XXIII Tropical Medicine Research Center & Pharmacology Meeting

Tropical Diseases and Hygiene at the Semiarid Brazil: Genomic, Microbiome, Metabolomic and Malnutrition

14 e 15/02/2020

LOCAL: CASA JOSÉ DE ALENCAR
FORTALEZA - CEARÁ

Realização

Universidade Federal do Ceará - UFC

INCT-IBISAB NUBIMED
Núcleo de Biomedicina

CAPES
Programa de internacionalização CAPES/Print

Apoio:

CNPq
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A. Introduction

The annual meeting in Tropical Medicine and Hygiene & Pharmacology is a regular scientific event, starting in 1996 and which in this year 2020 will complete its twenty-third edition. The event was created to bring together researchers, technicians and students at regional, national and international level interested in the scientific and technological development of research on tropical diseases that afflict populations in the Brazilian semiarid region. Initially the event was held with the effective participation of three groups of national researchers and three groups of international researchers. The groups of national researchers were led by professors Aldo AM Lima, Federal University of Ceará, Edgar M. Carvalho, Federal University of Bahia, and Selma Jerônimo, Federal University of Rio Grande do Norte. The international groups were led by the researchers, Richard L. Guerrant, University of Virginia, CHO, VA, Warren Johnson, Cornell University, NY and Lee W. Liley, University of California, Berkeley, CA. The annual event has covered topics of ongoing research and perspectives in several areas of tropical medicine such as leishmaniasis, leptospirosis, diarrheal diseases, tropical enteropathy, malnutrition, human T-lymphotropic virus (HTLV), human immunodeficiency virus (HIV), leprosy, tuberculosis, among others. This year we included the topic arboviruses (dengue, zika, chicungunha and yellow fever) as emerging and reemerging tropical diseases of regional, national and global importance in health. The event will be held in Fortaleza, CE, February 14-15, 2020. The event is open to the public of researchers, professors, undergraduate and graduate students, as well as technical personnel. Annually the event receives 40-60 people participating among regional, national, international researchers, students and technicians. The event has contributed to the development of research in tropical medicine and hygiene & pharmacology at the regional, national and international levels, maintaining research networks at the regional, national and international levels in areas of interest in public health facing the developing region in the Brazilian semiarid region. We also mention the effective participation and in the last seven years of two INCTs (Biomedicine- http://www.nubimed.ufc.br and Immunology of Tropical Diseases- inct.cnpq.br/web/inct-dt). National and international collaboration, such as the international exchange of graduate students and technicians, has been significant and innovative in the creation of long-term sustainable models of research networks (RECODISA: http://www.recodisa.ufc.br; MAL-ED: http://www.upcibimed.ufc.br/MAL-ED) and postgraduate degrees in Medical Sciences (Level 6 CAPES), Microbiology (Level 5 CAPES), Pharmacology (Level 6 CAPES) and Morphofunctional Sciences (Level 4 CAPES), as well as demonstrating its importance in national and global public health.

Figure 1 - Photo of the speakers and students participating in the II Annual Meeting of the Tropical Medicine Center in Salvador,

B. Brief history of previous editions

In the last forty years, initially two national research groups, led by professors Aldo AM Lima and Edgar M. Carvalho, developed in lines of research related to tropical medicine, leishimaniasis and diarrheal diseases & malnutrition, at Universidade Federal do Ceará and Universidade Federal Bahia, respectively. Subsequently, a third group led by Professor Selma Jerônimo from the Federal University of Rio Grande do Norte, also emerged in the line of research in leishimaniasis. From the beginning, these three research groups have maintained relevant and significant international collaboration with international groups led by researchers, Richard L. Guerrant, University of Virginia, CHO, VA, Warren Johnson, Cornell University, NY and Lee W. Liley, University of California, Berkeley, CA. The development and progress in these lines of research led to the aggregation of new researchers and the national and international financial support of these groups by national research funding agents, CNPq and CAPES, and internationally, such as the National Institute of Health (NIH), Bethesda, MD, most recently the Bill and Melinda Gates Foundation. The common environment of research related to tropical medicine in a region of national priority such as the region in the Brazilian semiarid, as well as the

created network of financing for these groups led to the formatting of this event, the most recent named Annual Meeting in Tropical Medicine Research Center & Pharmacology. The first annual meeting of the Tropical Medicine Research Center took place in mid-March 1996. **Figure 1** illustrates only the researchers participating in the II Annual Meeting of the Tropical Medicine Research Center in Salvador, BA, in March-1997. Note researchers Lee W. Liley (1st from left to right), Aldo AM Lima (3rd), Edgar M Carvalho (6th in the first line), Selma Jerônimo (8th in the first line), Richard Pearson, UVa, CHO, VA (1st. On the second line), and Warren Johnson (2nd. On the second line).

Figure 2 shows the team of speakers and undergraduate and graduate students participating in the XXII Tropical Medicine Research Center. The longevity of this event is based mainly on the regional, national and international interaction and collaboration of researchers linked to the lines of research in Tropical Medicine and Hygiene & Pharmacology. This interaction and collaboration has resulted in fruitful scientific and technological production, patents, training of human resources at postgraduate, postdoctoral, technical level, as well as allowing the institutional expansion of the International Collaboration Program through the CAPES-PRINT project (2018-2022). The results can be seen through the CVs of the leading researchers and collaborators. Two international research agreements between UFBA & University of Cornell and UFC, UFRGN & University of Virginia were developed over this period and today constitute international scientific and technological cooperation models for several other national and international institutions. Leading researchers today are recognized nationally and internationally in societies such as the Brazilian Society of Tropical Medicine, the American Society of Tropical Medicine and Hygiene, ASTMH and the Brazilian Academy of Sciences, ABC. This interaction allowed the development of several national and international patents, awards, medals and honors for the researchers participating in these annual events. The results of these researches have been impressive in Brazil and in the world and in the development of new prophylaxis and treatment of these tropical diseases mentioned in the preliminary program of this event of the XXIII Annual Meeting in Tropical Medicine and Hygiene & Pharmacology. As a result of the promotion of these annual and regular meetings, it was possible to develop two INCTs, Biomedicine and Immunology of Tropical Diseases, and two research networks, RECODISA (www.recodisa.ufc.br) and MAL-ED (www.mal-ed.fnih.org), nationally and internationally. The list of participants in these events and today formed through national and international graduate programs as evidenced in the leaders' CVs lattes, demonstrates the training capacity of human resources that had directly or indirectly promoted these annual and regular meetings of the Tropical Medicine Research Center. The quality of this training can be assessed through the majority,> 80% of graduates, are now professors and / or researchers from national and / or international institutions. It is important to mention that through these international collaborations it was possible for the international insertion of graduate programs.

Figure 2 - Photo of the speakers and students participating in the XXII Annual Meeting of the Centro de Medicina Tropical in Fortaleza, CE, November-2018.

Casa de José de Alencar

Av. Washington Soares, 6055 - José de Alencar
Fortaleza – CE – Brasil

Friday– February 14th

8:30 - 9:00 am	***Opening remarks***: ***José Cândido Lustosa Bittencourt de Albuquerque*** Rector, Federal University of Ceará ***Manassés Claudino Fonteles*** Emeritus Professor, Federal University of Ceará Member of the National Academy of Medicine ***Jorge Herbert Soares de Lira*** Pro-Rector Researcher and Postgraduation, Federal University of Ceará ***João Macedo Coelho Filho*** Director, Faculty of Medicine, Federal University of Ceará ***Pedro Jorge Caldas Magalhães*** Coordinator, PG-Farmacology, Faculty of Medicine, Federal University of Ceará ***Aldo Ângelo Moreira Lima*** Coordinator, XXIII TMRC & Pharmacology Meeting and Center of Biomedicine, Faculty of Medicine, Federal University of Ceará

PROGRAM

Friday – February 14th

Session I: Arboviruses in the semiarid Brazil: vector control and vaccines / Influenzae seasonality and vaccination calendar / Enteric infections.

Chairpersons*: Profs. José Xavier Neto / Ivo Castelo Branco, Federal University of Ceará.*

9:00 - 9:25 am	***Aedes* in focus to control and prevent outbreaks of arboviruses in the municipality of Cedro-CE, Brazil: population, health agents and government actions.** Ivo Castelo Branco, Federal University of Ceará
9:25 - 9:50 am	**Epidemiology of dengue fever in Brazil and the development of new vaccines.** Luis Carlos Rey, Federal University of Ceará
9:50 - 10:15 am	**Neurodevelopmental damage by *Zika* virus: from hydrocephalus to microcephaly.** José Xavier Neto, Federal University of Ceará
10:15 – 10:40 am	***Coffee Break***
10:40 - 11:05 am	**Influenza in pregnancy and childbirth in the Brazilian semiarid: the INFLUEN-SA study.** José Quirino Filho, Federal University of Ceará
11:05 – 11:30 am	**Enteric glia cells as a target to *Clostridioides difficile* infection.** Deiziane Viana Da Silva Costa, Federal University of Ceará
11:30-11:55 pm	**Enteroaggregative *Escherichia coli* enteric infections: virulence genes/genome and nutritional impact on cohort children from South America, Africa and Asia.** Alexandre Havt, Federal University of Ceará

Lunch: 11:55 – 13:30 pm

Session II: Enteric infections, environmental enteropathy and malnutrition: metabolomics, microbiome, pathobiology and pharmacology.

Chairpersons: *Profs. Aldo AM Lima / David Bolick.*

13:30 - 13:55 pm	**Modeling enteropathy or diarrhea with the top bacterial and protozoal pathogens: differential determinants of outcomes.** Richard L. Guerrant, University of Virginia, Charlottesville, VA, USA
13:55 -14:20 pm	***Campylobacter* spp murine enteric infection: microbiome, metabolomics and pathobiology.** David Bolick, University of Virginia, Charlottesville, VA, USA
14:20 -14:45 pm	**Tissue is tissue: duodenal biopsies and imaging to elucidate enteropathy in low-resource settings.** Sean R. Moore, University of Virginia, Charlottesville, VA, USA
14-45 -15:10 pm	**Impact of malnutrition on the intestinal microbiota: pharmacological basis of pre and probiotics therapeutics.** Aldo AM Lima, Federal University of Ceará

Coffee Break: 15:10-15:40 pm

Session III: Leishamaniasis a common endemic disease: epidemiology, clinical, immunology and genome.

Chairpersons: *Profs. Selma Jerônimo, Federal University of Rio Grande do Norte / Anastácio de Queiroz Sousa*, Federal University of Ceará.

15:40 -16:05 pm	**Leishmaniasis in the northeast of Brazil: epidemiology and genome.** Selma Jerônimo, Federal University of Rio Grande do Norte
16:05 - 16:30 pm	**Leishmaniasis and some of its atypical presentations.** Anastácio de Queiroz Sousa, Federal University of Ceará
16:30 - 16:55 pm	**Leishmaniasis and coinfections in dogs in the urban community of Fortaleza, Ceará, Brazil.** Adam L Lima, Ph.D. Student, State University of Ceará

Sala I - Saturday, February 15th

Session IV: Nutrition and Metabolomics applied to enteric infections, environmental enteropathy and malnutrition.

Chairpersons: *Profs. Jonathan Swann,* Imperial College London / Alexandre Havt, Federal University of Ceará.

9:00 - 9:25 am	**Characterizing the biochemical alterations associated with environmental enteric dysfunction using metabolomics.** Jonathan Swann, Imperial College London, UK
9:25- 9:50 am	**Metabolomics approach to explore new pathobiology associated with enteric infections.** Natasa Giallourou, Imperial College London, UK
9:50-10:15 am	**Helminthic infections and metabolomics exploration in cohort children in developing countries.** Gordana Panic, Imperial College London, UK
10:15-10:40 am	**Apolipoprotein E polymorphisms in children with heavy diarrhea burdens and implications for later development.** Reinaldo B. Oriá, Federal University of Ceará
10:40- 11:05am	**Dietary intake from complementary feeding is associated with intestinal barrier function and environmental enteropathy in Brazilian children from the MAL-ED cohort study.** Priscila N. Costa, Federal University of Rio Grande do Norte
11:05- 11:30am	**Energy and zinc intakes from complementary feeding are associated with the risk for undernutrition in children from South America, Africa and Asia.** Bruna LL Maciel, Federal University of Rio Grande do Norte

Sala II - Saturday, February 15th

Session V: Neuro gastrointestinal motility and diseases: nonerosive reflux disease, inflammation, mucosal integrity and pharmacology.

Chairpersons: *Profs. Marcellus P Souza / Armênio A Santos*, Federal University of Ceará.

10:00 - 10:25 am	**Translational research in nonerosive reflux disease from inflammation to impairment in mucosal integrity: Study of new mechanisms and new topically agents.** Marcellus Ponte e Souza, Federal University of Ceará
10:25- 10:50 am	**Expression of proteins associated with sarcopenia in reflux esophagitis.** Suliana Mesquita Paula, Federal University of Ceará
10:50-11:15 am	**Bile acids inhibit contractility of rat esophageal muscle.** Kalinne Gadelha, Federal University of Ceará
11:15-11:25 am	**Anti-inflammatory effect of Gaviscon® on oesophageal mucosa in murine model of non- erosive reflux disease.** Kerolayne de Melo Nogueira, Federal University of Ceará

10:00-12:00h - Coffee Break open during session.

Session VI: ***Posters during all coffee breaks Friday and Saturday***: *Young and Senior investigators.*
Chairpersons: Profs. Alexandre Havt / Aldo AM Lima, Federal University of Ceará.

Scientific Committee:

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9:00 - 9:25	**Integrated and-Health for Attacking Arboviruses in Ceará, Brazil** Ivo Castelo Branco Coelho; Henrique Pequeno; Miguel Franklin;. Ernesto T Lima Neto; Caio Cavalcanti Patology Department, School of Medicine, Federal University of Ceará

Background: Over the past 30 years, those responsible for the control and surveillance of epidemics, notably arboviruses, have been challenged to curb the process of infestation of mosquitoes of the genus *Aedes*, notably *aegyptus*, the main vector of dengue outbreaks and epidemics, Zika and Chikungunya. However, there are other viruses that pose a concrete threat to the country's public health. Considering that combating the vector is one of the most efficient ways of controlling these arboviruses, actions to combat mosquito outbreaks represent one of the main initiatives interfering in the development cycle from egg to adulthood, which lasts 7 days and can be born infected by dengue or Zika. About 80% of the outbreaks are in the home environment. Thus, it is imperative to involve the population in preventive actions that culminate in the elimination of these outbreaks, since no sphere of government can act fully in these places. The Aedes em Foco Project developed a series of strategies aimed at engaging the population in this work, as well as promoting to the government segments involved in vector control, a set of technological solutions aimed at the strategic performance of the current human resources mobilized, resulting in financial savings and structural, in addition to providing better indicators related to disease control in the city of Cedro-CE. **Methods**: As actions with the population, an application was developed for mobile devices that can be downloaded for free on smartphones, IOS or android, which can be manipulated individually or by groups, called brigades. The project developed an extension action in which students from the city of Cedro organize themselves in groups that visit schools, churches, homes, public and private buildings, raising public awareness of the problem of arboviruses and ways of prevention, in addition to promote the cleanliness of the containers. The brigade teams record the outbreaks found via the application, and these data, already with geolocation, are integrated into the official platform for endemic agents, responsible for vector control in the municipalities. **Results**: In this way, voluntary actions, genuinely from the population, are carried out in an integrated manner with the activities institutionalized by the local government. In order to increase adherence to this strategy, in addition to students, identification and training were carried out in some segments of society that are educators and influencers of opinions such as teachers, traders, service providers, religious. Educational content was also created in various media for the context of arboviruses. Thus, digital games, comic books, vector animations, distance courses, and three-dimensional simulators were offered free of charge to the population. Currently, Cedro endemic agents act in an innovative way towards their colleagues from all over the country. The digital mobile solution they use, allows the fieldwork record to be consolidated in real time. In this way, managers can make decisions in a much more appropriate time interval for the challenge of coping with mosquito outbreaks. Another important consequence of the implementation of this technology is that the data generated and stored on the platform allows a new perspective of the action of endemic agents. With the adoption of the system, the manager who supervises the activities of the teams in the field has a detailed view of their territory, being able to easily identify the most critical areas and direct their efforts to the most necessary areas at any given time. Thus, the tool offers managers a privileged condition of performance, enabling strategic and intelligent performance. **Conclusions**: The data analyzed from the 2019 cycles show a significant reduction in the number of outbreaks, which may provide a reduction in the risks of arboviruses in the city of Cedro this year. In their last year of work, the researchers of the Aedes em Foco Project expect to promote the consolidation of some solutions, with the respective validation of the results observed to date, and verification of the possible impacts generated by it.

9:25 - 9:50	**Epidemiology of dengue fever in Brazil and the development of new vaccines.** Luis Carlos Rey, Federal University of Ceará

Introduction: Dengue fever is considered one of the most widespread and harmfull vector-born diseases in worldwide scale. Four DEN viruses take turns in order to produce epidemic spikes of disease load. The development of effective dengue vacines is strongly needed since nearly 20% of the world is under risk or affected or affected every year. **Methods**: We performed a systematic review of epidemiological data and published information avaliable in worldwide web and medical literature in recent years using MESH terms. **Results**: After 2 years of low incidence of dengue cases (around 200k cases in 2017-2018), dengue piked with 1.5 million cases in 2019, 65% of them in the Southeast region of Brazil. The Northeast region, historically one of the most affected areas in Brazil, have shown in recent years a decline in dengue case reports. According to the serotype, DENV serotype 2 (DEN-2) is responsible for the abrupt increase in cases in the Southeast, while in the Northeast DEN-1 still remains predominant. We can predict a great epidemic in the Northeast in the coming years caused by the spread of DEN-2 nationwide. Sanofi Pasteur chimeric dengue vaccine DENVAX is the first registered product with good protection for severe disease and hospitalization for dengue, but is restricted to previous dengue-seropositive subjects. Takeda vaccine is being tested in phase 3 clinical trials, and preliminary results of two doses show good protection overall, however in previous dengue-naive participants the protection was lower for DEN-1 and DEN-2 or absent for DEN-3 serotype. DEN-4 efficacy in dengue-naives could not be assessed. A NIH-Butantan Institute single dose dengue vaccine is under ongoing phase 3 studies without efficacy results published so far. **Conclusion:** As dengue burden remains important and widespread, there is an urgent need for better dengue vaccines for population programs in epidemic areas as Southeast Asia, Africa and Latin America.

9:50 - 10:15	**Neurodevelopmental damage by *Zika* virus: from hydrocephalus to microcephaly.** Guedes, L.B. and Xavier-Neto, J. Medical Sciences – UFC; Department of Morphology

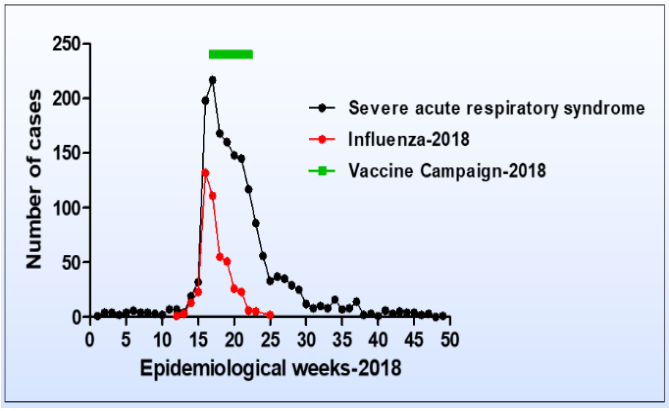
To understand the spectrum of neonatal abnormalities arising after infection with ZIKA virus (ZIKV) during early pregnancy, we performed a careful analysis of the anatomy of embryos exposed to the virus using Synchrotron X-ray micro tomography and an immunocompetent mouse model. Our three-dimensional reconstructions show that the volume of embryonic cerebrospinal fluid (eCSF) increases within the neural tube during early gastrulation to organogenesis stages, akin to the human second and fifth week of pregnancy. Our results illustrate the distinguishing sequence of events that triggers neurodevelopmental alterations associated with ZIKV infection in mammals. This stereotyped neuropathological sequence initiates with dysraphia, followed by hydrocephalus (primarily of the fourth ventricle), which could later lead to full-blown hydrocephalus and in rare cases in mice, to microcephaly. Our findings help to unravel the pathophysiology of neonatal ZIKA and will be potentially useful as landmarks to guide prognosis, windows for interventions as well as follow up decisions.

10:40 - 11:05	**Severe acute respiratory syndrome and influenza in pregnancy and childbirth in the Brazilian semiarid: the INFLUEN-SA study.**

José Q. Filho[1], Francisco S. Junior[1], Alberto M. Soares[1], Thaisy B.R. Lima[2], Sean R. Moore[3], and Aldo AM Lima[1], [1]Institute of Biomedicine, School of Medicine, Federal University of Ceará, [2]Epidemiological Surveillance Information System for Influenza, Ceará State Health Secretariat and [3]Division of Pediatric Gastroenterology, Hepatology & Nutrition, University of Virginia School of Medicine.

Background - Multiple studies show that influenza during pregnancy is associated with both preterm birth and low birthweight. Importantly, influenza vaccination of pregnant women has been demonstrated to reduce preterm birth by 30-70%. Previous work shows that seasonal influenza in Ceará in the Semi-Arid region of Brazil occurs 2 to 3 months earlier than in the South and Southeast. Furthermore, influenza strains in Fortaleza demonstrate higher parity with Northern hemisphere strains. *Despite these well-described epidemiological differences, all of Brazil is subject to the same vaccination schedule, hence pregnant women and their fetuses are inadequately protected against influenza in the tropical and equatorial regions of Brazil.* Using data science approaches, we will test the hypothesis that Brazil's current national policy targeting vaccination only during the months of April and May, using the Southern Hemisphere vaccine, inadequately protects against the harmful maternal-fetal effects, preterm birth and underweight, of influenza in the Semi-Arid and northern regions of Brazil. **Methods** - In Phase I, we propose to leverage existing Ceará state and national databases to confirm regional variations in associations between influenza and adverse birth outcomes (preterm birth and underweight) in Brazil. If Phase I is successful, in Phase II, we propose to reduce this burden by modeling and testing a modified vaccine schedule and formulation in pregnant women. We characterize the pregnant population with severe acute respiratory syndrome (SARS) by age, influenza virus and subtypes, incidence, deaths and historical lethality of cases in the state of Ceará, Brazil in the period 2013 - 2018. **Results** -In this historical series we had 3,297 cases of SARS, of which 145 (4%) were pregnant. The median age of SARS cases was 26 (15-44) years. Of the virus diagnosed cases of SARS (134), 43 (32%) were due to influenza. Of the 43 cases of influenza subtype 18 (42%) were A / H1N1 followed by 14 (33%) A H3 / seasonal, 8 (19%) influenza B and 3 (7%) A without subtype. Those of pregnant women with SARS were 3 (100%) deaths due to other viruses or etiologic agents not specified and 0 (0%) due to influenza. **Figure 1** shows the number of cases of SARS and cases of influenza in the year 2018. Observe the peaks of SARS and concomitant influenza between the 15-20 epidemiological weeks and the vaccination period immediately after the peaks. Children preterm birth and underweight were associated with SARS in pregnancy, 2,879.1 ± 783.57 g (p = 0.019) and 16 (27%) (p = 0.025) compared to controls pregnancy, 3,195.6 ± 572.61 g and 10 (13%), respectively. The 19 SARS per influenza in pregnancy decreased birth weight (3,029.0 ± 778.24 g) and increased percentage of preterm births 4 (21%) without reach significant P values (> 0.05). **Conclusions** -These data suggest that SARS causes maternal-fetal injury resulting in low birth weight and an increase in the proportion of preterm births. The data also show that the vaccination period is after the peak of cases of SARS and influenza showing the inadequate protection of SARS and influenza seasonality.

Figure 1



11:05 – 11:30	**Enteric glia cells as a target to *Clostridioides difficile* infection.** Costa DVS[1], Bolick D[2], Guerrant RD[2], Brito GAC[3*] ,Warren CA[2*] [1]Department of Physiology and Pharmacology, Faculty of Medicine, Federal University of Ceará, Fortaleza, Ceará, Brazil. [2]Division of Infectious Diseases and International Health, University of Virginia, Charlottesville, Virginia, United States. [3]Department of Morphology, Faculty of Medicine, Federal University of Ceará, Fortaleza, Ceará, Brazil. *equal contribution

Background: Increased risk of functional diarrhea has been reported in patients after *C. difficile* infection (CDI). Enteric glial cells (EGCs) regulate neuron function and contribute to inflammatory response. Endogenous adenosine is increased during tissue injury and inflammatory conditions. We investigated the effects TcdA and TcdB on expression of adenosine receptors in EGCs and the role of A2A, A2B and A3 on toxin-induced alterations in EGCs.

Methods: Rat EGC line CRL2690 (ATCC) was incubated with TcdA (50 ng/mL) or TcdB (1 ng/mL) alone or in combination with A2A agonist (ATL313), A2A antagonist (SCH-58261), A2B agonist (Bay60-6583), A2B antagonist (ATL801), A3 agonist (IB-MECA) or A3 antagonist (MRS1220) 1h prior toxins challenge. After 18h, EGCs were collected to evaluate gene expression of adenosine receptors or glial factors (*GDNF* and *S100B*) and *IL-6* by qPCR. Death cell was evaluated by a luminescence assay of annexin V expression and caspase 3/7 activity.

Results: TcdB and TcdA increased *A2A* and *A3* transcript, as well as decreased *A2B* transcript. A1 transcript was not affected by both toxins in EGCs (Rat EGC line CRL2690, ATCC). A2A agonist ATL313 decreased *IL-6* and *GDNF* expression, annexin V expression and caspase-3/7 activity induced by TcdA and TcdB in EGCs. A2A blocker SCH-58261 decreased *IL-6* expression induced by TcdA, but not by TcdB in EGCs. In addition, A2A blockade did not affect apoptosis induced by both toxins in EGCs. A2B agonist Bay60-6583 diminished *IL-6* transcripts in EGCs challenged by both TcdA and TcdB, but did not affect apoptosis. A2B blocker ATL801 unregulated *IL-6* in EGCs challenged with both toxins. A3 agonist IB-MECA downregulated *IL-6*, as well as reduced annexin V expression and caspase 3/7 activity in EGCs challenged with TcdA and TcdB. While A3 blocker MRS1220 did not alter *IL-6* upregulation induced by both toxins.

Conclusion: TcdA or TcdB downregulated A2B receptor and upregulated A2A and A3 in EGCs. Among these adenosine receptors, A2A receptor appears to play a regulatory role in the expression of *IL-6* and *GDNF* and apoptosis induced by TcdA and TcdB in EGCs. However, activation of A3 or A2B downregulated *IL-6* in EGCs exposed to both toxins.

11:30-11:55	**Enteroaggregative *Escherichia coli* enteric infections: virulence genes/genome and nutritional impact on cohort children from South America, Africa and Asia.** Alexandre Havt,, Lima, AAM. Federal University of Ceará

Enteroaggregative *E. coli* (EAEC) has been firstly recognized as a distinct pathogen by causing pediatric diarrhea among infants of Chile. But it is also well known for its role in persistent diarrhea in children in developing countries, as an emerged pathogen in outbreaks of acute diarrhea, as a causing agent of international travelers' diarrhea and among patients with HIV. Along years of study, many reports also indicated that, even though EAEC is very prevalent, its infection was not always associated with cases of diarrhea, but mostly related to subclinical infections, which could impair children growth, especially when it is happened among the first six months of age. But this information was not always consistent. The first indication that EAEC would impact children growth and would cause gut inflammation was reported in 1998. The MALED study, the world´s largest ever study of active surveillance for diarrhea confirmed that EAEC is one of the most prevalent pathogens among children of two years of age, but its real burden would be its impact of causing infant malnourishment. In addition, the MALED cohort study also indicated that when EAEC is associated with other pathogens, its presence among three or more pathogens worsen malnourishment, but the absence of EAEC among its co-pathogens does not cause malnutrition. Even though its important role in children growth impairment, our research group also described that EAEC infection among kids living in the semi-arid region of Brazil was still associated with diarrhea, showing that EAEC heterogenicity may be related with environmental aspects of the studied population but also would be associated with genetic features based on their content of virulence genes. Among our studies that investigated the importance of the presence of EAEC virulence genes and their impact on gut infection we could showed that the presence of most fimbria encoded genes would indicated a worsen infection, but when EAEC strains encoded the negative regulator of AggR (aar) the infection would be less virulent. And this was true for studies when cases were diarrhea or malnutrition. We all know now that among the pathogenic *E. coli* strains, EAEC persistently maintains its heterogeneous features and consistently amuses researchers from all over the world with its excellent ability of colonization and horizontal transfer of genes. These features mostly contribute to the exacerbation of other microbial virulence strategies and the occurrence of antimicrobial resistance.

13:30 - 13:55	**Modeling enteropathy or diarrhea with the top bacterial and protozoal pathogens: differential determinants of outcomes.** Richard L Guerrant, Center for Global Health and Division of Infectious Diseases and International Health, University of Virginia, CHO, VA, USA

Background: Clinically relevant "disease" is classically seen as specific unhealthy outcomes for the "host" as a result of host and environmental determinants. On the host side, genetics are the obvious key (be it inherited or 'epigenetic'), while the environment includes a wide range of "exposures" from physical characteristics (atmosphere, temperature, diet, antigens, toxins, *etc*) to microbiologic (including endogenous microbiota, environmental milieu or, for infectious diseases, exogenous "pathogens"). Understanding and ameliorating disease, or ideally preventing diseases or maintaining good 'health', requires an understanding of the causal relationships of the observed associations in clinical or field assessments. However, controlling relevant variables is key to experimental proof of causality, but is often difficult or impossible to achieve in humans. **Methods and Results**: Besides targeted, effective treatment (that is rarely sufficiently specific to be conclusive), experimental model systems have a critical role in establishing causal factors that impair health and are key to effective preventive or therapeutic interventions. Ideal "models" experimentally replicate the human conditions and their responses to interventions and range from *in silico* to *in vitro* or *in vivo* animal models. Likewise, defining a "disease" is critical to its recognition as well as to assessing interventions. For example, proper surveillance to determine disease outbreaks first involve a careful "case definition" whether we seek to understand an Ebola outbreak or to better detect, prevent and treat rheumatic fever. We have focused on the health impacts of common intestinal infections, especially in young, often undernourished children in impoverished settings worldwide. The obvious first enteric infectious "disease" that comes to mind is "diarrhea." However, other, potentially even more impactful outcomes of intestinal infections now being recognized include "environmental enteropathy" (to distinguish it from immunologic enteropathies, as we currently understand inflammatory bowel diseases, Crohn's Disease and ulcerative colitis; although celiac disease may be considered environmental but non-microbial). In order to propose "case definitions" to enable their study and amelioration, some of us have suggested a new set of terms, like "HAZdrop" (for the growth impairment or decline in height-for-age Z scores that so commonly occur in the first 2-3 years of life in impoverished areas) and even impairment of cognitive development (COGhit) that can be attributed to enteric infections (especially in early childhood, but also potentially in the elderly as well), or later life metabolic syndrome that can follow early life enteric infections, severe acute malnutrition and stunting (METsyn). Although each of these, and the virulence traits of the pathogens themselves, are profoundly influenced by the dietary environment, their assessments are arguably far more objectively measured than the frequency and consistency of material (feces) that come from one end of a very long, multifunctional tube (the gastrointestinal tract). **Conclusions**: It is both overt diarrhea as well as growth, weight gains, and measurable markers of enteropathy (histologic, barrier function or inflammatory biomarkers) that we have focused on 'modeling' in our murine models of enteric infections and their outcomes.

13:55 -14:20	**A Novel Mouse Model of Campylobacter jejuni Enteropathy: Pathobiology, Metabolome, Microbiome and Therapeutics** Bolick DT [1] , Fawad J [1], Costa DVS [2], Medeiros PHQS [3], Guerrant RL [2], Moore SR [1] [1] Division of Pediatric Gastroenterology, Department of Pediatrics, University of Virginia School of Medicine, Charlottesville, VA; [2] Division of Infectious Disease and International Health, University of Virginia School of Medicine, Charlottesville, VA; [3] Institute of Biomedicine, Federal University of Ceara, Fortaleza, CE, Brazil

Campylobacter infections are among the leading bacterial causes of diarrhea, 'environmental enteropathy' (EE), and growth failure worldwide. In developing countries, these repeated enteric infections often result in growth deficits and cognitive impairment in malnourished children. The lack of an inexpensive small animal model of enteric disease with Campylobacter has been a major limitation for understanding its pathogenesis, and for intervention or vaccine development. Here we describe a robust normal mouse model that can exhibit reproducible bloody diarrhea or growth failure, depending on the zinc or protein deficient diet and on antibiotic disruption of resident microbiota prior to infection. Several biomarkers and intestinal pathology in this model also mimic the clinical outcomes of malnourished children. This model opens new approaches to testing specific hypotheses regarding disease pathogenesis as well as vaccine development and other therapeutic interventions that are currently in progress. Here we demonstrate the usefulness of this model in testing passive antibody protection from Spirulina and protective microbiome manipulation using specific strains of bacteria isolated from the healthy human gut.

Campylobacter is common during pregnancy in developing countries, often with no obvious symptoms. There are currently no data on the effects of Campylobacter on the pregnant gut or on the development of the baby. To address this, we have adapted our mouse model to investigate the role of Campylobacter infections passed between generations. We have recently discovered that while antibiotic disruption of resident microbiota prior to Campylobacter infection is required in order to colonize mice, antibiotic pretreatment is not required for transmission to either breeder mates or subsequent offspring. In order to investigate this phenomenon, we have isolated Campylobacter from the stool of infected mice and then administered the isolated bacteria to mice without prior antibiotic treatment. These data combined with the transgenerational infection data will allow investigation into the significance of Campylobacter infection on the pregnant host and future generations.

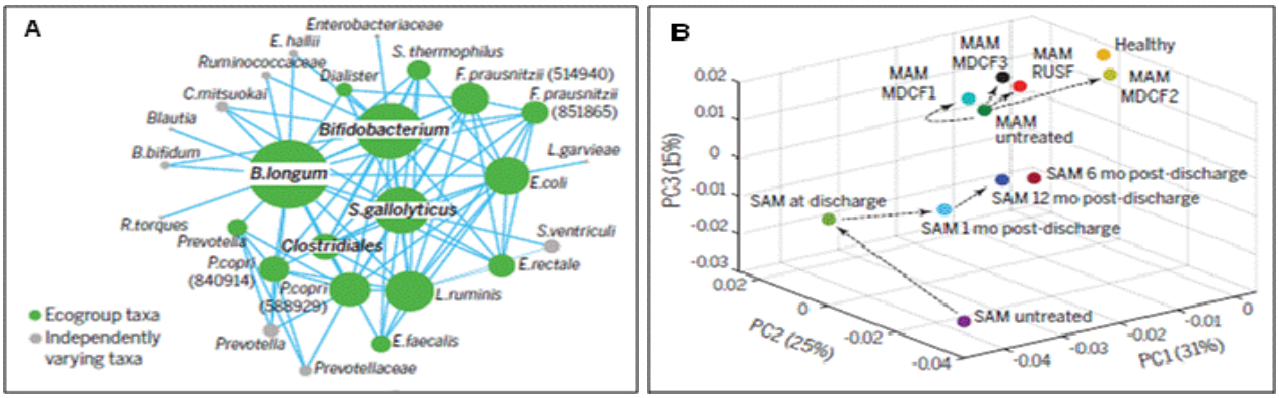
In summary, we have developed a highly reproducible mouse model of Campylobacter infection that has clinical outcomes that match those seen in human disease. Additionally, the same dietary deficiencies often seen in malnourished children, i.e. zinc/protein/micronutrient deficiencies have a direct impact on Campylobacter infection in mice. These findings extend to the pregnant mouse and transmission to subsequent generations are impacted by prior dietary status of the infected host. We hope that these insights into Campylobacter susceptibility will advance development of treatments against this major cause of diarrheal illness.

14:20 -14:45	**Tissue is tissue: duodenal biopsies and imaging to elucidate enteropathy in low-resource settings.** Sean R. Moore, Division of Pediatric Gastroenterology, Hepatology & Nutrition, University of Virginia School of Medicine.

Background: Environmental Enteropathy (EE), characterized by alterations in intestinal structure, function, and immune activation, is believed to be an important contributor to childhood undernutrition and its associated morbidities, including stunting. Half of all global deaths in children < 5 years are attributable to under-nutrition, making the study of EE an area of critical priority. **Methods and Results**: Community based intervention study, divided into two sub-studies, 1) Longitudinal analyses and 2) Biopsy studies for identification of EE features via omics analyses. Birth cohorts in Matiari, Pakistan established: moderately or severely malnourished (weight for height Z score (WHZ) <-2) children, and well-nourished (WHZ > -1) children. Blood, urine, and fecal samples, for evaluation of potential biomarkers, will be collected at various time points from all participants (longitudinal analyses). Participants will receive appropriate educational and nutritional interventions; non-responders will undergo further evaluation to determine eligibility for further workup, including upper gastrointestinal endoscopy. Histopathological changes in duodenal biopsies will be compared with duodenal biopsies obtained from USA controls who have celiac disease, Crohn's disease, or who were found to have normal histopathology. RNA-Seq will be employed to characterize mucosal gene expression across groups. Duodenal biopsies, luminal aspirates from the duodenum, and fecal samples will be analyzed to define microbial community composition (omic analyses). The relationship between histopathology, mucosal gene expression, and community configuration will be assessed using a variety of bioinformatic tools to gain better understanding of disease pathogenesis and to identify mechanism-based biomarkers. Ethical review committees at all collaborating institutions have approved this study. All results will be made available to the scientific community. **Conclusions**: Operational and ethical constraints for safely obtaining intestinal biopsies from children in resource-poor settings have led to a paucity of human tissue-based investigations to understand and reverse EE in vulnerable populations. Furthermore, EE biomarkers have rarely been correlated with gold standard histopathological confirmation. The Study of Environmental Enteropathy and Malnutrition (SEEM) is designed to better understand the pathophysiology, predictors, biomarkers, and potential management strategies of EE to inform strategies to eradicate this debilitating pathology and accelerate progress towards the 2030 Sustainable Development Goals.

14-45 -15:10	**Impact of malnutrition on the intestinal microbiota: pharmacological basis of pre and probiotics therapeutics.** Aldo AM Lima, Federal University of Ceará and MAL-ED Network Investigators (www.mal-ed.fnih.org/)

Background: The intestinal microbiota is important in the digestion of food, production of essential vitamins and protection against the colonization of enteric pathogens. UFC researchers in collaboration with researchers from the international research network MAL-ED (Malnutrition-Enteric Diseases; www.mal-ed.fnih.org/) (*Science 365, 140 2019*) studied the characteristics of the microbiota of healthy and malnourished children as well as their potential pharmacological use in the treatment of the vicious cycle enteric infection_malnutrition and tropical enteropathy, thus preventing complications such as deficiency in physical activity, education and cognitive development, as well as a reduction in child mortality in developing countries. **Methods**: Monthly fecal samples from children between 0 and 60 months of age were used in cohort studies and clinical interventions with nutrients in several geographic regions, Latin America (Peru and Brazil), Africa (South Africa and Tanzania) and Asia (Bangladesh) , India, Pakistan and Nepal), belonging to the MAL-ED network. The sequencing of genes present in the bacterial ribosome (region 4, V4 of 16S rRNA) in fecal samples were used to characterize the intestinal microbiota. A statistical model was developed to determine the interaction dynamics between the bacterial components of the microbiota and the potential bacterial ecogroup that defines healthy microbiota and with changes associated with child malnutrition. **Results**: The results revealed that a community of 15 bacteria (ecogroup) from the intestinal microbiota (**Figure 1A**) was consistent with a mature microbiota from the age of 20 months on in healthy children. This ecogroup of mature microbiota was also consistent with healthy children living in other geographic regions such as India and Peru. These results suggest that in general there is a network of bacterial colonies with organizational characteristics conserved in the intestinal microbiota. The definition of this mature microbiota ecogroup made it possible to compare and evaluate the structure and characteristics of the intestinal microbiota in children with moderate (MAM) and severe (SAM) malnutrition, in addition to making it possible to determine the effectiveness of treatment with nutritional diets in these altered microbiota (**Figure 1B**). As shown in **Figure 1B**, severely malnourished children treated with standard malnutrition diets have evolved to microbiota patterns similar to those with moderate malnutrition. Moderately malnourished children treated with a diet designed to recover the microbiota (MDCF2) had similar microbiota evolution to healthy control children. In another experiment using experimental pigs and without intestinal microflora, a consortium of cultured bacterial colonies from the mature microflora was introduced and then changed the exclusive breastfeeding diet to the standard Bangladeshi diet. This dietary change resulted in the typical development of the intestinal microflora identified at weaning. **Conclusions**: These results identified in healthy children an organization and interaction of bacterial colonies that form a



mature pattern of interaction and function, ecogroup or bacterial community, capable of maintaining and facing constant environmental challenges in the intestinal microflora. This finding allowed to identify and characterize different patterns in the intestinal microflora in children with moderate and severe malnutrition. Furthermore, these results showed that a diet designed for the recovery of the microbiota (MDCF2) is effective in recovering altered intestinal microflora in children with malnutrition.

15:40 -16:05	**Leishmaniasis in the northeast of Brazil: epidemiology and genome.** Selma Jerônimo, Federal University of Rio Grande do Norte

16:05 - 16:30	**Leishmaniasis and some of its atypical presentations.** Anastácio de Queiroz Sousa, Federal University of Ceará

16:30 - 16:55	**Canine Leishmaniasis and coinfections in urban community of Fortaleza, Ceará, Brazil.** Adam L Lima, Ph.D. Student, State University of Ceará Médico Veterinário, Mestre em Ciências Veterinárias (PPGCV-UECE), Doutorando em Ciências Veterinárias (PPGCV-UECE).

Leishmaniasis are anthropozoonosis caused by trypanosomes of *Leishmania* genus. There are more than 50 pathogenic species. Brazil is endemic country for *Leishmania infantum*, the etiological agent for zoonotic visceral leishmaniasis (VL) and dogs are one urban reservoir of many possible mammals reservoirs. There are several studies that correlates VL with dog seroprevalence for canine visceral leishmaniasis (CanL) and effort are made to diminish the number of reservoirs. In this scenario, dogs' comorbidities, especially tick-borne diseases, plays a role in CanL progression in longitudinal studies and susceptibility in cross-sectional studies. In 2013, we did find in bone marrow aspirates from 64/64 dogs provide by The Zoonosis Control Center (CCZ) from Fortaleza and Sobral city in Ceará State the morulae of *Ehrlichia* spp., a Gram-negative bacteria that infects mammals mononuclear cells. *Ehrlichia canis* is the etiological agent for Canine Ehrlichioses (CE). In another study, we found 64% positivity on CCZ dogs by PCR for *Ehrlichia canis* in 2016. During 2016 and 2017 we did one cross-sectional study and other longitudinal study to associate these two diseases. The cross-sectional study showed that dogs exposed for CE or Anaplasmosis are more likely to be infected by *Leishmania infantum* (RR: 1.55, P = 0.0135). The longitudinal study shows that dogs exposed to Anaplasmosis and CE are 2.69 more likely to be positive for *Leishmania* spp. (Adjusted RR: 1.68, 95% CI: 1.09–2.61, P = 0.019). Since the longitudinal study were conducted in USA, a country where CanL is vertically transmitted, we design a longitudinal study in Fortaleza-CE, an endemic city from Brazil-CE, to answer if CE have a "cause-effect" over CanL. On this new study started on 2018, we enrolled 660 households' dogs from two regions of Fortaleza. We are visiting these dogs once a year to collect blood samples and social-demographic information. Our partial results (cross-sectional from timepoint zero) show that overall positivity for CanL were 13% on DPP® test and 18% on IDEXX SNAP® test. We classified the families in seven stratums, being 01 more wealth and 7 the poorest and we found that higher stratums had higher positive dogs (2 = 0.00%, 3 = 9.57%, 4 = 12.11%, 5 = 17.39%, 6 = 16.00% and 7 = 30.77%). Dogs positive for CanL had a 76% positivity for CE, compared to 59% positivity in negative CanL animals. These results show a clear relation between CanL-CE. We expect to analyze data from three timepoints on the prospective-longitudinal study to determinate the real risk of infection for CanL in dogs exposed to CE, and if it is significant, we can use tick-borne diseases control tools to diminish CanL spread and therefore, reduce the urbans reservoir for VL.

9:00 - 9:25	**Characterizing the biochemical alterations associated with environmental enteric dysfunction using metabolomics.** Jonathan Swann, Imperial College London, UK

9:25- 9:50	**Metabolomics approach to explore new pathobiology associated with enteric infections.** Natasa Giallourou, Imperial College London, UK

9:50-10:15	**Helminthic infections and metabolomics exploration in cohort children in developing countries.** Gordana Panic, Imperial College London, UK

10:15-10:40	**APOE polymorphisms in children with heavy diarrhea and implications for later development: APOE4 as an antagonistic pleiotropic gene.** Reinaldo B. Oriá[1,2], Aldo Ângelo M. Lima[1], Richard L. Guerrant[1,3] [1]*Institute of Biomedicine, School of Medicine, Federal University of Ceara. Rua Coronel Nunes de Melo, 1315, CEP: 60430-270, Fortaleza, CE, Brazil.* [2]*Laboratory of Tissue Healing, Ontogeny and Nutrition, Department of Morphology, School of Medicine, Federal University of Ceara, Rua Delmiro de Farias S/N, CEP: 60416-030 - Fortaleza, CE, Brazil* [3]*Center for Global Health, P.O Box 801379, MR4, 409 Lane Road, Room 3148, University of Virginia, Charlottesville, VA, USA.*

A series of studies have shown that heavy diarrhea burdens and malnutrition in the first two years of life in children living in poor settings in the developing world have lasting deleterious effects on physical and cognitive development. Apolipoprotein E is key protein for reverse cholesterol transport to the liver to be metabolized and is also highly expressed in the brain with multiple immunoinflammatory effects (ApoE=protein; APOE=gene). The ApoE gene is polymorphic and has three common alleles, APOE2, APOE3 and APOE4, the latter has been recognized as a risk factor for Alzheimer's disease. Although much is known about the role of APOE4 with aging, few studies have addressed the role of APOE4 in early development. We have shown that APOE4 is relatively common in shantytown children living in Brazil and suggest that APOE4 has a protective role in cognitive development as well as weight-for-height in children with heavy burdens of diarrhea in early childhood, despite being a marker for cognitive decline with Alzheimer's later in life. APOE2 frequency was higher among children with heaviest diarrhea burdens during the first two years of life, as detected by PCR, raising the possibility that ApoE-cholesterol balance may be critical for growth and cognitive development under the stress of heavy diarrhea burdens. Recently, other studies further supported our results in Tsiname, Bolivia, an indigenous population with a high infectious load. In addition, APOE4 has been shown to improve fertility in women living in adverse environments. Altogether, these findings suggest that APOE4 behaves as an antagonistic pleiotropic gene, which may favor early life survival in disenfranchised environments, however, may later increase the risk for Alzheimer's disease.

10:40- 11:05	**Dietary intake from complementary feeding is associated with intestinal barrier function and environmental enteropathy in Brazilian children from the MAL-ED cohort study.** P.N. Costa[1], A.M. Soares[2], J.Q. Filho[2], F.S. Junior[2], R. Ambikapathi[3], E.T. Rogawski McQuade[4], R.L. Guerrant[5], L.E. Caulfield[6], A.A.M. Lima[3], B.L.L. Maciel[1] [1]Nutrition post-graduation program, Department of Nutrition, Federal University of Rio Grande do Norte, Natal, Rio Grande do Norte, Brazil. [2]INCT – Instituto de Biomedicina do Semiárido Brasileiro (IBISAB), Federal University of Ceará, Fortaleza, Ceará, Brazil. [3]Department of Public Health, Purdue University, West Lafayette, Indiana, USA. [4]Department of Public Health Sciences, University of Virginia, Charlottesville, Virginia, USA. [5]Center for Global Health, Division of Infectious Diseases and International Health, University of Virginia School of Medicine, Charlottesville, Virginia, USA. [6]Center for Human Nutrition, Department of International Health, The Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USA

Background: nutrition during the complementary feeding period provides nutrients and dietary compounds that influence intestinal health. Nevertheless, at this the time of complementary foods introduction there is an increased incidence of enteric infections among children. Repeated enteric infections, even when subclinical, can lead to intestinal barrier dysfunction and produce a condition called environmental enteropathy (EE). Recent data show that EE biomarkers are influenced by enteropathogen burden, sanitation and hygiene conditions, and socioeconomic status. However, it is not clear if EE could be additionally influenced by factors such as dietary intake. Therefore, our hypothesis is that the dietary intake from complementary feeding is associated with the biomarkers of intestinal barrier function and environmental enteropathy. **Objective:** The aim of this study was to assess the association between nutrient intake, intestinal barrier function, and environmental enteropathy (EE) among children in Fortaleza, Brazil. **Methods:** Among 233 children from the Brazilian site of the MAL-ED cohort study, nutrient intake was monthly collected from 9 to 15 months of age by 24 h dietary recalls. Nutrient intake was adjusted by within-person variance and energy intake. Nutrient intake was expressed as nutrient adequacy for each nutrient (energy, fiber, macronutrients, and micronutrients). Intestinal barrier function was assessed using the lactulose:mannitol (L/M) test and EE was determined as EE-score, which is a composite measure using fecal biomarkers concentrations (alpha-1-antitrypsin, myeloperoxidase and neopterin), both were measured at 15 months of age. Associations between nutrient intake, intestinal barrier function, and EE were determined using multiple linear regression. **Results:** there was adequate nutrient intakes (with the exception of fiber). Children showed impaired intestinal barrier function, and intestinal inflammation. The median L/M was 0.09 and the median EE-score was 5.0. There was a negative correlation between energy adequacy and L: M (ρ = -0.19, p <0.05) and between folate adequacy and NEO concentrations (ρ = -0.21, p <0.01). However, a positive correlation between thiamine adequacy and MPO concentration (ρ = 0.22, p <0.01) and between calcium adequacy and NEO concentration (ρ = 0.23; p < 0.01). The results from the regression models showed an inversely association between energy adequacy and L/M (β = -0.19, p = 0.02), which represents a protective effect of energy intake from complementary feeding on the intestinal barrier function. Moreover, fiber adequacy was inversely associated with the EE-score (β = -0.20, p = 0.04), indicating a beneficial effect of a higher fiber intake on reduction in the biomarkers of intestinal inflammation. **Conclusions:** these findings demonstrate that nutrient intake from complementary feeding is associated with intestinal barrier function and EE in Brazilian children.

11:05- 11:30	**Energy and zinc intakes from complementary feeding are associated with the risk for undernutrition in children from South America, Africa and Asia.** Bruna L. L. Maciel[1], Priscila N. Costa[1], José Q. Filho[2], Samilly A. Ribeiro[2], Francisco A. P. Rodrigues[2], Alberto M. Soares[2], Francisco S. Junior[2], Ramya Ambikapathi[3], Elizabeth T. Rogawski McQuade[4], Richard L. Guerrant[5], Laura Caulfield[3,6], Aldo A. M. Lima[2] [1]Nutrition post-graduation program, Department of Nutrition, Federal University of Rio Grande do Norte, Natal, Brazil; [2]*INCT – Instituto de Biomedicina do Semiárido Brasileiro (IBISAB),* Faculty of Medicine, Federal University of Ceará, Fortaleza, Brazil; [3]Department of Public Health, Purdue University, West Lafayette, Indiana, USA; [4]Department of Public Health Sciences, University of Virginia, Charlottesville, Virginia, USA; [5]Center for Global Health, Division of Infectious Diseases and International Health, University of Virginia School of Medicine; [6]Center for Human Nutrition, Department of International Health, The Johns Hopkins Bloomberg School of Public Health.

Background: Few studies have focused on quantitatively analyzing nutrients from infant diets, compromising complementary feeding evaluation and health promotion worldwide.

Objective: To describe dietary intake in infants from 9 to 24 months of age, determining nutrient intakes associated with the risk for underweight, wasting and stunting.

Methods: Usual nutrient intakes from complementary feeding were determined by monthly 24h recalls collected from 9 to 24 months of age in communities from 7 low- and middle-income countries: Brazil (n = 169), Peru (n = 199), South Africa (n = 221), Tanzania (n = 210), Bangladesh (n = 208), India (n = 227) and Nepal (n = 229), totalizing 1,463 children and 29,120 food recalls. Intakes were corrected for within- and between-person variance (ANOVA) and energy intake (residual method). Multivariable regression models were constructed to determine nutrient intakes associated with the development of underweight, wasting and stunting at 24 months of age.

Results: Children who were underweight at 24 months of age had lower intakes of energy, macronutrients and all of the 6 vitamins and 6 minerals analyzed. Wasting was associated with lower energy and protein intakes, 5 of the 6 vitamins and all of the 6 minerals assessed. Stunting was associated with lower lipids, higher fiber, and lower folate, vitamins A, C and calcium intakes. In the multivariable logistic regression models, lower energy and zinc intakes were associated with the development of underweight, wasting and stunting at 24 months. Assessment of energy and zinc prospectively from 9 to 24 months indicated these intakes were consistently lower in children with undernutrition.

Conclusions: Low intakes of energy and zinc in complementary feeding were associated with the risk for undernutrition. Data suggest these are complementary feeding characteristics to be improved cross-countries.

Keywords: child nutrition, dietary intake, nutrient intake, energy, zinc

Sources of support: The Etiology, Risk Factors and Interactions of Enteric Infections and Malnutrition and the Consequences for Child Health and Development Project (MAL-ED) was a collaborative project supported by the

Bill & Melinda Gates Foundation, the Foundation for the NIH, and the National Institutes of Health/Fogarty International Center.

10:00 - 10:25	**Translational research in NERD from inflammation to impairment in mucosal integrity: New mechanisms and new topically agents.** Marcellus H. L. P. Souza, Associate professor, MD, PhD. Department of Clinical Medicine, Federal University of Ceará, Brazil

Background: Microscopic inflammation and impairment of the esophageal epithelial barrier are two relevant mechanisms involved in the pathophysiology of the nonerosive reflux disease (NERD). We proposed to develop a novel murine model of NERD in mice, with microscopic inflammation and impairment in the epithelial esophageal barrier, in order to evaluated new mechanisms and new topically agents. **Methods and Results**: Female Swiss mice were subjected to the following surgical procedure: the transitional region between the forestomach and the glandular portion of the stomach was ligated, and a nontoxic ring was placed around the duodenum near the pylorus. The control group underwent sham surgery. The animals were euthanized and esophageal wet weight, macroscopic lesion, histopathological alterations, myeloperoxidase (MPO) activity, cytokine levels, transepithelial electrical resistance (TEER), and mucosal permeability was evaluated. The survival rate was 78% at 14 days, with mild loss in body weight. Surgery did not induce erosive esophagitis but instead induced microscopic inflammation and increased esophageal wet weight, IL-6, keratinocyte-derived cytokine (KC) levels, and MPO activity with maximal peak between 3 and 7 days and resolution at 14 days post surgery. Epithelial esophageal barrier was evaluated in operated mice at 7 and 14 days post surgery; a decrease in TEER and increase in the esophageal epithelial permeability were observed compared with the sham-operated group. In addition, the inhibition of acid secretion with omeprazole significantly prevented the esophageal inflammation and impairment of barrier function at 7 days post surgery. In other group of the mice, nonerosive reflux disease was surgically induced, and then the animals were killed 7 days post surgery. The experimental groups were: I, sham surgery (negative control); II, NERD untreated; III and IV, NERD + SB366791 or capsazepine (TRPV1 antagonists); and V, NERD + resiniferatoxin (for long-term desensitization of TRPV1). The esophagus was collected for western blotting and histopathology and for evaluation of wet weight, MPO, KC, TEER, and basal permeability to fluorescein. Compared to sham, NERD mice had increased esophageal wet weight and MPO and KC levels. The mucosa had no ulcers but exhibited inflammation. NERD mice showed mucosal TRPV1 overexpression, a more pronounced decrease in TEER, and increased basal permeability. Pharmacological modulation of TRPV1 prevented esophageal inflammation development, TEER changes by acidic exposure, and increase in esophageal permeability. Finally, we investigate a standardized biopolymer, cashew gum in human esophageal mucosa and mice with experimentally- induced non-erosive reflux disease (NERD). Human esophageal biopsies from NERD patients were collected to evaluate the mucosal protection of cashew gum through TEER, mucosal permeability, and mucoadhesiveness tests. A surgical model of NERD in mice was induced, and barrier functions followed by suggestive esophageal inflammatory hallmarks were evaluated. Pre-coating of cashew gum was effective in human esophageal mucosa by attenuating drop of TEER and mucosal permeability. Labeled- cashew gum adheres to human esophageal mucosa for up to 1□h. In animal studies, cashew gum improved parameters of barrier function (TEER and mucosal permeability) in distal esophagus mucosa. Cashew gum also promoted sequential support by reducing inflammatory hallmarks of esophageal damage. **Conclusions**: We develop an experimental model of reflux with a mild inflammation associated with an impairment in the mucosal integrity, we demonstrated that TRPV1 activation is an important mechanism involved in experimental reflux by modulate esophageal inflammation and mucosal integrity, and finally we showed that Cashew gum has a topical esophageal mucosal protection due to its mucoadhesiveness and anti-inflammatory profile.

10:25- 10:50	**Expression of proteins associated with sarcopenia in reflux esophagitis.** Paula SM, Alves MNM, Santos AA, Nobre-Souza MAN, Federal University of Ceará

Introduction: We have shown that patients with Gastroesophageal Reflux Disease (GERD) present crural diaphragm (CD) insufficiency, supporting the idea of a skeletal muscle deficiency in reflux esophagitis. By its turn, skeletal muscle atrophy is associated with changes of AKT phosphorylation (p-AKT), MURF-1 and atrogin levels. This study aims to show if these proteins are differently expressed in the CD muscles of patients with reflux esophagitis and if there is a relation with different grades of GERD. **Methods:** After approval by the local Ethical Committee, we obtained human CD biopsies from volunteers, 15 females and 10 males, aged between 25-62 years, at the time of antireflux laparoscopic surgery (esophagitis group - GERD) or gallbladder surgery (control group - CT). Esophagitis group was further divided in grades A (GERD-A), B (GERD-B) and C (GERD-C), according the Los Angeles scale. We studied different signaling pathways (AKT, p-AKT, MURF-1, Atrogin normalized by GAPDH). Data were shown as mean±SEM and compared by one-way ANOVA (*P<0,05). **Results:** Protein expressions of p-AKT and atrogin were not different between CT and GERD groups, however, MURF-1 expression of GERD-C group (1.05 ± 0.18; n=3) when compared to GERD-B group (0.27 ± 0.11; n=6). CT group was not different from GERD-A and GERD-B groups. **Conclusion:** Human CD muscles of GERD patients have detectable expression of key proteins of atrophy signaling pathways (AKT, p-AKT, MURF-1 and atrogin). Our results showed that CD of GERD-C patients had increased expression of MURF-1; a protein considered as a potential marker of skeletal muscle atrophy. Therefore, reflux esophagitis seems to be able to impact the hypertrophy/atrophy muscular pathways in the CD, especially in higher grades of GERD. Further studies will be necessary to clarify their respective cellular mechanisms.

Palavras-chaves:GERD, Crural diaphragm, atrophy.

Apoio financeiro: CAPES, CNPq.

10:50-11:15	**Bile acids inhibit contractility of rat esophageal muscle.** Kalinne Kelly Lima Gadelha,[1] Lucas Antonio Duarte Nicolau, [1] Daniel Sifrim,[2] Armênio Aguiar dos Santos, [1] Pedro Jorge Caldas Magalhães[1] [1] Department of Physiology and Pharmacology, School of Medicine, Federal University of Ceará, Fortaleza, CE, Brazil [2] Barts and The London School of Medicine and Dentistry, Queen Mary, University of London, London, United Kingdom

The upstream flow of gastric content into the oesophagus commonly causes symptoms with or without organ damage, a pathological condition that is known as gastro-oesophageal reflux disease (GORD). In humans, decreased contractility of the oesophageal body was concomitant to the occurrence of reflux symptoms. Abnormal motility patterns of the oesophageal body such as fragmented peristalsis, ineffective motility or absence of oesophageal contractility was found in GORD patients. In the present study, the effects of an acute exposure of rat esophagus to a content that simulates the gastroesophageal reflux were evaluated using experimental methods on isolated oesophageal tissues. Early studies showed that strips of rat oesophagus subjected to 30 min of luminal exposure to a test solution (medium containing pepsin and taurodeoxycholic acid – TDCA - at pH 1) responded less than strips maintained in the absence of pepsin and TDCA at pH 7.4. Preparations subjected only to a test solution at pH 1 (but without pepsin and TDCA) did not decrease the contractility in response to carbachol or KCl. In oesophageal preparations mounted following the circular orientation of the smooth muscle bundles, the G-protein coupled bile acid receptor (GPBAR1 or TGR5) agonist Oleanolic acid decreased the contractile response induced by a single concentration of carbachol, but the effect was significant after 45 min treatment. The results suggest that the TDCA present in the experimental refluxate is able to interfere with the oesophageal contractility in rats.

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11:15-11:25	**Anti-inflammatory effect of Gaviscon® on oesophageal mucosa in murine model of non- erosive reflux disease.** Nogueira KM, Sales TMAL, Neto JPC, Filho HBC, Nicolau LAD, Souza MHLP

Introduction: Among the disorders that affect the esophagus we have Gastroesophageal Reflux Disease (GERD), which is a chronic clinical manifestation, with significant prevalence and estimates suggest that this disease affects around 10-20% of the world population. One of the classes of drugs considered first-line for the treatment of GERD are the Proton Pump Inhibitors (PPIs), however, several studies have shown that about 60% of patients do not respond to treatment and have typical reflux symptoms (SCARPELLINI et al., 2016). Therefore, alginates are an alternative approach for the treatment of NERD symptoms as they are able to prevent the retrograde flow of gastric content. One example of this type of medication is Gaviscon®. These drugs represent therapeutic alternatives for symptomatic patients with GERD, including NERD. However, its anti-inflammatory potential has not yet been investigated. Objective: To investigate the anti-inflammatory effect of Gaviscon® treatment in mice with non-erosive reflux disease. **Methodology:** The project was submitted to the UFC Animal Research Ethics Committee (CEPA) and approved by Protocol 8556060619. For NERD induction, the protocol devised by Silva et al. (2017) was used. The animals will be divided into three groups: sham; NERD; NERD + omeprazole; and NERD + Gaviscon® and the mice will be euthanized on day 7 after surgery. Oesophageal samples will be collected for evaluation the inflammatory process by histopathological evaluation, oedema (wet weight), neutrophil migration (MPO activity), redox status (MDA concentration, SOD activity, GSH concentration), Th1 cytokines production (IL-1β, IL-6, IL-10, IFN-γ, KC, MCP-1, RANTES, and TNF-α), HIF2-α activation, NADPH expression and NF-κB activation. We also measured the mucosal integrity (tight junctions status, Transepithelial electrical resistance, mucosal permeability to fluorescein). **Results:** Animals with NERD present esophageal edema (34.45 ± 4.81mg/cm) when compared to the sham group. On the other hand, daily administration of Gaviscon significantly reduced the inflammatory parameter of edema ($P < 0.05$) by measuring wet weight (18.57 ± 2.78 mg/cm) as well as the group treated with omeprazole (13.80 ± 1.27 mg/cm). **Conclusion:** The result indicated that Gaviscon, which is already largely used, may have an anti-inflammatory activity and therefore protect the esophageal mucosa.

Fatos que Impactaram o Desenvolvimento Científico do Nubimed

Foto de Fev./1990. Em pé: Drs. Aldo AM Lima; e Barry Marshall (Nobel Laureate in Medicine 2005). Sentados Esq. p/ Dir.: Drs. Barry M Farr; Lúcia Libanês BC Braga; Richard L Guerrant; e Noélia L Lima.

1st TMRC & ICIDR 1997

Foto 24/Nov./2000. Esq. p/ Dir.: Drs. Leonard R Forte; Manasses C Fonteles; Manuel O Moraes Filho; Aldo AM Lima; Ferid Murad (Nobel Laureate in Medicine 1998); e Joel Larner (UVa).

4th TMRC & ICIDR 2000

6th TMRC & ICIDR 2002

III International Symposium
25th UFC & UVa Convênio - 2003

III Symposium in Biomediicne
25th UFC & UVa Convênio, 2003

10th TMRC & ICIDR 2006

XXII TMRC & Pharmacology Meeting
40th UFC & UVa Convênio, 2018

IV International Symposium
41th UFC & UVa Convênio, Dez.2019