

Re-emerging threat of *Trypanosoma cruzi* vector transmission in El Salvador, update from 2018 to 2020

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Supplementary Information	Supplementary Information is available at https://doi.org/10.1186/s40249-022-01008-5
Highlights	<ul style="list-style-type: none"> ◇エルサルバドル保健省やエルサルバドル大学等との国際共同研究プロジェクト ◇土壁住宅に住む貧困層にとっての脅威「シャーガス病」対策への画期的な一歩 ◇エルサルバドル全域での媒介昆虫調査により高リスク地域特定を達成、効率的対策に期待
概要	<p>国際共同研究グループは、シャーガス病の原因である媒介昆虫による感染リスクを評価するため、2年間にわたり中米エルサルバドル全域にて調査を行いました。</p> <p>シャーガス病は、WHO が定義する顧みられない熱帯病 (Neglected Tropical Diseases, NTDs) の一つであり、クルーズトリパノソーマ (<i>Trypanosoma cruzi</i>) と呼ばれる原虫に感染している媒介昆虫によりヒトに感染します。主にラテンアメリカで猛威をふるう感染症で、エルサルバドルは流行国の中でも特に感染者が多く深刻な状況であるにもかかわらず、媒介昆虫がどの程度、病原体に寄生されておりヒトへの感染源となっているかについて、十分な調査や報告がなされていませんでした。</p> <p>そこで本研究グループは、エルサルバドル全域で同国保健省や地方自治体等と協力し家屋への立ち入り調査を行い、媒介昆虫の侵入状況や感染率をもとに、リスクの高い地域を層別することができました。</p> <p>本研究成果により、今後特に感染リスクの高い地域を対象とした対策の効率化が期待できます。</p>
Description	<p><研究の背景></p> <p>シャーガス病は、WHO が指定する「顧みられない熱帯病」の一つです。主に世界の貧困層の間で流行しており、ラテンアメリカを中心として世界全域で600~700万人の感染者がいると推定され[*]、媒介昆虫が多く生息する土壁の家が多いエルサルバドルでは人口の約3%が罹患していると言われています。媒介昆虫は夜間などヒトが寝ている間に吸血し、その場で病原体を含む糞をし、それがヒトの体内に入り感染をもたらします。初期には症状が無いことも多く、慢性化した後に重い心臓疾患等を発症すると、治療法が無く死に至ります。</p> <p>主要な感染症対策は、媒介昆虫の排除および新規感染の抑制に注力することですが、エルサルバドルでの定期的な殺虫剤散布などのこれまでの対策は、明確なリスク層別検証を行わず、いわば、やみくもに実施されていました。媒介昆虫対策資源を効率的に配分するためにも、媒介昆虫の侵入と寄生に関する客観的リスク評価が必要とされていました。</p> <p>※WHO の報告による：https://www.who.int/health-topics/chagas-disease#tab=tab_1</p> <p><研究の内容></p> <p>本研究チームはエルサルバドル保健省や地方自治体と協力し、2018年9月から2020年11月まで、エルサルバドル全域で311の家屋を調査し、結果として107の</p>

	<p>家屋において媒介昆虫 <i>Triatoma dimidiata</i> (サンガメ 以下、<i>T. dimidiata</i>) 1,529 匹を採集、それらの糞を顕微鏡で観察し、病原体である <u><i>Trypanosoma cruzi</i></u> (以下、<i>T. cruzi</i>) の検出を行いました。感染が確認された糞サンプルについては DNA を抽出し、PCR 診断を実施しました。</p> <p>調査の結果、<i>T. dimidiata</i> の家屋侵入率は 34% で、<i>T. cruzi</i> の感染率は 10% でした。<i>T. dimidiata</i> はエルサルバドル全域に遍在していましたが、<i>T. cruzi</i> の感染率は地域によって異なり、14 ある地域の中でも特に 5 つの地域で高い感染率が確認できました。</p> <p><期待される効果・今後の展開></p> <p>本研究結果により、特に感染の高かった地域を対象に、継続的・選択的な媒介昆虫対策が可能となり、自治体による効率的な媒介昆虫対策資源の割り当て等への活用が期待されます。さらに本研究チームは、まだ明らかにされていないシャーガス病の病態解明へ向け、寄生虫のゲノム背景と疾病発症との関係について研究を行っています。</p>
<p>資金情報</p>	<p>本研究は、持続可能な開発のための科学技術研究協力 (SATREPS)、日本医療研究開発機構 (AMED、JP 21jm0110016)、および国際協力機構 (JICA) の支援を得て実施されました。</p>
<p>説明動画(Eng.)</p>	<p>https://youtu.be/Kj5_3i0WhSk</p>

‘エルサルバドル現地調査による成果: シャーガス病感染の高リスク地域を特定’. 大阪公立大学 https://www.omu.ac.jp/info/research_news/entry-01767.html. (参照 2022-08-16)

CASE STUDY

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Re-emerging threat of *Trypanosoma cruzi* vector transmission in El Salvador, update from 2018 to 2020

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Abstract

Background: Since the late twentieth century, Chagas disease gained global attention to suppress the vector burden as a main control strategy in endemic countries. In Central America, multi-national initiative successfully achieved significant reduction in the estimated disease prevalence as well as elimination of the region's principal vector species at the time in 2012. While the last decade has witnessed significant changes in ecosystem—such as urbanization and replacement of the main vector species—that can possibly affect the vector's habitation and residual transmission, the up-to-date vector burden in the region has not been evaluated thoroughly due to the cessation of active vector surveillance. The aim of this study was to update the risk of vector-borne *Trypanosoma cruzi* infection in El Salvador, the top Chagas disease-endemic country in Central America.

Methods: A nationwide vector survey was conducted in the domestic environment of El Salvador from September 2018 to November 2020. The selection of the houses for inspection was based on expert purposeful sampling. Infection for *T. cruzi* was examined by microscopic observation of the insects' feces, followed by a species confirmation using PCR. The data were analyzed using R software version 4.1.3. Proportion estimates with 95% confidence intervals were inferred using the Jeffrey's method provided under the epiR package.

Results: A total of 1529 *Triatoma dimidiata* was captured from 107 houses (infestation rate, 34.4%; 107/311) in all the fourteen departments of the country visited within the period; prevalence of *T. cruzi* infection was as high as 10% (153/1529). In the country, domestic *T. dimidiata* infestation was distributed ubiquitously, while *T. cruzi* infection rates varied across the departments. Five out of fourteen departments showed higher infection rates than the average, suggesting sporadic high-risk areas in the country.

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Conclusions: Our comprehensive study revealed substantial *T. cruzi* infection of *T. dimidiata* across the country, indicating potential active transmission of the disease. Therefore, strengthened surveillance for both vector and human infection is required to truly eliminate the risk of *T. cruzi* transmission in Central America.

Keywords: Chagas disease, Vector transmission, Triatomine, *Trypanosoma cruzi*, *Triatoma dimidiata*

Background

In Latin American countries, the infection of *Trypanosoma cruzi* predominantly occurs through the contact with contaminated feces of hematophagous triatomine bugs [1]. The vector burden reduction is considered the most effective strategy for Chagas disease control [2, 3]. Over the past decades, international control initiatives have put great efforts to suppress the domicile vector-borne transmission by combining indoor insecticides spraying, housing improvements and health education in many endemic countries [2].

In Central America, the Central American Initiative for Chagas Disease Control was launched in 1997 in coordination with Pan American Health Organization [4]. This multi-national initiative successfully achieved the certified elimination of *Rhodnius prolixus* in 2012, once considered as a major domestic vector species responsible for human infection [5, 6]. Consequently, the active transmission seemed interrupted in the region considering the reduction of estimated disease prevalence from 7% in the 1998 [4] to 1.3% in 2010 [1, 7]. Once recognized critically endemic, Chagas vector control in Central America is now considered under passive surveillance phase. The surveillance focuses on self-reported vector detection and spraying of residual infesting sites of triatomine bugs [2].

The transition of control program, however, may have resulted in neglecting the expansion of potential vector-borne *T. cruzi* transmission via emergence of a new vector species in the region: *Triatoma dimidiata*. This species was once found most prevalent in the sylvatic areas of Central America but now encountered widely in both rural and urban areas. This contrasts to *R. prolixus*, which was found exclusively in the domestic areas of rural residences [6]. After the elimination of *R. prolixus* from Central America, the main contributor to vector-borne transmission has become *T. dimidiata*. In this study, we aimed to assess current infected vector burden by conducting a nation-wide survey in El Salvador. El Salvador is known to be one of the most endemic countries in the Central American region, with fluctuating seroprevalence around 2% among blood donors from 2001 to 2011 [8].

Methods

Nation-wide survey

Nation-wide active surveillance of the insects in residential areas of El Salvador was conducted between September 2018 and November 2020 by experienced field investigators of Centro de Investigación y Desarrollo en Salud (CENSALUD, San Salvador) and Ministry of Health of El Salvador. Detailed information of survey sites can be found in the Additional file 1. The selection of the houses for inspection was based on expert purposeful sampling of the field investigators. Briefly, area selection for the survey was based on following two components: (1) the materials of the houses were reported to be adobe, mud wall (“bajareque” in local language), or other natural materials and (2) area where infestation of triatomines was reported by residents through the Ministry of Health. The “man-hour” technique was used for insect collection; a total of one hour (or an hour divided by the number of investigators, if more than two) was spent for triatomine inspection [9].

Vector identification and parasite detection

The insects’ species was determined morphologically by experienced entomologists at the CENSALUD laboratory. *T. cruzi* infection of the collected insects was determined by the conventional microscopic examination (under 400×) of insect feces mixed with normal saline solution. The routine surveillance of infected triatomine vector in the country through the Ministry of Health is completed by microscopic examination, mainly due to the shortage of instruments and resources to perform molecular diagnosis. Therefore, in this study, we adopted PCR diagnosis for molecular confirmation of *T. cruzi* species for the samples detected as positive under microscopic examination and a subset of microscopic negative samples to seek for the under-reported risk.

DNA extraction and PCR protocol

DNA extraction assay was performed using DNEasy Blood and Tissue kit (QIAGEN, Germany), following the manufacturer’s procedure provided in the kit. Briefly on tissue preparation, approximately 25 µg of tissue from the back of the triatomine was cut with a leaf of new scalpel for each sample. The cut portion of the tissue was placed in a 1.5 ml tube with 200 µl of tissue lysis buffer (Buffer

ATL, accompanied in the mentioned kit), followed by maceration with a homogenizer (Bio-Gen PRO200 Homogenizer, Pro Scientific, USA).

The PCR was conducted using 121 and 122 primers (121: 5'-AAA TAA TGT ACG GG(T/G) GAG ATG CAT GA-3', 122: 5'-GGT TCG ATT GGG GTT GGT GTA ATA TA-3') targeting specifically the kinetoplast minicircle DNA sequences [10, 11]. Each reaction was performed in a total of 25 µl reaction volume containing 0.5 µmol/L of each primer, 12.5 µl of GoTaq DNA polymerase (Promega, USA), 5 µl of DNA template, and additional distilled water to the final reaction volume. Briefly, the PCR condition was: 3 min at 94 °C for the denaturation, followed by 35 cycles of 30 s at 94 °C, 30 s at 55 °C, and 30 s at 72 °C with a final extension of 10 min at 72 °C. The amplified PCR product was detected at the size of 330 bp in the electrophoresis using 3% of agarose gel.

Data analysis

The data were entered into Microsoft Excel spreadsheets (Microsoft Corp., USA, 2010) and analyzed using R software version 4.1.3 (The R Development Core Team, R Foundation for Statistical Computing, Austria). Proportion estimates with 95% confidence intervals were inferred in defined populations using the Jeffrey's method provided under the epiR package [12]. This is one of the recommended methods for small-size datasets that provides reliable coverage and whose rationale has been described in detail elsewhere [13].

Results

A total of 1529 triatomines were captured in 107 houses and house annexes of all fourteen departments of El Salvador between September 2018 and November 2020. A total of 311 houses were visited during the period, indicating 34.4% of infestation rate. The materials of 107 houses infested with *T. dimidiata* were: adobe (56.1%, 60/107), mud wall (16.8%, 18/107), brick (14%, 15/107), and others (13.1%, 14/107) (See Additional file 2 for more details).

Morphological examination revealed *T. dimidiata* among all the triatomines captured in the study. During the investigation period, 68, 1413, and 48 were captured each year respectively (See Additional file 3 for details of triatomines). 10.0% of *T. dimidiata* (153/1529) revealed parasite positivity under microscopic examination. All microscopic positive specimens and a subset of 130 *T. dimidiata*, randomly picked from 1376 microscopically negative *T. dimidiata*, underwent DNA extraction of the midgut content, and were used as template DNA in the PCR assay. As a result, 100% of microscopic positive specimens revealed molecular confirmation of *T. cruzi*. In the subset, 6.9% (9/130, 95% confidence interval:

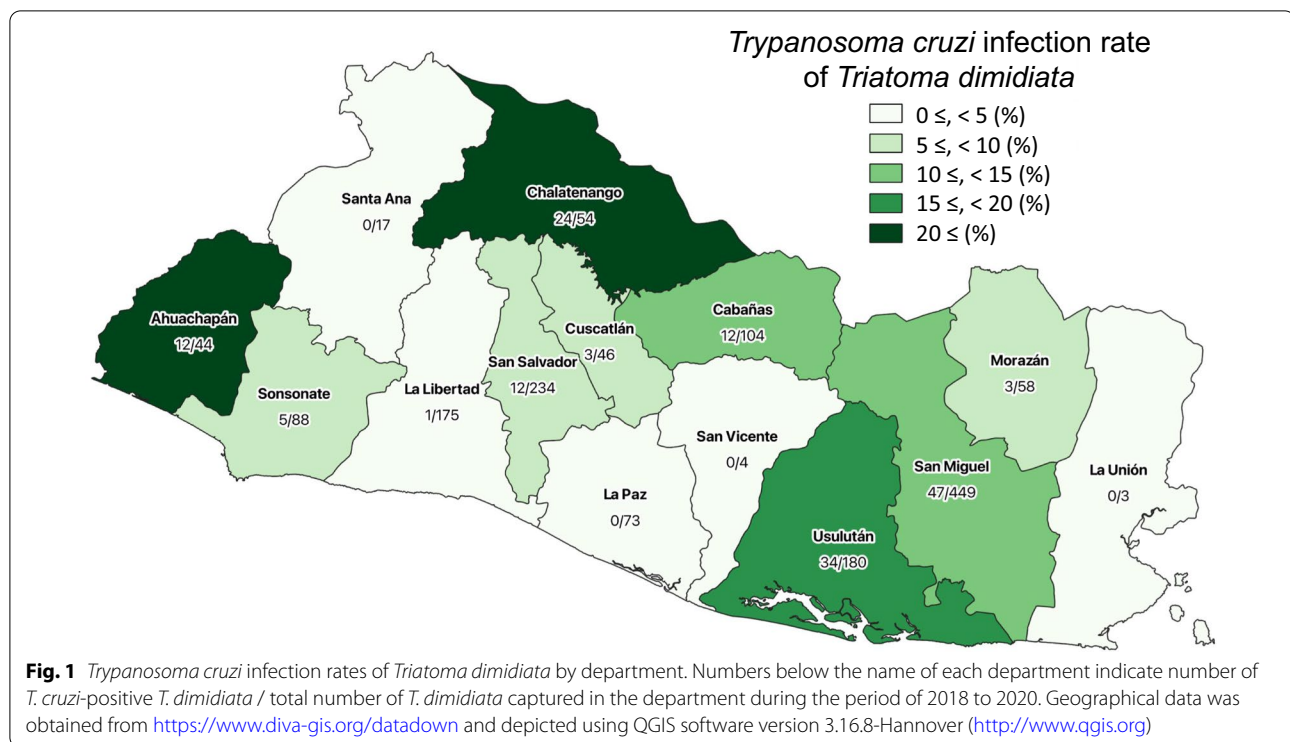
3.7–12.6%), identified positivity for *T. cruzi* by PCR. Considering these results, no less than 10.0% of *T. dimidiata* infested in domestic environment in El Salvador was *T. cruzi* positive by both microscope observation and PCR, indicating persistent vector-borne transmission risk in the region.

Geographical distribution of microscopic *T. cruzi*-positive *T. dimidiata* is shown in the country map (Fig. 1). Five out of fourteen departments showed higher infection rates than average of 10.0%, reflecting sporadic transmission risk in the country.

Discussion

T. dimidiata, the native triatomine species to the Central American region, was once encountered mainly in sylvatic forests [6]. Since the elimination of *R. prolixus*, the former vector species, *T. dimidiata* has enlarged its habitation to domestic areas [5, 6]. As this study warrants, *T. dimidiata* is now observed ubiquitously, whether urban or rural, in the domestic environment of urban and rural areas of El Salvador. Other studies also show that *T. dimidiata*, having adapted to the environment change of urbanization in rural areas, may be responsible for the increase in human-vector contact [2, 14]. After all, the emergence of *T. dimidiata* as the principal vector has become a serious issue in the era of passive vector surveillance. However, the underlying vector burden with the emerging triatomine species, *T. dimidiata*, has not received enough attention. In fact, once there was plenty of reports on the vector prevalence through program-based surveys in the late 1900s, now few investigations focus to evaluate the current situation of vector-borne risk in the Central American region [1, 15]. From the recent available studies conducted partially in two villages in El Salvador, the infestation rate was less than 10% in 2011 [16]. Our active surveillance nationwide resulted in high infestation rate, possibly resulting from the mentioned purposeful sampling method. In this study, we evaluated the triatomine infection by microscopic observation, followed by molecular confirmation using PCR. Although we observed the submicroscopic *T. cruzi* infection in triatomine feces, a few percent of potential PCR positivity in microscopic negative samples might have less impact on the surveillance by the microscopic detection since physiological and pathological significance of submicroscopic infection in triatomines are yet to be defined.

Recent spread of immigrants from endemic countries changed the epidemiology of the disease to an emerging health issue in non-endemic countries as well [17, 18]. Many emigrants travel from Central American countries to non-endemic countries every year. Continued vectorial contamination risk in this region shall



be recognized as potential health issues even in non-endemic countries [19].

Conclusions

In this study, we analyzed the current potential transmission risk of *T. cruzi* via emerging vector of the region, *T. dimidiata*. We conclude that the substantial vector-borne transmission risk still exists in this region; no less than 10% of triatomine bugs are potentially transferable of *T. cruzi* to humans. Therefore, strong surveillance for both vector and human infection shall be required to eliminate the risk of *T. cruzi* transmission in Central America. Understanding the prevalence of the *T. cruzi* infected triatomine bugs in each area is crucial in order to evaluate area-specific risk of vector-borne transmission. With this information, cost optimization of the vector surveillance strategy is possible by targeting the areas with especially high-risk of vector-borne *T. cruzi* infection, which is a significant consideration in low- and middle-income countries limited of human, material, financial and administrative resources.

Abbreviations

CENSALUD: Centro de Investigación y Desarrollo en Salud; DNA: Deoxyribonucleic acid; PCR: Polymerase chain reaction.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s40249-022-01008-5>.

Additional file 1. Details of survey area in this study.

Additional file 2. Wall materials of infested houses observed in this study.

Additional file 3. Details of triatomines captured in this study.

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Author contributions

MSR and YN equally contributed on study design, data acquisition, study implementation, analysis and implementation of data, major contribution to writing, read and approved final version. MC, KS, RG, and AG contributed equally on data acquisition, study implementation, analysis, read and approved final version. Yu Nakagama, YK, and CEA majorly contributed on study conception, study design, study implementation, analysis, implementation of data, substantially revised, read and approved final version. JRP, JER, WM, RCA, and JNS contributed equally on data acquisition, study implementation, read an approved final version. YM, KT, and YO contributed on interpretation of data, read and approved final version. All authors revised and commented on the manuscript and approved the final version. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests to disclose.

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