Distribution Agreement

In presenting this thesis or dissertation as a partial fulfillment of the requirements for an advanced degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis or dissertation in whole or in part in all forms of media, now or hereafter known, including display on the world wide web. I understand that I may select some access restrictions as part of the online submission of this thesis or dissertation. I retain all ownership rights to the copyright of the thesis or dissertation. I also retain the right to use in future works (such as articles or books) all or part of this thesis or dissertation.

Signature:

_____ _____

Lillian Chen Date

Inapparent Infection by Emerging Infectious Diseases During International Travel

by

Lillian Chen

Master of Public Health

Hubert Department of Global Health

Jessica K. Fairley, MD, MPH

Committee Chair

Kenneth G. Castro, MD

Committee Member

Matthew H. Collins, MD, PhD

Committee Member

Inapparent Infection by Emerging Infectious Diseases During International Travel

by

Lillian Chen

B.S. Biological Sciences

Georgia Institute of Technology

2017

Jessica Fairley, MD, MPH

An abstract of

A thesis submitted to the Faculty of the

Rollins School of Public Health of Emory University

In partial fulfillment of the requirements for the degree of

Master of Public Health

in Global Health

2019

Abstract

Background: Each year, high volumes of individuals travel around the globe, including many to tropical areas and developing countries where certain infectious diseases are endemic. Travel infection reporting and surveillance often fails to capture asymptomatic infections, though the majority of many common and important travel-related infections occur asymptomatically. At least two gaps need to be addressed: 1) The epidemiology of emerging infectious diseases in many travel destinations is poorly understood. 2) There is a lack of understanding of risks and rates of asymptomatic infection related to travel.

Objectives: 1) Assess incidence of symptomatic and asymptomatic infection in returned international travelers, 2) Define behaviors that increase or decrease risk for travel related infection.

Methods: In this pilot study, a prospective cohort of international travelers (ages 18 and above) was established at Emory's TravelWell Center. Patient demographics, travel history, upcoming itinerary, and planned protective measures were recorded in a questionnaire; exposures and symptoms were documented by a post-travel questionnaire. Arbovirus-reactive IgG was detected by ELISA in pre- and post-travel (≥28 days) serum specimens.

Results: 50 participants were recruited in the study (ages 21-71, median: 45.50) with most of the trips lasting 1-2 weeks and a primary purpose of leisure and sightseeing. Of the 28 participants that completed the post-travel follow-up, 13 had symptoms during or following their travel. The most common symptoms reported were diarrhea and abdominal pain (8, 62%). Having a trip of more than 2 weeks has a significant association with experiencing travel-related symptoms (RR: 4.38; 95% CI: 1.69, 11.33; p-value: 0.002) and graduate degree holders were significantly more likely to be ZIKV or DENV positive than those with college or below degrees (OR: 6.42; CI: 1.09; 37.74; p-value: 0.040).

Conclusions: Of returned travelers, 41% experienced at least one symptom associated with travel and 3 (6%) individuals seroconverted for ZIKV or DENV. Ongoing serologic evaluation of travelers will reveal incidence rates of asymptomatic flavivirus infection. Further work is needed to define the role of asymptomatic infection in traveler health. This prospective study of travelers coupled with biobanking and testing is a promising approach to understand global epidemiology of infectious diseases.

Inapparent Infection by Emerging Infectious Diseases During International Travel

by

Lillian Chen

B.S. Biological Sciences

Georgia Institute of Technology

2017

Jessica Fairley, MD, MPH

A thesis submitted to the Faculty of the

Rollins School of Public Health of Emory University

In partial fulfillment of the requirements for the degree of

Master of Public Health

in Global Health

2019

Table of Contents

Chapter 1: Introduction

Background

Global travel has exploded over recent years with 1.2 billion arrivals in 2015.[1] It is estimated that by 2030 there will be 2 billion international travelers, with most of them visiting developing countries.[2] Not only does travelling connect people to different locations around the globe, it aids in the spread of goods, services, and — as a drawback — contributes to the dissemination of communicable diseases. Global travel connects vulnerable populations and emerging diseases in ways never before. A pathogen can be carried in a human traveler throughout the course of their travels and be brought back to the traveler's home country and spread to the susceptible population. For example, people infected with the Zika virus abroad can return home and unknowingly transmit the infection to their sexual partners.[3]

For many common travel-related infections, symptoms that would prompt individuals to seek medical attention only develop in a minority of those infected. The current surveillance systems in place for arboviruses, ArboNET, rely on active reporting of cases from healthcare clinics and do not capture inapparent infections from returned travelers because these individuals do not typically seek medical attention after they return from their travels. Another syndromic surveillance system in place monitoring travel-related morbidity is the GeoSentinel. This data collection network was created by the International Society of Travel Medicine (ISTM) in 1995 in conjunction with the Centers for Disease Control and Prevention (CDC). Data from the 70 participating ISTM member travel/tropical medicine clinics are pooled together to detect geographic and temporal trends in morbidity among travelers, immigrants, and refugees.[4] A comprehensive literature review of travel-related illness found that between 6% and 87% of

travelers became ill during or after their travels. Angelo et al. highlighted that the travelers in the seven studies reviewed were most commonly affected by gastrointestinal symptoms including diarrhea, nausea, constipation, and abdominal cramps. Other moderately reported symptoms were fever, skin issues, and respiratory tract symptoms. Within the studies' follow-up period, between 8% and 55% of travelers sought medical care during travel or after travel.[5] It should be noted that inapparent infections are not unimportant infections. For example, studies have shown that inapparent infection are a major driver of forward transmission for vector-borne diseases such as dengue fever, where only about 30% of those infected show symptoms.[6] Thus, there is a gap in knowledge of the prevalence of inapparent infections in international travelers and the factors associated with acquiring these infections. To address these issues, we focus on the most common travel-related diseases: acute gastroenteritis diseases (AGE) and vector-borne diseases.

Problem statement

1. Infection is a common complication of international travel, with traveler's diarrhea being the most common.
2. The incidence of inapparent infection acquired during travel and its relationship to health is poorly understood.
3. Epidemiologic data are often weak and lacking for many endemic regions that are common travel destinations. Thus, the true health burden and risk attributable to infectious diseases for people living in or visiting these areas is underappreciated.

Purpose statement

The purpose of this study is to establish a framework for study of inapparent infection in travelers and to refine travel medicine practices that reduce the risk of travel-related infection as well as to more generally improve global health through enhanced infectious disease surveillance. Toward that end, we focus on the flaviviruses dengue and Zika and foodborne diseases and determine whether travelers experience inapparent infection by these pathogens of global health importance.

Research question

Do travelers experience inapparent travel-related flavivirus infections and if so, are there demographic or travel-related behaviors associated with these infections?

Significant statement

The current surveillance systems for emerging diseases such as arboviruses and for common travel-related infections such as AGE are limited. Likely, reported infections only represent the tip of the iceberg. This translational study aims to increase the capture of incident travel-related infections by using travelers as epidemiologic proxies to understand transmission of infectious diseases in places with poorly developed public health surveillance systems. The second aim is to link self-reported patient behavior data and serological data on rates of apparent and inapparent infection in return international travelers. Understanding patient attitudes and risk perceptions of acquiring travel-related diseases while abroad will better help healthcare professionals learn how to inform the public of such diseases and to create better surveillance systems to catch potential outbreaks faster.

Chapter 2: Literature Review

Infectious diseases and travel

Throughout history, human migration has aided the spread of infectious diseases to and from different geographic regions. When the Spaniards conquered the New World, they brought smallpox, which wiped out indigenous populations in the New World, both inadvertently and by intention.[7] More recently, there have been chikungunya outbreaks in Italy among returning international travelers as well as Ebola outbreaks in Africa from which individuals have been brought back to the United States and European countries for treatment.[8-10] As travel has become more convenient and affordable, the number of international travelers has dramatically increased in recent years. Currently, the annual number of international travelers has exceeded 1 billion people with more individuals traveling to developing countries.[1] Increasing numbers of travelers globetrotting, backpacking, and venturing off the beaten path raise the potential opportunities for encounter with emerging infectious diseases.

While travel can affect infectious disease transmission, the converse can also be true, particularly in the information age, in which health data and statistics for travel destinations may be easily accessible. The recent Zika virus epidemic, which caused an international public health emergency, is an example of the latter phenomenon. In addition to affecting travelers and newborns, the Zika virus also had tremendous social and economic impact. The first confirmed case of Zika virus infection in the Americas was reported in Brazil in 2015 and soon spread throughout Latin America.[11] The Zika epidemic was heavily reported and affected travelers' decisions to visit Brazil. For example, many athletes pulled out of the 2016 Olympics and travel to Latin America dramatically decreased, impacting South America's economy. The World Bank

Group estimates $3.5 billion US dollars in economic losses were suffered in the Latin America and the Caribbean region due to Zika.[12]

Inapparent infections

Networks like the GeoSentinel, TropNet, and ArboNET surveillance systems have been put in place to monitor travel-related infections. TropNet is The European Network for Tropical Medicine and Travel Health surveillance system that is comprised of a network of travel clinics for travel and tropical medicine in Europe.[13] The United States uses ArboNET to track arbovirus cases in the country with a focus on Zika, dengue, and chikungunya. Arbovirsues are a family of viruses that are transmitted by arthropods such as mosquitoes and ticks. Medically important arboviruses include yellow fever, West Nile, dengue, chikungunya, and most recently, Zika viruses.[14] However, surveillance systems like ArboNET only capture reported symptomatic cases, meaning inapparent infections going undetected.[15]

Currently, it is estimated that as many as 300 million inapparent dengue virus (DENV) infections with mild symptoms that go undetected by surveillance systems. Recent experiments with the vector, *Aedes aegypti,* have shown that humans with inapparent DENV infections are capable of transmitting the virus to new mosquitos. The study also showed that these inapparent human to mosquito transmissions account for a part in the transmission cycle and 88% of humans infected are inapparent infections.[15]

Zika infection is associated with Guillain-Barré syndrome, and most notably, congenital abnormalities in babies who are infected *in utero*. This virus is primarily transmitted by *Aedes* mosquitoes. Approximately 80% of those infected with Zika virus are asymptomatic.[16,17] Infected individuals can go back home and unknowingly spread the disease to their sexual

partners. A prospective study conducted in 2016 found that the virus persisted in semen up to 92 days after symptom onset.[3] Huits et al. conducted a prospective study of Belgian individuals traveling to Zika endemic regions highlighted that 49% of the participants reported an illness with nine cases of confirmed ZIKV infection and one asymptomatic case. Of the nine cases of participants who contracted the Zika virus, one returned from South America, six from Central America, and two from the Caribbean.[18]

Another common travel-related illness that affects travelers is acute gastroenteritis (AGE). AGE, which largely overlaps with traveler's diarrhea, is the leading cause of illness in return travelers who seek medical care and can be due to bacteria, viruses, or parasites. Bacteria that cause AGE include Enterotoxigenic *E. coli*, Enteroaggregative *E. coli,* and *Campylobacter jejuni.* However, norovirus is currently the pathogen that causes the most cases of clinically diagnosed AGE.[19] The low infectious dose and multiple modes of transmission of norovirus and its persistence in the environment makes it difficult to control the spread of the virus. The most common symptoms caused by norovirus includes diarrhea and vomiting. Vomiting has been documented to facilitate the transmission of the virus especially in enclosed spaces such as cruise ship where 97% of AGE related outbreaks have been linked back to norovirus. Currently, there are still knowledge gaps in the incidence of apparent and inapparent AGE caused by norovirus in travelers.[19]

Traveler behavior

Traveler knowledge is at the center of emerging infectious diseases issues. How travelers perceive their risk of contracting illnesses will affect how they seek care and use preventative services. Insight into how travelers view their risks for these diseases while traveling will

provide health professionals more guidance on how to give better advice in person and online.[20] Understanding traveler behaviors will help guide clinicians in pre-travel visits in advising travelers on potential travel-related symptoms as reported in Vilkman et al. The study noted that despite proper preventative measures such as patient education, vaccinations, and malaria prophylaxis, the majority (79%) of study participants reported illness during and/or after their travels.[21]

Chapter 3: Manuscript

Abstract

Background: Each year, high volumes of individuals travel around the globe, including many to tropical areas and developing countries where certain infectious diseases are endemic. Travel infection reporting and surveillance often fails to capture asymptomatic infections, though the majority of many common and important travel-related infections occur asymptomatically. At least two gaps need to be addressed: 1) The epidemiology of emerging infectious diseases in many travel destinations is poorly understood. 2) There is a lack of understanding of risks and rates of asymptomatic infection related to travel.

Objectives: 1) Assess incidence of symptomatic and asymptomatic infection in returned international travelers, 2) Define behaviors that increase or decrease risk for travel related infection.

Methods: In this pilot study, a prospective cohort of international travelers (ages 18 and above) was established at Emory's TravelWell Center. Patient demographics, travel history, upcoming itinerary, and planned protective measures were recorded in a questionnaire; exposures and symptoms were documented by a post-travel questionnaire. Arbovirus-reactive IgG was detected by ELISA in pre- and post-travel (≥28 days) serum specimens.

Results: 50 participants were recruited in the study (ages 21-71, median: 45.50) with most of the trips lasting 1-2 weeks and a primary purpose of leisure and sightseeing. Of the 28 participants that completed the post-travel follow-up, 13 had symptoms during or following their travel. The most common symptoms reported were diarrhea and abdominal pain (8, 62%). Having a trip of more than 2 weeks has a significant association with experiencing travel-related symptoms (RR: 4.38; 95% CI: 1.69, 11.33; p-value: 0.002) and graduate degree holders were significantly more likely to be ZIKV or DENV positive than those with college or below degrees (OR: 6.42; CI: 1.09; 37.74; p-value: 0.040).

Conclusions: Of returned travelers, 41% experienced at least one symptom associated with travel and 3 (6%) individuals seroconverted for ZIKV or DENV. Ongoing serologic evaluation of travelers will reveal incidence rates of asymptomatic flavivirus infection. Further work is needed to define the role of asymptomatic infection in traveler health. This prospective study of travelers coupled with biobanking and testing is a promising approach to understand global epidemiology of infectious diseases.

Introduction

Global travel has exploded over recent years with 1.2 billion arrivals in 2015.[1] It is estimated that by 2030 there will be 2 billion international travelers, with most of them visiting developing countries.[2] Global travel connects vulnerable populations and emerging diseases in ways never before. A pathogen can be carried in a human traveler throughout the course of their travels and be brought back to the traveler's home country and spread to the at-risk population. For many common travel-related infections, individuals do not seek medical attention unless they are exhibiting symptoms. Studies have shown that inapparent infection are a major driver of forward transmission for vector-borne diseases such as dengue fever It has been estimated that of the 390 million dengue infections per year, only 96 million of those cases show symptoms.[6] In addition, a comprehensive literature review of travel-related illness found that between 6% and 87% of travelers became ill during or after their travels. Angelo et al. highlighted that the travelers in the seven studies reviewed were most affected by gastrointestinal symptoms including diarrhea, nausea, constipation, and abdominal cramps.[5]

The purpose of this study is to investigate inapparent infection in international travelers through questionnaire instruments and serologic testing. To address these issues, we focus on the most common travel-related diseases: acute gastroenteritis diseases (AGE) and vector-borne diseases. We leveraged unique access to a large population of international travelers cared for at our university and sought to combine travel information, survey information on health perceptions and travel activities, as well as serologic data to better understand inapparent travel-related infections by focusing on flavivirus infection.

Methods

Population, sample, and study site

A mere 10 miles from the one of the busiest international airports in the world, Hartsfield-Jackson, is TravelWell Center. This travel clinic has been established since 1988 and provides approximately 2,000 pre-travel consultations and manages over 150 sick returned travelers annually. Therefore, this clinic has been chosen as the site for which the study enrolled its participants. Adult age 18 years and over seeking pre-travel consultation at Emory University Hospital Midtown (EUHM)'s TravelWell Clinic were screened and offered participation in the study. This study excluded immunocompromised individuals as well as individuals under the age of 18. The eligible population included travelers with international destinations who endorsed willingness to complete a follow up visit after their return at TravelWell Clinic or to Emory University's Hope Clinic. This study employed a convenience sampling approach due to efficiency of recruiting directly from pre-travel visits.

Research design

A prospective cohort of international travelers (ages 18 and above) was established. Upon consent, patient travel history, upcoming itinerary, and planned protective measures were recorded in a questionnaire. Pre-travel saliva and blood samples were also obtained. Participants were asked to return for the post-travel (≥28 days) to fill out the post-travel questionnaire detailing their exposures and symptoms as well donating saliva and blood samples. Arbovirus- and enteric pathogen-reactive IgG was detected by ELISA in pre- and post-travel serum specimens.

Data Collection Procedures

Patient data were collected through a pre-travel questionnaire packet the patients filled out themselves. The patients filled out the details of their upcoming trips, planned protective mosquito measures, water sources, and travel itinerary. Medical history, duration of travel, destination country, vaccine, and demographics were also obtained at the pre-travel visit. The post-travel questionnaire was administered by a study team member in the form of an interview. The questions were asked in an objective, non-leading manner such that answers were not suggested by the interviewer. Answers were recorded in the questionnaires based on subject-reported information. Patient responses from the questionnaires were entered in the online database, RedCap.

Blood samples were collected in 8.5mL serum separator tubes through via phlebotomy performed on a peripheral vein in the antecubital fossa. Blood was allowed to clot for 20 min at room temperature and then centrifuged for 10 minutes at 1000 x g at room temperature. Serum was stored up to one week at 4°C prior to transport or stored immediately at -80°C until analysis.

Serology

Serological assays were performed using Zika H/PF/2013[22] and the WHO reference strains for each of the four DENV serotypes[23]: DENV1 West-Pac 74, DENV2 S-16803, DENV3 CH54389, DENV4 TVP-360. Virus stocks were prepared in the C6/36 *Aedes albopictus* cell line (ATCC CRL-1660); infectious units were titrated on Vero cells (ATCC# CCL-81).

Antigen capture ELISA to detect human serum IgG binding to DENV or Zika was performed as previously described[24]. The anti-E protein mouse monoclonal antibody (mAb) 4G2[25] was used to coat 96-well plates and then blocked with 3% non-fat dairy milk. Flavivirus antigen (Zika or

DENV) was then captured during 1-hour incubation, and human IgG binding was detected with a goat anti-human IgG secondary Ab conjugated to alkaline phosphatase.

Neutralization titers (FRNT50) were determined by an abbreviated 96-well microFRNT similar to previously described[26,27]. Four 4-fold serial dilutions of serum in singlicate were mixed with approximately 50-100 focus-forming units of virus in DMEM with 2% FBS. The virus-antibody mixtures was incubated for 1 hour at 37°C and then transferred to a monolayer of Vero cells for infection for 2 hours at 37°C. OptiMEM overlay media (Gibco, 31985) supplemented with 2% FBS, 1% Anti-Anti and 5g (1%) carboxymethylcellulose was then added, and cultures were incubated for 40 hours (Zika), 48 hours (DENV2 and DENV4) or 52 hours (DENV1, DENV3). Cells were fixed with 70 µL of 4% paraformaldehyde for 30 minutes. 100 µL of permeabilization buffer was added for 10 minutes followed by 100 µL of blocking buffer (3% normal goat plasma) and left overnight at 4°C. Foci were detected by incubation with primary mAb 4G2 followed by addition of horseradish peroxidase-conjugated goat anti-mouse IgG secondary antibody. Foci were visualized with 60 µL of True Blue and counted with a user-supervised automated counting program on 2x-magnified images of micro-wells obtained on a CTL ELISPOT reader. Two naïve human plasma (NHS) controls were included on every plate to define 100% infection.

Statistical Analysis

Using SAS version 9.4, we conducted univariate and frequency procedures to determine demographic characteristics and symptoms among the study participants. Categorical variables were described by counts and percentages and continuous variables as minimum, maximum, and

median. Further, fit log binomial bivariate models to assess the associations between selected behaviors and characteristics with development of travel-related symptoms. Lastly, frequency procedures were used to determine pre-travel Zika and dengue related characteristics and seroconversion. Graphics were produced using Mapchart. All statistical analyses were conducted using an alpha level $p = 0.05$.

Ethical considerations

This project was approved by the Institutional Review Board of Emory University in the United States (IRB00103363). Informed written consent was performed for any individual before any study activities.

Results

Between 2018 – 2019, travel information was collected from 50 individuals (ages 21-71, median: 45.50) with half the trips lasting 1 – 2 weeks (n = 25, 50%). The demographics of the study population are displayed in Table 1. Of the 50 enrolled participants, 26 (53.1%) individuals having at least some advanced education beyond a bachelor's degree and 42 (84%) individuals were born in the United States. In this cohort, 21 (42.9%) had lived outside the United States for a period of 6 months or more. Figure 2 shows the regions in the globe where individuals traveled to and multiple regions were recorded if individuals marked multiple regions in travel itinerary. The countries in the Africa region were the most frequently visited (n = 24, 40%), followed by South America (n = 10, 17%), Central America/Caribbean (n = 6, 10%), Western Europe (n = 6, 10%), Southern Asia (n = 4, 7%), Middle East (n = 3, 5%), Eastern Asia (n = 3, 5%), Southeast Asia (n = 3, 5%), North America (n = 1, 2%). No travelers visited Eastern Europe or Oceania.

The region with the highest reported symptoms during the post-travel visit was South America (4, 27%) and Africa (3, 20%).

Even though the focus of this project was the detection of inapparent infections during travel, we noted that participants in our cohort did experience symptoms associated with travel at rates similar to what has been previously reported.[21] Twenty-seven individuals completed post-travel follow-up visits, with 11 (41%) of those reporting symptoms (Table 2). The most frequently reported symptom was diarrhea (n = 6, 22.2%), followed by abdominal pain (n = 4, 14.8%), and fatigue, nausea, and vomiting (n = 3, 11.1%). Table 4 shows the unadjusted risk ratios of experiencing travel-related symptoms with behaviors or demographic characteristics. We examined length of trip, and behaviors including: eating raw meat, raw seafood, street food, unpeeled or unwashed fruit, unpeeled or unwashed vegetable, and engaging in water-related activities, and demographic variables including: education level and age. We found having a trip of more than 2 weeks has a significant association with experiencing travel-related symptoms (RR: 4.38; 95% CI: 1.69, 11.33; p-value: 0.002). All other variables were not found to be statistically significant with the outcome.

Pre- and post-travel flavivirus serology was performed to determine whether interval infection had occurred in travelers. Table 3 depicts IgG ELISA results for ZIKV and DENV positivity for post-travel visit specimens. Of the post-travel serology results, 15 individuals tested positive for either Zika or dengue. Fourteen (66.7%) subjects were born in the United States and 5 (41.7%) had lived abroad for 6 months or more. Eleven (73.3%) graduate degree holders were flavivirus positive at the post-travel visit and were significantly more likely to be ZIKV or DENV positive than those who held college or below degrees (OR: 6.42; CI: 1.09, 37.74; p-value: 0.040). The other demographic unadjusted associations for birthplace status, age, or lived

abroad for 6 months or more were not significantly associated with flavivirus positivity at the post-travel visit. Of the two individuals who seroconverted for the dengue virus, one went to Senegal and did not recall getting mosquito bites, and the other went to Italy and Colombia and recalled getting mosquito bites. The individual who seroconverted for ZIKV went to Peru but did not recall getting mosquito bites. Figure 1 shows DENV and ZIKV seroconversion of individuals between their pre- and post-travel visit. Of those who completed both visits, 2 individuals seroconverted for DENV and 1 seroconverted for ZIKV.

Discussion

This translational study aimed to link serologic data and traveler behavior in international travelers from Atlanta, Georgia. This population consisted of mainly graduate degree holding individuals with the majority traveling to low- or middle-income countries. The two most traveled regions in this study population was South America and Africa. This prospective study followed a cohort of travelers and assessed them for inapparent and apparent symptoms is the start of integrating lab tests with clinical practice in travel medicine using a translational approach which includes both lab data and traveler demographics and behavior.

Our main finding, which reinforces previous research, is that length of travel is statistically associated with risk of experience travel-related symptoms. The longer the trip is, the higher the risk of an individual contracting a travel-related symptom, usually by ingestion. The most commonly reported AGE symptom in the cohort was diarrhea, which is consistent with previous literature indicating diarrhea is among the top travel-related symptoms that travelers report.[5,19] For example, a comprehensive literature review conducted by Angelo et al. looked at traveler demographics, trip specifics, ill travelers, and symptoms experienced, found that

between 43 – 79% of travelers who visited developing countries became ill and the most common reported symptom was diarrhea.[5] Another study, Vilkman et al., conducted a prospective travel study with post-travel follow-up of traveler behavior and symptoms experienced during and after their trip and found that long travel duration was also significantly associated with development of symptoms.[21]

Of the individuals with both pre-travel and post-travel information, one seroconverted for ZIKV and two seroconverted for DENV between pre- and post-travel time points. This rate of seroconversion is to be expected because it is a relatively rare event, particularly in this population which may not have the highest risk for exposure to mosquitoes that transmit flavivirus since these travelers are seeking pre-travel consultation and have received information on how to protect against mosquito bites. Another study in the literature also used pre and post-travel serology to identify seroprevalence for dengue, Zika, chikungunya, and West Nile virus in international travelers and found that 23 (14.7%) had IgM or IgG antibodies for at least one virus and of those seropositive individuals, 12 (52%) were asymptomatic.[8] While this study had more participants, we also found quite a bit of seropositivity in our cohort. Furthermore, our methods for detecting seroconversions and discerning between true infection and cross-reactive immunity elicited by flavivirus vaccines are robust, though these methods would only be an option for a limited number of labs with special BSL-2 virology capacity. Other travel clinics or labs may not have access to this method.

There are several limitations to this study. First, the sample size was relatively small and thus provided limited statistical power to the data analysis. The number of individuals who completed the post-travel visit was also few and thus made the cohort smaller when pre- and post-travel comparisons were made. Furthermore, many of the data depended on participant self-

reporting which has a degree of error to it. Some individuals may purposely provide inaccurate responses on the questionnaires, while some would have limited recall about details associated with their trip to provide the most accurate answers.

Since the sample population was a small number of travelers from TravelWell Center, the findings may not be applicable to travelers from other areas in Georgia or the United States. In addition, the population of individuals who visit Emory's TravelWell Clinic is only a small subset of the types of travelers who leave from Hartsfield-Jackson airport each year. A major limitation is the study population sample size was small and there was little variation across levels of variables so it's possible that associations exists between exposures and outcomes but there was not enough power to detect them. The next logical step would be to have a more well-rounded study aimed to include individuals from all socio-economic demographics, locations, and more subjects. Future directions would be to recruit more travelers from around Atlanta and Georgia and to have a bigger sample size. Even though there were limitations to our study, our data were still informative and supported previous literature. As numbers of international travelers continue to rise, translational studies such as this one will be able to help decrease the knowledge gap on traveler behavior and travel-related diseases.

Chapter 4: Conclusion and Recommendations

This pilot study looked at possible flavivirus seroconversions in travelers during their international trip. A total of three individuals seroconverted between their pre- and post-travel visit, two seroconverted for ZIKV and one for DENV. None of the travelers developed symptoms related and this seroconversion data would be unavailable to surveillance systems, had the individuals not participated in this study. This is because surveillance systems such as ArboNET are only able to capture symptomatic cases that are seen at travel clinics. This study marks one of the first steps to address the presence of inapparent infections missed by existing surveillance systems. It also gives travel clinics a chance to create a stronger partnership with government-sponsored surveillance systems and work to better characterize the burden of asymptomatic infectious which could lead to ongoing arbovirus infection transmission and use the information to prevent future outbreaks of flavivirus such as Zika and dengue virus. Another part of this study looked to link demographic and traveler behavior with AGE travel-related symptoms. Study findings show that there is a statistically significant association between length of travel and travel-related symptoms such as diarrhea and vomiting.

Future work would be to increase number of participants enrolled to increase statistical power to the analyses and incorporate multivariate models of risk. Efforts should also be made to recruit a more heterogeneous study population to ensure that the results can be more applicable to the general travel population. Our population was broad in the age range recruited but most travelers had high degrees of education and took precaution in their travel health by seeking pre-travel consultation.

References

1. Alizadeh A, Valentin B, Paulose H, Kester J, Glaesser D. Global travel patterns: an overview. *Journal of travel medicine.* 2017;24(4).
2. Fink D, Wani RS, Johnston V. Fever in the returning traveller. *BMJ.* 2018;360:j5773.
3. Gaskell KM, Houlihan C, Nastouli E, Checkley AM. Persistent Zika Virus Detection in Semen in a Traveler Returning to the United Kingdom from Brazil, 2016. *Emerging infectious diseases.* 2017;23(1):137-139.
4. GeoSentinel. The International Society of Travel Medicine. http://www.istm.org/geosentinel. Accessed March 31, 2019.
5. Angelo KM, Sotir MJ, Kozarsky PE, Ryan ET, Chen LH. What proportion of international travellers acquire a travel-related illness? A review of the literature. *Journal of travel medicine.* 2017;24(5).
6. Bhatt S, Gething PW, Brady OJ, et al. The global distribution and burden of dengue. *Nature.* 2013;496:504.
7. Pringle H. *How Europeans brought sickness to the New World.* 2015. https://www.sciencemag.org/news/2015/06/how-europeans-brought-sickness-new-world. Accessed April 12, 2019.
8. Loconsole D, Metallo A, De Robertis AL, Morea A, Quarto M, Chironna M. Seroprevalence of Dengue Virus, West Nile Virus, Chikungunya Virus, and Zika Virus in International Travelers Attending a Travel and Migration Center in 2015-2017, Southern Italy. *Vector borne and zoonotic diseases (Larchmont, NY).* 2018;18(6):331-334.
9. Shantha JG, Crozier I, Varkey JB, et al. Long-term Management of Panuveitis and Iris Heterochromia in an Ebola Survivor. *Ophthalmology.* 2016;123(12):2626-2628.e2622.

10. Otsuki S, Nishiura H. Reduced Risk of Importing Ebola Virus Disease because of Travel Restrictions in 2014: A Retrospective Epidemiological Modeling Study. *PloS one.* 2016;11(9):e0163418-e0163418.

11. Lowe R, Barcellos C, Brasil P, et al. The Zika Virus Epidemic in Brazil: From Discovery to Future Implications. *International journal of environmental research and public health.* 2018;15(1):96.

12. Chapter 11 - Economic Impact of Zika Virus. In: Qureshi AI, ed. *Zika Virus Disease.* Academic Press; 2018:137-142.

13. Lingscheid T, Kurth F, Clerinx J, et al. Schistosomiasis in European Travelers and Migrants: Analysis of 14 Years TropNet Surveillance Data. *The American journal of tropical medicine and hygiene.* 2017;97(2):567-574.

14. Huang Y-JS, Higgs S, Vanlandingham DL. Arbovirus-Mosquito Vector-Host Interactions and the Impact on Transmission and Disease Pathogenesis of Arboviruses. *Frontiers in microbiology.* 2019;10:22-22.

15. ten Bosch QA, Clapham HE, Lambrechts L, et al. Contributions from the silent majority dominate dengue virus transmission. *PLOS Pathogens.* 2018;14(5):e1006965.

16. Sampathkumar P, Sanchez JL. Zika Virus in the Americas: A Review for Clinicians. *Mayo Clinic Proceedings.* 2016;91(4):514-521.

17. Musso D, Gubler DJ. Zika Virus. *Clinical microbiology reviews.* 2016;29(3):487-524.

18. Huits R, Van Den Bossche D, Eggermont K, et al. Incidence of Zika virus infection in a prospective cohort of Belgian travellers to the Americas in 2016. *International Journal of Infectious Diseases.* 2019;78:39-43.

19. Lindsay L, DuPont HL, Moe CL, et al. Estimating the incidence of norovirus acute gastroenteritis among US and European international travelers to areas of moderate to high risk of traveler's diarrhea: a prospective cohort study protocol. *BMC infectious diseases.* 2018;18(1):605-605.

20. Rodrigues KMdP, Moreira BM. Preventing diseases in round-the-world travelers: a contemporary challenge for travel medicine advice %J Revista da Sociedade Brasileira de Medicina Tropical. 2018;51:125-132.

21. Vilkman K, Pakkanen SH, Lääveri T, Siikamäki H, Kantele A. Travelers' health problems and behavior: prospective study with post-travel follow-up. *BMC infectious diseases.* 2016;16:328-328.

22. Baronti C, Piorkowski G, Charrel RN, Boubis L, Leparc-Goffart I, de Lamballerie X. Complete coding sequence of zika virus from a French polynesia outbreak in 2013. *Genome announcements.* 2014;2(3):e00500-00514.

23. Collins MH, McGowan E, Jadi R, et al. Lack of Durable Cross-Neutralizing Antibodies Against Zika Virus from Dengue Virus Infection. *Emerging infectious diseases.* 2017;23(5):773-781.

24. de Alwis R, Smith SA, Olivarez NP, et al. Identification of human neutralizing antibodies that bind to complex epitopes on dengue virions. *Proceedings of the National Academy of Sciences of the United States of America.* 2012;109(19):7439-7444.

25. Henchal EA, Gentry MK, McCown JM, Brandt WE. Dengue Virus-Specific and Flavivirus Group Determinants Identified with Monoclonal Antibodies by Indirect Immunofluorescence. 1982;31(4):830-836.

26. Willcox AC, Collins MH, Jadi R, et al. Seroepidemiology of Dengue, Zika, and Yellow Fever Viruses among Children in the Democratic Republic of the Congo. 2018;99(3):756-763.

27. Swanstrom JA, Plante JA, Plante KS, et al. Dengue Virus Envelope Dimer Epitope Monoclonal Antibodies Isolated from Dengue Patients Are Protective against Zika Virus. *mBio*. 2016;7(4):e01123-01116.

Tables and Figures

Table 1 – Basic Traveler Demographics

Demographic variable	n (%)
Age in years, median (range)	45.5 (21, 71)
Education Level	
High School	1 (2%)
Some College	4 (8.2%)
College	18 (36.7%)
Graduate and beyond	26 (53.1%)
Missing	1 (2%)
Trip duration	
< 1 week	7 (14%)
1-2 weeks	25 (50%)
2-3 weeks	14 (28%)
4 weeks	3 (6%)
> 4 weeks	1 (2%)
Born in US	
Yes	42 (84 %)
No	8 (16 %)
Lived outside US for > 6 months	
Yes	21 (42.9%)
No*	28 (57.1%)
Missing	1 (2 %)
Total	50 (100%)

Table 2 – Symptoms during or up to one week after trip

Symptoms	n (%)
Travelers with symptoms	11 (41%)
Symptom	
Diarrhea	6 (22.2%)
Abdominal pain	4 (14.8%)
Fatigue	3 (11.1%)
Nausea	3 (11.1%)
Vomit	3 (11.1%)
Body ache	2 (7.4%)
Bleeding (gum, stool, urine)	1 (5.9%)
Fever	1 (5.9%)
Joint pain	1 (5.9%)
Other	3 (11.1%)
None	16 (59.3%)
Total post-travel cases	27 (100%)

Table 3. Demographics and unadjusted associations of Zika and dengue seropositivity

	ZIKV or DENV positive at post-travel visit						
	Yes	No	Total	OR	95% lower	95% upper	p- value
US born							
Yes	14 (66.7%)	7 (33.3%)	21	8.00	0.75	85.72	0.086
No	1 (20%)	4 (80%)	5	Ref			
Lived abroad for 6 months or more							
Yes	5 (41.7%)	7 (58.3%)	12	0.32	0.06	1.64	0.172
No	9 (69.2%)	4 (30.8%)	13	Ref			
Age							
18-35	6 (46.2%)	7 (53.8%)	13	Ref			
36-54	4 (66.7%)	2 (33.3%)	6	2.33	0.31	17.55	0.410
55+	5 (71.4%)	2 (28.6%)	7	2.92	0.41	20.90	0.287
Education							
College and below	3 (30%)	7 (70%)	10	Ref			
Graduate school	11 (73.3%)	4 (26.7%)	15	6.42	1.09	37.74	0.040

Figure 1 – DENV and ZIKV seroconversion between pre- and post-travel

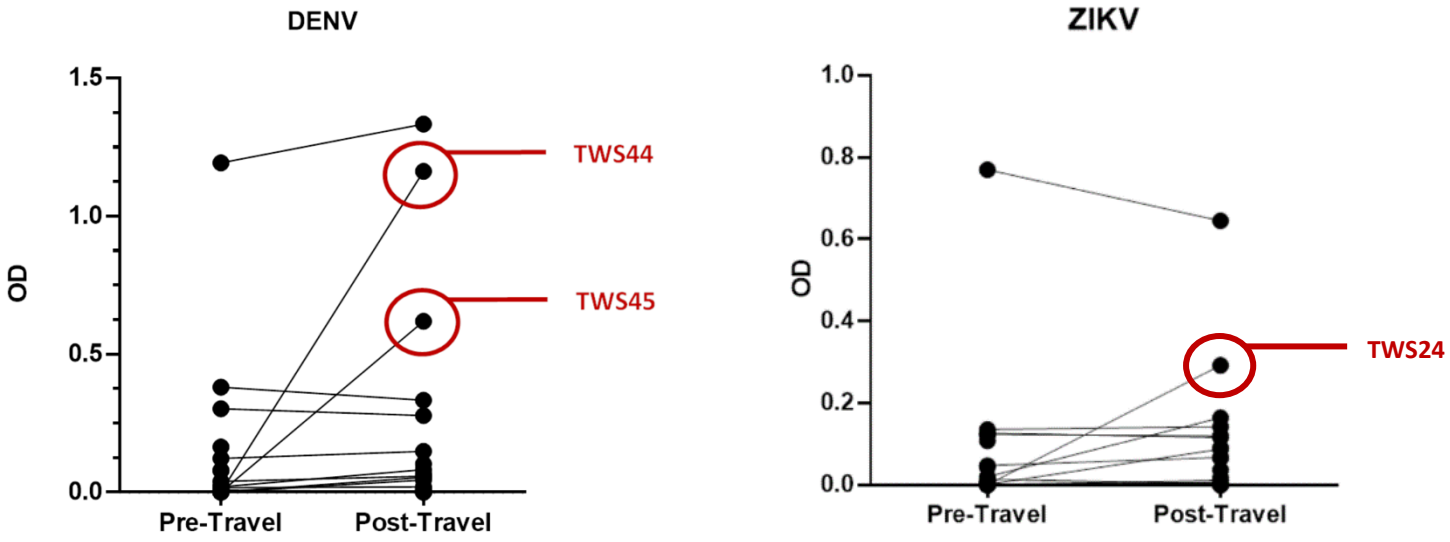

Figure 1. Pre- and Post-Travel flavivirus serostatus among US residents traveling internationally. Antigen capture ELISA was used to detect human IgG binding to DENV or ZIKV. Mean optical density (OD) of two technical replicates of flavivirus-naïve serum was subtracted from the mean OD of test specimens. Seroconversion is defined by a Post-Travel OD that is > 0.2 and ≥ 4x Pre-Travel OD. Labels for individual participants (DENV: TWS44, TWS45; ZIKV: TWS24) that exhibited seroconversion are superimposed on graphs.

Table 4. Unadjusted associations of experiencing travel-related symptoms among return travelers in 2018-2019 in Atlanta, Georgia

Behavior	Total (27)	Experienced travel-related symptoms (n = 9)	Did not experience travel-related symptoms (n = 18)	RR	Lower 95% CI	Upper 95% CI	p-value
Eating raw meat							
Yes	2	1	1	1.56	0.35	7.00	0.560
No	25	8	17	Ref			
Eating raw seafood							
Yes	7	3	4	1.43	0.48	4.23	0.520
No	20	6	14	Ref			
Eating street food							
Yes	4	2	2	1.64	0.52	5.23	0.401
No	23	7	16	Ref			
Eating unpeeled or unwashed fruit							
Yes	2	1	1	1.56	0.35	7.00	0.560
No	25	8	17	Ref			
Eating unpeeled or unwashed vegetable							
Yes	6	3	3	1.75	0.61	4.99	0.295
No	21	6	15	Ref			
Engaged in water-related activities							
Yes	10	2	8	0.49	0.12	1.90	0.299
No	17	7	10	Ref			
Education level							
Graduate school or beyond	16	6	10	1.38	0.43	4.36	0.589
College and below	11	3	8	Ref			
Age							
18-35	13	5	8	Ref			
36-54	7	1	6	0.37	0.05	2.59	0.317
55 and above	7	3	4	1.11	0.37	3.34	0.847
Length of trip							
More than 2 weeks	6	5	1	4.38	1.69	11.33	0.002
Less than 2 weeks	21	4	17	Ref			

Figure 2 – Travel destination distribution of TWS cohort. The bold numbers indicate the number of subjects that traveled to a certain region as ascertained in the pre-travel survey. Numbers in parentheses indicate the number of travelers endorsing any symptom at the post-travel visit. Destinations were available for all 50, and some travelers visited more than 1 region, total destinations = 60. A few subjects were lost to follow up, total number assessed for travel-related symptoms = 27.