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Chagas disease among travelers visiting friends and relatives to endemic areas: a descriptive study

Master's thesis

Catalina Salinas Cevallos, MD

Thesis director: Israel Molina Romero, MD, PhD

Thesis director

Israel Molina Romero, MD, PhD

Infectiouos Diseases attending physician at University Hospital Vall d'Hebron
Barcelona, Spain

Medical Director at PROSICS Barcelona
PROgrama de Salut Internacional de l'Institut Català de la Salut (PROSICS)
Barcelona, Spain

External teaching staff for the Official Master in Zoonoses and One Health
Universitat Autònoma de Barcelona
Barcelona, Spain

Dedication

To Antonio, my dear husband, for his unconditional encouragement and genuine interest in all my stories about bugs and infectious diseases.

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List of abbreviations

BP Before present years

CD Chagas disease

CDC Centers for Disease Control and Prevention of the United States

EMR Electronic medical record

GI Gastrointestinal

IQR Interquartile range

LA Latin America

VFR Visiting friends and relatives

USA United States of America

yo years old

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Abstract

Background: Chagas disease is regarded as a risk for travelers to continental Latin America. However this risk has not been quantified before. **Objective:** To determine the risk of *T. cruzi* infection among returning travelers visiting friends and relatives (VFRs) to endemic areas, to describe the demographic and travel characteristics, and to identify risk factors for acquiring *T. cruzi* infection. **Design:** prospective descriptive study. **Methods:** All available adults with laboratory proof of being free of Chagas disease before travel at University Hospital Vall d'Hebron in Barcelona, Spain from 2012 to 2015 were recruited for standardized questionnaire and measurement of *T. cruzi* antibodies in serum. **Results:** 60 participants were included in the study. All were immigrants from Central or South America living in Spain, who traveled to visit friends and relatives. 45/60 (75%) were female, the median age was 38.5 years old. The median duration of travel was 43.5 days (IQR 30-63.5 days). 35/60 (58%) visited rural areas. Fresh fruit juices and sugar cane were consumed by 36/60 (60%) of participants. Housing construction material prone to Reduviid bug infestation (adobe, palm tree-thatching, wooden or cane walls) were only met by 2/60 (3.3%). 59/60 (98%) of travelers VFR were negative for *T. cruzi* infection after travel to endemic areas, and one case 1/60 (2%) had discordant inconclusive serology. **Limitations:** The number of eligible patients was reduced by the amount of patients unreachable by telephone (313/633, 49.3%), and many were unable to participate due to strict working shifts, which may have selected for more females in our study. **Conclusions:** Among 60 travelers VFR to endemic areas we found no cases of Chagas disease. Travelers were proven to be free of *T. cruzi* infection prior to travel. This study provides the basis for future studies that aim to evaluate the risk of acquiring Chagas disease during travel to endemic areas to provide evidence for improvement of clinical recommendations and public health screening efforts.

Introduction

Concerns that international travel to Latin America may pose risk to acquire Chagas disease (CD) have been proposed and are part of health travel recommendations. *Trypanosoma cruzi* infection is endemic in 21 countries in Central and South America. In Europe and other non-endemic areas, CD is an emerging infection and much awareness has been raised recently due to its public health implications. However, the risk of acquiring CD in a single trip to endemic areas has not been quantified. Chagas disease has been reported among travelers before, but whether the infection was acquired prior or during to travel has not been established. The chronic indeterminate phase of CD can last for decades, therefore we think it is necessary to rule out prior infection in travelers of Latin American origin in order to quantify the risk of travel. This knowledge would represent the scientific evidence to support both pre-travel counseling and screening efforts. It may be possible that the risk of acquiring Chagas disease after travel is minimal, even among travelers visiting friends and relatives (VFR) who exhibit riskier behaviors. We analyze data from all available patients documented to be free of *Trypanosoma cruzi* infection prior to travel to endemic areas at University Hospital Vall d'Hebron in Barcelona, Spain from 2012 to 2015 as a pilot study that will serve as a guide for a larger scale study.

Trypanosoma cruzi is the causal agent of Chagas disease or American trypanosomiasis. It is named after Dr. Carlos Chagas, the Brazilian physician who described the parasite, its biological cycle, and clinical manifestations in 1909 during the construction of a railroad system in Brazil.[1] *T. cruzi* is a hemoflagellate protozoan, and its most common mode of transmission is through a bloodsucking vector. Reduviid insects from the genera *Triatoma, Panstrongylus,* and *Rhodnius* are the vectors most frequently implicated in human infection.[2] These vectors are widely distributed in the Americas from the southern part of the United States (US) to Chile and Argentina. Chagas disease has been present in the Americas as an enzootic disease for thousands of years. There is molecular evidence of *T. cruzi* infection in mummies from pre-Columbian era in the Andes region 9000 years before present (BP) and evidence of megacolon due to CD in a

mummy dated 1150 BP from what is now Texas, US.[3] The interaction between parasite and human hosts has evolved over time and has determined the epidemiological trends in endemic areas, but globalization is now determining epidemiology worldwide. The role that travelers to endemic areas play in the epidemiology of non-endemic areas is not fully understood.

The parasite and its life cycle

The life cycle of *Trypanosoma cruzi* begins when the vector becomes infected by having a blood meal from an infected mammal or human. The protozoa multiply in the insect's midgut as epimastigotes, and develop in the insect's hindgut as trypomastigotes which is the infective form. During the following blood meal, usually at night, infective trypomastigotes are eliminated in its feces. The infective parasites gain access to the mammal or human through skin breaks, mucous membranes, or conjunctivae, which completes its transmission cycle. Once inside its new host, *T. cruzi* is able to multiply as intracellular amastigotes similar to *Leishmania.* Inside the host cell, amastigotes differentiate into trypomastigotes which are then released into circulation once the cell ruptures. Thus, *Trypanosoma cruzi* spreads hematogenously to distant sites to begin new life cycles.[4]

Transmission and clinical manifestations

In addition to vectorial-borne, the other modes of transmission have gained relevance in a globalized world: blood transfusion, organ donation, congenital, and food-borne infection. Vectorial and oral transmission of *T. cruzi* are exclusive to the Americas. Infection related to blood products, donation of organs, and mother to child occur worldwide and have come to highlight the importance of screening at-risk populations. The socioeconomic burden of disease has motivated prevention initiatives in non endemic areas like Europe, Australia, Japan, and the United States. This review puts emphasis on vectorial and oral transmission due to their pertinence to travelers to continental Latin America.

Vectorial transmission of *Trypanosoma cruzi* is by far the most common mode of transmission of Chagas disease. It follows a sylvatic and a domestic cycle in which humans are incidental hosts, affected by their interactions with the environment and animals. When land is opened for human activities - may it be construction of railways, farming, or urbanization in enzootic areas - the vector adapts to living in human dwellings and feeding on domestic animals. Certain housing conditions, have been identified as risk factors because they provide crevices and nooks for Reduviid insects to thrive. Describing such conditions as "poor dwellings" may be an oversimplification. An entomologic survey in a periurban area of Arequipa, Perú found that 52% (194/374) of houses were infested with triatomines, of those 19.3% were infected with *T. cruzi*. Guinea pig pens (OR 1.69) and rabbit enclosures (OR 1.52) were identified as a risk factors for triatomine infestation. Adobe or stacked brick increased the likelihood of triatomine infestation (OR 2.52). Fully stuccoed walls was identified as a protective factor for both human dwellings and animal enclosures.[5] The presence of hens indoors was identified as a risk factor for *Triatoma infestans* in human dwellings by a study in rural communities in Santiago del Estero, Argentina.[6] Palm trees are also a preferred habitat for triatomine bugs, and palm leaves are often used for roof thatching. A study found that 27% of palm trees near human dwellings were infested by *Rhodnius ecuadoriensis,* in 3 different climatic areas of Ecuador.[7] The density of the vector is patchy throughout the Americas, some areas like "el Gran Chaco", a vast plain that includes parts of Argentina, Bolivia and Paraguay, have a much higher vector population and prevalence of Chagas disease in humans.

Briefly, the natural history of disease acquired by vectorial transmission includes an "acute phase" with a self-limited febrile illness. Physical signs that point to the site of entry of *T. cruzi* are the Romaña's sign (conjunctivae), and the "chagoma" (skin), which are present only in few patients. The very young and the immunocompromised hosts may present with severe forms of disease like meningoencephalitis and myocarditis. In most cases, the acute phase may pass largely unnoticed and resolves spontaneously in 4 to 8 weeks. It is followed by the "chronic indeterminate phase" where the patient remains asymptomatic for decades, but with low-level intermittent parasitemia and detectable antibodies. This phase is relevant to public health because

transmission can still occur despite lack of symptoms. A variable percentage, 30-40% of infected patients go on to develop the mega-syndromes of the "chronic phase". Cardiac abnormalities occur in ~30%, with biventricular dilatation, congestive heart failure, arrhythmias, are the most frequent. The gastrointestinal (GI) system is affected in ~10% with megaesophagus, dolichocolon, and megacolon. The largest patient-series from a non-endemic country (n=1274) was reported at our Institution.[8] A lower proportion of cardiac and GI compromise was found compared to patient-series from endemic areas, likely due to the younger age of migrants, and use of routine diagnostic studies for infected patients.

Oral transmission of *Trypanosoma cruzi* is responsible for outbreaks of massive parasitic infection leading to severe disease with high death rates. Unlike other food-borne infections, acute Chagas disease from oral transmission causes acute myocarditis with electrical conduction abnormalities and varying degrees of heart failure. Fever, facial edema, gingivitis, cervical lymphadenopathies, hepatomegaly, and skin rash have been described. Interestingly, acute Chagas from oral transmission does not always manifest with gastrointestinal complaints like diarrhea, and if present, is not a prominent symptom. The incubation time is 5 days and mortality is more common among young patients. Massive parasitemia is demonstrated by large numbers of circulating trypomastigotes of *T. cruzi* in peripheral blood smear as reported by several authors.[9] [10] Outbreaks of acute Chagas disease linked to foods and beverages have been described with increasing frequency in the Americas. They occur in areas not typically endemic due to long distance commerce of food.[11] The first outbreak (n=17) was reported in 1965 in Brazil and was presumed to be linked to vegetables contaminated with feces of marsupials. Since then, cases and outbreaks have been reported in Argentina, Brazil, Colombia, and Ecuador. The largest documented outbreak occurred in an urban school in Caracas, Venezuela in 2007. It affected 103 people of 1000 exposed to guava juice, of those infected 77 were students, 26 school employees, and 1 child died.[12] Other reported food vehicles of *T. cruzi* infection are sugar cane juice, açaí juice, palm wine, and wild game. In summary, oral transmission of Chagas disease can occur by accidental ingestion of the Triatomine insect, ingestion of Triatomine's

feces, direct consumption of infected wild game, foods contaminated by feces or urine of marsupials, or human breast milk.[13]

What is known about Chagas disease among travelers

Perception of risk of acquiring Chagas disease during travel to endemic areas is based on case reports of returning travelers with acute CD, and cases diagnosed on migrants of Latin American origin living in non-endemic countries. Risk assessment and quantification has not been fully established, thus current recommendations are empirical. The Yellow Book from the CDC (Center for Disease Control and Prevention of the US) is one of the preferred references on international travel for health professionals. Its current recommendation for travelers to Mexico, Central and South America focuses on general measures to avoid insect bites and food-borne disease. It states that "the risk to travelers is extremely low, but they could be at risk if staying in poor-quality housing or from consuming contaminated food or beverages in endemic areas."[14]

A thorough search of the literature on Chagas disease in returning travelers to Latin America yields two types of publications: isolated case reports, and descriptive data from Travel Medicine networks. One of the few examples, reports a case of acute Chagas disease in a 53 year old female traveler to Puerto Viejo, Costa Rica and diagnosed upon return to the US. This patient sought medical attention because of unilateral eye swelling (Romaña's sign) and high fever. A detailed evaluation found trypomastigotes of *T. cruzi* in peripheral blood smear, and cardiac abnormalities (diastolic left ventricular dysfunction, dilated left atrium, small pericardial effusion, etc) without other symptom that fatigue.[15] This case is notable because travel was short-term (3 weeks) and that particular region is not associated with a high incidence of CD. Presumably, not every case of acute CD in ill returning travelers merits publishing.

Regarding consolidated data from Travel Medicine networks, one of the most recent reports comes from the European Travel Network. Among 7408 returning travelers evaluated at 16 EuroTravNet sites across Europe in 2010, 60 cases of chronic Chagas disease were found.[16]

They defined two types of travelers: "travelers with long-term exposure abroad" included recent immigrants (evaluated for screening of infectious diseases as they enter the country) and long term expatriates (missionaries, volunteers, aid workers, researchers, and people staying abroad for business). "Short-term travelers" included: tourists, business travelers, and non-recent migrants or their descendants visiting friends and relatives (VFRs) in their countries, missionaries, volunteer workers, aid workers and researchers, students, military personnel on missions, and medical tourism. They found 58 cases of chronic CD among travelers with long-term exposure, which represent 9% of 639 recent immigrants seen in 2010. Among travelers with short-term exposure, 2 cases of chronic CD were found, which represent <1% of 942 VFRs in 2010. 58/60 cases were from Bolivia, 1/60 from Ecuador, and 1/60 from Paraguay. Cases came mostly from the site in Madrid.

A report from the GeoSentinel network on morbidity in travelers visiting Mexico and Central America evaluated 4779 ill travelers from December 1996 to February 2010. These population consisted of short-term travelers, mostly tourists with median duration of travel 17 days (interquartile range 8-43 days).[17] Only 1 case of acute Chagas disease was diagnosed in a 26 year old female from Canada who traveled to Mexico for tourism. It was unknown wether she received pre-travel advise from a medical professional. A review of the GeoSentinel surveillance network on illness in travelers VFR from 1997 to 2004 found no cases of CD.[18] This report included 502 travelers VFR whose destination was Latin America including the Caribbean region, which is not an endemic area.

Chagas disease in Spain and Europe

The current epidemiology of Chagas disease in Europe is determined by screening at blood banks, screening programs for the prevention of congenital disease, screening for organ donors and recipients, active surveillance of migrants of Latin American origin, and passive notification of cases from healthcare centers. The first cases of CD were reported in the 1980s, and the increasing number of cases has raised awareness of the importance of screening at-risk

population for the prevention of transmission. A recent meta-analysis from studies prior to 2004 on the prevalence of Chagas disease among Latin American migrants living in European countries found a pooled prevalence of 4.2%. The main contribution of this study was to compare these numbers with the estimated prevalence of CD in the Americas according to PAHO 2006 data. Migrants from Bolivia had the highest prevalence 18.1% whereas PAHO reports 6.75%, followed by Paraguay with 5.5% whereas PAHO reports 2.54%, and Nicaragua with 4.6% whereas PAHO reports 1.14.[19]

With the initial migratory flows from Latin America to Europe in the 1990's appeared the first cases of patients with Chagas disease diagnosed in European countries. Spain is the European country with the largest population of migrants from South American origin. However many changes in the migratory trends have occurred in past years. Unfortunately, not all measures for the prevention of transmission of CD are implemented across Europe, as is the case for prevention of congenital disease. It has already been recommended that up-to-date information is needed for adequate health-policy making. Given its public health implications, cases of Chagas disease need to be prevented, detected and treated.[20]

Chagas disease in the Americas

American trypanosomiasis or Chagas disease is a "neglected tropical disease" endemic to 21 countries in the Americas: Argentina, Belize, the Bolivarian Republic of Venezuela, Brazil, Chile, Colombia, Costa Rica, Ecuador, El Salvador, French Guyana, Guatemala, Guyana, Honduras, Mexico, Nicaragua, Panama, Paraguay, Peru, the Plurinational State of Bolivia, Suriname, and Uruguay. WHO estimates that 6 to 7 million people worldwide are infected, most of them in Latin America, and most people who suffer it are unaware.[21] The estimated number of people with *T. cruzi* infection in the Americas was 20 million in 1981.[22] The latest WHO epidemiological update on Chagas disease in Latin America estimates 5.8 million. Bolivia had the highest incidence due to vectorial transmission, followed by Mexico, Colombia, Peru, Ecuador, Guatemala, Argentina, El Salvador, Honduras, and Venezuela. The estimated

prevalence of infection per 100 habitants due to vectorial transmission was highest in Bolivia, followed by Argentina and Paraguay (all 3 are part of the Gran Chaco region), followed by Ecuador, El Salvador, and Guatemala.[23]

This great reduction in cases was achieved by multiple factors including spraying with residual insecticides. Examples of vector-control programs in Latin America are the Amazon (AMCHA), the Andean (IPA), the Central American (IPCA), and the Southern Cone (INCOSUR) Initiatives. Other factors include improvement of socioeconomic metrics like better housing conditions, home hygiene, blood donor screening, education and communication. This latest WHO epi-report used data from 2010 and did not include new cases due to oral transmission. This is particularly relevant for the countries in the Amazon basin and humid Andean valleys: Bolivia, Brazil, Colombia, Ecuador, French Guyana, Guyana, Peru, Suriname, and Venezuela (grouped as the Amazon Initiative AMCHA) which may have a large burden of disease secondary to oral transmission.

The risk of transmission by blood transfusion in Latin America is very low because of screening programs in most blood banks. Mexico was the country with lowest level of screening coverage in 2005, but improved its efforts and reached 92% coverage in 2012.[24] It is difficult to know certainly how many blood banks are not screening adequately since blood banks do not respond to a centralized institution in many countries across Latin America.

Objectives

The main objectives of this study are 1) to determine the frequency of *Trypanosoma cruzi* infection among returning travelers to endemic areas, 2) to describe the demographic and travel characteristics, and 3) to identify possible risk factors for acquiring *T. cruzi* infection and no *T. cruzi* infection.

Methods

Study design

This is a prospective observational study. It is the first report or pilot study of a multi-center version to be conducted in other centers across Barcelona and Spain. This study is written in accordance with the STROBE reporting guidelines for observational studies.[25]

Setting

This study recruited adult patients with a negative *Trypanosoma cruzi* serology test from a master list provided by the Microbiology Department at University Hospital Vall d'Hebron and Drassanes in Barcelona, Spain from January 2012 to December 2015. Serology had been originally requested as part of routine blood bank protocol; clinical workup of findings compatible with Chagas disease; prior to chemotherapy, biologicals, transplantation of organs as per protocol; or as screening of migrants of Latin American origin at the Tropical Medicine/ International Health Clinic. University Hospital Vall d'Hebron is the largest tertiary care center in Barcelona. Patients were recruited in the study from March to May 2017.

Participants

Eligible patients were all adults who had traveled to Central or South America after their latest negative *T. cruzi* serology test. Travel history was elicited by telephone conversation after a brief description of our research. Contact telephone numbers were obtained from the electronic medical record (EMR) at our institution. Possible outcomes of telephone calls were categorized into: "Yes travel", "No travel"; and "Untraceable". "Yes travel" patients were invited to participate in our study and an appointment was scheduled at the Travel Medicine/International Health Clinic, usually within the next 15 days. Reminders via text message were sent to assure

attendance. All participants provided a written informed consent, a standardized questionnaire, and a blood sample for *Trypanosoma cruzi* serology.

Exclusion criteria were assessed upon review of the medical notes on the EMR for each patient. They included: previous discordant *T. cruzi* serology, negative serology after successfully treatment for Chagas disease, and deceased patients. Discordant results were found when the latest serological test was negative, but prior tests were positive. This study is approved by the Ethics Committee for Clinical Research at University Hospital Vall d'Hebron.

Variables

The outcome variable for this study is the presence of *T. cruzi* antibodies from a blood sample provided by participants. Our institution performs two serological techniques for *Trypanosoma cruzi* antibody detection simultaneously as recommended by the World Health Organization (WHO) for the diagnosis of Chagas disease .[26] The use of *T. cruzi* serology testing is indicated in "the chronic indeterminate phase" of CD which is occurs after 8 weeks (56 days) from infection. Other diagnostic tools should be used for the "acute phase" which can last 4-8weeks from infection. Possible results are: positive to both antibodies which is diagnostic of *T. cruzi* infection, negative to both antibodies which rules out *T. cruzi* infection, or discordant serology when one antibody is positive and the other is negative.

A standardized questionnaire was designed to explore demographic characteristics, travel features, and possible behaviors and exposures associated with *T. cruzi* infection during travel. For statistical analysis we included the following quantitative variables: age, duration of travel. The following qualitative variables were included: sex, travel destination, reason for travel, rural areas, peridomestic farm animals, dogs/cats in or around the house, illness during travel, fever during travel. Risk factors of *T. cruzi* infection were Reduviid bug-bite, blood transfusion, consumption of either fresh fruit juices or sugar cane (gruped as beverages for analysis), and overnight stay at a house built of adobe or palm tree roofs or walls of cane or wood (grouped

together as house construction materials for analysis). The variable "reason for travel" was categorized into tourism, business, research or education, missionary or volunteer, and VFR, according to publications from GeoSentinel.[27] For diagnostic accuracy of the outcome variable, the period of time between travel and study participation was calculated for each participant. Please see ***Appendix 1*** for a model of the standardized questionnaire.

Data sources / Measurements

Efforts to encourage patients to come to clinic included reminder phone calls the day prior scheduled appointment; and text messages with date, time, and place of the appointment. The standardized questionnaire was filled out by an attending physician at the Tropical Medicine Clinic. Demographic data and date of previous *T. cruzi* serology test were available from the EMR. The rest of variables were elicited from the patient as questions during anamnesis, and recorded by the physician in a data collection sheet unique for each participant.

Blood samples from study participants were analyzed at the main Microbiology Laboratory at U.H. Vall d'Hebron. Laboratory personnel were not aware of this study, hence samples were analyzed as any other sample would be. Our institution uses two different serological methods performed in parallel in accordance to WHO recommendations. One method is an enzyme-linked immunosorbent assay (ELISA) with native (whole cell lysate) antigen for the detection of human antibodies to *T. cruzi* (ORTHO® *T. cruzi* ELISA Test System, Johnson & Johnson, High Wycombe, United Kingdom). Performance of this test was measured in different populations. The observed sensitivity of this test in high risk populations is 98.9% with a 95% exact confidence interval of 94.2-100%. The observed specificity in a high risk population study was 99% as reported by the manufacturer's package insert.[28] The other method is an ELISA with recombinant antigen (Bioelisa Chagas, Biokit, Lliçà d'Amunt, Spain) for the detection of total antibodies to *T. cruzi*. The package insert reports a sensitivity of 100% and specificity 97.4-100% in different studies.[29] As of February 2015, our Institution uses an ELISA with recombinant antigen from another manufacturer (Bioelisa Chagas, Vircell, Spain) with similar sensitivity and

specificity. Results are expressed as the index between the absorbance of the test serum and the threshold value. Interpretation of results was negative with an index <0.9, equivocal if 0.9-1.1, and positive is ≥1.1. The results of *T. cruzi* serology tests were available within 2-3 days.

Bias

Performance bias when applying the standardized questionnaire to participants was avoided because it was applied prior to taking the blood sample to check for *T. cruzi* antibodies. Therefore, interviewer were unaware of the outcome variable at this point. The same is applicable to the telephone calls. To avoid bias in variability of the data collection, only 4 physicians applied the standardized questionnaire, and only 1 investigator performed the telephone calls.

Study size

This is a pilot study. The sample size was a convenience sample, determined by all available eligible patients between 20212 and 2015 and the number of patients that participated by showing up in Tropical Medicine Clinic during the study period March to May 2017.

For the on-going multi center study, a sample size was calculated *a priori*. With a 95% confidence interval (CI), a population size of 2500, an expected proportion to have *T. cruzi* infection after travel to endemic areas of 3%, a desired precision for the CI of ±1.5%, and an estimated replacement rate of 40%, the sample size of 632 was calculated. The calculator for this estimation was the "Sample size and power calculator GRANMO" from the Municipal Institute of Medical Research, Barcelona, Spain.[30]

Quantitative variables

The date of travel and the date of study participation were known. A new variable was created to reflect the time lapsed between the exposure (travel to Latin America) and the date of new *T. cruzi* serology test. This was done to reflect the chronicity of infection and the suitability of the diagnostic method. Periods greater than 8 weeks (56 days) would be considered at as "chronic indeterminate phase" for participants with the outcome variable. The variable "Duration of travel" was measured in days, and was further categorized into <15 days, 16-30 days, 31-60 days, 61-90 days, 91-180 days, and >181 days. This was to reflect a possible effect between duration of exposure and infection with *T. cruzi*.

Statistical methods

Demographic and travel characteristics were analyzed using descriptive statistics. Linear regression between *T cruzi*-positive and *T cruzi*-negative to identify significant differences in demographic and travel characteristics. Excel was used for tabulation and descriptive analysis and DeduceR (R 3.3.1 GUI 1.68 Mavericks build) was used for linear regression model.

Results

Of the 633 patients with baseline negative *T. cruzi* serology assessed for eligibility, 140/633 (22.2%) did not travel, 313/633 (49.3%) were untraceable by telephone, and 58/633 (9.2%) met exclusion criteria. 122/633 (19.3%) patients were eligible for study participation because they did travel to Central or South America. Of those, 60 patients kept their appointments and were included in the study and analysis. Patients were untraceable for 6 reasons: 159 patients could not be reached by telephone despite 3 attempts; 86 patients had telephone numbers on record that no longer exist; 26 patients had a wrong telephone number on record; 7 patients had restricted in-coming calls; 20 patients did not have a medical record number in the EMR, and 15 patients had returned to their home country. Of the eligible patients, the main reasons for non-participation were the patient lived in a city different than Barcelona (3/122), inability to take time off work (56/122), and unwillingness to participate (3/122).

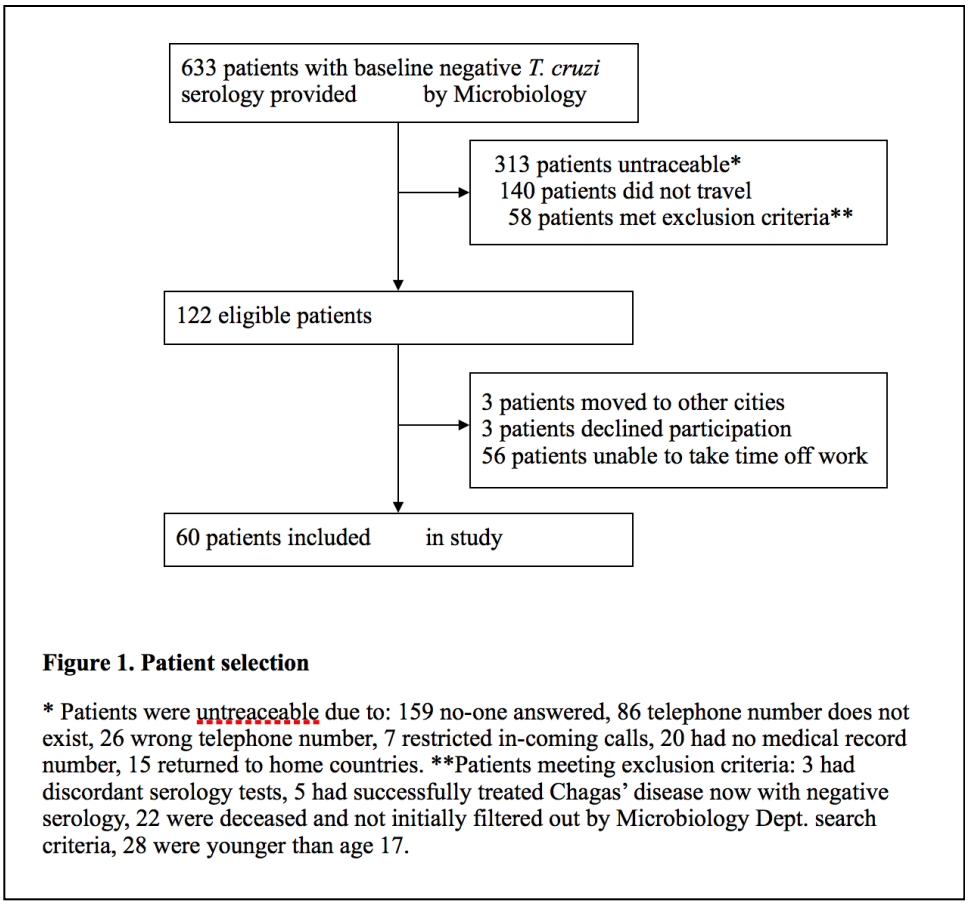

Figure 1. Patient selection

* Patients were untreaceable due to: 159 no-one answered, 86 telephone number does not exist, 26 wrong telephone number, 7 restricted in-coming calls, 20 had no medical record number, 15 returned to home countries. **Patients meeting exclusion criteria: 3 had discordant serology tests, 5 had successfully treated Chagas' disease now with negative serology, 22 were deceased and not initially filtered out by Microbiology Dept. search criteria, 28 were younger than age 17.

Regarding demographic characteristics, 45/60 (75%) were female and 15/60 (25%) were male patients. The youngest participant was 17 years old (yo) and the eldest was 78 yo. The mean age was 41 yo and the Standard Deviation was 11.36 years. The median age was 38.5 yo, the 25th percentile was 34 yo, and the 75th percentile was 47.5 yo. Please see **Table 1** for Demographic and Travel characteristics of the study population.

Descriptive data	Frequency[1]	%[2]
Demographic characteristics		
Female sex	45/60	75
Median Age in years	38.5	IQR 34-47.5
Travel characteristics		
Travel destination		
Bolivia	22	37
Ecuador	12	20
Colombia	6	10
Honduras	6	10
Perú	5	8
Argentina	3	5
Brazil	3	5
Nicaragua	1	2
Paraguay	1	2
Venezuela	1	2
Total	60	100
Reason for travel		
VFR	59/60	98
Business	1/60	2
Duration of travel		
< 15 days	6/60	15
16-30 days	15/60	25
31-60 days	21/60	35
61-90 days	9/60	15
91-180 days	4/60	6.6
>181 days	2/60	3.3
Visited rural areas	35/60	58
Peridomestic farm animals	32/60	53
Dogs in or around house	43/60	72
Cats in or around house	32/60	53
Fever during travel	9/60	15

Table 1. Demographic and travel characteristics of study population

[1] Absolute frequency for all variables except for "Age" which reflects the median value

[2] Percentage for all variables except for "Age" which reflects interquartile range

The country of birth and the country of travel destination were the same in all but 2 participants, 58/60 (96.7%). One case was born in Bolivia and visited friends and relatives in Argentina, the other was born in Colombia and visited Peru. The median duration of travel was 43.5 days (IQR 30-63.5 days). 90% of our study participants traveled between 2 weeks and 3 months. When asked about insect bites during travel 30/60 (50%) reported affirmatively, and while only 42/60 (70%) recognize the Reduviid-bug, none of the patients 0/60 (0%) reported being bitten by the Reduviid-bug. Given the exploratory nature of this study, the patients were interviewed with open questions like exposure to peridomestic farm animals during travel. The description of which farm animals they were exposed to was missing in 12/60 (20%).

Regarding risk factors directly associated with *T. cruzi* infection, please see **Table 2.** Patients that reported consumption of fresh fruit juices during travel were 42/60 (70%), and 23/60 (38%) reported sugar cane consumption. Those who consumed both "Beverages" implicated with *T. cruzi* infection were 36/60 (60%) of participants. Regarding housing conditions during travel, 11/60 (18%) patients stayed at a house built of *adobe;* 1/60 (2%) stayed at a house with palm tree roof; 3/60 (5%) stayed at a house with wooden walls; and 2/60 (3%) stayed at a house with cane walls. Zero participants received a blood transfusion during travel, and none reported being bitten by the Reduviid bug, colloquially known as "vinchuca".

Regarding the outcome variable, a negative *T. cruzi* serology test was obtained in 59/60 (98%) cases, and a discordant result in 1/60 (2%). None of the study participants had positive *T. cruzi* serology test.

Risk factor[1]	Frequency	%
Beverages[2]	36	60
House construction materials[3]	2	3.3
Beverages AND house materials	10	16.7
None	12	20
Total	60	100

Table 2. Risk factors for acquiring Chagas disease

[1] Not reflected on this table due to zero cases: Reduviid bug-bite, and Blood transfusion
[2] Refers to consumption of fresh fruit juices and sugar cane.
[3] Refers to adobe, roof made of palm trees, walls of wood, walls or cane, or any combination.

Discussion

Key results

Current Travel Medicine recommendations include Chagas disease as a risk for travelers to Central and South America. However no previous studies have tried to answer this question, thus risk for acquiring Chagas diseases during travel to endemic areas remains unknown. It is well described that travelers visiting friends and relatives are at higher risk for infections than tourists due to more risky exposures, less protection, and longer stays.[31] Among 60 adults who traveled to endemic areas to visit friends and relatives and were free of *T. cruzi* infection prior to travel, we found no cases of *T. cruzi* infection and found 1 patient with discordant serology.

A 78 year old female participant in this study had a discordant serology for *T. cruzi*. She was born in Buenos Aires, Argentina and lives in Spain for 17 years. Past medical history includes hypertension, obesity, cardiovascular accident, and non-ischemic dilated cardiomyopathy with ejection fraction 35% diagnosed in 2015. In January 2015, antibodies for *T. cruzi* were requested as part of diagnostic workup and results were negative. She was eligible for this study as she traveled to her home city in December 2015 for 40 days. Upon study participation in May 2017, she did not meet any risk factors associated with Chagas disease in the standardized questionnaire. *T. cruzi* serology test showed: positive for *T. cruzi* anti-recombinant antibody (index 1.64), and negative *T. cruzi* anti-native antibody (index 0.01). As per protocol, serology test was repeated in a new serum sample, and a second discordant result was obtained: positive *T. cruzi* anti-recombinant antibody (index 1.21), and negative *T. cruzi* anti-native antibody (index 0.01). *T. cruzi* PCR in blood was negative. These results make diagnosis inconclusive. Western blotting like the TESA-Blot (Biomérieux, RJ, Brazil) was evaluated as a confirmatory diagnostic tool in patients with inconclusive and discordant serology at our institution and helped establish the diagnosis of CD in half of them.[32] Unfortunately, the TESA-blot is not commercially available in Europe, and this patient will be followed up in clinic with repeat serology in 4-6 months.

This is the first study to evaluate the risk of acquiring Chagas disease from travel to continental Latin America. We exclusively enrolled patients documented to be free of *T. cruzi* infection prior to travel. We think this is a crucial precaution in order to avoid detecting patients who were infected but not diagnosed prior to travel. Previous publications have reported cases of Chagas disease among travelers without prior documentation of status of *T. cruzi* infection. EuroTravNet reported 60 cases of CD among travelers and migrants in 2010.[16] The majority of cases, 58 out of 60, were recent immigrants tested as part of screening of infectious diseases upon entry to Spain. The other 2 cases were non-recent migrants traveling to visit family and friends in Latin America. It would be incorrect to assume that CD was acquired during their latest travel to Latin America. These cases were basically patients in the chronic indeterminate phase of infection who were diagnosed during the study period. This phase can last for a lifetime in most patients, and they will remain asymptomatic, therefore it is crucial to determine the travelers' serological status prior to travel.

Travelers VFRs in this study were migrants of Latin American origin living in Spain for several years, middle aged (median 38.5 yo), female predominance (75%), who traveled to their home countries to visit friends and relatives. The median duration of travel was 43.5 days (IQR 30-63.5 days), which is much longer than the duration of travel of the ill returning tourist (median 17 days, IQR 8-43).[17] Other studies on illness in travelers VFSs have found a male predominance, mean age was similar at 38.9, and trip duration >30 days when compared to tourists.[31]

Exposure to well described risk factors was assessed with the standardized questionnaire. Consumption of fresh fruit juices and sugar cane is very popular in Latin America as was true for our population too. Guava, açaí juice and sucking on sugar cane have been implicated in outbreaks of food-borne Chagas disease.[10, 11, 12, 13] Fresh fruits and vegetables can be contaminated with the vector itself or its feces and be inadvertently ingested. With regards to the quality of housing conditions, only 12/60 (20%) of participants stayed at a house built of adobe, or palm tree-thatching, or cane walls. However, the majority (58%) reported visiting rural areas

and exposure to farm animals like chicken, guinea pigs, ducks, cows, pigs, goats, and others. This may be explained by better socioeconomic conditions in their home countries reflected in the improvement of building materials. Efforts to control de vectors have been successfully implemented in Latin America since the 1990s, with significant reduction of vectorial transmission in many countries and eradication in others.[23] Although 70% said they were able to recognize the "vinchuca" or Reduviid bug, 100% denied being bitten by it. Reduviid bugs bite at night when the human host is unaware, so this information just shows that the insect is familiar to most of our population.

Limitations

Telephone contact was necessary to assess for eligibility, which probably explains the large number of untraceable patients (313/633, 49.3%) we found. Presumably these patients do not keep the same telephone number for a long period of time. Of those who were reachable by telephone, half of eligible patients (62/122, 51%) were unable to come to the study site. Reasons for this include inability to get time off from work, several jobs that immigrants fill are shift which offer little or no flexibility. Female participants tend to work taking care of children or the elderly, and this gives them more time flexibility than their male counterparts. Also, there was no immediate benefit of participating in this study, so participants may have wanted to "save" work leaves for more "important" needs. Male immigrants' work schedule overlapped with the working hours of our study site, which may have introduced a selection bias to represent more females than males. Although the pre-test probability is low to begin with, we included all available data between January 2012 and February 2015 in this study.

Interpretation

Exposures during travel to endemic areas that were identified as risk factors for *T. cruzi* infection were consumption of fresh fruit juices and sugar cane, and housing materials prone to Reduviid bug infestation. Participants who met both of these risk factors were 10/60 (16.7%),

those who met either one risk factor were 38/60 (63.3%), and patients who did not meet any risk factor for CD were 12/60 (20%). None cases of *Trypanosoma cruzi* infection were acquired after travel to endemic areas. One patient had discordant serology results.

This is the first study to evaluate the risk of *T. cruzi* infection among travelers to Latin America taking the precaution to prove that participants were free of CD disease before travel. This study provides the basis for which future studies aiming to evaluate the risk of acquiring Chagas disease after travel to Central and South America. This knowledge would represent the scientific evidence to support both pre-travel counseling and screening efforts among migrants of Latin America living in non-endemic countries. The role that travel to endemic areas play in the epidemiology of CD in non-endemic areas is a relevant matter in our present globalized world.

Appendix 1

Data Collection Sheet - Standardized Questionnaire

HOJA DE RECOLECCION DE DATOS

NHC	
Fecha de consulta	
Fecha de serología previa	
Sexo (H=hombre / M=mujer)	
Edad (años)	
País / Ciudad de nacimiento	
Fecha de viaje (dd/mm/aaaa)	
Duración de viaje (días)	
País de destino	
Localidades que visitó?	
Motivo del viaje	
Visitó areas rurales? (si / no)	
Había animales de granja cerca de casa?	
Perros (1) / Gatos (2) en casa?	
Recuerda picaduras de insectos?	
Conoce la “vinchuca”?	
Tuvo picadura por vinchuca en este viaje? (si / no)	
Tomó zumos de frutas en este viaje?	
Consumió caña de azúcar durante este viaje?	
Conoce a alguien que tenga E. de Chagas? (si / no)	
Familiar?	
Vivía en la misma casa? (si / no)	
Amigo/conocido?	
Vivía en la misma casa? (si / no)	
Estuvo enfermo durante su viaje? (si / no)	
Fiebre? (si / no)	
Recibió transfusión de hemoderivados en el viaje?	
Se hospedó en casa de adobe?	
Se hospedó en casa con techo de palmera?	
Se hospedó en casa con paredes de madera?	
Se hospedó en casa con paredes de caña?	

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