French flight crews,

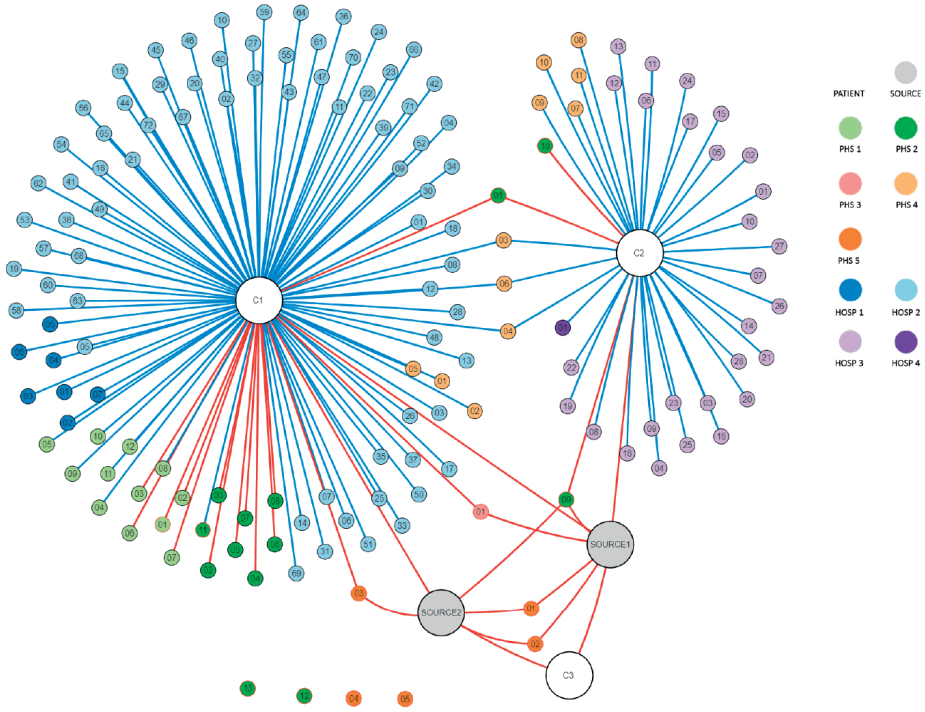

Figure Contact tree representing the high- and low-risk contacts in the Netherlands of the assumed source patients in Sierra Leone and of two Dutch healthcare workers diagnosed with confirmed Lassa fever, the Netherlands, December 2019 (n = 150).

the local and Dutch contacts of C1 and C2 who resided in Sierra Leone and the local contacts and secondary cases of the presumed source patients who resided in Sierra Leone.

Dedicated air transportation was arranged for five Dutch high-risk contacts and British contacts who still resided in Sierra Leone. Other contacts in Europe were identified, according to the stepwise backwards contact tracing starting with the air ambulance, in Germany (n = 5), the United Kingdom (UK) (n = 18), Denmark (n = 5) and Norway (n = 2). These contacts have been followed up by the corresponding national authorities, but further spread has not been reported.

Communication with a representative of the hospital in Sierra Leone was established and criteria were exchanged for the identification and monitoring of persons who had potentially been exposed locally. As the contact investigation had revealed contacts from other countries, authorities in Germany, the UK, Denmark and Norway were informed between 20 and 27 November using the Early Warning and Response System (EWRS) of the European Union (EU). On 20 November, an official notification was issued through

the EWRS and the World Health Organization Event Information Site. The International Health Regulations National Focal Points of Sierra Leone and Morocco were officially informed by the Dutch authorities.

DISCUSSION

Lassa virus is a single-stranded RNA virus belonging to the family *Arenaviridae. It* is endemic in several West African countries, in particular Sierra Leone, Liberia, Guinea and Nigeria, although cases had been reported only sporadically in the Tonkolili region (7,8). Rodents act as a reservoir and shed the virus in urine and droppings. Humans become infected through contact with contaminated rodent excreta, e.g. via objects or inhalation of aerosols. Human-to-human transmission is primarily nosocomial through patients' body fluids or contaminated fomites when PPE is not in place (5).

Lassa virus causes an estimated 300,000 infections per year worldwide with, in 80% of the cases, no or very mild symptoms and, in 20% of cases, severe disease (haemorrhages and multi-organ failure) (6,9). The case fatality is 15–20% in hospitalised cases (10). There is no evidence of human-to-human transmission from asymptomatic carriers, but well-designed studies to address this question are lacking (9,11).

This report shows that LASV can pose an infection risk during routine invasive hospital procedures involving patients in endemic areas, in particular on maternity wards as LASV has a high affinity for placenta and vascular tissues (7,12). Awareness of the local risks and implementation of standard precautions to reduce the risk of transmission of blood-borne pathogens are essential to prevent nosocomial transmission (8,13). As LASV infection is initially difficult to diagnose clinically, rapid and accurate differential laboratory diagnostics are crucial to initiate appropriate supporting care and to set up measures to prevent human-to-human transmission (9,10).

PEP with ribavirin was not advised for contacts in the Netherlands because the evidence on effectiveness is inconclusive while potential side effects can be severe (14,15). Favipiravir and experimental monoclonal antibodies (which have shown encouraging results in animal models) were procured, to be used upon clinical indication (16).

The psychosocial burden of the death of the Dutch healthcare worker and of the measures on the patients, contacts and their families was reported as considerable. Protocols are required that adequately balance the necessary containment measures and the psychosocial burden on patients, contacts and care providers (10).

The response teams in the involved countries in the EU and European Economic Area appeared to use different Lassa fever protocols regarding PEP and testing of asymptomatic contacts (personal communication, UK EWRS team, 23 November 2019). There is a clear need for evidence-based practices implemented in standardised policies across countries.

The Lassa fever response team of the Netherlands

In alphabetical order: Sandra Arend (LUMC), Timo Boelsums (PHS Rotterdam-Rijnmond), Carla Eger (LUMC), Gaby Franschman (PHS Kennemerland, Ambulance care), Corine GeurtsvanKessel (Erasmus MC), Martin Grobusch (AUMC location AMC), Geert Groeneveld (LUMC), Loes Jaspers (PHS Utrecht), Hetty Jolink (LUMC), Hans van den Kerkhof (RIVM), Suzanne Kliffen (RIVM), Kevin Kosterman (RIVM), Marijn Kramer (AUMC location AMC), Tessa Langeveld (RIVM), Jaap Maas (AUMC location AMC), Richard Molenkamp (Erasmus MC), Ton Oomen (RIVM), Bas Oude Munnink (Erasmus MC), Judith van Paassen (LUMC), Mariska Petrignani (PHS Amsterdam), Gini van Rijckevorsel (RIVM), Dorothee Rosskamp (RIVM), Janke Schinkel (AUMC, location AMC), Anja Schreijer (PHS Amsterdam), Loes Soetens (RIVM), Marloes Stradmeijer (PHS Hollands Midden), Marieke Timmer (RIVM), Jans Velzing (Erasmus MC), Leo Visser (LUMC), Jolanda Voermans (Erasmus MC), Albert Vollaard (RIVM), Ann Vossen (LUMC), Anne de Vries (PHS Kennemerland), Margreet te Wierik (RIVM), Hans Zaaijer (AUMC location AMC).

Conflict of interest

None declared.

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9

General discussion

GENERAL DISCUSSION

In the global village, with people, products and pathogens traveling worldwide, a pandemic of 'disease X' was expected to happen sooner or later. In the beginning of 2020, COVID-19 appeared to be the 'respiratory disease X'. Only six weeks after China's primary report of a pulmonary disease outbreak of unknown etiology, 450 cases of COVID-19 were already reported worldwide, including cases of autochthonous transmission in 13 countries in Asia, Europe and the Americas (1). France reported the first case in Europe, but Italy was probably the country where the initial European outbreak started (1, 2). Though several factors contributed to the extent of the initial outbreak in Italy, it seems no coincidence that Italy -being the country with the second -most number of tourist arrivals in Europe (after Spain) - had been a primary entry point for COVID-19 (2, 3).

In combating COVID-19, public health measures including travel restrictions were implemented in varying degrees by countries around the world. To what extent travel-related restrictions contributed to the prevention of the introduction of new COVID-19 variants of concern has to be studied. Preliminary results of a Dutch study suggests that new variants were still introduced in the Netherlands despite the travel restrictions (4).

The epidemiological studies in this thesis have all been conducted before the beginning of the COVID-19 pandemic. COVID-19 has highlighted the importance of travel medicine for both the individual traveler as for public health purposes once more. As individual health of travelers also benefits from public health measures other than COVID-19-restrictions (e.g., malaria control in endemic countries) as well as vice versa (e.g., yellow fever vaccination of travelers prevents virus introduction in non-endemic countries), an integrated approach of travel medicine and public health, would be beneficial. In line with this approach, most sections in this chapter start with a discussion of the main results of **chapter 2-8** related to the most recent epidemiology of the travel-related diseases studied, data relevant for the individual traveler. Subsequent sections will then zoom out and discuss these travel-related diseases in the context of public health. Recommendations aiming to protect the individual traveler from disease, based on findings from our studies complemented by recent literature, are summarized in table 1, those within the scope of public health are summarized in table 2.

Table 1 Conclusions on individual travel health arising from the research presented in this thesis and recommendations from our studies complemented by recent literature for guidelines and future research.

Chapter	Infection	Conclusions	Demographic group	Recommendations for guidelines and future research
2	Helminths: Schistosomiasis Strongyloidiasis Filariasis Toxocariasis	Risk of travel-related helminth infections was very low.	Long-term travelers*	Focus pre-travel health advice regarding helminth infections under study on long-term travelers most at risk (i.e., travel for work/education, and VFR/other) and travelers engaging in risky behavior, such as rafting in contaminated water.
		Post-travel eosinophilia was not a good marker for seroconversion.		Screening for eosinophilia should not be performed among asymptomatic travelers.
		Many participants found it difficult to fully adhere to preventive measures.		Explore if post-travel questionnaires can identify travelers most at risk, and in turn, target these travelers for a cost-effective post-travel screening program.
		A small number of participants were reportedly using medication during travel which could also be used to treat against helminth infections.		Investigate if preventive medication is of value in travelers most at risk.
		The majority of the travelers participating in our study were probably not at high risk of helminth infections.		Focus future travelers' studies on travelers most at risk, such VFR travelers or travelers for work/education.
				Investigate if simple and accessible tests like self-tests are effective in finding and ultimately treating infections.
3	Hepatitis E virus	There is a substantial risk of travel-acquired hepatitis E virus (HEV) infection (serologically confirmed). The highest incidence was found among travelers to Central America and the Caribbean, and Southern Asia.	Long-term travelers*	Explore whether the increased incidence of HEV compared to previous studies is due to increasing risk over time, use of more sensitive serological tests, or a combination of these factors.
		All travel-acquired HEV infections were asymptomatic or mild and they did not cause travel health problems.		Focus pre-travel health advice regarding HEV infections, in particular hygiene and use of safe water, on travelers most at risk for severe disease.

Table 1 Conclusions on individual travel health arising from the research presented in this thesis and recommendations from our studies complemented by recent literature for guidelines and future research. (*continued*)

Chapter	Infection	Conclusions	Demographic group	Recommendations for guidelines and future research
		Risk factors for infection could not be identified.		Identify travelers most at risk of infection. Explore in prospective studies risk factors for infection, and the induction of antibodies and their characteristics (e.g., cross-reacting, waning antibodies in relation to time since infection)
4, 7	Chikungunya virus	No chikungunya infections were found among long-term travelers who had traveled to the Americas prior its introduction in the region and the subsequent large epidemic in the Americas in 2014.	Long-term travelers[*]	Chikungunya virus have been spreading extensively, particularly in the Americas. Repeat studies at intervals to provide comparative data on changes in incidence over time.
		Chikungunya virus infections were found only during a reported outbreak in Suriname.	Dutch travelers to Suriname[#]	
		Risk factors for infection could not be identified.	Dutch travelers to Suriname[#]	Identify risk factors for travel-acquired infections as this can be beneficial to target travelers at increased risk, when vaccines become available in the future.
				In the absence of a vaccine, continue to advise travelers to take effective anti-mosquito measures, especially during outbreaks
5, 6, 7	Dengue virus	Seroprevalence of anti-dengue virus antibodies is very high among Dutch travelers born in Suriname, but low in Dutch travelers born in the Netherlands.	Long-term travelers[*] FGM from Suriname[$] Dutch travelers to Suriname[#]	Future Dutch vaccine policies for travelers should take into account that levels of pre-existing anti-DENV antibodies varies substantially between travelers from dengue endemic and non-endemic areas.
		The risk of travel-acquired dengue virus infection (serologically confirmed and/or additionally PRNT50 confirmed) was substantial, particularly among tourist travelers.	Long-term travelers[*] Dutch travelers to Suriname[#]	For travelers that do not meet vaccination criteria, continue to advise effective anti-mosquito measures.
			Dutch travelers to Suriname[#]	Future research assessing the incidence of DENV infections should focus on VFR travelers with limited exposure before migration and second generation migrants.

Table 1 Conclusions on individual travel health arising from the research presented in this thesis and recommendations from our studies complemented by recent literature for guidelines and future research. (*continued*)

Chapter	Infection	Conclusions	Demographic group	Recommendations for guidelines and future research
		No differences were found in the incidence rate of travel-acquired dengue virus infection between Asia, America, and Africa.	Long-term travelers*	Travelers to Africa, are well aware of the risks of malaria -the most diagnosed disease among travelers, but should be informed about dengue risk.
		Half of the participants with a serologically confirmed travel-acquired dengue virus infection reported no (typical) dengue symptoms.	Long-term travelers* Dutch travelers to Suriname#	
		One in ten of the participants with a travel-acquired dengue virus infection reported an episode of hospitalization during travel.	Long-term travelers*	Identify characteristics of travelers most at risk of a severe dengue virus infection. Explore the feasibility of including ill or hospitalized travelers in studies.
		Cross-reactivity of antibodies in the serological dengue virus test could not be ruled out; but no association was found with vaccination against other flaviviruses.	Long-term traveler* Dutch travelers to Suriname#	In future studies, consider to use additional PCR or antigen test diagnostics, such as self-tests and/or collection of dried blood spots, during travel to improve our insight into the reliability of serological test results of travel-acquired infections.
		Cross-protection between antibodies against different flaviviruses could also not be ruled out.	Dutch travelers to Suriname#	Explore potential (vaccine-induced) cross-protection, especially in absence of a disease specific vaccine.
				Explore whether (prolonged) high antibody levels are predictive for antibodies against multiple dengue viruses (e.g., predictive for protection against severe dengue) as an alternative to PRNT50
		Frequent use of DEET was not confirmed to be protective against dengue virus infections, possibly due to the methodological limitations of our study.	Dutch travelers to Suriname# Long-term travelers*	Evaluate methods other than daily or weekly reporting in travel diaries to measure DEET-use and explore whether other preventive anti-mosquito measures exist that are both effective and easier to adhere to, and are protective.
7	Zika virus	Zika virus infections were found soon after the reported primary introduction of the virus in Suriname.	Dutch travelers to Suriname#	In the absence of a vaccine, continue to advise travelers to take effective anti-mosquito measures, especially during outbreaks.

Table 1 Conclusions on individual travel health arising from the research presented in this thesis and recommendations from our studies complemented by recent literature for guidelines and future research. (*continued*)

Chapter	Infection	Conclusions	Demographic group	Recommendations for guidelines and future research
		Cross-reaction and cross-protection between (pre-existent) antibodies against different flaviviruses could not be ruled out.	Dutch travelers to Suriname[#]	In future studies, use additional PCR or antigen test diagnostics (such as self-tests and/or dried blood spots collection) during travel to increase the reliability of serological test results for travel-acquired infections.
8	Lassa virus	No seroconversions were found among over 150 high- and low risk contacts residing in the Netherlands.	Imported cases of Lassa Fever in the Netherlands	Evaluate experience from abroad with more targeted measures -such as a more stringent classification of contacts. If possible, incorporate the international collected data into (European) protocols to optimize the balance between necessary containment measures and the psychosocial burden on patients, contacts, and health care providers.

FGM: first generation migrants, VFR: travelers visiting friends and relatives., DEET: N,N-diethyl-meta-toluamide, HEV: hepatitis E virus

*Travelers from high-income countries to tropical and subtropical countries who traveled for 3 to 12 months between 2008 and 2011.

Travelers visiting friends and relatives and tourist travelers from the Netherlands who traveled up to 3 months to Suriname between 2014 and 2017.

$ First generation migrants from Suriname living in the Netherlands

Table 2 Public health considerations arising from the research presented in this thesis complemented by recent literature.

Chapter	Infection	Recommendations for future guidelines and research
2	Helminth infections: Schistosomiasis Strongyloidiasis Filariasis Toxocariasis	As the risk of acquiring helminths infections under study for most Dutch long-term travelers seems very low, explore the feasibility of a screening program at (healthcare) facilities at entry-points where travelers at increased risk of infection (migrants from endemic areas) arrive or return to in the Netherlands. As chronic infection can potentially cause severe complications, explore if screening for helminth infections at these locations is (cost)-effective.
3	Hepatitis E Virus	Continue to measure potential risk factors for travel-acquired HEV infection as establishing risk factors for acquisition or source of infection of HEV is beneficial for the identification of both travelers and people in endemic areas most at risk. This knowledge will contribute to tailored preventive recommendations and control measures. Development of a surveillance system for HEV in Europe would provide more insight into the incidence and determinants of circulating HEV in Europe, locally acquired and imported cases of HEV infections, and uptake and effectiveness of a vaccine once registered.
4-7	Arthropod-borne (arbo) viruses	To gain more insight in the import and further transmission of arboviruses among travelers: explore the use of alternative, easily accessible diagnostics such as self-tests in asymptomatic travelers or travelers with mild disease. As travelers can function as sentinels for disease or outbreak detection, consider whether it would be beneficial for Europe (or the global village) if confirmed dengue, Zika and chikungunya would become notifiable in the Netherlands. All travelers to subtropical and tropical areas should be informed about current risk of arboviruses and educated on how to ensure a mosquito-safe local environment during travel. As the habitat of vectors is expanding in Europe, strategies should be explored on how to reach and serve all travelers to risk areas in Europe, to provide advice on preventive measures.
8	Lassa virus	Develop and implement evidence-based uniform public health policies to prevent onward spread of Lassa virus from index cases to contacts across countries.

PART 1A: TRAVEL-ACQUIRED INFECTIONS AMONG LONG-TERM TRAVELERS TO (SUB)TROPICAL AREAS

Travel-acquired helminth infections & long-term travelers

In studies on helminth infections among ill returning travelers presenting at tropical medicine sites in predominantly high-income countries, most infections were contracted at the African continent (5, 6). A previous prospective study from 2006-2007 among Dutch short-term travelers found a low incidence of travel-related schistosomiasis, strongyloidiasis, filariasis and toxocariasis (6.4, 3.2, 1.1, and 1.1 per 1,000 person-months, respectively) (7). As the risk of helminth infections is associated with prolonged exposure, migrant travelers often represent a substantial part of diagnosed patients with an infection (5, 6). Whether long-term traveling is a risk factor for helminth infections per se is not known.

Recent epidemiological studies provided insights in (potential) risk factors and the seroprevalence of (previous) infection among selected groups residing in high-income countries. For example, contact with contaminated fresh water was generally found to be a risk factor for schistosomiasis and should be avoided. Adherence, however, appeared not to be easy: half of international students who studied in a schistosomiasis endemic country were exposed to fresh water (8, 9). The proportions of travelers infected with *strongyloides* spp, and the country of infection, differed between studies: in a Belgian serological study among asymptomatic soldiers returning predominantly from Africa, 1% had an infection, but in a Spanish multicenter hospital-based, cross-sectional study among migrants and long-term travelers who visited a local hospital for any reason, 9% had antibodies, with no substantial differences between participants originating or returning from Latin America and Africa. In a study from the USA among patients on a referral list for kidney transplantation, seroprevalence was almost 10% (10-12). Filarial infections were recently studied in a case report including a 25-year retrospective analysis of travelers of a reference travel clinic in Belgium. The researchers found 327 mostly symptomatic infections in 320 travelers, predominantly in long-term travelers and visitors/migrants from tropical countries, with a decreasing number of infections over time (13). Toxocariasis was recently studied retrospectively in a reference pediatric tropical disease unit in Spain. Antibodies were found in 5% of children screened for toxocariasis (14). Most of them had a Latin-American or Asian background, and nearly two-third of infections were asymptomatic.

To explore incidence and potential differences in risks among long-term travelers, in **chapter 4** we studied four helminth infections in travelers who attended the travel health clinic of the PHS Amsterdam: schistosomiasis, strongyloidiasis, filariasis and toxocaria-

sis. These infections were previously studied among short-term travelers (7). We found low numbers of travel-acquired infections, comparable to short term travelers, even though a substantial number of participants reported exposure while travelling such as swimming in fresh water (76%), drinking unboiled water from natural resources (38%) and walking bare-foot on humid soil (62%). Only a few participants at risk acquired antibodies against *Schistosoma spp* (2), *S.stercoralis spp* (1), filarial species (5), and against both filarial and *schistosoma spp* (1) respectively, corresponding with incidence rates of 1.5, 0.3, 2.6 and 0 per 1,000 person-months, respectively. Travel-acquired infection was associated with traveling for work/education and VFR/other. Though symptomatic helminth infection is often associated with eosinophilia, screening for eosinophilia to calculate the positive predictive value for the helminth infections under study, appeared not to be of diagnostic value. We therefore recommend not to use screening for eosinophilia as a diagnostic tool to evaluate exposure to these four helminth infections in asymptomatic travelers (7, 15, 16).

Our study confirms that the risk for Dutch healthy travelers to contract one of the studied helminth infections is very low, also in long-term travelers. Adjustments of current travel health advice towards helminth infections is therefore not recommended.

Case reports of schistosomiasis often describe cases who have engaged in risky behavior. Our study supports recent studies that adherence to non-risky behavior is difficult for many travelers (9). More understanding of risky behavior relative to the risk of infection would be helpful. Perhaps development of post-travel questionnaires about risky behavior can provide data about travelers most at risk. Such data could possibly be used to develop an algorithm to select travelers most at risk in which post-travel screening might be effective in tracing active infections.

Since three percent of our participants reported that they used medication that is also effective against (one of) the studied helminth infections, it would be interesting to study the feasibility and effectiveness of preventive medication in potentially high-risk travelers. Use of self-tests, which has become more common during the COVID-19 pandemic, could perhaps also be effective in post-travel screening and treating asymptomatic or mild infections.

Impact of public health measures on travel-acquired helminth infections

Since the World Health Assembly endorsed a resolution to seriously tackle schistosomiasis and soil-transmitted infections in 2001, much progress has been made in the control of these diseases, which possibly contributed to the low number of infections found in our study (**chapter 4)** (17). However, the 2020 elimination and coverage of pre-

ventive chemotherapy targets set by the WHO were only partly reached. The 2020 target for schistosomiasis was to eliminate schistosomiasis in specified endemic countries and to cover 75% of school-aged children with preventive chemotherapy, but elimination was reached in none of these countries - and coverage of chemotherapy among children reached 67%. For helminthiasis including strongyloidiasis the target was to have 75 endemic countries with 75% treatment coverage in children, but only 21 countries had 75% treatment coverage. For lymphatic filariasis the target was to fully eliminate the disease globally: elimination was reached in only 17 of 72 countries (24%). The COVID-19 pandemic caused a major drawback in number of people treated with preventive chemotherapy in 2020 (18). The WHO launched a 2021-2030 roadmap with -again- very challenging goals for 2030 (19).

The low incidences of the helminth infections we found in our study in **chapter 4** seems not compatible with the seroprevalence found in other recent studies performed in predominantly health-care settings (10-14). Apparently, our cohort did not include the travelers most at risk. Future traveler studies should focus on travelers most at risk, such as the VFR travelers and travelers for work or education.

As first generations migrants (residents born abroad) from helminth endemic countries will continue to migrate to Europe including the Netherlands, screening, and prevention of complications should be considered. In line with the recommendation, the authors of a Spanish study proposed that targeted systematic screening could reduce prevalence of severe complications of schistosomiasis (20). It would be useful to study whether screening of migrant travelers upon arrival in the Netherlands would be effective. Exploring whether it is feasible to combine this screening with the mandatory tuberculosis screening would be of interest. In addition, screening for selected helminth infections at health care facilities with a high proportion of migrant travelers such as ‘de Kruispost’, an NGO primary care facility for undocumented migrants, homeless and/or uninsured persons in Amsterdam, the Netherlands, merits further exploration (21).

Travel-acquired hepatitis E virus infections & long-term travelers

Though European residents are at risk for the endemic zoonotic genotype 3 hepatitis E infection, during travel they seem to be primarily at risk for fecal-orally transmitted hepatitis E (genotype 1 and 2) which is associated with fulminant disease in pregnant women, for which case fatality ratios around 26% have been reported (22, 23). The risk of hepatitis E infection for travelers is difficult to estimate and compare between travelers from different European countries for several reasons. First, the background seroprevalences vary between different areas in Europe, and also between travel destination areas outside Europe, with the highest estimated seroprevalence in local populations in Africa and Asia (22% and 16%, respectively) and the lowest in Oceania (6%) (24). Second, typing of the hepatitis E virus is only possible using PCR diagnostic tests during the viremic phase of acute infection, which is difficult in case of asymptomatic travelers. As infections are usually no longer active in returning and recovered travelers, the route of transmission is often unknown. Third, so far, studies have been inconclusive as to whether infection (or vaccination) induces persistent immunity against all serotypes, as waning levels of antibodies and re-infections have also been found (25). Also, the sensitivity of serological tests, used to study previous infections or seroprevalence, varied substantially (26, 27). Hence, studies that used less sensitive tests have probably underestimated the true risk of hepatitis E in travelers.

Recent GeoSentinel data from 70 travel and tropical medicine sites across 31 countries reported 165 symptomatic hepatitis E cases among travelers over 19 years; the majority (62%) of infections were contracted in South Central Asia, especially India, including one pregnant traveler, and no fatalities (28). In older studies, acute hepatitis was found in 1% of ill returning travelers to Israel and almost 40% of these were attributed to HEV infection and predominantly contracted during travel to India (84%), but no HEV infections were found in a prospective study from Israel among backpackers traveling at least 3 months, nor in a Dutch prospective study among 1206 short-term (<3 months) travelers (29-31). One vaccine -Hecolin® (HEV-239, genotype 1 based) is currently registered in China and Pakistan, and clinical trials with Hecolin® are ongoing (32-34). Though not registered in most countries, in specific situations, such as outbreaks, WHO recommends Hecolin® vaccination (33). Two other vaccine-candidates entered clinical trials, but one of these programs was discontinued more than a decade ago, due to budget and other issues (last publication in 2007) (35-37).

The Dutch study among short-term travelers and the backpacker study from Israel that found no hepatitis E seroconversions were conducted approximately 5 and 15 years ago using different serological tests (30, 31). We performed a comparable study among our cohort of long-term travelers in **chapter 5** using a serological test with a reported

sensitivity of 98% (30). In contrast to the two earlier studies, we found a considerable risk of hepatitis E infections (attack rate 3.7%; incidence 1.8 per 1,000 person-weeks). The highest rates were found in participants traveling to Central America and the Caribbean, Southern Asia and other parts in Asia excluding Southeast Asia, but no specific risk factors for infection could be identified. In our young and healthy cohort of long-term travelers, 50% of participants with a travel-acquired HEV infection experienced mild symptoms such as fever, nausea or vomiting during travel, but no HEV diagnosis or severe infections were reported during travel. We recommend exploring if the increased incidence we found compared to previous studies is related to long-term travel, an increase in risk over time, the use of more sensitive serological tests, or a combination of these factors.

Though our finding that infections mostly lead to asymptomatic or mild disease is comforting, little is known about risk factors for infection and severe disease, and in travelers with previous HEV infection. Prospective longitudinal studies evaluating infections and antibody levels over time in individuals with symptomatic and asymptomatic infections could provide more insight in disease severity, persistency of acquired immunity and potential genotype cross-protection. More research on the severity of HEV infections among specific groups, such as pregnant-, older-, and immunocompromised travelers is needed, especially as the number of travelers belonging to these groups has increased (38, 39). Until a registered vaccine or treatment will be available in Europe, we recommend particularly groups at increased risk of severe outcomes to adhere to preventive measures during travel.

Impact of public health measures on travel-acquired hepatitis E infections

The WHO recently launched the second Global Health Sector Strategy (GHSS) on viral hepatitis, 2022-2030. The GHSS aims to eliminate viral hepatitis by 203o.The estimated global burden caused by hepatitis B and C is much larger than that of hepatitis A and E, therefore GHSS focuses primary on prevention, testing and treatment of those two viral infections. As hepatitis E outbreaks in low-income countries are predominantly associated with contaminated water, the sixth goal of the Sustainable Development Goals –to ensure availability and sustainable management of water and sanitation for all- will be the main measure for battling the global burden of hepatitis E.

Most travelers from high-income countries have access to safe water while travelling. It is therefore striking that we found so many travel-acquired hepatitis E infections in our long-term travelers in **chapter 5.** In a recent hepatitis E seroprevalence study in Suriname, the authors noticed that contaminated water was probably not the mode of HEV transmission (40). This suggest that other routes of infection than drinking water

can contribute to hepatitis E infection as well, but risk factors for 'sporadic hepatitis E' infections other than those during an outbreak- are less well understood (41). Though the participants in our cohort did not report severe disease, more research, for example a case-control study, examining risk factors for sporadic cases of hepatitis E infection in travelers could help to identify the source of infection for both travelers and people living in endemic areas. This knowledge will contribute to tailored preventive recommendations and control measures. A more robust surveillance system of hepatitis E in Europe –as proposed by the European Centre for Disease Prevention and Control – might provide more insights in incidence and risk factors of circulating hepatitis E viruses in Europe, local acquired and imported cases of hepatitis E virus, as well as in the uptake and effectivity of a vaccine when registered (42).

Travel-acquired chikungunya virus infections & long-term travelers

Although since 2004 multiple chikungunya outbreaks have been described in Africa and Asia, and a large primary outbreak after introduction in the Americas in 2013 -which hit hard in the Caribbean and South America-, little is known about the risk of chikungunya virus infections among travelers. The only prospective study, among US participants traveling to the affected areas in the Americas during the is chikungunya epidemic in 2013-2015 found a chikungunya infection attack rate of 0.9% (43). EuroTravNet -a collaboration of travel and tropical medicine clinics in Europe and part of the GeoSentinel Global Surveillance Network- found from 2008 through 2012 51 chikungunya virus infections acquired in Africa or Asia among 32,136 ill (returning) travelers with a peak of 23 cases in 2010 (44). The European Centre for Disease Prevention and Control (ECDC) based on surveillance data of travel-related chikungunya cases (2012-2018) in 13 countries in the European Union, found 2,616 travel-related chikungunya virus infections (45). Most cases (72%) were reported during the 2014-2015 chikungunya epidemic in the Americas and the majority of these were indeed associated with infection in the Caribbean and South America (45). In a European study -after the epidemic in the Americas- among ill returned travelers between 2017 and 2019 from worldwide destinations and presenting with acute undifferentiated febrile illnesses to participating hospitals or travel clinics in Barcelona, Antwerp and Lausanne, chikungunya virus infections were found in 9/455 patients from all WHO regions except Europe. A third of these chikungunya patients returned from Africa (46).

At least 20 vaccines against chikungunya are currently in development, but only 3 vaccines so far have reported data from clinical (phase 1 or 2) trials in humans (47-50).

While in 2014-2015 the habitat of chikungunya virus expended tremendously after the primary introduction and outbreak in the Americas, we explored in **chapter 3** the prevalence and infection rate of travel-related chikungunya virus infections among long-term travelers participating in a our prospective cohort. All participants traveled before the 2014-2015 Americas epidemic and in only 0.5% of them antibodies before travelling were found, suggesting infection during previous travel. As expected, none of the participants who traveled to the Americas acquired an infection, but none of the other participants who traveled to Africa or Asia either.

Chikungunya and dengue are both predominantly transmitted by the mosquitoes *Aedes albopictus* and *Aedes aegypti*. The modeled global distribution of the two vectors was very similar in 2015 (51). Since we found multiple dengue virus infections in this cohort of long-term travelers during the same calendar period, little exposure to mosquitoes did not explain the absence of chikungunya virus infections. Other virus and/or mos-

quito characteristics likely influenced the differences in dengue and chikungunya virus infections. Our study confirmed earlier (limited) data that chikungunya virus infection is less common among travelers than dengue virus infections (52).

We recommend to repeat our prospective study among travelers with the aim to examine whether there have been changes in chikungunya virus incidence over time. Such a study could also provide insight in potential changes in the area of vector and virus, in particular in the Americas. This information is useful when generating a differential diagnosis of ill returning travelers. Identification of risk factors for (symptomatic) infection will also be of interest, as travelers at increased risk of infection will benefit most from preventive measures or vaccines, once they will be available.

Travel-acquired dengue virus infections & long-term travelers

In an analysis of EuroTravNet sentinel surveillance data from 1998 to 2018 -including over 100,000 ill-returned travelers seen at 25 European health centers-, 3,721 patients were diagnosed with dengue, including 29 patients with severe dengue (53). An increasing trend was observed in both number and proportion of dengue diagnoses over time (53). Dengue is among the top 10 of diagnoses in travelers from Asia and Latin America (53). A few prospective studies among short-term travelers found dengue attack rates ranging from 1 to 3% (54-57). The only other study among long-term (3-6 months) travelers from Israel found an attack rate of 6.7% (58). Incidence rates found in these studies varied between 6.7 and 30 per 1,000 persons-months of travel (54-58).

As pre-travel screening of travelers for risk factors for severe dengue infection -such as pre-existing anti-DENV antibodies, genetic polymorphisms in a specific Fc gamma receptor or human leucocyte antigens (HLA)-, is not common, predicting the individual risk of severe dengue both in migrants born in endemic countries and in travelers remains difficult (59). Currently, two (live-attenuated tetravalent) dengue vaccine are authorized by the European Medicines Agency (EMA): one (Dengvaxia®) was registered in 2018, and the other (Qdenga®) in December 2022 (60, 61). The Dengvaxia® vaccine can be counterproductive if used in dengue naïve persons, as it can increase the risk of severe dengue. The vaccine is therefore only registered for people aged 6-45 years who have (evidence of) a previous dengue virus infection (62-65). The indication is restricted to individuals living in EU areas where dengue disease is endemic, or to individuals in non-endemic settings who have a high probability of future exposure (60, 66). The Qdenga® vaccine is registered for adults and children from 4 years of age and older (61).

We studied dengue virus infection risk in our cohort of long-term travelers (**chapter 2)** and found a substantial attack rate of 6.5%, comparable to the study from Israel (58).

The higher rate compared to those found in studies among short-term travelers, is likely due to cumulative risk of long travel duration (54-57). The incidence rate in our study was comparable with incidence rates found in previous prospective studies among short- and long-term travelers (54-58). As increasing trends are observed in proportions of dengue diagnosis in the EuroTravnet sentinel study, we recommend repeating prospective studies to monitor changes in dengue incidence among both short and long-term travelers. Prospective longitudinal studies among vaccinated travelers from non-endemic countries are needed to investigate whether vaccination induces persistent protection against severe disease. Such studies will however be challenging as large numbers of participants are required. A study to investigate travelers' intent to vaccinate against dengue and its determinants, would be more realistic. The results of such studies will be useful for the development of an effective vaccination strategy.

Our study did not find statistically significant differences in incidence rates of dengue virus infections between travelers to the African, Asian, or Latin American continent. This suggests that travelers to the African continent should be more aware of dengue in the use of preventive anti-mosquito measures. Clinicians should consider screening for dengue in ill returning travelers with fever from Sub Saharan Africa when malaria -the most diagnosed illness in travelers from Sub Sahara Africa- is excluded (53).

Our study among predominantly seronegative participants confirms that many dengue virus infections are mild, as half of the participants with a travel-acquired infection reported no or mild non-specific symptoms, though 10% of the participants with evidence of a travel-acquired primary dengue virus infection required hospitalization during their trip. However, identification of determinants for hospitalization or severe disease was not possible due to small numbers. Characteristics of travelers most at risk for severe disease after primary infection should still be explored in future research. As the incubation period of dengue is short, many travelers with dengue will fall ill during travel and some of them will require hospitalization while abroad. As studies evaluating dengue at tropical medicine sites are limited to ill returning travelers, ideally a study should be designed that not only includes the limited number of participants recruited after return to their country of residence, but also recruits travelers with severe disease during their stay in high endemic destination areas.

Comparison of studies finding serology-based travel-related dengue virus infections is difficult, amongst others because of potential cross-reactivity, differences in tests used and interpretation of test results. As prospective research to dengue virus infections among travelers is scarce, more uniformity in the used (serological) diagnostic tests could be of value, both to increase the comparability between studies and to uniformly

follow trends. Also, diagnostics during travel, such as self-tests or collection of dried blood spots by the traveler, could be of complementary use to ascertain travel-acquired diagnosis.

PART 1B: TRAVEL-ACQUIRED INFECTIONS AMONG VFR AND TOURIST TRAVELERS TO SURINAME

Travel-acquired arboviruses & VFR and tourist travelers to Suriname

In the 20-year analysis of ill returning travelers attending European tropical medicine clinics, the proportion of dengue, chikungunya and Zika virus infection increased over time, but so did the proportion of VFR travelers from roughly 8% in 1998-2002 to 12% in 2013-2018 (53).The majority of viral hemorrhagic fever infections in this European study was caused by dengue virus (the proportion of VFR travelers was not reported) (53, 67).

In Surinamese migrants living in the Netherlands, several studies on blood- and sexually transmitted infections have been performed (68-70). Little is known however about previous and incident arboviral infections such as dengue virus and the recently introduced chikungunya and Zika virus infections -introduced in 2014 and 2015 respectively in Suriname and led to fierce epidemics during travel to Suriname-. Low numbers of dengue cases were reported in Suriname in the period 2014-2021 (range 1-563) with no cases of severe disease or death (71). As neighboring countries reported many cases, dengue cases are possibly underreported in Suriname (71). Several cases of Zika virus infections were described in returning (VFR) travelers from Suriname during the Zika epidemic (72, 73). A small prospective cohort study of Belgian travelers (mostly tourists) to the Americas in 2016 -also during the Zika epidemic- found a very high incidence rate (of 170 per 1,000 person-month) of travel-acquired Zika virus infection (74). In addition to (the development of) dengue and chikungunya vaccines, Zika vaccines are currently being developed. These seem to be most beneficial for women in their fertile age as the disease can cause severe birth defects. In a recent review 21 vaccine-candidates for a Zika virus vaccine were described: 20 were in phase 1 clinical trials and 1 in phase 2 (75).

To obtain more insight in previous infections in one of the largest ethnic migrant groups in Amsterdam, we studied pre-existing anti-dengue virus antibodies among first generation migrants from Suriname living in the Netherlands in **chapter 6 and 7,** and found that the prevalence in both cohorts was very high (69 and 81%). Remarkably, in the prospective cohort of travelers to Suriname in **chapter 7**, only a small proportion of participants with a previous infection remembered having previously been infected with dengue virus.

As the presence of antibodies alone is not conclusive for the number of previous dengue virus infections, and immunity is only achieved after multiple heterologous infections, a positive serological result is not conclusive for immunity, nor is it predictive for risk of severe disease. However, a seroconversion or a fourfold increase of antibodies in paired

sera can indicate a primary or subsequent infection, respectively. Ascertaining VFR travelers at risk of a secondary, potentially more severe disease, could be beneficial for identification of travelers eligible for vaccination and early supportive treatment in case of disease. Therefore, in **chapter 7** we prospectively estimated the attack and incidence rate of dengue virus infection among both VFR and tourist travelers to Suriname. We found one primary and one secondary dengue virus infection among 137 VFR travelers and 15 primary and one secondary infection among 344 tourist travelers. The overall incidence estimate -based on serology as well as on Plaque Reduction Neutralisation Test (PRNT50) results- was substantial (47 and 23 per 1,000 person-months, respectively), and the incidence was five time higher in Dutch tourist travelers compared to the VFR travelers. In contrast to many other infectious diseases, the risk for a travel-acquired dengue virus infection was thus much lower in the VFR traveler in our cohort. No severe dengue was reported, which coincides with clinical data that severe dengue occurs only in a minority of people at risk. The absence of severe infections leaves its risk factors still unelucidated (59). We found incidence rates during the estimated epidemic periods in Suriname of 19 for chikungunya and 86 and 83 (serologically or PRNT50 tested respectively) cases per 1,000 person-months of travel for Zika virus infections. No chikungunya, and only one Zika virus infection was found after the outbreak periods.

As antibodies against flaviviruses potentially cross-react in serological tests, and a new flavivirus (Zika) was introduced in Suriname during our study period, samples of participants with serologically determined travel-acquired DENV infection were additionally tested using a more accurate in-house Plaque Reduction Neutralization Test (PRNT50) to confirm infections found by enzyme-linked immunosorbent assay (ELISA). This provided new insights in potential cross-reactivity. Our findings suggest that cross-reactivity likely played a role in the serological results of pre-existing antibodies and possibly had influenced the findings of travel-acquired infections, although we did not find an association between infection and previous or additional flavivirus vaccinations. Other unknown or unmeasured factors might have played a role as well.

The PRNT50 test results could also support the hypothesis of cross-protection between flaviviruses. A remarkable 2-year decrease in dengue cases was reported throughout the Americas following the Zika epidemic in the Americas (76). Temporarily cross-protection of anti-Zika virus antibodies against dengue was suggested as a possible explanation for this decrease (77, 78). The high and broad activity against multiple DENV serotypes we found in nearly all PRNT50 tested VFR travelers, could have possibly protected them against secondary DENV infections, contributing to their low incidence. Additionally, they were possibly also cross-protected against ZIKV infection as none of them were infected either. We therefore recommend to further explore the potential role of cross-

protection between DENV and ZIKV, including the potential role of (future) vaccines when available for travelers. We also recommend future studies among VFR travelers to focus on travelers with limited previous exposure (i.e., migrated at young age and second generation migrants), assuming that these travelers have possibly less immunity against DENV serotypes. This could coincide with a different risk of infection and in disease severity.

Impact of public health measures on travel-acquired dengue, Zika and chikungunya infections

In the studies presented in **chapter 5 and 7**, we describe that the risk of acquiring the studied arboviral infections by travelers to subtropical and tropical areas is substantial. We did not investigate active infections in returning travelers. It is however likely that viremic travelers are regularly importing arboviruses upon return, perhaps even in our cohorts. Since the mosquito *Aedes Albopictus* was first described in 1979 in Europe, and *Aedes Aegypti* has re-established in 2005 on the Portuguese island Madeira, the habitat of these vectors of dengue, Zika and chikungunya has expended substantially in Europe (79-81). Introductions of dengue and chikungunya virus in summer periods -likely by viremic travelers- have already led to several outbreaks in the past two decades in southern Europe, with case numbers ranging from 1 to 2168 (82-84). In 2020, the first Dutch patients who acquired dengue while visiting the south of France (instead of a (sub)tropical country) were reported (85).

Using mathematical models, it has been estimated that between zero and 167 positive air-travelers import dengue virus into Europe each year, with the highest risk of dengue virus introduction to Germany, followed by France and the United Kingdom (86-88). Two Dutch studies set up to gain more insight into risks of arbovirus-importation have recently been completed, but results of projects measuring silent introductions are still pending (89, 90).

In the Netherlands, to prevent their establishment, the NVWA -the Netherlands Food and Consumer Product Safety Authority- actively surveys, controls and destroys incident findings of invasive *Aedes* mosquitoes (91, 92). The 'vectorial capacity' -a measure to indicate transmission potential among humans– so far is limited. The risk of an outbreak in the Netherlands is therefore considered very low (93). However, climate change could potentially influence mosquito abundance and thus the vectorial capacity. Preparedness to higher risk situations seems therefore justified (94).

Travel advice to increase awareness of dengue, chikungunya and Zika virus infections, including preventive measures and vector control measures- currently given to travelers

to tropical and subtropical countries- should be expanded to health care workers and travelers to southern Europe as well. New strategies on how to reach these travelers should be explored. One might consider mass or targeted media campaigns before the summer period. Travelers can also serve as sentinels of disease, as in the GeoSentinel and EuroTravNet studies, and prompt diagnosis and notification could assist in early detection of outbreaks abroad (95). Therefore, additional surveillance should be considered, such as changing voluntary notification into mandatory notification for confirmed cases of dengue, Zika and chikungunya virus contracted in continental European.

Most participants in our cohort of long-term travels (**chapter 2-5**) and travelers to Suriname (**chapter 7**) were willing to contribute to a return visit after traveling. During the COVID-19-pandemic, substantial numbers of travelers were willing to perform self-tests before traveling and upon return and voluntarily notified public health authorities in case of positive tests (96, 97). As especially dengue can be asymptomatic, strategies using self-collection could gain more insight into importation of arboviruses by asymptomatic patients, that would otherwise go unnoticed. Self-tests could be offered to returning travelers at the airport. As dengue tends to occur in peaks of infection -as we also found in our cohort of travelers to Suriname (**chapter 7**)-, offering tests to travelers from such seasonal outbreak areas would be particularly interesting.

Emerging arboviruses such as dengue, chikungunya and Zika cause enormous burdens of disease in endemic countries worldwide. Dutch travelers to endemic areas should not only be aware of their individual risks for disease, but also how to contribute to prevention of import of disease on return and safe environments for local populations when abroad, for example by properly dispose solid waste and empty or clean objects which can contain water to avoid mosquitos from breeding (98).

PART 2: PUBLIC HEALTH MEASURES TO PREVENT SPREAD OF IMPORTED HEMORRHAGIC FEVER

Although Lassa fever is endemic in several countries in West Africa, and the reservoir is found throughout Sub Sahara Africa, the number of travelers who contract Lassa fever has been remarkably low (99, 100). Only 33 travelers who contracted Lassa fever in West Africa and returning to (predominantly) high-income countries have been reported in nearly 50 years, with only 2 secondary cases in Germany -a physician and a mortician- who both treated Lassa patients without (proper) personal protective equipment prior to the diagnosis (101, 102). Symptomatic disease occurs in 1 of 5 infected people, with an observed case-fatality rate of 15% among hospitalized patients (100). The case fatality rate in reported symptomatic travelers seems to be even higher (35%), which is possibly explained by selection bias (as mild imported infections can go unnoticed), delays in diagnosis (as high-income countries are unfamiliar with the disease), or potential cross-protection in local populations (cross-protection from antibodies to other arenaviruses such as Mopeiavirus are described in animals) (102, 103). In 2016, the World Health Organization launched a Research and Development blueprint list -a global strategy and preparedness plan for diseases with epidemic potential but without (sufficient) available medical countermeasures-, and included Lassa virus as a priority pathogen (104). Currently three Lassa vaccine-candidates entered phase 1 clinical trials (105). Ribavirin, and the poorly available antiviral favipiravir, are the only therapeutic options so far, but clinical experience with both drugs is very limited and results are inconclusive (106). Thus, prevention of outbreaks largely depends on public health measures including correct use of personal protective equipment, and compliance with public health guidelines which contain stringent measures.

In 2019, a small outbreak of Lassa fever, consisting of a probable index case and four secondary cases, occurred in a rural hospital in Sierra Leone (107). Contact tracing led to the identification of 274 contacts of the identified cases in 7 different countries in Europe, the Americas and Africa (107). In **chapter 9**, we describe the 164 contacts in the Netherlands of the probable index case from Sierra Leone and two of the secondary cases -Dutch health care workers- who were evacuated while symptomatic and diagnosed in the Netherlands. Of the 164 identified contacts, 19 were classified as high-risk, 131 as low-risk and 14 as sporadic contacts. We also described the public health measures taken upon the diagnosis of Lassa fever in the two health care workers in the Netherlands. According to the International Health Regulation (2015), the Dutch national focal point promptly notified other involved national focal points and the WHO upon the primary identification of cases of this outbreak.

Despite the limited number of cases in this outbreak and no other secondary or tertiary cases across the borders from Sierra Leone, the high number of contacts worldwide demonstrated how easily a disease as Lassa could have spread in our interconnected world. The experience with Lassa fever in Europe is limited. National Lassa virus public health prevention guidelines of three of the four involved European countries were publicly available online (108-110). These guidelines were mostly similar, but also differed at some crucial points, including the use of post exposure prophylaxis, requirements for burial procedures, and classification in risk categories of exposed contacts (108-110). In the German guidelines, risk classification depends on both intensity of contact and the symptomatic phase of the patients at the time the contact occurred, in contrast to the guidelines of the Netherlands and the United Kingdom which are only based on intensity of contact (108-110). As demonstrated during the COVID-19 pandemic, in our interconnected world different control strategies between different countries can lead to confusion, questions and suboptimal adherence (111, 112).

The psychosocial burden of the measures taken for Lassa fever patients, their contacts and their families were perceived as 'considerable' by all people involved. We recommend to develop uniform European evidence-based guidelines, which balance both adequate containment measures and psychosocial impact for patients, contacts and health care providers, including sufficient practical suggestions and tools for risk communication (113).

CONCLUSIONS AND GENERAL RECOMMENDATIONS

Infectious diseases have emerged, declined, and re-emerged throughout history. In our interconnected world, with a rapidly growing world population, steadily increasing merchandise trade volumes and growing numbers of global travelers, the numbers of pathogens and the rate at which they could spread around the world have increased. The risk of introducing novel pathogens followed by further spread in susceptible populations, especially in dense urban areas, and the risk of epidemics and pandemics, has also increased (114). Individual measures that protect travelers, and public health measures are more and more interwoven; protecting the individual traveler is increasingly important for public health reasons, and global public health programs increasingly influence risks for travelers. As there are only a few mandatory measures for travelers established by the IHR, travelers should be more aware of their own role in the global village. The descriptive and analytical epidemiological studies in this thesis, provide either a beginning, a next step, or an update into the dynamics of specific infectious diseases that travelers can face during travel to subtropical or tropical areas and potentially bring home. The arboviruses dengue, chikungunya and Zika (re-)emerged and majorly expanded their habitat in past years and we observed substantial infection risks for travelers from the Netherlands. Risks however varied between diseases, groups of travelers and over calendar time of travelling, with increased risks during outbreak periods in destination areas (**chapter 4-7**). Successes of WHO initiatives and efforts to control the burden of disease caused by certain helminths in endemic counties have likely contributed to the low number of observed infections in our cohort of travelers visiting these countries. Especially as adherence to preventive measures is challenging for many travelers, awareness of helminth exposure risks remains important for travelers who might be involved in risk-taking behavior during travel, as chronic infections might cause severe complications (**chapter 2**). Previous prospective serological studies may have overestimated the incidence of dengue virus infection as we demonstrated using a plaque reduction neutralization test that cross-reacting antibodies against other flaviviruses can influence serological test results (**chapter 5-7**). The risk of dengue virus infection based on neutralizing antibodies was nevertheless still considerable (**chapter 7**). Conversely, newer diagnostics with a high reported sensitivity, provided us with higher incidence of travel-acquired hepatitis E infections compared to previous studies (**chapter 3**).

For most of the studied pathogens in this thesis, preventive measures are important because effective treatments or a vaccines are lacking. However, many promising vaccines are in development. The data collected in this thesis contributes to decisions about travelers at increased risk such as long-term travelers to dengue endemic areas

or travelers to outbreak areas who could be targeted for vaccination once they become available.

Promoting individual preventive behavioral measures is a substantial part of current travel health advice, but feasibility or perseverance to adhere seems challenging (**chapter 2-5, 9**). The effectiveness, for example, of the preventive use of DEET in our cohorts seems uncertain, possibly due to limited adherence (**chapter 5,7**). Our methods of measuring adherence to DEET-use had some limitations. Future travel medicine research should explore methods which better measure adherence to preventive measures and interventions that could increase compliance. In search of the optimal (combination of) behavioral preventive measures that people adhere to, travelers engagement is needed (113). Improving adherence to personal protective measures would be beneficial for both protecting the individual traveler and for the prevention of ongoing transmission and import of infectious disease after returning home.

As even small outbreaks can have enormous cross-border outbreak potential (**chapter 8**), prospective studies among travelers should not only focus on travel-acquired infection risk, but also on the risks of import of pathogens on return.

Returning travelers can also serve as sentinels for infectious diseases (outbreaks) abroad (as in **chapter 8**), but this usually concerns symptomatic travelers who seek health care. During the later phases of the COVID-19 pandemic, many asymptomatic Dutch travelers were also willing to voluntarily notify and confirm positive self-obtained test results to public health services (115). Also thousands of Dutch inhabitants participating voluntarily in influenza like illnesses surveillance (116). Research could explore how asymptomatic travelers could voluntarily contribute to surveillance of other infectious diseases as well. A promising initiative, the 'Infection Tracking in Travellers (ITIT)'-app is recently launched in Switzerland as part of a study that aims to identify profiles of travel-associated illness by collecting real-time health-data from participants traveling all over the world (117). The app is available in nine languages including Dutch. Such a large-scale study could help overcome our current challenges in research of travel-related infections in our increasing interconnected world with ongoing climate changes, by systematically collecting reliable diagnostics either performed by a clinician or the (a)symptomatic traveler themselves and providing more accurate data and changing trends of (arboviral) disease (118).

Whereas the logarithmic scale diagram published by Professor Steffen represents the incidence of vaccine preventable diseases in relation to each other, the data in this thesis estimates the incidence of diseases which are non-vaccine preventable (yet). The

Steffen'O'gram does not provide information on seasonal fluctuations or outbreaks, nor does it distinguish between specific travel groups. As travel medicine guidelines are regularly updated based on new insights in risks for specific diseases or specific groups at risk, it would be useful to develop a 'Steffen'O'gram 2.0': an interactive and more comprehensive universal risk assessment tool, in which all –vaccine-preventable and other- travel-related infectious diseases were listed in relation to each other. This tool could assist travel health specialists in visualizing a priori risks for individual travelers, and should be regularly updated depending on changing epidemiology and include information on the specific risk for different groups of travelers, and up-to-date information towards seasonal transmission and outbreaks. It should also incorporate risk estimates of travelers spreading (new) pathogens while traveling. Consensus on risk group definitions and diagnostic criteria should be established, and findings from new studies should continuously be included in the tool. International collaboration of stakeholders throughout our interconnected world would be required to realize and maintain such a universal risk assessment tool.

In the rapidly changing global village, it will be of utmost importance to continue studying risks in different groups of travelers. A universal risk tool can also reveal specific knowledge gaps, and therefore steer a research agenda. Due to ongoing climate change and autochthonous outbreaks of dengue and chikungunya including cross-border spread in Europe in recent years, future research should also include research to travelers risks within Europe, in particular travelers to southern Europe. To be able to conduct such studies, strategies on how to reach these travelers and engage them in these studies should be explored as they currently don't visit travel health clinics. Broadly gathered and coordinated published evidence will contribute to international uniformity of travel health recommendations, which might improve adherence to guidelines.

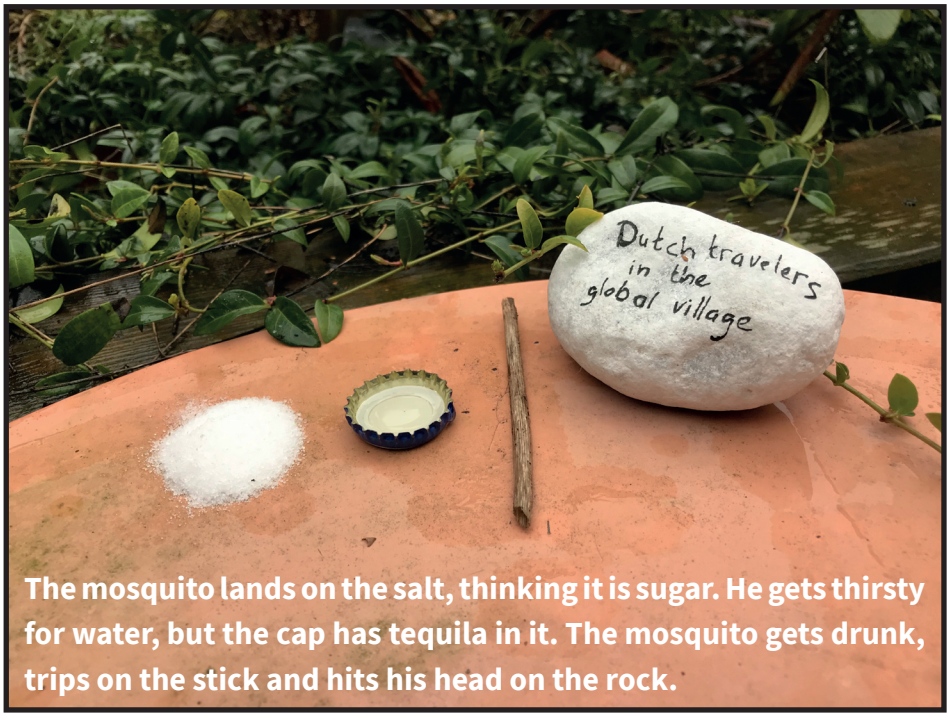

Figure 1 Mosquito trap.
Source: inspired by a frequently used online joke how to prevent mosquito-related diseases (119, 120).

"Things should be as simple as possible -
And no simpler"

Albert Einstein (121)

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A

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SUMMARY

Today, people worldwide are more connected than ever. Due to population growth, urbanization, technological developments, increased prosperity and travel, people from all over the world can directly connect with each other. In many aspects, the world seems to have turned into a large global village.

However, this globalization also has disadvantages towards infectious diseases. Due to the enormous number of travelers and international movement of products and animals, diseases or carriers of disease -such as mosquitos- can spread quickly and easily. For example, swine flu swiftly spread worldwide in 2009, just like chikungunya in 2013 and Zika did in 2015 after the first introduction on the American continent.

The globalization has fortunately also contributed to highlights in the combat against infectious diseases. For example, a global approach ensured that smallpox was eradicated in 1980 and polio had been almost eradicated in 2014. Major steps have also been taken in the battle against other vaccine-preventable diseases, such as tetanus or meningococcal disease. Substantial progress has also been made in fighting infectious diseases for which no preventive vaccine is available (yet), such as malaria. Due to globalization, public health and travel medicine are becoming intertwined.

Travel medicine aims to prevent diseases in travelers. A travel medicine advice is ideally standardized, but tailored towards the individual traveler. Each trip entails specific health risks, which depend both on the traveler themselves (presence of (chronic) illnesses, fitness, medication use, travel experience, vaccination history, reason for travel etc.) and the destination and characteristics of the trip. Moreover, tailored advice considers costs versus risk of infection, and the likelihood that a traveler adheres to a tailored advice is higher than to a general advice.

In order to achieve sound evidence-based protocols, it is necessary to increase knowledge of the risks of travel-related illnesses. Research into the risks of infectious diseases among local populations at travel destinations is often used, but their risk is generally not comparable to that in travelers. Studies among ill returned travelers presenting in hospitals or tropical outpatient clinic focus on ill travelers who seek care. They do not include mild or asymptomatic travel-acquired infections, nor the total number of travelers (which functions as the denominator in travel-related diseases). As a result, information about the true risk of travel-acquired infections is lacking.

Up-to-date epidemiological knowledge is required to develop and strengthen guidelines to reduce the acquisition and spread of travel-related infections within our global village. The aim of this thesis is to increase the epidemiological knowledge of travel-related infectious diseases, specifically those with no or limited vaccine and treatment availabilities. We focus on two travel groups, each with their own characteristics and risks during travel, who are well represented in Amsterdam: travelers who travel for three months or more -the 'long-term travelers'-, and travelers who travel to their country of origin to 'visit friends and relatives' -the VFR travelers-. In a prospective cohort of long-term travelers, we study the incidence of dengue, chikungunya, four helminth infections (schistosomiasis, strongyloidiasis, filariasis and toxocariasis) and hepatitis E (part 1a). In a previously collected cohort of first-generation Surinamese migrants, we examine the presence of antibodies against dengue. In a prospective cohort of VFR and tourist travelers to Suriname, we study the incidence of dengue, chikungunya and Zika (part 1b). We present a case with a severe travel-related illness imported into the Netherlands, and describe the risk assessment and public health measures taken to prevent the spread of travel-related infectious diseases (part 2).

Chapter 1 provides a general introduction into the global village, in particular to the positive and negative consequences of the worldwide interconnectedness related to prevention and control of infectious diseases: from eradication to sudden introduction and spread of infectious diseases. The aim of travel medicine in the Netherlands is described. Furthermore, travel groups with certain characteristics related to specific infection risks are discussed, including groups of travelers that are specifically well represented in Amsterdam.

In **chapters 2, 3, 4** and **5** we describe a prospective cohort of over 600 healthy long-term travelers who traveled between three and 12 months to (sub)tropical countries. From December 2008 through September 2011, adult travelers visiting the travel health clinic of the Public Health Service of Amsterdam before travel were eligible to participate. Participants donated blood pre- and post-travel and kept a weekly travel diary recording their itinerary, use of DEET (an insect repellant), presence of symptoms and physician visits during their trip.

In **chapter 2** we study the incidence of four helminth infections, namely schistosomiasis -formerly known as bilharzia-, strongyloidiasis, filariasis and toxocariasis. Before travel, 13 participants have evidence of previous infections (schistosomiasis (5), strongyloidiasis (3), filariasis (4), toxocariasis (1)). Post-travel, 10 travel-acquired infections among nine participants (schistosomiasis (3), strongyloidiasis (1), filariasis (6), toxocariasis (0)) are observed. Compared to tourists, participants who traveled for work or study,

VFR travelers and participants with reasons other than those mentioned are more often infected. We also study the positive and negative predictive value (PPV and NPV) of eosinophilia (indicating increased white blood cells of a specific type) on travel-related infection in post-travel donated blood. The PPV in our cohort is 0%, and the NPV is 98%. In summary, we conclude that the incidence of the four studied helminth infections is low. Routine screening for eosinophilia is of no diagnostic value in long-term travelers.

In **chapter 3** we focus on hepatitis (inflammation of the liver) E infections. Pre-travel, we find hepatitis E antibodies in 15% of participants. Nineteen new infection are acquired during travel, corresponding with an attack rate (AR) of 3.7%. The incidence of travel-acquired hepatitis E infection is 1.8 cases per 1,000 person-weeks. The travel diaries indicate no severe disease among infected participants, nor is any of the participants diagnosed with infection during travel. We discuss our results in the light of the test used in comparison to tests used in previous studies. We conclude that travel-acquired hepatitis E infection does not cause major health problems in mainly young and healthy travelers. Nonetheless, we recommend travelers to apply hygiene measures and to consume clean drinking water, particularly those at risk of severe disease (i.e., pregnant and immune-compromised travelers).

In **chapter 4** we study the arthropod-borne disease chikungunya. Our cohort of long-term travelers collected data and samples before the chikungunya epidemic of 2013/2014, when the virus was first introduced in and rapidly spread across the Americas. This provides a unique opportunity to examine the risk of infection among travelers prior to the major epidemic. Pre-travel, we find antibodies to chikungunya virus infection in three participants. No travel-acquired infections are found in any of the visited continents (Africa, Asia and/or Latin America). We recommend repeating the study, since chikungunya is currently more prevalent in the world.

In **chapter 5** we study the arthropod-borne disease dengue (break bone fever). Dengue virus is caused by a flavivirus, like yellow fever, Zika, Japanese encephalitis and tick-borne encephalitis. Antibodies against these other flaviviruses can potentially cross-react with a dengue antibody test which can result in false-positive test results and overestimates dengue risks. In our cohort, pre-travel we find antibodies against dengue virus in 3.2% of participants. Cumulative travel time of previous trips to (sub)tropical countries is associated with a previous dengue virus infection. Post-travel, we find dengue antibodies in 39 (6.5%) of participants, and estimate an incidence of travel-acquired dengue virus infection of 13.7 per 1,000 person-months of travel. Of the participants with travel-acquired infections, about half did not report any episodes of fever, while one in 10 is hospitalized during travel. Participants who reported having had symptoms

for two weeks or longer, and participants with two or more flavivirus vaccinations before the trip, more often have travel-acquired dengue virus infections. We conclude that the incidence of travel-acquired dengue virus infection in this group of long-term travelers is substantial, and higher compared to the short-term travelers (who were traveling for a maximum of three months) previously studied in Amsterdam.

In **chapters 6** and **7** we shift our focus to VFR travelers to Suriname, a popular travel destination for travelers from Amsterdam. With 349,022 residents (2016), Surinamese migrants are well represented in the Netherlands, especially compared to the population in Suriname itself (563,000 (2017)). In Amsterdam, 7% (63,359) of the population has a Surinamese migration background.

In **chapter 6** we investigate the prevalence of antibodies against dengue virus in a convenience sample of 400 first-generation Surinamese migrants living in the Netherlands. The presence of antibodies suggests that participants have had a previously acquired dengue virus infection. In this group with an average age of 52 years, 325 participants (81%) have evidence of a prior dengue virus infection. The time that participants have lived in Suriname before migration is a determinant for the presence of antibodies. Since dengue re-infections are associated with severe disease, further research is needed to evaluate whether these migrants have an increased risk of severe dengue in the event that they travel back to their country of origin.

In **chapter 7** we take a next step in our research to dengue. In a prospective cohort of 481 travelers -137 VFR travelers and 344 tourist travelers- to Suriname, we study the presence of antibodies against dengue virus prior to departure and the incidence and severity of travel-acquired dengue virus infections. The study offers both opportunities and challenges, as both chikungunya and Zika virus were first introduced in Suriname during the inclusion period of the cohort. These two arthropod-borne diseases are clinically similar to dengue, while antibodies against Zika virus can cross-react with serological dengue tests. Due to these introductions, we have broadened the focus of the study, and also study the incidence of travel-acquired chikungunya and Zika virus infections. Of the VFR travelers, 69% have antibodies against dengue virus, 14% against Zika virus and 3% against chikungunya virus before travel. Of the tourist travelers, 5% have antibodies against dengue virus, 0.6% against Zika virus and 0.3% against chikungunya virus. VFR travel is associated with a previous dengue virus infection. We find an incidence of travel-acquired infections in VFR travelers of 13.6 for dengue, 8.5 for Zika and 7.6 for chikungunya per 1,000 person-months of travel. Among tourist travelers, these figures are 67.8, 13.3 and 4.4 for per 1,000 person-months of travel, respectively. For dengue, we observe the highest overall incidence rate in 2016. For Zika and chikungunya, we ob-

serve the highest incidences during the estimated outbreak periods in Suriname, shortly after the introduction of these viruses in 2015 and 2014. As no participants experience a severe infection, it was not possible to obtain more insight in risk factors for severe dengue. An additional, more reliable Plaque Reduction Neutralization Test (PRNT50) identifies cross reactivity in a selection of samples which tested positive for the presence of pre-travel or travel-acquired anti-dengue or anti-Zika virus antibodies. PRNT50 confirms half of the dengue and three-quarters of the Zika travel-acquired infections, suggesting that the incidence rates for travel-acquired dengue and Zika infections for both VFR and tourist travelers were overestimated using serological (ELISA) tests. We conclude that in the absence of a vaccine, in particular tourist travelers run a substantial risk of dengue virus infections. Since cross-reactivity may have influenced our results, we recommend to additionally collect blood samples during travel (for example self collected 'dried blood spots') in future research, particularly in symptomatic travelers and participants traveling to outbreak areas to confirm infections with PCR tests.

In **chapter 8** we shift our perspective from infections acquired during travel to the spread of travel-related infections upon return. We describe two imported cases of Lassa fever in the Netherlands. This concerns two Dutch healthcare professionals working in Sierra Leone who most likely became infected while treating an infectious patient. They are evacuated -both symptomatic- to the Netherlands and diagnosed upon return. We describe the risk assessment of transmission to contacts of the (suspected) Lassa patients and the public health measures taken in the Netherlands to prevent further spread. In the Netherlands, 164 contacts are traced: 19 high-risk, 151 low-risk and 14 sporadic contacts. No secondary infections are found. As other contacts are also traced outside Sierra Leone and the Netherlands, foreign authorities involved abroad are informed using formal channels within the EU. The psychosocial burden of the public health measures taken are experienced as 'considerable' by all those involved. We find that different guidelines are used within the EU-countries involved, and that there is a strong need for evidence-based guidelines.

The main findings of the studies included in this thesis are summarized and related to the recent literature in **chapter 9**. In addition, their relevance is discussed in the scope of both travel medicine and public health. Recommendations for the improvement of travel medicine and public health guidelines, and suggestions for future research have been made. One of these research recommendations is to explore whether travelers can be more involved in the surveillance of travel-related diseases, for example by providing them digital tools and self-tests that can be used during travel. It is also recommended to involve travelers in behavioral research into preventive measures against mosquito bites that are both effective and easy to adhere to. The development of an international,

comprehensive universal risk assessment tool that can assist travel health professionals worldwide is recommended. This tool should provide visual insights into the risks of travel-related diseases, based on most recent epidemiological insights and tailored to the characteristics and plans of the individual traveler is recommended. Finally, It is also suggested to use internationally acquired experience in the public health guidelines for the prevention of transmission of viral hemorrhagic fever after introduction in non-endemic countries. These harmonized guidelines should be implemented internationally in order to increase compliance to taken public health measures.

SAMENVATTING

Vandaag de dag zijn mensen wereldwijd meer met elkaar verbonden dan ooit. Door onder andere populatiegroei, urbanisatie, technologische ontwikkelingen, toegenomen welvaart en reizen kunnen mensen uit allerlei uithoeken van de wereld direct met elkaar in contact komen. In veel aspecten lijkt de wereld veranderd te zijn in een groot mondiaal dorp.

Deze mondialisering heeft echter ook nadelen ten aanzien van infectieziekten. Door de gigantisch grote aantallen reizigers en internationaal verkeer van producten en dieren kunnen ziekten of dragers van ziekten -zoals muggen- zich snel en makkelijk verspreiden. Zo greep de Mexicaanse griep snel wereldwijd om zich heen in 2009, alsook chikungunya in 2013 en zika in 2015 na een eerste introductie op het Amerikaans continent.

De mondialisering heeft gelukkig ook bijgedragen aan hoogtepunten binnen de bestrijding van infectieziekten. Een mondiale aanpak heeft er bijvoorbeeld voor gezorgd dat pokken in 1980 uitgeroeid zijn en polio bijna uitgeroeid was in 2014. Ook ten aanzien van andere ziekten waarvoor een vaccin beschikbaar is -zoals tetanus of meningokokkenziekte-, zijn grote stappen in de bestrijding gemaakt. Dat geldt ook voor ziekten waar (nog) geen preventief vaccin voor beschikbaar is, zoals malaria. De mondialisering maakt dat publieke gezondheidszorg en reizigersgeneeskunde verweven raken met elkaar.

De reizigersgeneeskunde streeft naar preventie van ziekten bij individuele reizigers. Een reisgeneeskundig advies is idealiter gestandaardiseerd, maar vervolgens op maat gegeven aan elke reiziger. Elke reis brengt namelijk weer specifieke gezondheidsrisico's met zich mee, die zowel afhangen van de reiziger zelf (aanwezigheid van (chronische) ziekten, medicatiegebruik, reiservaring, vaccinatiegeschiedenis, reden voor reizen etc.) als van de bestemming en aard van de reis. Bovendien kunnen persoonlijke adviezen kosten ten opzichte van infectierisico mee wegen en is de kans dat een reiziger zich aan persoonlijke adviezen houdt groter dan aan een algemeen advies.

Om optimale en goed onderbouwde protocollen te ontwikkelen is het noodzakelijk om kennis van risico's op reis-gerelateerde ziekten te vergroten. Het gebruiken van onderzoek naar risico's op infectieziekten onder inheemse populaties op de reisbestemming lijkt voordehand liggend, maar het risico in deze populatie blijkt in de praktijk niet geheel vergelijkbaar te zijn met de risico's van reizigers. Onderzoeken onder zieke reizigers die na terugkomst in een ziekenhuis of bij een tropenpolikliniek terecht komen, geven met name een beeld van zieke reizigers die zorg hebben gezocht. Zij nemen echter niet

de reizigers met milde of asymptomatische infecties mee, noch het totaal aantal reizigers (wat fungeert als de noemer is in reis-gerelateerde ziekten). Daardoor ontbreekt informatie over het daadwerkelijke infectierisico tijdens de reis.

Voor het uitrollen en aanscherpen van richtlijnen om het oplopen en de verspreiding van reis-gerelateerde ziekten binnen ons mondiale dorp te verkleinen, is actuele epidemiologische kennis noodzakelijk. De doelstelling van dit proefschrift is het vergroten van de epidemiologische kennis van reis-gerelateerde infectieziekten, in het bijzonder van ziekten waar nog geen of beperkt vaccin en behandeling beschikbaar voor is. We focussen op twee groepen met ieder eigen kenmerkende reisrisico's en die ruim vertegenwoordigd zijn in Amsterdam: reizigers die drie maanden of langer op reis gaan -de 'langverblijvers'-, en reizigers die teruggaan naar het land van herkomst om vrienden en familie ('Visiting Friends and Relatives') te bezoeken, -de VFR-reizigers-. Door middel van prospectief onderzoek bij langverblijvers naar tropische en subtropische gebieden hebben we het voorkomen van dengue, chikungunya, een viertal parasieteninfecties (schistosomiasis, strongyloidiasis, filariasis en toxocariasis) en hepatitis E tijdens de reis onderzocht (deel 1a). Bij reizigers geboren in Suriname hebben we gekeken naar de aanwezigheid van antistoffen tegen dengue. Vervolgens hebben we het voorkomen van dengue, chikungunya en zika onderzocht bij reizigers naar Suriname die in Suriname of Nederland geboren zijn (deel 1b). We presenteren ten slotte een casus waarbij een ernstig reis-gerelateerde ziekte in Nederland werd geïmporteerd en beschrijven de risicobeoordeling en maatregelen genomen binnen de publieke gezondheidszorg om verspreiding van de reis-gerelateerde ziekte te voorkomen (deel 2).

Hoofdstuk 1 geeft een algemene introductie van het mondiale dorp met specifieke aandacht voor positieve en negatieve gevolgen van de globalisering op het voorkomen en de verspreiding van infectieziekten: van uitroeiing tot onverwachte introductie en verspreiding infectieziekten. Het doel van de reizigersgeneeskunde in Nederland wordt toegelicht. Ook worden groepen reizigers besproken met karakteristieken die gerelateerd zijn aan specifieke infectierisico's, inclusief groepen reizigers die ruim vertegenwoordigd zijn in Amsterdam.

In **hoofdstuk 2, 3, 4** en **5** beschrijven we een prospectieve cohort van ruim 600 gezonde langverblijvers –reizigers die tussen de drie en 12 maanden op reis zijn geweest- naar tropische en subtropische landen. Deelnemers aan het onderzoek bezochten in de periode tussen december 2008 en september 2011 het reizigers- en vaccinatiebureau van de GGD Amsterdam voorafgaand aan hun reis. Deelnemers stonden zowel voor als na de reis bloed af en hielden tijdens hun reis wekelijks een reisdagboek bij met bestem-

ming, gebruik van DEET (een insectwerend middel), eventuele symptomen van (infectie) ziekten en doktersbezoek.

In **hoofdstuk 2** onderzoeken we het voorkomen van een viertal parasieteninfecties, te weten schistosomiasis –voorheen bekend als bilharzia-, strongyloidiasis, filariasis en toxocariasis. Voor de reis vinden we 13 eerder doorgemaakt infecties (schistosomiasis (5), strongyloidiasis (3) filariasis (4), toxocariasis (1)). Na de reis worden er 10 nieuwe infecties gevonden in negen deelnemers (schistosomiasis (3), strongyloidiasis (1), filariasis (6),toxocariasis (0)). Vergeleken met toeristen worden deelnemers die voor werk of studie reisden, deelnemers die reisden naar het land van herkomst om familie en vrienden te bezoeken en deelnemers met een andere dan de genoemde reisredenen vaker geïnfecteerd. We onderzoeken ook in het bloedmonster dat na de reis is afgenomen, de positief en negatief voorspellende waarde (PVW en NVW) van eosinofilie (wat wijst op een verhoging van een specifiek type witte bloedcellen) op het hebben van een reisgerelateerd infectie. Symptomatische parasitaire infecties worden vaak geassocieerd met eosinofilie. De PVW in ons cohort is 0%, en de NVW is 98%. Samenvattend komen de vier onderzochte parasieteninfecties niet vaak voor. Het routinematig screenen op eosinofilie blijkt geen diagnostische voorspellende waarde te hebben in langverblijvers.

In **hoofdstuk 3** ligt de focus op hepatitis (leverontsteking) E infecties. Voorafgaand aan de reis vinden we hepatitis E antistoffen bij 15% van de deelnemers. Na de reis vinden we 19 nieuwe infecties, overeenkomend met een 'attack rate' (AR) van 3,7%. Het voorkomen van infectie tijdens de reis -de incidentie – is 1,8 gevallen per 1000 persoonsweken. Uit de gegevens verzameld in de reisdagboeken blijkt geen van de geïnfecteerde deelnemers een ernstige infectie gehad te hebben, noch is de diagnose al tijdens de reis gesteld. We bediscussiëren onze gevonden resultaten in het licht van de gebruikte test in vergelijking met tests die in voorgaande studies zijn gebruikt. We concluderen dat hepatitis E geen grote gezondheidsproblemen oplevert bij voornamelijk jonge en gezonde reizigers. Niettemin adviseren we reizigers hygiënemaatregelen toe te passen en schoon drinkwater te gebruiken, met name voor de kwetsbare groepen voor ernstige hepatitis E infecties (zwangere en immuun gecompromitteerde reizigers).

In **hoofdstuk 4** onderzoeken we de mug overdraagbare ziekte chikungunya. Ons cohort van langverblijvers heeft gegevens en monsters verzameld voor de chikungunya epidemie van 2013/2014, toen het virus voor het eerst werd geïntroduceerd en zich vervolgens snel verspreidde op het Amerikaans continent. Dit heeft een unieke kans opgeleverd om de chikungunya situatie vlak voor de grote epidemie te onderzoeken. Voorafgaand aan de reis vinden we in ons cohort antistoffen tegen chikungunya in drie deelnemers. Geen enkele reis-gerelateerde infectie wordt gevonden in de bezochte continenten (Afrika,

Azië en/of Latijns America). We concluderen dat het verstandig is om dit onderzoek te herhalen nu chikungunya zo veel meer voorkomt in de wereld.

In **hoofdstuk 5** ligt de focus op de mug overdraagbare ziekte dengue (knokkelkoorts). Dengue wordt veroorzaakt door een flavivirus, net zoals gele koorts, zika, Japanse encefalitis en tekenencefalitis. Antistoffen tegen deze andere flavivirussen kunnen mogelijk kruisreageren met een dengue antistof test, wat kan resulteren in vals positieve test resultaten en overschattingen van het dengue risico. In ons cohort vinden we voor de reis antistoffen tegen het denguevirus bij 3,2% van de deelnemers. De totale reisduur van eerdere reizen naar (sub)tropische landen is geassocieerd met een doorgemaakte infectie. Na de reis vinden we antistoffen tegen denguevirus bij 39 deelnemers (AR 6,5%) en schatten de incidentie van reis-gerelateerde denguevirus infectie op 13,7 per 1000 reispersoonsmaanden. In de groep van deelnemers met een reis-gerelateerde infectie rapporteert ongeveer de helft geen enkele episode van koorts, terwijl één op tien tijdens de reis met klachten wordt opgenomen in een ziekenhuis. Deelnemers die twee weken of langer klachten hebben gerapporteerd en deelnemers die voor deze reis twee of meer flavivirusvaccinaties hebben gekregen, hebben vaker reis-gerelateerde dengue. We concluderen dat de incidentie van reis-gerelateerde dengue in deze groep langverblijvers substantieel is en hoger vergeleken met de eerder in Amsterdam onderzochte kortverblijvers (die maximaal drie maanden op reis waren).

In **hoofdstuk 6 en 7** verleggen we onze focus naar VFR-reizigers naar Suriname,. een populaire reisbestemming van reizigers uit Amsterdam Met 349.022 inwoners (2016) zijn Surinaamse migranten ruim vertegenwoordigd in Nederland, zeker in vergelijking met de totale bevolking van Suriname zelf (563.000 (2017)). In Amsterdam heeft 7% (63.359) van de bevolking een Surinaamse migratieachtergrond.

In **hoofdstuk 6** onderzoeken we steekproefsgewijs het voorkomen van dengue antistoffen in een verzameling van bloedmonsters van 400 eerste generatie Surinaamse migranten die in Nederland wonen. De aanwezigheid van antistoffen suggereert dat deelnemers ooit eerder een denguevirus infectie hebben opgelopen. In deze groep met een gemiddelde leeftijd van 52 jaar blijken 325 deelnemers (81%) reeds eerder dengue gehad te hebben. De tijd die personen in Suriname hadden gewoond voor immigratie was een voorspeller voor de aanwezigheid van antistoffen tegen het denguevirus. Aangezien herinfecties van dengue in verband worden gebracht met ernstige dengue, zal verder onderzoek nodig zijn om te evalueren of deze migranten een verhoogd risico hebben op ernstige dengue mochten zij terug gaan reizen naar het land van herkomst.

In **hoofdstuk 7** nemen we een volgende stap in het onderzoek naar dengue. In een prospectief cohort van 481 reizigers -137 VFR-reizigers en 344 toeristen- naar Suriname bestuderen we voor vertrek de aanwezigheid van antistoffen tegen denguevirus en de incidentie en ernst van ziekte van infecties die tijdens de reis zijn opgelopen. Dit onderzoek kent kansen en uitdagingen door de primaire introductie van chikungunya- en zikavirus in Suriname tijdens de inclusietijd van het cohort. Deze twee mug overdraagbare ziekten vertonen klinisch veel overeenkomsten met dengue terwijl antistoffen tegen zikavirus ook kunnen kruisreageren met serologische dengue-testen. Door deze introducties verbreden we de focus van onze studie en onderzoeken we ook het voorkomen van reis-gerelateerde chikungunua- en zikavirus infecties. Voor de reis blijkt 69% van de VFR-reizigers reeds antistoffen te hebben tegen het denguevirus, 14% tegen zikavirus en 3 % tegen het chikungunyavirus. Van de toeristische reizigers blijkt 5% voor de reis antistoffen te hebben tegen denguevirus, 0,6% tegen het zikavirus en 0,3% tegen het chikungunyavirus. Een VFR-reiziger zijn is geassocieerd met een eerder opgelopen denguevirus infectie. We vinden een incidentie van reis-gerelateerde infectie bij VFR-reizigers van 13,6 voor dengue, 8,5 voor zika en 7,6 per voor chikungunya per 1000 reispersoonsmaanden. Voor toeristische reizigers zijn de getallen respectievelijk 67,8, 13,3 en 4,4 per 1000 reispersoonsmaanden. We vinden de hoogste incidentie in het jaar 2016 voor dengue. Voor zika en chikungunya vinden we de hoogste incidentie tijdens de geschatte uitbraakperiodes, kort na de introductie van deze virussen in Suriname in respectievelijk 2015 en 2014. Doordat geen enkele reiziger een ernstige infectie doormaakt is het niet mogelijk om meer inzicht te verkrijgen in de risicofactoren van ernstige dengue. Een aanvullende, meer betrouwbare Plaque Reductie Neutralisatie Test (PRNT50) identificeert kruisreacties in een selectie van bloedmonsters die voor de reis of reis-gerelateerd positief getest zijn op de aanwezigheid van anti-dengue- of anti-zikavirus antistoffen. PRTN50 bevestigt de helft van de dengue en driekwart van de zika reis-gerelateerde infecties en suggereert daarmee dat de incidenties van reis-gerelateerde dengue en zika voor VFR- en toeristische reizigers overschat worden bij gebruik van serologische (ELISA) tests. We concluderen dat in de afwezigheid van een vaccin, met name de toeristische reiziger een substantieel risico loopt op een denguevirus infectie. Aangezien kruisreactie van invloed kan zijn geweest op onze resultaten, adviseren we in toekomstig incidentie-onderzoek onder reizigers om extra bloed tijdens de reis te verzamelen (bijvoorbeeld middels een zelf-bloedafname waarbij bloed bewaard wordt via ‘dried blood spots’), om infecties te kunnen bevestigen middels een PCR test, in het bijzonder bij reizigers met klachten of reizigers naar een uitbraakgebied.

In **hoofdstuk 8** verleggen we ons perspectief van het oplopen van reis-gerelateerde infectieziekten naar de verspreiding van reis-gerelateerd infectieziekten bij terugkomst. We beschrijven twee import-casussen van lassakoorts. Dit betrof Nederlandse zorgme-

dewerkers werkzaam in Sierra Leone die zeer vermoedelijk geïnfecteerd raakten tijdens de behandeling van een infectieuze patiënt. Ze worden -beide symptomatisch- naar Nederland geëvacueerd en bij terugkomst gediagnosticeerd. We beschrijven de risico inschatting van de overdracht naar contacten van de (vermoedelijke) lassa patiënten en de genomen maatregelen in Nederland ter voorkoming van verdere verspreiding. In Nederland worden 164 contacten in kaart gebracht: 19 hoog risico, 151 laag risico en 14 sporadische contacten. Er worden geen secundaire infecties gevonden. Aangezien er ook andere contacten buiten Sierra Leone en Nederland zijn, worden betrokken buitenlandse instanties geïnformeerd via de formele kanalen binnen de EU. De psychosociale belasting van de genomen patiënt- en contactmaatregelen worden als aanzienlijk ervaren door alle betrokkenen. We concluderen dat richtlijnen nodig zijn waarbij een adequate balans gezocht wordt tussen de benodigde maatregelen om verspreiding te voorkomen en de psychosociale belasting die daarmee gepaard gaat. Verder constateren we dat alleen al binnen betrokken EU landen verschillende richtlijnen gehanteerd worden, en dat er sterk behoefte is aan wetenschappelijk gefundeerde richtlijnen.

Ten slotte worden de belangrijkste bevindingen van de studies in dit proefschrift in **hoofdstuk 9** samengevat en gerelateerd aan recente literatuur. Ook wordt hun relevantie besproken in het licht van zowel de reizigersgeneeskunde als de publieke gezondheid Er worden aanbevelingen gegeven om richtlijnen binnen de reizigersgeneeskunde en publieke gezondheidszorg te verbeteren en er worden suggesties gedaan voor verder onderzoek. Een van de onderzoekssuggesties is om te kijken of reizigers meer betrokken kunnen worden bij de monitoring van reis-gerelateerde ziekten door hen bijvoorbeeld van digitale hulpmiddelen en zelftesten te voorzien welke tijdens de reis gebruikt kunnen worden. Ook wordt aanbevolen om samen met reizigers op zoek te gaan naar gedragsmaatregelen om muggenbeten te voorkomen die zowel effectief zijn als makkelijk toe te passen door de reizigers. De ontwikkeling van een internationaal en universeel hulpmiddel wordt aanbevolen dat reisgeneeskundigen wereldwijd kan assisteren bij het vormen van een risicobeoordeling. Dit hulpmiddel zal visueel inzicht moeten kunnen verschaffen in die risico's van reis-gerelateerde infectieziekten, gebaseerd op de meest recente epidemiologische inzichten en afgestemd op de karakteristieken en plannen van de individuele reiziger. Ten slotte wordt de suggestie gemaakt om internationaal opgebouwde ervaring te gebruiken in de publieke gezondheidsrichtlijnen ter preventie van transmissie van virale hemorragische koorts in geval de ziekte wordt geïntroduceerd in landen waar de ziekte niet endemisch. Deze afgestemde richtlijnen zouden internationaal geïmplementeerd moeten worden ter bevordering van de compliantie aan genomen publieke gezondheidsmaatregelen.

ABBREVIATIONS

A.Albopictus	Aedes Albopictus (mosquito)
A.Aegypti	Aedes Aegypti (mosquito)
APOC	African Programme for Onchocerciasis Control
AR	Attack rate
AUMC, location AMC	Amsterdam University Medical Center, location Amsterdam Medical Center
B. malayi	Brugia malayi (roundworm)
Brd	borderline
B. timori	Brugia timori (roundworm)
C	Celsius
C (1/2/3)	Case
CDC	Centers for Disease Control and Prevention (United States of America)
CHIKV	Chikungunya virus
CI	Confidence Interval
CIb	Centre for Communicable Diseases (in dutch: Centrum Infectieziektebestrijding)
CO2	Carbon dioxide
COVID-19	Coranavirus Disease 2019
cVDPV	circulating Vaccine-Derived Poliovirus
DALYs	Disease-adjusted life-years
DEC	Diethylcarbamazine
DEET	N,N-diethyl-meta-toluamide
DENV	Dengue virus
DHF	Dengue Haemorrhagic Fever
DLI	Dengue-like illness
ECDC	European Centre for Disease Prevention and Control
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
EMA	European Medicines Agency
EMC	Erasmus Medical Center
EPI	Expanded Programme on Immunization
et al	And others
EU	European Union
EWRS	Early Warning and Response System
F	Female
FFP	Filtering Face-Piece
FGM	First generation migrant
Fig	Figure
FRNT	Focus Reduction Neutralization Test
FV	Flavivirus
GEE	Generalized Estimating Equation

GIDEON	Global Infectious diseases and Epidemiology Online Network
GPEI	Global Polio Eradication Initiative
GPELF	Global Programme to Eliminate Lymphatic Filariasis
HEV	Hepatitis E virus
Hosp	Hospital
HP	High positive
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IHA	Indirect hemagglutination assay
IHR	International Health Regulations
ILO	International Labour Organization
IR	Incidence rate
IRR	Incidence rate ratio
ITIT	Infection Tracking in Travellers (ITIT)
IQR	Interquartile range
JE	Japanese Encephalitis
LASV	Lassa virus
LCR	National Coordination Centre for Travelers Health Advice (in Dutch: Landelijk Coördinatie centrum Reizigersadvisering)
LUMC	Leiden University Medical Center
M	Male
M	Missing
MDA	Mass drug administration
MEC	Medical Ethics Committee
ml	Milliliter
mm	Millimeter
M. perstans	Mansonella perstans (roundworm)
M.ozzardi	Mansonella ozzardi (roundworm)
MRSA	Meticillin-resistant *Staphylococcus aureus*
M. strepocerca	Mansonella strepocarca (roundworm)
n	Number in sample size
N	Negative
NA	Not applicable
na*	% neutralization in post-travel sample: 100%
ND	Not done
no	Number
NPV	Negative predictive value
NTD	Neglected Tropical Diseases
NVWA	Netherlands Food and Consumer Product Safety Authority (in Dutch: Nederlandse Voedsel- en Warenautoriteit)
OEPA	Onchocerciasis Elimination Program for the Americas

OR	Odds Ratio
O.volvulus	Onchocerca volvulus (roundworm)
P	Positive
P	Prevalence
PAHO	Pan American Health Organization
P'bo	Paramaribo
PCR	Polymerase Chain Reaction
PEP	Post-exposure prophylaxis
PHEIC	Public Health Emergency of International Concern
PHS	Public Health Service
PM	Person-months
PPE	Personal Protective Equipment
PPV	Positive predictive value
POCT	Point of care testing
PR	Prevalence ratio
PRNT	Plaque Reduction Neutralization Test
p (value)	Probability value
RIVM	The National Institute for Public Health and the Environment (in Dutch: Rijksinstituut voor Volksgezondheid en Milieu)
R&D	Research and Development
RNA	Ribonucleic acid
RT-PCR	Reverse transcription polymerase chain reaction
RU	Relative Units
SARS	Severe Acute Respiratory Syndrome
SCI	Schistosomiasis Control Initiative
s/co	Signal-to-cutoff ratios
S. fülleborni	Strongyloides fülleborni (roundworm)
SGM	Second generation migrant
S. haematobium	Schistosoma haematobium (trematode worm)
S. intercalatum	Schistosoma intercalatum (trematode worm)
S. japonicum	Schistosoma japonicum (trematode worm)
SL	Sierra Leone
S. mansoni	Schistosoma mansoni (trematode worm)
S. mekongi	Schistosoma mekongi (trematode worm)
spp	Species
S. stercoralis	Strongyloides stercoralis (roundworm)
TBE	Tick-borne encephalitis
TBEV	Tick-borne encephalitis virus
T. canis	Toxocara canis (roundworm)
T. cati	Toxocara cati (roundworm)
TX	Texas

UK	United Kingdom
UMCU	University Medical Centre Utrecht
UN	United Nations
UNWTO	United Nations World tourism Organization
US(A)	United States of America
VFR	Visiting friends and relatives
WASH	Water, Sanitation, and Hygiene
W.bancrofti	Wuchereria bancrofti (roundworm)
WHA	World Health Assembly
WHO	World Health Organization
wk	Weeks
WMO	Medical Research Involving Human Subjects Act (in Dutch: Wet Medisch-wetenschappelijk Onderzoek)
WPV	Wilde polio virus
y	years
YF	Yellow fever
YFV	Yellow fever virus
ZIKV	Zika virus

PORTFOLIO

PhD training	Year	Workload (ECTS)
Courses		
AMC Graduate School, Amsterdam, the Netherlands		
-Practical Biostatistics	2014	1.1
-BROK (Basic course on Regulations and Organization for clinical investigators)	2016	1.0-*1.5*
-Infectious Diseases	2016	1.3
-Advanced topics in biostatistics	2018	2.1
-Scientific writing in English	2019	1.5
AUMC, location AMC, Amsterdam, the Netherlands		
-Basic course in native and foreign pathology for the general military physician (BIUPAMA) (selection of course)	2018	0.1
Netherlands Institute for Health Sciences (NIHES), the Netherlands		
-Epidemiology of infectious diseases (selection of courses)	2014	0.5
-Erasmus University Rotterdam: Logistic regression	2016	1.4
Netherlands School of Public & Occupational Health (NSPOH), the Netherlands		
-Basic statistics & SPSS	2012	0.6
-Public health: opportunities and effectiveness of prevention	2021	1.0
Public Health Service (GGD) of Amsterdam, the Netherlands		
-Weekly PhD educational hour (seminars, journal club, peer education, epidemiology class)	2014-2017	14.4
-Training coordinated regional incident-management procedure Coordinated Regional Incidence Response Procedure (GRIP)/ PHS disaster response plan (GROP), Public Health Service (GGD) of Amsterdam, the Netherlands	2016	0.1
-Crisis response team training, Public Health Service (GGD) of Amsterdam, the Netherlands	2016	0.2
-Multicenter (6 public health services) outbreak simulation, Public Health Service (GGD) of Amsterdam, the Netherlands	2016	0.1
Seminars, workshops		
-Annual seminar of infectious diseases research department, Public Health Service (GGD) of Amsterdam, the Netherlands	2015-2018	0.8
-Seminar Avian Influenza and Rabies, National Institute of Public Health and the Environment (RIVM), the Netherlands	2017	0.2
-Symposium HCV elimination in the Netherlands: lessons learned & challenges, Amsterdam, The Netherlands	2017	0.1
-Vaccinology Masterclass, Doorn, The Netherlands	2018	0.4

-29th Transmission symposium, National Institute of Public Health and the Environment (RIVM), The Netherlands	2019	0.2
13th National symposium Zoonosis, National Institute of Public Health and the Environment (RIVM), The Netherlands	2019	0.2
-Blue Monday, Netherlands School of Public & Occupational Health (NSPOH), Zeist, The Netherlands	2020	0.2
-30th Transmission symposium, National Institute of Public Health and the Environment (RIVM), The Netherlands	2020	0.2
-Webinar of the Dutch Working Party on Antibiotic Policy (SWAB), The Netherlands	2020	0.1
-Congress of the Umbrella Organisation for Public Health Physicians (Koepel Artsen Maatschappij + Gezondheid), The Netherlands	2021	0.2
-14th National symposium Zoonosis 'International cooperation in the control of zoonosis', National Institute of Public Health and the Environment (RIVM), The Netherlands	2021	0.2
-Seminar of the Dutch Association for infectious disease control (NVIB), Oisterwijk, The Netherlands	2021	0.4
-Symposium 'The good, the bad, and the Virus', Netherlands School of Public & Occupational Health (NSPOH)	2021	0.2
- Webinar of the Dutch Working Party on Antibiotic Policy (SWAB), The Netherlands	2021	0.1
15th National symposium Zoonosis (online), National Institute of Public Health and the Environment (RIVM), The Netherlands	2022	0.1

Poster preparations for international conferences

-"High prevalence of previous dengue virus infection among first-generation Surinamese immigrants in the Netherlands", 13th Conference of the International Society of Travel Medicine (CISTM13), Maastricht, The Netherlands (*poster presented by GJB Sonder)*	2013	0.5
-"A prospective study to chikungunya virus infection among Dutch long term travelers to (sub) tropical countries, 2008-2011"; 15th Conference of the International Society of travel Medicine (CISTM15), Barcelona, Spain *(poster presented by F Elfrink)*	2017	0.5
-"Helminth (schistosomiasis, strongyloidiasis, filariasis, toxocariasis) infections in Dutch long-term travelers, a prospective study, 2008-2011"; 15th Conference of the International Society of travel Medicine (CISTM15), Barcelona, Spain *(poster presented by GJB Sonder)*	2017	0.5

Oral presentations at international conferences

-"Dengue virus infection: incidence among long-term travelers"; 14th Conference of the International Society of Travel Medicine (CISTM14), Quebec, Canada	2015	0.5
-"Dengue, Zika and Chikungunya Virus Infections in a Prospective Cohort of Travelers to Suriname - 2014-2017"; 16th Conference of the International Society of Travel Medicine (CISTM16), Washington D.C., United States of America	2019	0.5

Attendance of (inter)national conferences

-3rd Euregional Maastricht Symposium on Immune Compromised Traveller (EUMICT), Maastricht, the Netherlands	2014	0.5

14th Conference of the International Society of Travel Medicine (CISTM14), Quebec, Canada	2015	0.8
16th Conference of the International Society of Travel Medicine (CISTM16), Washington D.C., United States of America		0.8
29th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), Amsterdam, The Netherlands	2019	0.7
-8th Northern European Conference on Travel Medicine (NECTM8), Rotterdam, The Netherlands	2022	0.6
Other		
-Media training, by Woordvoerders Etc, Baambrugge, the Netherlands	2017	0.6
-Media training, by Arian Kuil, Netherlands School of Public & Occupational Health (NSPOH), the Netherlands	2020	0.6
-Revision of the 'Rabies' guideline of the National Coordination Centre for Travelers Health Advice, The Netherlands	2018	1.0

Teaching	**Year**	**Workload (ECTS)**
Public Health Service (GGD) of Amsterdam, the Netherlands		
-Biweekly visits of medical students at department of Infectious Diseases	2015-2016	1.0

LIST OF PUBLICATIONS

Publications included in this thesis

FW Overbosch, A van den Hoek, J Schinkel, GJB Sonder. High prevalence of previous dengue virus infection among first-generation Surinamese immigrants in the Netherlands. **BMC infect Dis.** 2014 sep 10;14:493.

FW Overbosch, J Schinkel, I Stolte, M Prins, GJB Sonder. Dengue virus infection among long-term travelers from the Netherlands, a prospective study, 2008-2011. **PLoS One**. 2018 Feb 7;13(2):e0192193.

F Elfrink, FW Overbosch, J Schinkel, G Koen, GJB Sonder. Hepatitis E in long-term travelers from the Netherlands to subtropical and tropical countries, 2008-2011. **Emerg Infect Dis.** 2018 Jun;24(6):1055-1060.

FW Overbosch, T van Gool, A Matser, GJB Sonder. Low incidence of helminth infections(schistosomiasis, strongyloidiasis, filariasis, toxocariasis among Dutch long-term travelers, a prospective study, 2008-2011. **PLoS One.** 2018 May 30;13(5):e0197770.

FW Overbosch, F Elfrink, J Schinkel, GJB Sonder. No chikungunya virus infection among Dutch long-term travellers to (sub)tropical countries, a prospective 2008-2011. **BMC Infect Dis**. 2019 Feb 26;19(1):196.

FW Overbosch, J Schinkel, A Matser, G Koen, I Prange, M Prins, GJB Sonder. Dengue, chikungunya and Zika virus infections among Dutch travellers to Suriname: a prospective study during the introduction of chikungunya and Zika virus, 2014 to 2017. **Euro Surveill.** 2023;28(2):2200344.

FW Overbosch, M de Boer, KE Veldkamp, P Ellerbroek, CP Bleeker-Rovers, B Goorhuis, M v Vugt, A vd Eijk, T Leenstra, M Khargi, J Ros, D Brandwagt, M Haverkate, C Swaan, C Reusken, A Timen, M Koopmans, J v Dissel, Lassa fever response team of The Netherlands. Public health response to two imported, epidemiologically related cases of Lassa fever in the Netherlands (ex Sierra Leone), November 2019. **Euro Surveill.** 2020 Apr;25(15):2000265.

Other publications

FW Overbosch, SC Koeman, A van den Hoek, GJB Sonder, Dutch travel health nurses: prepared to prescribe? **J Travel Med** 2012 dec:19(6);361-5.

FST Suryapranata, FW Overbosch, A Matser, M Grobusch, MBB McCall, GGC van Rijckevorsel, M Prins, GJB Sonder. Malaria in long-term travelers: infection risks and adherence to preventive measures – a prospective cohort study. **Travel Med Infect Dis** 2022 Sep-Oct;49:102406.

LIST OF CONTRIBUTING AUTHORS

Author	Affiliations
Prof. dr. Bleeker-Rovers, CP	Department of Internal Medicine, Division of Infectious Diseases, Radboud university medical center, Nijmegen, the Netherlands
Dr. de Boer, MGJ	Department of Infectious Diseases, Leiden University Medical Center, Leiden, the Netherlands
Brandwagt, DAH	Department of Infectious Diseases, Public Health Service region Utrecht, Zeist, the Netherlands
Prof. dr. van Dissel, JT	National Institute for Public Health and the Environment, Centre for Communicable Disease Control, Bilthoven, the Netherlands Department of Infectious Diseases, Leiden University Medical Center, Leiden, the Netherlands
Dr. van der Eijk, AA	Department of Viroscience, Erasmus University Medical Center, Rotterdam, the Netherlands
Elfrink, F	Department of Infectious Diseases, Public Health Service of Amsterdam, Amsterdam, the Netherlands National Coordination Centre for Traveller's Health Advice, Amsterdam, the Netherlands
Dr. Ellerbroek, PM	Department of Infectious Diseases, University Medical Center Utrecht, Utrecht, the Netherlands
Dr. van Gool, T	Department of Internal Medicine, Division of Infectious Diseases, Tropical Medicine and AIDS, Academic Medical Center, the Netherlands Department of Medical Microbiology, Parasitology Section, Academic Medical Center, Amsterdam, the Netherlands
Dr. Goorhuis, B	Centre of Tropical Medicine and Travel Medicine, Amsterdam University Medical Center, location AMC, Amsterdam, the Netherlands
Dr. Haverkate, MR	National Institute for Public Health and the Environment, Centre for Communicable Disease Control, Bilthoven, the Netherlands
Dr. van den Hoek, JAR	Department of Infectious Diseases, Public Health Service of Amsterdam, Amsterdam, the Netherlands
Khargi, M	Department of Infectious Diseases, Public Health Service Hollands Midden, Leiden, the Netherlands
Koen, G	Department of Medical Microbiology, Laboratory of Clinical Virology, Amsterdam University Medical Centers, location Academic Medical Center, Amsterdam, the Netherlands
Prof. dr. Koopmans, MPG	Department of Viroscience, Erasmus University Medical Center, Rotterdam, the Netherlands
Dr. Leenstra, T	Department of Infectious Diseases, Public Health Service of Amsterdam, Amsterdam, the Netherlands
Dr. Matser, A	Department of Infectious Diseases, Research and Prevention, Public Health Service of Amsterdam, Amsterdam, the Netherlands Department of Internal Medicine, Amsterdam Infection & Immunity Institute (AIII), Amsterdam UMC, location Academic Medical Center, the Netherlands
Prange, I	Department of Infectious Diseases, Public Health Service of Amsterdam, Amsterdam, the Netherlands

Prof. dr. Prins, M	Department of Infectious Diseases, Research and Prevention, Public Health Service of Amsterdam, Amsterdam, the Netherlands Department of Internal Medicine, Amsterdam Infection & Immunity Institute (AIII), Amsterdam UMC, location Academic Medical Center, the Netherlands
Dr. Reusken, CBEM	National Institute for Public Health and the Environment, Centre for Communicable Disease Control, Bilthoven, the Netherlands Department of Viroscience, Erasmus University Medical Center, Rotterdam, the Netherlands
Ros, J	Department of Infectious Diseases, Public Health Service Kennemerland, Haarlem, the Netherlands
Dr. Schinkel, J	Department of Medical Microbiology, Laboratory of Clinical Virology, Amsterdam University Medical Centers, location Academic Medical Center, Amsterdam, the Netherlands
Dr. Sonder, GJB	Department of Infectious Diseases, Public Health Service of Amsterdam, Amsterdam, the Netherlands National Coordination Centre for Traveller's Health Advice, Amsterdam, the Netherlands Department of Internal Medicine, Amsterdam Infection & Immunity Institute (AIII), Amsterdam UMC, location Academic Medical Center, the Netherlands
Dr. Stolte, IG	Department of Infectious Diseases Research and Prevention, Public Health Service of Amsterdam, Amsterdam, the Netherlands Department of Internal Medicine, Amsterdam Infection & Immunity Institute (AIII), Amsterdam UMC, location Academic Medical Center, the Netherlands
Dr. Swaan, C	National Institute for Public Health and the Environment, Centre for Communicable Disease Control, Bilthoven, the Netherlands
Prof. dr. Timen, A	National Institute for Public Health and the Environment, Centre for Communicable Disease Control, Bilthoven, the Netherlands Athena Institute, VU University Amsterdam, the Netherlands
Dr. Veldkamp, KE	Department of Medical Microbiology, Leiden University Medical Center, Leiden, the Netherlands
Prof. dr. van Vugt, M	Centre of Tropical Medicine and Travel Medicine, Amsterdam University Medical Center, location AMC, Amsterdam, the Netherlands

AUTHOR'S CONTRIBUTIONS

Low incidence of helminth infections (schistosomiasis, strongyloidiasis, filariasis, toxocariasis) among Dutch long-term travelers, a prospective study, 2008-2011

FO enrolled participants, performed the statistical analyses and wrote the draft manuscript. TG was responsible for the parasitological analysis and the interpretation of the laboratory results. AM supervised statistical analysis. GS conceived the idea for the study, designed the project, and provided constructive comments. All authors revised and approved the final version.

Hepatitis E in long-term travelers from the Netherlands to subtropical and tropical countries, 2008-2011

FE enrolled participants, performed the statistical analyses and wrote the draft manuscript. FO made a substantial contribution in data collection and was involved in the revision of the manuscript. GK performed virological analysis. JS was responsible for the virological analysis and the interpretation of the laboratory results. GS conceived the idea for the study, designed the project, and provided constructive comments. All authors revised and approved the final version.

No chikungunya virus infection among Dutch long-term travellers to (sub)tropical countries, a prospective 2008-2011

FO enrolled participants, performed the statistical analyses and wrote the draft manuscript. FE made a substantial contribution in data collection and was involved in drafting the manuscript by providing constructive comments. JS was responsible for the virological analysis and the interpretation of the laboratory results. GS conceived the idea for the study, designed the project, and provided constructive comments. All authors revised and approved the final version.

Dengue virus infection among long-term travelers from the Netherlands, a prospective study, 2008-2011

FO enrolled participants, performed the statistical analyses and wrote the draft manuscript. TG was responsible for the parasitological analysis and the interpretation of the laboratory results. IS supervised statistical analysis. MP contributed substantially to conception of the work. GS conceived the idea for the study, designed the project, and provided constructive comments. All authors revised and approved the final version.

High prevalence of previous dengue virus infection among first-generation Surinamese immigrants in the Netherlands

FO performed the statistical analyses and wrote the draft manuscript. JS was responsible for the virological analysis and the interpretation of laboratory results. GS and AH conceived the idea for the study, designed the project, and provided constructive comments. All authors revised and approved the final version.

Dengue, chikungunya and Zika virus infections among Dutch travellers to Suriname: a prospective study during the introduction of chikungunya and Zika virus, 2014 to 2017

FO coordinated the project, analysed the data and drafted the manuscript, IP oversaw and contributed substantially to participant inclusion, GK and JS performed and supervised diagnostic tests, AM supervised statistical analysis, MP contributed substantially to conception of the work and GS supervised, conceived the project and received funding. All authors provided substantial contributions to the interpretation of the data and to subsequent drafts, and all approved the final version of the manuscript.

Public health response to two imported, epidemiologically related cases of Lassa fever in the Netherlands (ex Sierra Leone), November 2019

All authors (FO, MB, KEV, PE, CBR, BG, MvV, AvdE, TL, MK, JR, DB, MH, CS, CR, AT, MK, JvD) contributed to gathering and analysis of the information. FO, CR and AT drafted the manuscript and all authors (FO, MB, KEV, PE, CBR, BG, MvV, AvdE, TL, MK, JR, DB, MH, CS, CR, AT, MK, JvD) were involved in revising the manuscript. The members of the response team were involved in information provision and interpretation of data, as well as in the critical review of the manuscript.

DANKWOORD

"I love it when a plan comes together"

Vooruit, het heeft wat langer geduurd dan een A-team aflevering van een uurtje, maar er zijn toch veel overeenkomsten met de A-team en een proefschrift schrijven. De belangrijkste: je doet het niet alleen. De A-team niet, en ik ook niet. Zonder de geweldige hulp van alle fijne deelnemers, begeleiders, collega's, vrienden en familie was dit boekje nooit gelukt. Het was geweldig om te doen! Heerlijk dat het nu klaar is. Mijn dank aan jullie allen is onwijs groot!

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Janke en Gerrit, jullie hebben het gros van de labonderzoeken uit dit proefschrift van de 'langverblijvers' en 'dengue-onderzoek' verzorgd. Immer secuur en helder beschreven. Ik heb veel van jullie geleerd over de gebruikte testen en interpretaties, zowel met een klinische als public health blik. Ik heb de samenwerking altijd als zeer prettig ervaren,

en hoop dat er nog veel andere (reizigers)onderzoeken samen met jullie gedaan kunnen worden.

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ABOUT THE AUTHOR

Femke Overbosch was born on 9 November 1980, in Amsterdam, the Netherlands. Upon completing her secondary education at the Montessori Lyceum, Amsterdam, she initially enrolled in the bachelor program Medical technical informatics at the University of Utrecht. After a year she switched to Medical school at the University of Amsterdam. Following successful completion of the doctorate programs and various internships in different fields in Dutch hospital, she applied for an elective internship Internal Medicine at the Tijgerberg Hospital, Cape Town, South-Africa (2009). She obtained her medical degree in 2010. Her career started as a resident on the department of Internal Medicine at a peripheral hospital in Amsterdam (BovenIJ hospital), but she transitioned to pursue her passion in Public Health from 2011 onwards. At the department of Infectious Diseases at the Public Health Service of Amsterdam she gained experience as physician in travel health advice and infectious disease control. In 2014, she started her PhD research under supervision of Prof. Maria Prins and dr. Gerard Sonder, completing this thesis in 2023. In the meantime, she obtained the specialized medical profile of Communicable disease control physician KNMG (2022), for which she performed internships at the Public Health Service of Amsterdam, Public Health Service of Kennemerland, National Coordination Centre for Communicable Disease Control of the National Institute for Public Health and the Environment, the Amsterdam University Medical Center, location Academic Medical Center and MSD Medical Department in Haarlem. She aims to complete the medical specialization in public health to Public Health specialist, but will first relocate with her family to Switzerland for an adventure abroad. Femke is married to Jurgen and has two beautiful sons, Twan (10) and Stef (7).