SESSION 5: HUMAN RESPIRATORY PATHOGENS

Chair: ***Dr. Evans Amukoye.***

Organizer: *Dr. Wallace Bulimo.*

ORAL PRESENTATIONS

1. **The Molecular Evolution Of Two Internal Genes, M & Ns In Pandemic H1n1 Influenza A Virus**

Gachara, G.[1, 2], Mbithi, J. [1], Magana, J. [2], Symekher, S [2] Bulimo, W. [2],

[1]Dept of Medical Laboratory Sciences, Kenyatta University;
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Background: Influenza A viruses are constantly evolving through through genetic reassortment of their segmented. RNA genome. Most studies on the pandemic H1N1 virus has been on the HA and NA genes while internal genes has been less studied. The matrix (M) and non-structural (NS) gene proteins of influenza viruses have been identified as determinants of virulence and host range restriction. They are the only influenza virus RNA segments that are bicistronic.

Objective: To analyze the M and NS genes of Kenya isolates for their genetic variation and their evolution in comparison with the vaccine strain.

Methods: 36 virus isolates from diverse areas in the country isolated between July 2009 and January 2010 were selected and conventional PCR to amplify the whole NS and M gene performed using M13 primers. The resulting amplicons were then directly sequenced. A variety of bioinformatics tools were used for Phylogenetic analysis.

Results: The two genes were found to be highly conserved with nucleotide homology of 98-100%. At the amino acid level, the M1 protein was generally more conserved (92-100%) than the M2 protein (77-100%), while in the NS gene the NS1 proteins were more conserved than the NS2/NEP protein. The M gene showed no fixed amino acid changes over time in both proteins but amino acids at positions 5 and 9 in the M2 located in the extracellular domain were most mutable. The NS1 protein showed only one fixed amino acid change I123V which is a characteristic of clade 7 viruses. No fixed amino acid changes were noticed in the NEP.

Conclusion: This study provides an insight into the evolutionary process of the M and NS gene of pandemic H1N1 in the country. With only one fixed amino acid change noted, the study suggests that other genes in the pandemic strain may have lead to its adaptation in humans.

2. **The evolution of influenza surveillance in Kenya-past, present and future aspects**

Authors: Magana, J. ; Gachara, G.;- Gichogo, J; Symekher, S.

Abstract

This paper traces the evolution of influenza surveillance in Kenya since the first flu pandemic in 1918. The three pandemics of the last century in 1918, 1957 and 1968 prompted researchers to include influenza surveillance in their medical 'safari' that was common in these early years. These earliest sero-epidemiological studies provided the evidence that influenza was circulating within the community and provided the impetus to integrate influenza laboratory-based surveillance in the country with clinical surveillance. These early studies has currently been replaced by country-wide sentinel based surveillance and the serological assays in use then replaced by modern molecular based assays. This study summarizes the achievements, key lessons learnt, challenges and constraints faced, current status and future prospects in influenza surveillance in Kenya. It also presents the laboratory virological surveillance results and the trends observed in the country so far.

3. **Virologic Surveillance of Influenza and other Respiratory Viruses in Kenya**

Wallace D. Bulimo PhD, Kenya NIC Surveillance Team

AFHSC-GEIS Human Influenza Program at KEMRI; KEMRI Influenza Surveillance Program

Background: Prior to 2006, Kenya lacked laboratories and public health infrastructure to conduct sustained influenza and other respiratory viruses surveillance. However in July 2006, USAMRU-K DEID Program and KEMRI began collaborating to conduct surveillance for influenza and influenza-like-illness (ILI) in Kenya. This collaboration comprises of thirteen sentinel sites across the country. To support this surveillance, a well equipped National Influenza Centre (NIC) laboratory was established.

Objective: The objective of the surveillance network is to conduct surveillance of influenza and other respiratory viruses.

Methodology: All consenting patients ≥two months old meeting the WHO ILI case definition are eligible. Detailed questionnaires are used by study clinicians to capture patients' bio-data. Nasopharyngeal samples are collected, sent to the NIC in liquid nitrogen and viruses isolated in susceptible cell lines. Tests on isolates include serology, antigenic and genetic analyses.

Results: Respiratory viruses isolated include; Enteroviruses, HPIVs, hAdV, hRSV, HSV1 and Influenza A & B. All common subtypes of influenza A have been isolated. All pdH1N1 viruses isolated at the peak of the pandemic exhibited an overall HA protein identity of 97.9% - 99.1% compared to A/California/7/2009. They differed from the vaccine strain by having P83L S203T R223Q and I321V. A number of parallel mutations were observed. Changes were observed at three of the five antigenic sites. Regarding antiviral susceptibility, all pdH1N1 were resistant to adamantanes but sensitive to neuraminidase inhibitors (NI's). We observed that 63% of seasonal influenza A/H1N1 viruses isolated in 2008 were resistant to the NI's but were sensitive to adamantanes.

Conclusions: We have shown that respiratory viruses are of public health importance in Kenya. We have also shown that as elsewhere, in Kenya influenza viruses are continuously evolving hence the necessity for continuous surveillance. We have also shown that a successful virologic surveillance system in Kenya requires a multi-sectoral involvement.

4. **An overview of the Influenza Sentinel Surveillance in Kenya ; MOH/CDC Surveillance System.**

Authors Lebo E[1]; Njuguna H[1]; Emukule G[1]; Arunga G[2] Kalani R[3]; Waiboci LW [1] Mott J [1,4]; Muthoka P[3];Katz M[1],Breiman, R[1]

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[4]Influenza Division, CDC-Atlanta

Background: The epidemiology and burden of acute respiratory infections and influenza remains poorly defined in sub-Saharan Africa. The Centers for Disease Control-Kenya (CDC-K), conducts sentinel hospital based surveillance in collaboration with the Ministry of Public Health and Sanitation. The objective of the surveillance is to determine the epidemiology, risk factors and burden of Severe Acute Respiratory Illness (SARI), Influenza like Illness (ILI), and laboratory-confirmed influenza in Kenya. Methods Since October 2006, sentinel surveillance for hospitalized SARI and outpatient ILI was established at 11 sentinel surveillance hospitals. Epidemiological data, nasopharyngeal and oropharyngeal swabs were taken from patients who met the case definition for SARI and a proportion of patients meeting the case definition for ILI. Samples were tested for Influenza A, and B and subtypes either at the National Influenza Center or the CDC-K laboratory using real time RT-PCR. A proportion of the influenza positive samples were cultured and aliquots sent to the World Health Organization collaboration center (CDC- Atlanta) for characterization.

Results From 1 October 2006 to August 2011, 22,352 SARI and 14,205 ILI patients were detected through

the sentinel surveillance system. Of these patients, 19,911 (54.4%) were male and 23,291 (63.6%) were less than 5 years of age . 34,805 samples were tested for influenza and from these, 4,195(12%) were positive for Influenza. During 2008 and 2010 seasonal influenza A (H3N2) was the dominant influenza A virus subtype detected. Pandemic influenza A (H1N1) was dominant during 2009. Influenza type B co-circulated with influenza A during each of these years. The presence of any clinican diagnosed underlying chronic condition was only reported in 134 (3.1%) of influenza positive SARI cases, of which 92(68.7%) reported a chronic respiratory illness.

Conclusion: Sentinel surveillance is useful for monitoring the epidemiology and virology of both mild and severe influenza annually in geographically disparate locations. This information contributes policy-relevant data for decision-making in Kenya, and may serve as a platform to monitor the impact of influenza relative to other respiratory pathogens.

SESSION 5: POSTER PRESENTATIONS

1. **Molecular Characterization of HPIV1 in Infants Attending Mbagathi District Hospital, Nairobi, Kenya.**

Joshua K. Kiptinness[1] *, Fred Wamunyokoli*[2] *Eyako Wurapa*[3]*, Wallace Bulimo*[3]

[1]*Kenya Medical Research Institute, Nairobi, Kenya.*
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Background: Human parainfluenza virus type 1 (HPIV1) belongs to the Paramyxoviridae family. Viruses in this family have a lipid envelope and their genome consists of negative-stranded RNA. HPIV1 cause serious lower respiratory tract illness in infants and children which accounts for approximately 18% of all hospitalizations of pediatric patients with respiratory tract infection. HPIV1 strains isolated in the recent years demonstrate persistent antigenic and genetic differences compared to the Washington 1964 type strain, including differences between genotypes within the same epidemic and same geographic location.

Objective: To characterize HPIV1 HN gene and establish the phylogenetic relationship of Mbagathi isolates and those in genetic databases.

Methodology: 25 HPIV-1 stored isolates were amplified and isolated in $LLCMK_2$ cells and identified by direct Immunofluorescent antibody Assay. Real time RT-PCR and conventional RT-PCR was carried out on the positive isolates and nucleotide sequences determined. Multiple sequence alignment for the 21 positive isolates and 6 representative strains obtained from Gen Bank was carried out and a phylogenetic tree determined.

Results: Nucleotide sequence variation in the hemagglutinin-neuraminidase protein (HN) gene of 21 HPIV-1 isolates demonstrated unique substitution patterns involving 54 nucleotide positions (8/54 positions, approximately 40%) and 45 mutations in 21 substituted positions of 89 amino acids on the HN protein. There was one unique amino acid substitution which was in 9 of 21 isolates. Geographically all isolates were from the same region but had different clustering patterns.

Conclusion: Phylogenetic analysis of the HPIV-1 HN gene shows that isolates from a particular area tend to cluster together indicating that it is evolving by a slow progressive antigenic drift. The evolution of HPIV1 suggests that different parts of the HPIV1 genome may be under different evolutionary pressures. This study emphasizes the need for continued HPIV-1 molecular characterization not only in Nairobi but also in the whole country.

2. **Molecular Antiviral Susceptibility Testing of Influenza A Virus Isolates obtained in Kenya in the year 2008-2009.**

Meshack Wadegu[2] , Wallace D. Bulimo[2], Rachel A. Achilla[2], Janet Majanja[2], Silvanos Mukunzi[2], Finnley Osuna[1], James Njiri[2] , Benjamin Opot[2] , David C. Schnabel[2], Eyako K. Wurapa[2]

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Introduction: Antivirals play an important role in treatment and prevention of severe influenza infections. Amantidine and remantidine inhibit M2 protein of influenza A while oseltamivir and zanamivir inhibit NA of influenza A and B. M2 protein mediates influx of protons through the viral lipid membrane causing dissociation of the virus during virus entry. Binding of M2 inhibitors to M2 inhibits viral genome uncoating and RNP import into the nucleus. NA catalyzes removal of terminal sialic acid residues from viral and cellular glycoconjugates to facilitate virus release. NA also helps virus spread through circulation by removing sialic acids from cell surfaces. NA inhibitors interfere with release of progeny virus from infected cells preventing infection of new cells and halting the spread of infection. Mutations in M2 and NA proteins underpin these resistances at the molecular level. H274Y (H275 in NA1) change in the NA protein alters drug binding resulting in oseltamivir resistance. S31N substitution in the M2 domain determines antiviral resistance to M2 inhibitors. We investigated genetic characteristics of NA and M2 genes of influenza A viruses isolated in Kenya in 2008-2009 in relation to antiviral resistance.

Method: Nasopharyngeal specimen from outpatients ≥ 2 months old were screened by rtRT-PCR. Positive specimens were inoculated on MDCK cells. RNA extraction and amplification of M and NA genes was done followed by nucleotide sequencing of the amplified gene segments and sequences deposited in Gene Bank

Results and Discussion: In the study period we sequenced 12 influenza A (H1N1), 48 pandemic H1N1 and 36 influenza A (H3N2) M and NA genes. 58% of influenza A (H1N1) viruses had H275Y mutation but none had S31N change. All pandemic H1N1 and H3N2 strains had the S31N mutation in M2 protein. All H3N2 strains lacked H274Y mutation. These results conform to the global picture regarding influenza antiviral activity during the period.

Conclusion: Our results emphasize the unpredictable nature of influenza A viruses and need for continued surveillance of drug resistance patterns globally.

3. **Molecular And Phylogenetic Analysis Of The Hemagglutinin Gene Of Pandemic Influenza H1n1 2009 Viruses In Kenya**

Benjamin Opot, Finley Osuna, Meshack Wadegu, Rachel Achilla, Eyako Wurapa and Wallace Bulimo

Background: The hemagglutinin (HA) gene of 2009 H1N1 is derived from ‘‘classical swine H1N1’’ virus, which likely shares a common ancestor with the human H1N1 virus that caused the 1918 pandemic. While virulence of influenza virus is polygenic, the hemagglutinin (HA) protein is especially important in receptor binding and membrane fusion thus facilitating infection.

Objective: To describe the evolution of HA1 protein of the pandemic H1N1 influenza A virus in Kenya.

Methods: 3800 samples from ILI patients were collected between July 2009 and August 2010. The detection of pandemic H1N1 virus was done using real-time RT-PCR. The positive samples were then cultured in MDCK cells and confirmed using the HAI assay. 101 isolates representing the pandemic period were selected and conventional PCR to amplify the HA1 portion of the HA gene was performed and resulting amplicons directly sequenced. Bioinformatics tools were used for Phylogenetic analysis.

Results: The Kenyan isolates showed 97-99% amino acid identity with the vaccine strain and 95-100 % among themselves. Phylogenetic analysis of the translated amino acid sequences revealed the three clade specific mutations identified globally. These are P100S, S220T and V338I that characterize clade 1 and 7 viruses. Mutations were observed in all the HA epitope sites but were more dominant in the Ca site. Specific mutations were noticed in the 2010 isolates

including E391K, S468N and S202T/N. The V641/L mutation occurring in 29 isolates was unique to the Kenyan isolates but its significance has not been described.

Conclusions: The study shows there is greater divergence among the 2010 isolates in comparison with the vaccine strain. The more recent (2010) isolates seem to possess more mutations in the stalk region rather than the globular head. It has been showed that mutations may often be an evolutionary 'dead end' and have no much significance. It is thus important that laboratory surveillance continues to monitor the circulating viruses antigenically and genetically.

4. **Multiple and Single infections of influenza, RSV and human bocavirus during the post pandemic period**.

Samwel Lifumo Symekher [1], George Gachara [2], Carol Gikera [3], Jane Gichogu [1], Moses Rotich [1],Musa Ng'ayo [4], and Japhet Magana [1].

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Introduction: Acute respiratory infections (ARI) are leading causes of morbidity and mortality in children. Viruses have been recognized as predominant causative agents of ARI in them. Molecular testing has increased detection of pathogens.

Objective: To identify influenza, respiratory syncytial virus (RSV) and human bocavirus (HBoV) using molecular methods from patients recruited from an influenza sentinel surveillance in Kenya.

Methods: Oropharyngeal swabs from consenting patients with influenza like illness were collected, placed into cryovials with virus transport medium, and transported at $+4^0$C to the ARI-Unit, in Center for Virus Research, KEMRI. RNA and DNA were extracted from the samples. Influenza real time PCR was performed according to WHO protocols. Conventional PCR for RSV targeting the nucleocapsid and BCV targeting the NS1 gene were carried out using established protocols. The PCR products were observed in 2% agarose gel. Proportions of RSV positive and BCV samples were sequenced using the big dye technique.

Results: 297 samples were collected from the study sites. The most detected virus was RSV (n=140; 47.1%), followed by influenza viruses (n=66; 22.2%), and then HBoV (n=28; 9.4%). Of the influenza viruses detected, 64 (97%) were influenza A and 2 (3%) were influenza B. 133 patients infected by a single agent, 47 patients infected by two agents, and 4 patients infected by three agents. The sequence analysis of this study's RSV strains indicate clustering together of this study's isolates. For HBoV, the strains showed the clustering together with HBoV prototype strains ST1 and ST2 and other strains from Genbank.

Conclusions:234 viruses were identified from the patients recruited. Multiple infections were seen in 51 patients. Majority of this study's RSV clustered together on a different branch separate from other RSV indicating variability, while the BCV detected clustered together with other HBoV, indicating conservation.

5. **What influence did the H1N1 pandemic have on the temporal and seasonal distribution of other types circulating in Kenya?**

Janet Majanja[1], Wallace Bulimo[1], Rachel Achilla[1], Meshack Wadegu[1], Silvanos Mukunzi[1], Josephat Mwangi[1], James Njiri[1] and Eyako K. Wurapa[2].

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[2]U.S. Army Medical Research Unit – Kenya, Nairobi, Kenya.

Background: The Influenza sentinel surveillance network consisting of 8 sentinel surveillance sites located in District hospitals throughout the country has established that Influenza is a major cause of

respiratory illness in Kenya. Following the identification of the 2009 pandemic Influenza A/H1N1 virus in April 2009, the first Kenyan case was confirmed on 29th June, 2009 and thereafter widely detected in the sentinel surveillance specimens.

Objectives: The objective of the study was to determine the impact of the 2009 pandemic Influenza A/H1N1 virus on seasonal Influenza viruses in Kenya.

Methodology: Nasopharyngeal (NP) swabs were collected from consenting patients meeting the WHO Influenza like illness (ILI) case definition and transported to the National Influenza Centre in Nairobi. Detection of seasonal Influenza B, A/H1N1, A/H3N2, A/H5N1 and pandemic Influenza A/H1N1 viruses was carried out using Ag Path-ID™ One step Real Time polymerase chain reaction (RT PCR) reagents together with CDC Influenza virus RT PCR detection and characterization panels.

Results: 11 592 specimens were collected between January 2008 and July 2011. Of these, 1323 (11.4%) were positive for Influenza A and B viruses and their subtypes. In 2008, Influenza activity was highest between June and August. Influenza B was predominant (61.1%) followed by Influenza A/H3N2 (27.4%) with a constant circulation of seasonal A/H1N1 of 11.6% throughout the year. Influenza activity peaked from July to November 2009 with pandemic A/H1N1 dominating that year (37.2%) followed by Influenza B (34.2%). In 2010, Influenza activity was highest in July. Influenza A/H3N2 virus dominated (61.8%) with a constant circulation of Influenza B (20.1%) and pandemic A/H1N1 (17%) viruses and low levels of seasonal Influenza A/H1N1 virus (1%). The 2011 Influenza activity has so far been characterized by co-circulation of Influenza B (49.6%) and pandemic A/H1N1 (46.3%) viruses and some Influenza A/H3N2 (4.1%) with peak levels in February and March. No seasonal Influenza A/H1N1 virus has been observed.

Conclusions: The reduction and displacement of seasonal Influenza A/H1N1 is the most obvious effect of the pandemic Influenza strain while Influenza B continues to co-circulate with the pandemic virus. This information can be useful in vaccine strain selection.

6. Coinfections and Cocirculating RESPIRATORY VIRAL Pathogens during the H1N1 Outbreak of 2009 IN KENYA

Finnley Osuna, Wallace Bulimo, Rachel Achilla, George Gachara, Janet Majanja, Meshack Wadegu, Silvanos Mukunzi, James Njiri , Benjamin Opot, David Schnabel, and Eyako Wurapa.

US Army medical Research Unit - Kenya

Background: On June 29, the first case of pandemic H1N1 was confirmed in Kenya. Due to the pandemic, many patients with influenza-like illness (ILI) presented to hospitals for diagnostic screening. Although many were diagnosed with the pH1N1 infection, a big percentage tested negative.

Objective: To determine which respiratory viruses co-circulated with the pH1N1 virus in Kenya and the common co-infecting viruses.

Methodology: Eight public hospitals representing the entire country comprise the ILI sentinel surveillance sites. Nasopharyngeal swabs were collected from consenting patients meeting the ILI case definition. rRT PCR was used for identification of influenza A and B. For the other respiratory viruses, specimens were inoculated into susceptible cell lines and upon development of cytopathic effects (CPE's), frozen and analyzed by direct and indirect immunofluorescence assay, using the Respiratory Panel I Viral Screening and Identification kit (Chemicon International, Inc.).

Results: From August 2009 to August 2010, 3711 specimen were collected. Of these, 2669 (72%) tested negative while 1042(28%) were positive for at least one of the respiratory viruses tested. RSV was detected in 135 (13%), Enteroviruses in 52 (5%), PIV in 168 (16%), ADV in 123(12%), and HSV in 2 (0.2%) of all positive samples. Among influenza viruses, influenza A was detected in 360(34.5%) , and influenza B in 202 (19.3%) of all positive samples. PIV represented the largest group of non influenza respiratory viruses identified during the pandemic while Influenza B viruses were the most common co-circulating of all the respiratory viruses. Influenza B had the highest percentage of co infections with the pH1N1 virus followed by Seasonal influenza H1N1 and H3N2. There were no co infections with non influenza viruses.

Conclusion: These findings highlight the need to continue to improve respiratory virus detection assays

in addition to influenza testing for the clinical diagnosis and community surveillance of respiratory viral infections in the country.

7. **Subtypes for Health-care Associated Respiratory Virus infection in In-Patients at Kenyatta, New Nyanza and Mbagathi Hospitals in Kenya (September 2008 to August 2011)**

By Lilian Mayieka

Background: Health-care Associated Infections (HAI) occur in hospitalized patients in whom the infection was not present or incubating at the time of admission. Preliminary data from an ongoing study in KEMRI/CDC on HAI in three selected hospitals in Kenya indicates that of the most common viruses causing respiratory HAI(rHAI) were Human Respiratory Syncytial Virus (hRSV), Para influenza viruses (PIV), Influenza A viruses (Flu A), influenza B (Flu B) Adenoviruses and human metapneumovirus (hMPV)

Objectives: To identify the subtypes of RSV, Flu A and PIV circulating among hospitalized patients in Kenyatta National, New Nyanza Provincial and Mbagathi District Hospitals in Kenya from August 2009 to July 2011. hMPV

Methodology: All the Flu A specimens were sub typed for H1, H3 and pandemic H1 subtypes and PIV positive specimens were sub typed for PIV 1, 2 and 3 at the KEMRI/CDC laboratories by real time RT-PCR using pathogen specific primers. The RSV positive specimens were typed for A and B types using RSV multiplex primers at the KEMRI/Wellcome trust laboratories.

Results: A total of 287 samples collected by the HAI study, 48 (17%) were positive for PIVS, 42 (14.6%) of them were RSV positive, 25 (8.7%) were flu A positive, 35 (12%) were Adenovirus, only 10 samples (3.5%) were positive for hMPV and. Of the 42 RSV specimens that were typed, 3 (12%) specimens were RSV A, 11 (42%) specimens were RSV B while 12 (46%) specimens tested as both RSV A and B (co infection), and 16 samples did not type at all because of their late initial ct values. Of the 25 Flu A specimens, 11(44%) were pandemic H1 positive, 5(20%) were H3 and 8(36%) specimen were negative for all the subtypes tested, Of the PIV positive samples, 6 samples (12.5%) were positive for PIV-1, 6(12.5%) were PIV-2 and 36(75%) were PIV-3.

Conclusion: There was high number of co infection among the RSV positive patients (46%) and the number of RSV A positive was high than RSV B.Most of the Flu A positive patients had pandemic H1 and most of the PIV positive patients had PIV3.

8. **Prevalence of Acute Respiratory Infections caused by Influenza, RSV and Adeno viruses in Kenya in 2007-2008**

Julia Wangui (Msc)[1], D. James Nokes (PhD)[2], Eyako Wurapa (MD, MTM&H) [1] and Wallace Bulimo (PhD)[1]

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Background: Acute Respiratory Infection is one of the main causes of morbidity and mortality in children worldwide. Influenza viruses and Respiratory Syncytial Virus (RSV) are known causes of Acute Lower Respiratory Infections (ALRI) while Adeno viruses are causative agent of Acute Respiratory Infections (ARI). Winter epidemics in temperate regions result in 250,000 - 500,000 deaths per winter epidemic due to Influenza A whereas the RSV epidemic may cause 66,000 -199,000 deaths.

Objective: To determine the distribution of the viruses circulating in Kenya associated with ARI in children in March 2007 to February 2008

Methodology: Nasal Pharyngeal swabs (NP) were collected from children ≤60 months of age, with fevers of ≥38ºC, with a cough or sore throat. Samples were only obtained from patients after consent was granted by the guardian/parent. Only patients presenting with onset of illness within 72 hours were eligible. The samples were snap frozen before transportation to the National Influenza Center laboratory and stored at -

70°C. The study population was clustered into 5 geographical regions (Nairobi (NRB), Coast (CST), North Eastern (NEA), Western (WES) and Highlands (HLD). 384 samples were randomly selected from the total samples collected in 2007. Real time PCR was used to screen for Influenza A and B, RSV A and B and Adeno viruses.

Results: One or more viruses were detected in 179 of 384 samples screened (46.6%), with 32.0% positive for Influenza (26.8% A, 5.2%B), 11.2% RSV (4.7% for RSV A; 6.5 % RSV B), and 12.7% Adeno. 8.3% of the samples had multiple infections. The general prevalence of ARI due to Influenza, RSV and Adenoviruses amongst the patients who attended the outpatient clinic was highest during the months of June – August (21.4%). This was followed by the months of March – May (15.1%) and September – November (13.3%) The

Conclusions: ARI associated with Adeno viruses had the highest prevalence in the months during the dry season (December 2007 – February 2008) showing a prevalence of 3.1%, followed by RSV with 1.8% surpassing Influenza which had a prevalence 0.2% during this season.

9. **Molecular Detection of Human Metapneumoviruses circulating in Kenya in the year 2008.**

Rosemary Nzunza, Eyako Wurapa Wallace Bulimo.

US Army Medical Research Unit – Kenya

Background: Human metapneumovirus (HMPV) is a respiratory pathogen of the Pneumovirinae subfamily of the Paramyxoviridae, associated with acute respiratory tract infection (ARTI) in all age groups, especially young children, elderly subjects, and immuno-compromised patients. Two major surface antigens are expressed, the highly conserved fusion (F) protein, and the extremely diverse attachment (G) glycoprotein. The virus comprises two genetic groups, A and B. In Kenya, this virus has been isolated in hospitalized children and infants in a study that was done in Kilifi District Hospital. However, there is limited information on prevalence of human metapneumovirus (hMPV) strains circulating in all age groups.

Objective: To detect human metapneumoviruses from nasopharyngeal swabs specimen by real time RT-PCR.

Methodology: Nasopharyngeal swabs were collected from patients from 2months onwards who presented with Influenza like illnesses in our 8 sentinel surveillance hospitals. ILI was defined as fever >38ºC, cough or sore throat, onset of ILI within the previous 72 hrs. Total viral RNA was extracted and used for RT-PCR assay detect hMPV RNA genomic sequences using primers targeting the complete genome sequences of the F and G genes.

Results: We screened the specimens using real time RT–PCR. Between 1st January 2008 and 31 December 2008, 2964 patients were randomly recruited. A total 194 hMPV viruses were identified. Of these, 11 (6% positive) were from Mbagathi District hospital, 14 (3%) from Alupe District Hospital, 30 (5%) New Nyanza District Hospital, 17(5%) Malindi District Hospital, 7(4%) Port Reitz, 55(15%) Kisii District Hospital, 36(7%) Isiolo District Hospital, 24(7%) Kericho District Hospital. Generally all our regions posted higher than the 3% prevalence, published from the Kilifi Study.

Conclusions: This is the first study demonstrating the prevalence of hMPV infection in Kenya in all age groups. and suggests that hMPV infection is prevalent in infants and elderly patients with lower respiratory tract infection, in Kenya. However, more studies on the genetic and antigenic diversity of the strains should be done.

SESSION 5: Human Respiratory Pathogens

10. **Adenoviruses - A Kenyan Perspective**

Rachel Achilla[1], Wallace Bulimo[1], Janet Majanja[1], Meshack Wadegu[1], Silvanos Mukunzi[1], Josphat Mwangi[1], Julia Wangui[1], James Njiri[1], Benjamin Opot[1] and Eyako K. Wurapa[2].

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Abstract

Background: Human adenoviruses [HAdvs] mainly cause respiratory and gastroenteric diseases in humans. The viruses globally contribute 10% of acute respiratory disease in children below five years a major cause of morbidity and mortality. Human Adenoviruses circulating in Kenya have not been well studied. Adenovirus species associated with respiratory infection circulating in Kenya were analyzed.

Objectives: The objective of the study was to demonstrate circulation of adenoviruses in eight sentinel sites in Kenya

Methodology: Nasopharyngeal swabs were collected from consenting patients presenting with cough, sore throat, fever of 38 oC or above. Patients ≥2 months were eligible for the study. The study sites were eight district hospitals distributed across Kenya. The samples were inoculated into Hep2 cell line, observed for cytopathic effects and analyzed using an antigen specific immunoflourescent assay (IFA). Isolates were recovered from supernatants of cells staining positively by IFA.

Results: A total of 357 (3%) adenoviruses were isolated from the surveillance sites in Kenya over a three and half year period from a total of 11,592 samples. 204 (57%) of the positive isolates were from males and the remaining 43 % from females. Children aged five years and below were the most affected with 98% of the patients in this group. In 15% of the cases there were dual infections of adenovirus and other viruses. Three samples showed triple infection. Two of them had influenza A and enteroviruses and one had influenza A and Influenza B. There was a quadruple infection with influenza B, HSV and parainfluenza 3. The samples were isolated throughout the year and seasonality was not well established.

Conclusions: Adenoviruses are present in the Kenyan population and cause disease in children mostly below five years of age. Seasonality of the virus has not been demonstrated since the virus circulation pattern differs every year in the study period.

11. **Epidemiology of Human metapneumovirus in rural and urban populations of Kenya, 2006 - 2009**

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Background: *Human metapneumovirus* (hMPV) causes acute respiratory tract infections (ARTI) mostly in young children and the elderly with prevalence between 5-15% in various populations. There is paucity of data on the prevalence and genetic diversity of hMPV in Africa. This study investigated the incidence rates and subtypes of hMPV in an urban informal settlement and a rural community in Kenya.

Methods: Population-based surveillance for ARTI was conducted within two study areas: among two villages (population=28,000) in the Kibera informal settlement, Nairobi (population density=77,000/km^2) and in a rural community (population density=325/ km^2) in western Kenya called Lwak (population=23,000). Between October 2006 and September 2009, nasopharyngeal and oropharyngeal swabs collected from consenting patients meeting the case definitions for either severe acute respiratory infection (SARI) or influenza like illness (ILI), were tested for hMPV by real time RT-PCR. Incidence rate was calculated by the number of hMPV cases by person-years of observation (pyo) of residence per site. The *Human metapneumovirus* was isolated from RT-PCR-positive specimens through culture and hMPV subtypes determined by sequencing.

Results: Of 3377 specimens collected over the 3 year study period, 135 (4%) were hMPV positive; unadjusted incidence rates were 11.2/10,000 pyo in Kibera and 5.7/10,000 pyo in Lwak. Of the 135 hMPV cases, the highest proportion was in children ≤ 2 years old (36%). Incidence rates among this age group were also the highest with Kibera at 57.1/10,000 pyo and Lwak at 24/10,000 pyo. Those from Kibera were two times more likely to be infected by hMPV [RR=2, CI 1.21 – 5.02]; when compared with young adults, 18 – 34 years old, their rates were 10-fold [CI 3.14 – 44.07] higher in Lwak, and 8-fold [CI 4.46 – 19.52] higher in Kibera. From the isolates, subtypes A (28%) and B (72%) were identified in both sites.

Conclusion: The study showed higher incidence rates of hMPV in the densely populated urban site than in the rural area. Children ≤ 2 years were much more likely to be infected with hMPV than other age groups. Both hMPV subtypes are in circulation. This is the first documented study detailing incidence rates of hMPV in Kenya.