UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO

POSGRADO EN CIENCIAS BIOLÓGICAS

FACULTAD DE MEDICINA

BIOLOGÍA EXPERIMENTAL

CARACTERIZACIÓN PARASITOLÓGICA DE AISLADOS DE *TRYPANOSOMA CRUZI* Y ACTIVIDAD FENOLOXIDASA DE *TRIATOMA DIMIDIATA* DE TRES LOCALIDADES A DISTINTAS ALTITUDES EN EL ESTADO DE CHIAPAS, MÉXICO

TESIS

QUE PARA OPTAR POR EL GRADO DE:

DOCTOR EN CIENCIAS

PRESENTA:

DE FUENTES VICENTE JOSÉ ANTONIO

TUTORA PRINCIPAL DE TESIS: DRA. PAZ MARÍA SILVIA SALAZAR SCHETTINO
FACULTAD DE MEDICINA, UNAM
COMITÉ TUTOR: DR. ALEJANDO CÓRDOBA AGUILAR
INSTITUTO DE ECOLOGÍA, UNAM
DR. CUAUHTEMOC JUAN HUMBERTO LANZ MENDOZA
FACULTAD DE MEDICINA, UNAM

MÉXICO, CD. MX. JUNIO, 2017.

UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO

POSGRADO EN CIENCIAS BIOLÓGICAS

FACULTAD DE MEDICINA

BIOLOGÍA EXPERIMENTAL

CARACTERIZACIÓN PARASITOLÓGICA DE AISLADOS DE *TRYPANOSOMA CRUZI* Y ACTIVIDAD FENOLOXIDASA DE *TRIATOMA DIMIDIATA* DE TRES LOCALIDADES A DISTINTAS ALTITUDES EN EL ESTADO DE CHIAPAS, MÉXICO

TESIS

QUE PARA OPTAR POR EL GRADO DE:

DOCTOR EN CIENCIAS

PRESENTA:

DE FUENTES VICENTE JOSÉ ANTONIO

TUTORA PRINCIPAL DE TESIS: DRA. PAZ MARÍA SILVIA SALAZAR SCHETTINO
FACULTAD DE MEDICINA, UNAM
COMITÉ TUTOR: DR. ALEJANDO CÓRDOBA AGUILAR
INSTITUTO DE ECOLOGÍA, UNAM
DR. CUAUHTEMOC JUAN HUMBERTO LANZ MENDOZA
FACULTAD DE MEDICINA, UNAM

MÉXICO, CD. MX. JUNIO, 2017.

UNAM
POSGRADO
Ciencias Biológicas

COORDINACIÓN

Lic. Ivonne Ramírez Wence
Directora General de Administración Escolar, UNAM
P r e s e n t e

Me permito informar a usted que el Subcomité de Biología Evolutiva y Sistemática del Posgrado en Ciencias Biológicas, en su sesión ordinaria del día 24 de abril de 2017, aprobó el jurado para la presentación del examen para obtener el grado de **DOCTOR EN CIENCIAS** del alumno **DE FUENTES VICENTE JOSÉ ANTONIO** con número de cuenta **513024366**, con la tesis titulada **"CARACTERIZACIÓN PARASITOLÓGICA DE AISLADOS DE *Trypanosoma cruzi* Y ACTIVIDAD FENOLOXIDASA DE *Triatoma dimidiata* DE TRES LOCALIDADES A DISTINTAS ALTITUDES EN EL ESTADO DE CHIAPAS, MÉXICO"**, realizada bajo la dirección de la **DRA. PAZ MARÍA SILVIA SALAZAR SCHETTINO:**

Presidente:	DRA. MARGARITA CABRERA BRAVO
Vocal:	DR. JOSÉ HUGO AGUILAR DÍAZ
Secretario:	DR. ALEJANDRO CÓRDOBA AGUILAR
Suplente:	DRA. ANA ERIKA GUTIÉRREZ CABRERA
Suplente:	DR. CUAUHTÉMOC JUAN HUMBERTO LANZ MENDOZA

Sin otro particular, me es grato enviarle un cordial saludo.

A T E N T A M E N T E
"POR MI RAZA HABLARÁ EL ESPÍRITU"
Cd. Universitaria, Cd, Mx., a 08 de mayo de 2017

DRA. MARÍA DEL CORO ARIZMENDI ARRIAGA
COORDINADORA DEL PROGRAMA

POSGRADO EN CIENCIAS BIOLOGICAS
COORDINACIÓN

Agradecimientos

Al Posgrado en Ciencias Biológicas de la Universidad Nacional Autónoma de México y a todos los que integran este programa.

Al Consejo Nacional de Ciencia y Tecnología (CONACyT) por la beca 288696 otorgada para la realización de mis estudios de posgrado.

A la Dra. Paz María Salazar Schettino, por aceptarme como su alumno y dirigir esta tesis; gracias por sus consejos, apoyo y sobre todo por brindarme su amistad.

Al Dr. Alex Córdoba Aguilar, por ser parte fundamental a lo largo de todo este trayecto. Gracias por su confianza y por compartir ese amor por la ciencia.

Al Dr. Humberto Lanz Mendoza, gracias por sus consejos, sus observaciones en todos los tutorales y por enseñarme las técnicas para evaluar la respuesta inmune de insectos.

Agradecimientos a título personal

A las doctoras Martha, Margarita, Yoli, Ade y Glory por siempre recibirme con esa sencillez y calidez que las caracteriza. A Martita, Marichuy, Santiago, Mariana, Eli´s, a doña Evita; gracias a todos por hacer tan grata la permanencia en el Laboratorio de Biología de Parásitos.

Al Dr. Jhony Navat y al Dr. Miguel Moreno, por sus consejos, orientación y por compartir sus conocimientos conmigo, que sin duda enriquecieron este proyecto. A la Dra. Ana Erika Gutiérrez, por su dirección y consejos en la realización de uno de los artículos de este trabajo.

A mis amigos y colegas de la UNICACH: Dra. Lolyta, Dra. Adelina, Dr. Javier y Dr. Miguel, por sus enseñanzas y por verme como uno de los suyos. A Lalo y Evelyn por esas primeras salidas a campo en busca de chinches. A Chris y Freddy, por las charlas constructivas que fueron muy enriquecedoras. A mis compadres Viri y Rodrigo, gracias por abrirme siempre las puertas de su casa.

A Rocío Chavez Trejo, gracias por tu apoyo en todos los trámites del posgrado.

Agradecimientos técnicos

A la Dra. Any Laura Flores Villegas, Técnico Académico Titular "A" de Tiempo completo, adscrita al laboratorio Biología de Parásitos, Facultad de Medicina, UNAM por sus enseñanzas en el desarrollo de la técnica fenoloxidasa, la donación de ejemplares de *T. dimidiata* y su ayuda en el análisis estadístico.

Al Biól. Mauro Omar Vences Blanco, Técnico Académico Asociado "A", adscrito al Laboratorio de Biología de Parásitos, Facultad de Medicina, UNAM, por sus enseñanzas en el manejo y cuidado de triatominos.

A la M. en C. Yolanda Guevara Gómez, Técnica Académica Titular A, adscrita al Laboratorio de Biología de Parásitos, Facultad de Medicina, UNAM, por su apoyo para la realización y cuidado de medios de cultivo para *Trypanosoma cruzi*.

Al Dr. Armando Pérez Torres, del Departamento de Biología Celular y Tisular, por su apoyo en la realización de cortes histológicos de los ratones.

Al Biól. Jesús Díaz Gómez, Técnico Académico adscrito al Laboratorio Multidisciplinario Experimental y Bioterio de la Universidad de Ciencias y Artes de Chiapas (UNICACH), por su apoyo en el cuidado y manejo de animales de laboratorio.

Dedicatoria

A mi madre Amanda Vicente Aguilar, por darme la vida y enseñarme el camino del esfuerzo. Gracias a ti he llegado hasta aquí, este logro también es tuyo.

A mi esposa Nancy Gabriela Santos Hernández, por todo tu apoyo y estar siempre a mi lado; en las buenas y en las no tan buenas. Mejor compañera de vida no pude haber encontrado.

A mi hija Isabel De Fuentes Santos, que con tu existencia has traído una inmensa felicidad a mi vida. Te amo, mi pequeña.

A mi abuela, hasta donde estés…

ÍNDICE

LISTA DE FIGURAS Y CUADROS

RESUMEN

El parásito *Trypanosoma cruzi* es el responsable de la enfermedad de Chagas, una enfermedad endémica de América Latina con altas tasas de mortalidad y morbilidad en el humano. *T. cruzi* es transmitido por insectos hematófagos de la subfamilia Triatominae, la interacción del parásito con sus vectores es compleja y puede ser influenciada por factores bióticos y abióticos. Estudios con este parásito han observado un comportamiento biológico variable, lo cual se atribuye a diversos factores. En esta investigación se evaluó la relación de la altitud con la virulencia de *T. cruzi* y con la respuesta inmune de *Triatoma dimidiata*. Para ello, se colectaron triatominos infectados y no infectados a 300, 700 y 1400 msnm en el estado de Chiapas, México. La virulencia de T. *cruzi* fue medida en ratones hembras CD-1 mientras que la respuesta inmune de los triatominos fue medida como la actividad de la enzima fenoloxidasa (PO) después de la infección con el parásito. La virulencia de *T. cruzi* y la actividad de la PO en los insectos fue mayor a los 700 msnm, el análisis de la virulencia sugiere que a esa altitud la enfermedad es más severa. Por otra parte, la mayor actividad de la PO observada en insectos de esa misma altitud puede deberse a las condiciones ambientales favorables de esa zona para las funciones fisiológicas de *T. dimidiata*. Estos resultados indican que la altitud puede influir en la dinámica de la enfermedad de Chagas.

ABSTRACT

The parasite *Trypanosoma cruzi* is responsible for Chagas disease, an endemic disease in Latin America with high mortality and morbidity rates to human. *T. cruzi* is transmitted by hematophagous insects of the Triatominae subfamily, the interaction of the parasite with their vectors is complex and can be influenced by biotic and abiotic factors. Studies with this parasite have observed a variable biological behavior, which is attributed to several factors. In this research the relationship of altitude with the virulence of *T. cruzi* and with the immune response of *Triatoma dimidiata* was evaluated. For this, triatomines infected and uninfected were collected at altitudes of 300, 700 and 1400 masl in the state of Chiapas, Mexico. *T. cruzi* virulence was measured in CD-1 female mice while the immune response of triatomines was measured as the activity of the enzyme phenoloxidase (PO) after infection with the parasite. The virulence of *T. cruzi* and the activity of the PO in the insects was greater to 700 msnm, the analysis of virulence suggests that at that altitude the disease is more severe. On the other hand, the greater PO activity observed in insects of that same altitude can be due to the favorable environmental conditions of that zone for the physiological functions of *T. dimidiata*. These results indicate that altitude may influence the dynamics of Chagas' disease.

CAPÍTULO I

Introducción general

La enfermedad de Chagas fue descubierta en 1909 por el médico brasileño Carlos Justiniano Ribeiro das Chagas (Chagas, 1909). Este descubrimiento representó un hito en la historia de la medicina pues fue el mismo Carlos Chagas quien descubrió primero el agente causal, el transmisor y después algunas manifestaciones clínicas de la enfermedad. Desde entonces, esta parasitosis se ha convertido en un grave problema de salud pública en varios países de América Latina por su prevalencia estimada en ocho millones de personas infectadas (WHO, 2016), por la tasa de mortalidad anual de casi 12,000 enfermos (Cucunubá *et al*., 2016), por el registro de morbilidad de 44,000 casos por año (Moncayo y Silveira, 2009) y por el costo anual de 4,049 dólares por persona entre atención médica y pérdidas de productividad laboral (Lee *et al*., 2013).

De acuerdo con la Organización Mundial de la Salud (OMS), la enfermedad de Chagas está catalogada como una de las 13 enfermedades tropicales desatendidas en el mundo (Hotez *et al*., 2007). La relación de la enfermedad con los estratos sociales más pobres (Dias *et al*., 2008), podría explicar en gran parte la falta de atención de las autoridades de salud y gobierno en general. Mientras persistan condiciones precarias en los asentamientos humanos que faciliten el desarrollo de la enfermedad, como viviendas de mala calidad y poco acceso a sistemas de salud, esta zoonosis seguirá siendo un riesgo constante para muchas regiones del continente americano. Al estar involucrados factores económicos, políticos y sociales, la enfermedad de Chagas no solo es una enfermedad de la pobreza, sino también un ejemplo claro de cómo la ausencia o limitaciones de los

determinantes de la atención de salud, repercuten en la calidad de vida de las poblaciones humanas (Garrido-Pérez *et al*., 2010; Pinto-Dias, 2012).

El agente etiológico de la enfermedad de Chagas es el protozoario flagelado *Trypanosoma cruzi*, esta especie comprende un conjunto de cepas que circulan entre un hospedero vertebrado y uno invertebrado (Rassi *et al*., 2010). Dentro de los vertebrados, cerca de 100 especies de mamíferos pueden actuar como reservorios de *T. cruzi* (Noireau *et al*., 2009); mientras que los insectos hematófagos de la subfamilia Triatominae (Hemíptera; Reduviidae), conocidos popularmente como chinches besuconas o vinchucas, actúan como transmisores del parásito a través de las deyecciones (Jurberg y Galvâo, 2006). A lo largo de su ciclo de vida, *T. cruzi* cumple una serie de transformaciones que dependen del ambiente donde se encuentre. De esta manera se pueden diferenciar cuatro estadios que componen el ciclo de vida del parásito: tripomastigote metacíclico, tripomastigote sanguíneo, amastigote y epimastigote (ver Fig. 2.1 del capítulo 2). Los tripomastigotes son los responsables de la invasión de las células en el hospedero vertebrado, en tanto que los amastigotes representan la forma replicativa intracelular y crean nidos en diferentes tejidos. En pacientes humanos y estudios en animales de laboratorio es común encontrar diferencias significativas en cuanto a la velocidad y grado de replicación del parásito, así como en la invasión hacia diferentes tejidos y la mortalidad (Mejía y Triana, 2005; Suárez *et al*., 2009; Sánchez-Guillén *et al*., 2010; Espinoza *et al*., 2011). En conjunto, estos parámetros pueden llegar a definir la virulencia o comportamiento parasitológico de *T. cruzi* (Barreto, 1964; OMS, 1970).

Las diferencias en el comportamiento parasitológico suelen observarse de acuerdo a la región geográfica y cepa del parásito, un ejemplo que define bien lo anterior es que en

Centro y Norteamérica las complicaciones cardiacas son más frecuentes en comparación con las complicaciones digestivas que predominan en Sudamérica (Rassi, Jr *et* al., 2000; Rassi, Jr *et al*., 2009). Al parecer, esta heterogeneidad biológica puede estar asociada con la amplia diversidad genética que presenta *T. cruzi*, lo cual ha llevado a que las cepas sean clasificadas en diferentes grupos (Souto *et al*. 1996; Brisse *et al*. 2001; Zingales *et al*., 2009). Con estudios basados en la amplificación del gen mini exón de *T. cruzi* se ha llegado a determinar dos linajes filogenéticos principales: TCI con una aparente preferencia de invadir el músculo cardiaco; y TCII con una preferencia hacia esófago y megacolon (Brisse *et al*., 2001; Lages-Silva *et al*., 2006; Cassin-Duz *et al*., 2014). A pesar de que esta correlación ha sido ampliamente estudiada, aún no es posible esclarecer el porqué de tales diferencias (Manoel-Caetano y Silva, 2007)

Además de la variación genética se ha propuesto que otros elementos pueden estar relacionados con la virulencia de *T. cruzi*. Entre ellos, se sugiere que la respuesta inmune del hospedero es determinante en la replicación del parásito (Kayema y Takeda, 2010). El éxito evolutivo de *T. cruzi* se debe en gran parte a la capacidad que tiene de evadir los mecanismos inmunológicos del vertebrado, si bien la respuesta inmune controla la replicación, no logra eliminar la infección (Cardoso *et al*., 2015). Asimismo, existen factores dependientes e independientes del parásito y el hospedero que podrían influir en el comportamiento de *T. cruzi*. La variación en la virulencia ha dificultado el estudio del parásito y de la enfermedad de Chagas en general, y ha cuestionado su influencia en la distribución, control y tratamiento de la enfermedad (Bern *et al*., 2011; Mejía-Jaramillo *et al*., 2012; Chatelain, 2015).

Esta tesis se enmarca en entender los diferentes comportamientos del parásito. Así, en el capítulo II se analiza la virulencia de cepas de *T. cruzi* aisladas principalmente en México; al mismo tiempo se exponen y discuten los factores tanto biológicos como metodológicos que pueden estar relacionados con esta heterogeneidad. En este capítulo se evidencia una relación parásito-hospedero muy compleja y que está mediada por diversas causas como tamaño del inóculo, sitio de entrada de *T. cruzi* o triatomino del que se aisló el parásito. Estos factores llegan a jugar un papel muy marcado en el curso de la infección, donde incluso algunos pueden ser determinantes en la supervivencia del hospedero. Además, se enfatiza la necesidad de contar con metodologías estandarizadas que eviten discrepancias al determinar la virulencia de las diferentes cepas. Si se logra agrupar las poblaciones de *T. cruzi* en el aspecto parasitológico, genético, bioquímico y enzimático, se podrían crear grupos representativos que faciliten su estudio y en su momento poder crear vacunas contra la enfermedad (Guzmán-Marín *et al*., 1999).

Por otra parte, los epimastigotes son las formas de *T. cruzi* que representan la forma infectiva y replicativa en el insecto vector (Azambuja et al., 2005). Estas formas que surgen de la transformación de los tripomastigotes sanguíneos en el intestino de los triatominos, se anclan a la membrana perimicrovellosa y comienzan a multiplicarse por fisión binaria (Kollien y Schaub, 2000). Una vez que aumenta la población, algunos epimastigotes migran al recto donde se convierten a la forma infectiva para el vertebrado (tripomastigote metacíclico) que son expulsadas junto con las heces y orina (Vallejo et al., 2009). El hecho de que *T. cruzi* sufra transformaciones dentro del insecto, hace que los triatominos representen vectores biológicos del parásito, a diferencia de los vectores mecánicos que únicamente se limitan a transportar al patógeno sin que este se modifique o reproduzca. Es

claro que en el primer tipo de transmisión la interacción patógeno-vector es mucho más compleja, ya que las transformaciones del parásito responden al estrés que origina, en este caso, cada compartimento del tracto digestivo de los triatominos (Hamedi *et al*., 2015). Esta capacidad de adaptación es el resultado de procesos evolutivos que han tenido lugar desde hace posiblemente 99 millones de años (Texeira *et al*., 2009).

Durante su recorrido por el intestino de la chinche los parásitos pueden sufrir una reducción drástica en el tamaño de la población (Ferreira *et al*., 2016). Para que tenga éxito la transmisión a otro vertebrado, *T. cruzi* debe resistir y/o evadir componentes que son una desventaja para su establecimiento y reproducción. El conocimiento de los mecanismos que influyen en la interacción *T. cruzi*-triatominos se ha ido ampliando en años recientes, aunque aún falta mucha investigación para entender por completo esta relación, hasta hoy en día se sabe que incluso factores abióticos ejercen una influencia decisiva en los mecanismos de transmisión (Neves, 1971; Asín y Catalá, 1995). Para poder llegar a interrumpir el ciclo de vida del parásito en el insecto o crear estrategias de control basadas en esta interacción, es necesario conocer a fondo los factores implicados en el proceso de infección de *T. cruzi* en los triatominos.

Dado lo anterior, en el capítulo III se analizan y discuten estos factores bajo un contexto ecológico. Además, se proponen algunas líneas de investigación para coadyuvar a mejorar la comprensión de esta interacción. A lo largo de este capítulo, es posible asociar algunos factores bióticos como abióticos desde la entrada del parásito como formas sanguíneas hasta su expulsión como formas metacíclicas. Un aspecto interesante que merece ser resaltado es que la respuesta inmune de la chinche parece jugar un rol importante en la interacción. A pesar de esto son pocos los estudios enfocados a determinar

qué mecanismos inmunológicos se activan, lo que ha dificultado establecer cuál es su verdadera importancia en la relación.

El sistema inmune de los triatominos (y de los invertebrados en general) consta de dos componentes principales: el celular y el humoral. El primero es llevado a cabo por las células sanguíneas de los insectos conocidos como hemocitos, los cuales realizan procesos como fagocitosis, encapsulación, nodulación y diferenciación celular (Schmid-Hempel, 2005). Por su parte, el humoral está compuesto por péptidos antimicrobianos, especies reactivas de oxígeno y nitrógeno, óxido nítrico y la enzima de la fenoloxidasa (PO). La activación de la PO conlleva a una serie de reacciones en cascada que comienza con el reconocimiento del patógeno por células especializadas del insecto. Después del reconocimiento, la enzima no activada (zimógeno) profenoloxidasa (proPO) se activa y la enzima PO convierte la tirosina (que previamente fue sintetizado a partir de la fenilalanina) en DOPA. Posteriormente, la DOPA será convertida en Dopaquinona también por acción de la PO y después de varias reacciones se producirá melanina como producto final para encapsular al patógeno (Cerenius *et al*., 2008; González-Santoyo y Córdoba-Aguilar, 2011). La importancia de la cascada de la PO no solo radica en la producción de melanina, sino que a lo largo de su producción se liberan radicales libres que son tóxicos para los patógenos (Zhao *et al*., 2011). Además, se considera que la PO también participa en el proceso de reconocimiento de agentes extraños y que actúa como un indicador general de inmunocompetencia del insecto (González-Santoyo y Córdoba-Aguilar, 2011). La PO y otros mecanismos inmunes son costosos en términos energéticos y pueden verse afectados por el estado fisiológico del individuo (González-Santoyo y Córdoba-Aguilar, 2012) o por factores ambientales como la temperatura (Catalán *et al*., 2012).

En vista de la variación en el comportamiento de las poblaciones de *T. cruzi* y del poco conocimiento de la respuesta inmune de triatominos frente a la infección, el objetivo central del capítulo IV fue caracterizar el comportamiento de *T. cruzi* en ratones CD-1 y medir la actividad de la PO en triatominos infectados de tres diferentes altitudes. El factor altitud ha sido una variable poco estudiada en la enfermedad de Chagas a pesar de que se han observado variaciones en los cuadros clínicos, en la distribución de triatominos y en la tasa de infección (Noireau *et al*., 2005; Benítez-Alba *et al*., 2011; Pereira-Lopes *et al*., 2015). Las poblaciones de *T. cruzi* y de triatominos fueron colectadas a altitudes de 300, 700 y 1400 metros sobre el nivel del mar (msnm) en el estado de Chiapas, que se ubica en la región sureste de México. En esta zona la problemática de la enfermedad de Chagas no ha sido del todo esclarecida y se encuentra una de las especies más importantes en la epidemiología de la enfermedad, el vector *Triatoma dimidiata*. La importancia de esta especie en la transmisión de *T. cruzi* radica en su capacidad de invadir la vivienda humana y por ello, tiene más contacto con el humano (Salazar-Schettino *et al*., 2005). Los resultados de este capítulo muestran variación de la virulencia de *T. cruzi* y de la actividad PO de la chinche *T. dimidiata* con respecto a la altitud, en virtud de esto, se plantean hipótesis para explicar las causas de estas observaciones.

En general, esta tesis ofrece un panorama sobre la relación del parásito con sus hospederos vertebrados y sus insectos transmisores, y cómo diversos factores juegan papeles transcendentales en la dinámica de esta interacción tripartita. Asimismo, se propone a la altitud como un factor de riesgo en la transmisión de la enfermedad de Chagas en las poblaciones humanas, al menos en el sitio de estudio.

CAPÍTULO II

Factors associated with *Trypanosoma cruzi* virulence: a review of Mexican strains

J.A. De Fuentes-Vicente[1], A. E. Gutiérrez-Cabrera[2], A. Laura Flores-Villegas[1], Dolores G. Vidal-López[2], Martha I. Bucio-Torres[1], Margarita Cabrera-Bravo[1], Mariana del C. de Alba-Alvarado[1], Alex Córdoba-Aguilar[4] & Paz M. Salazar-Schettino[1]

[1]Departamento de Microbiología y Parasitología, Universidad Nacional Autónoma de México, Mexico City, Mexico, [2]CONACYT-Centro de Investigaciones Sobre Enfermedades Infecciosas, Instituto Nacional de Salud Pública, Cuernavaca, Morelos, Mexico, [3]Instituto de Ciencias Biologicas, Universidad de Ciencias y Artes de Chiapas, Chiapas, Mexico, [4]Departamento de Ecología Evolutiva, Universidad Nacional Autónoma de Mexico, Mexico City, Mexico.

Abstract

The parasite *Trypanosoma cruzi* is responsible for the American Trypanosomiasis, better known as Chagas disease. This parasitosis is a serious public health problem in endemic countries and it is estimated that there are about 8 million people infected in the world. The study of T. cruzi has been hampered by the biological heterogeneity that has, which causes diverse clinical manifestations in the human. This review attempts to give an overview of the parasitological aspects of strains of *T. cruzi* isolated mainly in Mexico and the influence of some methodological processes during their biological characterization in laboratory. With this, it is hoped that standardized methodologies will be created to allow the creation of representative groups based on their behavior within the host and thus, to help the understanding of the processes involved in the evolution of the lesions in the disease, to try to predict the evolution of Chagas disease and help create vaccines.

Keywords: *T. cruzi*, virulence, Mexican strains, Chagas disease

Introduction

Since the discovery by Carlos Chagas of the hemoflagellate *Trypanosoma cruzi* (phylum Euglenozoa; class Kinetoplastida; family Trypanosomatida), the causative agent of Chagas disease, in 1909 (Chagas, 1909), several studies have been conducted to elucidate the parasite behavior in a vertebrate host. Among other findings, a highly variable biological behavior (Gomés-Hernández *et* al., 2011; Guzmán-Marín *et al*., 2012) and a great genetic diversity across different regions in the Americas have been observed in *T. cruzi* (Zingales *et al*., 2009); these works demonstrated that the protozoan species consists in a set of strains that circulate among vectors, reservoirs, and humans throughout the continent (Guzmán-Marín *et al*., 1999; WHO, 2015). Several molecular analysis-based classification systems have been proposed (Souto *et al*., 1996; Blanco and Montamat, 1998; Garzón *et al*., 2002; Lages-Silva *et al*., 2006) to cope with the diversity of *T. cruzi* strains and facilitate their study. Among other classifications, biodems (Andrade and Magalhães, 1997), lineages (TCI and TCII) (Souto *et al*., 1996; Brisse *et al*., 2001), and discrete typifying units (DTUs) (Zingales *et al*., 2009) have gathered ample consensus. Recently, *T. cruzi* II was subdivided into five distinct groups or DTUs (IIa-IIe) (Flores-López and Machado, 2011). Currently, it is generally agreed that *T. cruzi* II strains (TcII-TcVI) are more pathogenic to humans than *T. cruzi* I strains (TcI); it has been proposed that *T. cruzi* II is a paraphyletic group, and that its divergence into distinct lineages is recent (< 3-million years) (Flores-López and Machado, 2011; Zumaya-Estrada *et al.,* 2012).

With this background, the heterogeneity of this parasite even in a same host is not surprising. In 1909, Chagas described two morphologically different types of circulating trypomastigotes, slender and stout, in cultures derived from a single parasite. Thirty-eight *T. cruzi* clones were obtained from eight isolates from chronic Chagasic patients (Blanco

and Montamat, 1996), demonstrating that these patients were bearing a mixed infection, either due to secondary infections or by a heterogeneous initial infection. The postulated heterogeneity in isolates from humans, mammals, and vectors has been analyzed before (Scott, 1981; Morel *et al.,* 1984; Breniere *et al.,* 1989).

Mexico is considered as an endemic country for Chagas disease (Salazar-Schettino *et al*., 2016); there is a wide variety of mammal reservoirs for *T. cruzi* (e.g. Salazar-Schettino *et al*., 1987; Domínguez-Vázquez and Espinoza-Medinilla, 1988; Salazar-Schettino *et al*., 1997), 32 species of parasite-transmitting triatomine insects (Salazar-Schettino *et al*., 2010), and reports of infected individuals in practically all Mexican States (Cruz-Reyes and Pickering-López, 2006). Among the 32 triatomine species reported to date, three species have a major epidemiological impact: *Meccus pallidipennis*, *Triatoma dimidiata,* and *T. barberi* (Fig. 2.1). Such vector diversity and abundance in Mexico could be due to the topographic and climatic conditions in the country. In fact, Mexico is the region in the Americas harboring the highest number of triatomine species involved in *T. cruzi* transmission (Salazar-Schettino *et al*., 2005; Salazar-Schettino *et al*., 2001).

The earliest studies on Chagas disease in Mexico were reported by Hoffman in 1928, describing the presence of the vector *T. dimidiata* in a Veracruz region; in 1940, Mazzotti reported the first cases of infected humans in the country (Hoffman, 1928; Mazzotti, 1940). Since then, several *T. cruzi* strains have been isolated in Mexico, and their virulence has been tested in laboratory animals, mainly in mice given their ease of handling and susceptibility to the disease (Rivera *et al*., 2000).

Virulence is defined as the degree of pathogenicity of a microorganism toward a host; in other words, it is the capacity of a microorganism to cause disease. Clinical manifestations of *T. cruzi* infection include symptomatology (WHO, 2015), parasitemia

(Silva *et al*., 2013), tissue damage (Tarleton, 2003; Melnikov *et al*., 2005), immune response (Espinoza *et al*., 2010; Espinoza *et al*., 2011), and mortality rate (Martins-Melo *et al*., 2007; Cucunubá *et al*., 2016); all these clinical signs can be influenced by strain constituents, by parasite passage among different triatomine species, or by host immunity, among other factors (Magalhães *et al*., 1996; Macedo *et al*., 2004; Manoel-Caetano *et al*., 2007, Guzmán-Marín *et al*., 2012).

The virulence of *T. cruzi* strains can be measured considering parameters like prepatent period, parasitemia, cellular tropism, and mortality; these factors were first proposed as key by Barreto (1964) and WHO (1980). This review is aimed to show and discuss, based on the aforementioned parameters, some biological and methodological factors that could influence the virulence of Mexican *T. cruzi* strains.

T. cruzi life cycle

The life cycle of *T. cruzi* alternates between an invertebrate vector and a vertebrate host (Noireau *et al*., 2009). During its development in both hosts, the parasite goes through four developmental stages: metacyclic trypomastigote, blood trypomastigote (infective forms), epimastigote, and amastigote (replicative form) in vectors and reservoirs, respectively (Kollien and Schaub, 2000). Hematophagous insects of the Triatominae subfamily (order Hemiptera; family Reduviidae) are responsible for *T. cruzi* vector transmission. The insect acquires blood trypomastigotes when feeding on blood from an infected mammal. After invading the insect foregut (stomach), blood trypomastigotes transform into epimastigotes a few hours after blood ingestion, establishing an infection in the vector (Azambuja *et al*., 2005). Epimastigotes migrate to the rear midgut, adhering to the perimicrovillar membrane of intestinal cells and reproducing by binary fission (Kollien *et al*., 1998; Alves *et al*., 2007). Epimastigotes migrate to the rectum and undergo metacyclogenesis, differentiating

into metacyclic trypomastigotes, which are expelled by the triatomine along with feces and urine; in turn, metacyclic trypomastigotes can infect a vertebrate host (Azambuja *et al*., 2005).

Metacyclic trypomastigotes can enter a vertebrate through the insect bite wound, as well as through skin lacerations, mucosae, or conjunctives (Silva-Neto *et al*., 2010). Once the parasite entered a vertebrate host, it can invade any kind of nucleated cell and transform into amastigote, which will multiply by binary fission to the point of lysing the host cell. Upon reaching the bloodstream, they will transform into blood trypomastigotes, which will either infect other cells and establish amastigote nests in different tissues, or be absorbed by a triatomine to complete its life cycle (Fig. 2.2).

Factors associated to *T. cruzi* virulence

Prepatent period

Prepatent period is defined as the period between infection and the time when parasite forms become observable in the host bloodstream (Cruz-Reyes and Camargo-Camargo, 2001). In general, the prepatent period of *T. cruzi* strains studied in Mexico vary from 6 to 10 days; such is the case of the Apodaca, La Cruz (Salazar-Schettino *et al*., 1978), Ninoa, and Querétaro (Espinoza *et al*., 2010) strains. However, Tay *et al*. (1980) reported that the Tetitlan strain (from Guerrero) required about 20 minutes after inoculation to transform into intracellular amastigotes, showing blood trypomastigotes in only two and a half hours. Thus, variations in this period could be indicative of the virulence of a parasite strain.

Shorter prepatent periods have been observed in several strains from Mexico and South America when the parasite is inoculated by the intraperitoneal route with respect to oral, nasal, and conjunctival routes (Tay *et al*., 1973; Malaquias *et al*., 2013). Recently, oral infection has attracted great attention, especially in South America, after accidental

ingestion of contaminated food or consumption of infected insects (Coura, 2006; Diaz & González, 2014). The longer prepatent periods characterizing this infection route could be associated to the role of gastric juice, since parasites could lose motility and even die because of the acidic medium (Neira *et al*., 2003). Although gastric juice is an extremely hostile environment, the parasite can establish an infection thanks to the gp82 surface glycoprotein, relatively resistant to peptic digestion in acid pH values (Yoshida, 2009). This adhesion molecule binds epithelial cells and induces Ca^{+2} mobilization, an essential event for parasite internalization (Ruíz *et al*., 1998; Toso *et al*., 2011).

The ability of *T. cruzi* to enter a mammal host through several routes increases the possibility of contact with human populations, since epidemiological risk sources are not limited to triatomine dejections, but include mechanisms relying on human actions. A clear example is conjunctival infection, in which the individuals themselves carry infected feces to the ocular zone. Nevertheless, the disease evolution and the host immune response could be critically affected by the site of infection (Barreto-de-Albuquerque *et al*., 2015).

On the other hand, it has been suggested that prepatent period could be shorter when *T. cruzi* blood stages are inoculated instead of the metacyclic forms found in insect feces; though inconclusive and scantily analyzed, this hypothesis is supported by some studies. Espinoza *et al*. (2010) observed 3- and 7-day prepatent periods after inoculating blood trypomastigotes of the Querétaro and Ninoa strains, respectively; similar results were reported by Gómez-Hernández *et al*. (2011) with Mexican strains characterized belonging to the TCI lineage. Conversely, Monteón *et al*. (2009) and Díaz-Gómez (2014) demonstrated prepatent periods longer than 10 days with metacyclic forms of strains isolated from *T. dimidiata* in Campeche, Nayarit, and Chiapas.

This phenomenon could be explained by a previous contact between blood trypomastigotes and the vertebrate immune system (Suárez *et al*., 2009). It has been hypothesized that freshly-inoculated blood forms, i.e. parasitic forms obtained from a vertebrate host, can better evade the host immune response. The mechanisms underlying the parasite ability to evade the immune response are mediated by enzymes like trans-sialidases (TS) (Nardy *et al*., 2016), which are expressed and released 20 times more in blood forms than in other parasite stages (Rubin-de-Celis *et al*., 2006), and by the capacity of transferring sialic acid from host glycoconjugates to mucin molecules on its own cell surface (Freire-de-Lima *et al*., 2015). It has been observed that TS facilitate parasite adhesion and invasion to vertebrate cells (Nardy *et al*., 2016), and also enhance the capacity of escaping the parasitophorous vacuole and invading the cell cytosol (Rubin-de-Celis *et al*., 2006). Thus, it has been postulated that a faster parasite replication occurs under these conditions, and consequently an earlier presence in the bloodstream is observed. Therefore, *T. cruzi* TS are considered a major virulence factor (Buschiazzo *et al*., 2012), and currently are under study as targets for future vaccines against Chagas disease (Hoft *et al*., 2007).

To understand the clinical picture of Chagas disease, experimental models have been implemented using animals with a proven susceptibility to the infection. Several studies in Mexico used female mice as animal model, due to their greater susceptibility (Salazar-Schettino *et al*., 1978; Sanchez-Guillén *et al*., 2006; Becerril-Flores *et al*., 2008; Mendoza-Rodríguez, 2015). However, males seem to be more susceptible in works using South American strains (Zúñiga *et al*., 1997; Zúñiga *et al*., 1998; Miccuci *et al*., 2010; Mena-Marín *et al*., 2012). Considering this, sex hormones could play a significant role in infection (Pérez *et al*. 2009; Miccuci *et al*., 2010). It has been reported that testosterone administration to animals favors an early replication of the parasite (Tartalini *et al*., 2011).

This hormone has a deleterious effect on host defensive mechanisms during the acute stage of the disease (Pérez *et al*., 2009), mainly affecting T and B lymphocyte and macrophage populations (Benten *et al*., 2004). In *T. cruzi*-infected young male rats, a testosterone concentration similar to that in uninfected adult rats has been observed, suggesting that the parasite is capable of altering the metabolism of this hormone. In fact, Carlos Chagas reported the presence of the parasite in animal testes in 1916 (Chagas, 1916; Pérez *et al*., 2009).

Several host-associated factors have been proved to modify *T. cruzi* virulence, but other variations depend on the biological and genetic traits of the strain under study (Albuquerque *et al*., 2008). The TCI parasite lineage predominates in Mexico, while the TCII lineage is prevalent in South America (Bosseno *et al*., 2002; Noireau *et al*., 2009). These genetic differences between *T. cruzi* strains should be considered when choosing the most appropriate animal model. Additionally, it is advisable to use young animals (for mice, weighing about 15-20 g), since some functions of the immune system, like thymus cellularity and lymphocyte dynamics, mature with growth and development (Revelli *et al*., 1987; Herrer and Díaz, 1995).

Parasitemia

This parasitological parameter is specific for the acute stage of Chagas disease. Benznidazole and nifurtimox, the drugs of election against the parasite, show the highest efficacy in this stage, since both nitroheterocyclic compounds are more active against extracellular *T. cruzi* forms (blood trypomastigote) than against intracellular forms (amastigote), causative of the chronic stage of the disease (Castro, 2014; WHO, 2016). The Tetitlan strain, isolated in Guerrero (Table 2.1), caused the highest parasitemia level (75×10^6 parasites/ml) for a Mexican strain and for any other strain isolated in the Americas.

High parasitemia levels have been observed when a large number of parasites are experimentally inoculated to animals. Mazzotti (1940) reported a higher number of trypanosomes in blood when assaying inoculum sizes ranging from 46 000 to 500 000 parasites. While these variations could be attributed to the pre-programmed number of divisions of the parasite in vertebrate cells (approximately 9 binary division cycles) (Andrade and Andrews, 2005), it is clear that the host immune response has a key role in controlling parasite replication (Basso, 2013).

Espinoza *et al*. (2010) reported varying parasitemia values with respect to antibody levels produced in response to experimental infections. The Ninoa strain, which elicited IgM and IgG2 antibodies in an early stage and IgG1, IgG2b, IgG3, and IgA antibodies 30 days later, exhibited lower parasitemia values (10^6 parasites/ml) than the Querétaro strain (2.9×10^6 parasites/ml); the latter elicited IgM and IgG2 antibodies only. Judging by the capacity of *T. cruzi* to block the host immune response, it seems clear that the Ninoa strain was not able to block this response, and in consequence its replication was compromised. Experiments in immunocompetent mice show that an absence of antibodies leads to a fail in controlling parasite replication and the disease (Kumar and Tarleton, 1998). Given their role in the infection, anti-*T. cruzi* antibodies are routinely used in blood bank screening to detect infected donors (Galavíz-Silva *et al*., 2009). This practice has successfully prevented an increase in the number of Chagas disease cases after blood transfusion in Mexico.

The capacity to efficiently block the host immune response depends on the virulence of the *T. cruzi* strain. Proteins like cruzipain, calreticulin, and TS play a role in this capacity. Some authors suggest that virulence could be related to the triatomine species from which the strain was first isolated, the site of collection, and the insect geographic distribution (Tay *et al*., 1969; Guzman-Marin *et al*., 2012). After these findings, the interest

on whether the different triatomine species could modulate parasite virulence raised (Tay *et al*., 1969; Guzman-Marin *et al.*, 2012; Little *et al*., 1996). A pioneering work on this subject was published by Tay *et al*. (1969); the author reported that the Tetitlan strain (first isolated from *T. mazzoti*) caused higher parasitemia values after passage through *T. infestans* and *T. barberi* with respect to the passage through *M. pallidipennis*. Similar results were obtained when a low-virulence strain isolated from *M. pallidipennis* increased its parasitemia values after passage through *T. barberi*. At the same time, a virulent strain isolated from the latter species reduced its parasitemia levels after passage through *M. pallidipennis* (Mendoza-Rodríguez, 2015). This suggests that *T. barberi* increases the virulence of *T. cruzi* strains. Four electrophoretic components of 82, 87, 96, and 103 kDa, not shared between both triatomine species, have been identified (Mendoza-Rodríguez, 2015). It would be important to characterize these components and determine their role in modulating parasite virulence.

In contrast with *T. barberi*, *T. dimidiata*, the main species in the Southeast region in Mexico, seems to decrease the virulence of *T. cruzi* strains, at least of the H4 and V strains, from Yucatán (Guzmán-Marín *et al*., 2012). Along with the lower susceptibility to the parasite in *T. dimidiata* with respect to other species (De Fuentes-Vicente *et al*., 2016a), this could explain the low number of reports on infected persons in the Southeast, where *T. dimidiata* is the only vector reported to date (Dumonteil and Gourbiére, 2004, Guzmán-Marín *et al*., 2012).

Although the mechanisms through which some triatomine species modulate *T. cruzi* biologic behavior are not known, it is likely that strain-vector coadaptation plays a significant role in transmission dynamics (Magalhães *et al*., 1996). Factors like carbohydrate composition in the parasite surface (Mello *et al*., 1996), the glycoproteic composition of the vector digestive tract (Antunes *et al*., 2013), the genetic composition of

both, and their shared evolutive history (Araujo *et al*., 2014) could participate in such adaptation. This is demonstrated by observing the differing degrees of susceptibility in triatomines to various parasite strains (Alves *et al*., 2000; Pérez-Rivero, 2010). Yet a poorly studied subject, the insect immune response could have some influence on *T. cruzi* virulence. Our research team found higher parasitemia values in CD-1 mice in certain Chiapas zones where *T. dimidiata* specimens exhibit a more intense immune response, particularly in the phenoloxidase enzyme (PO) activity (De Fuentes-Vicente *et al*., 2016b). On this regard, the possibility of a selection in the insect gut to ensure a successful infection to vertebrate hosts should be investigated. Given the wide intra- and inter-species variation in the insect immune response (Schmid-Hempel, 2005), it would be interesting to assess the components of this response and the molecules involved in the parasite-vector interaction with respect to their distribution patterns and evolutionary history.

Lectins, one of the most important molecule types in parasite-vector interaction, are found in triatomine midgut and have been proposed as parasite-anchoring molecules to the insect intestine (Basseri, 2002). The presence of lectins specific to sugars such as N-acetyl-D-mannosamine, α-N-acetyl-D-galactosamine, and α- and β-D-galactose has been studied in South American vectors like *Rhodnius prolixus* (Pereira *et al.*, 2011). In contrast, there is scarce information on the role of lectins in Mexican endemic vectors (Rivas-Medina, 2014). On this regard, it has been suggested that lectins specific for N-acetyl-D-galactosamine could be involved in parasite binding in vectors like *M. pallidipennis* and *T. barberi* (Rivas-Medina, 2014).

It should be mentioned that maintaining *T. cruzi* in laboratory conditions for extended periods seems to alter the parasite biological behavior. An increase in the number of blood trypomastigotes has been reported when *T. cruzi* strains are inoculated to mice

after being cultured for more than 10 months (Tay *et al*., 1973). However, other strains kept in culture for extended periods show a decrease in parasitemia levels instead (Becerril-Flores *et al*., 2008). Extended maintenance in culture media could cause genetic changes in the parasite, attenuating virulence. Culture in Liver Infusion Tryptose (LIT), the most widely used medium for *T. cruzi* maintenance, leads to a gradual adaptation of the parasite to an artificial environment where glucose levels are higher than those usually met by the flagellate in its evolutionary cycle (Ledezma *et al*., 2013). Thus, it has been suggested that *T. cruzi* life cycle should be simulated in the laboratory by alternate passage between triatomines and mice, thus expecting to maintain the biological properties of parasite strains (Contreras *et al*., 1994; Contreras *et al*., 1998; Ledezma *et al*., 2013).

Salazar-Schettino *et al.* (1978) indicated that passage through humans increases *T. cruzi* virulence. For instance, the Apodaca strain, isolated in a region where several cases of Chagas disease had been reported, showed higher parasitemia values than La Cruz strain, isolated in a zone where no Chagas disease case were reported before (Salazar-Schettino *et al*., 1978). Although humans are considered as an incidental host, it has been suggested that *T. cruzi* could have found in humans a better reservoir to ensure its continuity in nature. In fact, this human-parasite interaction dates back at least 9 000 years.

Cellular tropism

T. cruzi is capable of invading a wide range of vertebrate cells; however, the key receptors in parasite invasion events are still unknown (Fernandes and Andrews, 2012). The preference of *T. cruzi* strains for some specific host tissue has been widely studied. In general, there is evidence that the TCI lineage exhibits a cellular tropism for cardiac muscle, in contrast with the TCII lineage, which preferentially invades the intestine and esophagus (Botero *et al*., 2007; Andrade *et al*., 2002; Macedo *et al*., 2002). Reports in

Mexico on greater heart damage in human subjects and experimental animals could be related to a greater presence of the TCI lineage in the country (Bosseno *et al*., 2002). Although parasite genetics has been associated to different disease manifestations, a precise relationship cannot be established yet (Manoel-Caetano and Silva, 2009).

Various studies have reported that Mexican strains exhibiting high parasitemia values in mice induce a higher damage in cardiac muscle (Tay *et al*., 1973; Sánchez-Guillen *et al*., 2006; Becerril-Flores *et al*., 2008; De Fuentes-Vicente *et al*., 2016b). In the acute stage, when trypomastigotes are blood-borne, damage to cardiac muscle is mainly due to the effect on cardiac cells of parasite replication (Gutiérrez *et al*., 2009; Texeira, 2011); this could explain the relation between parasitemia and tissue damage. Three general pathogenic mechanisms have been described for Chagas disease: direct damage on myocardium and neurons due to parasitemia; microvascular alterations; and damage caused by the immune response. In time, these mechanisms lead to fibrosis and deterioration of the cardiac function. The former mechanism favors the development of cardiopathy. The presence and reproduction by binary fission of intracellular amastigotes in myocardial cells and the ensuing lysis result in the release of cell components and tissue destruction due to the inflammatory response (Texeira, 2011). This process occurs even when only parasite-related antigens or DNA have been detected (Jones, 1993; Marín-Neto, 2007; Marín-Neto, 2009). Another mechanism involved in myocardial damage involves microvascular anomalies that lead to myocardial ischemia; this process is caused by an increase in platelet adhesion to microvascular endothelium due to augmented endothelin levels, which in turn were triggered by the inflammatory process, along with neuraminidase production by blood trypomastigotes. This results in platelet thrombi and myocardial microinfarcts, with evident necrotic processes (Herrera, 2003). With respect to the elicited immune system response,

molecular mimesis and polyclonal activation are part of autoimmunity processes against proteins, cell components, neurons, and myocardial structures, as well as against β-adrenergic receptors, with the presence of auto-antibodies in serum from Chagasic patients (Manher, 2001). All of these mechanisms, activated by the mere presence of the parasite, have been involved in the pathogenesis of Chagasic cardiopathy lesions; thus, disorders in the autonomous nervous system, microvascular alterations, and the immune system response act together to develop fibrosis, the hallmark of chronicity (Marín-Neto, 2007).

On the other hand, *T. cruzi* has been reported as capable of invading brain tissues in experimental animals. Tay *et al*. (1969) observed that parasites invade the brain when parasitemia values are higher than 30×10^6 parasites/ml. This is in agreement with results reported by Salazar-Schettino *et al*. (1978), who observed amastigote nests in the brains from mice showing parasitemia values of 40×10^6 parasites/ml. Instead, we reported the presence of nests in the same organ with a strain from Chiapas which reached 22.57×10^6 parasites/ml, even though the number of nests observed was very low (De Fuentes-Vicente *et al*., 2016b). The presence of the parasite in the brain results in damage to the central nervous system and other cerebral components (Silva *et al*., 2010). These lesions may cause rear limb paralysis (Yarbuh *et al*., 2006), memory loss as seen in human patients (Chagas, 1916), and depressive disorders (Vilar-Pereira *et al*., 2012), among other conditions. These damages are reported as more severe in younger animals, due to the probable role of the blood-brain barrier (BBB) in the protection against *T. cruzi;* certain changes in BBB-associated receptors during adulthood seem to exert some protection against the parasite (Mata *et al*., 2012). In humans, about 9-36% of chronic Chagasic patients show evidence of cerebral infarctions (Silva *et al*., 2010).

T. cruzi also can infect skeletal musculature, reticuloendothelial tissue, spleen, and liver, albeit in a lesser degree (Mejía and Triana, 2005). Amastigote nests have been observed in lungs from BALB/c mice infected with the Mexican strains Albarrada, CH4, and Zarco (Melnikov *et al*., 2005), and in intestines from BALB/c mice infected with the Ninoa and Querétaro strains (Espinoza *et al*., 2011). It should be noted that amastigote nests found in the intestine of mice infected with both parasite strains were very small, only observable by immunofluorescence; this may explain the low number of reports on gastrointestinal infection by Mexican strains (Espinoza *et al*., 2011).

Resuming the issue of parasite maintenance in laboratory conditions, Becerril-Flores *et al*. (2008) observed that the number of organs invaded by several *T. cruzi* strains was significantly reduced (from 9 to 3 organs) after passages in LIT medium for one year. Maintenance in culture even resulted in the complete removal of 7 out of 11 evaluated strains. This decrease in tropism associated with parasite maintenance in culture was also observed by Tay *et al*. (1969), even though strains were maintained by alternate passages between mice and triatomines; therefore, other factors meriting study could be taking part in this decreased tropism. It is not known whether the triatomines used in these passages corresponded to the zones where parasites were isolated, since some degree of adaptation and sensitivity seems to exist between *T. cruzi* strains and triatomines from a same geographic region; this adaptation could influence the biologic characteristics of parasite populations (Schaub *et al*., 2000; García *et al*., 2007).

Mortality rate

Chagas disease causes about 12 000 yearly deaths (Cucunubá *et al*., 2016). Under natural conditions, the high mortality rate in humans is mainly associated to Chagasic myocardiopathy, usually with a poorer prognosis than dilation myocardiopathies with other

etiologies (Barbosa and Nunes, 2012; Ribeiro *et al*., 2012). Senior population is at a higher risk of death by Chagasic myocardiopathy, due to the disease chronic development and the extended infection time (Rísquez, 2009). While Chagasic myocardiopathy starts with parasite invasion and replication in cardiac muscle, death in acute-stage human patients is uncommon; nevertheless, death risk is higher among young children and immunosuppressed patients (e.g. HIV-infected subjects).

Mortality rates ranging from 0% to 100% have been reported in experimental infections. Some studies, like that published by Salazar-Schettino *et al*. (1978), support the relevance of parasite replication as a mortality modulator. In that study, the Apodaca strain, which reached the highest parasitemia levels, killed all subject mice. On the other hand, a 100% survival rate was reported with the La Cruz strain, which showed lower parasitemia values. This has been observed as well in other works with different Mexican strains (Barrera-Pérez *et al*., 2001; Gómez-Hernández *et al*., 2011) with some exceptions (e.g. Wallace *et al*., 2001). High survival rates were also observed with other strains from Oaxaca, Veracruz, Guerrero, and Campeche (Monteón *et al*., 2009). Curiously, the vector *T. dimidiata* abounds in those regions, and a high prevalence of Chagas disease in humans has been reported there (Ramsey *et al*., 2000; Salazar-Schettino *et al*., 2005; Cruz-Reyes and Pickering-López, 2006; Salazar-Schettino *et al*., 2010).

Besides *T. dimidiata*, *M. pallidipennis* seems to favor low mortality rates for several *T. cruzi* strains (Mendoza-Rodríguez, 2015). As mentioned above, the mechanisms underlying this phenomenon are not known. A common trait in both vector species is the capacity of entering human habitation and complete their life cycle indoors. Thus, humans become the main food source for the insect, and it could be hypothesized that this change in feeding habits results in a decrease of parasite virulence. This is not unreasonable, since a

similar phenomenon can be observed by exposing the parasite to avian blood in *T. dimidiata* gut; consequently, a higher survival rate is observed in infected mice (Calderón-Arguedas *et al*., 2003). Nevertheless, avian blood is known to be refractory to *T. cruzi* infection (Texeira *et al*., 2011). Taken together, this evidence indicates that parasite virulence could be associated not only to insect gut components, but also to the vector biologic behavior (Vallejo *et al*., 2009; García *et al*., 2010).

A parasite causing a low mortality rate in its host will result in a higher transmission probability, thus ensuring its permanence in nature (Wild *et al*., 2009). It is clear that in laboratory studies, factors like animal sex (Mena-Marín *et al*., 2012), inoculum size (Mazzotti, 1940), and animal age (Zúñiga *et al*., 2012) could affect mortality rate. The influence of parasite genetic variability on mortality in experimental infections has been little explored, and its effect is not clear. Espinoza *et al*. (2010) observed significant differences in the mortality rate of two genetically similar strains, Ninoa and Querétaro. On the other hand, significant differences in the mortality rate caused by two genetically distinct strains were found. One plausible explanation to this is that the adaptation of certain strains to a specific host could play a key role in parasite-host adaptation.

Conclusions and perspectives

This review evinces the biologic heterogeneity shown by the parasite protozoan *T. cruzi* in a vertebrate host. To date, this heterogeneity has prevented researchers to establish a concise classification system to facilitate the study of the parasite. Such heterogeneity could also explain the wide variety of clinical forms exhibited by American trypanosomiasis. While the main clinical picture in the chronic disease is cardiac muscle compromise, the digestive tract is involved in other cases, producing the 'mega' syndromes, megaesophagus and megacolon, and yet other cases report nervous system disorders only. One of the main

problems hindering the study of this parasitic infection is the question of whether the reported differences in clinical picture and response to treatment are indeed due to differences in the biologic behavior of the parasite. Currently, it is not known why *T. cruzi* is so variable, not only on the parasitological side, but also with respect to its genetics, biochemistry, and enzymology. This complexity could be closely related to the great number of reported vectors (132 species), each exerting a different kind of pressure on the flagellate. Besides, the wide variety of vertebrate hosts that *T. cruzi* is capable of naturally infecting (about 100 mammal species) further complicates the picture.

While it is true that experimental infections are not subject to environmental pressures that normally affect reservoirs, vectors, and the parasite (e.g. temperature and altitude), controlled infections provide an overview on the parasitological aspects of a *T. cruzi* strain in each region. Due to differences in their behavior, all strains isolated in different regions of the Americas should be biologically characterized, especially those strains for which humans act as a reservoir. Additionally, it is essential to standardize protocols to study *T. cruzi* strains in all research laboratories, given the discrepancies observed in direct comparisons of some parasite populations due to the different methodologies used.

Another interesting and poorly studied aspect of *T. cruzi* infection is the passage of isolates through different vectors; several components in triatomine gut could be influencing the biological and biochemical behavior of the parasite, not only its virulence. On this regard, Mexico is a diverse country, where widely-distributed endemic species may cohabit in a same region, causing different clinical manifestations. Undoubtedly, the role of triatomines in *T. cruzi* virulence underlies the previously reported heterogeneity of Mexican isolates. It should be noted that the digestive tract (anterior and rear midgut) of endemic

triatomine species has not been well studied, and very little is known on its protein composition.

Finally, herein we propose the characterization of all *T. cruzi* isolates to favor a real clinical application in patients from the regions where parasites were isolated. On this regard, there are very few studies in Mexico that, based on *T. cruzi* infections and clinical follow-up, have grouped patients into clinical categories (Salazar-Schettino, personal communication), in contrast with the several studies reported in South America (Sgammin, 1981). On a practical perspective, gathering more data on *T. cruzi* infections could be a useful resource to predict the disease evolution, the patient clinical and immunological response, and of course the most appropriate therapeutic options.

Acknowledgments

J. Antonio De Fuentes Vicente acknowledges the scholarship and financial support provide by the Consejo Nacional de Ciencia y Tecnologia (CONACYT). This paper constitutes a partial fulfillment of the doctoral work of a J. Antonio De Fuentes Vicente in the Doctorado en Ciencias Biologicas of the Universidad Nacional Autonoma de Mexico (UNAM).

References

Chagas C. Nova trypanosomiase humana. Estudos sobre a morphologia e o cyclo evolutivo de Schyzotrypanum cruzi, n. gen., n. sp., agente etiologico de nova entidade morbida do homem. Mem Inst Oswaldo Cruz. 1909; 1(2): 159-218.

Gómez-Hernández C, Rezende-Oliveira K, Nogueira G, Rocha L, Borges H, Martínez-Ibarra J, *et al*. Molecular characterization of *Trypanosoma cruzi* Mexican strains and their behavior in the mouse experimental model. Rev Soc Bras Med Trop. 2011; 44(6): 684-90.

Guzmán-Marín E, Jimenez-Coello M, Puerto-Solis M, Ortega-Pacheco A, Acosta-Viana Y. Influence of *Triatoma dimidiata* in Modulating the Virulence of *Trypanosoma cruzi* Mexican Strains. Interdiscip Perspect Infect Dis. 2012; doi:10.1155/2012/328091

Zingales B, Andrade SG, Briones MR, Campbell DA, Chiari E, Fernandes O, *et al*. A new consensus for Trypanosoma cruzi intraspecific nomenclature: second revision meeting recommends TcI to TcVI. Mem Inst Oswaldo Cruz; 104(7): 1051-4.

Guzmán-Marín E, Zavala-Castro J, Acosta-Viana M, Rosado-Barrera M. Importancia de la caracterización de cepas de *Trypanosoma cruzi*. Rev Biomed. 1999; 10(3): 177–84.

Souto RP, Fernandes O, Macedo AM, Campbell DA, Zingales B. DNA markers define two phylogenetic lineages of *Trypanosoma cruzi*. Mol Biochem Parasitol. 1996; 83(2):141–53.

Blanco A, Montamat EE. Genetic Variation among *Trypanosoma cruzi* populations. J Exp Zool. 1998; 282(2): 62-70.

Garzón EA, Barnabé C, Córdova X, Bowen C, Paredes W, Gómez E. *Trypanosoma cruzi* isoenzyme variability in Ecuador: first observation of zymodeme III genotypes in chronic chagasic patients. Trans R Soc Trop Med Hyg. 2002; 96(4): 378-82.

Lages-Silva E, Ramírez LE, Pedrosa AL, Crema E, da Cunha LM, Pena SD, *et al*. Variability of kinetoplast DNA gene signatures of *Trypanosoma cruzi* II strains from patients with different clinical forms of Chagas' disease in Brazil. J Clin Microbiol. 2006; 44(6): 2167-71.

Andrade SG, Magalhães JB. Biodemes and zymodemes of *Trypanosoma cruzi* strains: correlations with clinical data and experimental pathology. Rev Soc Bras Med Trop. 1997; 30: 27-35.

Brisse S, Verhoef J, Tibayrenc M. Characterisation of large and small subunit rRNA and mini-exon genes further supports the distinction of six *Trypanosoma cruzi* lineages. Int J Parasitol. 2001; 31(11): 1218-26.

Flores-López CA, Machado CA. Analyses of 32 loci clarify phylogenetic relationships among *Trypanosoma cruzi* lineages and support a single hybridization prior to human contact. PLoS Negl Trop Dis. 2011; 5(8): e1272.

Zumaya-Estrada F, Messenger L, López-Ordoñez T, Lewis M, Flores-López, Martínez-Ibarra A. North American import? Charting the origins of an enigmatic *Trypanosoma cruzi* domestic genotype. Parasit Vectors. 5: doi: 10.1186/1756-3305-5-226

Scott MT. The nature of immunity against *Trypanosoma cruzi* in mice recovered from acute infection. Parasit. Immunol. 1981; 3(3): 209-18.

Morel C, Goncalves A, Simpson L, Simpson AM. Recent advances in the development of DNA hybridization probes for the detection and characterization of *Trypanosoma cruzi*. Mem. Inst. Oswaldo Cruz. 1984; 79: 51-3.

Salazar-Schettino PM, Bucio-Torres MI, Cabrera-Bravo M, de Alba-Alvarado MC, Castillo-Saldaña DR, Zenteno-Galindo EA, *et al*. Enfermedad de Chagas en México. Revista de la Facultad de Medicina de la UNAM. 2016; 59(3): 6-16.

Salazar-Schettino PM, Bucio MI, de Haro I, Tay J, Alonso T. Reservorios y transmisores de *Trypanosoma cruzi* en el Estado de Oaxaca. Sal Publ Mex. 1987 29: 26-32.

Domínguez A, Espinoza E. Estudio de reservorios silvestres de *Trypanosoma cruzi* en el Estado de Oaxaca, México. Bol Chil Parasitol. 1988 43: 64-65.

Salazar-Schettino PM, Bucio Torres MI, Cabrera-Bravo M, Bautista J. First Case of Natural Infection in Pigs. Review of *Trypanosoma cruzi* Reservoirs in Mexico. Mem Inst Oswaldo Cruz. 1997; 92(4): 499-502.

Salazar-Schettino PM, Rojas-Wastavino, GE, Cabrera-Bravo M, Bucio-Torres MI, Martínez-Ibarra JA, Monroy-Escobar MC, *et al*. Revisión de 13 especies de la familia Triatominae (Hemiptera: Reduviidae) vectores de la enfermedad de Chagas, en México. J Selva Andina Res Soc. 2010; 1(1): 57-80.

Cruz-Reyes A, Pickering-López JM. Chagas disease in Mexico: an analysis of geographical distribution during the past 76 years – A review. Mem Inst Oswaldo Cruz. 2006; 101(4); 345–54.

Hoffmann CC. Nota acerca de un probable transmisor de la trypanosomiasis humana en el estado de Veracruz. Rev Mex Biol. 1928; 8:12-8.

Mazzotti L. Triatomineos de México y su infección natural por *T. cruzi*. Chagas Med 1940; 20: 95

Rivera I, Moreno E, González N, Lugo A. Caracterización de aislados de *Trypanosoma cruzi* del occidente de Venezuela. Rev Ecol Lat Am. 2000; 7(3): 1-10.

McCall LI, McKerrow JH. Determinants of disease phenotype in trypanosomatid parasites. Trends Parasitol. 2014; 30(7): 342-9.

Cummings KL, Tarleton RL. Rapid quantitation of Trypanosoma cruzi in host tissue by real-time PCR. Mol Biochem Parasitol. 2003; 129(1): 53-9.

Espinoza B, Rico T, Sosa S, Oaxaca E, Vizcano-Castillo A, Camallero, M. *et al*., Mexican *Trypanosoma cruzi* TCI Strains with Different Degrees of Virulence Induce Diverse Humoral and Cellular Immune Responses in a Murine Experimental Infection Model. J Biomed Biotechnol. 2010; doi:10.1155/2010/890672

Espinoza B, Solorzano-Domínguez N, Vizcaino-Castillo A, Martínez I, Elias-López AL, Rodríguez-Martínez JA. Gastrointestinal infection with Mexican TcI *Trypanosoma cruzi* strains: different degrees of colonization and diverse immune responses. Int J Biol Sci. 2011; 7(9): 1357-70.

Macedo A, Machado, C, Oliveira R, Pena S. *Trypanosoma cruzi*: genetic structure of populations and relevance of genetic variability to the pathogenesis of chagas disease. Mem Inst Oswaldo Cruz. 1999; 99(1): 1-12.

Magalhaes JB y Andrade SG. Estudo do comportamento de cepas do *Trypanosoma cruzi* após passagens em diferentes espécies de triatomíneos. Rev Soc Bras Med Trop. 1991; 24: 209-216.

Manoel-Caetano F, Silva AE. Implications of genetic variability of *Trypanosoma cruzi* for the pathogenesis of Chagas disease. Cad Saude Publica. 2007; 23(10): 2263-74.

Barreto, M. Reservorios do Trypanosoma cruzi nas América. Ver. Brás. Matar. 1964; 16: 527.

World Health Organization. Chagas disease: Guidelines for a standard protocol. Geneva: WHO; 1986.

Noireau, F., Diosque, P. & Jansen, M. *Trypanosoma cruzi*: adaptations to its vectors and its host factors. Vet Res. 2009; 40, (26): 1–23.

Kollien AH, Schaub GA. The development of *Trypanosoma cruzi* in triatominae. Parasitol Today. 2000; 16(9): 381-7.

Azambuja P, Ratcliffe N, García E. Towards an understanding of the interactions of *Trypanosoma cruzi* and *Trypanosoma rangeli* within the reduviid insect host *Rhodnius prolixus*. An Acad Bras Cienc. 2005; 77(3): 397-404.

Kollien A, Schaub G. *Trypanosoma cruzi* in the rectum of the bug *Triatoma infestans*: effects of blood ingestion by the starved vector. Am J Trop Med Hyg. 1998; 59(1): 166-70.

Silva-Neto M, Carneiro AB, Silca-Cardoso L, Atella G. Lysophosphatidylcholine: A Novel Modulator of *Trypanosoma cruzi* Transmission. J Parasitol Res. 2012; doi:10.1155/2012/625838

Cruz-Reyes A, Camargo-Camargo B. Glosario de términos en parasitología y ciencias afines. Instituto de Biología, Programa Universitario de Investigación en Salud, Universidad Nacional Autónoma de México, y Plaza y Valdés Editores. México, D.F. 2001: 347 pp.

Salazar-Schettino PM, Jiménez MJ, Tay J, Cárdenas RL. Estudio comparativo de la patogenicidad de cuatro cepas de *T. cruzi* en el ratón blanco. Rev Latinoamer Microbiol. 1978; 20: 51-57.

Tay 1980

Tay J, Gutiérrez M, Salazar P, Castillo M, Ortega-G M. Estudios sobre seis cepas mexicanas de *Trypanosoma cruzi.* Rev Inv Salud Publica. 1973; 33: 67-76.

Malaquias GB, Gruendling AP, Araújo S, Gomes M, Toledo M. Evolution of infection in mice inoculated by the oral route with different developmental forms of *Trypanosoma cruzi* I and II. Experimental Parasitology. 2013; 135: 511-7.

Coura JR. Transmissão da infecção chagásica por via oral na história natural da doençaa de Chagas. Rev Soc Bras Med Trop. 2006; 39: 113-117.

Díaz M, González C. Transmisión de Chagas agudo: transmisión oral de *Trypanosoma cruzi* como una vía de transmisión re-emergente. Rev Univ Ind Santander. 2014; 46(2): 177-88.

Neira I, Silva FA, Cortez M, Yoshida N. Involvement of *Trypanosoma cruzi* metacyclic trypomastigote surface molecule gp82 in adhesion to gastric mucin and invasion of epithelial cells. Infect Immun. 2003; 71(1): 557-61.

Yoshida N. Molecular mechanisms of *Trypanosoma cruzi* infection by oral route. Mem Inst Oswaldo Cruz. 2009; 104(1): 101-7.

Toso A, Vial F, Galanti N. Transmisión de la enfermedad de Chagas por vía oral. Rev Med Chile. 2011; 139(2): 258-66.

Barreto-de-Albuquerque J, Silva D, Pérez R, Berbert L, Santana E, Farias D, *et al*. *Trypanosoma cruzi* Infection through the Oral Route Promotes a Severe Infection in Mice: New Disease Form from an Old Infection? PLoS Negl Trop Dis. 2015; DOI:10.1371/journal.pntd.0003849

Monteón V, Godínez S, Cruz-Zetina G, Balmes J, López R, Hernández O. Caracterización biológica de aislados mexicanos de *Trypanosoma cruzi*: metaciclogénesis, parasitemia y resistencia contra Benznidazol. Rev Biomed. 2009; 20(3): 206-14.

Díaz-Gómez JM. Efecto de la infección por Trypanosoma cruzi en fetos de ratas. Tesis de licenciatura. Universidad de Ciencias y Artes de Chiapas, Tuxtla Gutiérrez, Chiapas, México. 68 pp.

Suárez N, Cabrera R, Cartagena L, Ayaqui R. Características biológicas de una cepa de *Trypanosoma cruzi* en un modelo murino y análisis de supervivencia. Rev Peru Med Exp Salud Publica. 2009; 26(2): 187-92.

Nardy AF, Freire-de-Lima CG, Pérez AR, Morrot A. Role of *Trypanosoma cruzi* Transialidase on the escape from host immune surveillance. Front Microbiol. 2016; 23(7): doi: 10.3389/fmicb.2016.00348.

Rubin de Celis S, Uemura H, Yoshida N, Schenkman S. Expression of trypomastigote trans-sialidase in metacyclic forms of *Trypanosoma cruzi* incrase parasite escape from its parasitophorous vacuole. Cellular Microbiology; 8(12): 1888-98.

Freire-de-Lima L, Fonseca LM, Oeltman T, Mendoca-Previato L, Previato JO. The trans-sialidase, the major *Trypanosoma cruzi* virulence factor: Three decades of studies. Glycobiology. 25(11): 1142-9.

Buschiazzo A, Muia R, Larrieux N, Pitcovsky T, Mucci J, Campetella O. *Trypanosoma cruzi* trans-sialidase in complex with a neutralizing antibody: structure/function studies towards the rational design of inhibitors. PLoS Pathog. 2012; 8(1): e1002474

Hoft DF, Eickhoff CS, Giddings OK, Vasconcelos JR, Rodrigues MM. Trans-sialidase recombinant protein mixed with CpG motif-containing oligodeoxynucleotide induces protective mucosal and systemic *Trypanosoma cruzi* immunity involving CD8+ CTL and B cell-mediated cross-priming. J immunol. 2007; 179(10): 6889-900.

Sánchez-Guille MC, Bernabé C, Tibayrenc M, Zavala-Castro J, Totolhua JL, Méndez-López J, *et al*. Trypanosoma cruzi strains isolated from human, vector, and animal reservoir in the same endemic region in Mexico and typed as T. cruzi I, discrete typing unit 1 exhibit considerable biological diversity. Mem Inst Oswaldo Cruz. 2006; 101(6): 585-590.

Becceril-Flores MA, Salazar-Schettino PM, Ramírez-Zamudio L. Analysis of variability of clones y subclones of *Trypanosoma cruzi* derived from Mexican strains by the behaviour in mice and culture cells. Res J Med Sci. 2008; 2(4): 166-174.

Mendoza-Rodríguez, MI. Caracterización biológica y bioquímica de cuatro aislados de *Trypanosoma cruzi*. Tesis de Licenciatura. Universidad Autónoma de México. 2015; 56 pp.

Micucci L, Bazan P, Fauro R, Baez A, Presti M, Triquel M, *et al*. Importancia del sexo huesped en el desarrollo de la infección por *Trypanosoma cruzi*. Rev Fac Ciencias Medicas. 2010; 67(2): 69-72.

Pérez AR, Pascutti MF, Fontanella G, Martin A, Tartalini V, Nocito A, *et al*. Influencia de la testosterona sobre la infección causada por *Trypanosoma cruzi*. Rev Med Rosario. 2009; 75: 84-92

Tartalini V, Fontanella G, Nocito A, Revelli S. Estudio preliminar de la miocarditis chagásica aguda experimental y su relación con la administración de esteroides sexuales. Insuf Card. 2011; 6(4): 156-64

Benten WP, Guo Z, Krucken J, Wunderlich F. Rapid effects of androgens in macrophages. Steroids. 2004; 69(9): 585-90.

Chagas C. Processos patogênicos da Tripanozomiase Americana. Mem Inst Oswaldo Cruz. 1916; 8:5-35.

Bosseno MF, Barnabé C, Magallón E, Lozaon-Kasten F, Ramsey J, Breniere SF. Predominance of *Trypanosoma cruzi* lineage I in Mexico. J Clin Microbiol. 2002; 40(2): 627-32.

Revelli S, Moreno H, Berra H, Valenti JL, Nocito AL, Amerio N, Morini JC. Influencia de la edad de la rata en la evolución de la infección con *Trypanosoma cruzi*. Medicina. 1987; 47: 360-6.

Mazzoti, L. Resultados obtenidos por la inoculación de ratones con pequeñas y grandes cantidades de *Trypanosoma cruzi*. Rev Inst Salub Enferm Trop México. 1940; 2: 181-7.

Andrade LO, Andrews NW. The *Trypanosoma cruzi*-host-cell interplay: location, invasion, retention. Nat Rev Microbiol. 2005; 3(10): 819-23.

Basso B. Modulation of immune response in experimental Chagas disease. World J Exp Med. 2013; 3(1): 1-10.

Cardoso M, Reis-Cunha JL, Bartholomeu D. Evasion of the immune response by *Trypanosoma cruzi* during acute infection. Front Immunol. 2015; 6: doi:10.3389/fimmu.2015.00659

Kuma S, Tarleton RL. The relative contribution of antibody production anc CD+8 T cell function to immune control of *Trypanosoma cruzi*. Parasite Immunol. 1998; 20(5): 207-16.

Tay J, Salazar-Schettino PM, Ontiveros D. Behavior of a strain of *Trypanosoma cruzi* in white mice after various transfer in different species of Triatomas. Rev Latinoam Microbiol Parasitol. 1969; 11(2): 79-89.

De Fuentes Vicente JA, Vidal-López DG, Schlie-Guzmán MA, Gutiérrez-Jiménez J. Tasa de infección y tiempo de defecación de los estadios ninfales de *Triatoma dimidiata* después de la infección experimental con *Trypanosoma cruzi*. Rev Biomed. 2016a; 27(3): 111-7.

Mello CB, Azambuja P, Garcia ES, Ratcliffe NA.Differential in vitro and in vivo behavior of three strains of *Trypanosoma cruzi* in the gut and hemolymph of Rhodnius prolixus. Exp Parasitol. 1996 82: 112-21.

Antunes LC, Han J, Pan J, Moreira CJ, Azambuja P, Borchers CH, Carels N. Metabolomic signatures of triatomine vectors of *Trypanosoma cruzi* unveiled by metabolomics. PLos One. 2013; 8(10): e77283.

Araújo CA, Waniek PJ, Jansen AM. TcI/TcII co-infection can enhance *Trypanosoma cruzi* growth in *Rhodnius prolixus*. Parasit Vectors. 2014; 7(94):doi: 10.1186/1756-3305-7-94.

Pérez-Rivero, JM. Estudio de la susceptibilidad de triatominos (Hemíptera; Reduviidae) mexicanos a *Trypanosoma cruzi* cepa NINOA. Tesis de Maestría. Instituto Politécnico Nacional. 2010: 44 pp.

De Fuentes-Vicente JA, Cabrera-Bravo MA, Enríquez-Vara JN, Bucio-Torres M, Gutiérrez-Cabrera AE, Vidal-López DG, Martínez-Ibarra JA, Salazar-Schettino PM, Córdoba-Aguilar A. Relationship between

altitud, triatomine (*Triatoma dimidiata*) immune response and virulence of *Trypanosoma cruzi*, the causal agent of Chagas disease. Med Vet Entomol.doi:10.1111/mve.12198.

Schmid-Hempel P. Evolutionary ecology of insect immune defenses. Annu Rev Entomol. 2005; 50: 529-51.

Basseri HR. Rol of lectins in interaction between parasites and the important insect vectors. Iranian J Publ Health. 2002; 31(2): 69-74.

Tay J, Gutiérrez M, Salazar-Schettino PM, Castillo M, Ortega M. Estudio sobre seis cepas mexicanas de *Trypanosoma cruzi*. Rev Inv Salud Publica. 1973; 33: 67-76.

Ledezma Y, Ledezma M, León M, Pineda W, Arteaga R, Navarro M, *et al*. Relación entre cambios en la expresión de proteasas y metaciclogénesis espontánea asociadas a las condiciones de mantenimiento de *Trypanosoma cruzi* en el laboratorio.Salus. 2013; 17(1): 56-67.

Contreras VT, De Lima AR, Zorrilla G. *Trypanosoma cruzi*: maintenance in culture modify gene and antigenic expression of metacyclic trypomastigotes. Mem Inst Oswaldo Cruz. 1998; 93:753-60.

Aufderheide AC, Salo W, Madden M, Streitz J, Buikstra J, Guhl F, *et al*. A 9,000-years record of Chagas disease. Proc Natl Acad Sci. 2004; 101(7): 2034-9.

Botero LA, Mejía MA, Triana O. Caracterización biológica y genética de dos clones pertenecientes a los grupos I y II de *Trypanosoma cruzi* de Colombia. Biomedica. 2007; 27(1): 64-74.

Teixeira AR, Hecht MM, Guimarao MC, Sousa AO, Nitz N. Pathogenesis of Chagas disease: parasite persistence and autoimmunity. Clin Microbiol Rev. 24(3): 592-630.

Marin-Neto JA, Cunha-Neto E, Maciel BC, Simoes MV. Pathogenesis of chronic Chagas hearth disease. Circulation. 2007; 115(9): 1109-23.

Herrera R, Díaz E, Pérez R, Chaín S, Sant-Yacumo R, Rodríguez E, *et al*. 2003. Estado protrombótico en estadios tempranos de la enfermedad de Chagas crónica. Rev Esp Cardiol. 2003; 56(4): 377-82.

Silva RL, Balarin MA, Correia D, Prata A, Rodríguez V. Familial analysis of seropositivity to *Trypanosoma cruzi* and of clinical forms of Chagas disease. Am J Trop Med Hyg. 2010; 82: 45-48.

Lugo de Yarbuh A, Colasante C, Alarcón M, Moreno E. Gastrocnemius skeletal muscle microvasculature and neuromuscular junction alterations in mice with experimental acute Chagas infection. Rev Cien. 2006; 26(6): 593-603.

Vilar-Pereira G, Silva AA, Pereira IR, Silva RR, Moreira OC, de Almeida, *et al*. *Trypanosoma cruzi*-induced depressive-like behavior is independent of meningoencephalitis but responsive to parasiticide and TNF-targeted therapeutic interventions. Brain Behav Immun. 2012; 26(7): 1136-49.

Mejía AM, Triana O. Genetic variability of *Trypanosoma cruzi* in blood and organsof infected mice determined by LSSP-PCR. Biomedica. 2005; 25(1): 76-86.

Melnikov VG, Velasco FF, Espinoza-Gómez F, Rodríguez FG, Dobrovinskaya OR. Pathologic changes in lungs caused by Mexican isolates of *Trypanosoma cruzi* in the acute phase of infection in mice. Am J Trop Med Hyg. 2005. 73(2): 301-6.

Cucunubá Z, Okuwoga O, Basañez M,Nouvellet P. Increased mortality attributed to Chagas disease: a systematic review and meta-analisys. Parasit Vectors. 2016; 9(42):Doi: 10.1186/s13071-016-1315-x .

Barbosa MM, Nunes MC. Estratificación del riesgo en la enfermedad de Chagas. Rev Esp Cardiol. 2012; 65(2): 17-21.

Ramsey JM, Ordoñez R, Cruz-Celis A, Alvear AL, Chavez V, et al. Distribution of domestic Triatominae and stratification of Chagas Disease transmission in Oaxaca Mexico. Med Vet Entomol. 2000; 14: 19-30.

Salazar-Schettino PM, Rojas-Wastavino GE, Cabrera-Bravo M, Bucio-Torres MI, Guevara-Gómez Y, García-de-la-Torre S. Epidemiología de la enfermedad de Chagas en Veracruz. Salud Publica de México. 2005; 47(3): 201-8.

Calderón-Arguedas O, Chinchilla M, García F, Vargas M. Variaciones biológicas de *Trypanosoma cruzi* (Kinetoplastida: Trypanosomatidae) asociadas con la ingestión de diferentes tipos de sangre por el vector (Hemíptera: Reduviidae). Parasitol Latinoam. 2003; 58(2): 3-10.

Wallace A, Ortíz S, Sánchez G, Villagra R, Muga M, Solari A. Studies on parasitemia courses and mortality in mice infected with genetically distant *Trypanosoma cruzi* clonets. Biol Resv. 2001; 34(2): 83-90.

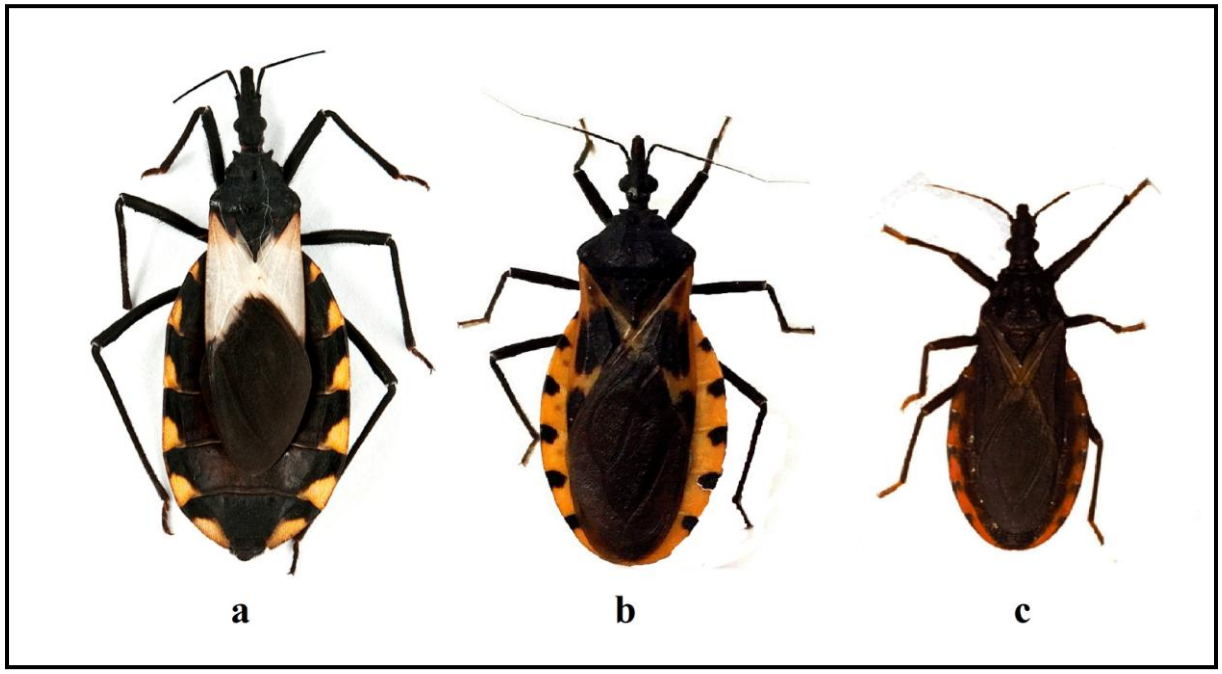

Figure 2.1 Species with greater epidemiological importance in Mexico. a) *Meccus pallidipennis*, b) *Triatoma dimidiata* y c) *T. barberi*

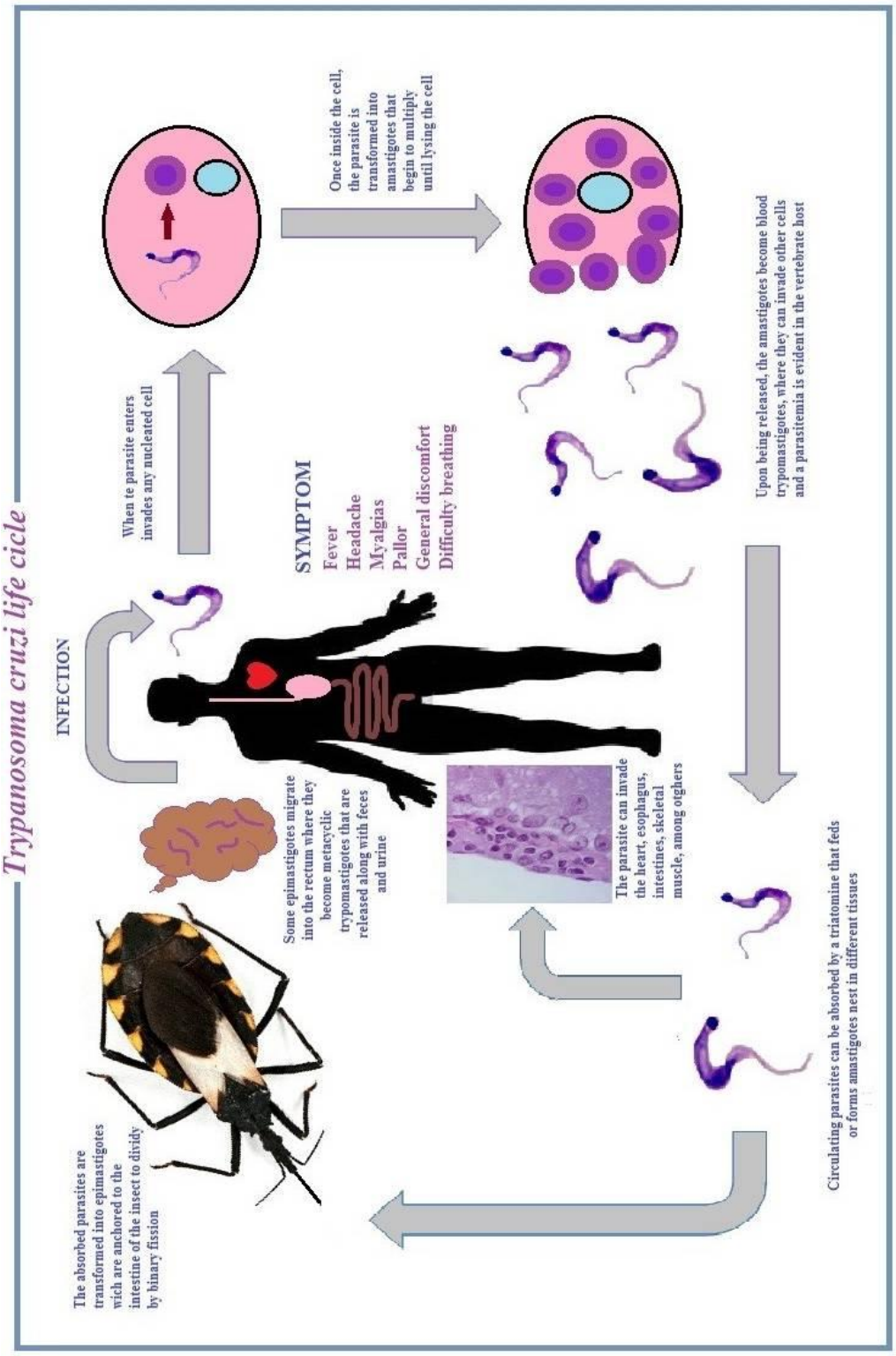

Figure 2.2 Life cycle of *Trypanosoma cruzi*

Table 2.1 Parasitological parameters (virulence) of some Mexican strains of *T. cruzi*

Strain	Isolation site	Obtained from	Used mammal	Prepatent period (days)	Peak parasitemia (par/mL)	Mortality (%)	Tissues invaded	Reference
Apodaca	Jalisco	Patient	Webster mice	6	$1x10^7$	100	Hearth, Skeletal muscle/Oesophagus/Liver/Brain/Lung	Salazar-Schettino *et al*. 1978
Santa Catarina	Jalisco	*T. barberi*	Webster mice	6	$3.5x10^7$	80	Hearth, Skeletal muscle/Oesophagus/Liver/Lung	
Zacoalco	Jalisco	*T. barberi*	Webster mice	3	$2.9x10_7$	0	Hearth/Skeletal Muscle	
La Cruz	Jalisco	*T. barberi*	Webster mice	9	$1.7x10^7$	0	Hearth/Skeletal Muscle/Oesophagus	
H4	Yucatan	Patient	Female NIH mice	8	$1.04x10^7$	50	Hearth/Skeletal muscle/oesophagus	Barrera Pérez *et al*., 2001
H5	Yucatan	Patient	Female NIH mice	8	$3.61x10^6$	8.33	Hearth/Skeletal muscle/oesophagus	
CH4	Yucatan	Patient	Male Balb-c mice	Unmentioned	$8.5x10^6$	50	Hearth/vessels/bronchi	Melnikov *et al*., 2005
Albarrada	Colima	Triatomine	Male Balb-c mice	Unmentioned	$2.2x10^6$	30	Hearth/vessels/bronchi	
Zarco	Colima	Triatomine	Male Balb-c mice	Unmentioned	$4.2x10^6$	30	Hearth/vessels/bronchi	

RyC-R	**Puebla**	***Peroyscus peromyscus***	**Female Balb-c mice**	**7**	**$174.5x10^4$**	**31.25**	**Hearth**	**Sanchéz-Guillén *et al*., 2006**
RyC-V	Puebla	*T. barberi*	Female Balb-c mice	7	$97.65x10^4$	10.4	Hearth	
RyC-H	Puebla	Patient	Female Balb-c mice	7	$55.3x10^4$	12.5	Skeletal muscle	
INC1	Oaxaca	Patient	Balb-c mice	10	6aprox.$7x10^5$	0	Not evaluated	Monteón *et al*., 2009
INC7	Veracruz	Patient	Balb-c mice	10	aprox$5.8x10^5$	0	Not evaluated	
INC9	Guerrero	Patient	Balb-c mice	10	aprox$5.3x10^5$	0	Not evaluated	
Camp7	Campeche	*T. dimidiata*	Balb-c mice	10	aprox$6.9x10^5$	0	Not evaluated	
Nayarit	Nayarit	*T. picturata*	Balb-c mice	10	aprox$2.4x10^5$	0	Not evaluated	
Ninoa	Oaxaca	Patient	Female Balb-c mice	7	$1.6x10^6$	0	Hearth	Espinoza *et al*., 2010
Qro.	Queretaro	*T. barberi*	Female Balb-c mice	3	$2.9x10^6$	100	Hearth	
Ninoa	Oaxaca	Patient	Female Balb-c mice	15	aprox$2x10^6$	0	Hearth/Duodenum/Jejunum/Ileum/colom	Espinoza *et al*., 2011
Qro.	Queretaro	*T. barberi*	Female Balb-c mice	15	aprox$3.7x10^6$	0	Hearth/Duodenum/Jejunum/Ileum/colom	

CGH1	**Jalisco**	***T. longipennis***	**Male mice**	**2-11**	**Not detected**	**6.6**	**Not evaluated**	**Gómez-Hernández *et al.*, 2011**
CGH2	Jalisco	*M. pallidipenis*	Male mice	2-11	Not detected	3.3	Not evaluated	
CGH3	Jalisco	*T. longipennis*	Male mice	2-11	$10x10^7$	3.3	Not evaluated	
CGH4	Jalisco	*M. pallidipenis*	Male mice	2-11	$4.6x10^6$	16.6	Not evaluated	
CGH6	Jalisco	*M. pallidipenis*	Male mice	2-11	$8x10^6$	6.6	Not evaluated	
KR1	Jalisco	*T. picturata*	Male mice	2-11	Not detected	10	Not evaluated	
Ninoa	Oaxaca	Patient	Male mice	2-11	$7.5x10^6$	20	Not evaluated	
INC5	Guanajuato	Patient	Male mice	2-11	$6.1x10^6$	53.3	Not evaluated	
ITR/MX/10/COP	Chiapas	*T. dimidiata*	Female Wistar Rat	12	$12x10^6$	0	Not evaluated	Díaz-Gómez, 2013
Morelos	Morelos	*M. Pallidipenis*	Femal CD-1 mice	18	$7.7x10^6$	30	Not evaluated	Mendoza, 2015
Mor/Tb	Morelos	*T. barberi*	Female CD-1 mice	17	$17x10^6$	60	Not evaluated	
Qro.	Queretaro	*T. barberi*	Female CD-1 mice	3	$29.8x10^6$	100	Not evaluated	
Qro./Mp	Queretaro	*M. pallidipenis*	Female CD-1 mice	3	$31x10^6$	100	Not evaluated	
TC 300	Chiapas	*T. dimidiata*	Female CD-1 mice	13	$15.66x10^6$	0	Hearth/Oesophagus/Gastrocnemius	De Fuentes-Vicente *et al*., 2016b
TC 700	Chiapas	*T. dimidiata*	Female CD-1 mice	13	$22.57x10^6$	6.66	Hearth/Brain/Oesophagus/Gastrocnemius	
TC 1400	Chiapas	*T. dimidiata*	Female CD-1 mice	15	$11.17x10^6$	3.33	Hearth/Oesophagus/Gastrocnemius	

CAPÍTULO III

Natural-born killers: factors underlying *Trypanosoma cruzi*-triatomine interactions

José A. De Fuentes-Vicente[1], Ana E. Gutiérrez-Cabrera[2], Paz M. Salazar-Schettino[1], A. Laura Flores-Villegas[1], Carl Lowenberger[3] & Alex Córdoba-Aguilar[4]

[1]Departamento de Microbiología y Parasitología, Universidad Nacional Autónoma de México, Mexico City, Mexico, [2]CONACYT-Centro de Investigaciones Sobre Enfermedades Infecciosas, Instituto Nacional de Salud Pública, Cuernavaca, Morelos, Mexico, [3]Department of Biological Sciences, Simon Fraser University, Burnaby, British Columbia, Canada, [4]Departamento de Ecología Evolutiva, Universidad Nacional Autónoma de Mexico, Mexico City, Mexico.

Correspondence: pazmar@unam.mx (Paz María Salazar-Schettino)

acordoba@iecologia.unam.mx (Alex Córdoba-Aguilar

Keywords: Chagas, triatomine bugs, *Trypanosoma cruzi*, evolution

Submitted in: Trends in Parasitology

Abstract

Chagas disease has devastating effects on humans and other mammals. For the disease to take place, the parasite, *Trypanosoma cruzi*, must infect blood-feeding triatomine bugs to finally reach mammal hosts. Since several species of triatomines and mammals are sylvatic, the Chagas life cycle is difficult to interrupt. This along with a lack of a cure, makes Chagas disease an almost unbeatable problem, rendering prevention programs as the main control. Since not all triatomine individuals can be infected with *T. cruzi*, one elemental yet unsolved question towards such prevention control is what makes a susceptible bug. We aim to answer this by merging findings linked to insect gut structure and microbiota, immunity, genetics, blood sources, ambient temperature and altitude to understand *T. cruzi*/triatomine interaction using a coevolutionary scenario. As expected, these abiotic and biotic factors interact with each other and provide varying fitness costs for bugs which can explain why infection takes place and how it varies in time and space. Major determinant factors are gut components and microbiota, blood sources and temperature. Although their close interaction has never been clarified, our analysis indicates that they must be considered for bug control programs as an alternative to insecticide use.

The killing couple

Several vector-borne parasites and pathogens are responsible for major public health issues, especially affecting socially and economically marginalized individuals [1]. This is the case of *T. cruzi*, the causative agent of Chagas disease [2] (Figure 1). This parasitic disease, considered as one of the 13 neglected tropical diseases in the world [3], is endemic in Latin America. Currently, an estimate of 7 million people are infected with *T. cruzi,* leading to approximately 22 000 annual deaths, throughout the Americas (Figure 2).

The vectors of *T. cruzi* are obligate hematophagous insects of the subfamily Triatominae, (Hemiptera: Reduviidae) that feed exclusively on vertebrate blood. More than half of 136 described species have been found infected with *T. cruzi* [4]. Triatomines acquire the parasite when they feed on the blood of an infected vertebrate host, and the parasites establish initially in the anterior region of the intestine and subsequently in the rectum of the insect where they replicate. As the insects feed they defecate on the host's skin. The feces contain infective metacyclic trypomastigotes that enter the host across mucous membranes or via micro abrasions produced after the host scratches the bite site (see life cycle of *T. cruzi* in Figure 1) [5,6].

The association between *T. cruzi* and triatomines is likely the result of a coevolutionary process, although this has not been formally postulated. Clarifying the mechanisms underlying these association would help us understand *T. cruzi*-vector dynamics, and will help devise strategies to interrupt parasite transmission to humans [7, 8]. Here we review biotic and abiotic factors influencing *T. cruzi*-vector interactions, using an evolutionary approach. Based on potential fitness costs for both actors, this should provide

novel elements to understand potential transmission that can be also used to design blocking strategies.

Bonnie and Clyde have just met: the insect gut as the first point of contact

When going through gut compartments, the parasite must cope with intestinal components and digestive enzymes that may influence its infection, establishment, and replication [9, 10]. The main site where an interaction of *T. cruzi* with triatomines is in the perimicrovillar membrane (PMM) [11]. This nonchitinous membrane surrounds microvilli in gut epithelial cells [12], and is comparable to the peritrophic membrane found in most other insects [13]. On entry, *T. cruzi* anchors itself to the vector's midgut via the formation of glycoconjugates with the PMM [14]; few *T. cruzi* epimastigotes are found adhered when the PMM is either under-developed or absent [15]. Triatomine control strategies could target this interaction by preventing the glycoconjugate production.

PMM synthesis and development is regulated by the neuroendocrine system [16]. The introduction of azadirachtin (a triterpenoid that blocks the production of the prothoracicotropic hormone) or insect decapitation alters the PMM structure [17, 18]. Both treatments produced a hostile condition for parasite development [19], but favorable conditions for *T. cruzi* development could be restored by a head transplant from a fed insect or ecdysone treatment [20].

It has been hypothesized that triatomines inherited and modified a set of digestive proteins from ancestral predator reduviids to lyse blood cells [21]. Whereas many hematophagous insects such as dipterans use trypsins in their digestion, triatomines use cathepsins [22]. More specifically, cathepsin D expression increases in *Rhodnius prolixus* and *Triatoma infestans* after ingesting *T. cruzi*-infected blood [23, 24]. Modulating the

insects' physiology to increase the rate of blood digestion (days 14-21 in *T. infestans* [25]) could reduce the time between feedings, and would increase the probability of *T. cruzi* being transmitted to a vertebrate host [26]. In fact, changes in behavior and feeding behavior of infected triatomines have been reported. For example, adult *Mepraia spinolai* finds hosts and defecates faster when infected, producing a more efficient parasite transmission [27]. This is not the case, however, for *R. prolixus*, whose mobility was reduced by infection [28]. On the other hand, cathepsin D secretion may speed up parasite development and metacyclogenesis [23, 29]. If recent data are confirmed that the parasite induces changes in its host to increase parasite fitness, we may hypothesize that *T. cruzi* "manipulates" the triatomine, becoming a part of the parasites' "extended genotype" [30].

A role for insect immune response: being tolerant to your manipulator?

In the face of *T. cruzi* infection, the expression of various insect immune effectors has been documented including lysozymes [24, 31], defensins [32-34], prolixicins [35], lectins [36, 37], nitric oxide [38], and components of the phenoloxidase (PO) cascade [39,40]. Notice that the activation of these components does not necessarily indicate that development and/or survival of *T. cruzi* will be affected or prevented. However, since *T. cruzi* mortality in the insect gut can be over 80% [41], and 100% in the insect's hemocoel, insect immune responses are functioning, albeit in an insufficient manner.

The signaling pathways of the innate immune response in triatomines have not been as well characterized as those in holometabolous insects. Activation of the IMD, Toll, and Jak-STAT pathways has been demonstrated in the digestive tract transcriptome of *R. prolixus* [42]. Many genes are known members of the Toll signaling pathway, but fewer genes were found associated with the IMD and Jak-STAT pathways [42]. Activation of the

Toll and IMD pathways relies on host Pattern Recognition Receptors recognizing Parasite Associated Molecular Patterns such as β-1,3-glucans, found on the cell wall of bacteria and fungi, and peptidoglycans found in Gram positive bacteria [43]. The relatively low abundance of IMD related transcripts could be due to the lack of Gram-negative bacteria in the GI tract, to which the IMD pathway responds. Alternatively, the recent publication of the *R. prolixus* genome [44] indicated that *R. prolixus*, and other related hemipterans such as the pea aphid (*Acyrthosiphon pisum*), the bedbug (*Cimex lectularius*) and the head louse (*Pediculus humanus*), appears to be missing key components of the IMD pathway, rendering it nonfunctional, or functioning at a very low level. It has been postulated that a reduction in the IMD pathway has been reduced to prevent the elimination of the obligate Gram-negative bacterial symbionts that are found in the GI tracts of these insects and on which these species rely for digestion [42]. Thus *T. cruzi* may reside in an area, the GI tract, which has a relatively low innate immune response compared with the hemocoel, solely to avoid being exposed to lethal insect defense factors. If *T. cruzi* is injected into the hemocoel of the vector, the parasites are quickly killed, indicating that the inefficient transmission of *T. cruzi* via fecal contamination is maintained because of the parasites' susceptibility to strong immune factors in regions of the insect outside the GI tract.

Activation of the PO enzyme has been observed in the gut [G. Favila-Ruiz, thesis, Universidad Nacional Autonoma de Mexico, 2016] and hemolymph (40) of *T. cruzi*-infected triatomines. It is, however, paradoxical that PO is activated in hemolymph of infected triatomines, where *T. cruzi* is not found under natural conditions. Nevertheless, this can be interpreted as a systemic response of the insect in the face of a general infection [45]. The expression of PO, or other innate immune components such as prolixicin does not eliminate the parasite from the intestinal tract, but may control the infection to some degree

[38]. Since mounting a PO-based immune response is very costly in energetic terms [46], we may hypothesize the pros and cons of mounting a strong, but costly, immune response in the gut against *T. cruzi*. The parasite may modulate the host's immune response to prevent being eliminated, or the insect may express immune factors that reduce parasite numbers to tolerable levels.

***T. cruzi* genetics: the script of a killer partner**

Recent molecular research has uncovered high genetic variability in *T. cruzi*. Souto *et al*. [47] showed a clear distribution of parasite strains in two main lineages, classified as *T. cruzi* I and II. Recently, a new consensus established a subdivision into six discrete typing units (DTUs): *T. cruzi* I-VI [48], however, the older division of two lineages is still retained.

TC I-IV DTUs show differences in distribution and in behavior inside the vertebrate host [49] which has prompted researchers to study the pathogenesis of *T. cruzi* DTUs based on these differences [50]. At the level of parasite-vector interaction, *T. cruzi* genetic structure is linked to the capacity of different strains to develop in a triatomine species [51, 52]. One clear example of this is the ability of TCI lineage to infect and replicate in *R. prolixus*, whereas the TCII lineage is rapidly eliminated [53]. Interestingly, both lineages can establish and complete their development in *R. colombiensis* and *T. brasiliensis* [54]. In fact, mixed parasitic infections (more than one *T. cruzi* strain or lineage) in a single vector are common in nature although one strain tends to predominate [55]. This is suggestive of competition among parasite lineages, a topic that has been completely overlooked. One might expect that a situation of relatively new competition among different DTUs infecting an insect would negatively affect insect fitness, in contrast to a situation in which the

different strains have had time to coevolve with the insect and each other, coexisting at equilibrium [56].

It is believed that an ancestral DTU gave rise to all current DTUs after at least two hybridization processes [57]. These hybridization processes may have been responsible for the differing degrees of parasite virulence and pathogenicity in mammals [56]. Similar to triatomines, the coexistence of different DTUs in mammals has been documented [58, 59], but whether this has affected strain pathogenicity is not known. While it is believed that different DTUs have a different geographic distribution [60], this picture is likely to be altered by climate change, range expansions, or accidental introduction of parasites to new areas. It will be interesting to determine which DTUs could modify their range in response to changes in vector and mammal expansion, as well as their impact on vector and host fitness (for the case of *Triatoma*, see below).

Kalifornia traveling partners: the role of gut microbiota

T. cruzi adaptation to the insect gut involves an intimate interaction with the triatomine microbiota [61]. Some characteristics of the intestinal microbiota of triatomines have been described through the sequencing of genes encoding the 16S rRNA, and have shown their diversity within each host is low, with only one or a few genera that are dominant; some bacterial genera appear to be specific to certain triatomines, such as *Rhodococcus* in *Rhodnius* and *Arsenophonus* in *Triatoma*. Previous studies showed that the presence of symbiotic organisms (staphylococci, streptococci, and *Serratia sp.,* among others) in the insect can affect parasite development and growth [62]. For instance, *Serratia marcescens* has a negative effect on the development of *T. cruzi* in *R. prolixus* [63], due to the action of the prodigiosin pigment (a bacterial secondary metabolite), which inhibits the complex III

of the parasite mitochondrial function and alters other key proteins of the cell cycle generating parasite lysis [64].

As mentioned above, *T. cruzi* is capable of modifying the PO-based immune response in the insect gut [39]. This effect may explain why *T. cruzi* presence leads to an increase in microbe diversity [65] although the mechanisms and fitness consequences for both parasite and insect are unclear. The homeostasis in the intestinal environment may also be altered by the numbers and microbial composition of the GI tract, and some combinations of bacteria could block the establishment of microorganisms that could affect *T. cruzi* development [66]. Triatomines have obligate bacterial symbionts that are passed from one generation to the next through coprophagy, and these are required to obtain essential nutrients missing in a blood-based diet [62]. Since an absence of these obligate symbionts prevents the insect development, targeting this requirement could address how to control natural triatomine populations (see Rosengaus *et al*., [67] for an example with termites).

Applying genetic engineering to symbionts of vector insects is a promising approach to block the development of pathogens and prevent their transmission to human populations [68]. For example, the obligate bacterial symbiont of *R. prolixus*, *Rhodococcus rhodnii,* was altered to express cecropin A, a pore-forming immune peptide. Introducing a modified *R. rhodnii* strain into a triatomine gut reduced *T. cruzi* population with no apparent harm to insect tissues [69]. Thus, paratransgenesis can be a valuable complement to eradicate Chagas disease [70].

A killer's menu: effect of blood sources

Triatomines feed on blood from a wide variety of vertebrate animals, both wild and domestic. This flexibility allows them to explore and exploit different habitats and contributes to maintaining infection cycles in both ecotopes [71]. In domestic or peridomestic habitats, insects can use dogs, chickens, cats, and humans as a food source [72]. However, the preference for a specific resource can influence the interaction with *T. cruzi*. For instance, the prevalence of Chagas disease is reduced in regions where triatomines feed solely on bird blood [73, 74]. Calderón-Arguedas *et al*. [75] found a higher survival rate of *T. cruzi*-infected mice previously exposed to bird blood from the gut of *T. dimidiata*. These findings suggest the possibility that the ornithophilic behavior of the triatomines acts as a mechanism that could modulate parasite virulence. Note that the presence of bird blood in the vector does not inhibit the development of *T. cruzi* in the gut [75]. Bird refractoriness to the infection is due to a lytic effect mediated by the alternate pathway of the complement system [76] which may also explain mammal refractoriness in a few mammal species in the wild [77]. Thus, although avian blood is not an effective barrier for *T. cruzi* development and transmission, it may significantly decrease the prevalence of human infection [75].

In addition to the feeding capacity on a wide variety of animals, triatomines can endure prolonged periods of starvation due to their very slow digestion [9]. This food deprivation implies fitness costs for the parasite as decreases its abundance in the gut, which increases after blood ingestion [78]. The consequences for infection in humans and domestic animals of such food-related condition dependence of the parasite, has not been studied. *In vitro* studies demonstrated that a peptide present in blood, α-D-globin, promotes parasite growth and development [79] which can be interpreted as the mechanism by which

blood can drive parasite's condition. For the triatomine, however, ingesting maximum blood volumes would increase its fitness possibly to balance the negative effects of *T. cruzi* [80]. Paradoxically, infection has been correlated with a significant decrease in blood consumption during feeding [81]. This may be a triatomine strategy to eliminate *T. cruzi* as reduced blood ingestion also reduces its access to α-D-globin, which is crucial for parasite development [79]. However, there must be a balance in how much blood can be consumed as too little blood would lead to a decrease in the size of the insect, delayed molting, and/or impaired reproduction and/or survival [81]. However, studies of infection and reduced blood consumption did not simultaneously consider development, reproductive success, and survival for both actors. If the reduction in blood consumption is not convenient for the parasite, it would be in the triatomine's interest to reach optimal values in these life history parameters.

It is believed that hematophagy in triatomines appeared about 85 million years ago, with a secondary role for other food sources such as hemolymph or feces from other individuals [21]. Strikingly, recently Díaz-Albiter *et al*. [82] observed the ingestion of sugar from artificial meals and phytophagous habits in laboratory experiments with *R. prolixus* fifth-instar nymphs. This rare report led to new perspectives in the plasticity of triatomine alimentary physiology. For example, it suggests that plasticity in food requirements may have allowed bugs to remain and endure such inhospitable environments as the Atacama Desert and still become a major health problem [83].

Summer of Sam: role of temperature and altitude

Temperature is a major determinant abiotic factor in *T. cruzi*-triatomine interactions [78]. The parasite completes its development in *T. infestans* between of 23-28 °C [84], but a

higher epimastigote density in the gut is observed at 28° C [78]. A modification of laboratory temperature results in different rates of parasite development in the vector gut [85], and even for parasite viability in dead triatomines [86]. Infected vectors can modify their ambient temperature preferences [87] although it is not clear whether such preference is host- or parasite-controlled as we are largely unaware of thermal adaptations in the parasite/triatomine interaction. We know that biochemical processes (e.g. molecular transport, enzyme activity, and protein structure) and are modified as temperature varies, resulting in a change in host metabolic rate [88] and, possibly, parasite development [89].

A different perspective of how temperature drives the killing couple is a macroecological one. Triatomines demonstrate niche preferences that are closely coupled with ambient temperature. For example, infected triatomines are more likely to occur in warmer months [90]. Ambient temperature can be crucial for both actors as a lower survival rate among *T. cruzi*-infected *R. prolixus* was found at intermediate temperatures, 24 and 27 °C (from a 21-30 °C range), which presumably explains why 24-27 °C is the preferred range in this species in the wild [91]. Elliot *et al.*'s study [91] suggests that temperature may be a major natural selection driver in infected bugs. In fact, these results can shed light on the evolution of thermal preferences during infection in triatomines in general.

Triatomines differ tremendously in their altitudinal distribution: from 100 to 1800 masl [92]. Since altitude is linked to ambient temperature, it is possible that differences in thermotolerance and thermopreference among triatomine species could constrain their altitudinal distribution and also their infection probability. A study with *Triatoma dimidiata* whose altitudinal range is wide but is more heavily concentrated at 600-800 masl [93], addressed such variation with parasite development. Parasites extracted from bugs at 600

masl developed in higher numbers and led to more acute symptoms in a mammalian host compared with parasites extracted from insects at 300 and 1200 masl [40]. Interestingly, vectors from 600 masl expressed a more intense immune response when experimentally infected with the parasite than those from the other two altitudes [40]. This study may imply that local adaptation of parasite and vectors can affect the virulence of the parasite in its mammalian host.

There is also a major gap in our understanding of parasite-vector distribution and clinical cases of Chagas disease. In fact, little is known about the influence of temperature and altitude in the dynamics of Chagas's disease prevalence, even though a correlation between some clinical manifestations [94] and the distribution and infection of triatomine vectors [93, 95] have been observed. For instance, megacolon symptoms caused by *T. cruzi* infection is common in inhabitants of Andean regions in South America, living above 3000 masl [94]. Similarly, in Mexico, higher parasitemias and a greater prevalence of myocardial damage were reported in mice infected with *T. cruzi* from an altitude of 700 masl, in contrast with parasites from 300 and 1400 masl [40].

From dusk till dawn: a coevolutionary history between *T. cruzi* and triatomines?

Several sources indicate a coevolutionary process between *T. cruzi* strains and triatomine species [96]. First, a single *T. cruzi* strain may show different development rates [97-98] and reproductive costs [57] depending on the vector species, suggesting species-specific variation in fitness costs for each combination of vector and parasite. Second, the distribution of *T. cruzi* lineages is related to the triatomine species found in different geographic zones [99]. For example, the TCI lineage is distributed from North America through South America, while the TCII lineage is restricted to South America [100]. These

patterns go beyond geography as TCI is associated with domestic cycles, while TCII is associated with both domestic and peridomestic cycles [48]. Clinically, there are also differences as TCI is linked with cardiomyopathies and TCIV to digestive syndrome [101]. Third, there is a trend in varying susceptibilities to infection in each parasite-vector species combination even within the same geographic region [71]. And finally, metabolites from different triatomines seem to drive *T. cruzi* strain evolution. These metabolites can be geographically variable given different blood sources in different locations [10].

It takes two to tango: concluding remarks

Knowing the factors that determine whether a vector will become infected is a key requirement for successful Chagas control programs [102]. Sadly, we still know very little about such factors (see Outstanding Questions). The emphasis on clinical aspects of Chagas disease may have precluded the development of broader research programs that include vector-parasite behavior, physiology, ecology and evolution. The factors we have discussed in this review (Figure 3) provide some ideas of how the "killing couple" takes place, but this information is still in its infancy. In terms of biotic factors, the infected mammals that serve to infect the triatomines differ significantly in their ability to develop the infection [77]. This variation could provide different rates of transmission in the infection and re-infection cycles. Abiotic factors such as population reduction strategies with insecticides have likely affected cycles of transmission by altering basic vector physiology due to the development of insecticide resistance by the vectors. How this selection pressure has indirectly selected *T. cruzi* is an open question, and must be addressed in a holistic synergistic manner rather than the more common approach of studying each factor individually. This can be solved using a network analysis approach where a multi-factor,

interactive analysis can be done for each region as the weight of each factor can be taken into account [95].

Chagas disease, and *T. cruzi*, will not disappear without the development of modern, long lasting surveillance programs, and cheap effective drugs to treat infected people. Even with new drugs, the cycle within the triatomines and sylvatic vertebrate populations may never be broken. For this reason, Chagas disease has been called "The New HIV/AIDS of the Americas" [103] with eerie similarities between people infected with *T. cruzi* and those infected in the early stages of the HIV epidemic. Both diseases disproportionately affect people living in poverty, and both are chronic conditions requiring prolonged chemotherapeutic treatment. As one of the major neglected tropical diseases of the Americas, we must address all factors of transmission dynamics to alleviate the morbidity and mortality associated with this parasitic disease, and understanding the ecological, physiological, and immunological factors that determine how and why insect vectors transmit this parasite are paramount to these objectives.

Acknowledgements

J. Antonio De Fuentes Vicente acknowledges the scholarship and financial support provide by the Consejo Nacional de Ciencia y Tecnologia (CONACYT), Mexico. To Vences-Blanco Mauro for the comments and suggestion in the preparation of this review.

References

Molyneux, D.H. (2006) Control of human parasitic diseases: context and overview. Adv. Parasitol. 61, 1-45

Parker, E.R. and Sethi A. (2011) Chagas disease: coming to a place near you. Dermatol. Clin. 29, 53–62

World Health Organization (2014) A Global Brief on Vector-Borne Disease. WHO, Geneva

Jurberg, J. and Galvão, C. (2006) Biology, ecology and systematics of Triatominae (Heteroptera: Reduviidae) vectors of Chagas disease and implications for human health. Denisia 19, 1096-1116

Azambuja, P. and Garcia, E.S. (2005) *Trypanosoma rangeli* interactions within the vector Rhodnius prolixus: a mini review. Mem. Inst. Oswaldo Cruz 100, 567–572

Silva-Neto, M.A. *et al*. (2010) Cell signaling during *Trypanosoma cruzi* development in Triatominae. The Open Parasitology Journal 4, 188-194

Garcia, E.S. (2007) Exploring the role of insect host factors in the dynamics of *Trypanosoma cruzi–Rhodnius prolixus* interactions. J. Insect Physiol. 53, 11–21

Vallejo, G.A. *et al*. (2009) Triatominae-*Trypanosoma cruzi/T. rangeli*: vector-parasite interactions. Acta Trop. 110, 137–147

Kollien, A.H. and Schaub, G.A. (2000) The development of *Trypanosoma cruzi* in Triatominae. Parasitology Today 16, 381-387

Antunes, L.C. *et al*. (2013) Metabolic signatures of triatomine vectors of *Trypanosoma cruzi* unveiled by metabolomics. PLoS One 8, e77283

Alves, C.R. *et al*. (2007) *Trypanosoma cruzi* attachment to perimicrovillar membrane glycoproteins on *Rhodnius prolixus*. Exp. Parasitol. 116, 44-52

Gutiérrez-Cabrera, A.E. *et al*. (2014) Development and glycoprotein composition of the perimicrovillar membrane in *Triatoma (Meccus) pallidipennis* (Hemiptera: Reduviidae). Arthropod. Struct. Dev. 43, 571–578

Gutiérrez-Cabrera, A.E. *et al*. (2015) Origin, evolution and function of the hemipteran perimicrovillar membrane with emphasis on Reduviidae that transmit Chagas disease. Bull. Entomol. Res. 106: 279-291

Silva-Neto, M.A. *et al*. (2010) Cell signaling during *Trypanosoma cruzi* development in Triatominae. The Open Parasitology Journal 4, 188-194

Kollien, A.H. *et al*. (1998) Modes of association of *Trypanosoma cruzi* with the intestinal tract of the vector *Triatoma infestans*. Acta Trop. 70, 127–141

Albuquerque-Cunha, J.M. *et al*. (2004) Effect of blood components, abdominal distension, and ecdysone therapy on the ultrastructural organization of posterior midgut epithelial cells and perimicrovillar membrane in *Rhodnius prolixus*. Mem. Ins. Oswaldo Cruz 99, 815–822

Nogueira, N.F. *et al*. (1997) Effect of azadirachtin A on the fine structure of the midgut of *Rhodnius prolixus*. J. Invertebr. Pathol. 69, 58-63

Cortez, M.R. *et al*. (2012) *Trypanosoma cruzi*: effects of azadirachtin and ecdysone on the dynamic development in *Rhodnius prolixus* larvae. Exp. Parasitol. 131, 363-371

González, M.S. and García, E.S. (1992) Effect of Azadirachtin on the development of *Trypanosoma cruzi* in the insect vector: Long-term and comparative studies. J. Invertebr. Pathol. 60, 201-205

González, M.S. *et al*. (1998) Role of the head in the ultrastructural midgut organization in *Rhodnius prolixus* larvae: evidence from head transplantation experiments and ecdysone therapy. J. Ins. Physiol. 44, 553-560

Otalora-Luna, F. *et al*. (2015) Evolution of hematophagus habit in Triatominae (Heteroptera: Reduviidae). Rev. Chil. Hist. Nat. 88, 1-13

Lehane, M. (2005) The biology of blood-sucking in insects. Cambridge University Press, Cambridge

Borges, E.C. *et al*. (2006) *Trypanosoma cruzi*: effects of infection on cathepsin D activity in the midgut of *Rhodnius prolixus*. Exp. Parasitol. 112, 130-133

Buarque, D.S. *et al*. (2013) Differential Expression Profiles in the Midgut of *Triatoma infestans* Infected with *Trypanosoma cruzi*. PLoS Negl. Trop. Dis. 5, e61203

Pinto, J. *et al*. (2012) Temporal differences in blood meal detection from the midguts of *Triatoma infestans*. Rev. Inst. Med. Trop. Sao Paulo 54, 83-87

Nouvellet, P. *et al*. (2013) The Improbable Transmission of *Trypanosoma cruzi* to Human: The Missing Link in the Dynamics and Control of Chagas Disease. PLoS Negl. Trop. Dis. 7, e2505

Botto-Mahan, C. *et al*. (2006) Chagas disease parasite induces behavioural changes in the kissing bug *Mepraia spinolai*. Acta Trop. 98, 219–223

Marliére, N. *et al*. (2015) Trypanosomes Modify the Behavior of Their Insect Hosts: Effects on Locomotion and on the Expression of a Related Gene. PLoS Negl. Trop. Dis. 9, e0003973

Waniek, P.J. (2014) Pathways of Insect Protein Digestion: Triatominae (Kissing Bugs). Entomol. Ornithol. Herpetol. 3, e109

Dawkins, R. 1999. The Extended Phenotype. The Long Reach of a Gene. Oxford University Press.

Ursic-Bedoya, R.J. *et al*. (2008) Identification and characterization of two novel lysozymes from *Rhodnius prolixus*, a vector of Chagas disease. J. Insect. Physiol. 54, 593-603

López, L. *et al*. (2003) Isolation and characterization of a novel insect defensin from *Rhodnius prolixus*, a vector of Chagas disease. Insect Biochem. Mol. Biol. 33, 349-447

Waniek, P.J. *et al*. (2011) *Trypanosoma cruzi* infection modulates the expression of *Triatoma brasiliensis* defl in the midgut. Vec. Born. Zoonot. Dis. 11, 845–847

Vieira, C.S. *et al*. (2016) Impact of *Trypanosoma cruzi* on antimicrobial peptide gene expression and activity in the fat body and midgut of *Rhodnius prolixus*. Parasit. Vectors 9, doi: 10.1186/s13071-016-1398-4

Ursic-Bedoya, R. *et al*. (2011) Prolixicin: a novel antimicrobial peptide isolated from *Rhodnius prolixus* with differential activity against bacteria and *Trypanosoma cruzi*. Insect. Mol. Biol. 20, 775-786

Pereira, M.E. *et al*. (1981) Lectins of distinct specificity in *Rhodnius prolixus* interact selectively with *Trypanosoma cruzi*. Science 211, 597-600

Mello, C.B. *et al*. (1996) Differential in vitro and in vivo behavior of three strains of *Trypanosoma cruzi* in the gut and hemolymph of *Rhodnius prolixus*. Exp. Parasitol. 82, 112-121

Whitten, M. F. *et al*. (2007) Differential modulation of *Rhodnius prolixus* nitric oxide activities following challenge with *Trypanosoma rangeli*, *Trypanosoma cruzi* and bacterial cell wall components. Insect Biochem. Molec. Biol. 37, 440–452

Castro, D.P. *et al*. (2012) *Trypanosoma cruzi* immune response modulation decreases microbiota in *Rhodnius prolixus* gut and is crucial for parasite survival and development. PLoS ONE 7, e36591

De Fuentes-Vicente, J.A. *et al*. (2016) Relationships between altitude, triatomine (*Triatoma dimidiata*) immune response and virulence of *Trypanosoma cruzi*, the causal agent of Chagas' disease. Med. Vet. Entomol. doi: 10.1111/mve.12198

Ferreira R. *et al*. (2016) Colonization of *Rhodnius prolixus* gut by *Trypanosoma cruzi* involves an extensive parasite killing. Parasitology 173, 434-443

Ribeiro, J.C. *et al*. (2014) An insight into the transcriptome of the digestive tract of the bloodsucking bug, *Rhodnius prolixus*. PloS Negl. Trop. Dis. 8, e2594

Boulanger, N. *et al*. (2006) Antimicrobial peptides in the interactions between insects and flagellate parasites. Trends Parasitol. 22, doi: 10.1016/j.pt.2006.04.003

Mesquita, R.D. *et al*. (2015) Genome of *Rhodnius prolixus*, an insect vector of Chagas disease, reveals unique adaptations to hematophagy and parasite infection. Proc. Natl. Acad. Sci. U S A. 112, 14936-14941

González-Santoyo, I. and Córdoba-Aguilar A. (2012) Phenoloxidase: a key component of the insect immune system. Entomol. Exp. Appl. 142, 1-16

Siva-Jothy, M. *et al*. (2005) Insect Immunity: An Evolutionary Ecology Perspective. Adv. Insect. Physiol. 32, 1-48

Souto, R.P. *et al*. (1996) DNA markers define two phylogenetic lineages of *Trypanosoma cruzi*. Mol. Biochem. Parasit. 83, 141–153

Zingales, B. *et al*. (2009) A new consensus for *Trypanosoma cruzi* intraspecific nomenclature: second revision meeting recommends TcI to TcVI. Mem. Inst. Oswaldo Cruz 104, 1051-1054

Guhl, F. and Ramírez, J.D. (2011) *Trypanosoma cruzi* I diversity: towards the need of genetic subdivision? Acta Trop. 119, 1-4

Manoel-Caetano, F.S. and Silva, A.E. (2007) Implications of genetic variability of *Trypanosoma cruzi* for the pathogenesis of Chagas disease. Cad Saúde Pública 23, 2263–2274

Pinto, A.S. *et al*. (1998) Compared vectorial transmissibility of pure and mixed clonal genotypes of *Trypanosoma cruzi* in *Triatoma infestans*. Parasitol. Res. 84, 348-353

Campos, R. *et al*. (2007) Susceptibility of *Mepraia spinolai* and *Triatoma infestans* to different *Trypanosoma cruzi* strains from naturally infected rodent hosts. Acta Trop. 104, 25-29

Mejía-Jaramillo, A.M. *et al*. (2009) *Trypanosoma cruzi*: Biological characterization of lineages I and II supports the predominance of lineage I in Colombia. Exp. Parasitol. 121, 83-91

Roa, L.A. *et al*. (2013) Vectorial capacity of *Rhodnius colombiensis* to transmit *Trypanosoma cruzi* I y *T. cruzi* II. Rev. Aso. Col. Cienc. Biol. 25, 22-30

Campos-Soto, R. *et al*. (2016) Interactions between *Trypanosoma cruzi* the Chagas´ disease parasite and naturally infected wild *Mepraia* vectors of Chile. Vector Borne Zoonotic Dis. 16, 165-171

Dobson, A. (1985) The population dynamics of competition between parasites. Parasitology 91, 317-347

Peterson, J. *et al*. (2015) Broad patterns in domestic vector-borne *Trypanosoma cruzi* transmission dynamics: synanthropic animals and vector control. Parasit. Vectors 8, doi:10.1186/s13071-015-1146-1

Rozas, M. *et al*. (2007) Coexistence of *Trypanosoma cruzi* genotypes in wild and periodomestic mammals in Chile. Am. J. Trop. Med. Hyg. 77, 647-653

Brenière, S. *et al*. (2016) Over six thousand *Trypanosoma cruzi* strains classified into discrete typing units (DTUs): attempt at an inventory. PLoS Negl. Trop. Dis. 10, e0004792

Carrasco, H.J. *et al*. (2012) Geographical distribution of *Trypanosoma cruzi* genotypes in Venezuela. PLoS Negl. Trop. Dis. 6, e1707

Faria de Mota, F. (2012) Cultivation-independent methods reveal differences among bacterial gut microbiota in triatomine vectors of Chagas disease. PLoS Negl. Trop. Dis. 6, e1631

Gumiel, M. *et al*. (2015) Characterization of the microbiota in the guts of *Triatoma brasiliensis* and *Triatoma pseudomaculata* infected by *Trypanosoma cruzi* in natural conditions using culture independent methods. Parasit. Vectors 8, 10.1186/s13071-015-0836-z

Azambuja, P. *et al*. (2004) Isolation of *Serratia marcescens* in the midgut of *Rhodnius prolixus*: Impact on the establishment of the parasite, *Trypanosoma cruzi*, in the vector. Exp. Parasitol. 107, 89-96

Genes, C. *et al*. (2011) Mitochondrial dysfunction in *Trypanosoma cruzi*: the role of *Serratia marcescens* prodigiosin in the alternative treatment of Chagas disease. Parasit. Vectors 4, doi: 10.1186/1756-3305-4-66

Diaz, S. (2016) Triatomine bugs, their microbiota and *Trypanosoma cruzi*: asymmetric responses of bacteria to an infected blood meal. Parasit. Vectors 9, doi: 10.1186/s13071-016-1926-2

Garcia, E.S. *et al*. (2010) Interactions between intestinal compounds of triatomines and *Trypanosoma cruzi*. Trends Parasitol. 26, 499-505

Rosengaus, R.B. *et al*. (2011) Mate preference and disease risk in *Zootermopsis angusticollis* (Isoptera: Termopsidae). Physiol. Ecol. 40, 1554-1565

Taracena, M.L. *et al*. (2015) Genetically modifying the insect gut microbiota to control Chagas disease vectors through systemic RNAi. PLoS Negl. Trop. Dis. 9, e0003358

Fieck, A. *et al*. (2010) *Trypanosoma cruzi:* synergistic cytotoxicity of multiple amphipathic anti-microbial peptides to *T. cruzi* and potential bacterial hosts. Exp. Parasitol. 125, 342-347

Jose, C. *et al*. (2013) Recombinant *Arthrobacter* β-1, 3-glucanase as a potential effector molecule for paratransgenic control of Chagas disease. Parasit. Vectors 6, doi: 10.1186/1756-3305-6-65

Gorchakov, R. *et al*. (2016) *Trypanosoma cruzi* infection prevalence and bloodmeal analysis in triatomine vectors of Chagas disease from rural peridomestic locations in Texas, 2013-2014. J. Med. Entomol. 53, 911-918

Castillo-Neyra *et al*., (2015) The potential of canine sentinels for reemerging *Trypanosoma cruzi* transmission. Prev. Met. Med. 120, 349-356

Quintal, R. and Polanco G. (1977) Feeding preference of *Triatoma dimidiata* in Yucatan, Mexico. Am. J Med. Hyg. 26, 176-178

Christensen, H. *et al*. (1988) Host feeding profiles of *Triatoma dimidiata* in peridomestic habitats of Western Panamá. Am. J. Trop. Med. Hyg. 38, 477-479

Calderon-Arguedas, O. *et al*. (2003) Variaciones biológicas de *Trypanosoma cruzi* (Kinetoplastda: Trypanosomatiade) asociadas con la ingestión de diferentes tipos de sangre por el vector *Triatoma dimidiata* (Hemiptera: Reduviidae). Parasitol. Latinoam. 58, 3-10

Bern, C. *et al*. (2011) *Trypanosoma cruzi* and Chagas' Disease in the United States. Clin. Microbiol. Rev. 24, 655-681

Martínez-Hernández, F. *et al*. (2014) Follow up of natural infection with *Trypanosoma cruzi* in two mammals species, *Nasua narica* and *Procyon lotor* (Carnivora: Procyonidae): evidence of infection control? Parasit. Vectors 7, doi: 10.1186/1756-3305-7-405

Asin, S. and Catalá S. (1995) Development of *Trypanosoma cruzi* in *Triatoma infestans*: influence of temperature and blood consumption. J. Parasitol. 81, 1-7

Garcia, E.S. *et al*. (1995) Induction of *Trypanosoma cruzi* metacyclogenesis in the hematophagous insect vector by hemoglobin and peptides carrying alpha-D-globin sequences. Exp. Parasitol. 81, 255-261

Sandoval CM. *et al*. (2013) Demographic fitness of *Belminus ferroae* (Hemiptera: Triatominae) on three different hosts under laboratory conditions. Mem. Inst. Oswaldo Cruz 108, 854–864

Botto-Mahan, C. *et al*. 2006. Chagas disease parasite induces behavioural changes in the kissing bug *Mepraia spinolai*. Acta Trop. 98, 219-223

Díaz-Albiter, H. *et al*. (2016) Everybody loves sugar: first report of plant feeding in triatomines. Parasit. Vectors 9, doi: 10.1186/s13071-016-1401-0

Aufderheide, A.C. *et al*. (2004) A 9,000-year record of Chagas disease. Proc. Natl. Acad. Sci. USA. 101, 2034-2039

Neves, D.P. (1971) Influência da temperatura na evolução do *Trypanosoma cruzi* em triatomíneos. Rev. Inst. Med. Trop. 13, 155-161

Guzmán-Marín E. *et al*. (1994) La temperatura como factor de diferenciación de *Trypanosoma cruzi* en *Triatoma dimidiata*. Rev. Biomed. 33-37

Guzmán-Marín E. *et al*. (1999) Viabilididad del *Trypanosoma cruzi* en *Triatoma dimidiata* muertos. Enf. Infed. Miocrobiol. 19, 113-115

Hinestroza, G. *et al*. (2016) Behavioral fever response in *Rhodnius prolixus* (Reduviidae: Triatominae) to intracoelomic inoculation of *Trypanosoma cruzi*. Rev. Soc. Bras. Med. Trop. 49, 425-432

Abram, P. *et al*. (2016) Behavioural effects of temperature on ectothermic animals: unifying thermal physiology and behavioral plasticity. Biol. Rev. doi: 10.1111/brv.12312

Pérez-Morales, D. *et al*. (2017) Ultrastructural and physiological changes induced by different stress conditions on the human parasite *Trypanosoma cruzi*. Cell Stress Chaperones 22, 15-27

Hernandez, J.L. *et al*. (2010) Indicadores de infestación, colonización e infección de *Triatoma dimidiata* (Latreille) (Hemiptera: Reduviidae) en Campeche, México. Neo. Entomol. 39, 1024-1031

Elliot, S.L. *et al*. (2015) *Trypanosoma cruzi*, etiological agent of Chagas disease, is virulent to its triatomine vector *Rhodnius prolixus* in a temperature-dependent manner. PLoS Negl. Trop. Dis. 9, e0003646

Carcavallo, R. (1999) Climatic factors related to Chagas disease transmission. Mem. Inst. Oswaldo Cruz 94, 367/369

Benítez-Alba, J.I. *et al*. (2012) Distribución de triatominos (Heteroptera: Reduviidae) asociados a la vivienda humana y posibles zonas de riesgo en seis estados de la República Mexicana. BIOCYT 5, 227-240

Pereira-López, G *et al*. (2013) Length and caliber of the restigmoide colon among patients with Chagas disease and controls from areas at differ rent altitudes. Rev. Soc. Bras. Med. Trop. 46: 746-751

Rengifo-Correa, L. *et al*. (2017). Understanding transmissibility patterns of Chagas disease through complex vector–host networks. Parasitology 12, 1-13

García, E.S. *et al*. (1984) Molecular biology of the interaction *Trypanosoma cruzi*/invertebrate host. Mem. Inst. Oswaldo Cruz 29, 33-37

Alejandre-Aguilar, R. *et al*. (1993) Comparative study of the susceptibility of 5 triatomine species (Insecta: Reduviidae) to *Trypanosoma cruzi* infection. Rev. Latinoam. Microbiol. 35, 201-206

Magalhaes, J.B. *et al*. (1996) *Trypanosoma cruzi* strains: behavior after passage into authoctonous or foreign species of triatomine (biological and biochemical patterns). Rev. Inst. Med. Trop. São Paulo 38, 23-28

Cardinal, M.V. *et al*. (2008) Molecular epidemiology of domestic and sylvatic *Trypanosoma cruzi* infection in rural northwestern Argentina. Int. J. Parasitol. 38, 1533-1543

Noireau, F. *et al*. (2009) *Trypanosoma cruzi*: adaptations to its vectors and its host factors. Vet. Res. 40, 1–23

Ibáñez-Cervantes, G. *et al*. (2013) Identification by Q-PCR of *Trypanosoma cruzi* lineage and determination of blood meal sources in triatomine gut samples in México. Parasitol. Int. 62, 36-43

Dumonteil, E. *et al*. (2002) Geographic distribution of *Triatoma dimidiata* and transmission dynamics of *Trypanosoma cruzi* in the Yucatan peninsula of Mexico. Am. J. Trop. Med. Hyg. 67, 176-183

Hotez, P.J. *et al*. (2012) Chagas disease: "The new HIV/AIDS of the Americas". PLoS Negl. Trop. Dis. 65, e1498

Schilman, P.E. and Lazzari, C.R. (2004) Temperature preference in *Rhodnius prolixus,* effects and possible consequences. Acta Trop. 90, 115-122

Gorla, D.E. (2002) Variables ambientales registradas por sensores remotos como indicadores de la distribución geográfica de *Triatoma infestans* (Heteroptera: Reduviidae). Ecol. Austral. 12, 117-127

Tay, J. et al. (2008) Estudios del ciclo biológico de *Triatoma pallidipennis* (Stat 1872) y otros aspectos sobre su biología. Rev. Fac. Med. UNAM. 51, 56-5

Becerril, M.A. et al. (2010) Riesgo de transmisión de *Trypanosoma cruzi* en el municipio Metztitlán, Estado de Hidalgo, México, mediante la caracterización de unidades domiciliares y sus índices entomológicos. Neo. Entomol. 39, 810-817

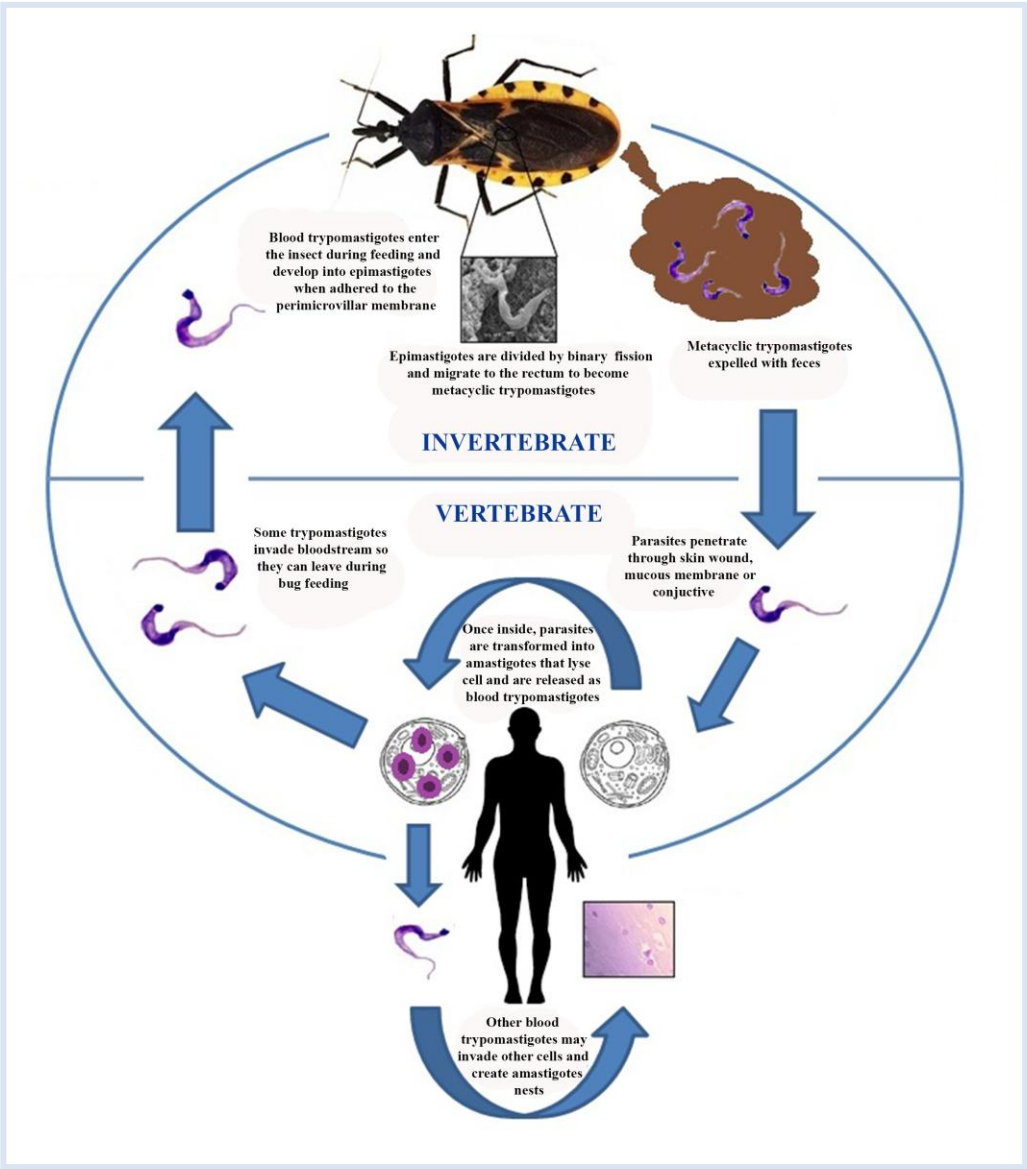

Figure 3.1. Life cycle of *Trypanosoma cruzi* that include triatomine and vertebrate hosts.

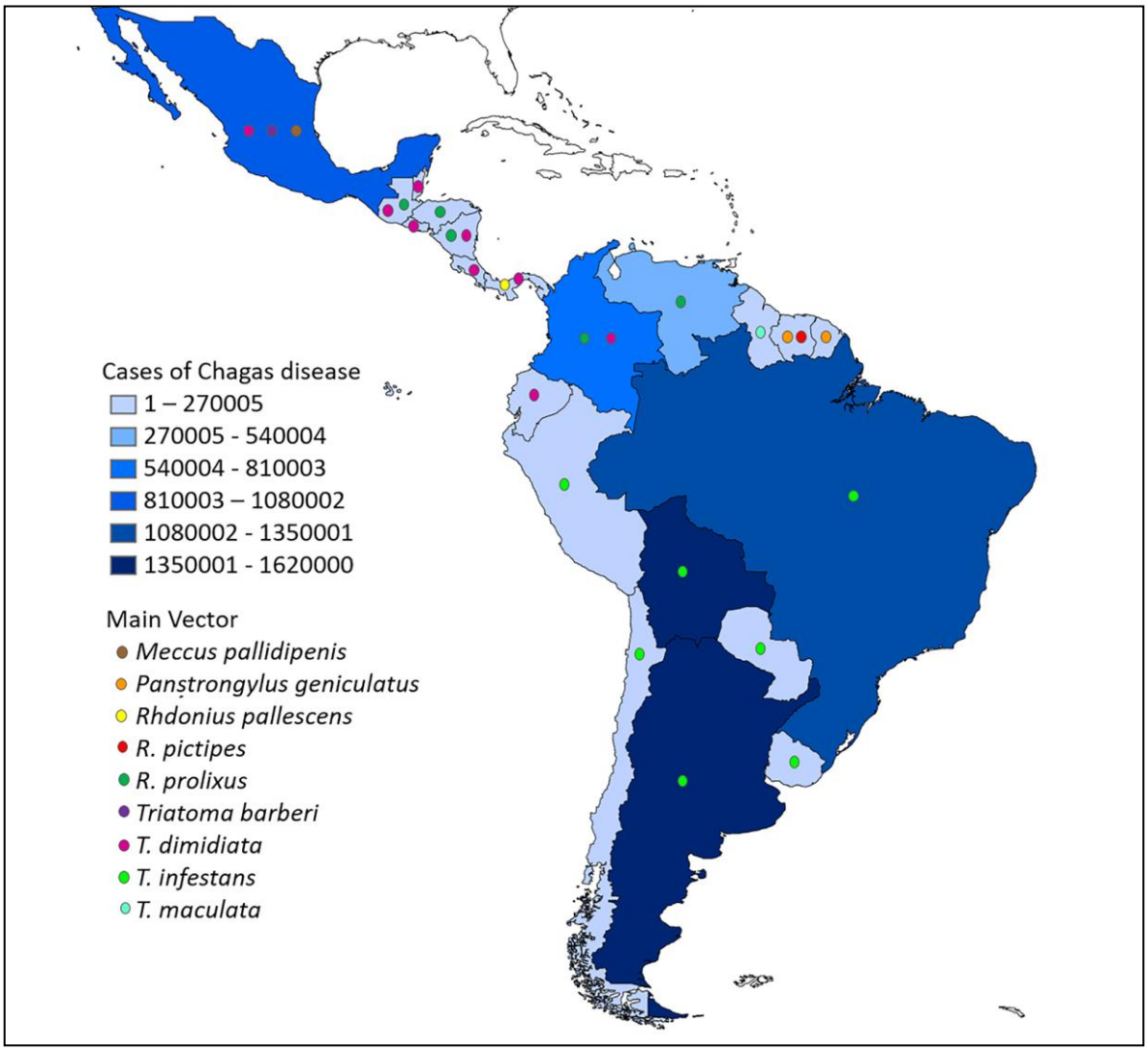

Figure 3.2 Distribution of Chagas disease and main vectors by country in Latin America

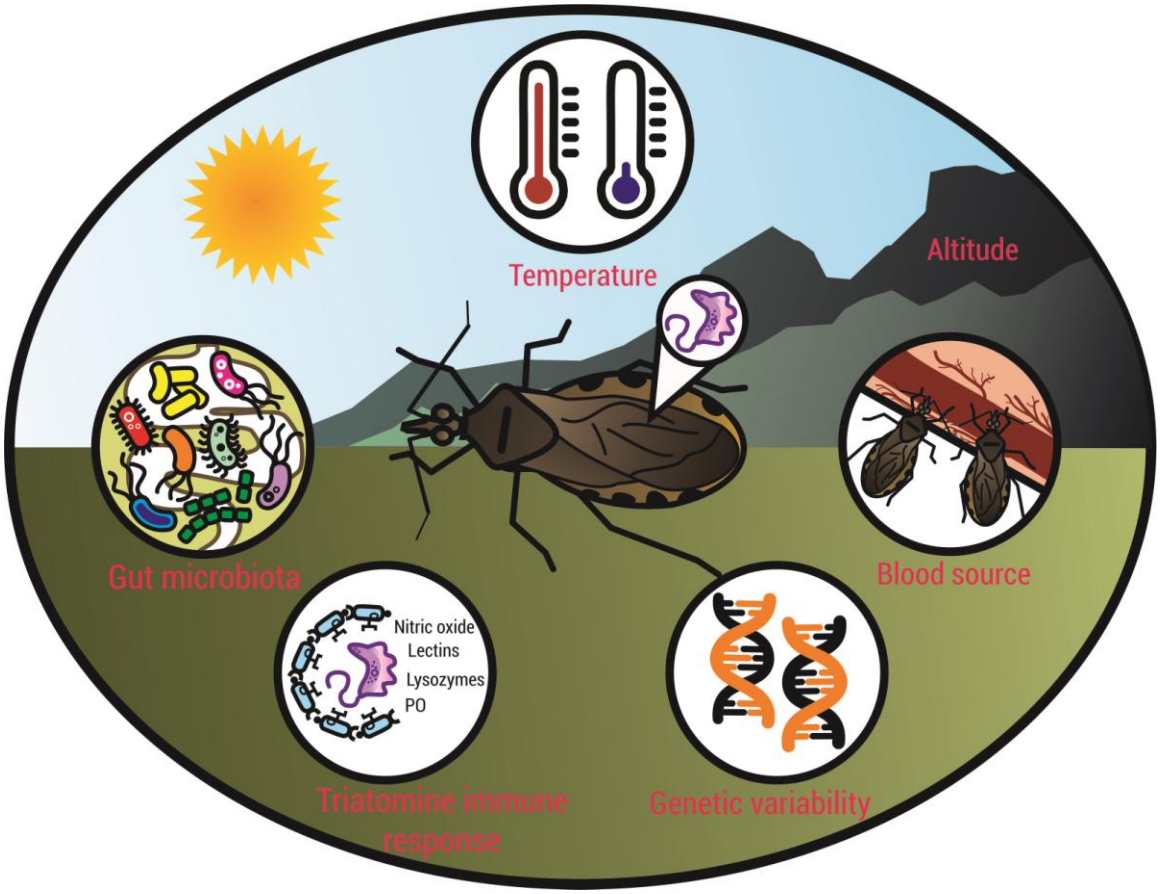

Figure 3.3 Abiotic and biotic factors underlying *T. cruzi*-triatomine interactions. Abiotic factors include temperature and altitude while biotic factors include blood sources, triatomine immune ability and gut microbiota. These factors interact with *T. cruzi* genetic variability that explain whether a triatomine can be an infective host

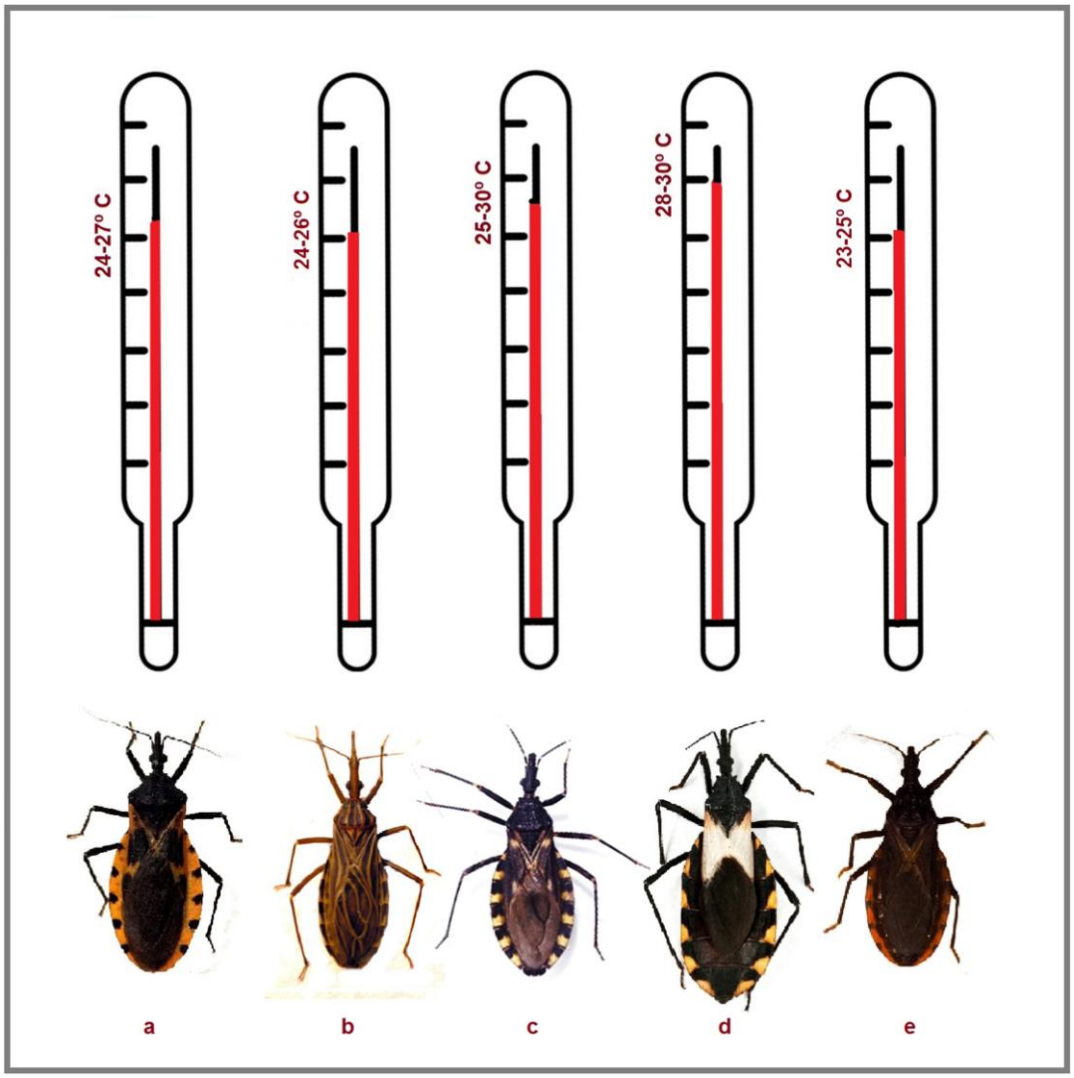

Figure 3.4 Thermal preferences (in °C) of five of the most important triatomine species in terms of Chagas infection risk. These different temperatures reflect species-specific, niche-related characteristics where triatomines perform better in terms of survival and reproduction. a) *Triatoma dimidiata* [90], b) *Rhodnius prolixus* [104], c) *T. infestans* [105], d) *Meccus pallidipennis* [106] and e) *T. barberi* [107].

Medical and Veterinary Entomology

Medical and Veterinary Entomology (2017) **31**, 63–71 doi: 10.1111/mve.12198

CAPÍTULO IV

Relationships between altitude, triatomine (*Triatoma dimidiata*) immune response and virulence of *Trypanosoma cruzi*, the causal agent of Chagas' disease

J. A. DE FUENTES-VICENTE[1], M. CABRERA-BRAVO[1], J. N. ENRÍQUEZ-VARA[2], M. I. BUCIO-TORRES[1], A. E. GUTIÉRREZ-CABRERA[3], D. G. VIDAL-LÓPEZ[4], J. A. MARTÍNEZ-IBARRA[5], P. M. SALAZAR-SCHETTINO[1] and A. CÓRDOBA-AGUILAR[2]

[1]Departamento de Microbiología y Parasitología, Universidad Nacional Autónoma de México, Mexico City, Mexico, [2]Departamento de Ecología Evolutiva, Universidad Nacional Autónoma de México, Mexico City, Mexico, [3]CONACYT-Centro de Investigación Sobre Enfermedades Infecciosas, Instituto Nacional de Salud Pública, Cuernavaca, Morelos, Mexico, [4]Laboratorio Experimental y Bioterio, Instituto de Ciencias Biológicas, Universidad de Ciencias y Artes de Chiapas, Mapastepec, Mexico and [5]Departamento de Desarrollo Regional, Centro Universitario del Sur, Universidad de Guadalajara, Guadalajara, Mexico

Abstract. Little is known about how the virulence of a human pathogen varies in the environment it shares with its vector. This study focused on whether the virulence of *Trypanosoma cruzi* (Trypanosomatida: Trypanosomatidae), the causal agent of Chagas' disease, is related to altitude. Accordingly, *Triatoma dimidiata* (Hemiptera: Reduviidae) specimens were collected at three different altitudes (300, 700 and 1400 m a.s.l.) in Chiapas, Mexico. The parasite was then isolated to infect uninfected *T. dimidiata* from the same altitudes, as well as female CD-1 mice. The response variables were phenoloxidase (PO) activity, a key insect immune response, parasitaemia in mice, and amastigote numbers in the heart, oesophagus, gastrocnemius and brain of the rodents. The highest levels of PO activity, parasitaemia and amastigotes were found for *Tryp. cruzi* isolates sourced from 700 m a.s.l., particularly in the mouse brain. A polymerase chain reaction-based analysis indicated that all *Tryp. cruzi* isolates belonged to a *Tryp. cruzi* I lineage. Thus, *Tryp. cruzi* from 700 m a.s.l. may be more dangerous than sources at other altitudes. At this altitude, *T. dimidiata* is more common, apparently because the conditions are more beneficial to its development. Control strategies should focus activity at altitudes around 700 m a.s.l., at least in relation to the region of the present study sites.

Key words. *Triatoma dimidiata*, *Trypanosoma cruzi*, altitude, amastigotes, Chagas' disease, parasitaemia, phenoloxidase.

I

Correspondence: Paz M. Salazar-Schettino, Departamento de Microbiología y Parasitología, Facultad de Medicina, Universidad Nacional Autónoma de México, 04510 Mexico City, Mexico. Tel.: +52 55 5623 2381; Fax: +52 55 5623 2382, E-mail: pazmar@unam.mx; Alex Córdoba-Aguilar, Departamento de Ecología Evolutiva, Instituto de Ecología, Universidad Nacional Autónoma de México, 04510 Mexico City, Mexico. Tel.: +52 55 5622 9003; Fax: +52 55 5622 8995; E-mail: acordoba@iecologia.unam.mx

Introduction

Parasitic diseases transmitted by arthropods represent significant causes of death around the world (Buarque *et al.*, 2013), and include dengue, malaria, leishmaniasis and Chagas' disease [World Health Organization (WHO), 2014]. The last of these is categorized as an acute public health problem in Latin America as a result of its broad distribution and high prevalence, with an estimated nine million people infected (Schofield *et al.*, 2006; Noireau *et al.*, 2009).

Chagas' disease is caused by the protozoan *Trypanosoma cruzi* (Parker & Sethi, 2011), a parasite that circulates in the blood of over 150 species of mammal and is transmitted by haematophagous hemipterans of the subfamily Triatominae (Hemiptera: Reduviidae), commonly known as assassin bugs or kissing bugs (Noireau *et al.*, 2009). Since its first parasitological, clinical and epidemiological descriptions, the occurrence of Chagas' disease has been related to environmental factors that include temperature, precipitation, vegetation, humidity, latitude and altitude (Cruz-Reyes & Pickering-López, 2006; Mischler *et al.*, 2012). In fact, some studies have tried to find links between these factors and the biology of triatomine vectors (Rocha *et al.*, 2001; Heger *et al.*, 2006; Martínez-Ibarra *et al.*, 2008; Mendes-Pereira *et al.*, 2013). For example, it has been reported that differences in altitude cause variations in the distribution of triatomines (Noireau *et al.*, 2005), in the colonization and infection of the insect vectors (Ramsey *et al.*, 2000), and in some clinical manifestations of Chagas' disease (Pereira-Lopes *et al.*, 2013). Of course, these abiotic factors are unlikely to be the sole factors explaining triatomine biology and distribution as they may interact with biotic factors. It is known that *Tryp. cruzi* may negatively affect the fitness of triatomines through changes in fecundity (Fellet *et al.*, 2014), development and survival (Schaub, 1989; reviewed by Flores-Villegas *et al.*, 2015). It is highly likely that such negative effects are also modulated by abiotic variables. For example, *Tryp. cruzi* development within the triatomine *Triatoma infestans* became faster as the ambient temperatures experienced by the insect increased (Asin & Catalá, 1995). By contrast, the parasite induced higher mortality in the triatomine *Rhodnius prolixus* (Hemiptera: Reduviidae) at ambient temperatures of 24–27 °C in comparison with more extreme temperatures of 21 and 30 °C (Elliot *et al.*, 2015). Because triatomines are ectotherms, they can be expected to demonstrate a close temperature-dependent physiological response and thus it can be hypothesized that, in field conditions, interactions between abiotic and biotic factors will influence the effects of *Tryp. cruzi* infection in triatomines and, ultimately, hosts.

Given the relationships of abiotic and biotic factors with both *Tryp. cruzi* development and infection patterns, and triatomine distribution, one very general hypothesis is that varying geographic factors associated with *Tryp. cruzi* distribution may drive triatomine immune responses and infectivity in mammal hosts. An exploration of these possible relationships may deepen understanding of the occurrence of Chagas' disease. For example, if such relationships were identified, further studies might elucidate why infectivity is dependent on specific abiotic factors and clarify the associated risk to human populations. Additionally, these insights would help to explain the ecological context of the disease, which is a subject that has received little attention (Abad-Franch *et al.*, 2009; Telleria & Tibayrenc, 2010; Nouvellet *et al.*, 2015).

The insect immune system consists of cellular and humoral components. Whereas the cellular component is carried out by haemocytes (phagocytes, encapsulation and nodulation), in the humoral component different factors are secreted in the circulatory system (Eleftherianos & Revenis, 2011). Factors of particular importance include reactive oxygen species (ROS), reactive nitrogen species (RNS), antimicrobial peptides and the phenoloxidase (PO) cascade. This last response is initiated by the recognition of foreign elements and the activation of the PO enzyme, which converts phenols to quinones. By a non-enzymatic pathway, quinones continue their transformation until they become melanin, which can encapsulate foreign agents (González-Santoyo & Córdoba-Aguilar, 2012). However, the PO pathway can also give rise to other key immunological mechanisms (González-Santoyo & Córdoba-Aguilar, 2012). For this reason, PO has been suggested as a general indicator of immunocompetence (Mucklow *et al.*, 2004; González-Santoyo & Córdoba-Aguilar, 2012).

The present study analyses the relationships among an altitudinal gradient (as a broad-scale indicator of geographic variation) in a Mexican community (Chiapas State), the immune response of *T. dimidiata* measured as the activity of PO, and the virulence of *Tryp. cruzi* in a mammal host. For this purpose, uninfected triatomines were collected at different altitudes and infected with *Tryp. cruzi* from corresponding altitudes. Health services personnel from Chiapas State had previously indicated that at these sites *T. dimidiata* is present and cases of Chagas' infection exist. These personnel had also provided verbal (e.g. non-measured) indications that suggested altitudinal variation in *Tryp. cruzi* infectivity. Assuming that this altitudinal variation represented one explaining factor, three steps were carried out. Firstly, PO activity was measured in the triatomines to determine whether there were immunological differences according to altitude that might also explain *Tryp. cruzi* infectivity. Secondly, the virulence of the parasite was investigated in experimentally infected mice to compare the number of resulting parasites and the number of amastigotes lodged in different mouse organs according to the altitude of *Tryp. cruzi* origin. Thirdly, the issue of whether *Tryp. cruzi* isolates from different altitudes correspond to distinct lineages was investigated as, if this were the case, the effects of *Tryp. cruzi* might also differ (reviewed by Manoel-Caetano & Silva, 2007). These different steps were then merged to support a discussion of the relevance of altitude and associated factors in the triatomine immunity elicited and *Tryp. cruzi* infectivity.

Materials and methods

Insects

Triatoma dimidiata adults were collected from three sites at distinct altitudes in Chiapas, Mexico: (a) at the town of Benito Juárez in the municipality of Berriozábal (300 m a.s.l.; 16°58′00″ N, 93°20′30″ W); (b) at El Paraíso in the municipality of Copainalá (700 m a.s.l.; 17°53′33″ N, 93°00′21″ W), and

(c) at Independencia in the municipality of San Juan Cancuc (1400 m a.s.l.; 17°08′24″ N, 92°22′15″ W) (Figure S1). Local health services personnel had already provided information on the presence of both *T. dimidiata* and Chagas' disease infection. Mean temperature and relative humidity (RH) at these sites are, respectively: 30 ± 2 °C and 50% at Benito Juárez; 26 ± 1 °C and 70% at El Paraíso, and 18 ± 2 °C and 85% at Independencia. After 1 day of collection, all samples were taken to the Laboratory for Parasite Biology on the Mexico City campus of the Universidad Nacional Autónoma de México, for analysis. Parasite presence was determined through the analysis of fresh faeces of *T. dimidiata* using optical microscopy (magnification ×40). Samples of faeces were obtained by exerting pressure on the insect abdomen at 24 h after capture, which represented the amount of time required to transport the collections to the laboratory. After this analysis, the insects were maintained alive by providing access to mouse blood.

Trypanosoma cruzi *isolates*

Trypanosoma cruzi parasites were isolated from the rectal blister caused in female CD-1 mice by naturally infected triatomines. Mice were supplied by the vivarium of the Faculty of Medicine of the Universidad Nacional Autónoma de México. Faeces were gathered from three infected triatomines collected at a given altitude, and a pool of these faeces was mixed with phosphate-buffered saline (PBS) at pH 7.2. Each parasite isolate was denominated according to the altitude of the region from which it was sourced (TC300, TC700 and TC1400) and inoculated intraperitoneally in five mice (Instituto Nacional de Salud, 2005).

Assays of PO activity in T. dimidiata *infected with* Tryp. cruzi

Fifteen uninfected insects from each altitude (a total of 45) were infected with the *Tryp. cruzi* isolate from the same altitude; these groups represented the experimental groups. For this, previously infected mice were placed in immobilizers and offered to the triatomines for feeding until satiation. After 5 days, the insect haemolymph was extracted with a 1-mL syringe by puncturing the membrane region that separates the coxa and trochanter (Espinosa-de-Aquino, 2012). The haemolymph was immediately collected in Eppendorf tubes and mixed with PBS of NaCl (pH 7.2) at a ratio of 1 : 2. During extraction, haemolymph was maintained on dry ice. As controls, 15 uninfected insects from each altitude were handled and maintained in the same way as the experimental insects except that these were not infected. Control groups were confirmed to be uninfected by examining their fresh faeces for *Tryp. cruzi* using optical microscopy (×40); only those with faeces from which *Tryp. cruzi* was absent were used.

Quantification of proteins

As recommended for sample standardization (Moreno-García *et al.*, 2013), proteins were quantified using the commercial Pierce BCA Protein Assay Kit (Thermo Fisher Scientific, Inc., Rockford, IL, U.S.A.). In a 96-well plate (Costar 96; Sigma-Aldrich Corp., St Louis, MO, U.S.A.), 10 µL of haemolymph (from each altitude and from infected and uninfected triatomines) and 40 µL of PBS were placed in each well, with each isolate assayed in duplicate. To each well, 150 µL of the reactive provided by the kit was added and then incubated at 37 °C for 30 min with an aluminium cover. Afterwards, plate absorbance was measured using an enzyme-linked immunosorbent assay (ELISA) reader (ELX 800; BioTek Instruments, Inc, Winooski, VT, U.S.A.) at 562 nm. Using the absorbance data from the samples and a standard concentration curve constructed by utilizing albumin, the total protein concentration in the sample was calculated using regression analyses (Moreno-García *et al.*, 2013). Thirty micrograms of protein were used per sample.

Phenoloxidase activity

For each of the groups representing the three altitudes ($n = 15$ for each), PO activity was measured spectrophotometrically by adding 100 µL of L-DOPA substrate (dihydroxyphenylalanine, 4 mg/mL; Sigma-Aldrich Corp.) to 30 µg of protein from each sample in a 96-well plate. The plate was covered and incubated at 37 °C for 20 min. Afterwards, readings were taken every 15 min for 2 h with an ELISA reader at 492 nm. As a blank, 50 µL of PBS and 50 µL of L-DOPA were used. To show PO activity, the absorbance data [= average rate of change; for example, Cornet *et al.* (2009)] during the 2 h of the assay were used to construct the slope of the curve for each group. The experiment was carried out in duplicate to give a total of 30 samples for each altitude.

Trypanosoma cruzi *virulence*

The virulence of each *Tryp. cruzi* altitudinal isolate was measured using infected female CD-1 mice (weighing 18–20 g). At 20 days post-infection, a count was made in a Neubauer chamber of the parasites per mL of peripheral blood of mice used to isolate the parasite. Once this estimate was obtained, 15 mice were infected per isolate with 1×10^6 of blood trypomastigotes through intraperitoneal inoculations. Response variables, established by Barreto (1964) and the WHO (1986), were registered during a period of 37 days after infection (with only one replica). During the prepatent period, a daily check was made by direct microscopic examination (at magnification ×4) of the peripheral blood of mice. For this, samples were obtained by making a small cut in the distal part of the mouse tail. During the period of parasitaemia, parasites per mL (par/mL) were quantified by counting them in a Neubauer chamber using 10 µL of peripheral blood at a dilution of 1 : 10 with sodium citrate (3.8%). To evaluate mortality, daily records were kept until the mice were killed at 37 days. To determine cellular tropism, tissues from the heart, brain, gastrocnemius and oesophagus of mice were assessed. For this purpose, the organs were fixed in 10% formaldehyde until use. Afterwards, 10 slices (each 10 µm thick) per organ were obtained and dyed with haematoxylin and eosin (H&E) stain. For each slice, 100 fields were viewed under microscopy (×100).

Typing of Tryp. cruzi *isolates*

To determine whether the lineage of the *Tryp. cruzi* isolates from the three altitudes were different or similar, part of the intergenic region of *Tryp. cruzi* mini-exon genes was used (Souto *et al.*, 1996). For this, DNA of the isolates was extracted using a modified protocol phenol-chloroform isoamyl alcohol (Espinoza & García, 2003). A pool of three primers (IDT) [5′-GTGTCCGCCACCTCCTTCGGGCC (TCI, group 1-specific), 5′-CCTGCAGGCACACGTGTGTGTG (TCII, group 2-specific), and 5′-CCCCCCTCCCAGGCCAC ACTG (TC, common to groups 1 and 2)] was used for amplification of the mini-exon genes. Amplification reactions were performed in a final volume of 25 µL containing 12 µL of Go Taq Green Master Mix 2X, 10 µL of nuclease-free water, 0.4 µM of each primer, and 20 ng of *Trypanosoma* DNA. Cycle amplification was performed using a MyGene MG96G thermal cycler (Hangzhou LongGene Scientific Instruments Co. Ltd, Hangzhou, China) under the following conditions: 5 min at 94 °C, followed by 27 cycles of 40 s at 94 °C, 40 s at 61 °C, and 1 min at 72 °C, and a final elongation of 5 min at 72 °C. Polymerase chain reaction (PCR) products (300 bp for TCII and 350 bp for TCI) were analysed in a 2% agarose gel.

Statistical analysis

Differences in PO activity between the experimental and control groups for each altitude were analysed using two-way analysis of variance (ANOVA). Data for PO activity were previously transformed using the Box–Cox procedure. This transformation indicated that the normality (Shapiro–Wilk test, $W = 0.982$, $P = 0.089$), as well as the homogeneity of variance (Fligner–Killeen test, $\chi^2 = 10.177$, d.f. = 5, $P = 0.0703$) of the PO data complied with the assumptions for parametric tests. For ANOVA, the predictor variables were altitude (300, 700 and 1400 m a.s.l.), group (experimental and control groups), and the interaction between these two variables. Post hoc Tukey tests were then used to determine significant differences between group combinations. The virulence of the parasite was analysed using an ANOVA with altitude as the predictor variable and the number of parasites on day 29 as the response variable. Given that the three groups differed in peak, the best logistical solution was to use the nearest day to the peak for the three groups, which was day 29. Previous to analysis, data were normalized using a cubic root transformation (Shapiro–Wilk test, $W = 0.975$, $P = 0.09$) and homogeneity of variance (Fligner–Killeen test, $\chi^2 = 3.933$, d.f. = 2, $P = 0.139$) assumptions. As there were very few amastigotes in the brain, comparisons between amastigote levels for the three altitudes were made for the three remaining organs (heart, oesophagus and gastrocnemius) using a two-way ANOVA. Amastigote data were normalized using a square root transformation to fulfil parametric test assumptions (Shapiro–Wilk test, $W = 0.988$, $P = 0.348$) and homogeneity of variance (Fligner–Killeen test, $\chi^2 = 9.953$, d.f. = 8, $P = 0.268$). For this analysis, organ and isolate were entered as predictor variables, and amastigotes as the response variable. Tukey's test was used to determine which groups showed significant differences. All analyses were carried out using R Version 3.2.3 (R Core Team, 2015).

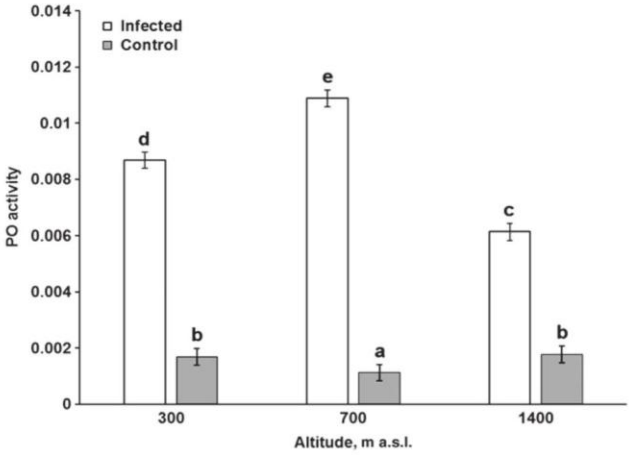

Fig. 1. Changes in phenoloxidase (PO) activity in relation to the altitude of *Triatoma dimidiata* collection and the different experiment and control groups. Values are expressed as the mean ± standard error. Distinct letters indicate significant differences (Tukey's test, $P < 0.05$).

Results

Phenoloxidase activity in T. dimidiata *in relation to altitude and group*

Significant differences were found in PO activity as a function of altitude ($F_{2,129} = 12.316$, $P = 0.001$), experimental group ($F_{1,129} = 1813.839$, $P = 0.001$), and the interaction of these two variables ($F_{2,129} = 64.838$, $P = 0.001$). The post hoc comparisons and visual inspection of the values of these combinations indicated that for each of the three altitudes, infected groups had higher values for PO activity than their corresponding control groups (Fig. 1). Within the experimental groups, the highest values for PO activity were those for 700 m a.s.l., followed by those for 300 m a.s.l. and finally those for 1400 m a.s.l. (Fig. 1). Within the control groups, the lowest values for this parameter were those for 300 m a.s.l., and there was no difference between the groups representing the other two altitudes (Fig. 1).

Virulence of Tryp. cruzi

The periodic observations of the peripheral blood of experimental animals allowed the determination of the prepatent periods of the three isolates, which oscillated between 13 and 15 days. Based on circulating trypanosomes, there was a continuous increase in parasitaemia for each of the three isolates. With an inoculum of 1×10^6 parasites, the isolate sourced from 700 m a.s.l. showed a peak of 22.57×10^6 par/mL at 33 days post-inoculation (p. i.). Isolate TC300 reached a peak of 15.66×10^6 par/mL at day 29 p. i., whereas isolate TC1400 reached a peak of 11.17×10^6 par/mL at day 27 p. i. (Fig. 2). The analysis at day 29 p. i. indicated significant differences

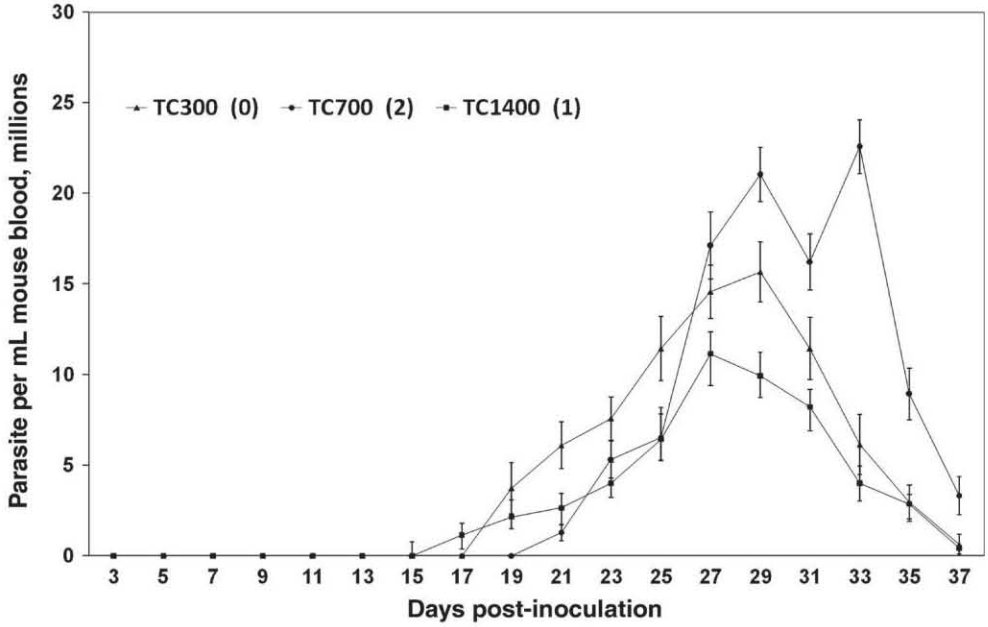

Fig. 2. Time curves of parasitaemia in female CD-1 mice infected with three isolates of *Trypanosoma cruzi* sourced from different altitudes (TC300, 300 m a.s.l.; TC700, 700 m a.s.l.; TC1400, 1400 m a.s.l.). Values are expressed as the mean ± standard error. Numbers in parenthesis indicate the quantity of mice per group that died during the experiment.

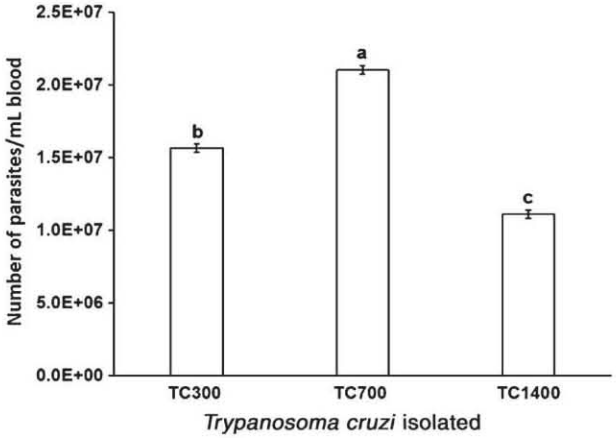

Fig. 3. Parasitaemia in female CD-1 mice inoculated with *Trypanosoma cruzi* from different isolates sourced from different altitudes (TC300, 300 m a.s.l.; TC700, 700 m a.s.l.; TC1400, 1400 m a.s.l.). Values are expressed as the mean ± standard error. Distinct letters indicate significant differences (Tukey's test, $P < 0.05$).

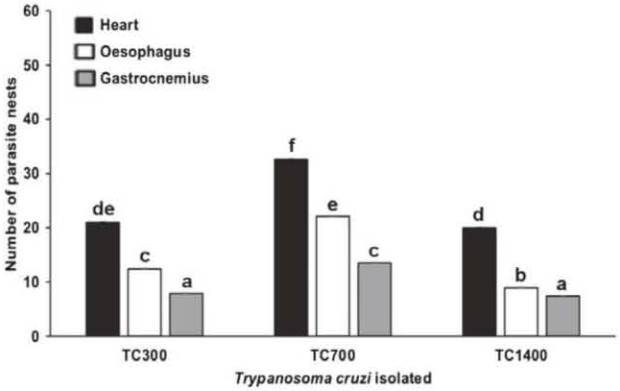

Fig. 4. Numbers of *Trypanosoma cruzi* amastigotes in mouse organs by organ and altitude of source of isolate (TC300, 300 m a.s.l.; TC700, 700 m a.s.l.; TC1400, 1400 m a.s.l.). Values are expressed as the mean. Standard errors are not apparent because the values were extremely low. Distinct letters indicate significant differences (Tukey's test, $P < 0.05$).

between groups ($F_{2,85} = 264.61$, $P < 0.0001$), whereby isolate TC700 showed the greatest level of parasitaemia, followed by isolate TC300 and finally isolate TC1400 (Fig. 3). The mortality recorded during the 37 days of the experiment was low: in the 90 mice infected, only three deaths were observed during the entire experiment, including two from isolate TC700 and one from isolate TC1400.

Nests of amastigotes were found through dissection of the four different organs tested. (Examples from TC700 are shown in Figure S2) It is noteworthy that only isolate TC700 was able to invade the brain (in four insects). Indeed, this isolate produced the greatest number of amastigotes ($F_{2,126} = 897.56$, $P = 0.001$) (Fig. 4). Additionally, all three isolates resulted in cellular tropism with greater frequency in the cardiac muscle than in the other organs (Fig. 4). Nests of amastigotes were observed to a lesser degree in the oesophagus, followed by the gastrocnemius and brain ($F_{2,126} = 1640.94$, $P = 0.001$). The interaction between isolates (by altitude) and organs was significant ($F_{4,126} = 24.24$, $P = 0.001$). The interaction indicated that, for each organ, the level of amastigotes was highest for the isolate sourced from 700 m a.s.l. (Fig. 4).

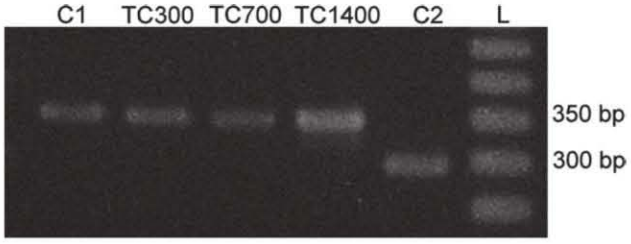

Fig. 5. Amplification of polymerase chain reaction products from the analysis of mini-exon genes according to the altitude at which *Trypanosoma cruzi* isolates were sourced (TC300, 300 m a.s.l.; TC700, 700 m a.s.l.; TC1400, 1400 m a.s.l.). C1 indicates TCI lineage control (Qro strain); C2 indicates TCII lineage control (Y strain). L indicates the 50-bp ladder. Note that both control strains have been typified previously (Espinoza *et al.*, 2010).

Lineage of Tryp. cruzi *isolates*

Amplification of the mini-exon genes with the three isolates of *Tryp. cruzi* (TC300, TC700 and TC1400) resulted in a PCR product of 350 bp (Fig. 5). This confirms that the three populations belong to the same TCI lineage.

Discussion

The overall results demonstrate that the highest values for triatomine PO activity, and for the number of parasites and organs invaded, were found with *Tryp. cruzi* from 700 m a.s.l. In addition, the isolates from the three altitudes indicated that all *Tryp. cruzi* populations belong to the same strain I. The activation of PO in triatomines infected with *Tryp. cruzi* is not a novel event (e.g. Mello *et al.*, 1995; Ursic-Bedoya & Lowenberg, 2007; Castro *et al.*, 2012; Espinosa-de-Aquino, 2012). For example, PO activity increases after infection of the triatomine *R. prolixus* with *Tryp. cruzi* (Castro *et al.*, 2012) in a similar way to that found in the present study. It is not clear whether PO acts directly against the parasite or is part of a systemic response of *R. prolixus* faced with an invasion. In fact, rather than the increase in PO leading to a reduction of the parasite in the insect, the exact opposite occurs (Castro *et al.*, 2012). It has even been proposed that both *Tryp. cruzi* and the related parasite *Trypanosoma rangeli* are capable of dealing successfully with an increase in PO levels (Flores-Villegas *et al.*, 2015). If this is true, then it is likely that the insect does indeed detect the presence of the parasite, but is incapable of eliminating it. Another possibility is that the rise in PO is a systemic effect that facilitates other immune pathways to eliminate the parasite. For example, the activation of PO leads to the activation of highly toxic compounds (e.g. 5,6-dihydroxyndole) against viruses and parasitoid wasps (Zhao *et al.*, 2011).

It is still unclear whether the fitness cost of *Tryp. cruzi* infection to triatomines (Fellet *et al.*, 2014; Flores-Villegas *et al.*, 2015) is related to an efficient insect immune reaction against the parasite. Infection by the parasite is known to lead to the production of other immune components such as defensin *Def1*, which can reduce but not eliminate the parasite (Araujo *et al.*, 2006). Perhaps the best evidence of an efficient action against the parasite is that of nitric oxide and superoxide (Whitten *et al.*, 2001). However, it is difficult to establish a link between the production of PO and that of nitric oxide or superoxide because they are involved in distinct cascades (Rivero, 2006; González-Santoyo & Córdoba-Aguilar, 2012). Studies related to any defensive effect against *Tryp. cruzi* by triatomines are sorely needed.

One novel aspect of the present study with regard to PO and the parasite is the environmental context (limited to the altitudinal gradient). Phenoloxidase activity was highest in insects collected at 700 m a.s.l. In this zone in the present study, the average temperature is 26 ± 1 °C and RH is 70% (Instituto Nacional de Estadística y Geografía, 2016). These ranges are appropriate for the optimum physiological development of *T. dimidiata* (Reyes & Angulo, 2009; Reyes-Novelo *et al.*, 2011). Such appropriateness can be linked to the effects of stressful conditions on the immune response in these animals. The immune response is known to be energetically costly to produce and hence its maintenance must be balanced with regard to other costly functions (Sadd & Schmid-Hempel, 2009). Environments that are less favourable may cause an energetic imbalance that negatively affects the immune response (e.g. Zhivotovsky *et al.*, 1996). For example, suboptimal environments can lead to immune down-regulation as a result of a lack of resources for the host (e.g. Bowden, 2008). One such example refers to a cold season or environment, which, together with low availability of food, can negatively affect immune response and survival (e.g. Demas & Nelson, 1998). For *T. dimidiata*, it is possible that the environment at 700 m a.s.l. is better for achieving a more robust immune response than that at 1400 m a.s.l. This may refer to local adaptation, given that *T. dimidiata* at the altitude of 700 m a.s.l. may be able to strengthen its immune capacity more than *T. dimidiata* at other altitudes (e.g. Karl *et al.*, 2010). A second explanation is that, at 700 m a.s.l., the supply of pathogens presents a greater challenge to which *T. dimidiata* responds more intensely. This supposes that at other altitudes the pressure exerted by pathogens is less intense [for a similar idea, see Moller & Rózsa (2005)]. A third explanation is that *Tryp. cruzi* at 700 m a.s.l. promotes a more intense immune response as a form of manipulation of the host (Damian, 1997). Each of these explanations warrants further investigation. Furthermore, although the only 'controlled' variable in the present study was altitude, there are certainly other abiotic and biotic factors involved, some of the most important of which, based on other insect studies (Schmid-Hempel, 2005), may be temperature and food.

With regard to virulence and infectivity, the isolate from 700 m a.s.l. showed different patterns in comparison with the other two isolates, although all *Tryp. cruzi* collections from the different locations were of the same strain (TCI). However, judging by other studies (Higo *et al.*, 2004), it would not be surprising to find genetic variation in geographical regions that are relatively close to one another, as were the present study sites. In fact, even the same *Tryp. cruzi* strain can give rise to different symptoms as a result of large genetic differences (e.g. Del Puerto *et al.*, 2010). Thus, it is likely that genetic differences may underlie the differences in isolates sampled from the different altitudes, as evidenced by: (a) different organ tropism, with TC300 more likely to invade brain tissues in comparison with isolates sourced

from the other altitudes; (b) a prepatent period of 13–15 days unlike the 8–12 days reported for the H4 and H5 strains of *Tryp. cruzi* in Yucatán (Barrera-Pérez *et al.*, 2001), a state near Chiapas, and, to a lesser extent, (c) mortality, whereby the TC700 isolate led to two of the three mouse deaths.

By contrast, the level of parasitaemia has been related to the invasion of *Tryp. cruzi* in tissues (Tay *et al.*, 1973; Salazar-Schettino *et al.*, 1978; Sánchez-Guillén *et al.*, 2006). This relationship is in accordance with the present results, given that the isolate that showed the greatest parasitaemia (that from 700 m a.s.l.) also demonstrated the greatest amount of amastigote nests in the organs tested. The preference for amastigote nests in cardiac muscle among the three isolates coincides with the majority of strains evaluated in Mexico (Marie-France *et al.*, 2002), which have been related to the lineage *Tryp. cruzi* I, which predominates mainly in the central and northern parts of the American continent (Noireau *et al.*, 2009). Indeed, this variability has hindered efforts to establish adequate parameters for classification and taxonomy, which are key to effective parasite study and control (Guzmán-Marín *et al.*, 1999).

In conclusion, the present study sheds light on the role of one abiotic factor (i.e. altitude) related to the origin of *Tryp. cruzi* in the pathogen–host interaction under natural conditions. Understanding of which factors are masked by such altitudinal differences should certainly support the development of strategies for the prevention of Chagas' disease. In fact, studies on the collection and prediction of niche ecology in the state of Chiapas indicate greater densities of *T. dimidiata* in zones at 800 m a.s.l. (Benítez-Alba *et al.*, 2012). Given that the present results show the isolate from 700 m a.s.l. to be the most infective, altitudes of 700 and 800 m a.s.l. seem to represent a greater risk to corresponding human populations. However, the most common cases of seropositivity in Chiapas come from altitudes of around 1340 or < 300 m a.s.l. (Mazariego-Arana *et al.*, 2001). This suggests the existence of other factors that may explain why there are not necessarily more cases of infection with *Tryp. cruzi* at 700 m a.s.l., although the virulence of this parasite is greater at that altitude.

Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: DOI: 10.1111/mve.12198

Figure S1. Locations of collection of three different groups of *Triatoma dimidiata* and isolates of *Trypanosoma cruzi*.

Figure S2. Amastigote nests in the (A) heart, (B) oesophagus, (C) gastrocnemius and (D) brain of female CD-1 mice inoculated with *Trypanosoma cruzi* (isolate TC700). Original magnification ×100.

Acknowledgements

JAdF-V received a CONACyT doctoral grant and logistical support from the Posgrado en Ciencias Biológicas, Universidad Nacional Autónoma de México (UNAM). This study is a partial requirement for the doctoral work of JAdF-V in the Posgrado en Ciencias Biológicas, UNAM. This study was partially supported by the Programa de Apoyo a Proyectos de Investigación e Innovación Tecnológica (PAPIIT; grant no. IN203115).

References

Abad-Franch, F., Monteiro, F.A., Jaramillo, N., Gurgel-Goncalves, R., Stehling, F.B. & Diotaiuti, L. (2009) Ecology, evolution, and the long-term surveillance of vector-borne Chagas disease: a multi-scale appraisal of the tribe Rhodniini (Triatominae). *Acta Tropica*, **110**, 159–177.

Araújo, C.A., Waniek, P.J., Stock, P., Mayer, C., Jansen, A.M. & Schaub, G.A. (2006) Sequence characterization and expression patterns of defensin and lysozyme encoding genes from the gut of the reduviid bug, *Triatoma brasiliensis*. *Insect Biochemistry and Molecular Biology*, **36**, 547–560.

Asin, S. & Catalá, S. (1995) Development of *Trypanosoma cruzi* in *Triatoma infestans*: influence of temperature and blood consumption. *Journal of Parasitology*, **81**, 1–7.

Barrera-Pérez, M.A., Rodríguez-Felix, M.E., Guzmán-Marín, E. & Zavala-Velazquez, E.D. (2001) Biological behaviour of three strains of *Trypanosoma cruzi* from Yucatan, Mexico. *Revista Biomedica*, **12**, 224–230.

Barreto, M.P. (1964). Reservatórios do Trypanosoma cruzi nas Américas. *Revista Brasileira de Malariología e Doenças Tropicais*, **16**, 527–552.

Benítez-Alba, J.I., Huerta, H. & Téllez-Rendón, J.L. (2012) Distribution of triatomines (Heteroptera: Reduviidae) associated with human habitation and potential risk areas in six states of the Mexican Republic. *BIOCYT*, **5**, 327–340.

Bowden, T.J. (2008) Modulation of the immune system of fish by their environment. *Fish and Shellfish Immunology*, **25**, 373–383.

Buarque, D.S., Braz, G.R., Martins, R.M. *et al.* (2013) Differential expression profiles in the midgut of *Triatoma infestans* infected with *Trypanosoma cruzi*. *PLoS One*, **8**, 1–9.

Castro, D.P., Moraes, C.S., González, M.S., Ratcliffe, N.A., Azambuja, P. & García, E.S. (2012) *Trypanosoma cruzi* immune response modulation decrease microbiota in *Rhodnius prolixus* gut and is crucial for parasites survival and development. *PLoS One*, **7**, e36591.

Cornet, S., Biard, C. & Moret, Y. (2009) Variation in immune defence among populations of *Gammarus pulex* (Crustacea: Amphipoda). *Oecologia*, **159**, 257–269.

Cruz-Reyes, A. & Pickering-López, J.M. (2006) Chagas disease in Mexico: an analysis of geographical distribution during the past 76 years – A review. *Memórias do Instituto Oswaldo Cruz*, **101**, 345–354.

Damian, R.T. (1997) Parasite immune evasion and exploitation: reflections and projections. *Parasitology*, **115**, 169–175.

Del Puerto, R., Nishizawa, J.E., Kikuchi, M. *et al.* (2010) Lineage analysis of circulating *Trypanosoma cruzi* parasites and their association with clinical forms of Chagas disease in Bolivia. *PLoS Neglected Tropical Diseases*, **4**, e687.

Demas, G.E. & Nelson, R.J. (1998) Photoperiod, ambient temperature, and food availability interact to affect reproductive and immune function in adult male deer mice (*Peromyscus maniculatus*). *Journal of Biological Rhythms*, **13**, 253–262.

Eleftherianos, I. & Revenis, C. (2011) Role and importance of phenoloxidase in insect homeostasis. *Journal of Innate Immunity*, **3**, 28–33.

Elliot, S.L., Rodrigues, J. de O., Lorenzo, M.G., Martins-Filho, O.A. & Guarneri, A.A. (2015) *Trypanosoma cruzi* etiological agent of Chagas disease, is virulent to its triatomine vector *Rhodnius prolixus* in a temperature-dependent manner. *PLoS One*, **9**, e0003646.

Espinosa-de-Aquino, W.B. (2012) Estudio de moléculas del sistema immune de *Triatoma pallidipennis* infectados con *Trypanosoma cruzi*. Master's Thesis. Universidad Nacional Autónoma de México, Mexico City.

Espinoza, E. & García, E. (2003) *Manual de laboratorio de genética*. ECOSUR-San Cristóbal de las Casas. El Colegio de la Frontera Sur, Tapachula.

Espinoza, B., Rico, R., Sosa, S. *et al.* (2010) Mexican *Trypanosoma cruzi T. cruzi* I strains with different degrees of virulence induce diverse humoral and cellular immune responses in a murine experimental infection model. *Journal of Biomedicine and Biotechnology*, **2010**, 890672.

Fellet, M.R., Lorenzo, M.G., Elliot, S.L., Carrasco, D. & Guarneri, A.A. (2014) Effects of infection by *Trypanosoma cruzi* and *Trypanosoma rangeli* on the reproductive performance of the vector *Rhodnius prolixus*. *PLoS One*, **9**, e105255.

Flores-Villegas, A.L., Salazar-Schettino, P.M., Córdoba-Aguilar, A. *et al.* (2015) Immune defence mechanisms of triatomines against bacteria, viruses, fungi and parasites. *Bulletin of Entomological Research*, **105**, 523–532.

González-Santoyo, I. & Córdoba-Aguilar, A. (2012) Phenoloxidase: a key component of the insect immune system. *Entomologia Experimentalis et Applicata*, **142**, 1–16.

Guzmán-Marín, E., Zavala-Castro, J., Acosta-Viana, M. & Rosado-Barrera, M. (1999) Importancia de la caracterización de cepas de *Trypanosoma cruzi*. *Revista Biomedica*, **10**, 177–184.

Heger, T.J., Guerin, P.M. & Eugster, W. (2006) Microclimatic factors influencing refugium suitability for *Rhodnius prolixus*. *Physiological Entomology*, **31**, 248–256.

Higo, H., Miura, S., Horio, M. *et al.* (2004) Genotypic variation among disease lineages of *Trypanosoma cruzi* and its geographic aspects. *Parasitology International*, **53**, 337–344.

Instituto Nacional de Estadística y Geografía (INEGI) (2016) Espacio y Datos de México. http://www.beta.inegi.org.mx/app/mapa/espacioydatos/ [accessed on 12 March 2016].

Instituto Nacional de Salud (INS) (2005) *Manual de Procedimientos de Laboratorio para el Diagnóstico de la Trypanosomiasis Americana (Enfermedad de Chagas)*. Series de Normas Técnicas No. 26. INS, Lima.

Karl, I., Hoffmann, K.H. & Fischer, K. (2010) Cuticular melanisation and immune response in a butterfly: local adaptation and lack of correlation. *Ecological Entomology*, **35**, 523–528.

Manoel-Caetano, F.S. & Silva, A.E. (2007) Implications of genetic variability of *Trypanosoma cruzi* for the pathogenesis of Chagas disease. *Cadernos de Saúde Pública*, **23**, 2263–2274.

Marie-France, B., Barnabé, C., Magallón-Gastelum, E. *et al.* (2002) Predominance of *Trypanosoma cruzi* Lineage I in México. *Journal of Clinical Microbiology*, **40**, 627–632.

Martínez-Ibarra, J.A., Salazar-Schettino, P.M., Solorio-Cibrián, M. *et al.* (2008) Influence of temperature and humidity on the biology of *Triatoma mexicana* Hemiptera: Reduviidae: triatominae under laboratory conditions. *Memórias do Instituto Oswaldo Cruz*, **103**, 719–723.

Mazariego-Arana, M.A., Monteón, V.M., Ballinas-Verdugo, M.A., Hernández-Becerril, N., Alejandre-Aguilar, R. & Reyes, P. (2001) Seroprevalence of human *Trypanosoma cruzi* infection in different geographic zones of Chiapas, Mexico. *Revista da Sociedade Brasileira de Medicina Tropical*, **34**, 453–458.

Mello, C.B., García, E.S., Ratcliffe, N.A. & Azambuja, P. (1995) *Trypanosoma cruzi* and *Trypanosoma rangeli*: interplay with hemolymph components of *Rhodnius prolixus*. *Journal of Invertebrate Pathology*, **65**, 261–268.

Mendes-Pereira, J., Sila, P., Vieira, A., Morales, A., Bomfim, R. & Gurgel-Goncalves, R. (2013) Climatic factors influencing Triatominae occurrence in Central-West Brazil. *Memórias do Instituto Oswaldo Cruz*, **108**, 335–341.

Mischler, P., Kearny, M., McCarroll, J.C., Scholte, G.C., Vounatsou, P. & Malone, J.B. (2012) Environmental and socio-economic risk modeling for Chagas disease in Bolivia. *Geospatial Health*, **63**, 59–66.

Moller, A.P. & Rózsa, L. (2005) Parasite biodiversity and host defenses: chewing lice and immune response of their avian hosts. *Oecologia*, **142**, 169–176.

Moreno-García, M., Córdoba-Aguilar, A., Conde, R. & Lanz-Mendoza, H. (2013) Current immunity markers in insect ecological immunology: assumed trade-offs and methodological issues. *Bulletin of Entomological Research*, **103**, 127–139.

Mucklow, P.T., Vizoso, D.B., Jensen, K., Refardt, D. & Ebert, D. (2004) Variation in phenoloxidase activity and its relation to parasite resistance within and between populations of *Daphnia magna*. *Proceedings of the Royal Society of London, Series B, Biological Sciences*, **271**, 1175–1183.

Noireau, F., Carbajal-de-la-Fuente, A.L., Lopes, C.M. & Diotaiuti, L. (2005) Some considerations about the ecology of Triatominae. *Anais da Academia Brasileira de Ciências*, **77**, 431–436.

Noireau, F., Diosque, P. & Jansen, M. (2009) *Trypanosoma cruzi*: adaptations to its vectors and its host factors. *Veterinary Research*, **40**, 1–23.

Nouvellet, P., Cucunubá, Z.M. & Gourbiére, S. (2015) Ecology, evolution and control of Chagas disease: a century of neglected modelling and a promising future. *Advances in Parasitology*, **87**, 135–191.

Parker, E.R. & Sethi, A. (2011) Chagas disease: coming to a place near you. *Dermatologic Clinics*, **29**, 53–62.

Pereira-Lopes, G., Ferreira-Silva, M.M., Ramos, A.A., Moraes-Souza, H., Prata, A. & Correia, D. (2013) Length and caliber of the rectosigmoid colon among patients with Chagas disease and controls from areas at different altitudes. *Revista da Sociedade Brasileira de Medicina Tropical*, **46**, 746–751.

Ramsey, J.M., Ordoñez, R., Cruz-Celis, A. *et al.* (2000) Distribution of domestic Triatominae and stratification of Chagas disease transmission in Oaxaca, Mexico. *Medical and Veterinary Entomology*, **14**, 19–30.

R Core Team (2015) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna. http://www.R-project.org/ [accessed on November 2015].

Reyes, M. & Angulo, V.M. (2009) Ciclo de vida de *Triatoma dimidiata* Latreille, 1811 (Hemíptera: Reduviidae) en condiciones de laboratorio: producción de ninfas para ensayos biológicos. *Biomédica*, **29**, 119–126.

Reyes-Novelo, E., Ruiz-Piña, H.A., Escobedo-Ortegón, J. & Barrera-Pérez, M.A. (2011) Biología y ecología de *Triatoma dimidiata* (Latreille, 1811), algunos aspectos de estudios. *Dugesiana*, **18**, 11–16.

Rivero, A. (2006) Nitric oxide: an antiparasitic molecule of invertebrates. *Trends in Parasitology*, **22**, 219–225.

Rocha, D.S., Jurberg, J., Carcavallo, R.U., Presgrave, O.A., Cunha, V. & Galvao, C. (2001) Influence of temperature and humidity on the nymphal development of *Rhodnius prolixus*. *Revista de Saúde Pública*, **35**, 400–406.

Sadd, B.M. & Schmid-Hempel, P. (2009) Perspective: principles of ecological immunology. *Evolutionary Applications*, **2**, 113–121.

Salazar-Schettino, P.M., Jiménez, J., Tay, J. & Cárdenas-Ramírez, L. (1978) Estudio comparativo de la patogenicidad de cuatro cepas de *T. cruzi* en el ratón blanco. *Revista Latino-Americana de Microbiología*, **20**, 51–57.

Sánchez-Guillén, M.C., Barnabé, C., Tibayrenc, M. *et al.* (2006) *Trypanosoma cruzi* strains isolated from human, vector, and animal reservoir in the same endemic region in Mexico and typed as *T. cruzi* I, discrete typing unit 1 exhibit considerable biological diversity. *Memórias do Instituto Oswaldo Cruz*, **101**, 585–590.

Schaub, G.A. (1989) Does *Trypanosoma cruzi* stress its vectors? *Parasitology Today*, **5**, 185–188.

Schmid-Hempel, P. (2005) Evolutionary ecology of insect immune defenses. *Annual Review in Entomology*, **50**, 529–551.

Schofield, C.J., Jannin, J. & Salvatella, R. (2006) The future of Chagas disease control. *Trends in Parasitology*, **22**, 583–588.

Souto, R.P., Fernandes, O., Macedo, A.M., Campbell, D.A. & Zingales, B. (1996) DNA markers define two phylogenetic lineages of *Trypanosoma cruzi*. *Molecular and Biochemical Parasitology*, **83**, 141–153.

Tay, J., Gutiérrez, M., Salazar-Schettino, P.M., Castillo, M. & Ortega, M. (1973) Estudio sobre seis cepas mexicanas de *Trypanosoma cruzi*. *Revista de Investigación en Salud Pública*, **33**, 67–76.

Telleria, J. & Tibayrenc, M. (2010) *American trypanosomiasis: One Hundred Years of Research*. Elsevier, London.

Ursic-Bedoya, R.J. & Lowenberg, C.A. (2007) *Rhodnius prolixus*: identification of immune-related genes up-regulated in response to pathogens and parasites using suppressive subtractive hybridization. *Development and Comparative Immunology*, **31**, 109–120.

Whitten, M.M., Mello, C.B., Gomes, S.A., *et al.* (2001). Role of superoxide and reactive nitrogen intermediates in *Rhodnius prolixus* (Reduviidae)/*Trypanosoma rangeli* interactions. *Experimental Parasitology*, **98**, 44–57.

World Health Organization (1986) *Chagas Disease: Guidelines for a Standard Protocol*. WHO, Geneva.

World Health Organization (2014) A Global Brief on Vector-Borne Disease. WHO, Geneva.

Zhao, P., Zhiqiang, L., Strand, M.R. & Jiang, H. (2011) Antiviral, anti-parasitic, and cytotoxic effects of 5,6-dihydroxyindole (DHI), a reactive compound generated by phenoloxidase during insect immune response. *Insect Biochemistry and Molecular Biology*, **41**, 645–652.

Zhivotovsky, L.A., Feldman, M.W. & Bergman, A. (1996) On the evolution of phenotypic plasticity in a spatially heterogeneous environment. *Evolution*, **50**, 547–558.

Accepted 11 August 2016
First published online 18 October 2016

CAPÍTULO V

Discusión general

A poco más de 100 años de su descubrimiento, la enfermedad de Chagas sigue siendo una de las principales causas de discapacidad solo después de las enfermedades respiratorias, las diarreas y el SIDA (Garrido-Pérez *et al*., 2010). Si bien la prevalencia de la enfermedad disminuyó considerablemente durante la década de los noventas gracias a programas de control de los vectores, todavía quedan muchos obstáculos para el control efectivo de la enfermedad (Noireau *et al*., 2009).

Una de las principales dificultades es la amplia heterogeneidad que presenta *T. cruzi* en el aspecto parasitológico, lo cual se traduce en que algunas cepas son más virulentas que otras. Ha sido propuesta la clasificación del parásito en tres biodemos principales tomando como variables el pico máximo de parasitemia, la mortalidad de los ratones infectados y el tropismo celular, siendo el grupo I el que está representado por las cepas más virulentas (Andrade y Magalhães, 1997). En México se han reportado parasitemias muy variables y mortalidades que van desde el 0 hasta el 100 % de los animales infectados (Tabla 2.1), esto sugiere que en nuestro país podrían estar circulando los tres biodemos del parásito. La variación en el comportamiento intenta ser explicada por la variabilidad genética que ha mostrado *T. cruzi* con diversos marcadores moleculares (Junqueira *et al*., 2005), pero surgen cuestionamientos cuando cepas genéticamente similares muestran diferentes grados de virulencia (Espinoza *et al*. 2010). Incluso, se han observado diferencias en el comportamiento de cepas que pertenecen a zonas geográficas muy cercanas entre ellas (Salazar-Schettino *et al*. 1978).

Parte de los resultados generales de esta tesis muestran la primera evidencia de que el comportamiento parasitológico de *T. cruzi* puede estar relacionado con la altitud. La mayoría de los estudios de enfoque altitudinal se han centrado en evaluar la distribución de los triatominos, y se concluye que la mayoría de las especies se observan en zonas tropicales y subtropicales que van desde los 100 hasta 1800 msnm (Carcavallo, 1999). Sin embargo, existen especies en México como *T. barberi* que puede ser encontrada hasta los 2400 msnm (Salazar-Schettino *et al*., 2005) o como *T. infestans* en Bolivia que se encuentra en zonas que superan los 4000 msnm (Carcavallo, 1999). En referencia a la enfermedad, la altitud parece influir en algunas manifestaciones clínicas en el humano (Pereira-Lopes *et al*., 2013), pero en general se carecen de reportes que exploren otras manifestaciones durante la infección. Debido a la escasa información resulta difícil determinar las causas de la dependencia entre la virulencia de *T. cruzi* y la altitud, por lo que se hace necesario seguir investigando para intentar responder este fenómeno.

Resulta interesante el hecho de que tanto la virulencia de *T. cruzi* como la respuesta inmune de triatominos (medida como la actividad de la PO) sean más intensas a la misma altitud, es decir a 700 msnm. En relación a la virulencia, el periodo prepatente de los tres aislados fue similar con un lapso de 13-15 días, lo cual demuestra una replicación lenta del parásito y representan periodos más largos que lo reportado para otras cepas evaluadas en México, como CGH3 y Qro. que mostraron periodos prepatentes de 2 y 3 días, respectivamente (Espinoza *et al*., 2010; Monteón *et al*., 2009). Otras cepas del sureste del país han tenido periodos similares a los que aquí se reportan y curiosamente se han obtenido de zonas donde se encuentra la misma especie de transmisor que en Chiapas, *T. dimidiata* (Barrera-Pérez *et al*., 2011; Monteón *et al*., 2009). Esto es importante ya que de

acuerdo con investigaciones de Guzmán-Marín *et al*. (2012), *T. dimidiata* puede ser una especie que provoque la disminución de la virulencia de *T. cruzi*. Es conocida la capacidad que tiene *T. dimidiata* de explotar diversas fuentes alimenticias (Sasaki *et al*., 2003), entre ellas la sangre de gallina (e.g. Farfán-García y Angulo-Silva, 2011), la acción refractaria que tiene la sangre de aves a la infección por *T. cruzi* (Teixeira *et al*., 2011) podría estar ocasionando la disminución de la virulencia. En la naturaleza, por ejemplo, algunos estudios epidemiológicos han señalado una menor prevalencia de la enfermedad de Chagas en zonas donde los triatominos tienen un comportamiento ornitofílico (Quintal y Polanco, 1977; Christensen *et al*., 1988).

A partir del inicio de las parasitemias en los ratones de este experimento, el parásito estuvo presente en la sangre circulante de los tres grupos hasta el día 37 cuando fueron sacrificados para estudios histopatológicos. No obstante, al día 33 post-infección (*pi*) se registró el pico de parasitemia más elevado de $22.57x10^6$ par/ml en el grupo infectado con TC700, mientras que el pico más bajo se presentó en el grupo inoculado con TC1400 con $11.17x10^6$ par/ml. La replicación del parásito está relacionada con la capacidad de evadir el sistema inmune del vertebrado, a pesar de que muchos parásitos son destruidos en la vacuola fagocítica al inicio de la infección, los que logran dividirse lisan la célula y pasan al torrente sanguíneo donde invaden cualquier otra célula del cuerpo (Texeira *et al*., 2011; Dos Reis *et al*., 2012). En el torrente sanguíneo *T. cruzi* puede bloquear el sistema del complemento mediante la participación de proteínas como la calreticulina, o bien escapar de otros componentes séricos al invadir con mayor facilidad las células del hospedero con ayuda de proteínas como las transialidasas (Rubín de Celis *et al*., 2006). Estos y otros mecanismos del parásito para escapar del sistema inmune (Cardoso *et al*.,

2015) representan una forma de adaptación biológica para sobrevivir en el medio inmunológicamente hostil del hospedero (Palau, 2000).

Es razonable pensar que una mayor cantidad de parásitos en la sangre circulante del hospedero aumenta las posibilidades de *T. cruzi* de ser adquirido por un triatomino al alimentarse, lo cual implica entonces que en la zona donde se aisló TC700 habría un mayor riesgo de transmisión del parásito. Lo anterior es apoyado por los resultados de Mazariego-Arana *et al*. (2001), quienes reportaron una prevalencia de la enfermedad de Chagas del 50 % en la comunidad de Nueva Jerusalem en Chiapas, lugar que está situada a aproximadamente 600 msnm. Por el contrario, en ocho comunidades localizadas en la costa del estado la prevalencia fue de apenas el 1.2 % y en la zona de Mesochiapas, donde se encuentran altitudes desde los 1000 a los 1500 msnm, no se encontraron casos seropositivos. Esta mayor prevalencia de la enfermedad en humanos podría estar ocasionando que el parásito sea más virulento, ya que de acuerdo a Salazar-Schettino *et al*. (1978), el paso de *T. cruzi* por el hombre parece aumentar su virulencia. Sin embargo, esto también podría relacionarse con los datos de colecta y predicción de abundancia de *T. dimidiata* en el estado, los cuales sitúan una mayor presencia del transmisor en altitudes cercanas a los 800 msnm (Benítez-Alba *et al*., 2001). Para completar esto sería importante evaluar la infección natural de triatominos de las diferentes altitudes, lo cual se espera sea mayor a 700 msnm.

Otro aspecto a resaltar es que a pesar de que el aislado TC700 produjo mayores parasitemias que TC300 y TC1400, la mortalidad durante todo el experimento fue baja, pues únicamente murieron dos ejemplares infectados con TC700 y uno con TC1400. La mortalidad en animales experimentales se ha relacionado con altas parasitemias durante la

etapa aguda (Zúñiga *et al*., 2012), aunque no siempre es el caso (Gómez-Hernández *et al*., 2011), en humanos la muerte durante esta fase es poco común y ocurre con mayor frecuencia en personas inmunocomprometidas. Como se ha mencionado anteriormente, la mortalidad observada con cepas mexicanas en laboratorio ha sido muy variable y existen cepas con altos porcentajes de mortalidad como Qro. (Espinoza *et al*., 2010; Mendoza-Rodríguez, 2015) y Apodaca (Salazar-Schettino, *et al*., 1978). Es importante para un parásito no matar a su hospedero ya que eso significaría eliminarse a sí mismo, por lo que algunas hipótesis sugieren que los parásitos evolucionan hacia un menor daño a su hospedero (menor virulencia) con el fin de mantener su ciclo en la naturaleza (Rico-Hernández, 2011), aunque también la baja mortalidad que observamos podría ser consecuencia de la disminución en la virulencia provocada por *T. dimidiata*, lo cual se ha discutido anteriormente.

Como se ha comentado, en México el corazón es el órgano más afectado y la ocurrencia se debe a la mayor presencia del linaje TCI del parásito en el país, el cual muestra un tropismo preferente hacia este tejido (Bosseno *et al*., 2002). Lo anterior concuerda con nuestros resultados ya que los análisis moleculares (amplificación del gen mini-exón) determinaron que los tres aislados pertenecen al mismo linaje (TC1) y los estudios histopatológicos evidenciaron una mayor invasión al corazón de los ratones, sin embargo TC700 produjo más nidos que los otros dos aislados. La presencia y replicación del parásito en el tejido cardiaco, así como una respuesta inmune exacerbada y al parecer un proceso de autoinmunidad, son los responsables del deterioro de la función cardiaca que pueden conducir a la muerte súbita del paciente (Barbosa y Nunes, 2012), no obstante, la hipótesis de la autoinmunidad se mantiene en controversia debido a las dificultades

experimentales, controles incompletos o inadecuados y falta de evidencia de este mecanismo (Kierszenbaum, 2005). Aunado a esto, TC700 fue el único aislado que tuvo la capacidad de invadir el cerebro de algunos ejemplares, esta capacidad ha sido poco reportada y posiblemente las altas parasitemias sean un factor determinante para esta invasión (e.g. Tay *et al*., 1969; Salazar-Schettino *et al*., 1978). Las lesiones en el sistema nervioso central pueden provocar parálisis de las extremidades traseras (Yarbuh *et al*., 2006) o pérdida de la memoria (Chagas, 1916), a pesar de esta invasión aún se desconocen los mecanismos que le permiten al parásito atravesar la barrera hematoencefálica. Hasta este punto es claro que el aislado TC700 es más virulento que TC300 y TC1400 en relación a los parámetros observados, lo que implica un mayor riesgo para las poblaciones humanas que se encuentran en esa zona. De acuerdo con la clasificación de los biodemos propuesta por Andrade (1985), los tres aislados pertenecen al biodemo III, el cual se caracteriza por incluir poblaciones con una multiplicación lenta, picos de parasitemia entre los 20-30 días y una baja mortalidad.

Con respecto a la actividad de la PO, la infección con *T. cruzi* produjo la activación de la enzima en los triatominos de diferentes altitudes. Como se expuso previamente, los insectos de 700 msnm mostraron una mayor actividad enzimática en la hemolinfa; si bien *T. cruzi* tiene un hábitat restringido únicamente al intestino del vector y no traspasa el hemocele (Kollien y Schaub, 200), la hemolinfa se caracteriza por ser un componente que media la mayoría de los procesos fisiológicos del insecto y tiene contacto con la mayoría de los órganos (Nation *et al*., 2008), por este motivo su estudio puede ser un buen indicador de estrés en los individuos. Estudios previos han reportado la activación de varios mecanismos de defensa después de la infección con *T. cruzi* (e.g. Whitten *et al*., 2007; Waniek *et al*.,

2010) e incluso también en la hemolinfa (Mello *et al*. 1996), pero hasta el momento no se ha reportado que esto ocasione la eliminación del parásito.

Una explicación de la respuesta vía PO es que el sistema inmune de la chinche reconozca y ataque al parásito pero no sea lo bastante eficaz como para eliminar la infección. Esto podría sugerir entonces que al igual que en el vertebrado, *T. cruzi* tiene la capacidad resistir la respuesta inmune del invertebrado y mantener una infección de por vida como sucede en los mamíferos (Dos Reis *et al*., 2011). Al menos con *T. rangeli* se ha observado que este es capaz de resistir y suprimir la actividad de la PO en la hemolinfa del vector *R. prolixus* (Gomes *et al*., 2003), debido a algunas similitudes como morfológicas y antigénicas (Urdaneta-Morales y Tejero, 1992) no es aventurado pensar que *T. cruzi* tiene la capacidad de soportar las respuestas inmunológicas. Si esto es cierto, la identificación de los factores clave en la resistencia al sistema inmune de los triatominos representa un enfoque prometedor para bloquear el establecimiento del parásito. Sin embargo, existe también la posibilidad de que el parásito tenga la capacidad de modular la respuesta inmune de la chinche, como se ha observado con el clon Dm28 en *R. prolixus* (Castro *et al*., 2012).

Está ampliamente aceptado que la capacidad de montar una respuesta inmune en los insectos depende mucho de la disponibilidad de recursos, ya que su activación es altamente costosa en términos energéticos (Schmid-Hempel, 2003). Es probable que la mayor actividad observada en los triatominos de 700 msnm se deba a que estos tienen una mayor disponibilidad de recursos para montar una respuesta inmune. Los datos ambientales de esta zona muestran las condiciones preferenciales para *T. dimidiata*, los cuales consisten en temperaturas entre 24-26 °C y una humedad cercana al 70 % (Reyes-Novelo *et al*. 2011).

En contraste, las zonas de estudio a altitudes de 300 y 1400 msnm mantienen condiciones de 18° y 30° C de temperatura, respectivamente (INEGI, 2016), e implica que en ellas las chinches también tienen que gastar recursos en maximizar su adecuación al estar en ambientes estresantes, de manera que lo destinado a la respuesta inmune es menor. Esta relación entre el sistema inmune y el ambiente son parte del estudio de una nueva rama de la biología conocida como ecoinmunología (Schulenberg *et al*., 2009).

Al mantenerse el parásito de por vida en el insecto, sería interesante evaluar por cuánto tiempo se mantiene activada en particular la enzima PO y si llega un momento en el que los niveles de la actividad se mantienen bajos, al grado de que la interacción se convierte en una asociación equilibrada. Esto indica que evolutivamente ambos han llegado a relacionarse de tal forma que no ocurre una sobreexplotación, de hecho la mortalidad de las chinches generalmente no se ve afectada, por lo que de manera convencional *T. cruzi* era considerado un parásito no patógeno para los triatominos. Sin embargo, estudios recientes muestran que la infección puede tener algunos efectos negativos en las chinches como retraso en las mudas (Elliot *et al*., 2015) y disminución en la tasa de reproducción (Fellet *et al*., 2014), lo cual es un costo en adecuación para el triatomino. Si la disminución en la tasa de reproducción es a causa del montaje de la respuesta inmune por la presencia del parásito, parece evidente que existe una correlación negativa entre la respuesta inmune y algunas características de historia de vida de estos insectos (en este caso reproducción), lo que comúnmente se denomina "trade-offs" o disyuntivas (Redman *et al*., 2016). De acuerdo con modelos de asignación de recursos en insectos, las disyuntivas surgen debido a la competencia por uno o más recursos limitantes,

y que ciertos mecanismos de señalización regulan la asignación de dicho recurso entre procesos reproductivos e inmunológicos (Schewenke *et al*., 2016).

A juzgar por el hecho de que la virulencia de *T. cruzi* y la respuesta inmune de las chinches fueron mayores a la misma altitud, se podrían poner a prueba las siguientes hipótesis: 1) es posible que una respuesta inmune más intensa de la chinche ocasione que el parásito se vuelva más virulento o 2) que la respuesta inmune de la chinche sea un reflejo de la virulencia de *T.* cruzi. La primera hipótesis tiene como fundamento la hostilidad que puede generar el sistema inmune de los triatominos, por lo cual el parásito necesitaría de estrategias que pueden ocasionar que se vuelva más virulento para el vertebrado; para el caso de la segunda hipótesis, se ha planteado que los niveles medios de defensa de una población hospedadora es en realidad el reflejo de la virulencia media de los parásitos en esa población (Martin *et al*., 2001). Debido a la gran diversidad de especies transmisoras y cepas del parásito, es posible que estos y otros eventos puedan estar ocurriendo en la naturaleza de manera particular, y dependan quizá de las adaptaciones evolutivas que tengan ciertas cepas a un transmisor en específico.

Entender cuáles son los mecanismos que ocasionan una mayor agresividad de *T. cruzi* o que se presenten más casos de la enfermedad en el humano, ayudaría a establecer zonas que sean consideradas de mayor riesgo e implementar programas de control dirigidos a esos lugares.

CAPÍTULO VI

Conclusiones generales

Los resultados obtenidos en esta investigación muestran que el comportamiento parasitológico de *T. cruzi* puede variar con respecto a la altitud; en las diferentes zonas de estudio que corresponden a tres diferentes altitudes, 300, 700, y 1400 msnm, se pudo constatar que los parásitos obtenidos de una localidad situada a 700 msnm se comportaron más virulentos en el ratón, lo cual fue observado con parámetros parasitológicos como periodo prepatente, parasitemia, mortalidad y tropismo celular. De igual manera, los estudios de la respuesta inmune de los triatominos indicaron que se presenta una mayor actividad de la PO contra el parásito en los insectos de la misma altitud (700 msnm). En conclusión, se establece que las poblaciones humanas cercanas a 700 msnm en esa región y donde existe la presencia de la chinche, hay un mayor riesgo de contagio y de que la enfermedad sea más severa, además, los resultados de la respuesta inmune abren nuevas perspectivas para el estudio de la interacción parásito-vector.

Literatura citada

Amparyup P, Charoensapsri W, Tassanakajon A. 2013. Prophenoloxidase system and its role in shrimp immune responses against major pathogens. Fish Shellfish Immunol 34(4): 990-1001.

Andrade SG, Magalhães JB. 1997. Biodemes and zymodemes of *Trypanosoma cruzi* strains: correlations with clinical data and experimental pathology. Rev Soc Bras Med Trop 30: 27-35.

Andrade SG. 1985. Morphological and behavioural characterization of *Trypanosoma cruzi* strains. Rev Soc Bras Med Trop 18(l): 39-46.

Asin S, Catalá S. Development of *Trypanosoma cruzi* in *Triatoma infestans*: influence of temperature and blood consumption. J Parasitol 81(1): 1-7.

Azambuja P, Ratcliffe N, García E. 2005. Towards an understanding of the interactions of *Trypanosoma cruzi* and *Trypanosoma rangeli* within the reduviid insect host *Rhodnius prolixus*. An Acad Bras Cienc 77(3): 397-404.

Barbosa M, Nunes MC. 2012. Estratificación del riesgo de la enfermedad de Chagas. Rev Esp Cardiol 65(2): 17-21.

Barrera-Pérez MA, Rodríguez-Féliz ME, Guzmán-Marín E, Zavala-Velázquez J, Dumonteil E. 2001. Biological behaviour of three strains of *Trypanosoma cruzi* from Yucatan, Mexico. Rev Biomed 12(4): 224-30.

Barreto, M. Reservorios do Trypanosoma cruzi nas América. Ver. Brás. Matar. 1964; 16: 527.

Benítez-Alba JI, Huerta H, Téllez-Rendón JJ. 2012. Distribución de triatominos (Heteroptera: Reduviidae) asociados a la vivienda humana y posibles zonas de riesgo en seis estados de la República Mexicana. BIOCYT 5(17): 227-240.

Bern C, Kjos S, Yabsley MJ, Montgomery SP. 2011. *Trypanosoma cruzi* and Chagas disease in the United States. Clin Microbiol Rev 24(4): 655-681.

Bosseno MF, Barnabé C, Magallón E, Lozaon-Kasten F, Ramsey J, Breniere SF. 2002. Predominance of *Trypanosoma cruzi* lineage I in Mexico. J Clin Microbiol 40(2): 627-32.

Brisse S, Verhoef J, Tibayrenc M. 2001. Characterisation of large and small subunit rRNA and mini-exon genes further supports the distinction of six *Trypanosoma cruzi* lineages. Int J Parasitol 31(11): 1218-26.

Cacarvallo, RU. 1999. Climatic factors related to Chagas disease transmission. Mem Inst Oswaldo Cruz 94(1): 367-9.

Cardoso M, Reis-Cunha JL, Bartholomeu D. 2015. Evasion of the immune response by *Trypanosoma cruzi* during acute infection. Front Immunol. 6: doi:10.3389/fimmu.2015.00659

Cardenas-Ramírez L, Tay J, Salazar-Schettino PM. 1975. Cambios histopatológicos en el ratón por cepas mexicanas de *Trypanosoma cruzi*. Rev. Inv. Salud Pública México. 35: 131-153.

Cassin-Duz AL, de Abreu P, Mendes B, Aguiar-Soares R, Oliveira-Cardoso JM, Bitencourt F, *et al*. 2014 The TcI and TcII *Trypanosoma cruzi* experimental infections induce distinct immune responses and cardiac fibrosis in dogs. Mem Inst Oswaldo Cruz 109(8): 1005-1013.

Castro, D.P. *et al*. (2012) *Trypanosoma cruzi* immune response modulation decreases microbiota in *Rhodnius prolixus* gut and is crucial for parasite survival and development. PLoS ONE 7, e36591

Catalán TP, Wozniak A, Niemeyer HM, Kalergis AM, Bozinovic F. 2012. Interplay between thermal and immune ecology: effect of environmental temperature on insect immune response and energetic costs after an immune challenge. J Insect Physiol 58(3): 310-317.

Cerenius L, Lee BL, Soderhall K. 2008. The –proPO-System: pros and cons for its role in invertebrate immunity. Trends Immunol 29(6): 2636-271.

Chagas C. 1909. Nova trypanosomiase humana. Estudos sobre a morphologia e o cyclo evolutivo de Schyzotrypanum cruzi, n. gen., n. sp., agente etiologico de nova entidade morbida do homem. Mem Inst Oswaldo Cruz 1(2): 159-218.

Chagas C. 1916. Processos patogênicos da Tripanozomiase Americana. Mem Inst Oswaldo Cruz 8:5-35.

Chatelain E. 2015. Chagas disease drug discovery: toward a new era. J Biol Scree 20(1): 22-35.

22. Cucunubá Z, Okuwoga O, Basañez M,Nouvellet P. 2016. Increased mortality attributed to Chagas disease: a systematic review and meta-analisys. Parasit Vectors 9(42):Doi: 10.1186/s13071-016-1315-xda Academia Brasileira de Ciências, 77, 431–436.

Dias JP, Bastos C, Araújo E, Mascarenhas AV, Martins-Neto E, Grassi F, *et al*. 2008. Acute Chagas disease outbreak associated with oral transmission. Rev Soc Bras Med Trop 41(3): 296-300.

Dos Reis D, Monteiro WM, Bossolani GD, Teston AP, Gomes ML, de Araujo SM, *et al*., 2012. Biological behaviour in mice of *Trypanosoma cruzi* isolates from Amazonas and Paraná, Brazil. Exp Parasitol 130(4): 321-9.

Elliot SL, Rodriques Jde, Lorenzo MG, Martins-Filho OA, Guarneri AA. 2015. *Trypanosoma cruzi*, etiological agent of Chagas disease, is virulent to its triatomine vector Rhodnius prolixus in a temperature-dependent manner. PLoS Negl Trop Dis 9(3): e000364e

Espinoza B, Rico T, Sosa S, Oaxaca E, Vizcano-Castillo A, Camallero, M. *et al*., 2010. Mexican *Trypanosoma cruzi* TCI Strains with Different Degrees of Virulence Induce Diverse Humoral and Cellular Immune Responses in a Murine Experimental Infection Model. J Biomed Biotechnol. doi:10.1155/2010/890672

Espinoza B, Solorzano-Domínguez N, Vizcaino-Castillo A, Martínez I, Elias-López AL, Rodríguez-Martínez JA. 2011. Gastrointestinal infection with Mexican TcI *Trypanosoma cruzi* strains: different degrees of colonization and diverse immune responses. Int J Biol Sci 7(9): 1357-70.

Fellet MR, Lorenzo MG, Elliot S, Carrasco D, Guarneri AA. 2014. Effects of infection by *Trypanosoma cruzi* and *Trypanosoma rangeli* on the reproductive performance of the vector *Rhodnius prolixus*. PLoS ONE 9(8): e105255

Ferreira RC, Kessler RL, Lorenzo MG, Paim RM, Ferreira L, Probst CM, *et al*. Colonization of *Rhodnius prolixus* gut by *Trypanosoma cruzi* involves an extensive parasite killing. Parasitology 173(3): 434-443.

Garrido-Pérez SM, Hernández-Meléndrez E, Rodríguez-Cabrera A. 2011. La enfermedad de Chagas como un rezago social en salud. Rev Cubana Salud Publica 37(1): 159-174.

Gomes SA, Feder D, García ES, Azambuja P. 2003. Suppresion of the prophenoloxidase system in *Rhodnius prolixus* orally infected with *Trypanosoma rangeli*. J Insect Physiol 49(9): 829-37.

Gómez-Hernández C, Rezende-Oliveira K, Nogueira-Nascentes GA, Rocha-Batista L, Borges-Kappel H, Martínez-Ibarra, JA, *et al*. 2011. Molecular characterization of *Trypanosoma cruzi* Mexican strains and their behavior in the mouse experimental model. Rev Soc Bras Med Trop 44(6): 684-90.

González-Santoyo I, Córdoba-Aguilar A. 2012. Phenoloxidase: a key component of the insect immune system. Entomol Exp Appl 142:1-16.

Guzmán-Marín E, Zavala-Castro J, Acosta-Viana M, Rosado-Barrera M. 1999. Importancia de la caracterización de cepas de *Trypanosoma cruzi*. Rev Biomed 10(3): 177–84.

Hotez P, Molyneux D, Fenwick A, Kumaresan J, Sachs S, Sachs J, Savioli. 2007. Control of neglected tropical disease. N Engl J Med 357: 1018-1027.

Junqueira AC, Degrave W, Brandao A. 2005. Minicircle organization and diversity in *Trypanosoma cruzi* populations. Trends Parasitol 21(6): 270-2.

Jurberg J, Galvão C. 2006. Biology, ecology, and systematic of Triatominae (Heteroptera, Reduviidae), vectors of Chagas disease, and implications for human health.Denisia 50: 1096-1116.

Kierszenbaum F. 2005. Where do we stand on the autoimmunity hypothesis of Chagas disease? Trends Parasitol 21(11):513-6.

Kollien AH, Schaub GA. 2000. The development of *Trypanosoma cruzi* in triatominae. Parasitol Today 16(9): 381-7.

Kollien, A.H. and Schaub, G.A. 2000. The development of Trypanosoma cruzi in Triatominae. Parasitology Today 16: 381-387

Lages-Silva E, Ramírez LE, Pedrosa AL, Crema E, da Cunha LM, Pena SD, *et al*. 2006. Variability of kinetoplast DNA gene signatures of *Trypanosoma cruzi* II strains from patients with different clinical forms of Chagas' disease in Brazil. J Clin Microbiol 44(6): 2167-71.

Lee B, Bacon KM, Bottazzi ME, Hotez PJ. 2013. Global economic burden of Chagas disease: a computational simulation model. Lancet Infect Dis 13(4): 342-348.

Lugo de Yarbuh A, Colasante C, Alarcón M, Moreno E. 2006. Gastrocnemius skeletal muscle microvasculature and neuromuscular junction alterations in mice with experimental acute Chagas infection. Rev Cien 26(6): 593-603.

Manoel-Caetano F, Silva AE. Implications of genetic variability of *Trypanosoma cruzi* for the pathogenesis of Chagas disease. Cad Saude Publica. 2007; 23(10): 2263-74.

Martin TE, Moller AP, Merino S, Clobert, J. 2001. Does clutch size evolve in response to parasites and immunocompetence? Proc Natl Acad Sci USA 98: 2071-6.

Mazariego-Arana MA, Monteón MV, Ballinas-Verdugo MA, Hernández-Becerril N, Alejandre-Aguilar R, Reyes PA. 2001. Seroprevalence of human *Trypanosoma cruzi* infection in diferent geografic zones of Chiapas, Mexico. Rev Soc Bras Med Trop 34(5): 453-8.

Mejía AM, Triana O. 2005. Genetic variability of *Trypanosoma cruzi* in blood and organsof infected mice determined by LSSP-PCR. Biomedica 25(1): 76-86.

Mejía-Jaramillo AM, Fernández GJ, Montilla M, Nicholls R, Triana-Chávez O. 2012. Biomedica 32(2): 196-205.

Mello CB, Azambuja P, García ES, Ratcliffe NA. 1996. Differential in vitro and in vivo behavior of three strains of *Trypanosoma cruzi* in the gut and hemolymph of *Rhodnius prolixus*. Exp Parasitol 82: 112-21

Mendoza-Rodríguez, MI. Caracterización biológica y bioquímica de cuatro aislados de *Trypanosoma cruzi*. Tesis de Licenciatura. Universidad Autónoma de México. 2015; 56 pp.

Moncayo A, Silveira AC. 2009. Current epidemiological trends for Chagas disease in Latin America and future challenges in epidemiology, surveillance and health policy. Mem Inst Oswaldo Cruz 104(1): 17-30.

Monteón V, Godínez S, Cruz-ZetinaG, Balmes J, López R, Hernández O. 2009. Caracterización biológica de aislados mexicanos de *Trypanosoma cruzi:* metaciclogénesis, parasitemia y resistencia contra Benznidazol. Rev Biomed. 20(3): 206-14.

Nation JL. 2008. Insect physiology and biochemistry. CRC Press, University of Florida, USA: 522 pp.

Neves DP. 1971. Influência da temperatura na evolução do *Trypanosoma cruzi* em triatomíneos. Rev Inst Medicina Tropical 13: 155-161.

Noireau, F., Diosque, P. & Jansen, M. 2009. *Trypanosoma cruzi*: adaptations to its vectors and its host factors. Vet Res 40(26): 1–23.

Palau MT. 2000. Relación hospedero-parásito *Trypanosoma cruzi*. MVZ-Córdoba 5(1): 33-7.

Pereira-López G, Ferreira-Silva MM, Ramos AA, Moraes-Souza H, Prata A, Correira D. 2013. Length and caliber of the restigmoide colon among patients with Chagas disease and controls from areas at different altitudes. Rev Soc Bras Med Trop 46: 746-751.

Pinto-Dias JC. 2012. Tendencias sociales de la enfermedad de Chagas para las próximas décadas. Salud Colectiva 8(1): 39-48.

Rassi AJr, Rassi A, Little WC. 2000. Chagas hearth disease. Clin Cardiol 23(12): 883-889.

Rassi AJr, Rassi A, Marín-Neto JA. 2010. Chagas Disease. Lancet 375(9723): 1388-1402.

Redman E, Wilson K, Cory J. 2016. Trade-offs and mixed infections in an obligate-killing insect pathogen. J An Ecol 85: 1200-9.

Reyes-Novelo E, Ruíz-Piña HA, Escobedo-Ortegón J, Barrera-Pérez MA. 2011. Biología y ecología de *Triatoma dimidiata* (Latreille, 1811), algunos aspectos de estudio. Dugesiana 18(1): 11-16.

Rico-Hernández G. 2011. Evolución de interacciones parásito-hospedero: coevolución, selección sexual y otras teorías propuestas. Rev UDCA 14(2): 119-30.

Rubin de Celis S, Uemura H, Yoshida N, Schenkman S. 2006. Expression of trypomastigote trans-sialidase in metacyclic forms of *Trypanosoma cruzi* incrase parasite escape from its parasitophorous vacuole. Cellular Microbiology 8(12): 1888-98.

Salazar-Schettino PM, Jiménez MJ, Tay J, Cárdenas RL. 1978. Estudio comparativo de la patogenicidad de cuatro cepas de *Trypanosoma cruzi* en el ratón blanco. Rev Latinoamer Microbiol 20: 51-57.

Salazar-Schettino PM, Rojas-Wastavino GE, Cabrera-Bravo M, Bucio-Torres MI, Guevara-Gómez Y, García-de-la-Torre S. Epidemiología de la enfermedad de Chagas en Veracruz. Salud Publica de México. 2005; 47(3): 201-8.

Sánchez-Guille MC, Bernabé C, Tibayrenc M, Zavala-Castro J, Totolhua JL, Méndez-López J, *et al*. 2006. *Trypanosoma cruzi* strains isolated from human, vector, and animal reservoir in the same endemic region in Mexico and typed as T. cruzi I, discrete typing unit 1 exhibit considerable biological diversity. Mem Inst Oswaldo Cruz 101(6): 585-590.

Schmid-Hempel P. 2005. Evolutionary ecology of insect immune defenses. Annu Rev Entomol 50: 529-51.

Schmid-Hempel, P. 2003. Variation in immune response defence as a question of evolutionary ecology. Proc Biol Sci. 270(1513): 357-66.

Schulenburg H, Kurtz J, Moret Y, Siva-Jhoty MT. 2009. Introduction. Ecological immunology. Philos Trans R Soc Lond B Biol Sci 364(1513): 3-14.

Schwenke RA. 2015. Reproduction-Immunity trade-off in insect. Annu Rev Entomol 61: 239-256.

Souto RP, Fernandes O, Macedo AM, Campbell DA, Zingales B. 1996. DNA markers define two phylogenetic lineages of *Trypanosoma cruzi*. Mol Biochem Parasitol 83(2):141–53.

Tay J, Salazar-Schettino PM, Ontiveros D. 1969. Behavior of a strain of *Trypanosoma cruzi* in white mice after various transfer in different species of Triatomas. Rev Latinoam Microbiol Parasitol 11(2): 79-89.

Teixeira A, Gomes C, Lozzi S, Hecht M, de Cassia A, Monteiro P, *et al*. 2009. Environment, interactions between *Trypanosoma cruzi* and its host, and health. Cad Saude Publica 25(1): 32-44.

Texeira AR, Hecht MM, Guimarao MC, Souza AO, Nitz N. 2011. Pathogenesis of Chagas disease: parasite persistance and autoimmunity. Clin Microbiol Rev 24(3): 592-630.

Urdaneta-Morales S, Tejero F. 1992. *Trypanosoma rangeli* (Tejera; 1920): observations upon pleomorphism. Mem Inst Oswaldo Cruz 87: 511-516.

Vallejo, G.A., Guhl, F., Schaub, G.A. 2009. Triatominae *Trypanosoma cruzi/T. rangeli*: Vector-Parasite Interactions. Acta Tropica 110: 137-147.

Waniek PJ, Jansen AM, Araújo CA. 2011. *Trypanosoma cruzi* infection modulates the expression of *Triatoma brasiliensis* defl in the midgut. Vec Born Zoonot Dis 11: 845–7

Whitten M, Sun F, Tew I, Schaub G, Soukou C, Nappi A, Ratcliffe N. 2007. Differential modulation of *Rhodnius prolixus* nitric oxide activities following challenge with *Trypanosoma rangeli*, *T. cruzi* and bacterial cell wall components. Insect Biochem. Molec Biol 37: 440–52.

World Health Organization. Chagas disease: Guidelines for a standard protocol. Geneva: WHO; 1986.

Zingales B, Andrade SG, Briones MR, Campbell DA, Chiari E, Fernandes O, *et al*. 2009. A new consensus for Trypanosoma cruzi intraspecific nomenclature: second revision meeting recommends TcI to TcVI. Mem Inst Oswaldo Cruz 104(7): 1051-4.

Zúñiga C, Binder N, Palau MT, Larenas J, Vergara U. 2012. Edad del hospedero en la evolución de la infección experimental con *Trypanosoma cruzi* en un modelo murino. Rev Ibero-Latinoam Parasitol. 71 (1): 23 – 33.