CICLO CARLOS CHAGAS DE PALESTRAS

8ª EDIÇÃO

100+11: O TEMPO NÃO PARA

LIVRO DE RESUMOS

Instituto Oswaldo Cruz 120 ANOS
Ministério da Saúde FIOCRUZ Fundação Oswaldo Cruz
MINISTÉRIO DA SAÚDE
PÁTRIA AMADA BRASIL GOVERNO FEDERAL

LIVRO DE RESUMOS

Ciclo Carlos Chagas de Palestras – 8ª edição

100+11 ANOS DA DESCOBERTA DA DOENÇA DE CHAGAS: O TEMPO NÃO PARA, MESMO QUE SEJAMOS FREADOS PELA SINDEMIA DE COVID-19

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

Organizadores: Ângela Junqueira**,** Marli Lima, e Joseli Lannes

Prezados participantes,

Neste ano, que celebramos 120 anos do Instituto Oswaldo Cruz e da Fundação Oswaldo Cruz, o Ciclo Carlos Chagas de Palestras (CCCP) atinge sua 8ª Edição com o tema **"100+11: o tempo não para, mesmo que sejamos freados pela sindemia de Covid-19"**, sendo pela primeira vez apresentado de forma *online*. Se por um lado não nos encontramos, por outro temos a oportunidade de participação à distância, que se reflete no recebimento de resumos de trabalhos desenvolvidos em diversos estados do Brasil e do exterior.

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, assim como manter uma pauta viva de discussão sobre a doença de Chagas diante dos desafios a serem enfrentados. Este ano, celebramos o primeiro ano do "Dia Mundial das Pessoas Acometidas pela doença de Chagas - 14 de abril", um reconhecimento pela Assembleia Mundial de Saúde da Organização Mundial de Saúde, 2019, visando maior visibilidade e enfrentamento das necessidades dos portadores da doença de Chagas. Nos últimos 7 anos, a reunião anual do CCCP foi uma oportunidade para reunir pesquisadores da Fiocruz e de outras Instituições, nacionais e internacionais. Refletimos sobre os desafios da pesquisa na doença de Chagas e criamos ambiente propício a interações entre pesquisadores e destes com portadores da doença de Chagas. Foi neste fórum que, em 2016, lançou-se a ***RioChagas***, primeira Associação de Portadores da Doença de Chagas do Rio de Janeiro, desde então presente em nossas reuniões, como uma representação das Associações de Portadores da Doença de Chagas que formam a FINDECHAGAS.

Neste ano de desafios pela pandemia de Covid-19, recentemente reconhecida por ser um sindemia, condição que igualmente se aplicaria à doença de Chagas, reafirmamos nossa disposição em continuar fazendo o que acreditamos, em nome da ciência e da formação de nossos estudantes e pesquisadores. Particularmente, nos encontramos preocupados sobre os efeitos de comorbidades e da co-infecção por Covid-19 dos portadores da doença de Chagas, um dos temas que abordaremos. Agradecemos aos participantes pelo interesse em nosso evento e aos palestrantes pela generosidade de compartilharem seu conhecimento. Agradecemos, também, aos avaliadores de resumos que contribuíram para a indicação das apresentações orais (CE-IOC em 13 de novembro). Todos os resumos estão disponíveis *online,* na *página do IOC,* no *Campus Virtual da Fiocruz* e no *Research Gate*. Agradecemos, de forma especial o essencial apoio logístico do Núcleo de Eventos e do Jornalismo do IOC.

Nestes momentos em que lidamos com tantas incertezas, vislumbramos a oportunidade de fortalecer a solidariedade, conhecer a resistência e a resiliência. Renovamos uma vez mais as esperanças na força da democracia institucional e 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 Ângela Junqueira, Marli Lima e Joseli Lannes

Ciclo Carlos Chagas de Palestras - 100+11: O tempo não para

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

Organizadoras: Ângela Junqueira, Marli Lima e Joseli Lannes – IOC/Fiocruz

12/11

9:00h – Abertura – Presidente da Fiocruz Dr Nisia Trindade Lima, Vice-Presidente de Pesquisa e Coleções Biológicas Dr Rodrigo Correa-Oliveira, Diretor do IOC Dr José Paulo Leite, Presidente da Associação RioChagas Sra Josefa de Oliveira, Organizadoras do CCCP (falas de 3 minutos)

9:30h

Dr. Pedro Albajar-Vinas – OMS, Genebra, Suíça – "Doença de Chagas: Cenário epidemiológico, conquistas e desafios para os próximos 10 anos"

Debatedor: Dr. Rodrigo Correa-Oliveira

10:30hs

Dr. Ezequiel Zaidel – Departamento de Cardiología, Sanatorio Güemes, Buenos Aires /Argentina – "Desafio do convívio doença de Chagas, comorbidades e Covid-19"

Debatedor: Dr. Alejandro Hasslocher-Moreno

13/11

9:00-10:00h

Mini-palestras por jovens pesquisadores – 5 resumos a serem selecionados dos resumos recebidos (10 minutos apresentação e 5 minutos de discussão)

10:00h – Centro de Estudos do IOC

Dra. Andrea Silvestre – INI Evandro Chagas/Fiocruz, Rio de Janeiro, Brasil – "A doença de Chagas congênita: situação atual e desafios – o enfrentamento via UNITAID"

Debatedores: Dr Pedro Albajar-Vinas e Dra Luciana Ribeiro Garzoni

Comissão Avaliadora de Resumos

Ângela Junqueira / IOC

Andrea Alice da Silva / UFF

Antônia Ribeiro / IOC

Catarina Macedo Lopes / IOC

Cleber Galvão / IOC

Constança Britto / IOC

Daniel Gibaldi / IOC

Danielle Grynszpan / IOC

Fernando Genta / IOC

Isabela Resende Pereira / UFF

Jacenir Mallet / IOC

Joseli Lannes / IOC

Juliana De Meis / IOC

Katia Calabrese / IOC

Maria da Gloria Bonecini / INI

Maria de Nazaré Correia Soeiro / IOC

Mariana Waghabi / IOC

Marli Lima / IOC

Mauro Mediano / INI

Michelle Barros IAM

Natalia Nogueira / UERJ

Otacílio Moreira / IOC

Otília Sarquis / IOC

Roberto Saraiva / INI

Rubem Menna-Barreto / IOC

Solange de Castro / IOC

Teresa Cristina Gonçalves / iOC

Virginia Maria Barros de Lorena / IAM

Muito obrigado a todxs!

Resumos Selecionados para Apresentação Oral

13 de novembro - 9:00hs – Centro de Estudos Especial

A avaliação dos resumos, considerando coerência título/conteúdo, clareza, originalidade e qualidade, foi feita por 3 avaliadores independentes especialistas na temática. Eles receberam o título e o texto (omitiu-se autores e filiações), deram notas e indicaram 1 resumo (1 estrela) para apresentação oral. A síntese dos dados, levou à seleção abaixo de resumos para apresentação oral (marcados com 2 ou 3 estrelas):

#13
Area:Cell biology and parasite/host cell interaction; Chemotherapy (drugs and etiological treatment scheme). **Cardiac cell culture approaches as drug screening protocols for Chagas disease**. Ludmila Ferreira de Almeida Fiuza, Denise da Gama Jaen Batista, Daniela Nunes, Otacílio Moreira, Cynthia Cascabulho and Maria de Nazaré Correia Soeiro.

#29
Area: Pathology / Pathogenesis and Clinical Aspects. **Evaluation of CXCL9 / MIG and CXCL10 / IP-10 chemokines in serum of patients with cardiac and indeterminate clinical forms of chronic Chagas disease.** Michelle da Silva Barros; Diego José Lira Torres; Leyllane Rafael Moreira; Kamila Kássia dos Santos Oliveira; Tiago Ribeiro de Arruda; Maria Glória Aureliano Melo; Sílvia M. Martins; Cristina Carrazzone; Wilson de Oliveira Júnior; Clarice N. Lins de Morais, Virginia Maria Barros de Lorena.

#35
Area: Parasite (genetic, molecular, biological and morphological diversity). **Identification of *Trypanosoma cruzi* ribose-5-phosphate isomerase inhibitors for Chagas disease chemotherapy: from an *in vitro* approach into the wilderness of exploring protein sequence-structure-function diversity.** Sophia Azevedo, Rafael Ferreira, Mayla Abrahim, Marcos Catanho, Ana Carolina Guimarães and Teca Galvão.

#37
Area: Vector, transmission cycles, ecology and biodiversity. **Catalytic residues in the Murein Endopeptidase of *Rhodnius prolixus*.** Pussenti, A. C. H., Genta, F.A.

#42
Area: Vector, transmission cycles, ecology and biodiversity. **Ecological Niche Modeling in the identification of potential areas of expansion of the *Trypanosoma cruzi* enzootic cycle in *Didelphis aurita* of the Atlantic Rainforest.** Raphael Testai de Souza, Marinez Ferreira de Siqueira, Diogo Souza Bezerra Rocha, Ana Maria Jansen, Samanta Cristina das Chagas Xavier.

Resumos

Abstracts

Area: Behavior (Vector or Host)

#1 ★

Memory deficit in chronic experimental Chagas disease: effects of benznidazole therapy

Glaucia Vilar-Pereira[1], Leda Castaño Barrios[1], Andrea Alice Silva[2], Angelica Martins Batista[1], Isabela Resende Pereira[1], Otacílio Cruz Moreira[3], Constança Britto[3], Hilton Antônio Mata dos Santos[4,5], Joseli Lannes-Vieira[1]

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[4]Faculdade de Farmácia, UFRJ
[5]Laboratório de Análise e Desenvolvimento de Inibidores Enzimáticos e Laboratório Multiusuário de Análises por RMN, UFRJ

Memory deficit has been associated with chronic Chagas disease, a neglected tropical disease caused by the protozoan parasite *Trypanosoma cruzi*. In degenerative diseases, memory loss has been associated with increased oxidative stress, revealed as enhanced lipid peroxidation, in the cerebral cortex. Benznidazole (Bz), a trypanocidal drug efficient to reduce blood parasite load in the acute and chronic phases of infection, showed controversial effects on heart disease progression, the main clinical manifestation of Chagas disease. Thus, we evaluated whether chronically *T. cruzi*-infected C57BL/6 mice present memory deficit assessed by the novel object recognition task at 120 days postinfection (dpi). Next, we tested the effects of Bz therapy (25mg/Kg/day) on memory evocation, trying to establish a relation with parasite load and oxidative stress in the central nervous system (CNS). At 120 dpi, *T. cruzi*-infected mice showed memory impairment, compared with age-matched non-infected controls. Bz therapy (from 120 to 150 dpi) hampered the progression of memory loss and, moreover, reversed the object recognition memory impairment. Further, Bz therapy reduced parasite load in the CNS tissue. At 120 and 150 dpi, lipid peroxidation was increased in the hippocampus and cortex tissue extracts. Notably, Bz therapy reduced levels of lipid peroxidation in the cerebral cortex. In summary, experimental chronically *T. cruzi*-infected mice show memory impairment and Bz therapy improved memory loss in association with reduction of parasite load and oxidative stress in the CNS. Thus, our results open a new perspective to improve the quality of life of Chagas disease patients.

Financial support: FAPERJ, CNPq, PAEF/IOC, INCT-Vacinas

Area: Behavior (Vector or Host)

#2

Studies on the impact of occasional phytophagy in the development of *Rhodnius prolixus**

Thiago Christian da Silva Ribeiro[1,2], Fernando Ariel Genta[1,*]

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2 - Universidade do Estado do Rio de Janeiro
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Chagas disease is a chronic and severe disease caused by the flagellated protozoan *Trypanosoma cruzi*. The classic form of transmission happens by hematophagous insects we call triatomines, through contact with infected feces and urine in the bite area, mucous membranes or regions of the eyes. Triatomines are a large and diverse group, and are characterized as obligatory hematophagous. The association of these insects with plants is considered a side effect derived from the habitat preference of their vertebrate hosts. However, recent studies have shown that triatomines can also consume sugar and nutrients from fruits. We described for the first time the ingestion of sugary solutions in several species of triatomines, which have always been considered in general strict hematophagous. However, many aspects related to the importance of sugary food in kissing bugs are unknown. The knowledge of the feeding behavior of these insects can be the ground for the development of new control strategies. The goal of this project is to test the impact of occasional fruit eating on the fitness of *Rhodnius prolixus*. That may result in improved procedures for raising kissing bugs in the lab and to the development of baits for triatomines.

Supported by Faperj, CNPq, CAPES and Fiocruz
*this is the original version of the abstract

Area: Behavior (Vector or Host)

#3

Description of sexual behavior *Rhodnius prolixus* Stål 1859 (Hemiptera, Reduviidae)

Thiago P. Machado[1], Simone P. C. Freitas[2] & Jacenir R. Santos-Mallet[1,2]

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[2] Fundação Oswaldo Cruz, Escritório Piauí/PI.

The study of insect vectors has driven research on their mating and reproduction behaviors since this information is directly related to the dispersion and colonization potential of these animals. The objective of this work was to study the aspects of *Rhodnius prolixus* sexual behavior. The insects used were provided by the laboratory insectary and were previously separated in nymphs of 5th instar and kept virgin for the observation of the following aspects: attempt to mate, female rejection, time of copulation and post-copulation. The pairs were placed in Petri dishes where 65 couples were matched, of which 13 copulated. The behavior of the couples was described from the moment they entered the experiment. Males were the first to initiate interaction with females, harassing their partners to gain access to the genitalia, grabbing or chasing them, for example. The males tried to copulate on average 1.7 times. When the females were not interested in mating, they compressed their abdomens or fled the male's contact field, for example. During the copulation, the couples remained static, but some females moved around the Petri dishes with the male attached to their genitalia. After mating, the male kept the female to prevent her from mating again. Our preliminary data indicates that the copulation of *R. prolixus* lasts an average of 35 minutes, an interval of time that is in line with what is expected for the genus according to works in the literature.

Financial support: CAPES

Area: Cell biology and parasite/host cell interaction; Chemotherapy (drugs and etiological treatment scheme)

#4

Effect of Selenium treatment on cerebral microvascutopathy observed during acute experimental Chagas disease

Beatriz Matheus de Souza Gonzaga[1], Vanessa Estato[2], Hugo Caire[2], Anissa Daliry[3], Samuel Horita[1], Daniela Beghini[1], Tania Araujo-Jorge[1], Luciana Garzoni[1]

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Chagas disease (CD) affects 7 million people worldwide and in Brazil approximately 3 million people are infected by *Trypanosoma cruzi*. The main clinical manifestation in the chronic phase is cardiomyopathy. However, central nervous system changes can also occur including stroke, meningoencephalitis, and cognitive damage. Studies show that 25% of stroke cases in patients with CD are not related to cardioembolism and could be associated with alterations of cerebral microcirculation. Recently, our group demonstrated that *T. cruzi* acute infection (Y strain) leads to cerebral microvasculopathy in Swiss Webster mice as consequence of endothelial dysfunction, capillary rarefaction and increased leukocytes rolling and adhesion. Currently, treatment options are benznidazole (BZ) or nifurtimox, drugs with trypanocidal effects that presents a cure rate of 80% in the acute phase and 20% in the chronic phase. Therefore, it is necessary to develop therapeutic strategies that contribute to the improvement of clinical manifestations of CD both in the acute and chronic phases, regardless of the trypanocidal effect. Selenium is a micronutrient capable of reducing risks of cardiovascular and neurodegenerative diseases. It is also beneficial in the treatment of cardiomyopathy in acute and chronic phases of CD, as previously demonstrated by our group in experimental model of CD. In this study, we evaluated in experimental model of acute CD, the effect of Se in monotherapy or in association with BZ on cerebral microvasculopathy. Male Swiss Webster mice were inoculated intraperitoneally with 10^4 *T. cruzi* trypomastigote forms (Y strain). After 24 hours, the animals were treated orally with 4 ppm sodium selenite, 50 or 100 mg/kg/day BZ and 4 ppm Se associated with 50 mg/kg/day BZ for 14 consecutive days. We evaluated cerebral microcirculation by intravital microscopy and cerebral blood flow through fluxometry. Our results demonstrate that Se in monotherapy impaired cerebral microvasculopathy, preventing functional capillary rarefaction and the increase in leukocyte rolling observed in untreated animals. Treatments with BZ alone or in combination with Se were able to prevent the reduction of parasitemia and mortality, also prevented: functional capillary rarefaction, the increase of leukocyte rolling and adhesion, and the reduction of cerebral blood flow, observed in infected and non-treated animals.

Area: Cell biology and parasite/host cell interaction; Chemotherapy (drugs and etiological treatment scheme)

#5

Effect of new triazoles as inhibitors of P2X7 receptor with action against *Trypanosoma cruzi*: a preliminary research

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Background: Chagas disease (CD), caused by the protozoan *Trypanosoma cruzi*, is a neglected disease, according to the World Health Organization (WHO). In CD, purinergic signalization (ATP and adenosine) actives inflammatory response. Millimolar ATP concentration due to cellular damage can activate the P2X7 receptor and promote pro-inflammatory cytokine release and cell death. There are several selective antagonists to this receptor; however, they were not effective in clinical trials. Thus, we propose to study new prototypes with action against *T. cruzi* and antagonist activity upon receptor P2X7. A series of triazoles (TD1-TD17) with selective action against P2X7 receptors and low toxicity towards mammalian have been tested against *T. cruzi in vitro*.
Methods: *In vitro* cell cytotoxicity was tested using peritoneal macrophages of Swiss mice plated on transparent 96-well plates and kept for 24 hours at 37 ºC with an atmosphere of 5% CO2. Then, the cells were incubated with 100 µM triazole derivatives. After 24 hours, the resazurin colorimetric assay was performed. Alternatively, peritoneal macrophages were plated for 24 hours on black bottom plates, and after compound exposure, 2 µL of propidium iodide (PI) solution were added for 5 minutes, and fluorescence was read using a spectrophotometer.
Results: The compounds TD6, TD8, TD9, and TD12 reduced, respectively, 5%, 7%, 10% and 6% the viability of the mammalian cells; however, the other compounds did not interfere with the viability of the peritoneal macrophages. When performing the uptake test with PI assay, only TD8 and TD12 induced 40% to 50% of dead cells. Moreover, death induction was low compared to other compounds, even at their maximum concentration, indicating these compounds exhibited low toxicity.
Conclusions: Triazoles prototypes exhibited low toxicity in mammalian cells. However, the initial data against epimastigotes form of *T. cruzi* indicated that TD series did not reduce parasite viability. However, new tests will be realized against *T. cruzi*.

Financial support: CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior), CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico).

Area: Cell biology and parasite/host cell interaction; Chemotherapy (drugs and etiological treatment scheme)

#6

In Vitro, In Silico, and In Vivo Analyses of Novel Aromatic Amidines against *Trypanosoma cruzi*

Camila Cardoso Santos, Joint PhD at Biologia Parasitária in Fiocruz advised by Dr Maria de Nazaré Correia Soeiro

Camila C. Santos1; Jéssica R. Lionel1; Raiza B. Peres1; Marcos M. Batista1; Patrícia B. da Silva1; Gabriel M. de Oliveira1; Cristiane F. da Silva1, Denise G. J. Batista1, Sandra, Maria O. Souza2, Carolina H. Andrade3, Bruno J. Neves3, Rodolpho C. Braga3, Donald A. Patrick4, Svetlana M. Bakunova4, Richard R. Tidwell4 and Maria de Nazaré C. Soeiro1#
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Five bis-arylimidamides were assayed as anti-*Trypanosoma cruzi* agents by in silico, *in vitro* and *in vivo* approaches. None was considered to be a pan-assay interference compound. They had a favorable pharmacokinetic landscape and were active against trypomastigotes and intracellular forms, and in combination with benznidazole, they gave no interaction. The most selective agent (28SMB032) tested *in vivo* led to a 40% reduction in parasitemia (0.1 mg/kg of body weight/5 days intraperitoneally) but without mortality protection. *In silico* target fishing suggested DNA as the main target, but ultrastructural data did not match.

Area: Cell biology and parasite/host cell interaction; Chemotherapy (drugs and etiological treatment scheme)

#7 ★

Novel 3-nitro-1,2,4-triazole derivatives with potent activity against *Trypanosoma cruzi* in an intracellular model*

Cheyene Almeida Celestino[1,2] Frederico Silva Castelo Branco[1], Rodolfo Rodrigo Florido França[1,2], Patrick Antunes do Nascimento[1,3], Álvaro José Romanha[4†], Silvane Maria Fonseca Murta[4], Policarpo Ademar Sales Júnior[4] e Núbia Boechat[1].

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Chagas disease is caused by the parasite *Trypanosoma cruzi* and has two pathological phases: acute and chronic, but only the former acute is curable. In Brazil, only benznidazole (BNZ), a 2-nitroimidazolic drug is approved for the chemotherapy of this disease, however, it presents a number of side effects.[1] For such reason, our group has been working on the discovery of novel anti-*T. cruzi* compounds. A target that has been studied for the development of new drug candidates is the ergosterol biosynthesis, which is the main membrane sterol present in *T. cruzi*, as well as in fungi.[2] Some ergosterol biosynthesis inhibitors that was initially developed as antifungals, have been evaluated against *T. cruzi* and have shown promising results.[3] Posaconazole, a triazolic compound and the main representative of this class, was able to cure in both phases in an animal model, but it was not successful in clinical trials.[3] On the other hand, compounds containing the nitrotriazole moiety proved to be more potent and less toxic than their corresponding triazolic analogues and, in addition, the former is capable of acting both as an inhibitor of ergosterol biosynthesis and as a substrate for nitroreductase enzymes, making such substances able to act as a multi-target.[4] Thus, in this work, we designed five 3-nitro-1,2,4-triazolic derivates with the scaffold of compounds developed by our group that previously proved to be more than 100-fold more potent than BZD.[5] These novel compounds were synthesized through a simple and inexpensive synthetic route comprising four steps. The pharmacokinetic properties of the compounds were predicted *in silico* and showed that these substances can present a good human intestinal absorption profile (> 93%), in addition to better permeability in Caco-2 (> 0.65 cm/s) than BZD (0.65 cm/s). No compound showed potential to act as a P-glycoprotein substrate, which can reduce the chances of resistance due to efflux. The *in vitro* evaluation against *T. cruzi* was performed in an intracellular assay on mouse fibroblast L929 cells infected by trypomastigote and amastigote forms of the Tulahuen strain of the parasite. The preliminary results show that these derivatives are all remarkably more potent than the reference drug BZD (IC_{50} = 1.52 µM) with submicromolar IC_{50} in the range of 0.04-0.91 µM and high selectivity indexes.

Financial support: CAPES, CNPq e FAPERJ.

References: **(1)** World Health Organization (WHO), Available in: http://www.who.int/mediacentre/factsheets/fs340/en/. Accessed in 25/11/2019. (2) LEPESHEVA, G.I. *et al*. *Advances in Parasitology*, v. 75, p. 65-87, 2011. (3) URBINA, J. A. *Journal of Eukaryotic Microbiology*, v. 62, p. 149-156, 2015. (4) Papadopoulou, M. V. *et al*. *Bioorganic & Medicinal Chemistry*, v. 23, p. 6467-6476, 2015. (5) Castelo-Branco, F. S. Tese (Doutorado em Química), Instituto de Química, Universidade Federal do Rio de Janeiro, Rio de Janeiro, 2016.

*this is the original version of the abstract

Area: Cell biology and parasite/host cell interaction; Chemotherapy (drugs and etiological treatment scheme)

#8 ★

Alternative therapies for Chagas disease: *in vitro* studies

Gabriela Rodrigues Leite1, Denise da Gama Jaén Batista1, Patrícia Bernardino da Silva1, Alexis Murillo Carrasco2, Lucas Freitas de Freitas3, Ademar B. Lugão3, Rosemeire A. Silva2, Maria de Nazaré Correia Soeiro1

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3 Instituto de Pesquisas Energéticas e Nucleares, IPEN, Brazil

Chagas disease is characterized by a chronic progressive inflammatory heart disease associated or not to digestive disorders. Benznidazole (BZ) and nifurtimox are effective only in the initial stage of infection and induce severe side effects (de Araújo et al., 2020). The synthetic peptide, with sequence CTHRSSVVC (peptide A – pep A) mimics the CD163 molecule present in monocytes, macrophages and neutrophils, playing a role in acute phase processes. Peptide fingerprinting on human and animal atherosclerotic tissues showed that Pep A binds atheroma plaques in carotid biopsies of human patients and knockout mice for LDL receptors and submitted to high fat diets (Silva et al.,2016). Among other biological roles, CD163 acts as innate immune sensor upon infectious microorganisms, modulating the inflammatory response and the establishment of infection. Thus, our purpose was to identify new therapeutic antiparasitic drug options for CD as well as assess the potential effect of inflammatory mediators that may contribute to regulate the unbalanced cardiac inflammatory environment. The *in vitro* assays were done using peritoneal macrophages stimulated or not with thioglycolate. The results showed that pep A and pep C (control peptide with scrambled sequences CHVSVRTSC) were not toxic to the mammalian cell cultures, displaying LC50>500 µM, while macrophages from unstimulated and stimulated animals treated with BZ gave LC50=333.47±165.18µM and >500µM, respectively. Next, against intracellular forms of *T. cruzi* (Tulahuen strain, DTU VI) in L929 cell lines, two approaches were conducted: (a) previous treatment of mammalian host cell before infection and (b) exposure of already infected host cells with the studied molecules. Regardless the approach used, pep A (50 µM) reduced up to 20-30% the parasite load, while BZ given at 10µM, after the infection establishment, gave ≥90% of decrease. Simultaneously, we carried out experiments with macrophages obtained from mice stimulated or not with thioglycolate using the same protocols. Our preliminary data showed that unstimulated and stimulated macrophages treated with pep A, before the parasite interaction (Y strain, DTU II) showed inhibition of 62 and 68 %, respectively, in the infection index as compared to untreated cultures. After the establishment of the infection, the addition (at 50 µM) of pep A gave maximum of 36 and 35% of reduction in the parasite load, upon both non-stimulated and stimulated macrophages. BZ just showed a reduction in the parasitic load on post-treatment, with results between 97% (unstimulated) and 99% (stimulated) of effectiveness, confirming literature data. Pep A failed to induce trypanocidal effect against bloodstream trypomastigotes (Y strain), while BZ gave EC50=12 µM. Additional studies are needed to explore and confirm the present findings using pep A (and Pep C) alone or in association with BZ.

Support by: Fiocruz, CNPq, FAPERJ, FAPESP

References:

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Area: Cell biology and parasite/host cell interaction; Chemotherapy (drugs and etiological treatment scheme)

#9

New 2-nitroimidazole derivatives with promising dual activity against *Trypanosoma cruzi and Mycobacterium tuberculosis*

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Chagas disease (CD) is caused by *Trypanosoma cruzi* (*Tc*) and affects about 7 million people worldwide, being the parasitic disease that display higher death rates in the Americas. There are only two drugs available for the chemotherapy of CD: benznidazole (Bnz) and nifurtimox. However, both drugs have low efficacy in chronic phase and present several side effects. Another illness that requires of novel drugs development is tuberculosis (TB), caused by *Mycobacterium tuberculosis* (*Mtb*). In fact, TB is one of 10 top causes of death worldwide and kills 1.5 million of people by causing 10 million cases every year. Recently, a new drug namely delamanid (DMN), was also incorporated to treatment of multidrug-resistant (MDR-TB). However, some strains of *Mtb* are resistant to it. As could be seen by the structure of Bnz and DMN, the nitroimidazole group is an important pharmacophore present in drugs for both diseases. Another heterocyclic system with high importance in CD and TB is the 1*H*-1,2,3-triazole nucleus reported in various bioactive substances. Our tentative to identify hits with dual potential for treating these serious diseases considerate that they are endemic in South America and coinfection was already reported in Colombia, Argentine and Brazil. Then, a dual active drug would strengthen the integrated combat of these diseases, reducing the cost with health assistance. In this sense, we developed five news hybrids with potential dual activities against TB and CD, with basis on pharmacophores units from antituberculosis and antichagasic drugs or leads. The synthesis of the *N*-alkylated 2-nitroimidazole derivatives was initiated using 2-niroimidazole and epichlorohydrin as starting material. The final products were obtained after four steps of linear synthesis with moderate yields. The *in vitro* anti-Trypanosoma evaluation of products were carried out in an intracellular model using L929 cell line infected with trypomastigotes and amastigote forms of *Tc* of *β*-galactosidase-transfected Tulahuen strain. Their antimycobacterial activity evaluated against *Mtb* strain H37Rv. All tested derivatives proved to be non-cytotoxic against mammalian cells. The most promising compound in relation to *in vitro* activity against *Tc* was 1-(2-nitro-1*H*-imidazol-1-yl)-3-(4-(4-pentylphenyl)-1*H*-1,2,3-triazol-1-yl)propan-2-one with IC_{50}= 3.2 μM. This one was also active against isoniazid and rifampicin-resistant strain of *Mtb* (INH/RIFR-*Mtb*) with a MIC= 16.3 μM, being 3.5-fold more potent than the reference drug isoniazid (MIC= 58,4 μM). For such reasons, it was a good prototype for further development of new compounds with potential activity against both CD and TB.

Acknowledgments: Farmanguinhos, FIOCRUZ, FAPERJ and CNPq.

Area: Cell biology and parasite/host cell interaction; Chemotherapy (drugs and etiological treatment scheme)

#10

Profile of cytokines produced by peripherical blood mononuclear cells infected *in vitro* with a Colombian strain of *Trypanosoma cruzi* and treated with benznidazole.

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The drug recommended for the treatment of Chagas disease in Brazil is benznidazole (Bz), however, its effectiveness varies according to the stage of the disease, dosage, age, sensitivity of the strains to the drug, as well as the genetic background of host. Therefore, the synergism between the action of Bz and the immune response of patients may be important for successful treatment. Our study evaluated the profile of cytokines produced by peripheral blood mononuclear cells (PBMC), infected *in vitro* with a strain of *Trypanosoma cruzi* that has resistance to Bz, the Colombian strain (CCol), and treated with Bz at a concentration of 1µg / mL (BZ1) and 2µg / mL (BZ2). PBMC from individuals without the disease (n = 11) were obtained through density gradient centrifugation by Ficoll Hypaque PLUSTM and deposited in 48-well plates (1 x 10^6 cells / mL) for adhesion of adherent cells/monocytes for 1h. Then, non-adherent cells/lymphocytes were removed and reserved, and CCol trypomastigotes were added (2.5 x 10^5 parasites / well) for 2h. The parasites that did not go inside were washed away and the lymphocytes were replaced. Co-culture was performed in 24 hours, 5 and 10 days, and after the established time, culture supernatants were collected and cytokines (IL-2, IL-4, IL-6, IL-10, IFN-γ and TNF) by cytometric sphere matrix (CBA). Our results showed that CCol induced a strong inflammatory response by increasing IFN-γ and TNF in 24 hours, being statistically significant when compared to controls without infection. However, after 5 days of cultivation, TNF production declined, and IL-10 increased, being statistically significant, when compared to controls without infection. Thus, we believe that CCol initially induces an aggressive inflammatory profile, but that over time, with the increase in IL-10, a balance occurs between host and *T. cruzi*, thus reducing exacerbated inflammation. In addition, cultures that received Bz treatment had levels of cytokine production similar to the culture conditions only infected with CCol, which suggests that, regardless of Bz concentration, the drug was unable to modify cytokine production in cells infected with CCol.

Area: Cell biology and parasite/host cell interaction; Chemotherapy (drugs and etiological treatment scheme)

#11

In vitro prospection of Imatinib derivatives for the treatment of Chagas disease

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Chagas disease is a tropical neglected illness caused by the parasite *Trypanosoma cruzi*. It was discovered in 1909 by Carlos Chagas and for the past 50 years the frontline drugs have been the nitroderivatives, benznidazole (BZ) and nifurtimox. Both are poorly active when administered at the later chronic phase and induce several side effects. Thus, there is an urgent need to identify novel drug candidates for this serious parasitosis. One promising strategy that has been proposed is the repurposing approach that uses pharmacological agents already used for the treatment of other illnesses and that may share common mechanism of action and cellular targets, reducing costs and time in the pipeline of drug development. Imatinib (IMB) is a competitive tyrosine kinase inhibitor used mainly in the treatment of chronic myeloid leukemia. Due to the recent findings reporting the moderate activity of IMB against *T. cruzi* (EC_{50}= 33.6μM) (Simões-Silva *et al*., 2017), our group has been screening novel IMB derivatives and presently the *in vitro* activity upon intracellular (Tulahuen strain) and bloodstream forms (Y strain) of 8 new derivatives will be reported. Except for one (cpd 3), all compounds were active upon intracellular forms of *T. cruzi*, displaying potency either similar or superior to BZ, achieving 100% of parasite death up to 10 μM after 96 hours of incubation. These compounds were also tested against bloodstream trypomastigotes (BT), and most presented high trypanocidal activity. With exception of cpd 1, the IMB derivatives reached 100 % of parasite lysis after 2 hours of exposure (up to 50μM), while BZ was inactive after this period of drug exposure. Cpd 3 was one of the most promising, reaching, after 2 h of drug exposure, the lowest EC_{50} and EC_{90} values (4±2 μM and 5.4±0.1 μM, respectively). After 24 h of incubation, Cpd3 gave EC_{50} and EC_{90} values lower than 3.5±0.1 μM. When their toxicity was tested against L929 cells, most compounds reached LC_{50} > 100 μM. Regarding selectivity, cpd 7 gave the best results, with selectivity index > 60 upon intracellular forms. Further studies are underway to optimize these compound series aiming to contribute for a future drug development for Chagas disease.

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Area: Cell biology and parasite/host cell interaction; Chemotherapy (drugs and etiological treatment scheme)

#12

Investigation of the cruzain dynamic behavior in its apo form and complexed to benzimidazole inhibitors*

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Chagas disease (CD) is caused by the parasite *Trypanosoma cruzi*, presenting alarming rates of infection and mortality. However, there are only two drugs used to treat this disease, nifurtimox, and benznidazole. Although effective in the acute phase of the disease, these drugs are unsatisfactory in the chronic phase. In the search for new therapies, the cruzain enzyme (CRZ) of *T. cruzi* has been described as a therapeutic target since its inhibition results in a putative treatment in all stages of CD. Thus, Ferreira and collaborators (2014) have identified some CRZ inhibitors of the benzimidazole class with trypanocidal activity. Nevertheless, the structural and dynamic basis for CRZ inhibition, promoted by these inhibitors, is still unclear. Hence, the scope of this work is to investigate, at the atomic-molecular level, the CRZ inhibition mechanism, based on four inhibitors (BZ1-4) selected in the work of Ferreira and collaborators. The complex between CRZ and BZ1 was extracted from the Protein Data Bank (PDB code: 3KKU) while the complexes formed between BZ2-BZ4 were built using the molecular docking technique in the AUTODOCK 4.2 software. Thus, the predicted lowest energy complex of each system was selected as the starting structure in the molecular dynamics simulations. The simulations were performed through the GROMACS 5.1.8 software package, using the CHARMM27 force field, the TIP3P water model, counterions to neutralize the total charge, and constant temperature and pressure of 310 K and 1.0 atm, respectively. Electrostatic and Lennard-Jones interactions were considered within a cut-off value of 1.4 nm. The SETTLE and LINCS algorithms were used to restrict water and chemical bonds, respectively. Finally, the energy minimization step was performed using the steepest descent method. The time-step value for the integration was defined as 2.0 fs, and all the systems were simulated during 100 ns using the NpT ensemble. Overall, this study suggests the inhibitors bind to the CRZ through the H-bond interactions, mainly by the residues Gln19, Gly23, Trp26, Ser64, Asp161, and Trp184. Moreover, the enzyme has not changed its structural composition and compactness, even with the complexation of the inhibitors. However, the network map analyses show that the inhibitors decrease the correlation among CRZ Cα atoms, increasing the number of atomic communities and promoting a disruption in the helix-1 macro dipole.

Acknowledgments: CNPq, CAPES, and FAPERJ.
*this is the original version of the abstract

Area: Cell biology and parasite/host cell interaction; Chemotherapy (drugs and etiological treatment scheme)

#13 ★★

Cardiac cell culture approaches as drug screening protocols for Chagas disease

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More than a century after being reported by Carlos Chagas, Chagas disease (CD), a neglected illness caused by the protozoan *Trypanosoma cruzi,* still remains a serious global public health problem. The available first line treatment is restricted to two nitroderivative drugs, benznidazole (Bz) and nifurtimox, which exhibit limited efficacy and trigger side effects, justifying the search for alternative therapies. Recently was demonstrated a lack of correlation between clinical trials outcomes using inhibitors of lipid biosynthesis and their previous pre-clinical studies. Among other raised hypothesis and discussions, one was related to the importance of revisiting screening protocols *in vitro* and *in vivo* for new drug candidate for CD aiming to reduce translation gaps (Chatelain & Ioset, 2018). In this sense, three-dimensional cell cultures (such as cardiac spheroids) have been claimed to represent a more feasible and accurate protocol to screen new drugs. Presently, three-dimensional (Garzoni et al., 2009) and 2-D (Meirelles et al., 1986) cultures of cardiac cells were used to compared and validate *in vitro* screening protocols of antiparasitic compounds. Our data revealed that regarding cardiotoxicity profile, both 2D and 3-D systems gave similar results when different compound classes were investigated the azole CYP51 inhibitor VNI (Guedes-da-Silva, 2015; Guedes-da-Silva *et al.*, 2017) and Bz, demonstrated a LC50 values ranging from > 100 µM to > 200 µM, respectively. The bisarylimidamide DB766 [(Batista et al., 2010) showed a moderate cardiotoxic profile with values ranging from 51.6 and 41.7 µM for 2 and 3-D systems cultures. While, for the toxic AIA 28SMB032 (Santos *et al.*, 2018), spheroids demonstrated higher sensibility (about 3 times) as compared to 2D cultures. The molecular analysis (qPCR) confirmed *in vitro* efficacy of Bz during the infection of 3D cultures, reaching at 10 µM about 90% of parasitism reduction, similarly as 2D. The analysis of other compounds efficacy and toxicity is underway as well as the evaluation of inflammatory mediators releases after infection and drug administration into 2D and 3D matrices in order to explore more evidences related the advantages of using cardiac organoids in drug discovery programs to identify promising therapeutic alternatives for CD.

Suported by Fiocruz, CNPq, CAPES and FAPERJ

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Area: Cell biology and parasite/host cell interaction; Chemotherapy (drugs and etiological treatment scheme)

#14

Study of the trypanocidal action of natural products from Jurubatiba Restinga – RJ

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Since ancient times, humanity has been looking for medicines to relieve pain and cure various diseases. Substances of natural origin are continuously studied to have a safer and more effective treatment, and plant species have been a prime target for the search for new therapeutic agents. In this context, a vast number of natural products have shown activity against *Trypanosoma cruzi,* a flagellated protozoan that causes Chagas disease (CD) that affects approximately 6 to 7 million people worldwide. The current treatment for Chagas disease (CD) is based solely on two nitroderivative substances, Nifurtimox (Nf) and benznidazole (Bz), both introduced in the medical clinics about 50 years ago, and considered unsatisfactory mainly due to their low efficacy and high toxicity. In this sense, there is a great urgency for the development of new therapies. Several research groups have been studying the potential of natural products as a source of new active substances against the parasite. Thus, the main objective of this project was to evaluate *in vitro* extracts and fractions of plants derived from Restinga de Jurubatiba-RJ over *T cruzi*. For this, we carried out an initial screening against epimastigote and trypomastigote forms of 30 natural products derived from Jurubatiba restinga plants, all at a concentration of 100 µg/mL. From this assay, 5 natural products were identified with good to moderate trypanocidal activity (CE_{50} 53.67 - 105 µg/ mL) both epimastigote and trypomastigote forms. We also evaluated the toxicity against peritoneal macrophages by resazurin reduction and LDH enzyme release assay. Our results indicated low or moderate toxicity (5 – 48%) for most products tested, with special attention to EMPFA. We also evaluated extracts of *M. vittoriana* and the isolated substance centraterine against epimastigote forms. The results obtained from the resazurin reduction assay indicated trypanocidal potential for all products, with particular attention to centraterina, which obtained an EC50 of 308 ng/mL. However, assays on peritoneal macrophages revealed high toxic potential for all extracts and for centraterine. Therefore, our results together demonstrate the presence of substances with therapeutic potential in Jurubatiba restinga's plant species.

Key words: Chagas disease, *Trypanosoma cruzi*, toxicity, natural products.
Financial support: CAPES

Area: Cell biology and parasite/host cell interaction; Chemotherapy (drugs and etiological treatment scheme)

#15 ★

Novel nitrotriazoles with possible two mechanism of action showed high selectivity and potent activity against *Trypanosoma cruzi*

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Although it is the parasitic disease that mostly kill in endemic areas and even after 111 years of its discovery, a curative treatment for the chronic phase of Chagas disease is still unavailable, as the only two approved drugs, benznidazole (BZD) and nifurtimox, have substantial efficacy only in the acute phase[1]. The etiological agent *Trypanosoma cruzi*, like fungi, has ergosterol as the main membrane sterol, so its biosynthesis route via inhibition of the CYP51 enzyme is a well-studied target in the search for new active substances against this parasite[2]. The triazolic ergosterol biosynthesis inhibitor (EBI) posaconazole was able to cure animals in both acute and chronic phases[3], however it failed in clinical trials[4]. In fact, EBI showed to be very potent but not effective. Another class of substances with potent anti-*T. cruzi* activity is the 3-nitrotriazoles which proved to be more potent and selective than their corresponding analogues with the 2-nitroimidazolic and triazolic nuclei[5]. Moreover, 3-nitrotriazolic derivatives can act by two distinct mechanisms, either via inhibition of CYP51, such as the triazolic EBI, and as substrates of the nitroreductase enzyme, such as BZD[5]. Thus, in this work we developed a series of novel 3-nitrotriazolic derivatives through of a simple and inexpensive four-steps synthetic route. The *in vitro* biological evaluation was carried out in intracellular model with amastigote and trypomastigote forms of *T. cruzi* (Tulahuen strain) using L929 murine fibroblasts as mammalian host cells. All substances showed potent activity against *T. cruzi* with submicromolar values of IC_{50} and IC_{90}, all of which proved to be more potent than BZD. The most potent substance in the series presented IC_{50} = 0.0085 μM and IC_{90} = 0.05 μM, and were, approximately, 180-fold and 90-fold more potent than BZD (IC_{50} = 1.52 μM and IC_{90} = 4.47 μM), respectively. In addition, such compounds presented high selectivity indexes (SI), being the most selective with a SI >2,222, with is approximately at least 2-fold higher than that of BZD (SI = 1,390).

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Area: Cell biology and parasite/host cell interaction; Chemotherapy (drugs and etiological treatment scheme)

#16

Evaluation of the effect of physical exercise on mitochondrial and oxidative metabolism in *Trypanosoma cruzi*-infected mice

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Chagas disease (CD), caused by the protozoan *Trypanosoma cruzi*, is a serious health problem in Latin America. This disease has two clinical phases: acute, which has a high number of circulating parasites, and chronic, characterized by parasitemia low or subpatent. The chronic phase can be asymptomatic (indeterminate) and last decades. About 30% of cases evolve to a symptomatic chronic form, in which cardiac damage is frequently observed, however digestive involvement or behavioral alterations can also be detected. In the cardiac form, heart failure is the main cause of death in CD, the main symptoms being fatigue, dyspnea, and progressive impairment of the functional capacity of the heart. However, little is known about the mechanisms related to the progression of the cardiac form of CD. Currently, non-pharmacological strategies, such as physical exercise, appear as a complementary approach to the treatment of several cardiomyopathies, being recommended as an adjunct to the rehabilitation of heart failure of any etiology. Physical exercise improves immune response and increase antioxidant defenses of cardiac tissue. In a study with patients with chronic symptoms of CD, aerobic exercise brought benefits such as improvement in the echocardiographic parameters and the quality of life. This type of activity is not recommended in individuals in the acute phase, as it exacerbates the inflammatory process. However, there is a lack of knowledge of the effect of physical exercise on the indeterminate form of the chronic phase of CD. In this scenario, we aim to assess the effect of moderate exercise on metabolism mitochondrial and oxidative in mice in the chronic phase of infection with 100 or 500 *T. cruzi* strain Y (type II) parasites. Our data demonstrated that BALB/c mice are more susceptible to infection, showing a peak of parasitemia and greater number of inflammatory cells in the cardiac tissue than C57BL/6. Both models studied showed electrical changes 120 days post-infection (dpi), however such changes were not observed 180 dpi. At 180 dpi, echocardiographic analyzes demonstrated a decrease in the left ventricular ejection fraction of infected animals. In the cardiac and skeletal tissues of infected animals we observed a decrease in the consumption of mitochondrial oxygen, accompanied by an increase in reactive oxygen species at 120 and 180 dpi. Compared with non-trained, trained *T. cruzi*-infected C57BL/6 mice presented less inflammatory infiltrates and increased oxygen consumption in the cardiac tissue. To better assess the effect of physical exercise on chronic phase of CD, we are repeating the experiment with a greater amount of animals, aiming to carry out other analyzes such as cytokine dosage, parasitic load by qPCR in addition a split time (150 dpi) for analysis electrocardiographic.

Keywords: *Trypanosoma cruzi*, exercise, mitochondria.
Supported by**:** FAPERJ, CNPq and FIOCRUZ.

Area: Diagnosis, Epidemiology

#17

Application of real-time PCR technique on samples suspected of acute Chagas disease infection

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Chagas disease (CD) is caused by the protozoan *Trypanosoma cruzi* and affects millions of people worldwide. The choice of laboratory techniques for diagnosis is dependent on the clinical phase in which the carrier is. In cases of suspected acute infection, the diagnosis is made through direct parasitological techniques and, in inconclusive cases, IgM anti-*T. cruzi* serology can be used. However, these techniques have limitations and molecular methods appear as alternatives for diagnosis confirmation. Thus, the objective of this study was to compare the results of the real-time PCR technique (qPCR), using the TcSAT-IAM system, developed in SRDC/IAM/Fiocruz-PE for the *T. cruzi* nuclear DNA target, with those of the classical techniques for the diagnosis of acute CD. Samples of 77 individuals exposed to the outbreak that occurred in 2019, in the State of Pernambuco, were tested, being 39 individuals considered cases, according to laboratory diagnostic criteria and/or clinical-epidemiological. The sensitivity of the TcSAT-IAM system was 53.85%, with a confidence interval (CI) of 38.57-68.43, and the specificity was 94.74% (CI 82.71-98.54); positive and negative predictive values were 91.3% and 66.67%, respectively; accuracy of 74% and agreement considered moderate. Among the cases, 28 were classified by laboratory criteria, with qPCR presenting agreement in 16 (57.14%) of them, and 12 (42.86%) discordant. However, these 12 individuals had, on average, 20.33 days of antiparasitic treatment at the time of sample collection to be directed to qPCR. Considering the 11 cases classified by clinical-epidemiological criteria, all obtained negative results for parasitological and serological tests. However, when these samples were submitted to qPCR, 5 (45.45%) had positive results, and 6 (54.54%) had negative results. But, it is important to note that 2 of these individuals had already started antiparasitic therapy at least 8 days before the collection. The data demonstrate the importance of sample collection prior to treatment and the development of a composite gold standard in which the data from the qPCR are considered along with those from direct parasitological and serological tests. The relevance of the molecular technique for rapid diagnosis capable of assisting medical decisions and antiparasitic therapy is highlighted.

Keywords: *Trypanosoma cruzi*; Diagnosis; Real time PCR.
Financial support: Serviço de Referência em Doença de Chagas/IAM/Fiocruz-PE

Areas: Diagnosis, Epidemiology

#18 ★

Rapid Lateral Flow Immunochromatographic Test (RLFT) in the serological diagnosis of *Trypanosoma cruzi* infection in *Canis familiaris*

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For being very exposed to *Trypanosoma cruzi* infection, the domestic dog (*Canis familiaris*) has already been proposed as an efficient sentinel animal in areas where there is a risk of infection for humans. Serological diagnosis is the method of choice to assess the extent of dispersion of the wild transmission cycle of this parasite and, consequently, the risk of human disease. The use of chimeric parasitic antigens results in a sensitive and specific diagnostic test in contrast to crude antigens. Our objective is to evaluate the performance of the Rapid Lateral Flow Immunochromatographic Test (RLFT) for the diagnosis of natural infection of *Canis familiaris* by *T. cruzi*. Based on studies on chimeric proteins to make the diagnosis more accurate, two proteins (IBMP-8.1 and IBMP-8.4) with the best performance in the Immunoenzymatic Assay (ELISA) were tested for use in a RLFT platform with two diagnostic bands. A panel with 277 serum samples from domestic dogs was evaluated: (n = 129) positive for *T. cruzi* in the serological diagnosis; samples with positive blood culture for *T. cruzi* (n = 18); pattern of recognition of different DTUs and negative samples (n = 130). RLFT was also challenged against samples of dogs with proven infection by other trypanosomatids: *Leishmania infantum*, *Trypanosoma rangeli*, *Trypanosoma caninum* and *Crithidia* sp., and samples from dogs infected by: *Anaplasma* sp., *Dirofilaria immitis* and *Erlichia* sp., in order to assess cross reaction (n = 19). The sensitivity was 97.5% and specificity 85.6%, according to the ROC curve. Of the 18 samples with positive blood culture for *T. cruzi*, 16 were positive in the rapid test (88.9%). RLFT was efficient in recognizing infection by DTUs (TcI, TcIII and TcIII / TcV). No cross reactions were observed in infections by *L. infantum*, *T. rangeli*, *T. caninum* and *Dirofilaria immitis*. However, the test cross-reacted with *Crithidia* sp., *Anaplasma* sp. and *Erlichia* sp. RLFT showed an agreement of 0.89 and a coefficient of 0.76, considered substantial (Kappa). The intensity pattern of the bands was directly proportional to the serological title. RLFT was sensitive to detect infection by different *T. cruzi* DTUs and specific when compared to infections by other trypanosomatids. RLFT for *T. cruzi* will enable rapid preventive actions to be taken in places with or without notification of Chagas disease and will benefit mainly those locations where access to a more complex laboratory test is limited.

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Areas: Diagnosis, Epidemiology

#19

Assessing the entomo-epidemiological situation of Chagas disease in rural communities in the state of Piauí, Brazilian semi-arid region

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Background: In northeastern Brazil, the wild nature of *T. cruzi* vectors has challenged control actions. In this region, the species *Triatoma brasiliensis* complex, *Triatoma pseudomaculata* and *Rhodnius nasutus* are the main vectors of *T. cruzi*. These species are able to recolonize the domestic environment after insecticide spraying, bringing back the risk of *T. cruzi* transmission to domestic animals and eventually humans. **Objectives**: This study aims to describe the entomological and epidemiological scenario of Chagas disease in rural communities in the state of Piauí. **Methods**: A cross-sectional study (n=683 individuals/244 dwellings) was carried out to obtain serum samples, sociodemographic data and triatomines in 12 rural communities. **Results**: The overall seropositivity rate of the 12 rural communities was 55/683 (8.1%). Among the research subjects, 74 (10.8%) had already undergone serological tests for Chagas disease. Among these, 19 had received positive results in the past and 36 did not know their seropositive status for Chagas disease. There were no positive examinations among the 307 subjects <30 y of age. Chagas disease prevalence rates were significantly higher in older groups, reaching 24/70 (34.3%) and 9/23 (39.1%) among subjects ages 61–75 and >75 y, respectively. The rates were similar between males and females (7.7% and 8.4%, respectively). Among the 244 households investigated, 50 (20.5%) had at least one positive resident. During visits 1474 triatomines were captured, 1331 (90.3%) of which were found in peridomestic structures such as chicken coops, pens, heaps of tiles and firewood, while the remaining 143 (9.7%) were found inside the houses. The proportion of immature instars (nymphs) among the insects captured in the peridomestic structures and inside the houses was 71.4% and 73.4%, respectively, demonstrating the process of colonization of human dwellings by Chagas disease vectors. Among the insects collected, 1286 (87.2%) were classified as belonging to the *T. brasiliensis* complex, 158 (10.7%) as *Triatoma sordida*, 27 (1.8%) as *T. pseudomaculata* and 3 (0.2%) as *Triatoma melanica*. *T. cruzi* infection rates in insects were 0.5% by light microscopy and 0.9% by culture in NNN/LIT medium. Five cultivated isolates were submitted to molecular genotyping, three of which were identified as *T. cruzi* I and two as *T. cruzi* II. **Conclusions**: Although no vector transmission currently occurs, prevalence rates are high in adults and the elderly. Insect surveillance and control activities should not be discontinued in an environment favorable to the perpetuation of house colonization by triatomines.

KEYWORDS: Brazil, Chagas disease, Triatoma, *Trypanosoma cruzi*
Financial support: Instituto Oswaldo Cruz (IOC) / Fiocruz PIAUÍ

Areas: Diagnosis, Epidemiology

#20 ★

Towards a highly accurate *in vitro* diagnostic method for chronic Chagas disease: engaging *Trypanosoma cruzi* genetic intraspecificity with novel recombinant chimeric antigens

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Chagas disease (CD) is caused by a highly diverse parasitic hemoflagellate called *Trypanosoma cruzi*. As a result of this unusual genetic and phenotypic intraspecificity, there are no gold-standard methods for diagnosing chronic CD. To address this genetic diversity, chimeric antigens, composed of conserved and ubiquitously expressed epitopes of the parasite, were employed to identify specific anti-*T. cruzi* antibodies. We have produced four recombinant chimeric antigens (IBMP-8.1, IBMP-8.2, IBMP-8.3 and IBMP-8.4), which have already been evaluated in phase I, II and III trials utilizing ELISA for human samples. Among them, IBMP-8.1 and IBMP-8.4 were recently selected by Bio-Manguinhos to compose the new rapid test to be used by the Brazilian national health provider (SUS). Here, we bring together the results of phase I, II and III studies demonstrating the IBMP-8.1 and IBMP-8.4 antigens robustness in CD diagnosis. The recombinant chimeric antigens were expressed in *Escherichia. coli* BL21-Star vector and purified by chromatographic methods. In order to assess the diagnostic performance, we used human samples from endemic and non-endemic settings from Latin American countries (Argentina, Bolivia, Brazil and Paraguay) and Spain. Furthermore, the antigens were assessed regarding their reproducibility, cross-reactivity and performance towards samples obtained by different parasitic lineages (DTU) infections. Cross-reaction were analyzed considering samples from several infectious-parasitic diseases. A total of 7,875 serum samples were used to evaluate the diagnostic performance of IBMP proteins. Both antigens demonstrated a high AUC value (> 99.9%), indicating that they can efficiently distinguish positive from negative samples. In addition, we found a high sensitivity (IBMP-8.1: 97.7%; IBMP8.4: 99.4%), specificity (IBMP-8.1: 99.9%; IBMP-8.4: 100%) and accuracy (IBMP-8.1: 99.4%; IBMP-8.4: 99.8%) values, as well as a perfect agreement between the reference standard tests and IBMP (k □ 1.0). No statistical difference was observed when the samples were stratified by the region of origin, indicating that the molecules are able to diagnosis CD regardless of the infecting strain. With respect to the cross-reactivity, only 13 (0.65%) and 4 (0.20%) out of 1,999 samples from unrelated diseases were reactive for IBMP-8.1 and IBMP-8.4 chimeric proteins, respectively. These findings indicate a negligible cross-reaction, including a large sample of sera with well-defined leishmaniasis diagnosis (cutaneous and visceral). The antigens maintained their performance characteristics regardless of geographic origin or parasite lineage. Furthermore, the IBMP chimeric antigens can be safely applied in the diagnosis of Chagas disease in settings where *Leishmania* and *T. cruzi* are considered co-endemic.
Financial support: INOVA Fiocruz, FAPESB, FACEPE, FINEP, CAPES, CNPq

Areas: Diagnosis, Epidemiology

#21

Analysis of RNA as a molecular marker of *Trypanosoma cruzi* viability during the interaction with the invertebrate host

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Chagas disease is a neglected tropical illness, caused by the flagellated protozoan *Trypanosoma cruzi*, that involves a social and economic problem in several countries, especially in Latin America. Regarding the detection of *T. cruzi* infection in vertebrate and invertebrate hosts, tools based on PCR or qPCR assays are useful for qualitative or quantitative molecular diagnosis and presents several advantages over optical microscopy, considered the classic method for the analysis of triatomines. Previously, our group standardized an accurate multiplex TaqMan qPCR assay to estimate the parasite load in triatomines infected with *T. cruzi*. Nevertheless, a recurring question concerns *T. cruzi* DNA detection/quantification in triatomines since the DNA amplification could not differentiate between live and dead parasites. On the other hand, the RNA, due to it is short-lived and labile, can be considered a potential molecular marker of viability of pathogens in hosts as well as in food products. Until now there are few reports comparing the application of molecular diagnostic tools that effectively differentiate between viable and nonviable parasites, especially when it comes to *T. cruzi* evaluation. Therefore, this work aimed to develop a quantitative real time PCR with reverse Transcription (RT-qPCR) to determine the load of viable *T. cruzi* in triatomine samples, evaluating the differences between qPCR targeting DNA or mRNA (cDNA) in midgut samples of *Rhodnius prolixus* artificially-infected with *T. cruzi* (Dm28c). The RT-qPCR assay targeted the triatomine 12S subunit ribosomal RNA, as an internal control, and the *T. cruzi* GAPDH (TcGAPDH) mRNA, a gene constitutively expressed among *T. cruzi* from TcI to TcVI, and between all the evolutive stages of the parasite. The results generated herein confirmed the improved performance of the RT-qPCR assay, with increased PCR efficiency and dynamic range for both targets, in addition to a high sensitivity and specificity. The ability of the RT-qPCR assay to detect viable *T. cruzi* cells in *R. prolixus* was compared with the qPCR multiplex assay. Besides that, the time during which both molecules can be detected both in samples containing feasible parasites and in samples containing contents of lysed *T. cruzi* after boiling treatment was also compared. Taken together the results show that, while RNA is shortly degraded after parasite lysis, DNA presented a stability for a longer period. Thus, this methodology has a potential to be explored for quality control of food products and the investigation of Chagas disease oral outbreaks, through the consumption of açai *in natura*.

Keywords: *Trypanosoma cruzi*, *Rhodnius prolixus*, molecular diagnosis, RT-qPCR, qPCR

Areas: Diagnosis, Epidemiology

#22

Molecular diagnosis of trypanosomatids infection in wild canids skin samples

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Studies regarding trypanosomatid infection in blood samples of free-living wild animals, especially canids, are rare. Consequently, little is known about the role of this group in the transmission cycle of these parasites. *Trypanosoma caninum* and *Leishmania infantum* have been described infecting the skin of domestic dogs; however, no trypanosomatid species have been detected on wild canid skin. The aim of this work was to evaluate the presence of trypanosomatids on skin samples of wild canids through molecular diagnosis. The samples were collected from free-living individuals from a region of cattle ranches in the municipality of Cumari, Goiás, Cerrado biome, between 2013 and 2014. We analyzed 63 skin samples from three species: *Cerdocyonthous* (n = 39), *Lycalopex vetulus* (n = 16), and *Chrysocyon brachyurus* (n = 8), deposited in LABTRIP tissue bank. All samples were extracted using the Wizard Genomic DNA Purification Kit (Promega®) and subjected to nested-PCR for the partial region of the 18S rDNA gene specific to the family Trypanosomatidae. Positive samples were purified using the kit Illustra GFX (GE Healthcare®) and sent for sequencing. The sequences were analyzed using BLAST/GenBank. Three *C. thous* samples were positive in the nested PCR with amplification patterns between 600 and 700 bp, resulting in a prevalence of 4.8% (3/63) and 7.7% (3/39), considering the total samples and only the species, respectively. One of the samples was characterized as *Trypanosoma cruzi* DTU TcII with 99.78% identity and 100% coverage in BLAST. We were unable to identify the species in the other two samples; nevertheless, they were considered positive for trypanosomatids. The presence of trypanosomatid DNA on *C. thous* skin sheds light on two aspects of the wild transmission cycle puzzle: (i) it may indicate the potential transmissibility of the species, given that its presence could imply the parasite availability to the vector in the peripheral blood and (ii) the potential broader distribution of TcII, considering that we have performed hemoculture in the same individuals and did not isolate the parasite. The higher sensitivity of the molecular method and the TcII finding reinforces the hypothesis that *this genotype could have ashort period of parasitemia and, therefore, is hidden in nature. Trypanosoma cruzi* DTU TcI and TcIII were described infecting the blood of other wild canids as well as TcII in domestic dogs. However, this is the first time that TcII is described infecting a wild canid demonstrating, once again, that this genotype infects different wild mammal species.

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Areas: Diagnosis, Epidemiology

#23

Evaluation of the sensitivity of DNA extraction of *Trypanosoma cruzi* in blood clots samples

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Trypanosoma cruzi is a heteroxenous parasite whose biological cycle is composed of hosts mammals and triatomines. Among the biological materials used to diagnose the infection of this parasite in mammals, the blood clot (BC), that is little used and, in most times, discarded, has potential for molecular diagnosis. Recent works demonstrate success with the use of the clot for diagnosis, but the sensitivity of DNA extraction using this material is not yet known. The aim of this study was to evaluate the sensitivity of the detection of *T. cruzi* DNA from BC under experimental conditions. *T. cruzi* DTU II (strain MHOM/BR/1957/Y) was expanded in biphasic culture medium NNN/LIT+10%SFB for Neubauer chamber count. The concentrations of 1x104, 1x105 and 1x106 parasites in 1ml rat blood of the species *Rattus novergicus* were tested. The blood+culture mixture of *T. cruzi* DTU II was maintained at 4ºC/4 hours for BC formation. The BC and sera were separate by centrifugation. The DNA from BC was extracted from a 0.08g fragment by "*Salting Out*" method and submitted to nested-PCR for the partial region of the 18S rDNA gene, with variations in the protocol - 8.5µl and 12.5µl of the GoTaq enzyme (Promega®), 16pmol of each primer and total DNA concentrations ranging from 141.8ng/µl to 1583.5ng/µl. The experiment was performed in duplicate. All samples analyzed were positive using both protocols of nested-PCR. Samples of clots in which it mixed with the blood 1x104, 1x105 and 1x106 parasites/ml were positive with a total DNA concentrations from 1257.5; 1225.5 and 354.5ng/µl, respectively. One of the DNA sample (BC fragment of blood+1x106 parasite/ml mixture) was negative in the both nested-PCR reaction protocols, however the same sample was positive in the second experiment when another BC fragment was used. This demonstrates that the success of the DNA extraction of clot is not only related to the concentration of parasites in the sample, but also in the BC fragment used for extraction. The BC proved to be an adequate material for detection of *T. cruzi* DNA in the concentrations of parasites here tested. Lower concentrations of parasites will still be used for the knowledge of the *T. cruzi* DNA detection minimum in clot samples. The use of clot as a source of DNA and molecular tools will contribute to the diagnosis of *T. cruzi* infection in mammals complementing the serological and parasitological diagnosis.

Area: Education, Information

#24

Playful teaching in learning about Chagas disease: Experience report

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Chagas disease is a neglected tropical disease caused by the hemoflagellate protozoan *Trypanosoma cruzi*. Even after more than 110 years of its discovery, the disease remains as an important public health problem worldwide, with more than 12 million chronic carriers in the Americas and Brazil, affecting mainly the low-income population who do not have access to information or prophylactics measures. Thus, the objective of this work was to analyze the effectiveness of the ludic instruments used for the children and infant-juvenile public in learning about Chagas disease. It is a descriptive study of the type Experience Report with ludic approach about the forms of transmission, biological cycle, and evolutionary forms of *T*. *cruzi*, as well as clinical manifestations of the disease. The target audience were children and adolescents from public schools of children and elementary schools in Recife-PE, that were monitored by students and collaborators at the National Week of Science and Technology 2019 that took place at the Instituto Aggeu Magalhães, FIOCRUZ Pernambuco. During the event, there was a didactic and illustrative explanation for all the students of kindergarten and elementary school. Afterwards, the students were able to reinforce and evaluate learning through the various playful activities, addressing the contents on clinical, evolutionary, transmission and prevention forms of the disease. Board games were used, visualization of evolutionary forms of the parasite in the optical microscope, reading of an educational comic books, memory game through questions and answers. Children and young people had voluntary participation in all activities and one of our speakers was dressed in a barber costume, which caught the public's attention and motivated participation in all proposed activities. The games and play are part of the children's universe and promote motivation for elementary school students, so the realization of playful activities in this area contributes to better construction of knowledge in early childhood education and infant-juvenile, being the children and young people multipliers of information. Therefore, ludic teaching is important and effective in building knowledge and valuing teaching-learning in early childhood and elementary education.

Keywords: Chagas disease, Playful teaching, Education.

Area: Education, Information

#25

Experience in a research laboratory with knowledge about the insect triatominae and the parasite *Trypanosoma cruzi*: A report of professional experience in the Scientific Vocation Program

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Research activities on Chagas disease enable to work in a practical and integrated way contents of different curricular disciplines constituting a path to discovering the skills and vocation of young people in the scientific world. In the light of scientific knowledge about triatomines and *Trypanosoma cruzi* parasite, routine methodologies research and diagnosis of Chagas disease, can constitute a student's first experience in a laboratory in health and research area, providing life and professional experiences. The objective here is to present the theoretical-practical activities common in Chagas disease area that were developed together with the Scientific Vocation Program of the Joaquim Venâncio Polytechnic School, Fiocruz. The beginning of the experience occurs with the Provoc team itself, which teaches biosafety and good conduct at Fiocruz. Together with the institutional policy, the first contact consisted of presentation of the team and internal training of good laboratory practices with visit to the laboratory and demonstration class on equipment and glassware used in the laboratory. Subsequent weekly theoretical-practical meetings followed these approaches: study of external morphology and use of dichotomous key for insects; differentiation of phytophagous, predatory and blood-sucking stink bugs; study of the main morphological differences of triatomine genera; observation of the life cycle using educational plaques and living in colonies; assembly and conservation of specimens for collection; observation of internal morphology and research of *T. cruzi* in insect intestines; observation of *T. cruzi* forms from culture isolates and maintenance in culture medium; preparation of medium and solutions; and importance of biological collections for the study of Biodiversity with a visit to the Entomological Collection of the Oswaldo Cruz Institute – Fiocruz. The methodologies make it possible to work Biology and Chemistry contents, with greater affinity in high school, raising students' desire to work with Biomedicine. From the experience in the laboratory, interest in parasitology has been aroused, focusing on the molecular biology of the parasite and on knowledge about the vector species involved in the transmission of Chagas disease in Rio de Janeiro, a theme that is intended to be explored in the coming years of Provoc. In conclusion, programs like Provoc which through integrated actions of Education and health, act in the transfer of scientific knowledge as well as in formation of young scientists aligned with the quality policies for the promotion of public health, can strengthen the entomological surveillance services in a proactive way to control the transmission of neglected diseases.

Keywords: Education; Chagas disease; Scientific Vocation Program.

Financial support: CNPq; IOC / Fiocruz.

Area: Education, Information

#26 ★

FluorArte Workshop in Expresso Chagas 21: An ArtScience strategy to discuss Chagas disease using fluorescent images*

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Chagas disease (CD) is a neglected disease caused by *Trypanosoma cruzi* and transmitted by a reduviidae bug. In acute phase, unspecific symptoms can be observed. The chronic phase can be symptomatic or not and the main clinical manifestation is the cardiomyopathy. The absence of symptoms in 70% of infected people, make it an invisible disease resulting in absence of public policies that warrant access to health systems for those people. For that reason, strategies that reach affected people, empowering them to know the disease and to fight for their rights, are powerful. In this context, scientists of Oswaldo Cruz Institute, together with people of “Rio Chagas Association” created the “Expresso Chagas 21” an imaginary train that refers to the train where Carlos Chagas discovered CD in 1909. An itinerant social technology integrates art and science for education in non-formal teaching spaces, health promotion and active search of infected people. Here we will describe the workshop FluorArt that integrated the activities of “Expresso Chagas 21” during the expedition in north of Minas Gerais (Brazil). FluorArt workshop is based on ArtScience strategy, which by cooperation and creativity stimuli, contributes to solve concrete problems. FluorArt promotes observation of images related to CD obtained from scientific papers, discussion, and creation of “fluorescent” images. The preparation of the FluorArt workshop involved meetings with the team of expedition, bibliographic survey using the descriptors "immunofluorescence + chagas" and "immunofluorescence + *Trypanosoma cruzi*" and construction of materials. We consulted approximately thirty academic articles from Google, of which six images were selected based on criteria such as sharpness, color, and direct analogies with CD for construction of a six-side dice. After signature by the participants of ethical permission terms, the FluorArt was carried out in 5 steps: i) personal presentation; ii) dice game followed by the question “What do you think this image might be?”; iii) painting of the image interpretation with phosphorescent colors; iv) revelation of painting in a booth with black light; v) final dialogue about the painting, its relationship with CD and a brief presentation of original fluorescent images in posters. The workshop was well accepted by the participants, it was able to stimuli creativity based on 13 creativity skills of Bernstein, and was innovative by presenting potential to education, creativity stimuli, health promotion, and improvement of access to health of a vulnerable population exposed to CD.

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*this is the original version of the abstract

Area: Education, Information

#27 ★

Chagas Express XXI: an educational social technology

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From 2015 to 2018, patients affected by Chagas disease (CD) experienced at Fiocruz some of our ArtScience workshops in a course intitled “Talking about Chagas with ArtScience”. They then asked researchers to bring health contents to people living in the socioeconomic vulnerable endemic areas. As an answer to this challenge, we developed the itinerant ArtScience exhibition called “Chagas Express XXI”. The aim of the present study was to describe and analyze this unique experience developed in Brazil, that fuses ArtScience practical workshops build about a neglected disease (CD). It articulates workshops, exhibitions, games, practical laboratory activities and conversation/ participation rounds, with relevant content for endemic areas with prevalence of chronic cases or risk of acute cases. Chagas Express XXI was developed by researchers and students at Fiocruz and CD patients who participate at the Rio Chagas Association. The artistic concept configured the Chagas Express XXI in the format of a train station as an entrance and exit, followed by a set of six “wagons” forming an imaginary train with various playful activities. Identified at the station, participants were sensitized to the exhibition and follow the thematic wagons: (1) Associations, (2) Innovations & Laboratory, (3) Discoveries & Play, (4) Home & Environment, (5) Well-Being and (6) Your Voice. During the nine days of exhibition, Chagas Express XXI engaged more than 2.000 people and 56% asked for blood testing (CD diagnostic test). We observed a high percentage of positivity. From the 1.100 adults tested, 20% were diagnosed as positive case to CD. We obtained another very important result: the registration of more than 600 participants interested on collaborate and participate in civil organizations of health forum, such as the proposed “Nuclei of health promotion” and new local “CD Associations”. The public attending Express Chagas XXI answered some questions about CD. A high percentage of the participants have already heard about CD (84%) and personal experience dealing with the disease in the family is common (22-46%%). An alarming result was the extremely high percentage of people who did not know about the possibility of treating CD (81%). All these actions taken together, brought visibility to issues surrounding CD, with its 4 dimensions: biomedical, epidemiological, economic-social and political-cultural and greater engagement of interested people. Express Chagas XXI acts as an educational social technology that emerged from an integration of research, education and extension disseminating information through dialogic message between academia and society.

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Area: Education, Information

#28
Scientific production in chagas: a look from the scielo collection

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As an aggravated health problem, Chagas disease and an endemic disease in Brazil, it is considered as one of the main public health problems in the world. Even with investments and investments or the number of infections related to the disease continues to be reported in recent decades, due to factors such as rapid and disordered increase in urbanization, access to universal basic sanitation and quality. In such a diverse scenario of demands, the importance of the role of public policies is undeniable, especially those that guide or invest and estimate research. In this sense, the knowledge of scientific production in relation to this condition is important to know the profile of these researches in wounds, made available for free access. A source created to search for this production was the Brazil Collection from the Scientific Electronic Library (SciELO). The main results pointed to a growth from 1998, having a biomedical character of the research carried out in the country; a pattern of scientific production in its majority, in co-authorship; incipience of scientific production on scientific subjects in the area of Applied Social Sciences and treatments as the most important sub-theme for reproduction in the production area.

Keywords: Scientific production. Neglected diseases. Chagas disease. SciELO.

Area: Education, Information

Resumo Tardio / Late Abstract

Symposium on Science, Art and Citizenship: education and information on Chagas Disease

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Chagas disease is a tropical parasitic disease caused by the flagellate protozoan *Trypanosoma cruzi*. Considered a neglected tropical disease or disease of poverty, Chagas is endemic in 21 countries of the Americas. Therefore, education and scientific dissemination are needed to obtain greater visibility and thus promote engagement with citizenship actions. In 2018, the first event of the Symposium on Science, Art and Citizenship was aimed to promote discussion and shed light on education, diagnosis and treatments for Chagas disease. Initially, we created the network on the fanpage (Facebook) and then a YouTube Channel and broadcast the event live. Patients, people affected by Chagas disease, that constitute the Rio Chagas Association, Fiocruz researchers and students participated in the Symposium. We reached 746 people in the live broadcast and 50 people attending the physical event. 60% of the participants have heard about the event through our fanpage, 20% by acquaintances of the Association and 20% by the researchers involved in the project. The participants heard about the event through our fanpage (60%), the Rio Chagas Association (20%) and the researchers involved in the Symposium (20%). The public profile was academic, master and doctorate students, specialization, undergraduate students, researchers and from the communities. The participants motivations into the event were: (1) to acquire knowledge; (2) to improve their research and project and (3) to make Chagas Disease visible. Thus, the Symposium on Science, Art and Citizenship acted as a strategic source for promoting health education actions, being considered a place for knowledge exchange, making the topic more evident through the dissemination of citizenship, education and information on Chagas Disease.

Supported by: FAPERJ/ CNPq
Keywords: Chagas disease; Symposium; Science; Art and Citizenship

Area: Pathology / Pathogenesis and Clinical Aspects

#29 ★★

Evaluation of CXCL9 / MIG and CXCL10 / IP-10 chemokines in serum of patients with cardiac and indeterminate clinical forms of chronic Chagas disease

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FUNDERS: Proep / Facepe / SRDC

Chagas disease is considered one of the most important infectious-parasitic diseases related to poverty, being included in the group of neglected diseases. The immune response against *Trypanosoma cruzi* has been shown to be very important in the evolution process of the different clinical manifestations of chronic Chagas disease, being chemokines important mediators involved in maintaining the inflammatory process, which may be associated with a poor prognosis of chronic disease. Therefore, the present study aimed to relate the levels of chemokines CXCL9 / MIG and CXCL10 / IP-10 in serum of chronic carriers of the cardiac and indeterminate clinical forms of chronic Chagas disease. Individuals with Chagas disease were divided into three groups: indeterminate (n = 46), mild cardiac (n = 32) and severe cardiac (n = 29), through clinical examinations. A group of individuals without infection was included (NEG). The chemokines were measured in serum samples, using the Cytometry Bead Array technique. Data analysis was performed using the FCAP Array software (BD). The levels of CXCL9 / MIG and CXCL10 / IP-10 were significantly higher in infected individuals when compared with the NEG group, which shows the involvement of these chemokines in the inflammatory process of chronic Chagas disease. In humans, there is no consensus on the role of CXCL9 / MIG and CXCL10 / IP-10 in the development of Chagas heart disease. On the other hand, individuals with severe heart disease showed lower levels compared to individuals with other clinical forms, although it was not statistically significant. This suggests a possible mechanism for regulating inflammation in individuals with severe heart disease, which is important to investigate in future studies.

Area: Therapy (immunotherapy, cellular therapy and others)

#30 ★

Investigation of the antioxidant and antichagasic potential of luteolin: analysis of structure-activity relationships

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Chagas disease is a tropical disease with worldwide reach that affects millions of people, especially in Latin America, where it is endemic. This disease is endemic in 21 countries in Latin America and has mortality rates ranging from 7 to 10,000 deaths per year. The drugs available are obsolete and have low and high toxicity. In this context, the development of capable and safe drugs for Chagas disease is an important demand, especially in endemic countries like Brazil. Since oxidative stress is involved in various parasitic diseases, such as Chagas disease in this work were investigated antioxidant and antichagasic potential of luteolin, a compound of natural origin. The antichagasic activity was evaluated by *in vitro*, *in vivo* and *in silico* models, whose investigated molecular target was the inhibition of the cruzain enzyme of *T. cruzi*, using a standard of luteolin (Sigma-Aldrich, USA). Initially, the cytotoxicity was determined by methylthiazolyldiphenyl-tetrazolium bromide (MTT) method and the viability of *T. cruzi* Y strain (epimastigote) were evaluated after treatment by counting the total number of live parasites, taking into account the flagellar motility, using Neubauer's camera and optical light microscope. The compound luteolin showed activity against epimastigote with IC50 of 56,5±1.12 µM. The cruzain enzyme (PDB ID: 3KKU) was used in pharmacodynamic studies, whose enzyme inhibition results were rationalized by molecular docking, carried out in the program Gold 5.2. Luteolin exhibited activity against cruzain of 21.48±1,23 µM, showing to be a promising compound. In the molecular docking simulations, luteolin exhibited a good binding to cruzain due to the hydrogen bond between the compound and with residues in catalytic cavity. To measure the antioxidant activity that was used hydrogen peroxide (H2O2) scavenging activity (EC50=16.62± 0.22 µM) and nitric oxide (NO) scavenging activity (EC50= 15.01±0.13 µM). Molecular coupling analyzes were used to understand possible mechanisms of antioxidant action, especially by inhibiting nitric oxide synthase (PDB ID: 6NGJ), for which hydrogen bonding interactions between luteolin and Val 677, Trp 678 and Ser 334 residues were observed, in addition to a Pi-Pi stacking interaction with Trp 678 residue, which explain the activity observed in *in vitro* assays.

Keywords: Luteolin; *Trypanosoma cruzi; Cruzain;* Molecular docking.
Acknowledgment**:** This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001. Just as the authors thank the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

Area: Therapy (immunotherapy, cellular therapy and others)

#31

Experimental combination therapy using benznidazole and amiodarone in mouse model of *Trypanosoma cruzi* acute infection

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Chagas disease (CD), caused by *Trypanosoma cruzi*, affects about 6 to 7 million people in Latin America being the cardiomyopathy a clinical manifestation most associated with mortality in acute patients, due to compromised electrical and mechanical cardiac function. The etiological treatment of CD is restricted to benznidazole (**Bz**) and nifurtimox (**Nif**), which have therapeutic failures, side effects and low efficacy in chronic patients. Thus, combined therapies emerge as an important tool in the treatment of CD, because they allow the reduction of the dose and time of treatment with **Bz**, reducing side effects, and modulating other components of the disease, giving a better prognosis to the patient. In this sense, amiodarone (**AMD**) one of the most efficient antiarrhythmic drugs currently available and prescribed to patients with CD it is to consider a candidate to improve the etiological treatment of CD, due its already demonstrated trypanocidal activity *in vitro* and *in vivo,* using a murine model. Moreover, the study published in 2015, BENEFIT, showed that the only subgroup of chronic patients, who showed improvement in the clinical parameters evaluated, was composed of individuals treated with **AMD** in association with **Bz**. However, the effectiveness of **AMD** in DC is still poorly studied, as well as its interaction with drugs used in etiological treatment. In this context, this project aims to fill the gaps related to the real efficacy of the combination therapy of a suboptimal dose of **Bz** and **AMD** during the acute phase of CD in experimental model, as well as, determining the *in vitro*: activity, interaction, selectivity and possible mechanisms of action. In the present study, we observed that the *in vitro* interaction of these drugs is classified as additive against bloodstream trypomastigotes and intracellular amastigote forms of *T. cruzi* (Y strain). Furthermore, the combined treatment of these two drugs in primary cultures of mouse embryo heart muscle cells infected with *T. cruzi* partially reversed the cytoskeletal damage caused by the infection. Lastly, using a murine model of acute phase of CD, we observed that the **Bz / AMD** combination reduced: the number of circulating parasites, the mortality rate, and the deposition of collagen and inflammation in cardiac tissue. This combination also improved the electrocardiographic profile of the animals, with a decrease in the P wave duration and reduction of arrhythmias events.

Financial support: FAPERJ, CAPES e CNPq.

Area: Therapy (immunotherapy, cellular therapy and others)

#32

TGF-β neutralization improves cardiac conduction system during the chronic phase of Chagas disease in a murine experimental model

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Chronic chagasic cardiomyopathy (CCC) is the most important and frequent clinical manifestation of chronic Chagas disease. During CCC, the parasite remains inside the cardiac cells, leading tissue damage, involving extensive inflammatory processes and irregular fibrosis. Some molecules act in the fibrosis formation, but one in particular plays a key role in the fibrogenic process inducing extracellular matrix synthesis: the transforming growth factor-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. Thus, the main goal of this study is to evaluate the effect of a neutralizing antibody anti-TGF-β (1D11) during the chronic phase of Chagas disease in a murine experimental model. Female mice were infected with trypomastigotes of the Colombian strain of *T. cruzi* and were monitored electrocardiographically to observe the cardiac conduction system. After 120 dpi, the treatment with anti-TGF-β (10mg/kg) was initiated in two different schemes: single dose and once a week up to 150 dpi. We observed that the infection altered the cardiac electrical conduction: decreasing the heart rate, increasing the PR interval and the duration of the P wave. The anti-TGF-β treatment reestablished the electrocardiographic profile of the infected animals. Moreover, the treatment with anti-TGF-β decreases the collagen deposition in the heart of infected animals, supporting an effective strategy to reverse fibrosis. These data are promising and the therapeutic effects of 1D11 suggest a new possibility to treat cardiac fibrosis in the chronic phase of Chagas heart disease by TGF-β inhibitors.

Supported by: INSERM / Fiocruz / CNPq / FAPERJ / DECIT
Keywords: Chagas disease; fibrosis; TGF-beta

Area: Parasite (genetic, molecular, biological and morphological diversity)

#33

Biodiversity and phylogenetics of a lipid droplet kinase of *Leishmania* and *Trypanosoma*

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Introduction: Leishmaniasis and Chagas disease are neglected diseases caused by protozoan parasites of the Trypanosomatidae family. Available treatments against these diseases present several problems such as high toxicity and selection of resistant strains besides a low cure efficiency in the case of Chagas disease. Accessible data of *Leishmania* and *Trypanosoma* in public databases, alongside studies using genetic tools, have improved our knowledge of parasite biology and drug resistance. Phylogenetics aims to study the relationships among macromolecules as well as different taxonomic groups over evolutionary time. This area of Life Sciences has several applications in ecology, biodiversity, health, and others. Phylogenetic inference, for example, allows functional prediction of genes and proteins that have not yet undergone experimental evaluation. A lipid droplet kinase (LDK), which is known to be involved in lipid biogenesis, has been studied in *T. brucei*. To date, no phylogenetic study of LDK in Trypanosomatidae has been described. Thus, our main goal is to reconstruct and evaluate the LDK molecular phylogeny of *Leishmania* and *Trypanosoma*.
Methodology: Molecular sequences from *Leishmania* and *Trypanosoma* were retrieved from TriTrypDB and UniProt. Sequence similarity searches were carried out using BLAST. Gene organization and protein architecture of potential homologs were accessed using different databases and computational tools. Amino acid signatures were revealed by PROSITE. Sequence alignments and evolutionary tree reconstruction were performed in MEGA. Tree annotation and functional prediction of genes and proteins were accomplished based on the literature and database information. KinBase was used to access protein kinase classification.
Results: Potential homologs of LDK in *Leishmania* and *Trypanosoma*, identified by BLAST and Pfam searches, vary considerably in length despite the conservation of their protein architecture. According to Pfam, LDK has a single domain (PF00069) in its architecture, which is present in serine/threonine kinases. PROSITE reveals a total of 49 LDK critical residues including active sites and pos-translational modification sites, which are grouped into five different categories. LDK from *Leishmania infantum* (LINF_280026600) belongs to a hypothetical orthologous group (OG5_148826) with 11 members in *Leishmania* and *Trypanosoma* as identified in OrthoMCL. Domain sequence alignments and phylogenetic trees support their evolutionary relationships. KinBase indicates that LDK belongs to the CAMK group. Such database provides information for CAMK homologs in *L. major* (8 proteins) and human (17 proteins). These proteins have quite diverse architectures. We intend to study these proteins more deeply.

Keywords: neglected disease, parasite biology, protein kinase, phylogenetics, molecular evolution, function prediction.
Financial Support: FAPEMIG, INOVA Fiocruz, and PIBIC/CNPq.

Area: Parasite (genetic, molecular, biological and morphological diversity)

#34 ★

Genome-wide mutagenesis and multi-drug resistance in American trypanosomes induced by the frontline drug benznidazole

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Chagas disease is caused by the protozoan parasite *Trypanosoma cruzi* and affects 5–8 million people in Latin America. Although the nitroheterocyclic compound benznidazole has been the front-line drug for several decades, treatment failures are common. Benznidazole is a pro-drug and is bio-activated within the parasite by the mitochondrial nitroreductase TcNTR-1, leading to the generation of reactive metabolites that have trypanocidal activity. To better assess drug action and resistance, we sequenced the genomes of *T. cruzi* Y strain (35.5 Mb) and three benznidazole-resistant clones derived from a single drug-selected population. This revealed the genome-wide accumulation of mutations in the resistant parasites, in addition to variations in DNA copy-number. We observed mutations in DNA repair genes, linked with increased susceptibility to DNA alkylating and inter-strand crosslinking agents. Stop-codon-generating mutations in TcNTR-1 were associated with cross-resistance to other nitroheterocyclic drugs. Unexpectedly, the clones were also highly resistant to the ergosterol biosynthesis inhibitor posaconazole, a drug proposed for use against *T. cruzi* infections, in combination with benznidazole. Our findings therefore identify the highly mutagenic activity of benznidazole metabolites in *T. cruzi*, demonstrate that this can result in multi-drug resistance, and indicate that vigilance will be required if benznidazole is used in combination therapy.

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Area: Parasite (genetic, molecular, biological and morphological diversity)

#35 ★★

Identification of *Trypanosoma cruzi* ribose-5-phosphate isomerase inhibitors for Chagas disease chemotherapy: from an *in vitro* approach into the wilderness of exploring protein sequence-structure-function diversity

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The enzyme ribose-5-phosphate isomerase of *Trypanosoma cruzi* (TcRpi) may be a suitable drug target for Chagas disease, as it shares no significant sequence and structural similarity to its human analog, HsRpi, and is essential for parasite survival. In previous work, we used computational methods to identify compounds that potentially inhibit TcRpi activity without affecting HsRpi. In the present work, we aim to clone, express, and purify recombinant TcRpi and HsRpi enzymes to test their inhibition profiles with compounds identified by virtual screening. While cloning the TcRpi gene from the Y and CL Brener strains for protein expression in *Escherichia coli*, we identified six protein sequences displaying several amino acid substitutions when compared to the reference sequence of *T. cruzi* CL Brenner. Preliminary results suggest that one of these variants is less efficient than what has been reported in the literature. This result, together with the different sequences we observed, raised questions about the diversity of TcRpi sequence and function in *T. cruzi* populations. To address these issues, we BLAST searched for TcRpi gene variants deposited in GenBank and TriTrypDB databases, identifying four additional sequences. Some of the substitutions are at or near sites that have been previously implicated in enzymatic properties. Thus, we used homology modelling to generate 3D models of the non-redundant sequences found in the databases and of the variants we identified. The models will be used in docking assays and molecular dynamics simulations to evaluate if the amino acid substitutions could change protein-ligand interaction. We are also using comparative genomics to map the diversity of TcRpi sequences in parasites isolated from naturally infected organisms, such as *Triatoma infestans*, guinea pigs, and dogs. Ultimately, we hope to contribute to the understanding of the relationship between the genetic and functional diversity observed in TcRpi, and any impact on its use as a therapeutic target.

Area: Parasite (genetic, molecular, biological and morphological diversity)

#36 ★

Genotypic diversity of *Trypanosoma cruzi* in chronic patients born and resident in the State of Pernambuco

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Chagas disease (CD) is a neglected endemic disease that affects about 6-7 million people worldwide. CD has the flagellate protozoan *Trypanosoma cruzi* as its etiological agent and it is characterized by two phases: the acute phase, with patent parasitemia, and the chronic phase, with low and intermittent parasitemia. CD has different clinical outcomes with varying degrees of severity. Our goal is to address the participation of the parasite in this process in a cohort from Pernambuco State (PE). Here, we performed (i) molecular diagnosis by conventional polymerase chain reaction (cPCR) for kinetoplast DNA (kDNA) and (ii) genotypic characterization of *T. cruzi* into discrete typing units (DTU). The study was conducted on a group of 346 patients born and still reside in PE with two positive sorological tests for CD evaluated at the Ambulatório de Doença de Chagas e Insuficiência Cardíaca do Pronto Socorro Cardiológico de Pernambuco (PROCAPE/UPE). According to the clinical form of the disease, chronically *T. cruzi*-infected patients were classified as undetermined (57), cardiac (232), digestive (15) and cardio/digestive (42). Venous blood was collected and used to obtain whole blood and/or serum. DNA isolation of theses samples was performed by commercial kit using silica column. Using two independent analysis, the amplification of kDNA by cPCR showed 37.3% positivity (129/346). The cPCR positive samples (129) are undergoing genotypic characterization by cPCR/qPCR and use of appropriate algorithms. So far, 15 clinical samples have been analyzed and we have characterized DTUs in 11 samples: TcVI (6 patients), TcII (1 patient), TcI+TcVI (1 patient), inconclusive TcII/VI (1 patient) and, for the first time in PE State, TcI DTU (2 patients). We thus confirmed the viability of the clinical samples and the possibility of genotypic characterization of the cPCR positive samples. After completing the molecular analysis, we expect to contribute to the knowledge of the geographic distribution of *T. cruzi* DTUs in the state of Pernambuco.

Financial support: CAPES, CNPq, FAPERJ

Area: Vector, transmission cycles, ecology and biodiversity

#37 ★★

Catalytic residues in the Murein Endopeptidase of *Rhodnius prolixus*

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INTRODUCTION: *Rhodnius prolixus* is a blood-sucking triatomine that has a high vector capacity. It is a prominent vector for *Trypanosoma cruzi* in Latin America. *T. cruzi* is the etiologic agent of Chagas disease, a neglected endemic disease that mainly affects the population with low socioeconomic status. There is an important relationship between the midgut microbiota, the insect's physiology, and the relationship with the trypanosomatid. Murein endopeptidase has been described in the midgut of *R. prolixus* through the phenomenon of horizontal transfer of a bacterial protease gene with a role in the digestion of insect vectors, which may have a fundamental role in the physiology of triatomines. This was the first description of horizontal transfer of a bacterial protease gene with role in the digestion of insect vectors.
GOAL: Evaluate the conservation of the catalytic residues of *R. prolixus* murein endopeptidase.
METHODS: The murein endopeptidase sequence expressed in the insect's midgut (RPRC003168 gene, Vector Base code) was aligned with bacterial sequences and the amino acids important in enzyme function were manually inspected. The study was done using bioinformatics tools, including the VectorBase, BLAST, Merops and BioEdit.
RESULTS: The protein encoded by *R. prolixus*, belongs to the family of murein endopeptidases (M74 family of peptidases), and has catalytic residues conserved. It can be seen that all conserved residues with proven involvement in the catalysis were observed in the amino acid sequence of the protein encoded by the insect's genome. These are five histidine residues (H110, H113, H206, H209, H211; numbering according to the *Escherichia coli* MepA protein), responsible for the coordination of a Zinc atom, and two aspartic acid residues (D118 and D120).
CONCLUSIONS: Catalytic residues are conserved in the murein endopeptidase gene expressed in the midgut of *R. prolixus*. Thus, the enzyme in the midgut of this vector may have a catalytic function, contributing to the digestive physiology of the vector.

Key Words: *Rhodnius prolixus*, murein endopeptidase, bioinformatics tools
Acknowledgements: CNPq, FAPERJ, and Fiocruz

Area: Vector, transmission cycles, ecology and biodiversity

#38 ★

Biological activity of the oleoresin of *Copaifera multijuga* against *Rhodnius prolixus*

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Chagas disease is one of the Neglected Tropical Diseases (NTD) and the main form of transmission in Latin America is through contact with the feces / urine of the insect host of the protozoan *Trypanosoma cruzi*, which feeds on the blood of mammals. Contamination can occur by defecation/ urination of triatomines close to the bite area when the person affected smears it into the bite, the eyes, mouth or some skin break, by consumption of contaminated food (oral) or less frequently, by blood transfusion, organ transplantation, laboratory accidents and congenital. It's important to know that there is no vaccine for Chagas Disease and the best method to prevent it is through vector control [1]. In addition, many species of insects have developed resistance to insecticides [2], so plants have become an alternative in research since they are sources of bioactive compounds and can still provide lower production costs. *Copaifera multijuga* Hayne is distributed in the states of Amazonas, Rondônia and Pará [3] and in traditional folk medicine it's used due its anti-inflammatory, healing, antiseptic properties and others [4]. In this context, a commercial oleoresin from *Copaifera multijuga* Hayne was 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 300º at 4ºC/min. Helium was the carrier gas (1mL/min). The substances were identified by Wiley data system library of the equipment. A test against the triatomine *Rhodnius prolixus* in the fifth stage by applying the solutions directly to the insects was made at the concentrations of 150 mg/mL, 5x dilution, 10x dilution and 100x dilution and the mortality monitored by 72 hours. As the control solution, pure solvent was used under the same conditions that the oleoresin. Analysis through GC-MS showed the presence of β-Caryophyllene, α-Copaene and Germacrene D as major components, in accordance with the literature of this species. In the end of the experiment there was no mortality in control, however, 55% of deaths were observed in the concentration of 150mg/mL, 50% in the 5x dilution, 25% in 10x dilution, and 0% in 100x dilution. These results indicate that the oleoresin is promising and active against the triatomine *Rhodnius prolixus.* Further tests will be carried out.

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Area: Vector, transmission cycles, ecology and biodiversity

#39

Ecological segregation of *Triatoma brasiliensis* and *Triatoma pseudomaculata* in man-made habitats: resource partitioning or interspecies competition?

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Triatoma brasiliensis and *T. pseudomaculata* are vectors of *Trypanosoma cruzi*, the agent of Chagas disease, in the Caatinga of northeastern Brazil. In the wild, these species occupy different ecotopes and exploit different hosts. The ground-dwelling *T. brasiliensis* is associated with rocky outcrops (and cacti in some areas) often shared by rodents, whereas *T. pseudomaculata* is arboreal and appears to feed mainly on birds. Both species, however, can infest man-made habitats, where the possibility arises for competition over key resources – shelter and blood. We determined *T. brasiliensis* and *T. pseudomaculata* occurrence/co-occurrence in 418 man-made ecotopes (91 dwellings) to investigate species-segregation patterns and to probe two candidate underlying processes: (a) resource partitioning (*T. brasiliensis* preferring mineral or rodent-occupied ecotopes and *T. pseudomaculata* preferring vegetal or fowl-occupied ecotopes) and (b) true interspecies competition (each species effectively avoiding the other). We used generalized linear mixed models (GLMMs) to identify correlates of species-specific occurrence including the other species' co-occurrence. Both species co-occurred in four ecotopes (0.96%). *T. brasiliensis* (overall occurrence 12.9%) was more common than *T. pseudomaculata* in mineral ecotopes (17.9% *vs*. 7.2%) and in ecotopes used by rodents (74.1% *vs*. 11.1%); the top-performing *T. brasiliensis* GLMM estimated a strong positive effect of rodent availability on bug occurrence (βrodent = 3.59±0.58 SE). In contrast, *T. pseudomaculata* was overall rarer (7.1%) and occurred more often than *T. brasiliensis* in vegetal ecotopes (13.6% *vs*. 6.8%) and in ecotopes used by fowl (19.3% *vs*. 6.4%); the top-performing *T. pseudomaculata* GLMM estimated the effect of fowl presence on bug occurrence as βfowl = 2.33±0.48 SE. GLMMs revealed no evidence that one species' occurrence affected the probability that the other co-occurred in the same ecotope, after adjusting for ecotope traits (including spatial clustering) and host availability. These findings suggest that our study species maintain spatial segregation in human environments by preferentially associating with the vertebrates they exploit in nature. We conclude that resource partitioning, and not true interspecies competition, likely drives ecological segregation of *T. brasiliensis* and *T. pseudomaculata* in man-made habitats. Elucidating the mechanisms underlying resource partitioning will require experimental approaches.

Keywords. Competition, Species interactions, Ecological segregation, Triatominae.
Funding. Vice-Presidência de Pesquisa e Coleções Biológicas (VPPCB), Fiocruz, Brazil.

Area: Vector, transmission cycles, ecology and biodiversity

#40

Preliminary Ultrastructural Results of the Antennas of *Triatoma brasiliensis brasiliensis* and *Triatoma sherlocki*

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It is known that the species of the *Triatoma brasiliensis* complex are vectors of *Trypanosoma cruzi*, the etiological agent of Chagas disease, and have different levels of epidemiological importance. The antenal phenotype has been used to analyze genera, species, and populations of triatomines. However, little is known about the details of the antenna structures of the different species that make up the complex. To perform comparative morphological characterization, scanning electron microscopy images of male and female antennae of *Triatoma brasiliensis brasiliensis* and *Triatoma sherlocki* will be used. The preliminary results of this study showed that bristles-type sensilae - with mechanoreceptor function - were found in both analyzed species and in all antennal segments, which corroborates with the literature. Trichoid sensillae, which are chemoreceptors, were found only in the flagella of the two species, however, *T. b. brasiliensis*, had the highest number. Species of triatomines that are capable of colonizing different habitats have many chemoreceptors in their antennae, while those that are adapted to only one have little or no chemoreceptor. Only in *T. b. brasiliensis* coeloconic sensillae, with thermohydroreceptor function, sensitive to humidity were observed. Campaniform sensilae (mechanoreceptors) were seen with great incidence in the first antenal segment (scap) of *T. sherlocki* and, in *T. b. brasiliensis*, were found in a smaller amount in the first and second antennal segments. Studies indicate that this sensila works as a proprioceptor that monitors cuticular stress, in addition to detecting temperature, humidity and Co2 concentration. In this preliminary analysis, it was possible to show qualitative and quantitative differences in the sensilla in the two species studied: *T. b. brasiliensis* presented sensilas that are consistent with its ability to inhabit different ecotopes, *T. sherlocki*, which preferably inhabits the wild environment and rarely invades homes. All species of the *T. brasiliensis* complex are being analyzed comparatively. Their ability to occupy different ecotopes will be correlated to the results of the characterization of the antennal sensillae, thus bringing greater understanding to the evolutionary and eco-epidemiological issues of this group of *T. cruzi* vectors.

Support: CNPq, Fiocruz.

Area: Vector, transmission cycles, ecology and biodiversity

#41

Biological Aspects of Triatomines from Artifical Ecotopes in the Municipality of São João do Piauí (PI)

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The municipality of São João do Piauí (PI) is located in the Northeast region of Brazil, on the banks of the Piauí River. The municipality showed an incidence of 7.6% in acute cases of Chagas disease between the years 2012-2016. This condition is caused by the parasite - *Trypanosoma cruzi*, transmitted by triatomines. Such vectors have nocturnal habits, live in wild and rural areas and are hematophagous. This research was carried out in the municipality of São João do Piaui in October 2018 and aimed to survey the fauna of triatomines, the natural infection of species collected in the field and describe their ecotopes. The study was carried out in two rural locations in the municipality, Lagoa da Serra and Poço do Rego. For the collections, the active search method was used. We found 249 triatomines, 242 in Lagoa da Serra and seven in Poço do Rego, among them females, males and all stages of nymphs, of the species *Triatoma braziliensis braziliensis* and *Triatoma braziliensis macromelasom.* Triatomines have been found inside the houses and in peridomestic structures, such as corrals, chicken coops, heaps of tiles and wooden blocks. The natural infection rate was 2.2% in Lagoa da Serra and 0.5% in Poço do Rego. In this work it was possible to perceive the need for a vector surveillance and control program in the municipality, so that there is a reduction in the presence of the vector in homes.

Keywords: Triatomines – *Trypanosoma cruzi* – ecotopes

Area: Vector, transmission cycles, ecology and biodiversity

#42 ★★★

Ecological Niche Modeling in the identification of potential areas of expansion of the *Trypanosoma cruzi* enzootic cycle in *Didelphis aurita* of the Atlantic Rainforest

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Although Ecological Niche Modeling (MNE) is widely used for the distribution of mammals and vectors, it is rarely directed to the study of parasitology. Parasitism is a phenomenon that involves the interaction among different species and occurs in a certain space (environment). It is a complex, multivariable phenomenon in which the components exert selective pressure on each other with different results. The host that suffers selective pressure due to environmental conditions, being also the environment of the parasite, and exerts selective pressure on it. Thus, it is necessary to include cartographic studies to understand the parasite/host/environment triad. The *Didelphis aurita* possum is endemic to the Atlantic Rainforest biome, is one of the ancestral hosts of *Trypanosoma cruzi*, capable of maintaining all *T. cruzi* genotypes, a parasite characterized by its extreme heterogeneity. They are nomads and generalists and live in places with high rates of anthropic action. As a hypothesis, it is proposed that the occurrence of infection of these *T. cruzi* genotypes is directly related to environmental variables. The objective is to model the distribution of *D. aurita* and the infection by *T. cruzi* (positive blood culture) in the Atlantic Rainforest, to predict its areas of occurrence and transmission. Two MNE models were generated: *D. aurita* distribution model and *T. cruzi* transmissibility areas model, in response to climatic factors. Of the 19 bioclimatic variables (BIO1-19) with a resolution of 1 km, available on the WorldClim v.1.4 website, 6 less correlated ($-0.7 \leq \alpha \leq 0.7$) were selected: BIO2, BIO4, BIO8, BIO9, BIO13 and BIO15. Four models were generated using the Cross-validation methodology for partitions (80% for training and 20% for testing), with algorithms: Bioclim, Maxent, SVMe and SVMk. True Skill Statistic (TSS) was used to compare performance between models, values > 0.6 indicate good statistical quality. The best performances in TSS were obtained by Maxent and SVMe. For the transmissibility model, the TSS values varied from, [0.75-0.985] and [0.595-0.999], while for *D. aurita* distribution they were, [0.683-0.88] and [0.577-0.824], respectively. The models were integrated to identify areas of intersection between them, resulting in two final models. All analyzes were performed using the R programming language and thematic maps using QGIS software version 3.4.0. From the generated models, it was possible to map the areas with the greatest climatic suitability for potential distribution of *D. aurita* and hot areas of *T. cruzi* transmission in the Atlantic Rainforest.

Funding institutions: CNPq, CAPES e FAPERJ.

Area: Vector, transmission cycles, ecology and biodiversity

#43

Chromatic and morphometric aspects of nymphs from *Triatoma costalimai* and *Triatoma jatai*: The state of the art

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Triatoma costalimai and *Triatoma jatai* are rupestrian species found in the Cerrado, with records at home. The occurrence in sympatry of both species in municipality of Paranã - TO show the importance of knowing structures that can help in differentiating between themselves. Here are presented the main chromatic and morphometric differences of nymphs of these species. Five and 30 nymphs were used, respectively, from each instar, captured in the municipalities Aurora do Tocantins and Paranã. Observations were performed using a stereomicroscope with a millimeter eyepiece. Eighteen variables were measured(mm), recommended for taxonomy of nymphs of triatomines, related to the length and/or width of the body, head, pronotum and antennae. A descriptive analysis of the results based on mean(X) and standard deviation(S), as well as paired comparisons between species for each stage were performed with test t. The main chromatic differences were shown in the general aspect of the body, in the 4th antennal segment and in the connective spots. In *T. costalimai*, N1-N3 showed general dark brown appearance with a beige abdomen and N4-N5 black with dark brown abdomen. The N1-N3 of *T. jatai* were greyish, N4 dark gray and N5 black. The four segments of the antenna varied with the brown tint. In *T. costalimai*, connective spots seen from N3 showed a pattern of light and dark brown colors and in some specimens red. In *T. jatai* it was restricted to yellow, black and gray. The morphometric results showed that the overlap in body size and some structures made it difficult to identify the nymphal stage of species just by this variable. Body size was larger in *T. costalimai* than in *T. jatai* in all stages ($p < 0.01$). In the comparison between species, N3 nymphs only could be differentiated in relation to body size, length of the post-ocular region and interocular distance ($p < 0.01$). In other stages, significant differences were observed between species for length of head, neck, ante-ocular region, 4th antennal segment, width of anterior pronotum border, ($p < 0.01$). The results showed characters that can assist in the identification of these species' nymphs. However, the chromatic variations were exclusively qualitative, and the morphometric variations showed an overlap in size within the stages. The description of the morphology, hairiness and geometric morphometry of the head/thorax may contribute to refine the taxonomic identification of nymphs of these species. This knowledge can be used by entomological surveillance and Chagas disease control programs.

Keywords: Morphology, Morphometry, Nymphs, Triatominae, Taxonomy.
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Area: Vector, transmission cycles, ecology and biodiversity

#44

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

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The control of Chagas disease (CD), caused by the protozoa parasite *Trypanosoma cruzi*, focuses on the eradication of insect vectors proven to be adapted to human households. Despite advances in the research of biological behavior of triatomines, little is known about the impact of parasite infection on the vector's fitness. This study aims to identify species of the *Triatoma rubrovaria* subcomplex by molecular taxonomy and to analyze their vector potential in the dynamics of parasite transmission in the Pampa biome. Different aspects related to the vectorial capacity were evaluated, such as food source, infection rate and parasite genotyping. In parallel, *T. rubrovaria* bionomic parameters after *T. cruzi* TcVI infection were experimentally analyzed. The insects were fed on mice infected with TcVI at the peak of parasitemia. Feeding and dejection behaviors were observed. The counting of parasites in the feces of *T. rubrovaria* was performed at 30-, 60- and 90-days post-infection (dpi). At 30 dpi, the presence of epimastigote, intermediate and metacyclic trypomastigote forms was detected; the infectious forms were observed, mainly, at 60 dpi. Specimens of *T. rubrovaria* infected with *T. cruzi* showed higher ingestion of blood and feeding efficiency compared to uninfected controls, and the frequency of stings was higher in the mice's head region, suggesting a modulation of vector behavior by the parasite. In addition, we obtained the first record of coprophagy and kleptohematophagy in *T. rubrovaria*. This behavior can increase the possibility of ingestion and transmission of parasites among individuals of this species. A total of 1,724 triatomines were collected in Rio Grande do Sul (RS), of which 936 were used for the molecular analysis. A *T. cruzi* infection rate of 2.8% (26/936), a parasitic load variation of 1.5×10^1 to 2.3×10^7 parasite equivalents/ intestine and the presence of TcI, TcV and coinfection by TcI + TcIV were observed. The food sources identified were (in descending order of frequencies): humans, sheep, goat, chicken, bullock, opossum, mice and dog. The results obtained suggest *T. rubrovaria* as a potential vector of *T. cruzi* in RS, presenting bionomic parameters associated with its vector capacity very similar to the primary vector *Triatoma infestans*, especially when infected, alerting to the importance of constant entomological surveillance in the studied area.

Support: CAPES, CNPq, FAPERJ

Area: Vector, transmission cycles, ecology and biodiversity

#45 ★

Wing morphological changes in *Triatoma brasiliensis brasiliensis*: biotic and abiotic factors influence of artificial and natural ecotopes in areas from Caicó municipality, Rio Grande do Norte, Brazil

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Triatoma brasiliensis brasiliensis is considered one of the most important vectors of *Trypanosoma cruzi* in semiarid areas of the Northeast. In the state of Rio Grande do Norte, the Seridó region is transforming due to the intense process of desertification caused, mainly, by anthropic impacts on the Caatinga biome. Studies on the alar metric variability of *T. b. brasiliensis* in this area are non-existent, making it necessary for the knowledge of the phenotypic responses of this subspecies to biotic/abiotic factors. In this context, we evaluated the possible influence of the parasite and the ecotope on the wing morphology of *T. b. brasiliensis*. Active and passive searches for the insect were carried out in partnership with the Health Surveillance Department in natural and artificial ecotopes from five villages. Natural infection was determined using primers 121 and 122 for the amplification of *T. cruzi* kDNA. The morphological variability of *T. b. brasiliensis* was evaluated using geometric morphometry. Differences in centroid size (CS) between groups were tested using a one-way ANOVA and Student t-test. The statistical significance of shape variations was estimated by the Bootstrapping resampling test (1000 cycles) and Wilks Lambda test, using the XYOM software. A total of 952 *T. b. brasiliensis* were captured, corresponding to 9 populations. The morphometric analyzes of the wings revealed that there was no influence of *T. cruzi* on the CS and the shape, but significant differences in the alar CS between sexes, ecotopes and localities were detected. These indicating a possible influence of the type/availability of the food source, the effect of the population density, in addition to probable isolation by distance/physical barrier between populations. The homogeneities in the TC (most populations) and the shape between villages reflect the similarities of macro-environmental conditions, as these are in the same municipality with the same climatic pattern as the Caatinga biome. On the other hand, the differences in shape related to sex and the ecotope in two of the studied villages indicate the need for further studies to measure the microclimate variables of the ecotopes occupied by these populations.

Support: CNPq, Fiocruz, CAPES

Area: Vector, transmission cycles, ecology and biodiversity

Resumo Tardio / Late Abstract

Influence of *Serratia marcescens* and *Rhodococcus rhodnii* on the humoral immunity of *Rhodnius prolixus*

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Chagas disease is a human infectious disease widespread in the Americas caused by the parasite *Trypanosoma cruzi* and transmitted mainly by triatomine vectors. In the invertebrate host, such as it occurs in *Rhodnius prolixus*, *T. cruzi* development success depends on the parasite strain and its component of bacterial gut microbiota. The microbiota is essential to offer vitamins and nutrients, to maintain the insect immune homeostasis, and to protect the insect from a pathogenic microorganism infection. Herein, we analyzed the effects of the Gram-negative *Serratia marcescens* and the Gram-positive *Rhodococcus rhodnii* bacteria on the immune responses of *R. prolixus* nymphs treated with antibiotics and infected with *S. marcescens* or *R. rhodnii*. The oral insect antibiotics treatment with the mixture of ampicillin, penicillin, and hygromycin added to the blood meal, reduced these two bacteria species population in the digestive tract without caused any alteration on the mortality and ecdysis compared to the control group. The antibiotic-treated insects and infected with *S. marcescens* presented reduced antibacterial activity against *Staphylococcus aureus* and phenoloxidase activity in hemolymph samples. It also had lower nitric oxide synthase (*NOS*) gene expression and higher expression of the antimicrobial peptide defensin C gene (*DefC*), in the fat body. The gene expression of *S. marcescens* infected insect presented higher *DefC*, lower prolixicin (*Prol*), and *NOS* levels in the anterior midgut samples when compared to control insects. However, the antibiotic-treated insects infected with *R. rhodnii* had increased antibacterial activity against *Escherichia coli* and low activity against *S. aureus*, higher phenoloxidase activity in the hemolymph, and low *NOS* expression in the fat body when compared to control insects. In the anterior midgut samples, these insects presented higher *NOS*, defensin A (*DefA*), and *DefC* expression and low *Prol* expression in contrast to control insects. Therefore, the *S. marcescens* and *R. rhodnii* modulate the insect immune responses differently. The *R. prolixus* immune modulation observed not only in the midgut but also systemically in the fat body by these two bacterial symbionts might be crucial to the *T. cruzi* and *Trypanosoma rangeli* development and transmission and needs further investigation.

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