SESSION 2: THE ENTEROVIRUSES

Chair: ***Dr. Peter Borus.***

Organizer: ***Mr. James Nyangao.***

ORAL PRESENTATIONS

1. **Identification Of Diarrhoea Causing Viral Agents And Molecular Characterization Of Group A Rotaviruses From Children In Mukuru Slums Nairobi**

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Background: Enteric viruses are recognized as the most important etiologic agents of gastroenteritis the root cause of diarrhea contributing 50 – 70%, and a major worldwide menace responsible for childhood morbidity and mortality. Rotaviruses, noroviruses, enteric adenoviruses and astroviruses have been found to be the major enteric viruses responsible for this diarrheal disease.
Objective: The aim of this study was to investigate the prevalence of these enteric viruses as agents of children diarrhea, in and around Mukuru slums in Nairobi Province, and to molecularly characterize group A rotaviruses.
Methodology: Stool samples were collected at medical laboratories in Reuben medical center and St. Mary's health center from 340 children less than five years of age and suffering from gastroenteritis. They were screened for rotavirus, enteric adenovirus, astrovirus and noroviruses Gi and Gii using RT-PCR. The main human rotavirus genotypes (G1, G2, G3, G4 and G9 and P[8], P[4], P[6], and P[9]) were determined using established and adapted reverse transcriptase PCR-based genotyping methods.
Results: Rotaviruses were detected in 24%, enteric adenovirus - 2.7%, *norovirus Gii* - 1.7% and astrovirus 1.5% of the samples analyzed. Rotaviruses and adenoviruses were detected throughout the period of study, while astroviruses and noroviruses were absent in different months. In rotavirus, three P genotypes were detected: P[8] (60%), P[6] (22.9%), P[4] (11.4) and their relative incidence varied over the 15 months of this study. G1, G9 and G1 were also detected at 40.5 %, 32.4% and 21.6% respectively. G3/ G9 mixed types were detected at 5.4%.

Conclusion: Rotaviruses, enteric adenoviruses, noroviruses Gii and astroviruses are important causes of acute gastroenteritis in Mukuru slums Nairobi, where rotaviruses are the most effective. A combination of three P and G rotavirus genotypes are circulating among children in Mukuru slums, thus monitoring of the genotypic changes should be encouraged for effective future vaccination.

2. **Rotavirus infection in young children and characterization of the isolated strains in Kenya [Detection of G12P[6] strain]**

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Introduction: Rotavirus is the most common cause of severe diarrhea worldwide causing up to 600,000 deaths globally in children under the age of five. Public health interventions to provide clean water and improved sanitation are unlikely to decrease the incidence of this disease, vaccines have been developed as the first line strategy for prevention.

Objective: To determine the burden of Group A Rotaviruses in young children admitted due to

gastroenteritis at Kenyatta National Hospital and characterization of the strains detected.

Methodology: Stool samples were collected from young children under five years admitted due to acute gastroenteritis at Kenyatta National Hospital, Nairobi, Kenya from August 2006 to May 2010. A total of 2,789 stool samples collected during this period were processed and tested for Group A Rotavirus by ELISA. Rotavirus dsRNA was extracted from 285 positive specimens and subjected to reverse transcriptase polymerase chain reaction(RT-PCR). VP7 and VP4 genotyping was performed in a multiplex-PCR reaction with primers as previously described by Gouvea et al., (1990) and Gentsch et al., (1992), respectively.

Results: Rotavirus was detected in over 40% of all the cases. Children between the ages 9 – 12 months being the most affected. Rotavirus serotype G1 predominated (35.4%), followed by G9 (20.4%) then G3(16.8%),G2 (7.4)% then G8 (1.1%) and finally one sample was found to be the emerging G12 strain. It was observed that 17.5% of the strains could not be typed by the primers used. For the VP4 genotypes, P[4] predominated (24.2%) followed by P[6] (17.5%) and P[8] (11.2%) and 2.8% were mixed infections. 42.5% of the strains could not be typed to determine the VP4 genotypes.

Conclusion: Rotavirus infection was found to be over 40% of the cases. G1 serotype was the most abundant strain. For the first time in Kenya, a rotavirus G12P[6] strain was detected. It will be important to introduce a rotavirus vaccine in the immunization program of the country to help in reducing this high burden of rotavirus disease.

SESSION 2: POSTER PRESENTATIONS

1. **Detection and Distribution of Human Enteroviruses In Kenya (July 2006 - July 2011)**

Silvanos Mukunzi[¶], Rachel Achilla[¶], David Schnabel[¶], Janet Majanja[¶], Josephat Mwangi[¶], Meshack Wadegu [¶], Finnley Osuna[¶],James Njiri[¶], Benjamin Opot[¶] Eyako Wurapa[¶] and Wallace Bulimo[¶].

Background: Human *Enterovirus* genus belongs to the family of *Picornaviridae*. They are highly diverse, comprising of over 100 serotypes grouped into five species namely; HEV A, HEV B, HEV C ,HEV D and Poliovirus. Infection with this group of RNA viruses produce a myriad of clinical conditions varying from asymptomatic or mild febrile illness to fatal cases of myocarditis and infections of the central nervous system. Outbreaks of human enterovirus infections with significant morbidity and mortality have been reported worldwide thus necessitating need for laboratory surveillance.

Objective: To describe the human enterovirus virological surveillance in Kenya from year 2006 to 2011.

Methods: Nasopharyngeal swabs were collected and transported to the NIC laboratory on weekly basis for processing as part of routine national surveillance for ARI. Enterovirus detection was performed by isolation in cell culture (RD cell-line) and confirmed using indirect IFA assay.

Results: A total 369 samples representing 2.5% of all assayed samples were confirmed positive for enteroviruses. Out of these, 250 samples (67.8%) tested positive for Pan-enterovirus, 75(20.3%) for Enterovirus, 5 (1.4%) for Echovirus and 39(10.6%) for Coxsackievirus. The detections were observed throughout the study period. Samples testing positive were drawn from all sites comprising the program surveillance network spread across the country.

Conclusion: The study confirms circulation of human enterovirus strains across the country, and which occurs throughout the year as is the case in tropical climates. The high detection frequency of pan-enterovirus may be due to mutations and recombinations occurring in the viruses. Continuous surveillance of these viruses using more sensitive methods such as molecular methods is therefore necessary as the information generated is important in understanding the epidemiology of viral infections and early detections of emergence and spread of new or more pathogenic serotypes such as EV 71 and poliovirus.

2. **Molecular Characterization of Poliovirus among Acute Flaccid Paralysis samples received in KEMRI Polio Laboratory**

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Background: Poliovirus is the pathogen responsible for paralytic poliomyelitis and is classified into three serotypes (PV1, PV2, and PV3) based on the pattern of neutralization by monoclonal antibodies specific for each serotype. The two basic cornerstones of the initiative for poliomyelitis eradication are massive OPV immunization and sensitive poliovirus surveillance. Surveillance consists of investigation of acute flaccid paralysis cases and virological studies of isolates originating from clinical specimens.

Objectives: To classify poliovirus types present in samples suspected to have poliovirus

Methods: Virus Samples: All PCR isolates were from supernatants of isolated clinical samples cultured in L20B cell (a mouse cell line) that were suspected to contain poliovirus.
PCR procedure: The viral RNA in the cell culture supernatant was converted to complimentary DNA in a reverse transcription PCR step then amplified in a conventional PCR reaction using Taq polymerase. PCR products were resolved by gel electrophoresis. Primers used had specificity for the enterovirus group and Sabin type-specific for each of the three serotypes. This resulted in characterization of poliovirus isolates and serotype identification of the isolates.

Results: 140 samples were characterized using PCR, all the samples tested positive for vaccine derived poliovirus (Sabin)-among them 19 were sabin type 1, 61 were sabin 2 and 55 were serotype 3. Eight of the samples were a combination-sabin (1+3) were 3, sabin (2+3) were 4 and sabin (1+2) was 1. Among the samples received 6 samples were from Kenya, 57 were from Somalia and 77 were from Southern Sudan.

Conclusion: The reported data indicate that vaccine-derived viruses may make their way through narrow breaches and evolve into transmissible pathogens even in adequately immunized populations like Kenya as long as the neighboring war torn areas like Somalia and southern Sudan still report them.

3. **Molecular genotyping of rhinovirus in Kilifi and the role of the identified serotypes in the epidemiology of the common cold**

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Background: Human rhinoviruses (HRV) are the most frequent cause of the common cold and are associated with chronic obstructive pulmonary disease exacerbation, asthma exacerbation, and wheezing in children. HRVs are classified in the genus *Enterovirus* of the diverse family *Picornaviridae*. Although data on the epidemiology and impact of HRV is available in developed countries, little data is available for developing countries. The epidemiology of HRV is complex and up to 20 strains of HRV can circulate throughout a community during a single season. Furthermore, the prevailing HRV strains differ significantly from place to place and from season to season.

Objective: Investigate the HRV species circulating in Pingilikani, 2009, an out-patient setting, in Kilifi District.

Method: Nasopharyngeal flocked swabs were collected from children aged 1month to 9 years presenting with upper respiratory tract infection

(URTI) between Jan-April 2009. Of 299 samples, 79 were determined to have HRV by real-time RT-PCR. For these HRV positives, RNA was extracted, PCR carried out on the VP4/ VP2 junction, the resultant products sequenced and analysed using BioEdit and MEGA5 softwares. Statistical analysis was done in STATA version 11.

Results: The sequencing success was 69% (54/79), with 31% (25/79) being un-typed. All three species of HRV were identified and distributed in the proportions 39% (21/54) HRV-A, 19% (10/54) HRV-B and 43% (23/54) HRV-C. The study found the proportion of HRV-B 19%, a significant higher proportion compared to the KDH study which was 5% (exact = 0.003, 95% CI 1.6-6.8). A few viruses from species A and B clustered closely with strong bootstrap values >90%

Conclusion

The study has been able to describe the HRV species in circulation causing URTI over a short time interval (6 months) in an outpatient rural setting. The trend of proportionality observed here (where HRV-A and HRV-C dominate) have also been reported elsewhere. The close monophyletic clustering of some viruses suggested an 'epitype' kind of infection.