22nd Tropical Medicine Research Center & Pharmacology Meeting

PHARMACOLOGY AND INFECTIOUS DISEASES: NEW MOLECULAR PATHWAYS

November, 23-24th, 2018

Fortaleza, Ceará - Brasil

Oceani Beach Park Hotel

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XXII Tropical Medicine Research Center & Pharmacology Meeting: International Program CAPES_Print

Pharmacology and Infectious Diseases: New Molecular Pathways

Word of Management Committee:

The annual meeting of the **XXII Tropical Medicine Research Center** is a regular annual scientific event, which began in 1996 and will be in its twentieth year in 2018. The event was created to bring together researchers, technicians and students at national and international levels interested in the scientific and technological development of research on tropical diseases that afflict populations in the region in the Brazilian semi-arid 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 Edgar M. Carvalho, Federal University of Bahia, Selma Jerônimo, Federal University of Rio Grande do Norte, and Aldo AM Lima, Federal University of Ceará. International groups were led by researchers, Warren Johnson, Cornell University, NY, Richard L. Guerrant, University of Virginia, CHO, VA and Lee W. Liley, University of California, Berkeley, CA. In this edition the event is entitled "***Infectious Diseases and Pharmacology: new molecular pathways***". In this subject, we will cover topics of ongoing research and perspectives in several areas of tropical medicine, motility and neurogastroenterology, such as enteric infections, environmental enteropathy, malnutrition, leishmaniasis, diseases affecting gastrointestinal motility, neurogastroenterology and pancreatitis. The event will be held at ***Oceani Beach Park Hotel, Aquiraz, CE, November 23-24, 2018***. The event is free and open to researchers, teachers, undergraduate and graduate students as well as technical staff. Annually the event receives from 40-100 people participants from national, international researchers, students and technicians. The event has contributed to the development of research in tropical medicine at national and international level, maintenance of two networks, one at the national level and another international research on the topics mentioned. We also cite the effective participation and in the last eight years of two ***INCTs (Biomedicine and Immunology of Tropical Diseases)***. National and international collaboration, such as the exchange of post-graduate students and technicians, was significant and in this sense a long-term model was created for this type of scientific and technological activity. We are currently supporting the recent project approved at the Pro-Rectory of UFC and already under execution called ***CAPES-Print International*** with the effective participation of collaboration between groups of national and international researchers from the ***Federal University of Ceará, State University of Ceará, University of Virginia, VA, USA, Imperial College London, UK, Queen Mary University of London, UK, Center for Diarrheal Diseases Research, ICDDR, B, Dhaka, Bangladesh, and University of Liverpool***. From this collaboration and interaction were benefited and created new institutes of research, postgraduate courses national and international.

XXII Tropical Medicine Research Center & Pharmacology Meeting: International Program CAPES_Print

Pharmacology and Infectious Diseases: New Molecular Pathways

Palavra do Comitê Gestor:

A reunião anual do **XXII Tropical Medicine Research Center** é um evento anual científico regular, com início no ano de 1996 e que neste ano de 2018 completará sua vigésima segunda edição. O evento foi criado no sentido de congregar pesquisadores, técnicos e estudantes ao nível nacional e internacional interessados no desenvolvimento científico e tecnológico da pesquisa em doenças tropicais que afligem as populações na região no semiárido brasileiro. Inicialmente o evento foi realizado com a participação efetiva de três grupos de pesquisadores nacionais e três grupos de pesquisadores internacionais. Os grupos de pesquisadores nacionais foram liderados pelos professores Edgar M. Carvalho, Universidade Federal da Bahia, Selma Jerônimo, Universidade Federal do Rio Grande do Norte, e Aldo AM Lima, Universidade Federal do Ceará. Os grupos internacionais foram liderados pelos pesquisadores, Warren Johnson, Cornell University, NY, Richard L. Guerrant, University of Virginia, CHO, VA e Lee W. Liley, University of California, Berkeley, CA. Nesta edição o evento tem como título "***Doenças Infecciosas e Farmacologia: novas vias moleculares***". Neste teme, abordaremos tópicos de pesquisas em andamento e de perspectivas em diversas áreas da medicina tropical, motilidade e neurogastroenterologia, como infecções entéricas, enteropatia ambiental, desnutrição, leishimaniose, doenças que afetam a motilidade gastrointestinal, neurogastroenterologia e pancreatite. O evento será realizado no ***Oceani Beach Park Hotel, Aquiraz, CE, 23-24 Novembro de 2018***. O evento é grátis e aberto ao público de pesquisadores, professores, alunos de graduação e pós-graduações, bem como pessoal técnico. Anualmente o evento recebe de 40-100 pessoas participantes dentre os pesquisadores nacionais, internacionais, alunos e técnicos. O evento tem contribuído para o desenvolvimento da pesquisa em medicina tropical ao nível nacional e internacional, manutenção de duas redes, uma ao nível nacional e outra internacional de pesquisas nos tópicos mencionados. Citamos ainda a participação efetiva e nos últimos oito anos de dois ***INCTs*** (***Biomedicina e Imunologia das Doenças Tropicais***). A colaboração nacional e internacional, como o intercâmbio de estudantes de pós-graduações e técnico, foi significativa e neste sentido foi criado modelo de longa duração para este tipo de atividade científica e tecnológica. Citamos atualmente o suporte do projeto recente aprovado na Pro-Reitoria de Pós-Graduação da UFC e já em execução denominado ***CAPES-Print International*** com a participação efetiva de colaboração entre grupos de pesquisadores nacionais e internacionais provenientes da ***Universidade Federal do Ceará, Universidade Estadual do Ceará, University of Virginia, VA, USA, Imperial College London, UK, Queen Mary Univesity of London, UK, Center for Diarrheal Diseases Research, ICDDR, B, Dhaka, Bangladesh, e University of Liverpool***. Desta colaboração e interação foram beneficiados e criados novos institutos de pesquisas, cursos de pós-graduações nacional e internacional.

XXII Tropical Medicine Research Center & Pharmacology Meeting

Pharmacology and Infectious Diseases: New Molecular Pathways

Oceani Beach Park Hotel
Av. dos Golfinhos, 771 - Porto das Dunas,
Aquiraz - Ceará, Brazil

November 23th – 24th, 2018

Friday– November, 23th

8:30 - 9:00 am	**Opening remarks**: *Henry de Holanda Campos*, Rector, Federal University of Ceara *Valéria Goes Ferreira Pinheiro*, Director, Faculty of Medicine, Federal University of Ceara *Pedro Jorge Caldas Magalhães*, Coordinator, PG-Farmacology, Faculty of Medicine, Federal University of Ceara *Aldo Ângelo Moreira Lima*, Coordinator, 22nd TMRC Meeting and Center of Biomedicine, Faculty of Medicine Federal University of Ceara

PROGRAM

Friday – November, 23th

Session I: Motility, Neurogastroenterology and Pancreatitis

Chairperson*: Prof. José Xavier Neto, Federal University of Ceara*

9:00 - 9:30 am	New aspects of pathophysiology of the gastro-esophageal reflux disease. *Daniel Sifrim*, Queen Mary, University of London
9:30 - 9:50 am	A murine model of laryngeal inflammation induced by GERD: effect of topical prottective agents. *Thiago Menezes*, M.Sc. student, Federal University of Ceara
9:50 - 10:10 am	Esophago-Gastric junction motility and sarcopenia. *Vicente, M.*Sc. student, Federal University of Ceara
10:10 - 10:30 am	Impairment of rat esophageal muscle contractility associated with experimental non-erosive esophageal mucosal damage. *Kalline Gadelha*, M.Sc. student, Federal University of Ceara
10:30 - 11:00 am	Discusssion I
11:00 - 11:20 am	Visceral pain: the purinergic system a new therapeutic target in acute pancreatitis *Deyssen KF Bezerra*, Federal University of Ceara
11:20 - 11:40 am	Acute Pancreatitis: Mechanism of intracellular calcium signaling from *Ximenia Americana* *Patricia S Pantoja*, M.Sc. student, Federal University of Ceara
11:40 - 12:10 pm	Discusssion II

Coffee Break: available during the meeting
Lunch: 12:30 - 13:30 pm

Session II: Proteomic and Metabolomic Applied to Pharmacology & Infectious Diseases

Chairperson: *Prof. Manassés C Fonteles*, Federal University of Ceara

13:30 - 14:00 pm	Metabolome biomarkers exploration for malnutrition and diarrheal diseases. *Jonathan R Swann*, PhD, Imperial College London, London, UK
14:00 - 14:15 pm	Detection of lactulose and mannitol as biomarkers using HPLC-PAD and LC-MS/MS to study gut function. *Francisco Adelvane de P Rodrigues*, Federal University of Ceara
14:15 -14:30 pm	Ginger metabolites inhibit *Staphylococcus aureus* virulence factors. *James A Silva*, Federal University of Sergipe
14:30 -15:00 pm	Leishmaniasis a common endemic disease that seems to be rare and complicated. *Anastácio de Queiroz Sousa*, Federal University of Ceara

15-00 -15:20 pm	Vaccine for treating leishmaniasis in dogs can help curb spread to humans. *Adam L Lima*, Ph.D. Student, State University of Ceara

Coffee break: 15:20-15:40 pm

Session III: From Enteric Infection to Therapeutics: A Translational Model for Science Application

Chairperson: *Prof. Alexandre Havt*, Federal University of Ceara

15:40 -16:10 pm	Uroguanylin and guanylin peptides: from enterotoxigenic *E. coli* infection to experimental therapeutics. *Manassés C. Fonteles*, State University of Ceara
16:10 - 16:30 pm	Modeling diet and pathogen specific enteropathy and diarrhea: a novel murine model of *Shigella flexneri* infection. *Pedro Henrique S Quintela*, Ph.D. Student, Federal University of Ceara
16:30 - 16:50 pm	Molecular diagnostics of enteropathogens association with subclinical and clinical infections in the MAL-ED birth cohort study. *Alexandre Havt*, Federal University of Ceara
16:50 - 17:30 pm	Capes_Print International Program at UFC Henry de Holanda Campos, Rector, Federal University of Ceará Antônio Gomes S. Filho, Pró-Reitor de Pesquisa e Pós-Graduação Federal University of Ceará Internationalization in translational and epidemiological research in neurogastroenterology Round Table Discussion **Chairpersons** - Profs. Marcellus HLP Souza and Aldo AM Lima, Federal University of Ceará

Saturday, November 24th

Session IV: Causes, Pathogenesis, Genetics and Biomarkers Studies of Malnutrition & Enteric Diseases I

Senior and Young Investigators Session

Chairperson: *Prof. Aldo A. M. Lima*. Coordinator of the Institute of Biomedicine/ Federal University of Ceara

Dr. Francisco A P Rodrigues, Federal University of Ceara

9:00 - 9:30 am	Gut bacteria that prevent growth impairments transmitted by microbiota from malnourished children *Tahmeed Ahmed*, M.D., Ph.D. Center for Diarrheal Diseases Research, ICDDR, B Dhaka, Bangladesh
9:30- 9:45 am	Clinical impact of virulence-related genes from *Shigella/Escherichia coli* enteroinvasive pathotype infection in children from Semiarid Brazilian region *Mariana Bona*. PhD student in Medical Science - Federal University of Ceara
9:45-10:00 am	Antimicrobials resistance of enteroaggregative *Escherichia coli* strains isolated from nourished and malnourished children from Fortaleza, Ceara, Brazil *Marília SMG Amaral*, PhD student in Medical Microbiology - Federal University of Ceara
10:00-10:15 am	Use of carbohydrate biomarkers by LC-MS/MS for assays of intestinal permeability *Lyvia MVC Magalhães*, PhD student in Medical Sciences - Federal University of Ceara
10:15- 10:30am	*Campylobacter jejuni* virulence genes and immune-inflammatory biomarkers association with growth impairment in children from Northeastern Brazil *Herlice do Nascimento Veras*. PhD student in Microbiology, Federal University of Ceara

10:30-10:50 am -Coffee Break

Session V: Causes, Pathogenesis, Genetics and Biomarkers Studies of Malnutrition & Enteric Diseases II

Senior and Young investigators session

Chairperson: Prof. Aldo AM Lima, Coordinator of the Institute of Biomedicine, UFC

10:50- 11:10am	Virulence related genes and co-enteropathogens associated with clinical outcomes of enteropathogenic *Escherichia coli* infections in children from Brazilian semiarid region *Ana KS Santos*, PhD student in Medical Microbiology - Federal University of Ceara
11:10-11:40am	Enteropathogens association with gut function, immune-inflammatory responses and growth development: the MAL-ED birth cohort study *Aldo AM Lima* Coordinator, INCT-Biomedicine, Federal University of Ceara
11:40 pm	Meeting Close

Scientific Committee:

Aldo AM Lima, M.D., Ph.D., Federal University of Ceara

Alexandre Havt, Ph.D., Federal University of Ceara

Armênio A Santos, M.D., Ph.D., Federal University of Ceara

José Xavier Neto, M.D., Ph.D, Federal University of Ceara

Marcellus HLP Souza, M.D., Ph.D., Federal University of Ceara

Pedro JC Magalhães, Ph.D., Federal University of Ceara

SUMMARY

Friday– November, 23th

9:00 - 9:30	**New aspects of pathophysiology of the gastro-esophageal reflux disease** *Daniel Sifrim*, Queen Mary, University of London

9:30 - 9:50	**A murine model of laryngeal inflammation induced by GERD: effect of topical prottective agents** *Thiago Meneses Araújo Leite Sales*, M.Sc. student, Federal University of Ceara The present study should standardize an experimental model of laryngeal inflammation in mice with GERD and investigate mucosal protective agents in order to prevent inflammation. The GERD model was surgically induced in Swiss mice (30-35g) by pylorus substitution and gastric fundus ligation. The control group was Sham (false operated). The animals were sacrificed 3 and 7 days postoperatively. The experimental groups consisted of Group I: Sham, II: GERD, III: Omeprazole (an IBP: 40 mg / kg, i.p.), IV Alginate (10 mg / kg). Wet weight and myeloperoxidase (MPO) of the esophagus and larynx were analyzed. Basal transepithelial electrical resistance (TEER) of the larynx was evaluated with exposure of a KREBS pH 7.4 solution and permeability was assessed after baseline TEER on exposure of a KREBS pH 7.4 + fluorescein (376 Da) solution. Surgery induced GERD without erosion but with changes in weight and MPO activity in the esophagus on days 3 and 7. Sham intervention did not cause esophageal inflammation. When assessing the larynx, it was observed that the levels of wet weight and MPO were altered only on day 3. The impairment of the laryngeal epithelial barrier was assessed using the Ussing chamber technique, on days 3 and 7 post-surgery, and a decrease in TEER and increased permeability in animals with GERD on day 3, compared to the Sham group were observed. The model at day 3 was selected for the following experiments. Inhibition of acid secretion with omeprazole and oral use of alginate prevented laryngeal inflammation (wet weight and MPO), TEER decrease, and increased permeability, compared with the GERD group. Thus, we conclude that a model of laryngeal inflammation in mice with GERD can be implemented and validated, and we demonstrated that the treatments with omeprazole and alginate were beneficial to prevent laryngeal inflammation as well as impairment of the integrity of the epithelial barrier.

9:50 - 10:10	**Esophago- Gastric junction motility and sarcopenia** *Vicente, M.*Sc. student, Federal University of Ceara Sarcopenia is a process closely linked to aging and losing mass, performance and muscle function. The diaphragm is a striated muscle and its crural portion is an important component of the esophagogastric junction (EGJ). Based on these assumptions, this study aimed to verify the association between the body solution and a motor function of JEG in elderly people. This is a transversal and descriptive study of a quantitative approach, performed on elderly human from the Geriatrics Outpatient Clinic of the Walter Cantídio University Hospital. The sample, composed of 17 volunteers, was submitted to a clinical evaluation for the assessment of typical and atypical symptoms of Gastroesophageal Reflux Disease, physical examination for the collection of anthropometric measurements, evaluation of body composition by dilution of deuterium in saliva, evaluation of motility esophageal evaluation of EGJ by high-resolution esophageal manometry (HRM), maximal inspiratory pressure (MIP) measurement by manovacuometry, in addition to ambulatory 24-h impedance-pH measurements. The research protocol was approved by the Research Ethics Committee of the Federal University of Ceará (UFC) and the patients signed the Free and Informed Consent Term. After analyzing the body composition, the participants were divided into two groups: Lower % of body water (n = 9) and Higher % of body water (n = 8). The groups were similar in age and gender. There was no difference in the scores of the symptom questionnaires, nor for esophageal motility variables. The variables EGJ axial displacement (DS), contractile integral (CI), Maximum JEG Pressure (MPÁX), EGJ Contractility Index (CI-EGJ) and Total EGJ Activity (AT-JEG) were statistically different between groups. Thus, despite some limitations like the size and type of the sample, this study may conclude that the elderly people with low muscle mass present alterations in the motor function of the EGJ.

10:10 - 10:30

Impairment of rat esophageal muscle contractility associated with experimental non-erosive esophageal mucosal damage

Kalline Kelly Lima Gadelha, M.Sc. student, Federal University of Ceara

Kalinne Kelly Lima Gadelha,[1] Francisco José Batista-Lima, [1] Daniel Maia Nogueira de Oliveira, [1] Emanuella Feitosa de Carvalho, [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 present study investigated whether the experimental simulation of duodeno-gastro-esophageal reflux alters the contractile responsiveness of rat esophageal strips. Following 30 min of luminal exposure to a solution at acid pH that contained pepsin and taurodeoxycholic acid (TDCA), isolated strips of the rat esophagus and gastroesophageal junction were subjected to contractile or relaxing stimuli. Acid challenge decreased the responsiveness of esophagus strips to contractile stimulation, especially in esophageal preparations that were mounted following the circular orientation of the muscularis externa layer. The contractility of longitudinal preparations of the rat esophagus appeared less susceptible to the deleterious effects of acid challenge. In contrast, the responsiveness of ring-like preparations from the gastroesophageal junction to contractile stimulation was unaltered by acid challenge. TDCA decreased the responsiveness of circular esophageal preparations to KCl, an effect that was exacerbated by luminal acidity. Contrarily, whereas the relaxant ability of the rat esophagus did not change, acid challenge increased the relaxant efficacy of sodium nitroprusside and isoproterenol in strips of the gastroesophageal junction. A significant decrease in transepithelial electrical resistance (TEER) was seen when the esophageal mucosa was challenged at pH 1 but not at pH 4. Treatment with alginate blunted the deleterious effects of acid challenge on TEER and the responsiveness of esophageal preparations to KCl. The present findings support the notion that luminal acidity is an important factor that disrupts barrier function in the esophagus to allow the diffusion of noxious agents, such as bile acid, that alter the contractile status of the esophagus.

11:00 - 11:20	**Visceral pain: the purinergic system a new therapeutic target in acute pancreatitis** *Deysen KF Bezerra* – Federal University of Ceara **Dor visceral: o sistema purinérgico como um novo alvo terapêutico na pancreatite aguda.** Um dos sintomas mais importante da pancreatite é a dor abdominal, que em geral sinaliza a necessidade de atendimento nos serviços de saúde. Muitos aspectos da fisiopatologia desse fenômeno ainda não estão compreendidos. Nesse trabalho foi avaliado o envolvimento dos receptores purinérgicos e da via NLRP 3 na dor pancreática. Para isso, a pancreatite aguda foi induzida em camundongos *Swiss* (25-30g) por meio de duas injeções intraperitoneais de etanol (1,35 g/Kg), associado ou não ao POA (150 mg/Kg), com intervalo de 1h. A modulação da nocicepção foi avaliada pelo método de Von Frey. A participação dos receptores purinérgicos foi investigada através do uso de antagonistas, o PPADs (inespecífico para P2) e o BBG (seletivo para P2X7), administrados por via intravenosa, 30 min antes da avaliação da 24h após a indução da pancreatite. O efeito do BBG sob o SNC foi avaliado mediante a administração intratecal deste fármaco. A influência de componentes do inflamossoma NLRP 3 foi avaliada através do uso de camundongos geneticamente modificados para Caspase-1, NLRP 3, ASC, IL-1R e IL-18, sendo, para isso, utilizados animais C57BL (20-25g). O bloqueio inespecífico de receptores purinérgicos atenuou essa hipernocicepção (etanol + POA + PPADS 12,5 mg/Kg: 9,38 ± 2,30 g, etanol + POA: 4,4 ± 0,45 g), e este efeito ainda foi presente quando administrado um bloqueador específico de P2X7 (etanol + POA + BBG 50 mg/Kg: 8,14 ± 0,50 g, etanol + POA: 3,35 ± 0,49 g). Houve manutenção do efeito anti-nociceptivo com a administração do BBG por via intratecal (etanol + POA + BBG: 8,47 ± 0,92 g, etanol + POA: 3,52 ± 1,08 g). Foi demonstrada que a deleção de componentes do inflamossoma NLRP 3 (Caspase-1, NLRP 3, ASC, IL-1R e IL-18) reduziu a hipernocicepção visceral, mostrando que essa via exerce papel importante nesse fenômeno. Concluimos que a hipernocicepção na pancreatite aguda envolve a participação dos receptores purinérgicos, ocorrendo, pelo menos parcialmente, por meio do envolvimento do inflamossoma NLRP 3, sendo uma promissora via terapêutica de modulação da dor pancreática.

11:20 - 11:40

Acute Pancreatitis: Mechanism of intracellular calcium signaling from *Ximenia americana's* bark

Patrícia S Pantoja, Federal University of Ceara

Patricia da Silva Pantoja
Fabricia da Cunha Jácome Marques
Samara Rodrigues Bonfim Damasceno
Maria Gonçalves Pereira
David Neil Criddle
Pedro Marcos Gomes Soares

Acute pancreatitis is an inflammation in pancreas that the ethiology hasn't known yet. Calcium and pre-activation enzymes are one of the mechanisms involved in pathology. **Objective:** Evaluate the calcium signaling from *Ximenia americana's* bark in mechanism of acute pancreatitis. **Methods:** Male Swiss mice (25-30g) and CD1 mice was used to in vivo and in vitro experiments. In vivo, the pancreatitis was induced by Na-TC surgery model with 5 groups: sham, saline, taurocholate and dose-response curve for TPL from *Ximenia americana's* bark. The treatment occured in two doses in 1h and 13h after induction and sacrifice in 24h after injection of bile acid. The acinar cells were isolate with CSLPA collagenase to do in vitro experiments. The dye as FLUO-4, TMRM was used to do analyses for calcium signaling and mitochondrial depolarization, respectively. Pre-treatment has done with fraction II from *Ximenia americana's* bark 1h before to do the analyses and the lesion agent has put 30 min before. The statistical analysis has done by prism graph. **Results:** TPL showed significative difference in Na-Tc surgery model for 30mg/Kg in all patterns of histology scores. In vitro, the fraction II from *Ximenia americana's* bark showed significative reduce for calcium signaling and mitochondrial depolarization with TLCS, CCK and POA. Besides, the tapsigargin with F II ameliorate the calcium intracellular in acinar cell. The docking with arabinose suggests the F II is modulating ORAI1 chanel and it is responsible for effect in calcium signaling. The experiments with D-(-)Arabinose was significative in tapsigargin model and reduced calcium signaling in TLCS, CCK and POA induced.

13:30 -14:00	**Metabolome biomarkers exploration for malnutrition and diarrheal diseases** *Jonathan R Swann*, PhD, Imperial College London, London, UK

14:00 -14:15

Detection of lactulose and mannitol as biomarkers using HPLC-PAD and LC-MS/MS to study gut barrierfunction

Francisco Adelvane de P Rodrigues, Federal University of Ceara

The lactulose:mannitol (L:M) diagnostic test is frequently used in field studies of environmental enteropathy (EE). However, heterogeneity in test administration and disaccharide measurement has limited the comparison of results between studies and populations. We aim to assess the agreement between L:M measurement between high-performance liquid chromatography with pulsed amperometric detection (HPLC-PAD) and liquid chromatography-tandem mass spectrometry (LC-MSMS) platforms. Samples were obtained in a clinical study of 69 children from northeastern Brazil. We assessed the intra- and inter-assay precision and recovery by HPLC-PAD of several sugars at the concentration of 0.1 mM: glucosamine, mannitol, melibiose and lactulose, inositol, sorbitol, glucose and lactose. The precision variation was <2.5% for intra-assay and <4.1% for inter-assay for all sugars. The percent of recovery of all sugar probes ranged from 86-104%. HPLC-PAD and LC/MS showed similar precision, variation, and slightly higher percent of recovery with HPLC-PAD method. The accuracy of lactulose and mannitol (0.01, 0.03, 0.1 and 1 mM) concentrations measured by HPLC-PAD and LC/MS showed highly significant (*P<0.0001*) linearity with r^2 > 0.9940 for both sugars. Urine samples in the clinical study showed high precision, recovery and accuracy, as well as highly significant correlations (P<0.0001) for percent (excretion/ingestion) of lactulose, mannitol. L/M ratio excretion in urine by HPLC-PAD and LC/MS. Both analytical platforms are accurate to assess lactulose and mannitol in urine samples to evaluate intestinal permeability in field studies in children.

Key words: Intestinal permeability, Barrier Function Markers, Enteropathy, Massas spectrum.

14:15 -14:30

Ginger metabolites inhibit *Staphylococcus aureus* virulence factors

James A Silva, Federal University of Sergipe
Departamento de Farmácia, Universidade Federal de Sergipe, Lagarto, Sergipe – Brasil

Ginger is a medicinal herbal that has been used since antiquity in countries as China, India and Greece, as food spice and remedy to treat many diseases. Among others therapeutic actions the ginger presents antioxidant, anti-inflammatory and antimicrobial activity[1]. Strong antimicrobial activities were found for the main ginger compounds (gingerols) against oral pathogens: *Porphyromonas gingivalis, P. endodontalis, Prevotella intermedia*[3] and against *Helicobacter pylori*[4] with MICs ranging from 1.6 to 30 □g/mL. Despite this, there is not much data available regarding the antimicrobial activities of ginger or its metabolites. Antimicrobial resistance is considered a growing global health problem due to the loss of efficacy of the first line of antibiotics. In this way, additional treatment strategies are needed to improve clinical response and to reduce resistance to antibiotics. One of these strategies is the inhibition of the virulence factors produced by the microorganisms. They are important therapeutic targets as they are used as strategy to escape from the host defense system by increasing infection and damage in patients tissues[5,6]. Based on this and the fact that the search for inhibitors of bacteria virulence factors in the *Z. officinale* species has never been studied, the objective of this work was to evaluate the inhibitory potential of the main metabolites against important virulence factors of *S. aureus*: lipases, DNAses and biofilm formation.

1. Semwal et al. Phytochem., v. 117, p. 554, 2015.
2. Bellik et al. Asian Pac. J. Trop. Dis. v. 4(1), p. 40-44, 2014.
3. Park et al. Phytother. Res. 22, 1446–1449.
4. Zhang et al. Modern Food Sci. Technol. 29, 1259–1261.
5. Kiodrowski et al. PLOS ONE, v. 9, n. 4, p. 1-13, 2014.
6. Katrin et al. J. Infect. Disease, v. 210, n. 3, p. 473- 482, 2014.

14:30 -15:00	**Leishmaniasis a common endemic disease that seems to be rare and complicated** *Anastácio de Queiroz Sousa*, Federal University of Ceara

15-00 -15:20	**Vaccine for treating leishmaniasis in dogs can help curb spread to humans** *Adam L Lima*, Ph.D. Student, State University of Ceara Visceral leishmaniosis (VL) is one of the three main presentations of leishmaniasis. It is a severe disease in humans, causing fever, hepatosplenomegaly and if untreated death. In Brazil, it is caused by dimorphic protozoa *Leishmania infantum* being transmitted by the bite of its biological vector, an infected sand-fly (*Lutzomyia longipalpis*) and it is one of the five countries that concentrate more than 90% from VL cases in the world. Brazil has characteristics that provide a unique epidemiology: climate, social-economics, and dogs as the main urban reservoirs. Dogs affected by *L. infantum* can host the parasite for years without clinical signs of canine visceral leishmaniosis (CanL) tricking the diagnostic tests for several months, being able to spread this zoonotic disease. Several papers from Brazil stated that human cases are related to the incidence of CanL in endemic urban areas. The current Brazilian eradication and control of visceral program have not achieved success in the past decades. New tools must be studied and developed to reduce cases of VL including actions on the reduction on canine cases to reduce population risk. In this scenario, one vaccine has been tested to prevent disease and clinical presentation of CanL in already infected animals. This concept is accepted by those who show that non-clinical CanL is less likely to infect a sand-fly than a severe CanL. In our study, we show that vaccination in infected dogs reduced the risk of progression to clinically overt leishmaniasis by 25% in asymptomatic dogs (RR: 1.33 95% C.I. 1.009–1.786 p-value: 0.0450). Receiving vaccine vs. placebo reduced all-cause mortality in younger asymptomatic dogs by 70% (RR: 3.19 95% C.I.: 1.185–8.502 p-value = 0.0245). and that vaccination in infected-healthy dogs can be a tool for Leishmania control. Following this data, we observed that comorbid infections induce progression of visceral leishmaniasis and started a study in Fortaleza to determinate the Relative Risk (RR) of acquiring CanL being infected by tick-borne disease. This epidemiology-focus study will provide us with more science on the control of CanL, consequently providing us tools to decrease VL.

15:40 -16:10

GUANYLIN AND UROGUANYLIN: RECENTS ADAVANCES ON KIDNEY AND GUT PHYSIOLOGY

Manassés Claudino Fonteles (Fonteles, MC); Lucília Maria Abreu Lessa (Lessa, LMA)

In the early 1980s, studies with, E coli thermostable toxin (Sta), demonstrated that in addition to promoting secretory diarrhea, it also promoted several renal effects such as diuresis, natriuesis and caliuresis. Activation of a GC-C receptor was involved in these mechanisms of STa, so the investigators came to suspect an endogenous ligand for this receptor could promote similar effects of STa. Some years later, the guanylin peptides were identified as the endogenous ligand for this receptor. Our research group was one of the first to suggest this hypothesis, when we studied the eftect of E. coli Sta enterotoxin and 8-bromo cyclic gmp in perfused rat kidneys, where we observed intense natriuresis and caliuresis.

The Guanylin peptides have less aggressive effects than those of STa, and are produced in the intestine in response to an increase in the luminal concentration of NaCl and promote renal effects in the control of electrolytic homeostasis. It has therefore been postulated that they are part of a gut-renal axis for the control of hydroelectrolytic homeostasis. Uroguanilin is the main peptide of this family of molecules, having more expressive effects than the others.

Our group has demonstrated along the last decades several findings about uroguanylin and guanylin renal effects and signaling mechanisms. Our data, by using isolated perfused kidney method showed that Uroguanylin and Guanylin elicit direct effects on the kidney by increasing Na^+, Cl^- and K^+ excretion in a time- and dose-dependent fashion. Their mechanisms of action may be both GC-C dependent and independent mechanisms (other GC receptors). Synergism between ANP and UGN/Guanylin has also being demonstrated. Besides, our findinds demonstrated that GC-C, but not GC-A receptor is upregulated by high salt diets.

Moreover, by using in vivo stationary technique we have demonstrated that uroguanylin (UGN) significantly inhibits NHE3-mediated bicarbonate reabsorption, which may leads to natriuresis. These effects were associated with increase on NHE3 phosphorylation (inhibition) status in proximal tubule apical membrane, probably by a crosstalk mechanism between the cGMP and cAMP interaction. Our microperfusion studies also had shown that Uroguanylin inhibits H^+-ATPase activity in distal tubules by a PKG dependent pathway, and it was observed an increase in potassium secretion in distal segments perfused with the peptide, which was linked to MAXI-K channel activation.

Over the past years much has been discovered about the regulation of renal fluid and electrolyte excretion. However, there are still many mechanisms involved in this process, that requires better understanding. During the last decades, we have published some substantial data about the renal effects and signaling mechanisms of Uroguanylin and Guanylin, which corroborate with the hypothesis that they play a role in Fluid and Electrolyte Balance and may interact with ANP.

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16:10 - 16:30

Modeling diet and pathogen specific enteropathy and diarrhea: a novel murine model of *Shigella flexneri* infection

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Recent large multicenter studies have re-evaluated enteropathogens that cause diarrhea, 'silent' growth impairment and cognitive deficits in children from developing countries. Our group has been modelling these infections (*Shigella flexneri*, *Campylobacter jejuni*, enteroaggregative *E. coli* - EAEC, enteropathogenic *E. coli* - EPEC, enterotoxigenic *E. coli* - ETEC, Cryptosporidium and Giardia) in mice using specific diets and antibiotics. *Shigella* shows the highest overall diarrhea and poor growth burdens in children. We present a novel murine model of *S. flexneri* infection, in which we also investigated the role of zinc deficiency (ZD) and tested the efficacy of the vaccine CVD 1208S-122 (attenuated *S. flexneri* 2a strain). C57BL/6 mice fed either standard chow or ZD diets were pretreated with an antibiotic cocktail and received *S. flexneri* strain 2457T orally. Antibiotic pre-treated mice showed higher *S. flexneri* colonization than non-treated mice, with ZD promoting persistent colonization. Infected nourished mice showed diarrhea, significant weight loss, intestinal epithelial damage and increased intestinal inflammation with subsequent recovery, while infected ZD mice had similar, but chronic outcomes. Urine metabolomics showed biochemical changes related to energy and inflammatory responses in nourished mice, but not in ZD. Zinc supplementation reduced intestinal inflammation and stool shedding in ZD infected mice. For the vaccine testing, C57BL/6 mice received the vaccine intranasally in three weekly doses prior to infection with *S. flexneri* four weeks later. Vaccinated mice showed significant protection against diarrhea and weight loss induced by *S. flexneri* and transiently reduced stool shedding. Serum anti-LPS *Shigella* IgG antibodies levels were increased in the vaccinated mice when compared to infected unvaccinated mice. In conclusion, young antibiotic-treated mice provide a new model of oral *S. flexneri* infection, with ZD promoting persistent infection. These findings support the CVD 1208S-122 vaccine to be further tested in clinical trials of protection against *S. flexneri*.

16:30 - 16:50	**Molecular diagnostics of enteropathogens association with subclinical and clinical infections in the MAL-ED birth cohort study** *Alexandre Havt*, Federal University of Ceara A multisite birth cohort study (MAL-ED) was conducted from 2009 to 2014 by the efforts of 8 different countries to evaluate, the etiology, burden and associated clinical characteristics of childhood diarrhea. However most of the diagnostic methods used were not molecular data-based. The same Diarrheal and non-diarrhea stool samples from MAL-ED were reanalyzed using a TAQman array card (quantitative PCR) developed to simultaneously diagnose 29 enteropathogens among bacteria, virus and protozoa. Children aged 0 to 2 years were from Dhaka, Bangladesh; Vellore, India; Bhaktapur, Nepal; Naushero Feroze, Pakistan; Venda, South Africa; Haydom, Tanzania; Fortaleza, Brazil; and Loreto, Peru. The quantitative data were used to calculate population-level pathogen-specific attributable burdens, derived stringent quantitative cutoffs to identify etiology for individual episodes, and created etiology prediction scores using clinical characteristics. We analyzed 6625 diarrheal and 30 968 non-diarrheal surveillance stools from 1715 children. Overall, 64.9% of diarrhea episodes could be attributed to an etiology by quantitative PCR compared with 32.8% using the original study. Viral diarrhea was more common than bacterial and parasitic diarrhea. Ten pathogens accounted for 95.7% of attributable diarrhea: shigella, sapovirus, rotavirus, adenovirus 40/41, enterotoxigenic *Escherichia coli*, norovirus, astrovirus, *Campylobacter jejuni*or *C. coli*, cryptosporidium and typical enteropathogenic E coli. The amount of 86.2% of the attributable incidence for Shigella was non-dysenteric. A prediction score for shigellosis was more accurate (sensitivity 50.4%), and specificity (84.0%) than current guidelines, which helped us to recommend treatment only of bloody diarrhea to cover Shigella sensitivity (14.5%) and specificity (96.5%). Quantitative molecular data improved the management of children diarrhea of low-income settings showing that quantitative PCR better estimates childhood diarrhea pathogen-specific burdens than regular microbiology methods. Most predominant causes were viral diarrhea, with an important burden of sapovirus. However, Shigella presented the highest overall burden and high prevalence at the second year of life.

16:50 - 17:30	**Internationalization of the UFC in translational and epidemiological research in neurogastroenterology** Aldo AM Lima, Alexandre Havt, Armênio A Santos, José X Neto, Marcellus HLP Souza, Pedro JC Magalhães, and Pedro MG Soares Center of Biomedicine and INCT-Biomedicine, Faculty of Medicine, CAPES_Print International Program, Federal University of Ceara, The internationalization of research in Biomedicine at the Faculty of Medicine (FM), Federal University of Ceará (UFC) began 40 years ago with the University of Virginia, USA (UVa) through Prof. Aldo AM Lima, when isolating heat-stable enterotoxin (STa) from *Escherichia coli* from diarrhoeal stools of children from Pacatuba, CE. The analysis of STa action in the perfused kidney led to the discovery of an endogenous ligand for the guanylate cyclase C (GC-C) receptor, guanyline and uroguanylin. We then proceed to study the dynamics of enteric infections, malnutrition, microbiome, genome, metabolome and environmental enteropathy, generating knowledge about the morbidity and etiologies of enteric infections as well as their impacts on the growth and neuro-cognitive development of children from poor communities. This resulted in the MAL-ED (Malnutrition Enteric Disease) research network funded by the Bill & Melinda Gates Foundation. This interchange led to the development and application of a molecular biomarker test, carbohydrates, to evaluate the intestinal barrier function, adopted by MAL-ED network. Via INCT-Biomedicine (http://inct.cnpq.br/web/inct-ibisab) we created the Center of Chromatographies and Mass Spectrometry, FAMED, UFC with capacity to measure analytes, nutrients, metabolites and drugs with accuracy, sensitivity and specificity. The collaboration INCT-Biomedicine and Chemical Department at UFC & Imperial College London, UK, is now expanding this expertise in microbiomics and its functional translation to human health. The UFC has thus formed more than 60 researchers, whether postdoctoral, graduate students or technicians, and publishing more than 151 scientific papers. We also established an exchange (Sciences without Borders), with Prof. Daniel Sifrim, Queen Mary University of London, UK, validating the concept of insufficiency of the crural diaphragm in gastroesophageal reflux disease (GERD), which can be improved by respiratory physiotherapy. Studying the esophageal permeability of rodents and patients with GERD, we showed the protective role of natural products (cashew gum) against acid exposure. The UFC thus formed more than 8 researchers and published 15 scientific papers. Via also Science without Borders, we launched an exchange with Prof. David Criddle, University of Liverpool, UK, studying the dynamics of intracellular calcium of pancreatic acini under confocal microscopy. The UFC formed 6 researchers and published 2 scientific papers. We will now expand our international exchange to Imperial College London, UK and to University of Oslo, Norway (Prof. Akhtar Hussain, Visiting Scholar in FM-UFC).

SUMMARY

Saturday, November 24th

9:00 - 9:30	**Understanding the role of gut microbiota in childhood acute malnutrition** Tahmeed Ahmed MBBS, PhD Senior Director, Nutrition & Clinical Services Division, icddr,b Professor, Public Health Nutrition, BRAC University Dhaka, Bangladesh tahmeed@icddrb.org Severe acute malnutrition (SAM) and moderate acute malnutrition (MAM) are major causes of childhood mortality worldwide, affecting 17 million and 52 million under-five children. Although treatment of SAM is available, its coverage is still poor and even among those treated, relapse is not uncommon. Recent evidence suggests a role for gut microbiota in the pathogenesis and the response to treatment of SAM. We studied the gut microbiota of children suffering from severe and moderate acute malnutrition in Bangladesh. Children with SAM were studied during the acute phase, nutritional rehabilitation and follow up in icddr,b Hospital, Dhaka. During the nutritional rehabilitation phase, the children were randomized to either ready-to-use therapeutic food (RUTF) or a combination of local diets (*khichuri* and *halwa*). Children with MAM were randomly selected from a birth cohort in a slum settlement and so were healthy controls. Gut microbiota were identified using 16S rRNA datasets generated from monthly fecal samples obtained from the healthy control children. 'Relative microbiota maturity index' and 'microbiota-for-age Z-score' were developed from a model developed from the age-discriminatory bacterial species identified in the healthy and acutely malnourished children. The index and the Z-score compare maturation of an acutely malnourished child's fecal microbiota relative to healthy children of similar chronological age. Our results indicate that SAM is associated with relative immaturity of the gut microbiota. Moreover, treatment with either RUTF or the local diets is associated with incomplete recovery of the gut microbiota. Similarly, MAM is also associated with immaturity of the gut microbiota, the degree of immaturity correlating with the severity of malnutrition. The immaturity of the gut microbiota in acute malnutrition has an important role in metabolic and immunologic perturbations that result in suboptimal response to therapeutic measures.

9:30- 9:45

Clinical impact of virulence-related genes from *Shigella/Escherichia coli* enteroinvasive pathotype infection in children from Semiarid Brazilian region

Mariana Bona. PhD student in Medical Science - Federal University of Ceara

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Shigella/Enteroinvasive *Escherichia coli* (EIEC) pathotype is a major enteropathogen associated with diarrhea and malnutrition in children from developing countries. This study aimed to correlate *Shigella*/EIEC virulence-related genes (VRGs) with clinical symptoms and nutritional status in children from the Brazilian semiarid region. We designed a case-control study of community diarrhea in six cities of the Brazil semiarid region with 1200 children aging 2-36 months. Standardized questionnaire was applied for collecting sociodemographic, nutritional status and clinical information of the children. DNA samples were extracted from stools and diagnosed for *Shigella*/EIEC using PCR-based approaches. Positive samples were tested for 28 VRGs using four multiplex PCRs. Intestinal inflammation was determined by measuring fecal myeloperoxidase (MPO). *Shigella*/EIEC pathotype was detected in 5% of the children and was significantly associated with diarrhea. The genes *sen* (encoding *Shigella* enterotoxin 2), *ipgB2*, *ipgB1* (both encoding type 3 secretion system-T3SS effectors that modulate actin filament), and *ospF* (encoding a T3SS effector involved in suppression of host responses) were further associated with diarrhea in *Shigella*/EIEC positive children. Among children presenting diarrhea, *virA* gene (encoding a T3SS effector that promotes microtubule destabilization) was associated with fever, while *virB* (encoding a major transcriptional activator) was associated with low height-for-age z-score. In addition, these VRGs were associated with increased fecal MPO. These data reinforce the impact of *Shigella*/EIEC on diarrhea in children from Brazilian semiarid region and highlighted the contributions of specific virulence genes for its pathobiology.

Keywords: *Shigella*/EIEC pathotype; virulence genes; child diarrhea

9:45-10:00

Antimicrobials resistance of enteroaggregative *Escherichia coli* strains isolated from nourished and malnourished children from Fortaleza, Ceara, Brazil

Marília SMG Amaral, PhD student in Medical Microbiology - Federal University of Ceara

Marília Silveira Maia Gurgel do Amaral; Ana Karolina Silva dos Santos; Herlice do Nascimento Veras; Mariana Duarte Bona; Geovana Mesquita Sampaio; Thiago Miranda de Freitas; Mara de Moura Gondim Prata; Pedro Henrique Quintela Soares de Medeiros; Natália Kelly Fernandes de Menezes Melo; Alexandre Havt; Aldo Ângelo Moreira Lima.

Enteroaggregative *Escherichia coli* (EAEC) is an enteric pathogen which is associated with different clinical outcomes, from subclinical to clinical infections. EAEC shows high prevalence, especially in children under five years of age and there is a lack of data of antimicrobials resistance at the primary care and community-based study. This case-control study aimed to determine the antimicrobials resistance of EAEC strains among cases (malnourished children) and controls (nourished children) at Fortaleza-CE, Brazil. The study included 402 children aged 6-24 months, with 201 cases and 201 controls. Cases were defined as children with weight-for-age z score (WAZ) < -2, while controls were defined as WAZ > -1. Fecal samples were collected and evaluated for EAEC diagnosis genes (*aaiC* and *aatA*) by polymerase chain reaction (PCR). The positive samples were tested by antimicrobial susceptibility test (AST) by diffusion disc for 12 antimicrobials: ceftriaxone, ertapenem, aztreonam, ceftazidime, ceftazidime/clavulanic acid, cefoxitin, ciprofloxacin, sulfamethoxazole/trimethoprim, ampicillin, ampicillin/sulbactam, gentamicin and azithromycin (Etest), three samples were insufficient for the AST. Multidrug resistance was defined as resistance against three or more antibiotics from different groups. EAEC was diagnosed in 23.3% (97/402) of the population, 55 cases and 42 controls. The phenotypes of resistance against at least one drug and multidrug resistance were 63.83% (60/94) and 22.3% (21/94), respectively. The highest resistance rate was for ampicillin - 58.5% (55/94), followed by the sulfamethoxazole-trimethoprim combination - 55.3% (52/94). In contrast, moderate and low resistance rates were against azithromycin with 24.4%, (23/94) and ampicillin-sulbactam with 7.4% (7/94), respectively. One sample was positive for extended spectrum beta-lactamase (ESBL). There was no statistical association between antimicrobial resistance and malnutrition (P=0.287, OD=1.609, IC 95% 0.6873-3.765). In conclusion, these results showed the high prevalence of EAEC infections in children and present high rates of antimicrobials resistance at Fortaleza-CE, Brazil.

10:00-10:15

Use of carbohydrate biomarkers by LC-MS/MS for assays of intestinal permeability

Lyvia Maria V C Magalhães, Phd student in Medical Science
Federal University of Ceara(UFC).

A wide variety of biomarkers have been proposed to analyze changes in intestinal permeability. The lactulose / mannitol test (L / M) consists of a non-invasive test involving the oral administration of both carbohydrates, followed by measurement of these compounds in the urine testing by mass spectrometry coupled with liquid chromatography (LC-MS / MS) provides the ratio of lactulose and mannitol in the urine simultaneously and is one of the most sensitive techniques available. This study aimed to perform analytical validation of an LC-MS/MS method for L/M test. Lactulose, mannitol and sorbitol solutions at the concentrations of 50, 100, 500, 1000, 1500 and 2000ng / ml were used for the validation of the test, using Acetonitrile (ACN) and H2O (50% / 50%) as diluent. The chromatographic separation was performed using the HILIC-ZIC® column, in a flow of 300µL / m and an injection volume of 20µL. The ionization was made by negative mode electrospray and the time for each analysis was 10 minutes. The steps of validation included: Calibration, MRM (Multiple Reaction Monitoring), FIA (Flow Injection Analysis), Method Acquisition and Quantification. Linear correlation coefficients were greater than 99%. The inter-experiment analytical recovery of the sugars at the concentration of 500ng / mL was: lactulose (6.62%, 2.34%, 8.60%), mannitol (8.75%, 4.81%, 4.32%) and sorbitol (8.73%, 6.16%, 15.81). The limits of detection (LOD) for lactulose, mannitol and sorbitol were 63.07ng / mL, 123.00ng / mL and 200ng / mL, respectively, while the limits of quantification (LOQ) for lactulose, mannitol and sorbital were 210.23, 410.02 and 666.66 ng / mL, respectively. The results obtained are satisfactory and showed that the proposed method is suitable for dosing lactulose, mannitol and sorbitol, allowing distinction of molecules with the same molecular mass, such as mannitol and sorbitol.

10:15- 10:30

Campylobacter jejuni virulence genes and immune-inflammatory biomarkers association with growth impairment in children from Northeastern Brazil

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Campylobacter spp. have been associated with anthropometric Z-score decrements, but the role of specific virulence genes associated with these outcomes has not been explored. This study aimed to investigate whether specific *Campylobacter jejuni* virulence-related gene and immune-inflammatory biomarkers are associated with malnutrition in children from Northeastern Brazil. A case-control study was performed in Fortaleza, Brazil. Children aging 6–24 months were characterized as malnourished (cases) if weight-for-age Z-score (WAZ) ≤ 2 and as nourished (controls) if WAZ ≥ 1. DNA samples were extracted from stools and screened for *C. jejuni/coli* by real-time PCR. A subsequent *C. jejuni*-specific PCR was employed and positive samples were evaluated for 18 *C. jejuni* virulence genes by using four multiplex PCRs. *C. jejuni* was detected in 9.71% (33/340) of the children's samples, being 63.63% (21/33) from nourished and 37.37% (12/33) from malnourished children. The *cadF*, *iamA*, *cheW*, and *sodB* genes were the most frequent genes (100%, 90.9%, 87.9%, and 75.8%, respectively), while some others (*ceuE*, *jlpA*, *pldA*, and *pVir*) showed low rates (all below 6%). Malnourished children were significantly associated with infection with *C. jejuni* strains lacking *cdtB* gene (active subunit of cytolethal distending toxin) and harboring *flgE* gene (flagellar hook protein). These strains were also associated with children presenting increased serum SAA and sCD-14, but decreased IgG anti-LPS. These data reinforce the impact of *Campylobacter jejuni* infection on children without diarrhea and highlight the contribution of a specific virulence gene profile, *cdtB*(−)*flgE*(+) and increased systemic response in malnutrition children.

10:50- 11:10

Virulence related genes and co-enteropathogens associated with clinical outcomes of enteropathogenic *Escherichia coli* infections in children from Brazilian semiarid region: a case-control study of diarrhea

Ana Karolina Silva dos Santos, PhD student in Medical Microbiology - Federal University of Ceara

Ana Karolina S. Santos[1], Pedro Henrique Q. S. de Medeiros[1], Mariana D. Bona[1], Mara M. G. Prata[1], Marília S. M. G. Amaral[1], Herlice N. Veras[1], Rafaela C. Pankov[1], Samilly A. Ribeiro[1], Paloma A. Cavalcante[1], Thiago M. Freitas[1], Rafhaella D. G.Gondim[1], Daniel M. N. de Oliveira[1], Natália K. F. M. Melo[1], Alexandre Havt[1], Aldo A. M. Lima[1].
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Background: Enteropathogenic *Escherichia coli* (EPEC) is a major cause of diarrhea-related deaths in children from developing countries and presents high genetic variability. This study aimed to characterize EPEC infections regarding diarrhea and nutrition-related outcomes in association with microbial virulence related-genes (VRGs) distribution and copathogens in children from the low-income Brazilian semiarid region. *Methods:* A cross-sectional case-control study of diarrhea was conducted in 1,191 children aged 2-36 months old from the Northeast Region of Brazil. Stool samples were collected from each child, as well as clinical, epidemiological and anthropometric data. A broad molecular evaluation of enteropathogens from the stools was performed and EPEC positive samples were further investigated for 18 VRGs using five multiplex-PCRs. *Results:* EPEC was detected in 28.2% (337/1,191) of the study population in similar proportions among cases and controls. Typical EPEC (tEPEC) infections were more associated with diarrhea than atypical EPEC (aEPEC), while aEPEC infections presented higher prevalence. Total EPEC infections were associated with decreases on height-for-age Z-score (HAZ), when compared with children without EPEC infections. In addition, aEPEC infections were associated with lower weight-for-height Z-score values than tEPEC infections. The VRG *ler*, a negative regulator of the locus of enterocyte effacement (LEE), was associated with absence of diarrhea within aEPEC positive children; *espB*, a major component of the type 3 secretion system (T3SS), was associated with diarrhea within tEPEC positive children; the presence of pro-colonization genetic markers - the combination *cesT*(+)*espP*(-) and the *map gene* - was associated with undernutrition; and *Campylobacter* spp., Norovirus and enteroaggregative *E. coli* (EAEC) coinfections were associated with increased clinical severity in EPEC infected children. *Conclusion:* These data identified tEPEC association with diarrhea cases and specific VRGs of EPEC (*ler*, *espB, cesT* and *map* genes), and *Campylobacter* spp., Norovirus and EAEC as major contributors of diarrhea and undernutrition in children from the the low-income Brazilian semiarid region.

11:10-11:40	**Enteropathogens association with gut function, immune-inflammatory responses and growth development: the MAL-ED birth cohort study** Aldo AM Lima, Alexandre Havt, Margaret N Kosek, Elizabeth T Rogawski, James A Platts-Mills, Tahmeed Ahmed, Richard L Guerrant, Eric R Houpt and the MAL-ED Network Investigators Federal University of Ceará, University of Virginia and The MAL-ED Network Instituitions Diarrhea diseases mortality and morbidity remain an important worldwide public health problem. Recent work have focus on the host responses to frequent enteric infections which alter the gut in several ways that interfere with health status of the host even in the absence of diarrhea. Impaired linear growth affects more than 30% of children in low-resource settings. This chronic undernutrition is associated with high mortality, poor cognitive and physical development, as well as school performance in children under five years old. We evaluate the role of enteropathogens and gut function biomarkers in growth faltering up to 2 and 5 years old children across eight countries sites in the etiology, risk factors and interactions of enteric infections and malnutrition and the consequences for child health and development (MAL-ED) birth cohort study. In order to evaluate the association of intestinal inflammation and linear growth failure we used the following biomarkers, neopterin (NEO), alpha-anti-trypsin (AAT), and myeloperoxidase (MPO) in asymptomatic stool samples from 537 children. These tests results were significantly associated with impaired linear growth. The disease score developed using these biomarkers showed that children with highest score grew 1.08 cm less than children with lowest score over the 6-month period following the tests and controlling for diarrheal diseases. Enteropathogens were evaluated with these tests, including also urinary lactulose:mannitol test (L:M ratio) and plasma alpha-1-acid glycoprotein (AGP). *Campylobacter* spp., enteroaggregative *E. coli* (EAEC) were associated with higher concentrations of MPO and AAT, while *Giardia* was associated with lower concentrations of MPO, NEO and ATT. L:M ratio were usually higher, specially with Campylobacter spp, atypical enteropathogenic *E. coli* (aEPEC), enteroinvasive *E. coli* (EIEC), *Shigella* spp, *Cryptosporidium* spp. and *Giardia*. The rarer pathogens such as EIEC, *Shigella*, *P. shigelloides* and *Y. enterocolitica* were also associated with increased MPO and decreased NEO concentrations. These models assumed additive effects of pathogens as more than one pathogen was detected. Recent evaluation of enteropathogens using new molecular methods showed association of *Shigella*, EAEC, *Campylobacter* spp. and *Giardia* with impaired linear growth, which was sustained during the first 2 years of life, and in some cases up to 5 years of life. These data showed consistent association of enteropathogens with gut dysfunction and subsequent linear growth impairment in children from the MAL-ED birth cohort study. In the perspective of this new quantitative qPCR approach to determine the etiologies of subclinical and clinical enteric infection plus close stool samples collected to biomarkers tests available, we will refine the association between enteropathogens, gut functions and damage biomarkers, local and systemic inflammation and growth development.