SESSION 1: ARBOVIROLOGY- NOVEL ANIMAL VIRUSES AND VHF

Chairman: *Dr. Lillian Musila.*

Organizers: *Dr. Rosemary Sang.*

ORAL PRESENTATIONS

1. Arboviruses and Viral hemorrhagic fevers as emerging threats to health and biosecurity in East Africa

Dr. Lillian A. Musila
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Abstract
In the past decade, there has been an increase in the awareness of arboviral diseases and hemorrhagic fevers as a public health issue in East Africa. This increase is attributable to sustained entomological and human surveillance, application of more robust diagnostic tools and platforms and increased funding for these emerging diseases by local and international agencies. Re-emergence of diseases such as Yellow fever, Dengue, the ever-looming threat of Rift Valley Fever Virus act as constant reminders of the potential health and economic impact of these diseases. Detection of arboviruses in their arthropod vectors and serological evidence of the exposure of human populations in Kenya to several arboviruses expose the extent of underdiagnosis of these diseases. The detection, handling and storage of the select agents Rift Valley Fever and Crimean Congo Hemorrhagic Fever viruses are of concern due to their bioterrorism potential. Current challenges include the need for more enhanced biological containment facilities, limited availability of diagnostic tools and limited understanding of the epidemiology of these endemic pathogens. Implementation of a regional strategy for surveillance, improved diagnostics, control and management of these diseases is paramount.

2. Distribution, Diversity and Abundance of mosquito vectors for arboviruses in ecologically distinct regions of Kenya

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Background: Arboviruses, transmitted by arthropods, cause clinical syndromes in humans ranging from febrile illnesses to hemorrhagic fevers. Spatial and temporal distribution of vectors influence the epidemiology of arboviruses such as Rift Valley fever (RVF), West Nile (WN), Yellow fever (YF), Dengue (DEN), Chikungunya (CHIK) and Onyong' nyong' (ONN) fever, all of which are prevalent in Kenya. We investigated the abundance, distribution and diversity of vectors for arboviruses in selected regions of Kenya in order to understand the epidemiology of arboviral diseases, regional risks for transmission and for future planning of effective control strategies.

Methodology: Mosquitoes were sampled in twelve regions from 2006 - 2010 using CO_2 baited CDC light traps and human landing collections and identified to species morphologically and pooled by species and sex to a max of 25 specimens/ pool.

Results: Over 520,000 mosquitoes were collected and identified into 11 genera and 99 species or species groups, 25 of them vectors of arboviruses actively transmitted in Kenya. The genus Culex was the most diverse, followed by Aedes. However, Aedes mosquitoes were overall most abundant. Ae. mcintoshi and *Ae. ochraceus*, were abundant in North Eastern province while *Mansonia uniformis* and *Ma. africana* predominated in Rift Valley province. Both regions were foci of the 2006-2007 RVF outbreak and the species implicated as main vectors respectively. *Ae. ochraceus, Ma.* africana and *Ma. uniformis* were also sampled in Nyanza province, a non RVF endemic area while Ae. circumluteolus was sampled mostly in Budalangi in Busia. *Ae. aegypti*, a vector of DEN, CHIKV, and YFV, was predominant in Rabai, Coast

province, where dengue and chikungunya are endemic. Few *Ae. aegypti* were collected from Western province where these diseases have also been identified, and N. E. province which has no transmission history. *Ae. simpsoni* and *Ae. africanus*, both vectors of sylvatic YFV, predominated in Coast and Nyanza respectively. The abundance of WNV vectors, such as *Culex quinquefasciatus,* in Rift Valley and Nyanza point to the risk of WNV transmission in these regions.

Conclusions: Arbovirus vectors are well distributed throughout Kenya both in regions with previous history of arbovirus outbreaks and where transmission to humans has not been reported, suggesting that a combination of factors influence disease epidemiology. This highlights potential for emergence and re-emergence of viral diseases in these vulnerable populations. Therefore there is need to map countrywide species distribution and abundance so as to plan focused control measures since there is correlation between vector population and disease outbreaks.

3. **MassCode for Pan-Arbovirus Surveillance in East Africa**

Dr. Jandouwe Villinger

Abstract
We have developed a high throughput multiplex pan-arbovirus PCR diagnostic platform for surveying East African mosquito and tick populations. We employ an emerging Mass Spectrometry based diagnostic platform that can identify up to 30 targets by multiplex PCR using primers labeled with distinct MassTags. We have designed a MassTag PCR panel with universal primers for *alphavirus, Sindbis, phlebovirus, nairovirus, orthobunyavirus, flavivirus, Thogoto* and D*hori.* In a second tier, we will reamplify samples that tested positive for a family of arboviruses in the MassTag PCR panel using the same universal primers without MassTags in singleplex PCR. We will identify specific viruses based on sequence composition by high resolution melting (HRM) analysis of PCR amplicons. We will similarly employ HRM analysis to differentiate and identify known and unknown viral variants, bloodmeal hosts, and vector populations. This technology allows us to develop an early warning system for predicting and managing future arbovirus outbreaks, and has the potential to detect unknown arboviruses in known arbovirus groups.

4. **Arbovirus Exposure is common in Western Kenya**

Matilu Mwau[1], Toru Kubo[4], Silvia Kadima[1], Lucy Okubi[1], Joyce Ngoi[1], Samson Muuo[1], Vincent Okoth[2], Shingo Inoue[3], Allan Ole Kwallah[1], Mercy Chahilu[1], Norman Demba[1], Janet Awando[1], Sheila Kageha[2], Olivia Wesula[2] and Kouichi Morita[4]

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Background: Several arboviruses are endemic in Kenya. They may be an important cause of fever, and some have caused epidemics in the past. In spite of this, an accurate estimate of the magnitude of the problem has not been attempted in most areas.

Objectives: The overall objective of this study was to determine the seroprevalence of selected arboviruses in, and socio-demographic characteristics of, clients with fever visiting health facilities in Western Kenya

Methodology: Febrile patients who consented in Kitale, Endebbes and Andersen Hospitals were recruited. Malaria and typhoid were ruled out. Questionnairea were used to collect sociodemographic data. Blood was tested for Chikungunya, Yellow Fever, Dengue and West Nile virus antibodies using an Indirect IgG+M+A+D ELISA and Plaque Reduction Neutralisation Tests were done in ELISA positive samples.

Results: We screened 1578 febrile patients. The mean age was 31.6y with a median of 28y. 1357 samples were screened for Chikungunya; 317(23.35%) were ELISA positive. 1379 were screened for Dengue, 53 (3.84%) were positive. 1370 were screened for West

Nile Virus, 112 (8.18%) were positive. Plaque Reduction Neutralisation was conducted for all ELISA positive and borderline samples. At 1:40 dilutions, complete neutralization of Chikungunya virus occurred in 127/305 samples, of Dengue Virus in 6/63 samples, and of West Nile Virus in 12/110 samples. One sample neutralized Chikungunya virus completely at >1:2560 dilutions. Dual seropositivity occurred. Women were at a higher risk of Chikungunya exposure (OR= 1.5, $p<0.05$). The risk of seropositivity increased with age.

Conclusion and recommendations: Arbovirus exposure, especially to Chikungunya is common in Western Kenya. There is an urgent need to isolate and characterize these viruses, and to strengthen epidemic preparedness.

SESSION 1: POSTER PRESENTATIONS

1. **A Reverse Transcription Real-Time Loop Mediated Isothermal Amplification Assay For The Rapid Detection Of Yellow Fever Virus**

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Background: Yellow fever (YF), a mosquito-borne flavivirus, is a viral haemorrhagic disease in Africa and South America where it is endemic. Detection of Yellow Fever Virus (YFV) in Africa remains a challenge due to lack of highly specific tests at peripheral healthcare facilities. Kenya is a hotbed of YFV hence the development of a rapid detection tool that is adaptable to resource poor settings will improve diagnostics.

Objective: To develop and optimize a rapid detection RT-LAMP assay for YFV for use in resource poor settings.

Methodology: Vaccine strain, YFV (17D), was propagated in Vero cells at 37 °C until cytopathic effect was observed. Viral RNA was then extracted from the infected culture fluid. Both RT-PCR using YFV known specific primers and RT-LAMP using newly designed primers were performed. RT-LAMP was done isothermally at 62 °C using a real-time turbidimeter which allowed detection within 2 hrs. Agarose gel electrophoresis was used to view end-point results of the conventional YFV RT-PCR.

Results: Specificity of RT-LAMP and RT-PCR assays was determined using RNA from other related flaviviruses (WNV, DEN1-4, JEV) as well as RVF and CHIKV. Only YFV RNA was detected by the two methods. In addition, the RT-LAMP assay had a detection limit of 0.1 PFU while RT-PCR had a detection limit of 1 PFU.

Conclusion: The developed YFV RT-LAMP assay is optimized for use isothermally at 62 °C making it possible to use resources available in peripheral healthcare facilities such as water baths, heat blocks or turbidimeters. The assay showed higher sensitivity using YFV 17D but further work using local isolates will enhance its applicability in screening outbreak field samples.

2. **Characterization and Isolation of Chikungunya Viral antigen for development of ELISA diagnostics**

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Abstract

Background: Chikungunya (CHIK) virus is a potentially deadly disease that periodically cause outbreaks and pose a huge threat due to their increased global re-emergence necessitating continuous

surveillance of vectors, human and animal cases. CHIK fever is a viral disease transmitted to humans by the bite of infected *Aedes aegypti* mosquitoes. CHIK fever remains undiagnosed or is often confused with malaria and typhoid, because of the non-specific nature of clinical signs and lack of readily available laboratory testing. The virus was first isolated in 1953 in Tanzania. The most recent outbreaks have been reported from India and various Indian Ocean islands including Comoros, Mauritius, Reunion, Seychelles and re-infection of the virus occurred in Re-union Island and India in March 2010.

Objectives: This study focused on characterization of CHIK comoros strain as an antigen for development of ELISA reagents for detection of CHIK viral infections.

Methodology: The virus titer was determined using plaque assay and molecular analysis of virus done by Reverse transcriptase –polymerase chain reaction (RT-PCR).

Results: The envelope region 1 of the 5 independent vials of virus were amplified by RT-PCR, expected band size of 787bp was achieved by presence of bands on the 2% agarose gel. phylogenetic analysis showed 3 strains from Comoros clustered together with other Comoros strains and were closely related to Re-Union and Lamu strains of CHIK. The other 2 strains clustered together with the CHIK S-27, an African strain.

Conclusion: The African strains were clustered separately from the Indian ocean islands, showing genetic variation as the outbreak spread from Lamu to Comoros then Re-uonion Island. Plaques of different sizes were observed in the plaque assays suggest a co-circulating strain/virus in the Comoros Island isolate. This is an interesting finding that is being investigated further.

3. Alphavirus prevalence observed through mosquito surveillance in semi arid areas of Kenya.

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Background: Alphaviruses are diverse group principally mosquito-borne RNA viruses that cause diseases in humans worldwide. In Africa they include Chikungunya, O'nyong-nyong and Sindbis viruses and cause febrile illnesses with encephalitis and/or arthritis. They are of significant public health concern and have caused major outbreaks in Eastern Africa in the recent past.

Objective: To determine the presence and circulation of arboviruses, and the associated vectors responsible for their maintenance and transmission.

Methodology: Mosquitoes were trapped using CO_2-baited CDC light traps from December 2009 to June 2010 in 6 sites in Ijara and Marigat districts during the wet seasons. They were identified to species, pooled (25 mosquitoes per pool) and homogenized in minimum essential medium. Homogenates clarified by centrifugation were inoculated in VERO cells monolayers. Cultures were incubated at 37°C and observed daily for cytopathogenic effects (CPE).Cultures showing CPE were harvested and viruses identified by RT-PCR and sequencing.

Results: To date, over 92,000 mosquitoes have been collected, identified into 37 species and pooled into 4,382 pools.11 Ndumu virus (NDUV) isolates have been detected from pools of *Ae. mcintoshi* (7), *Ae. ochraceus* (1) and *Ae. tricholabis* (2) all from Ijara and An. pharoensis (1) from Marigat. Semliki forest virus (SFV) was isolated from *Ae. ochraceus* (3) and *Ae. tricholabis* (2) from Ijara. One isolate of sindbis (SINV) was detected from *Cx. antenattus* from Marigat.

Conclusion: This results demonstrate that SFV, SINV and NDUV all alphaviruses known to cause febrile illness with arthritis and encephalitis are prevalent circulating among mosquito species in the two semi arid regions of Kenya and could account for some of the febrile illnesses of unknown etiology observed in these areas. While NDUV was found in both sites, SFV was detected in Ijara and SINV in Marigat only.

Control strategies to prevent alphavirus transmission should target the three mosquito genera.
NB: AVID Consortium is a consortium of institutions implementing the Arbovirus Incidence and Diversity project based at ICIPE, and including Kenya wildlife service, Department of veterinary services, Kenya agricultural research institute, International livestock research institute and Kenya medical research institute.

4. **Exposure To Arboviruses Is Common In Children In Alupe, Western Kenya**

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Background: Arboviruses cause acute febrile illnesses that mimic malaria or typhoid.

Primary objective: To determine the seroprevalence of select arboviruses and the socio-demographic and clinical features of exposed children in two health facilities in Alupe.

Methodology: This was a cross sectional survey of children aged 1-12 years visiting Alupe District Hospital and KEMRI Alupe clinic. Informed consent was sought and a questionnaire administered. Children were examined, and their blood tested for CHIKV, YFV, DENV and WNV using an indirect ELISA for IgG+IgM+IgD+IgA. Malaria and Widal tests were done for those with fever. HIV testing was optional. Data was analysed using Stata/IC 10.1 for Macintosh.
Results: 656 children (340 females and 316 males) were recruited. The mean age was 58.1 months. 52.9% of the children were below school-going age. Common complaints were: feeling sick (42.07%), fever (39.48%), nausea and vomiting (27.44%) and stomachache (22.10%). Common examination findings included rash (26.06%), swollen nodes (21.37%) and throat inflammation (22.78%). HIV prevalence was 1.14%. 48.16% malaria and 78.16% Widal tests were positive. In total, 27.3% had some exposure to arboviruses. 9.6%, were exposed to WNV, 8.99% to DENV, 5.55% to CHIKV and 4.42% to YFV. 26.2% of the 206 who tested positive for malaria also were seropositive for either YFV, DENV, CHIKV or WNV. Arbovirus seropositivity rates increased with age. Being female, attending school, dehydration, photophobia, joint pain and swelling, recent mosquito bites and headaches were associated with an increased risk of seropositivity but not significantly so.

Conclusion: Children in Alupe are exposed to WNV, CHIKV, YFV and DENV. Seropositivity rates increased with age and in females. Co-infection with malaria and arboviruses is present. Further research should be done to isolate and characterise arboviruses. Community based surveys will determine the real magnitude of arbovirus exposure and their role in causation of fever.

5. **Sero-prevalence of Crimean Congo Hemorrhagic Fever Virus in Out-patients attending Sangailu and Ijara Health Centres Kenya**

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Background: Crimean Congo Hemorrhagic Fever (CCHF) is a tick-borne viral disease with high mortality reported in Africa, Asia, South East Europe and Middle East. The majority of human cases are pastoralists who come in contact with animals infested with *Hyalomma*
species of ticks which are the vectors of CCHF virus.

Objective: To determine the level of exposure of human population to CCHFV in outpatients attending Sangailu and Ijara Health Centres, Kenya

Methodology: A total of 517 human serum samples were collected from patients presenting with febrile illness or including fevers of unknown origin at Sangailu Dispensary and Ijara Health Centres. These samples were screened for the presence of anti-CCHF IgG using CCHF IgG VECTOR BEST ELISA Kit.

Results: The results indicate 18.6% of the total sera were positive for anti-CCHF IgG of which 64.6% were those seen at Sangailu and 35.4% seen at Ijara Health Centres. The sero-prevalence of CCHFV in Sangailu was 23% and 13.7% in Ijara. Overall, 52.1% those testing positive were females and 47.9% were males. In Sangailu health centre, 51.6% of those testing positive were females and 48.4% males. In Ijara, 52.9% of those testing positive were females and 47% males. Median age of those testing positive was 29 years. Ant-CCHF IgG positive farmers constituted 29.3%, housewives 17.9% and 7.7 businessmen. Out of those testing positive for anti-CCHF IgG, 18.8% had contact with goats, 20% with camels and 20.1% with donkeys.

Conclusion: This study indicates that age, location and contact with donkeys are risk factors to CCHF virus exposure. This study confirms the circulation of CCHF virus amongst human populations in Ijara District and therefore surveillance programs should be sustained to enhance early detection of this virus that has potential to cause outbreaks of severe disease.

6. **Assessment Of Diversity Of Ndumu Virus Strains Isolated From Mosquitoes From Four Districts In Kenya**

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Background: Ndumu virus belongs to the *Togaviridae* family, genus *alphavirus*. Alphaviruses are mosquito-borne RNA viruses that cause a variety of diseases worldwide & are prone to genetic transformation which may change their virulence. Therefore, monitoring and detection of genetic diversity is important.

Objectives:
General Objective: Characterization of Ndumu virus isolates from Garissa, Ijara, Busia and Baringo districts in Kenya.

Specific Objective:To investigate the genetic diversity of Ndumu virus isolates from Garissa, Ijara, Busia and Baringo districts in Kenya.

Methods: The virus isolates were cultured in Vero cells and monitored for cytopathic effects (CPE). The isolates caused (CPE) on cell culture on day 1 post inoculation. The infected cells were harvested and the supernatant was used to isolate total RNA after which Reverse transcriptase polymerase chain reaction was performed using primers specific for the envelope gene (E1) to confirm the identity of the virus. This was followed by pyro-sequencing of the whole genomes of the isolates using 454 sequencer. A mapping assembly of the sequence reads from the 454 sequencer was done using GS Runmapper. Nucleotide and amino acid sequence alignment was done using Muscle version 3.7 software. Molecular Evolutionary Genetics Analysis software version 4.0. was used for phylogenetic analysis.

Results: The Ndumu virus isolates from Baringo, Busia and Garissa districts showed almost no genetic variation, only one isolate from Ijara was distinct. The study has facilitated in the identification of single nucleotide polymorphisms (SNPs) within Ndumu virus genome. Such SNPs may change the protein coding sequence and affect the virus' virulence and/or host susceptibility over time.

Conclusion: This study has shown that the Ndumu virus isolates from Busia, Baringo and Garissa exhibited minimal genetic diversity despite the geographic distance separating them. The Ndumu virus isolate from Ijara district was the most different from all the rest.

7. The Baculovirus Expression Vector System As A Model For Demonstrating The Susceptibility Of Rift Valley Fever Virus Genome To RNA Interferance

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Abstract

The Baculovirus Expression Vector System (BEVS) is based on high levels of heterologous gene expression using a recombinant Autographa californica multiple nuclear polyhedrosis virus (AcMNPV), which relies on the l*epidopteran* species *Spodoptera frugiperda* and *Trichoplusia ni* as insect hosts. Rift Valley Fever virus (RVFV) (genus: Phlebovirus; family: Bunyaviridae) infects both humans and animals. The main aim of the present study was to determine the susceptibility of RVFV S-segment RNA transcripts to RNA-induced gene silencing using the BEVS in Sf21 insect cell lines. RNA interference (RNAi) is an RNA-dependent gene regulatory mechanism by which double-stranded RNA (dsRNA) induces gene silencing by targeting complementary mRNA for degradation.

RVFV genomic RNA was isolated from the Kenyan Garrisa 002 RVFV isolate obtained from the 2006/2007 epidemic. This was used to amplify full-length S-segment cDNA and cloning of a multiplex construct shRNA expression cassette comprising short cDNA fragments of the RVFV genome. The Green Fluorescence Protein (GFP) was cloned into the pIZ/V5-His vector to be independently expressed as a study control. The full-length genomic cDNA of S segment and shRNA transcription cassette were cloned into the donor vector pFastBac Dual^{TM} under the control of the baculovirus late promoters P_{p10} and P_{PH} respectively, and transposed into the bacmid in DH10Bac E. coli cells to generate a recombinant AcMNPV baculovirus. The pIZ/V5-His-GFP clone was transfected into Sf21 insect cells, which were subsequently infected with the recombinant AcMNPV baculovirus. The monitoring of RNA interference was achieved by detecting the progressive decline in GFP expression, as the experimental control, using fluorescence microscopy as well as semi-quantitative RT-PCR of both GFP and S-segment transcripts. The BEVS can be used to optimize clones for shRNA transcription and for the synthesis of siRNA in large quantities under the control of the powerful baculovirus promoters P_{p10} and P_{PH}.