CICLO CARLOS CHAGAS DE PALESTRAS

10ª EDIÇÃO

100+13: O TEMPO NÃO PARA A INDEPENDÊNCIA DE PRODUÇÃO DE MEDICAMENTO ESPERA PROCLAMAÇÃO

LIVRO DE RESUMOS

IOC
Instituto Oswaldo Cruz

Ministério da Saúde
FIOCRUZ
Fundação Oswaldo Cruz

Ciclo Carlos Chagas de Palestras - 100+13: O tempo não para
A independência de produção de medicamento espera proclamação

Organizadores – IOC/Fiocruz
André Roque, Joseli Lannes, Tania Araújo-Jorge e Rubem Menna-Barreto

Neste ano, em que comemoramos os 122 anos do Instituto Oswaldo Cruz e da Fundação Oswaldo Cruz e também celebramos o bicentenário da Independência do Brasil, o Ciclo Carlos Chagas de Palestras (CCCP) realiza sua 10ª Edição com o tema **"100+13: o tempo não para: A independência de produção de medicamento espera proclamação".**

No terceiro ano da pandemia de Covid-19, já temos vacinas e são conhecidas as medidas não farmacológicas de proteção, mesmo assim optamos por evento em formato remoto o*nline*, que permite acesso de nossas palestras e discussões de forma ampliada. pelo Canal do IOC no Youtube **https://www.youtube.com/canalioc**

Criado em 2013, o CCCP objetiva apoiar o calendário de eventos internacionais na "Semana de Chagas", da Federação de Portadores da Enfermidade de Chagas – FINDECHAGAS. Nos últimos anos, o CCCP se inclui no calendário de celebrações do *"Dia Mundial das Pessoas Acometidas pela doença de Chagas - 14 de abril*", criado pela Assembleia Mundial de Saúde da Organização Mundial de Saúde, 2019, visando maior visibilidade e enfrentamento das necessidades dos portadores desta doença.

As reuniões anuais do CCCP têm sido oportunidades para reunir pesquisadores da Fiocruz e de outras Instituições, nacionais e, muitas vezes, internacionais, criando ambiente propício a interações entre pesquisadores e estudantes e destes com portadores da doença de Chagas, em particular da ***RioChagas***, Associação de Portadores da Doença de Chagas do Rio de Janeiro.

No CCCP22, nossa 10ª Edição, que ocorre em **07 e 08 de abril**, tivemos inscrição de participantes e submissão de resumos via *Campus Virtual da Fiocruz.* Este evento dará oportunidade para discussão sobre os principais achados contemporâneos e os desafios futuros da pesquisa científica em doença de Chagas, sobretudo, no IOC e na Fiocruz, considerando o cenário científico atual no contexto nacional e internacional. Teremos como foco a discussão de temas como a fronteiras do conhecimento da ecologia de Tripanossomatídeos e terapias farmacológicas e não farmacológicas para a fase crônica da doença de Chagas. Por último, abordaremos o gargalo da produção continuada e independente do principal medicamento em uso, Benznidazol, considerando, em particular, a formulação pediátrica.

Esperamos que todos aproveitem o conhecimento aqui apresentado nos resumos de trabalhos e nas palestras e discussões nestes dias **07 e 08 de abril**.

Renovamos uma vez mais as esperanças na força da democracia para mudarmos a nossa sociedade através da educação, da cultura e da ciência e tecnologia. Reafirmamos a necessidade de fortalecimento do nosso Sistema Único de Saúde (SUS), uma necessidade de resposta aos desafios de saúde atuais e futuros." Uma vez mais recorremos à frase mote de Oswaldo Cruz "***Não esmorecer para não desmerecer*"**, que nos guia.

Muito obrigado a todos

André Roque, Rubem Menna-Barreto, Tania C Araújo-Jorge e Joseli Lannes

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10ª EDIÇÃO

100+13: O TEMPO NÃO PARA
A INDEPENDÊNCIA DE PRODUÇÃO DE
MEDICAMENTO ESPERA PROCLAMAÇÃO

Programa Final

Ciclo Carlos Chagas de Palestras - 100+13: O tempo não para
A independência de produção de medicamento espera proclamação

Webinar – CanalIOC do Youtube
https://www.youtube.com/canalioc

Organizadores – IOC/Fiocruz
André Roque, Joseli Lannes, Tania Araújo-Jorge e Rubem Menna-Barreto

07/04

Manhã
9:00h – **Abertura** (falas de 3 minutos)
Presidente da Fiocruz Dra. Nísia Trindade Lima – **a confirmar**
Vice-Presidente de Pesquisa e Coleções Biológicas Dr Rodrigo Correa-Oliveira
Diretora do IOC Dra. Tânia C. de Araújo-Jorge,
Presidente da Associação RioChagas – Sra. Josefa de Oliveira
Organização do CCCP: Dr. André Roque, Dra. Joseli Lannes, Dr. Rubem Menna-Barreto

9:30h-11:30hs
Fronteiras do conhecimento da ecologia de Tripanossomatídeos
9:30hs - 9:50hs - O caráter multidisciplinar da ecologia dos tripanossomatídeos: lacunas e desafios - Dra. Ana Maria Jansen
9:50hs - 10:10hs - Surpresas que as ferramentas moleculares nos têm proporcionado - Dra. Maria Augusta Dario
10:10hs - 10:30hs - Sintaxe espacial da Ecologia dos tripanossomatídeos - Dra. Samanta Xavier
10:30hs - 10:50hs - Revelando incertezas amostrais da ecologia dos tripanossomatídeos - Msc. Raphael Testai, doutorando BCS
10:50hs - 11:30hs - Debate

Tarde
13:30hs – 15:00hs – Parte 1

Terapias farmacológicas para a fase crônica da doença de Chagas do pré-clínico ao clínico
13:30hs – 13:50hs: Multiterapia para a cardiopatia chagásica crônica: estudos pré-clínicos e desafios para cruzar o “vale da morte” – Dra. Joseli Lannes/IOC
13:50hs – 14:10hs: Por que é importante o tratamento etiológico na Forma Indeterminada da doença de Chagas? – Dr. Alejandro Marcel Hasslocher-Moreno/INI
14:10hs – 14:30hs: Selênio na terapia da forma cardíaca da doença de Chagas – Dra. Tania Araújo-Jorge/IOC
14:30hs - 15:00hs - Debate

Tarde
15:00hs – 16:30hs – Parte 2

Terapias não-farmacológicas para a fase crônica da doença de Chagas do pré-clínico ao clínico
15:00hs – 15:20hs: Estabelecimento do modelo de doença de Chagas crônica indeterminada: insights do efeito do exercício físico sobre cardiomiopatia – Dr. Rubem Menna-Barreto
15:20hs – 15:40hs: Determinantes da capacidade funcional e impacto do exercício físico nos aspectos físicos e mentais de pacientes com cardiomiopatia chagásica – Dr. Henrique Silveira Costa/UFMG
15:40hs – 16:00hs: Acolhimento do paciente afetado pela doença de Chagas: muito além do tratamento – Dra. Cristina Carrazzone/PROCAPE/UPE
16:00hs - 16:30hs - Debate

08/04

Manhã
9:00 - 10:00h

Mini-palestras por jovens pesquisadores – **4 resumos** selecionados dos resumos recebidos (8 minutos apresentação e 3 minutos de discussão)

10:00h - 12:30hs – Centro de Estudos do IOC

Um poeta nos visita – Momento de Poesia com Antonio Orlando Nomeriano (5-10min)

Mesa redonda: Em busca da independência de produção de medicamento e da formulação pediátrica
20 min: **título a confirmar** – Dr. Pedro Albajar/OMS
20 min: Acesso ao tratamento para Doença de Chagas no Brasil: perspectivas para a sustentabilidade da oferta de Benznidazol – Dra. Clara Alves/MSF
20 min: Mejora del acceso al tratamiento de la infección por *T. cruzi*: del diagnóstico descentralizado a la búsqueda de nuevas opciones terapéuticas" – Dra. Andrea Marchiol/DNDi e Dra. María Jesus Pinazo/DNDi
20 min: Perspectivas de produção de IFA para produção de Benznidazol na Fiocruz – Dra. Núbia Boechat/FarManguinhos

Debatedores: Dr. Jorge Souza Mendonça – Diretor de FarManguinhos/Fiocruz
Dra. Tania C. de Araújo Jorge – Diretora do IOC/Fiocruz

Momento de Poesia

O AMOR E O AMAR

Amor é tudo receber e o todo dar,
É sentimento puro e permanente,
O que mesmo findo é pra sempre,
Em corações incontidos por amar.

Amor é orar e vigiar, rir de soluçar,
É voltar-se à natureza ferozmente,
Banquete para corações ardentes,
De seres transcendendo por amar.

Amor é subir montanhas a cantar
Agarrado ao descuidado coração,
Sublime contentar-se em só amar

Amor é o perder-se em desvendar
Do fugaz que tanto preza devoção,
Da lucidez e desatino, que é amar.

Antonio Orlando Nomeriano
Deixa eu lhe dizer uma coisa ...
1ª Edição, Rio de Janeiro, RJ - Drago Editorial, 2019

Comissão Avaliadora de Resumos

André Roque / IOC
Andrea Alice da Silva / UFF
Anissa Daliry / IOC
Catarina Macedo Lopes / IOC
Cleber Galvão / IOC
Constança Britto / IOC
Cynthia Machado Cascabulho / IOC
Daniel Adesse / IOC
Daniel Gibaldi / IOC
Fernando Genta / IOC
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Mariana Waghabi / IOC
Marli Lima / IOC
Michelle Barros / IAM
Natalia Nogueira / UERJ
Otacílio da Cruz Moreira / IOC
Otília Sarquis / IOC
Roberto Ferreira / IOC
Roberto Saraiva / INI
Rubem Menna-Barreto / IOC
Solange Lisboa de Castro / IOC
Virginia Maria Barros de Lorena / IAM

Muito obrigado a todos e todas!

Resumos Selecionados para Apresentação Oral

08 de abril - 9:00hs – Centro de Estudos Especial

Abstract 1- Nucleoside analogues as an alternative for the treatment of Chagas Disease: in vitro and in vivo analysis
Ludmila F. de A. Fiuza, Denise G. J. Batista, Roberson D. Girão, Fabian Hulpia, Serge Van Calenberg, Maria de Nazaré C. Soeiro

Abstract 2- Transforming growth factor beta neutralization reduces Trypanosoma cruzi infection and improves the cardiac performance
Roberto Rodrigues Ferreira, Rayane da Silva Abreu, Glaucia Vilar-Pereira, Wim Degrave, Marcelo Meuser-Batista, Nilma Valéria Caldeira Ferreira, Elen Mello de Souza, Joseli Lannes-Vieira, Tania C de Araújo-Jorge, Mariana Caldas Waghabi

Abstract 3- Epidemiological importance of species of the subcomplex Triatoma rubrovaria through analysis of vectorial competence and food source
Thaiane Verly; Stephanie Costa; Jasiel Santos Junior; Nathanielly Lima; Mirian Pereira; Francisco Odencio; Carlos Moreira; Jacenir Mallet; Márcio Pavan; Constança Britto

Abstract 4- Landscape modeling applied to Trypanosoma cruzi transmission cycles in two Chagas disease outbreak areas in Acre, Amazon
Felipe de Oliveira, Ana Maria Jansen, Matheus Pinheiro Ferreira, Samanta Cristina das Chagas Xavier

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Menção Honrosa

Physical exercise promotes a reduction in cardiac fibrosis in chronic indeterminate form of experimental Chagas disease.
Yasmin Pedra-Rezende, Juliana M. Barbosa, Ana Cristina S. Bombaça, Luiza Dantas-Pereira, Daniel Gibaldi, Glaucia Vilar-Pereira, Hílton A. M. Santos, Isalira P. Ramos, Natália L. Silva-Gomes, Otacilio Moreira, Joseli Lannes-Vieira, Rubem F. S. Menna-Barreto

Area: Behavior of hosts

Blood meal source diversity and *Trypanosoma cruzi* lineages infection in triatomines from Caatinga by PCR-Sanger and NGS approaches

Oliveira QM1; Silva MCR1; Iñiguez AM1*

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American trypanosomiasis or Chagas disease (CD) in humans, caused by the protozoan *Trypanosoma cruzi,* occurs in several species of mammals, but the transmission network is also supported by other non-host vertebrates that serve as blood meal sources (BMS) of hematophagous vectors. Methodologies that allow specific taxonomic identification are essential to know the species involved, and their potential role, in the dynamics of transmission. This study aims to investigate the diversity of BMS and *T. cruzi*/DTUs infection in triatomines from the Caatinga biome involved in CD. *Triatoma brasiliensis* (n=49) from Currais Novos municipality, Rio Grande do Norte, were examined. DNA was extracted from the intestinal contents of the insects and PCR, Sanger method (SM), and Next Generation Sequencing (NGS) were applied using 12S rDNA and *cyt*b markers. BMSs were identified in 38/49 insects, including 10 different vertebrate species by SM. Humans, domestic cat and pig, cattle, mouse, were revealed mostly in domestic and peridomestic ecotopes, and 4 different species of wild rodents were identified in the wild. NGS method detected from 39.541 to 66.822 reads by triatomine, contributing with a temporal variable in the local transmission. Comparing a bug specimen fed on a domestic cat, as demonstrated by SM, NGS analysis showed more 8 vertebrate species served as BMS. *Trypanosoma cruzi* infection was verified with 59% of infection rate by TcI and TcII, peridomestic ecotope show higher taxa (44,8%). Cattle and pigs play an important role in the maintenance of triatomines in the peridomicile. Rodents could be considered as a link between the peridomicile and the wild environment, keeping *T. cruzi* transmission close to humans. Sensitivity of NGS makes it possible to identify multiple BMSs demonstrating a succession of events and a possible transmission network of the parasite among possible hosts. The study provides new data for understanding the ecology of CD in the Caatinga biome.

Fomento: CNPq; FAPERJ; CAPES

Area: Cell Biology and Parasite/Host Cell Interaction

Physical exercise promotes a reduction in cardiac fibrosis in chronic indeterminate form of experimental Chagas disease

Yasmin Pedra-Rezende1,2, Juliana M. Barbosa1, Ana Cristina S. Bombaça1, Luiza Dantas-Pereira1,2, Daniel Gibaldi2, Glaucia Vilar-Pereira2,3, Hílton A. M. Santos4,5, Isalira P. Ramos6, Natália L. Silva-Gomes7, Otacilio Moreira7, Joseli Lannes-Vieira2,*, Rubem F. S. Menna-Barreto1,*

1 Laboratório de Biologia Celular, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil, 2 Laboratório de Biologia das Interações, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil, 3 Instituto Brasileiro de Medicina de Reabilitação, Rio de Janeiro, Brazil, 4 Faculdade de Farmácia, Universidade Federal do Rio de Janeiro, Rio de Janeiro,Brazil,5Laboratório de Análise e Desenvolvimento de Inibidores Enzimáticos e Laboratório Multiusuário de Análises por RMN, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil, 6Laboratório de Cardiologia Celular e Molecular, Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil,7 Laboratório de Biologia Molecular de Doenças Endêmicas, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil

Chagas disease (CD), caused by the protozoan *Trypanosoma cruzi*, is a neglected illness endemic in Latin America and affects 6 to 7 million people worldwide. The mechanisms related to disease progression are still unknown. The etiological treatment has limited effectiveness in chronic CD, thus new therapeutic strategies are required. In this context, the practice of physical exercise has been widely advocated to improve the quality of life of CD patients. In the chronic indeterminate form (CIF), the most frequent clinical CD manifestation, the effect of exercise on disease progression is unknown. In this work, we aimed to evaluate in a CIF model, the effect of physical exercise on cardiac tissue. To establish the CIF model, BALB/c and C57BL/6 mice were infected with 100 and 500 trypomastigotes of the Y *T. cruzi* strain. Compared with BALB/c mice, C57BL/6 mice showed lower parasitemia peak, mortality rate, and less intense myocarditis. Thus, C57BL/6 mice infected with 500 parasites were used to the subsequent analysis. At 120 and 150 dpi, reduced heart rate and slight prolonged corrected QT and QRS intervals were detected, which were normalized at 180 dpi, characterizing the CIF. Thus, Y-infected mice were submitted to an exercise program on a treadmill for 4 weeks (from 150 to 180 dpi), with a gradual increase in speed from 6 to 20 m/min five times per week with a 30-60 min daily training session. At 180 dpi, the physical exercise neither worsen clinical parameters of infected mice nor impacted cardiac mitochondrial (n= 8) and oxidative metabolism (n=5), compared with sedentary mice. At 120 and 180 dpi, no differences were observed in the serum cytokine levels (n=8), supporting that a crucial biomarker of systemic inflammatory profile was absent and not impacted by exercise. Trained Y-infected mice (n=5) showed similar parasite load and inflammatory cells but reduced cardiac fibrosis ($p<0.0001$), in comparison with sedentary Y-infected mice (n=6). Therefore, our data support that physical exercise promotes beneficial changes, suggesting that this intervention may prevent cardiac changes induced by *T. cruzi* infection.

Supported by: FAPERJ, CNPq and FIOCRUZ.

Area: Cell Biology and Parasite/Host Cell Interaction

Treatment with benznidazole decreases the parasite load of adipose tissue infected with *Trypanosoma cruzi*

Leyllane R. Moreira[1]; Ana C. Silva[2]; Amanda V. Nascimento[2]; Cíntia N. da C. Oliveira[2]; Victor V. A. de Souza[2]; Michelle da S. Barros[2]; Kamila K. dos S. Oliveira[2]; Diego J. L. Torres[1]; Claudeir D. da S. Júnior[1]; Ana K. A. Soares[3]; Karina L. A. Saraiva[2]; Milena de P. Cavalcanti[2]; Virginia M. B. de Lorena[1,2].
1- (UFPE).
2- (Fiocruz-PE)
3- (FAV).

Adipose tissue (AT) for a long time was considered just an energy reserve, however, it has emerged as a potential reservoir of infection, contributing to the persistence of parasites, such as *Trypanosoma cruzi*, which causes Chagas disease. Since AT acts as a reservoir of infection, the effectiveness of treatment with Benznidazole (Bz) may be questionable, since AT could act as a barrier to the action of the drug, being one of the factors associated with treatment failure. In this context, our study investigated *in vitro* the efficacy of the drug in human adipose-derived stem cells (ADSC), differentiated into adipocytes, infected by *T. cruzi* and treated with Bz, as well as evaluated the immunomodulation caused by the treatment. For this, we performed the differentiation of ADSC in AT, which was submitted to infection by *T. cruzi* and the addition of treatment with Bz (1µg/mL), at times of 24, 48, 72 hours and 4 days. After the treatment time, the AT was removed to quantify the parasite load through real-time PCR and the culture supernatants were collected for the measurement of cytokines (IL-2, IL-6, IL-10 and TNF) by CBA. All the results obtained were submitted to the normality test and then to the Friedman test. Conclusions were taken at a significance level of 5%. The results obtained showed that the parasite load increased over time, reaching its peak between 72h and 4d, nevertheless, in the culture conditions that were treated with Bz, the parasite load decreased. Cytokines were measured after 72h of treatment, and the cytokine IL-6 presented high levels in the culture conditions infected with *T. cruzi*, though the treatment did not change the production of the cytokine in a statistically significant way. Therefore, we suggest that although Bz cannot entirely overcome the infection after 4 days, treatment in infected TA has a beneficial role in reducing the parasite load.

Keywords: Adipose Tissue, *Trypanosoma cruzi*, Benznidazole

Area: Chemotherapy (drugs and etiological treatment scheme)

Drug repositioning in vitro assays of novel drug candidates for Chagas disease

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Chagas disease (CD) caused by the protozoan *Trypanosoma cruzi* is a silent illness that has two clinical phases. In the acute phase, the infected individuals are usually asymptomatic or display flu-like symptoms, and due to their immune response, the parasitism is controlled but not extinguished and they move to the second phase, the chronic stage. In this later phase, 30-40% of the carries may develop cardiac and or digestive progressive alterations resulting in death. The therapy of CD relay on two old nitroderivative drugs that are poorly effective during the chronic stage and cause several side effects, which can lead to treatment abandonment. These factors justify the search for new drug candidates with superior efficacy and safe profile than the existing options. As also faced by other neglected tropical disease, CD receive few supports for drug discovery and development justifying repositioning approaches using drugs already marketed to treat other diseases, aiming to reduce costs and time. In this sense, presently three compounds were evaluated against *T.cruzi* infection in vitro: a benzimidazole (an anthelmintic acting on microtubule polymerization); an imidazole (fungicide acting on lipid biosynthesis and tetracycline (a broad-spectrum antimicrobial agent with antimalarial effect); besides a riminophenazine derivative (inductor of reactive oxygen species (ROS)), respectively named LIDEB-1; LIDEB-5; LIDEB-6 and LIDEB-10. The analysis of the cytotoxicity profile performed in L929 cell lines demonstrated low to moderated toxicity (LC50 values ranging from 27.0+-8.2 to >400uM, while the reference drug (benznidazole – Bz) was >400uM. Next, dose-response assays were performed using L929 cell lines infected with trypomastigote forms of the Tulahuen strain (transfected with the B-galactosidase gene). After 96 h of incubation the results showed that LIDEB-5 gave a very promising effect reaching EC50 values = 0.091 + 0.03 uM while Bz gave 2± 0.4 uM. LIDEB-5 also reached an outstanding selectivity on intracellular forms (SI = 262). These data stimulated further studies that are underway aiming to contribute for a future therapeutic alternative for Chagas disease.

Supported by CNPq, FAPERJ, Fiocruz and Universidad Nacional de La Plata.

Area: Chemotherapy (drugs and etiological treatment scheme)

Experimental combination therapy with benznidazole and amiodarone in a mouse model of chronic *Trypanosoma cruzi* infection.

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7Laboratório de Cardiologia Celular e Molecular, Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil.
8 Laboratório de Biologia Molecular de Doenças Endêmicas, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil.

Chagasic chronic cardiomyopathy (CCC) is the main clinical manifestation of Chagas disease (CD) caused by *Trypanosoma cruzi*. Etiological treatment of CD is limited to benznidazole (**Bz**) and nifurtimox, which have a high percentage of treatment failure among patients with chronic CD. Amiodarone (**AMD**) is the safest and most effective antiarrhythmic drug used to treat CCC and its association with **Bz** led to a reduction in hospitalizations and risk of death related to cardiovascular events. However, the exact mechanisms of action of **AMD** on CCC are poorly understood, as well as its interaction with drugs used in the etiological treatment of CD, which is the gap that this project aims to fill. In this study, we tested the *in vivo* effects of **Bz** and **AMD** in a preclinical mouse model of CCC, using female C57BL/6 mice infected with *T. cruzi* of Colombiana. Thus, Colombiana-infected mice were submitted to oral treatment for 30 consecutive days (from 120 to 150 dpi) in monotherapy and in combination. The trypanocidal effect of treatment was evaluated by Pizzi-Brener and RTqPCR methods. In addition, we evaluated the cardiac function of animals, using the electro-echocardiogram and the reduction of inflammation and fibrosis in cardiac tissue, through histopathology and quantification of cytokine levels by flow cytometry with the CBA kit (BD). We find that the combination of **Bz/AMD** reduced parasite burden and parasitemia in the same rate of the **Bz** in monotherapy (**Bz** versus **Bz/AMD**; $p>0.05$). The group treated with **Bz/AMD** was the only therapeutic regimen tested that was able to significantly reduce the concentration of TNF in cardiac tissue and plasma, compared to infected and untreated control group ($p<0.05$). Consistent with the reduction of TNF, we also observed in cardiac tissue (**a**) a reduction in the deposition of fibronectin and collagen, (**b**) an increased integrity of gap junctions, and (**c**) a reduction in the production of reactive oxygen species (ROS) in *T. cruzi*-infected mice treated with **Bz/AMD**. In addition, **Bz/AMD** improved ventricular function in infected and treated mice, as this evidenced by the increase in ejection fraction and left ventricular systolic output, since there was no statistical difference between **Bz/AMD** group and uninfected control group ($p>0.05$). In conclusion our findings study highlights that **AMD**, alone or combined with **Bz**, could be a potential candidate for repositioning and combining drugs to achieve a more efficient therapeutic protocols in the management of CCC patients.

Financial support: FAPERJ, CAPES e CNPq

Area: Chemotherapy (drugs and etiological treatment scheme)

Experimental validation of *in silico* analysis findings regarding pharmacokinetic properties of Imatinib derivatives identified as possessing *in vitro* anti-*Trypanosoma cruzi* activity

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According to the World Health Organisation, Chagas disease (CD) affects over 6 million individuals worldwide and belongs to the group of neglected tropical diseases, lacking research funding and manpower. Since current treatments are lacking both in efficacy and safety, new drugs are urgently needed but since it mainly affects impoverished populations, it still attracts low investments from the main pharmaceutical companies. To circumvent these limitations, drug repositioning has been approached. This strategy consists of testing drugs already employed in the treatment of other illnesses in preclinical assays on the protozoan CD agent, *Trypanosoma cruzi*. Once a promising drug is identified, its molecular structure is refined producing derivatives/analogues which are further tested to determine whether the molecular alteration has yielded a positive outcome. A previous study using derivatives of Imatinib (IMB), a drug used in the treatment of myeloid leukaemia, motivated the synthesis of novel 23 derivatives to be screened against *T. cruzi*. In parallel to the *in vitro* testing, *in silico* analysis was also performed using the Swiss ADME platform. However, since *in silico* results are not directly linked to empirical data we took the opportunity to validate some of the *in silico* findings by evaluating their solubility in 2:1 serial dilutions (from 400µM to 10µM) using cell culture medium as a vehicle. These solutions were observed under light microscopy to ascertain the presence of precipitate, which would classify them as insoluble at a certain concentration. However, if the precipitate had not sedimented after 24h of incubation at 37°C, the compound was considered “virtually soluble”. The Swiss ADME platform works with 3 different algorithms which estimate solubility. While all 3 models underestimated the solubility of the compounds, the ESOL algorithm produced closer results. The Swiss ADME platform also has 6 different algorithms to estimate the partition coefficient. As there is an inverse relation between the partition coefficient and water solubility, these algorithms were also compared to the experimental results. Our findings showed that the algorithm which displayed the closest relation with the observed experimental data was iLOGP. In addition, a qualitative analysis of the electrostatic surface map of these compounds using the program “Avogadro” also gave evidence of a positive correlation between the presence of electron removal groups, particularly halogens of low atomic mass, and an increase in solubility of the tested compounds.

Supported by: FIOCRUZ, CNPq, FAPERJ, CAPES

Area: Chemotherapy (drugs and etiological treatment scheme)

Nucleoside analogues as an alternative for the treatment of Chagas Disease: *in vitro* and *in vivo* analysis

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Considered a serious public health problem and listed among the 20 neglected tropical diseases, Chagas disease (CD) described by Carlos Chagas more than a century ago, is caused by the protozoan *Trypanosoma cruzi*. About 6 million individuals are infected, however only 1% have access to the available treatments, which are restricted to two old nitroderivative drugs, Benznidazole (Bz) and Nifurtimox. Both require long-term treatment, produce serious adverse effects, in addition to low efficacy in the late chronic phase. Therefore, experimental chemotherapy studies are essential to identify accurate methodologies and more selective and efficient alternative therapies. Nucleoside analogues represent a promising therapeutic option given the parasite's inability to perform de novo purine biosynthesis, requiring hosts elements. Data from our group demonstrate the excellent antiparasitic activity *in vitro* (submicromolar scale) confirmed by *in vivo* proof-of-concept of some nucleoside derivatives. Thus, the present work aims to follow phenotypic studies with these compounds, using different parasite forms and strains, under different culture mammalian cells matrices (2 and 3D), following *in vivo* analysis. Nucleotide analogues named FH11706, FH8513 and FH10714 were screened *in vitro* on intracellular forms (Tulahuen strain in L929 cell lines) and the data showed potent antiparasitic activity with EC_{50} between 0.2 – 1.1 µM, in addition to selectivity indexes >100 for all tested compounds. These results stimulated further studies on the other form relevant to human infection – bloodstream forms (strain Y). The findings revealed EC_{50} values ranging from 3.9 - 13.13 µM. In view of the excellent activity of the compounds in 2D cell culture model, further analysis was performed on a 3D matrix using cardiac spheroids infected with *T.cruzi* (Y strain). The light microscopy quantification of the parasite load showed that after 168h of incubation, the analogues at 10 µM (corresponding to EC_{90} of Bz) greatly reduced the spheroid parasitism reaching 94% of decline. The promising *in vitro* results justified *in vivo* analysis under a mouse model acute *T.cruzi* infection (Swiss male mice – 18/ 20g – infected with 10_4 bloodstream forms of Y strain, n ≥ 3 per group). The data revealed that oral treatment with 25 mg/kg FH11706, FH8513 and FH10714 (1x/day – 5 days) suppressed the parasitemia peak at levels comparable to Bz, without showing noticeable animal toxicity up to 50mg/kg. New assays will be conducted *in vitro* and *in vivo* to further evaluate the activity of nucleoside analogues in combination with Bz, in addition to studies using mixed parasite infection, and thus contribute to the identification of new therapies for CD.

Supported by: Fiocruz; CNPq; CAPES and FAPERJ.

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Area: Chemotherapy (drugs and etiological treatment scheme)

PHENOTYPIC AND PHARMACOLOGICAL STUDIES OF NEW NITRO COMPOUNDS UPON TRYPANOSOMA CRUZI

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Chagas disease (CD) affects more than 6 million people worldwide, mainly in endemic areas of Latin America but due to globalization, it has spread across European countries, Asia and North America, with an average of 14 thousand deaths per year. CD has been identified for over a hundred years, but pharmacological developments are long overdue. Benznidazole (Bz) and nifurtimox, two old nitroderivatives introduced at the clinic more than 50 years ago, are the only available drugs but both induce side effects and present low efficacy for the later chronic phase, justifying multidisciplinary partnerships to develop more safe and active therapies. In this context, two compounds, a nitro derivative (AI/L2/65) with dual mode of action - acts as a prodrug by releasing the toxic nitro free radicals, also an inhibitor of ergosterol synthesis and a sulfonamide derivative (AI/L3/34) an inhibitor of ergosterol synthesis – were assayed against different Trypanosoma cruzi strains belonging to distinct parasite DTUs (Y, Tulahuen strain, DTU II and VI, respectively). Also, their toxicity against mammalian cells was assessed besides some pharmacological aspects by in silico, in vitro and in vivo studies. No toxicity profile upon L929 cells was noticed up to 400 µM. The findings showed high activity against intracellular forms with EC50 values ≥ 1 µM. Against bloodstream trypomastigotes both displaying EC50 >25 µM. In silico analysis corroborated in vitro tests demonstrating plasma (> 180 min) and cytosolic (> 90 min) metabolic stability in mice liver cells (1 and 6 mL / min / g, respectively). Permeability tests showed that both compounds have also good permeability profile in Hep G2 cells (> 100 µM). In vivo murine models showed T max of 2 h and T ½ of 271.98 min. Our findings suggest the promising effect of nitro compounds in order to contribute to the development of new drugs for CD.

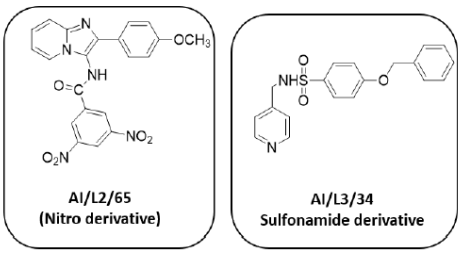

Figure 1: Molecular structures of compounds assayed against different strains of Trypanosoma cruzi.

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Area: Clinical Aspects

Clinical and epidemiological profile of the digestive form of Chagas disease in a cohort of patients followed up at the Evandro Chagas National Institute of Infectious Diseases (INI) - Fiocruz

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Introduction: The digestive form (DF) of Chagas disease (CD) is the clinical form of chronic CD characterized by the presence of megaesophagus and/or megacolon. Among all the clinical forms, DF is the least frequent and, according to the region and population characteristics, can vary from 9 to 41%.

Objective: To identify the prevalence, epidemiological, clinical characteristics, and mortality of patients with DF of CD regularly followed in the INI-Fiocruz.

Method: Retrospective observational study, consisting of patients with CD followed up at outpatient center at INI-Fiocruz, from July 1986 to December 2021.

Results: Among 2,201 patients, 253 (11.5%) had DF during their admission at INI-Fiocruz (baseline). Of those, 152 (60.1%) had megaesophagus, 57 (22.5%) had megacolon, and 44 (17.4%) had both syndromes. Most cases were from the states of Bahia (n=72; 28.5%) and Minas Gerais (n=51; 20.2%). Patients with DF were older (56.8+12.3 vs 46.9+12.4; p<0.001), with a greater percentage of women (61.3% vs 51.3%; p=0.003), non-white race (60.5% vs 49.3%; p=0.001), and illiterate (27.3% vs 19.7%; p=0.005) in comparison to those without DF. No differences could be observed between the transmission routes of Chagas disease nor the origin of patients from endemic areas while comparing carriers with and without DF. There were 77 new cases of DF during the follow-up (48 [62.3%] women and 29 [37.7%] men), totalizing 330 DF cases. The cumulative incidence of DF was 4.0%. The mean baseline age of new cases was 48.3+10.9 years. From the total 330 DF cases, there were 77 (23.3%) losses during the follow-up period and 119 deaths (36.1%). Sixteen deaths were directly related to DF (13.4%), 5 (4.2%) by megaesophagus and 11 (9.2%) by megacolon.

Conclusion: Megaesophagus was the most prevalent clinical presentation of DF in the INI-Fiocruz cohort. Older age, women, non-white race, and illiteracy were more frequent in patients with DF in comparison to those without DF. Megacolon was the main cause of death from DF. Strategies aiming to cope the morbidity and mortality of DF should be included for improvements in clinical outcomes and quality of life of patients with CD.

Area: Clinical Aspects

Indeterminate Form of Chagas Disease

Alejandro Marcel Hasslocher-Moreno (INI-Fiocruz)

The indeterminate form (IF) of Chagas disease (CD) is constituted by a latent period that begins soon after the end of the acute phase. It is the most prevalent clinical form of CD and is characterized by the absence of symptoms and normality on physical examination, chest radiography, electrocardiogram (ECG) and contrast-enhanced radiological examinations of the esophagus and colon. Although a normal ECG is an essential criterion in the classic definition of IF, some patients may have nonspecific electrocardiographic changes that do not define chronic Chagas' heart disease (CCHD). Thus, the isolated presence of: sinus arrhythmia; QRS electrical axis deviation to the left; low QRS voltage, secondary ventricular repolarization change; sinus bradycardia ≥40 beats/min; sinus tachycardia; left anterior hemiblock; first-degree right or left branch block; first-degree atrioventricular block; single ventricular extrasystole; and migratory pacemaker may be present in individuals with IF. Other complementary propaedeutic methods, such echocardiogram (ECHO); exercise stress test; 24-hour Holter; non-invasive autonomic tests; cardiac scintigraphy; hemodynamic studies; magnetic resonance imaging; and even endomyocardial biopsy, can show changes that do not mischaracterize the IF, since healthy individuals, without *Trypanosoma cruzi* infection, can present the same pattern.

Individuals with IF may evolve to CCHD. Many factors are involved in the risk of progression, including age, male gender, geographic origin, parasite load, *Trypanosoma cruzi* strain and its “discrete typing units” (TcI–TcVII), genetic aspects of the host, the severity of the initial acute infection, the exposure to reinfection in areas with sustained vector transmission, nutritional status, presence of comorbidities, social context, the quality of life of individuals, among other aspects. IF generally has a good prognosis, with mortality equivalent to that of the general population. The follow-up of IF individuals should be maintained at the primary care level and annual ECG is recommended to detect any progression to CCHD, which is estimated at around 1.9% per year. In IF, the presence of altered ECHO can mean risk for cardiovascular events, translating into a worse prognosis when compared to individuals with normal ECHO.

Regarding the etiological treatment with trypanocidal drugs, it is strongly recommended in carriers of IF and young treated adult patients progress less to CCHD when compared to untreated patients. Patients with IF usually have comorbidities that become more frequent as they age. Systemic arterial hypertension, dyslipidemia, diabetes and, less frequently, coronary artery disease predominate. The control of these diseases may prove to be fundamental for the secondary prevention of CCHD.

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Area: Clinical Aspects

Laboratory-acquired Chagas disease: a case report

Alejandro M. Hasslocher-Moreno (INI-Fiocruz)

Introduction: Accidental infection by *Trypanosoma cruzi* (*T. cruzi)* can occur through different ways, including during the use of sharp instrument while handling of infected animals in research laboratories.

Objective: To describe the evolution of a case of accidentally acquired Chagas disease (CD).

Clinical Description: On August 31, 2020, a 46-year-old woman sought the service of INI-Fiocruz for suspected *T. cruzi* infection. She reported that on August 11, during a laboratory procedure. She had a needle stick injury in the right hand while handling a *T. cruzi*-infected animal. She did not report bleeding or pain at that time. Thirteen days later, she noticed the appearance of an erythematous macula in the right palm and started to present intense asthenia, generalized myalgia, headache, fever, and pain in the right axillary hollow. Physical examination showed that she was in good general condition but with an “inoculation chagoma” in the palmar region. Benznidazole (BZN) 400mg daily was started immediately.

Laboratory Tests: Direct search for *T. cruzi* in blood smear was positive; Initial serology for CD showed non-reactive ELISA (index reactivity [IR] - zero), non-reactive chemiluminescence (ChLIA), negative immunochromatographic assay (ICA) and indirect immunofluorescence (IFI) = 1/80. Hemogram showed normochromic and normocytic anemia, low hemoglobin (11.5 g/dl) and hematocrit (32.9%), leukopenia (total leukocytes/mm^3 - 2550), and thrombocytopenia (platelets/ mm^3 - 79,000). Increased liver enzymes (AST- 115 U/L, ALT-106 U/L). High ferritin levels (1000 ng/ml), and normal troponin.

Cardiological Exams: Electrocardiogram presented sinus tachycardia. Echocardiogram and Holter-24h were normal.

Follow-up: The patient evolved with prompt remission of symptoms and vanishing of the skin lesion. She completed 70 consecutive days of BZN with no adverse reactions. Direct search for *T. cruzi* by light microscopy was negative on the third day of BZN use. Weekly serial serologies showed that Elisa seroconverted (RI = 2.3) on the 34th day of etiological treatment, reaching the highest IR value on the 54th day, when these values decreased, becoming negative in the sixth month after treatment. IFI increased the titers (1/1280) on the 34th day of treatment, maintaining high titers until the 62nd day post-treatment. Unfortunately, we were unable to perform the IFI until the end of the follow-up. ChLIA and ICA remained unreactive throughout.

Conclusion: The patient progressed satisfactorily with a dramatic positive response to BZN, achieving parasitological, serological, and clinical cure within 6 months. Elisa and IFI proved to be valid for diagnosis and post-treatment monitoring, while ChLIA and ICA were not able to detect acute CD.

Area: Clinical Aspects

Prevalence between blood group systems and clinical forms of Chagas disease

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Introduction: Tissue immunogenetic markers may influence *Trypanosoma cruzi* tropism for different organs. ABO and other blood group systems express a variety of tissue carbohydrate antigens that influence the susceptibility or resistance to diseases.
Objective: To evaluate the association of blood group systems and the clinical forms of Chagas disease (CD).
Methods: Descriptive cross-sectional study which included patients with CD followed at the outpatient clinic of the INI-Fiocruz, between 2013-2016. Information about red blood cell phenotyping (ABO, Rh, Kell, Kidd, Duffy, MNS), clinical forms of CD (indeterminate, cardiac, digestive, and mixed), and demographic data were collected. The comparison of categorical variables according to the different groups was performed using Pearson's chi-square test with Yates' correction and chi-square test for trend.
Results: A total of 619 patients (60.1±12.1 years old, 56.9% female) were included in the study. Most patients younger than 45 years old were born in the states of Ceará (36.5%), Paraíba (18.9%) and Rio de Janeiro (12.2%), while most of the patients older than 65 years old were born in the states of Bahia (30.0%), Minas Gerais (25.0%) and Pernambuco (16.8%). The distribution of the ABO blood system types differed between patients with the indeterminate and digestive forms (p=0.008), with regard to group B and AB. There were no differences in the other blood system types.
Conclusion: When considering the differences in erythrocyte phenotypes found in different studies, it is important to highlight that the population of Brazil is one of the most heterogeneous in the world. Considering this scenario, in this study, the phenotypic frequency for the ABO blood group system is in agreement with previously published data for the blood donor population in Brazil. Except for the comparison between patients with indeterminate and digestive forms, in relation to the ABO blood system types (B and AB), there is no evidence that the prevalence of different blood group systems is different between the clinical forms of CD.

Area: Clinical Aspects and genetic polymorphisms

Influence of angiotensin converting enzyme I/D gene polymorphisms in progression of Chagas' heart disease in a cohort of Brazil

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Chagas disease (CD) is a neglected endemic disease that affects about 7 million individuals worldwide. It is caused by the protozoan parasite *Trypanosoma cruzi.* A few decades after infection, 30% of patients develop chronic Chagas cardiomyopathy (CCC), with electrical changes and heart failure (HF) in 10% of cases. It is suggested that the severity of CCC is related to (i) parasite persistence, (ii) histopathological and functional changes in the heart, and (iii) systemic inflammatory profile. Moreover, alterations in the expression of the renin-angiotensin-aldosterone system components have been shown in CCC. A functional insertion (I)/deletion (D) polymorphism of the angiotensin-converting enzyme (ACE) gene was associated with plasma enzyme activity and angiotensin II generation. Knowing that ACE inhibitors are used in CD and have beneficial effects, we can suggest the impairment of this axis in Chagas cardiomyopathy. In the present study, we evaluated the ACE rs4646994 I/D polymorphism in the development of HF, performing a case-control study in a study group of 402 patients residing in Northeast Brazil with positive serology for CD. Genotyping was performed by PCR. The groups were compared using unconditional logistic regression analysis and adjusted for non-genetic covariates for age, sex, and trypanocidal treatment. Patients were classified as non-cardiac (stage A; 109) and mild (stage B1; 161) and severe (stage C; 132) forms of Chagas' heart disease. HF was defined using Framingham criteria with two major or one major and two minor criteria and left ventricular ejection fraction (LVEF), which was determined by a physician blinded to the protocol number, using Vivid 3. Patients with ACE D (C vs A: OR = 1.9; P-value = 0.08; C vs A + B1: OR = 1.6; P-value = 0.12) were not associated with Chagas' heart disease. However, we saw better LVEF in patients with genotype II when compared to DD and DI genotypes. We emphasize that LVEF is an important prognosis factor in CCC and one of the markers to assess HF. Therefore, genetic variants need to be further explored and may contribute to a better management of patients with CD.

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Area: Diagnosis

Association of classical and molecular techniques for therapeutic follow-up in Chagas disease

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Caused by the protozoan Trypanosoma cruzi, Chagas disease (CD) affects millions of people worldwide. Laboratory techniques for diagnosis are dependent on the clinical stage. In cases of infection in the chronic phase, the diagnosis is made using techniques that detect IgG anti-*T. cruzi*. However, immunological methods do not allow monitoring therapeutic failure. Thus, molecular methods emerge as alternatives for diagnostic confirmation and a tool for therapeutic follow-up. The aim of this study was to follow, by real-time PCR (qPCR), 26 individuals treated for CD during the acute phase of the infection. The individuals were considered cases of acute infection, according to laboratory and/or clinical-epidemiological diagnostic criteria recruited after an outbreak of CD in Ibimirim city and were followed for approximately 24 months after treatment. The TcSAT-IAM system, developed at SRDC/IAM/Fiocruz-PE to *T. cruzi* nuclear DNA target, was used in association with classical serological monitoring techniques. After 24 months of treatment, 14 (53.84%) were reactive for IgG anti-*T. cruzi* and, of these, 12 (85.71%) had decreased antibody levels when compared to titers before treatment. As for the qPCR performed approximately 2 months after exposure to the parasite, of the 26 individuals, 12 (46.15%) had samples collected before or up to 3 days after the start of treatment and had a parasite load detectable by the TcSAT-IAM system. All the other patients already had, on average, 22 days of treatment when they had samples collected and directed to molecular diagnosis. Two months after treatment, all subjects tested negative for the detection of *T. cruzi* DNA. Approximately 24 months after exposure to the outbreak, only 1 (0.71%) individual tested positive for the detection of *T. cruzi* DNA. This demonstrates the relevance of using qPCR for diagnosis and therapeutic follow-up in CD, together with classical techniques, reinforcing the importance of correlating the results of different diagnostic methodologies.
Keywords: *Trypanosoma cruzi*; Diagnosis; Real time PCR.

Area: Education/Information

Talking about Chagas disease with ArtScience: the Internacional Symposium of Science, Art and Citizenship 2021

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The Science, Art and Citizenship Symposium has been taking place since 2002, involving different themes, one of which is Chagas Disease, whether in scientific theater, videos or lives. During the pandemic, the modality of the Symposium is in a virtual way, so for the visibility of the Chagas Disease, an international Symposium was organized, focusing on education and information activities about Chagas disease in May 2021, with an international focus of the experiences in Bolivia and Spain: Communities and Science in Iberoamerica. We created a google forms for the registrations and performed analysis using the Youtube analytics tool. We obtained 36 formalized registrations, 30 from Brazil, 5 from Spain and 1 from Nicaragua. 72.8% of the audience were master and doctorate students, 8.3% researcher, 8.4% high school teacher, 2.8% elementary school teacher, and 8.3% higher education teacher. The motivation to participate in the Symposium presented by them were: knowledge of the Art Science group in Brazil and the development of international actions talking about Chagas disease and, the development of actions in indigenous communities. The Symposium activity was performed live on the Science, Art and Citizenship Network Youtube Channel. We received a total of 308 views, almost three times more than the total capacity of the Emmanuel Dias auditorium at Fiocruz. 80% of the audience were female. 61.6% subscribed to the channel. The audience age range was 45 to 64 years. We use only organic dissemination with 75% coming from whatsapp and 17% from institutional e-mails. We observed importantes actions in research: a qualitative research carried out in Bolivia that resulted in a Art Science video called "A Vida de Severina" and, actions in teaching, research and extension: the “Art Science Research Project” focused on transnational social space developed about Chagas "Barcelona La Caixa Living Lab" involving multidisciplinary team and activities with Coalision Chagas presentation, which was created in 2012 with a group of 6 partners, ISGlobal, CEADES, DNDI, Mundo Sano Foundation, Baylor College of Medicine and Texa's Children Hospital that come together to provide access to comprehensive care with emphasis on diagnosis and treatment, expanding the avenues of communication and visibility of the Chagas disease and affected people. Taken together, our results show the current scenario of Art science actions in Chagas disease research and extension and, the importance of creating opportunities to provide visibility to these activities in Brazil and other countries, connecting those who are involved in the cause.

Keywords: Chagas Disease; Symposium on Science, Art science, Citizenship.

Funding: IOC (Instituto Oswaldo Cruz) e CAPES.

Area: Education/Information

The FluorArte Workshop adapted to the virtual environment to discuss Chagas disease during the COVID-19 syndemic

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Chagas disease is a neglected disease caused by *Trypanosoma cruzi* in which the absence of symptoms in 70% of infected people makes it invisible. Educational strategies on this subject are necessary, however, during the COVID-19 syndemic, face-to-face actions developed by our laboratory were interrupted in compliance with health standards. FluorArte Workshop is an activity that integrates art and science for education in non-formal spaces of education, health promotion and Chagas disease scientific dissemination. FluorArte Workshop was presented face-to-face in 5 cities in Minas Gerais (Brazil) engaging 435 participants and during the COVID-19 syndemic it should be adapted to the virtual modality. Here, we describe the methodological challenge of transforming the workshop entitled FluorArt to the remote version. FluorArt's conception was based on the ArtScience strategy, inspired by the fluorescence microscopy technique, and promotes the observation of images related to Chagas' disease obtained from scientific works. The workshop was based on: dialogue; prior knowledge; creativity; and the process of building new knowledge by the participants. FluorArte Workshop were adapted to the virtual environment following the steps: i) register the participant's personal data on the site; ii) drawing one of the six images in a virtual wheel and answering the question "Observe the drawn image and share what do you think that it could be?"; iii) carrying out the activity of finding the painting that corresponds to the images and iv) viewing a poster with all the explanations of the images. The wheel with the images and the activity of finding the corresponding painting were created for free on the website "Wordwall". The website has not been officially launched, but it has been available for testing on the internet since November 2021. The page was accessed by 99 people in 2021 and the workshop was tested by 22 people. The face-to-face activity was validated regarding the relationship with ArtScience, aligning it with the thirteen cognitive categories for the development of creative capacity and we also intend to validate the virtual workshop. Preliminary qualitative results showed that the FluorArt virtual version is being well accepted by the participants and we strong believe that it will contribute to education, scientific dissemination and, health promotion in Chagas disease in the context of the COVID-19 syndemic.

Financial Support: FIOCRUZ, CAPES and CNPq

Area: Epidemiology

Challenges for incorporating Chagas disease into primary health care routines in the northeastern semi-arid region

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The semi-arid region of northeastern Brazil is the dispersion epicenter of some vectors of Chagas disease, representing one of the regions with the highest prevalence rates in a global scale. In this region, chemical vector control is challenged by the existence of wild stocks of vector insects and continuous post-treatment reinfestation. Our recently published serological and entomological surveys carried out in the southeastern region of the state of Piauí, in the semi-arid region, did not identify positive tests in subjects under 30 years old, despite high frequency of intradomiciliary colonization with *Triatoma brasiliensis*. Nevertheless, seroprevalence rates in the region increases in parallel with age, reaching more than 35% in persons over 60 years of age. These data demand the immediate incorporation of Chagas disease into primary health care routines within the scope of the Family Health Strategy, with the objective of allowing access to serological diagnosis and the initial classification of the disease stage and clinical form, including identifying subjects in which the use of the trypanosomicidal drug benznidazole would be indicated. We are working with the local administration and with the Central Public Health Laboratory (LACEN – Piauí) to sensitize health workers to include Chagas disease testing in all patients who undergo routine laboratory tests, with emphasis on those enrolled in the diabetes mellitus and hypertension programs. A referral flow for secondary and tertiary complexity health care also needs to be implemented.

Area: Epidemiology

Socio-epidemiological factors and comorbidities associated with Chagas disease manifestations in two urban reference health care centers in Rio de Janeiro, Brazil

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Chagas Disease (CD) is considered a neglected tropical disease that remains as an important public health issue in Latin America. This study aims to analyze the association between socio-epidemiological factors and comorbidities with clinical manifestation of CD among patients from two reference healthcare centers in the city of Rio de Janeiro, Brazil.
This is a cross-sectional study carried out between 2016-2020. Participants with confirmed CD diagnosis from both sexes aging > 18 years old were eligible to participate. Data collection was based on multidimensional questionnaire that included socio-epidemiological, demographic, and clinical variables. Outcomes were CD clinical forms (indeterminate, digestive, cardiac and cardio-digestive forms) and the stages of the cardiac form classified according to the II Brazilian Consensus on Chagas Disease. Statistical analyses were based on univariate and multivariate logistic regression performed at IBM SPSS (v.23).
A total of 985 patients (65±11 ys; 59.5% women) were included. Higher age and Brazilian birth states (Minas Gerais and Bahia) were independently associated with greater chance of CD cardiac form. Higher age and men were independently associated with greater chance of digestive form. Patients with arterial hypertension and diabetes were less likely to have the digestive form when compared to patients without arterial hypertension or without diabetes, respectively. In terms of CD severity, men had a greater chance of having more severe cardiac presentation than women. Those from Minas Gerais and Bahia states had a greater chance to have B1 or B2 stages. On the other hand, patients with dyslipidemia were less likely to have the cardiac stages C or D and follow-up length showed to be shorter for those patients with more severe cardiac stages (C or D) of CD.
Our results allowed concluding that some characteristics could play an important role as social markers on the clinical manifestation of CD and reinforce its epidemiology dynamics and complexity. Research in CD needs to consider the particularities of the affected population, its environment and migratory history which are crucial information for directing public health policies, resource allocation, development of prevention and control strategies, and specialized care for this group.

Area Therapy (immunotherapy, cellular therapy and others)

Transforming growth factor beta neutralization reduces *Trypanosoma cruzi* infection and improves the cardiac performance

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Chronic Chagasic cardiomyopathy (CCC), a progressive inflammatory and fibrosing disease, is the most prominent clinical form of Chagas disease, culminating in heart failure and high rates of sudden death. During CCC, the parasite remains inside the cardiac cells, leading tissue damage, involving extensive inflammatory response and irregular fibrosis. Some molecules act in the fibrosis development, but one in particular plays a key role in the fibrogenic process inducing extracellular matrix synthesis: the transforming growth factor-β (TGF-β). TGF-β is also involved in the development of Chagas cardiomyopathy with increased serum levels of this cytokine and activation of its signaling pathway in the cardiac tissue, resulting in increased expression of extracellular matrix proteins, which characterizes the fibrosis. Inhibition of TGF-β signaling pathway attenuates *Trypanosoma cruzi* infection, preventing the development of cardiac damage, during the acute phase of Chagas disease, in an experimental model. The aim of this study was to investigate the effect of 1D11, a neutralizing antibody to all three isoforms of TGF-beta, on *T. cruzi* infection: in vitro and in vivo. To this end, cardiomyocytes were seeded for 24h, incubated with trypomastigotes and treated with 1D11 (100ug/ml). C57BL/6 mice were also infected with *T. cruzi* (10^2 parasites from the Colombian strain) and, after 120 dpi, treated with 1D11(10mg/kg). In the present study, we show that the addition of 1D11 greatly reduces cardiomyocyte invasion by *T. cruzi*, in vitro. Further, the treatment significantly reduces the number of parasites per infected cell. In a murine experimental model, the *T. cruzi*-infection altered the cardiac electrical conduction: decreasing the heart rate, increasing the PR interval and the P wave duration. The treatment with 1D11 reversed this process, improving the cardiac performance and reducing the fibrosis of the cardiac tissue. Taken together, these data further confirm the major role of the TGF-beta signaling pathway in both *T. cruzi*-infection, in vitro and in vivo. The therapeutic effects of 1D11 are promising and suggest a new possibility to treat cardiac fibrosis in the chronic phase of Chagas' heart disease by TGF-β neutralization.

Key words: Chagas disease; TGF-beta and 1D11.

Financial support: CAPES; CNPq and FAPERJ.

Area: Vector, transmission cycles, ecology and biodiversity

Biological activity of *Aniba puchury*-minor (Lauraceae) essential oil against *Rhodnius prolixus* (Hemiptera: Reduviidae)

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According to the World Health Organization (WHO), Chagas disease is considered a Neglected Tropical Disease and it is estimated that between 6 and 7 million people are infected. One of the ways of transmission to humans is through contact with feces/urine of the host contaminated with the protozoan *Trypanosoma cruzi* and since there is no vaccine available for the population, the best form of prevention is to develop strategies directly related to the vector [1]. In this context, studies with natural products, including medicinal plants, have become increasing, because plants are sources of bioactive compounds. As example, there is *Aniba puchury*-minor (Mart.) Mez, a plant native to Brazil that occurs in the northern region of the country [2] and is normally used in folk medicine to treat diarrhea and indigestion [3]. To achieve the objectives of this research, the essential oil (EO) from the leaves of A*. puchury*-minor was obtained by hydrodistillation for 2 hours and analyzed by Gas Chromatography coupled to Mass Spectrometry (GC-MS) in an Agilent equipment (6890N, 5973N) and electron ionization mode at 70 eV. The temperature of DB-5MS column varied from 50ºC to 300ºC at 4ºC/min. Helium was the carrier gas (1mL/min). The substances were identified by the Wiley data system library of the equipment. In order to investigate the effects of the EO in the triatomine *Rhodnius prolixus* mortality and feeding, a test was carried out by applying 2 µL of the solutions directly in the thorax of fifth instar insects at the concentrations of 1:5, 1:10 and 1:100 dilutions. As the control solution, pure DMSO was used under the same conditions. The results of GC-MS analysis showed the presence of 1,8-cineole (8.6%), sylvestrene (7.8%) eugenol (6.8%) and sabinenne (6.0%) as major compounds. Regarding blood meal phagoinhibition, percentages of 100, 85 and 90 were observed in the treated triatomines with the dilutions of 1:5, 1:10 and 1:100 of EO, respectively. For mortality, values of 28.5% and 6.7% were found in the treatment with 1:5 and 1:10 dilution, respectively, with no deaths on the other treatments. These results indicate that the essential oil is promising and directly affects the diet of the triatomine *R. prolixus*. Further tests will be carried out.

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Area: Vector, transmission cycles, ecology and biodiversity

Epidemiological importance of species of the subcomplex *Triatoma rubrovaria* through analysis of vectorial competence and food source

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The control of Chagas disease, which is caused by *Trypanosoma cruzi*, focuses mainly on the elimination of vectors with proven adaptation to human dwellings. Although domiciled insect vectors are the most important epidemiologically, autochthonous vectors constantly collected in anthropic environments pose a risk of transmission of the parasite to humans. This study proposed to identify species of the subcomplex *Triatoma rubrovaria* through molecular taxonomy and to analyze their vector potential on the transmission of the parasite in the Pampa biome. Different aspects related to vectorial capacity were evaluated, such as food source, infection rate and parasitic genotyping. In parallel, bionomic parameters of *T. rubrovaria* after infection by *T. cruzi* were analyzed experimentally. A total of 1.724 triatomines were collected in Rio Grande do Sul, of which 927 insects had DNA from their intestines extracted for molecular analysis. The phylogeny of the subcomplex grouped the 92 samples successfully sequenced for the Cyt b fragment. Of the sequenced samples, 19 (20.7%) were identified as *T. carcavalloi*, 17 (18.5%) as *T. circummaculata* and 12 (13.04%) as *T. rubrovaria*. The remaining samples were grouped into five clades without reference sequence. We observed an infection rate by *T. cruzi* of 2.8% in the field, with parasitic load variation ranging from $1.5 \times 10_1$ to $2.3 \times 10_7$ parasite equivalents/intestine and the presence of TcI, TcV and coinfection by TcI + TcIV. Twelve species of mammals were identified, in addition to birds and insects, being *Homo sapiens* the most frequently detected food source (73.5%), followed by *Gallus gallus* (33.1%). For vector competence analyses, N5 nymphs of *T. rubrovaria* and *T. infestans* were fed on mice infected with *T. cruzi* VI, in laboratory conditions. We compared the presence and number of evolutionary forms of the parasite in the excreta of both species of triatomines at 30, 60 and 90 days post-infection. *Triatoma rubrovaria* and *T. infestans* presented similar results in infection rates and of *T. cruzi* TcVI metacyclogenesis. Regarding vectorial behavior, we confirm that the triatomine tends to move away from the bite site after the blood meal. Interspecific differences were observed in the volume of ingested blood and in the proportion of individuals who excreted after blood feeding, revealing the higher feeding efficiency and rate of *T. infestans* dejection. The volume of blood ingested and the bite behavior of *T. rubrovaria* seem to be influenced by TcVI infection. Infected specimens tend to ingest ~25% more blood and to bite more frequently the host's head. The results obtained here suggest the urgent need for revising the taxonomy of the group. Furthermore, us analyzes show that *T. rubrovaria* is a potential vector of *T. cruzi*, having bionomic parameters associated with its vectorial capacity similar to the primary vector *T. infestans*, especially when infected, thus alerting to the importance of constant entomological surveillance in the studied areas.

Support: CAPES, CNPq, FAPERJ

Area: Vector, transmission cycles, ecology and biodiversity

Landscape modeling applied to *Trypanosoma cruzi* transmission cycles in two Chagas disease outbreak areas in Acre, Amazon

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Trypanosoma cruzi (Kinetoplastea, Trypanosomatidae), primarily a wild enzootia is the etiologic agent of Chagas disease (ACD). Currently, most human infections occur orally, mainly in the Amazon. Understanding the variables that modulate this complex transmission system is essential to define control measures. In order to understand the environmental variables of *T. cruzi* transmission in the wild, multi-temporal images (RapidEye satellite, 5 meters spatial resolution) were used to map and classify changes in land use and cover, we used the Random Forest algorithm in RStudio version 4.0.3. Landscape metrics Land cover, Edge length and Overall core area were obtained using LecoS plugin from QGIS v3.10.6 software. Abundance and richness of wild mammals were evaluated by: Shannon index, Simpson, Equitability and Beta diversity (Turnover and Nestedness). The risk model was generated by logistic regression for two areas of ACD outbreak in Acre in the municipalities: Marechal Thaumaturgo (2019) and Rodrigues Alves (2016). In Rodrigues Alves, where the greatest environmental changes have been observed, areas with increased edge effect were correlated with lower diversity of small mammal species (Shannon = 1.59791, Simpson = 0.7956104 and Beta diversity = 0.75). *Proechimys steerei* (n=23), a generalist and resilient species, predominated in both municipalities. Both areas showed an enzootic cycle of *T. cruzi*: in Marechal Thaumaturgo the infection rate of wild mammals was 35% (25/70). In three specimens of *Hylaeamys perenensis* infection by TcII DTU was observed. From a dog, *T. cruzi* DTU TcIV was isolated, showing that transmission of *T. cruzi* is taking place near areas of human use. In Rodrigues Alves, 37% (10/27) of wild animals were infected with *T. cruzi* and DTU TcI was isolated from *Philander canus*. Dogs showed 32% (17/52) of seropositivityn and three DTU TcI isolates were obtained. The presence of infected dogs proved to be a signal for the occurrence of a cycle of transmission of *T. cruzi* among wild mammals in the surroundings. The model showed that the variables: proximity of houses to forest areas and presence of dogs infected by *T. cruzi* signal the presence of sylvatic transmission and therefore, the risk of transmission of *T.cruzi* to humans (R2=0.92; Overall = 94.84%; AIC 25.25). ACD outbreaks in Acre have been shown to be associated with environmental degradation and the use of multi-temporal imaging and monitoring of the distribution of infection in dogs reveals the risk for new areas of ACD cases/outbreaks in the area.

Keywords: *Trypanosoma cruzi*, Remote Sensing, Random Forest, Landscape Ecology,Amazon Forest
Financial support: FIOCRUZ/IOC, FAPERJ, CNPq, CAPES and IME.

Area: Vector, transmission cycles, ecology and biodiversity

Molecular Detection of Blood Meal Source up to 3 Months Since the Last Meal: Experimental Starvation Resistance in Triatomine

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The identification of Blood Meal Source (BMS) in hematophagous vectors contributes to better understand the ecology of hemoparasite transmission. Those insects can endure long periods of time without without feeding, waiting for a favorable setting. Although this represents an important behavior observed in those groups, such as triatomines, little is known about how time can affect BMS detection, especially considering such extended periods of time. To comprehend how this behavioral phenomenon can impact molecular detection, we submitted two groups of *Rhodnius robustus* to increasing periods of starvation under experimental conditions. The first group of 150 of specimens, including N5 and adults, were artificially fed up on *Mus musculus* and 10 specimens were weekly removed, weighed and kept at -20ºC until BMS molecular identification. The second group, 5N (n = 30) and adults (n=30) were separated in crystallizers of 5 individuals each, in a total of 12 groups, weighted weekly until death. To assess weight loss, all triatomines were weighted before any procedures and monitored individually each week. To achieve BMS molecular detection, the abdomen of all *R. robustus* specimens was removed and its DNA extracted using *DNeasy Blood and Tissue* (Qiagen). For molecular detection, 12S rDNA marker was used according to described conditions and for endogenous control of PCR amplification, a DNA mini-barcode approach using *cox*1 marker was applied. Correlations analyses were performed using chi-square calculations with a significant index set at $\alpha = 0.05$. For comparisons of weight loss and DNA concentration, the arithmetical mean of groups by week was considered. We observed that the recovering of blood meal source occurred until the 13th week of artificial starvation, with a direct correlation between weight and positive blood meal source. No significant difference in weight between nymphs and adults after feeding was seen, although nymphs displayed better starvation resistance to death when compared to adults, surviving 27 weeks. The study brought new insights to the understanding of *Trypanosoma cruzi* transmission by *R. robustus* in nature environment, with a temporal perspective.

Area: Vector, transmission cycles, ecology and biodiversity

Populations of *Triatoma brasiliensis brasiliensis* Neiva, 1911 Analyzed through Geometric Morphometry, on a 102 years' timescale.

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Triatoma brasiliensis brasiliensis Neiva, 1911 is one of the most important vectors of Chagas disease in the semiarid regions of Northeast Brazil. The risk imposed by *T. b. brasiliensis* to the populations, due to constant invasions and/or colonization, leads to the need for constant monitoring activities and the understanding of its evolutionary process. In this context the present study aims to verify possible morphological changes in specimens of *T. b. brasiliensis* collected in different states of Northeast Brazil, on a 102 years' time scale. To this end, specimens of *T. b. brasiliensis* from the Entomological Collection of the Oswaldo Cruz Institute (CEIOC) and in the Collection of Triatomines of the Oswaldo Cruz Institute (CTIOC) were analyzed through geometric morphometric analysis. The insects used were grouped, which culminated in the formation of 8 groups for the study, comprising specimens from 1912 to 2014. Anatomical reference points were determined on the insects wings, and subsequently submitted to a generalized Procrustes analysis. The results suggested that the specimens did not change their phenotype. When inserting examples of *Triatoma infestans* (Klug, 1834) in the morphological space of *T. b. brasiliensis*, the two species did not overlap. With this unprecedented study covering more than 100 years, we conclude that the wing shape of *T. b. brasiliensis* remained morphologically stable in spite of microevolutionary and environmental changes. One could suppose that due to the climatic and geographic stability in the main area infested by *T. b. brasiliensis* represented by the semiarid areas of "sertão" no external forces has been acting favoring changes in the morphology of this species. However, in the limitrophe geographic areas of "sertão" if detailed studies are performed the scenario could be distinct than the results here obtained.

Key words: Morphology; Chagas Disease; Vectors; Wing Shape.