Epidemiology Ecology and Social-Economics of Disease emergence in Nairobi (ESEI) is a project that has been implemented in Nairobi city for the last five years. In this newsletter I would like to review the public health and demography and economic threads of the research Project. At the outset, I wish to state that this is not sharing of the results obtained, as this is an on-going activity. The project uses E. coli as an exemplar to understand the processes and the pathways of pathogen introduction in the population through animal source foods. Nairobi consumers obtain animal source foods from a varied number of pathways. It is imperative to understand these pathways by studying product value chains. These value chains are key to linking consumers to livestock and therefore the risk of transmission of microflora between them. Value chain analysis includes describing, mapping (directional) understanding the governance and upgrading of the value chain. Red meat, poultry and milk value chains were targeted for this analysis. Sampling will be done at particular nodes of the value chains to isolate, phenotypically and genotypically characterize E. coli. Additionally, antimicrobial resistance profiles and genes associated with the resistance will be determined. To understand the mobility of the isolates between different animal and human populations is being undertaken by whole genome sequencing.

The project has also undertaken to understand the spatial distribution of E. coli among the different cross-sections of population in Nairobi. We are seeking to understand whether socio-economic status influence the spatial distribution or how keeping livestock or contact with livestock may influence this distribution. Nairobi was divided into 7 economic zones based on income and a total of 99 households from 33 sub-locations are being sampled. Of the three households one has large livestock, one no livestock and one small livestock. The 99 households residents are interviewed, clinical examination, and food consumption and economic data collected as well as fecal samples from the livestock and humans, food, environmental and water samples from surface pools collected for E. coli isolation and characterization.

Sample analysis takes place in two laboratories at the University of Nairobi that analyses all the livestock and environmental samples and Kenya Medical Research Institute analyses human samples. Synthesis of the data will help answer the questions set out under these two threads.
Well, time has flown since we sampled the first household in the 99 households study. On 7th June we visited our 66th household, meaning that after 8 months we are now two thirds of the way through. The project is taking us to all parts of Nairobi, as the maps illustrate. The field teams normally spend Monday to Wednesday collecting data, then use Thursdays and Fridays to recruit new households to the study, meet with local chiefs and county officials, give feedback to participants and keep on top of all the other jobs, such as vehicle maintenance, stock-keeping, accounting and paperwork. The wildlife team regularly go out on evenings and weekends to set and check traps for rodents and bats (who inconveniently refuse to venture out during normal working hours!) In some areas it has occasionally been necessary to conduct the study interviews in the evening, when participants return from work. Having to be flexible to fit around our human and animal participants’ needs, plus the perennial problem of Nairobi traffic, means early starts and long days.

The laboratory teams also come in for their share of hard work. Even with motorbike couriers, samples normally do not arrive at the labs until the afternoon, especially when large households are sampled. To process all these samples takes time. Each sample is first incubated in an enrichment broth, then undergoes two rounds of purification on a special type of agar which selects for E. coli, before being cultured on a more general agar prior to freezing the bacteria for storage. As you may imagine, this is several days’ work – each step takes at least 24 hours – and of course the bacteria don’t stop growing at weekends! Timing of steps is crucial, to ensure that pure colonies can be selected for storage. Later on, batches of isolates are revived and a number of biochemical tests are performed, to check that the bacteria we send to the UK for sequencing really are the E. coli that we are interested in. Once we are reasonably sure that what we have is an E. coli, they have to be regrown once more so that they can be sent to ILRI, where the DNA is extracted to send to the sequencing facility at Oxford.

So as you might imagine, it is extremely gratifying to finally start to see some of the results of all this hard work. Dr. Melissa Ward recently visited the teams in Nairobi and brought with her some of the first outputs of the sequencing to show us. In return, we took her along to see the sampling in action, in one of the slum sites. Melissa said, “It really brings the project to life, to see exactly how all the data and the samples are collected. Now, when I sit at my computer, I can really understand where it’s all coming from.” For us, it was equally exciting to get some tantalising glimpses of what the final dataset might look like and what kind of patterns we may be able to identify from the phylogenetic structure and genomic data. We’re not giving anything away at this stage – but we can tell you that we definitely have E. coli – and lots of it!

### Selected student profiles

**Emma Heming DeAllie** is a MRes in Clinical Sciences student from the University of Liverpool with a BSc in Psychology. Collaborating with the Urban Zoo Project, her current project is focused on antimicrobial susceptibility testing in E. coli.

**Maria Garza** is a Veterinarian and holds an MSc in One Health from the Royal Veterinary College and London School of Hygiene and Tropical Medicine. With experience in the field of livestock infectious diseases, she is currently working as a researcher for RVC in diverse research projects and currently collaborating with the Urban Zoo Project.

**Three Interns** from the Animal Health & Industry Training Institute (AHITI) are currently attached at the Urban Zoo Project providing technical support in the 99 household study component and the campylobacter poultry project in Nairobi.
As we approach the final quarter of the 99 household study, it is a pleasure to be asked to reflect on the wildlife sampling component of this study. The wildlife sampling team has come a long way since its inception in September 2015, when we were all relative novices in trapping Nairobi’s diverse array of wildlife species. We have had some long days and sleepless nights, but to their credit, the enthusiasm of those involved has never wavered.

A typical day for the wildlife team starts at 5am, when we embark on bird sampling. To ensure we follow best practice for all of our trapping we collaborate with experts at the National Museums of Kenya, and in the mornings Titus Imboma (an ornithologist from the museums) helps us set up an array of mist nets, aimed at trapping birds as they fly in proximity to the household and livestock-keeping areas of each compound. Once caught, each bird is placed in a paper bag to collect a faecal sample, before a number of other body measurements and biological samples are collected. Such opportunistic sampling is a common philosophy among wildlife disease ecologists, and additional samples provide an important resource for future epidemiological work. We next check the rodent traps – we use live-capture Sherman traps which are set throughout the house, livestock-keeping facilities and the household compound. Any rodents that we catch are transported back to the lab at ILRI, where they are humanely euthanized and subjected to a post-mortem examination (PME). This procedure is used to permit the collection of fresh faeces and organ samples which are stored frozen and in formalin. The latter ensures that tissues from these animals are preserved for histopathological interpretation, should the need arise in the future. As dusk settles over Nairobi, the sampling team heads back to the house to trap bats.

The techniques used to trap bats are very similar to those for birds; very fine mist nets are suspended between fly-ways where bats seek their food (either insects or fruit depending on the species of bat). Due to their propensity to bite, bats present a challenge to remove from the nets and restrain during sampling, but with the appropriate techniques and equipment (i.e. tough gloves!) they can be safely held to collect measurements and samples. We sacrifice a maximum of two bats each night, which are taken back to the lab at ILRI for PME. The rest are sampled live, and released unharmed. When we encounter a bird or bat roost, we use tarpaulins spread underneath the roost in order to collect pooled faecal samples representative of the individual animals using the roost.

Something that has become evident as we move from house-to-house, navigating Nairobi’s maze of leafy suburbs, high-rise apartments and river-side slums, is the sheer diversity of wildlife habitat present in this city. This is reflected in the number of species (birds, rodents, bats, primates and carnivores) we have sampled to date (see table 1). All of these species inhabit different ecological niches which likely govern their levels of interaction with humans and livestock; as an example one would expect very different levels of interaction between house rats that scavenge on animal feed and sunbirds that rely on nectar. How this translates to the risk of disease transmission is something we hope to shed light on by studying the genetic diversity of E. coli in these wildlife, and comparing it to those from humans, livestock and the environment.

**Table 1: Taxa and species sampled to-date**

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Total No. Sampled</th>
<th>No. of species sampled</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birds</td>
<td>320</td>
<td>29</td>
</tr>
<tr>
<td>Bats</td>
<td>28</td>
<td>7</td>
</tr>
<tr>
<td>Rodents</td>
<td>70</td>
<td>5</td>
</tr>
<tr>
<td>Primates</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Carnivores</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

By James Hassell

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The 99 Households Study is part of the Urban Zoo Project which is a joint project between scientists from Kenya and the UK. We are interested in how diseases can be transmitted between animals and people living in close contact in a city environment.

The 99 Household study aims to collect in-depth information from 99 families from 33 different neighbourhoods stratified by socio-economic status across the whole of Nairobi. We are testing humans, animals and the home environment for bacteria that can be shared and spread between them.
Human, food and environmental data are among the wide range of data collected within the 99 households. The data are often collected by Clinical Officers. Human sampling involves among others, individual consenting to participate, questionnaire interviews administration, general physical examination and anthropometric measurements, biological data collection and offering feedback and health education on the outcome of the laboratory based investigations. Two sets of structured questionnaires are administered; a general household and individual participant questionnaires. Biological data that is collected includes fecal samples and nasal swabs. Fecal samples are assessed for E. coli and campylobacter bacteria while nasal swabs are assessed for antimicrobial resistance. Collection and transportation of human samples from the field to laboratories involves sterile techniques.

Like human sampling, sterile steps are also observed during food and environmental data collection. Only livestock sourced foods are collected in the study. A sample of meat, milk and a wipe of egg shells if available, are collected. Sterile wipes of kitchen working surfaces such as chopping boards as well as kitchen door knobs are also collected. Environmental samples are collected using sterile boot socks. Normal saline-wet boot socks are worn and environmental samples collected by walking around the area surrounding the household as well as surfaces within livestock pens if available. Whirl pack bags are used in transportation of environmental samples. Water samples from water puddles, boreholes or storage water tanks are also collected as environmental samples. Subsamples of food and environmental samples are marked with a red dot to identify those going for whole genome sequencing and a blue dot on those being analyzed for campylobacter. All collected data are de-identified using barcode numbers to enhance participant and sample anonymity.

On completion of data collection, participants in the household are either given Albendazole or Mebendazole anthelminthic depending on age. Anyone found to be clinically ill is offered a prescription. If they are seriously ill a written referral letter to the nearest and most preferred health facility for further management is offered. Laboratory outcomes are communicated back to individual participants within two to three weeks of data collection. This is accompanied by health education with emphasis on how to maintain proper hygiene as well as interaction with livestock. Like many other community studies, our study is not devoid of challenges. Some of the challenges encountered involve heavy traffic. As investigators, we have to sometimes anticipate early morning starts. Participants which means rescheduling the day to collect data. Others include withdrawal from participation and inability to access household heads especially in high income settings.

**Sampling Kibera chickens- a look at urban farming in its most innovative**

Under the **Urban Zoo** umbrella, we have been sampling chicken farms as well as chicken meat retailers in Kibera, Nairobi, in order to investigate the prevalence of a food-borne pathogen, *Campylobacter*. Kibera, said to be the largest urban slum in Africa, is a surprising, challenging and rewarding environment to work in. The constantly evolving environment illustrates urban farming in its most inventive form. Densely populated and very low-income, the urban landscape goes from shiny newly-built roads, public toilets and other community spaces, often sponsored by donors, to muddy alleyways with open sewers and precarious living spaces.

Livestock is part of everyday life. Goats roam everywhere - some even took a nap under our car – as well as chickens, ducks, and sometimes even camels. People are keen to discuss their farming arrangements and projects, or laugh at our interest for the local chickens (kieni hitu) which seem so uneventful to them. As sampling is ongoing, results for *Campylobacter* presence are not yet available. This bacteria, common in chickens, yet not harmful to them, can lead to severe diarrhoea in humans, especially children. Poultry in Kibera often sleep in houses; kids and chickens run alike in courtyards; we have found chicken-raising pens on a shelf, behind doors, above some roofs and in other unexpected places. With such a diverse interface between humans and chickens, it will be valuable to determine the presence of *Campylobacter* and better understand related public health risks.

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