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Date: Friday, March 4, 2016
Time: 12:45-14:15
Room: Hall 3 (Posters & Exhibition)

Improvement of DNA extraction from human biopsies for a microbiome metagenomic approach

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Background: The last few years have seen an accelerated expansion of microbiome studies rendering a clear picture of its major role in human health and disease. Several international studies have shown that the human gut microbiome consists of four enterotypes; however, these studies have not included Latin American population and have not analyzed differences according to diet that can be important microbiome modifiers. On the other hand, few studies have addressed the eye microbiome and microbiome diversity is unknown. In Mexico, Irritable Bowel Syndrome (IBS) and Dry Eye Syndrome are very common disorders and it has been considered that the microbiome is implicated in both of them. Therefore, it is highly important to study the microbiome in these disorders in our population.

Methods & Materials: Microbiome composition will be determined by next generation sequencing. Sample standardization is reported herein: DNA extraction was performed from 4 colonic and 1 conjunctiva biopsies, collected at the HGM and the IOCV, respectively. The samples were preserved in RNA later (Ambion) at room temperature and were processed within 3 h of collection. Tissue disruption was conducted with proteinase K enzymatic treatment in buffer AL (QIAamp DNA mini Kit, Qiagen) at 56°C for 90 min. The extraction was carried out according to the manufacturer's protocol. The obtained DNA was analyzed with NanoDrop2000 (Thermo Scientific) for DO 260/280nm ratio and for DNA concentration estimation. Also, an agarose gel electrophoresis was run to visualize the DNA integrity.

Results: This protocol allowed us to obtain an average DNA quantity of 6μg from the colonic biopsies and 4μg per conjunctiva-biopsy with a DO 260/280nm greater than 1.8 and no visible DNA degradation base on literature.

Conclusion: The data suggest that this protocol is suitable for obtaining genomic DNA from colonic and conjunctival biopsies for next generation sequencing.

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Urinary Neutrophil Gelatinase-Associated Lipocalin (NGAL) might be an independent marker for anticipating scar formation in children with acute pyelonephritis

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Background: Urinary tract infections (UTIs) are the most serious common bacterial infections among young children. It may affect kidneys that classified as acute pyelonephritis (APN) and may lead to renal parenchymal involvement and scarring with high prevalence rate (15-60%) among children. This study aimed to assess the urinary concentrations of neutrophil gelatinase-associated lipocalin (NGAL) in patients with APN to diagnose those with potency to scar formation

Methods & Materials: Children who were admitted with a diagnosis of APN were enrolled and divided into 2 groups; APN with scar and APN without scar. Urinary levels of NGAL and its ratio to creatinine (Cr) levels were measured in the acute phase of infection. A receiver operating characteristic (ROC) curve was generated to allow calculation of cut-off values

Results: Sixty-one children were enrolled across the 2 groups: group 1 consisted of 16 patients (all female); group 2, 38 children (36 female and 2 male). Urinary levels of NGAL were significantly higher in APN with scar than in APN without scar ($p = 0.037$). For comparison of groups 1 and 2, the cut-off values were measured as 7.32 ng/mL, sensitivity; 81.3% and specificity;66%.

Conclusion: Evaluation of urinary NGAL levels may help us to identify children with APN who are at risk of developing renal scarring

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Elucidation of the role of non-structural viral protein (W) of Newcastle disease virus

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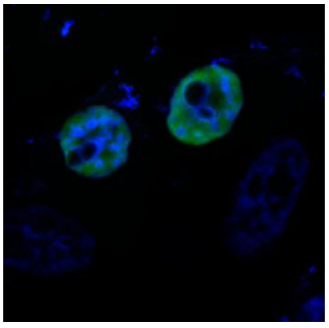
Background: Newcastle disease (ND) is a highly contagious disease of birds infecting more than 250 avian species across the world.

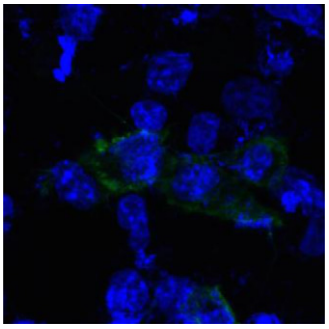
In India, ND is an economically important and endemic poultry disease. ND is mostly fatal in infected flocks and the currently available vaccines are ineffective. It is caused by Newcastle disease virus (NDV) belonging to family *Paramyxoviridae*. NDV is an enveloped virus carrying a negative-sense, single-stranded RNA genome with six genes arranged in tandem coding for six structural proteins: nucleocapsid (NP) protein, phosphoprotein (P), matrix (M) protein, fusion (F) protein, hemagglutinin-neuraminidase (HN) protein and large polymerase (L) protein. Additionally NDV expresses two non-structural (NS) proteins, V and W, by co-transcriptional (mRNA) editing of P gene via polymerase stuttering mechanism. Insertion of a single, non-templated G residue results in V protein and insertion of two G residues leads to W protein. These two NS proteins share common N-terminal with P protein and vary at their C-terminal. The NS proteins are not packaged in the virion but expressed only when the virus is actively replicating in the host. While the role of V protein has been extensively studied and reported to be anti-interferon, the function of W protein remains elusive. Our current study is attempted to answer the following questions: Could W mRNA and/or W protein be key factor(s) for viral replication and transcription and/or help evade host immune response?

Methods & Materials: We performed sequence analysis of W protein by bioinformatics. We conducted localization and mutation studies on W protein.

Results: Our preliminary study on sequence analyses of W protein revealed a stretch of basic amino acid residues in the C terminal indicating probable nuclear localizing signal. Our subcellular localization studies confirmed localization of P and V in cytoplasm while W protein predominantly localized in nucleus. By mutation studies we have identified the amino acid residues responsible for nuclear localization of W protein.



Vero cells were transfected with HA tagged W, a nonstructural protein of Newcastle Disease Virus. Cells were fixed and permeabilized 1 day later. Confocal image here shows the nuclear localization of W. Nuclei are stained with DAPI in blue.



Vero cells were transfected with HA tagged V, a nonstructural protein of Newcastle Disease Virus. Cells were fixed and permeabilized 1 day later. Confocal image here shows the expression of V in cytoplasm. Nuclei are stained with DAPI in blue.

.....GAHGRAPKRGTTNVRLNSREVNPA
AETVRKDRRTKSRPPLETRAQTRTQH
IMDNGRSHNYQLVQPLMLSDQGRAKT
IPLYLRIMSSHL.

Alignment of aminoacid sequence at the C-terminal domain of W protein showing the basic amino acid rich residues (Bolded and underlined) as the probable the nuclear localizing signal.

Conclusion: The stretch of basic aminoacid residues at the C terminal region of W protein is important for its localization into the nucleus. Our future direction is towards understanding the role of W protein in the nucleus.

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Community acquired Staphylococcus aureus infection in previously healthy neonates in Argentina

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Background: Community-Acquired S aureus (CA-*Sa*) resistance are changing in the last ten years in Argentina, increasing methicillin-resistant *S aureus* (MRSA) strains. First case in our neonatal unit was diagnosed in 2006. The objective was analyze clinical, epidemiological, microbiological and outcome features of neonates with CA-*Sa* infection

Methods & Materials: Prospective observational study. We included previously healthy patients (p) $\leq$ 30 days of age admitted in the neonatal unit with CA-*Sa* infection from 2006 to 2014. We defined CA-*Sa* infection based on CDC guidelines.

Results: We included 37 healthy neonates with CA-*Sa* infection. Twenty nine (78%) were born by vaginal labor. Mean gestational age was 39 weeks (r: 35-41), weight 3279 grams (r: 2500-4450). Patients had less than 3 birth hospital days in 95% of cases. Contact with parental soft tissue infections were present in 38%, 65% of them were associated with maternal forunculosis. Nineteen p (51%) were female. Mean age at admission was 18 days (r: 4-30). Thirty four p (92%) had skin and soft tissue involved at admission, with celulitis, bullous impetigo, pustulosis, chest, facial/neck, inguinal and goin abscesses and mastitis. Fifteen p (40%) also had invasive disease: sepsis, omphalitis, osteoarthritis, orbital cellulitis, cerebral abscess, meningitis, liver abscess and necrotizing pneumonia. Only 3p with invasive disease (osteoarthritis: 1p, otitis:1p and pleuropulmonar infection: 1p) didn't have skin and soft tissue manifestation at admission. Positive culture was obtained in 33p (89%) from purulent effusion, and 4 (11%) from blood culture alone. Between 37 CA-*Sa* infections, 27 (72%) were MRSA and 11% were clindamycin resistant too. All patients were treated with antibiotics and in 24p (65%) surgical drainage was performed. Thirty four