Summary

Activities described under this aim will focus on understanding the movement of emerging pathogens including Ebola and Lassa viruses in West Africa, and Chikungunya in Kenya, and screening for corona viruses to identify susceptible animals throughout the seasons. The described activities will initially focus on the identification of animals susceptible to viral hemorrhagic using a non-invasive sampling technique in which hematophageous insects including ticks, flies and mosquitoes will be used a "syringes" for sampling animals blood to screen for the pathogen of interest. Seroprevalence and prevalence of the above pathogens will then be monitored in animals previously implicated in the transmission or amplification of these pathogens as well as susceptible animals species identified using the non-invasive sampling techniques. Sampling of blood as well as immune-privilege sites (testes, brain, eyes, central nervous system) will be performed to increase the likelihood of pathogen detection by qRT-PCR. Finally, the pathogen prevalence data will be combine with environmental factors such as vegetation type, average monthly precipitation and temperature, farming harvest period to identify any seasonal factors facilitating virus movement between susceptible animal clusters. The impact of susceptible host biology including population density, breeding period, departure of juvenile animals from the colonies on virus movement will also be assessed.

Objectives: The goals of this aim are to 1) identify wild and domestic animals susceptible to Ebola and Lassa viruses infection; to 2) document the year-round prevalence and seroprevalence of these virus in susceptible animals and vectors, and to 3) combine the prevalence data with geographical, seasonal information to develop a model to predict the likelihood of spill-over events into the human population.

AIM 1: Monitoring and predicting emerging pathogens (CCHF, RVF, Monkeypox and filoviruses) circulation in susceptible animals

Activities described under this aim will focus on understanding the movement of emerging pathogens including Ebola and Lassa viruses (another pathogen for Kenya?), in susceptible animals throughout the seasons in Nigeria, Kenya??. The described activities will initially focus on the identification of animals susceptible to the above pathogens using a non-invasive sampling technique described thereafter. Seroprevalence and prevalence of the above pathogens will then be monitored in animals previously implicated in the transmission or amplification of these pathogens as well as susceptible animals species

identified using the non-invasive sampling techniques. Sampling of blood as well as immune-privilege sites (testes, brain, eyes, central nervous system) will be performed to increase the likelihood of pathogen detection by qRT-PCR.

Objectives: The goals of this aim are to 1) identify wild and domestic animals susceptible to Ebola and Lassa viruses infection; to 2) document the year-round prevalence and seroprevalence of these virus in susceptible animals and vectors, and to 3) combine the prevalence data with geographical, seasonal information to develop a model to predict the likelihood of spill-over events into the human population.

Approach.

Susceptible species identification: hematophageous insects including ticks, flies and mosquitoes will be used a "syringes" for sampling animals blood to screen for previous Ebola virus exposure by RT-PCR and serology respectively [7,8]. The used of blood-sucking flies as flying syringes to sample wild animals has been reported. Using this technique, a large number of species ranging from birds to reptible and mammals (including human, elephants, bovine) could be sampled [8]. We have since adapted this technique to use not only flies but ticks and mosquitoes to sample an even larger number of animal species. Ticks, flies and mosquitoes will be collected monthly over a 12-months period. After individually homogenizing engorged mosquitoes, flies and ticks, viral RNA will be extracted from pools of insect homogenate. Ebola, and Lassa will be detected by qRT-PCR from these extracts. Individuals insects from the RT-PCR positive pools will be tested separately and the origin of the blood meal from RT-PCR positive insects will be identified by DNA barcoding [9,10]. Both Negative and positive controls will be included in all performed RT-PCR experiment. Sequencing of some RT-PCR positive samples will be performed to validate RT-PCR results.

Prevalence and seroprevalence studies: Following identification of new animal species susceptible to Ebola or Lassa infection, the prevalence and seroprevalence of these pathogens will be assessed in the target species. Animal species previously implicated in the transmission and maintenance of these diseases will also be screened for pathogen specific antibodies by ELISA and the presence of pathogen by qRT-PCR. For instance,

Filovirus (Ebola and Marburg) prevalence and seroprevalence will be assessed in bats, NHP, wild boar. Whenever possible, blood as well as immune privileges sites such as the brain, testes, eyes and spinal cord will be collected from wild animals.

. Sampling of insects and vertebrate will occurs during a 12 month period as the prevalence of Ebola and Lassa virus in susceptible animals is likely to varies throughout the year due to seasonal factors as illustrated with Lassa virus frequency in its animal reservoir [12].

Anticipated results:

The proposed activities is expected to identify novel animal species susceptible to Ebola, or Lassa infection. The use of three distinct types of hematophageous insects (ticks, flies and mosquitoes) for sampling of wild and domestic animals will increase our ability to survey a large number of species in the sampled area hence increasing the probability of identifying infected animals.

This aim of our proposal will yield a better understanding of the breath of animals susceptible to Ebola, or Lassa infection, as well as the insect vector involved in the transmission of some of these pathogens. The local capacity building will promote continue surveillance of these pathogens beyond the funded period. The improve knowledge of the movement of Ebola, or Lassa in susceptible animals and/or vectors will help reduce risky behaviors and identify populations at higher risk of spillover. Prophylactic interventions such as immunization campaign targeting these high-risk population could then be envisioned. In addition, these populations could be warned of any increase in Ebola, or Lassa prevalence in local wildlife to reduce the probability of spillover event.

Finally, the diverse complete genome of CCHF, RVF, Ebola, Marburg or Monkeypox obtain will help the design of pan-specific or species/clades specific primers and probes for qRT-PCR based diagnostic or surveillance. As such, this aim will significantly increase the detection of these pathogens not only in Central Africa but in every affected countries.

Pitfalls and solutions:

In the unlikely event that our insect-based surveillance is unable identify novel animal species susceptible to Ebola, or Lassa. Seroprevalence and prevalence information will still be available for animal species previously implicated in the transmission and maintenance of these pathogens. Pathogen prevalence is generally low in susceptible animals. However, sampling of immune privilege sites where virus persist for months following viral clearance from the blood stream will significantly increase our ability to detect infected animals. Furthermore seroprelavence studies in susceptible animals will also be measured. Although indirect, the presence of antibodies against Ebola, or Lassa will inform on the circulation of these pathogens in susceptible animals. It is worth noting that antibodies against *ebolavirus have been previously detected in approximately 1 and 5% of bats in the DRC/Gabon borders, Philippines and Bangladesh* [10–13] .

It is worth pointing out that year-round sampling throughout the dry and rainy seasons rather than sporadic sample collection will also improve our ability to detect acutely infected animals and provide critical clues on seasonal factors affecting Ebola, or Lassa prevalence in susceptible animals.

Aim 1b: Characterizing and modeling of the effects of environmental factors on the ecology of emerging viruses

Background: Both the LASV natural reservoir and the fruits bat species believe to harbor ebolavirus also have a wide geographical distribution in Sub-Saharan [10,32]. However, index cases of LASV and EBOV outbreak have been limited to specific hotspot. Field studies on LASV have demonstrated a huge heterogeneity in LASV prevalence among cluster of M. natalensis, even within individual villages [21].

Objectives:

The objective of this aim is to identify seasonal and geographical factors facilitating or inhibiting the movement of Ebola, or Lassa within susceptible species and to develop computer model to predict the likelihood of spill-over events into the human population.

Approach.

The prevalence and seroprevalence of Ebola, or Lassa in all susceptible animal species gathered year-round as part of AIM 1a will be plotted against environmental factors such as vegetation type, average monthly precipitation and temperature, farming harvest

period to identify any seasonal factors facilitating virus movement between susceptible animal clusters. The impact of susceptible host biology including population density, breeding period, departure of juvenile animals from the colonies on virus movement will also be assessed.

INNOVATION

The innovation in this program lies in the incorporation of non-invasive surveillance techniques during field studies, the reinforcement of local capacity that will result in year-round sampling capabilities.

Non-invasive surveillance: insect-based animal surveillance will by-pass the difficulties associated with sampling the vast diversity of wild animals present in rural areas Africa.

Year-round sampling: The reinforcement of local capacity for field studies resulting from providing both equipment and training will allow for year-round sampling . This will increase our ability to identify Ebola, or Lassa susceptible animals by accounting for any seasonal variation in pathogen prevalence in its natural reservoir. In addition, year-long sampling will identify any seasonal impact on Ebola, or Lassa shedding from susceptible animals and transmissibility to human hosts.

Protocol for capture, sampling of wild and domestics animals.

Rodents will be captured using baited Sherman rat traps while bats will be captured using mist nets. Large Sherman traps will be used in order to capture a variety of rodent species. Domestic species (Pigs and Livestock) will be manually restrained and sampled. Monthly targeted sampling of additional species identified using non-invasive surveillance techniques, will also be carried out. Such sampling will only be carried out on dead animals and/or animals for which the technical team has both the technical skills and legal rights and permissions for sampling such species. Adequate training in the humane capture, euthanasia and sampling of animals will be provided so as to meet all the ethical and legal requirements of the participating countries.

All wild animals will be anesthetised using isoflurane and bled (IV or intra-cardiac), the inactivated blood and tissues will be screened for CCHF, RVF, Ebola, Marburg or Monkeypox using RT-PCR. After sampling, all rodents will be euthanized by inhalation overdose.

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