International Livestock Research Institute

Training course report

Sampling and characterization of mosquito vectors of Rift Valley fever in Uganda

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Background

In Uganda, like most countries in sub-Saharan Africa, around 70% of all households keep at least one kind of livestock (including poultry). Livestock production is primarily a family business, but only a fraction of the food produced is used for home consumption. Most of it is sold at local markets which are mainly informal. Livestock production is limited by pathogens and losses faced by smallholder farmers due to disease or death of animals. This, in turn, threatens food security, availability of well-balanced diets and the overall livelihoods of animal keepers. Diseases transmitted between animals and humans (zoonoses) such as brucellosis, tuberculosis and Rift Valley fever (RVF) threaten the health of producers, processors and consumers. Improved animal health, therefore, directly contributes towards improved livelihoods and human health through better diets and fewer zoonotic diseases. Knowledge and awareness about animal diseases, their risks and intervention options are limited in Uganda. Because of this, health risks for humans due to zoonoses are not commonly recognized. For many of the animal health problems, there are effective solutions at hand (for example, vaccines). However, lack of infrastructure and institutions in low- and middle-income countries does not allow last-mile delivery of solutions or implementation of disease control. Lack of awareness on the benefits vaccines results in unwillingness to purchase them.

To address some of these challenges, a new program called *Boosting Uganda's Investment in Livestock Development* (BUILD Uganda) has been developed. The program has four components: (1) support ongoing campaigns to eradicate peste des petits ruminants, (2) control zoonotic diseases, focussing initially on RVF, (3) control antimicrobial resistance and (4) improve veterinary public health at the point of slaughter.

The RVF component aims to minimize the impacts of the disease by improving capacities for surveillance and response to outbreaks at national and community levels though better risk prediction, implementation of evidence-based disease control policies and improving levels of awareness on the disease. RVF is caused by a virus that mainly affects livestock and has the capacity to infect humans. It is spread between animals (mostly cattle, sheep, goats and camels) via many species of mosquitoes (for example, *Culex* spp., *Aedes* spp. and *Mansonia* spp.) or via contact with infected tissues. In animals, the disease manifests as widespread abortions and mortalities in the young while in humans, the disease occurs as a mild flu-like syndrome in most cases or a severe haemorrhagic fever and even death in a few cases. The virus was first identified in 1931 on a farm in the Rift Valley of Kenya and since then outbreaks have been reported in many countries in sub-Saharan Africa and the Middle East.

Key activities that will be implemented under the RVF component are

- serological screening and molecular characterization of circulating RVF strains collected during outbreaks in Uganda (2016) and Kenya (2018) and stored at the International Livestock Research Institute (ILRI), the National Animal Disease Diagnostics and Epidemiology Centre (NADDEC) in Uganda, the Uganda Virus Research Institute, the Kenya Ministry of Agriculture, Livestock and Fisheries and the Kenya Medical Research Institute (KEMRI);
- epidemiological studies to determine risk factors for RVF outbreaks in the country; and
- socioeconomic studies with livestock keepers to assess their knowledge about the disease, its impact and potential entry points for vaccine introduction including aspects such as the role of gender on exposure patterns and implementation of disease control efforts.

The RVF component is being implemented in Isingiro, Sembabule, Kabale, Butebo, Budaka and Soroti districts.

Training summary

Objectives

The RVF project team comprising researchers from ILRI, the Uganda Ministry of Agriculture, Animal Industries and Fisheries (MAAIF), the Kenya Medical Research Institute (KEMRI), the Uganda National Agricultural Research Organization (NARO) and ILRI designed and implemented a 10-day training course for selected district entomologists and vector control officers on 26 November to 5 December 2019. The objective of the training course was to build capacities for surveillance of RVF vectors. The training course was aimed to improve the existing knowledge and tools for veterinary entomology in MAAIF given that the trainees enrolled had good skills for sampling tsetse flies, ticks and other vectors of veterinary importance but not mosquitoes. Participants were taken through pre- and post-training assessments. This report has, therefore, been structured into four sections: (1) pre-training assessment, (2) training on mosquito sampling and characterization, comprising theoretical and practical sessions, (3) post-training assessment and (4) recommendations.

Pre-training assessment

Twenty-two trainees were identified from the RVF project districts prior to the training course. The pre-training assessment identified baseline knowledge and skills of the participants as well as the needs of their host institutions regarding entomological surveillance. Information obtained from this exercise was used to guide the training program. Questionnaires determined basic quantitative review of capacities, knowledge and needs:

- 80% of the trainees (n = 18) had attained at least a bachelor's degree while 20% had a diploma.
- 75% of the trainees had participated in entomological surveys. Out of the 15 trainees (75%) who had experience in entomological surveys, only five (33%) had been exposed to mosquito sampling and identification.
- Only 30% of the trainees (n = 7) had access to a stereo microscope; these were staff from NADDEC, the National Livestock Resources Research Institute (NALIRRI) and Mbarara District Local Government.
- Overall, 50% of the trainees had received professional training after graduation.
- 60% of the trainees had an interest in understanding vector biology, epidemiology of vector-borne diseases and the role of an entomologists in the control of these diseases.

Mosquito sampling and characterization

The first three days covered theoretical background on arboviruses, vector biology, morphology of mosquitoes, sampling tools and biosafety measures that should be observed while sampling mosquitoes. The practical sessions involved setting mosquito traps in pre-identified field sites, collecting the catches and sorting and identification of mosquitoes based on mosquito identification keys. Participants worked in groups of four. The trainees were also trained on how to identify suitable mosquito breeding sites for setting traps.

Theoretical sessions

An outline of the topics covered during these sessions is given below.

General overview on arboviruses: Under this topic, a definition of arboviruses as well as the general symptoms associated with arboviruses, their diagnosis, prevention and control measures were discussed. Examples of arboviruses introduced were yellow fever, dengue, chikungunya, West Nile, RVF, o'nyong'nyong and Zika viruses (all mosquito-borne) and Crimean-Congo haemorrhagic fever virus (tick-borne). Historical perspectives of these arboviruses, transmission cycles and drivers of virus emergence were also covered. It was noted that it is important to consider co-circulation of arboviruses during RVF outbreaks. This was emphasized through the example of the 2006–07 RVF outbreak in northeastern Kenya in which over 23 different arboviruses were isolated from different mosquito species. It was also noted that during surveillance, other vectors or potential vectors of arboviruses may be identified. The example of recent emergence of *Aedes vittatus* in Mombasa was noted; this species is a competent vector of dengue, Zika and chikungunya viruses and its adaptation to new breeding habitats and changing population dynamics may contribute to transmission and circulation of these viruses during outbreaks. This may

significantly alter the epidemiology of diseases. Important vectors of arboviruses of public health significance were also covered as well as the challenges associated with their control.

Introduction to RVF virus vectors and vector biology: The global distribution of RVF virus (thought to be present in 32 countries) was discussed. It was also noted that RVF virus has so far been isolated from over 40 mosquito species globally, both known and potential vectors. Primary and secondary vectors of RVF virus were also introduced. It was mentioned that the distribution of vectors influences the distribution of the virus, as has been observed in Kenya. The breeding habitats of RVF virus, primary vectors and the transmission cycle, including species succession in *dambos* following heavy rains, were covered. Also, the host feeding preference of the primary vectors as determined by blood meal analysis of blood-fed mosquitoes collected during the 2006–07 RVF outbreak in northeastern Kenya was also presented. In that study, a large proportion of blood meals was obtained from sheep and goats. This was expected, given that these animals constituted the largest proportion of livestock species kept in the area.

Introduction to surveillance and field sampling tools: Timing and approaches for the surveillance of mosquitoes was covered. It was clarified that approaches used were often determined by the life stage of the mosquito being targeted. That is, if the primary interest was to collect larvae, specific tools would be needed as opposed to if the surveillance targeted adults. For the participants to better understand this discussion, the life cycle of mosquitoes was presented.

For sampling adult mosquitoes, participants were informed that there was a range of specific and more general tools that could be used. Traps that were available included Centers for Disease Control and Prevention (CDC) light traps, Biogents (BG)-Sentinel traps, human landing catch, resting boxes and backpack/Prokopack aspirators. An explanation was given on how each of the trapping tools could be used and the respective mosquito species that each trapping tool could sample more efficiently. Comparative data on mosquito catches between BG sentinel and CDC light traps were provided based on recent studies conducted in coastal Kenya. It was noted that BG traps proved to be more efficient than CDC light traps in collecting a wide range of species in large numbers. Tools for sampling mosquito larvae were presented as well as some of the challenges that were expected during surveillance.

Vectorial capacity: A definition of vectorial capacity was presented using examples from work by Agha et al. (2017)[1] using *Aedes aegypti* from Mombasa, Nairobi and Kisumu in relation to dengue and the work of Turell et al. (1992)[2] with *Aedes triseriatus* in relation to RVF virus transmission.

Transportation of mosquitoes: In this session, approaches used for transportation of mosquitoes from the field to the site laboratory and the use of triethylamine to knock down live mosquitoes before sorting (separation of mosquitoes from other insects) were discussed. The importance of shipment from the site laboratory to the main laboratory while maintaining a cold chain was emphasized. Entomological sample transportation, acceptance and rejection documents (specimen transport inventory sheet) and specimen tracking forms were presented while explaining the need to maintain accountability for each sample collected.

Mosquito morphology: The trainees were taught how to distinguish mosquitoes from other insects using the mouth parts. It was also explained that mosquitoes have three distinct body parts (head, thorax and abdomen). Distinguishing male from female mosquitoes by looking at the morphology of the antennae (bushy/plumose in males and normal in females) was also discussed. Characteristics of anopheline and culicine mosquitoes (eggs, larvae, pupae and resting behaviour) and distinguishing different mosquito genera using mouthparts (pulps and proboscis of similar or varying length) and tip of the abdomen (pointed in the genus *Aedes*, rounded in the genus *Culex* and rounded and upturned in the genus *Monsonia*) was introduced. This required detailed description of the different parts of a mosquito (head, thorax, abdomen and legs) which are significant in taxonomy. Similarly, the use of wings and wing venation, scaling pattern on the entire mosquito body and presence or absence of hairs or bristles were introduced.

Mosquito identification (taxonomic keys): Definition and explanation of the dichotomous keys in identifying mosquitoes to their species level was discussed. Trainees were also taken through examples of how to use

[1] Agha, S.B., Tchouassi, D.P., Bastos, A.D.S. and Sang, R. 2017. Dengue and yellow fever virus vectors: seasonal abundance, diversity and resting preferences in three Kenyan cities. *Parasites and Vectors* 10: 628. https://doi.org/10.1186/s13071-017-2598-2

[2] Turell, M.J. 1992. Virus-dependent mortality in Rift Valley fever, eastern equine encephalomyelitis, and chikungunya virus-inoculated mosquito (Diptera: Culicidae) larvae. *Journal of Medical Entomology* 29(5): 792–795. https://doi.org/10.1093/jmedent/29.5.792

the dichotomous keys, namely, simplified keys to selected mosquito genera of adult mosquitoes and keys for identification of adult female mosquitoes associated with RVF virus, dengue, yellow fever and chikungunya.

Practical sessions

The trainees were taken to various field sites to identify suitable trapping sites. Some of the preferred places were those that had experienced RVF outbreaks in the past. However, this exercise also aimed to limit distances covered from the training venue given the limited time that was available for the work. The timing of trap deployment and collection was discussed. Hands-on demonstrations were given on how these two activities could be conducted. Training on the use of global positioning system to take coordinates was also done during trap deployment. Catches collected from the traps were used to demonstrate mosquito sorting and identification. For this work, the trainees were distributed into four groups and each group given a mosquito to use for identification using the dichotomous key. They were guided on how to identify the mosquitoes to species level. Tubes were labelled with identified and unidentified mosquitoes (area abbreviation/site number/pool number) and preserved in the dry shipper.

Trainees were guided through the processes of pinning freshly collected mosquitoes and subsequently correctly identifying them to species level. Approximately 20 species of mosquitoes were identified during the exercise: *Aedes mcintoshi, Culex theileri, Aedes sudanensis, Anopheles ziemani namibiensis, Culex univittatus*, *Aedes cumminsii, Mansonia africana, Culex vansomereni, Culex annulioris, Culex rubinotus, Aedes aegypti, Aedes simpsoni, Anopheles gambiae, Culex tigripes, Culex antennatus, Culex poicilipes, Coquillettidia fascopenatta* and *Eretmapodite chrysogaster*.

Post-training evaluation

A post-training assessment was done to (i) evaluate the relevance and effectiveness of the training in building capacities in surveillance of RVF vectors, (ii) determine if participants had adequately attained their training expectations in order to inform the post-training action plans, (iii) document any challenges, lessons and best practices at this stage and (iv) comment on any areas that require improvement or adjustment to enhance project performance and realize expected results.

A questionnaire was administered asking trainees to answer the questions on the assessment areas by indicating by a score of 1 to 5 on the various aspects of the training. The rating of each score was as follows: 1: very bad, 2: inadequate, 3: sufficient, 4: good and 5: very good. The results from the questionnaire are shown in Table 1.

Table 1. Results of the post-training assessment

Question	Score				
	Very bad	Inadequate	Sufficient	Good	Very good
Was the length of the course appropriate for the subjects to be covered?			5%	60%	35%
Was the course material adequate?			20%	40%	40%
How would you grade the theoretical sessions in terms of educational value?				15%	85%
How would you grade the practical sessions in terms of educational value?				20%	80%
Was the laboratory work useful?				10%	90%
How would you grade the field work?				25%	75%
Was the venue of the course adequate?			10%	10%	80%
Was there cooperation among trainees?			10%	55%	35%
How would you rate the organization and logistics?				55%	45%
How would you rate the usefulness of the course for your role as an entomologist?				15%	85%

Further training and mentorship

The trainees will be engaged in routine mosquito surveillance activities with support from the trainers in the course of the program implementation. This will enable them to hone the acquired skills and knowledge. It is expected that by the end of the program, they will have had adequate exposure to enable them run entomological surveillance with limited supervision. Multiple refresher training courses will also be conducted in the course of the program. Trainees were asked to include any other comments they thought were useful to the training. These are indicated below.

- The training was generally very useful and created awareness on RVF and other zoonotic diseases among participants.
- All participants should get the necessary equipment for use in their respective districts.
- There is need to invite one vector control officer and one entomologist from each district for better decision-making and to strengthen the One Health approach.
- The next training course should be in districts selected for RVF work.
- The project should support or set up laboratories in districts.
- The project should train more entomologists.
- The project should organize quarterly refresher field and laboratory training sessions for participants.
- There is need for further training on mosquito identification using the larvae.
- The training was very educative and provided a very strong foundation for mosquito identification and characterization.

Recommendations

- Conduct a quick refresher course for the trainees when their memories of what they learned are still fresh.
- One topic that was not adequately covered due to time constraints was larval sampling and rearing in the insectary for subsequent identification. This should be covered during the next training.
- Mosquito identification was covered using pinned specimens. There is need to take the trainees through the process of sorting and identifying unpinned mosquitoes straight from the petri dishes.
- Solar powered weather data loggers would do better in place of a conventional rain gauge and thermometer. This would eliminate the need to hire someone committed to taking daily readings. This is especially so since there is need to factor in weather patterns several weeks to months leading to the sampling period.
- More training should be done on mosquito processing for virus isolation as surveillance does not end with vector identification.
- The project should purchase the following items for routine surveillance work in the districts:

Equipment	Catalogue number	Vendor/manufacturer
BG-2 Sentinel Trap	2880	Bioquip
Catch bags	2880C	Bioquip
Sealed gel electrolyte batteries (12v)	2863	Bioquip
Microscope stage	6188	Bioquip
Olympus dissecting microscope	SZ61	Olympus
Swiss style forceps	4531	Bioquip
Swiss style forceps	4532	Bioquip
Featherweight Forceps	4750	Bioquip
Larval dippers with wooden handle	1132	Bioquip
Larval trays	1426A	Bioquip
Larval trays	1426b	Bioquip
Larval trays	1426C	Bioquip
CDC miniature light traps	2836BQ	Bioquip
Dry ice dispensers/igloos	2811	Bioquip
Sealed gel electrolyte batteries (6v)		Bioquip
Replacement bag and collecting cup	2801B	Bioquip
6v/12v battery chargers	2865	Bioquip
HOBO weather data logger		

Agenda

Date and time	Session	Trainer
26 November 2019		
0900–0930	Introduction of the training objectives	Dan Tumusiime
0930–1200	Pre-training assessment	Dan Tumusiime
1200–1300	BUILD Uganda project (RVF component)	Bernard Bett
1300–1400	Lunch	
1400–1500	Drivers, ecology and historical perspectives focussing on Uganda	Dan Tumusiime
1500–1600	Role of vector surveillance on RVF control	Patrick Abila
27 November 2019		
0900–1000	Discuss the transmission cycle of the virus: host and vectors involved	Bernard Bett
1000–1030	Break	
1030–1130	Vector control: integrated pest management approaches	Patrick Abila
1130–1300	Introduction to vectors of RVF	Joel Lutomiah
1300–1400	Lunch	
1400–1600	Arboviruses in general	Joel Lutomiah
28 November 2019		
0900–1030	Vectorial capacity I	Joel Lutomiah and Sheila Agha
1030–1100	Break	
1100–1300	Vectorial capacity II	Joel Lutomiah and Sheila Agha
1300–1400	Lunch	
1400–1600	Conduct field visits to identify potential breeding sites for mosquitoes and set the meteorology measuring devices, i.e. rain gauge and minimum/maximum thermometer	All
29 November 2019		
0900–1030	Introduction to trapping and surveillance tools	Joel Lutomiah
1030–1100	Break	
1100–1200	Collection of metadata: how to capture and download global positioning system coordinates, ecological/meteorological data, data collection tools and formats	Patrick Abila and Richard Alingu
1200–1300	Appropriate transportation of materials from the field to the district laboratory for taxonomy, and then from the district laboratory to the national reference laboratory: maintaining the cold chain in all these stages	Joel Lutomiah and Patrick Abila
1300–1400	Lunch	
1400–1600	Field visits to set traps	All
30 November 2019		
0700–0800	Collect catches and set traps	
0900–1030	Mosquito morphology	Patrick Abila
1030–1100	Break	
1100–1300	Taxonomy 1: distinguishing genera (use pinned mosquitoes from the Uganda Veterinary Research Institute). The focus is how the teams can use identification keys	Joel Lutomiah and Patrick Abila
1300–1400	Lunch	
1400–1600	Collect catches and set traps	All
1 December 2019		
0700–0800	Collect catches and set traps	
0900–1030	Taxonomy 1	Joel Lutomiah
1030–1100	Break	
1100–1300	Sample processing: pooling, labelling the tubes, linking pools to metadata, storage, preparation of homogenates for laboratory analysis (virus detection)	Joel Lutomiah
1300–1400	Lunch	
1400–1600	Field work	All
2 December 2019		
0700–0800	Collect catches and set traps	All
0900–1030	Taxonomy 2: species identification	Joel Lutomiah
1030–1100	Break	
1100–1300	Sample processing: pooling, labelling the tubes, linking pools to metadata, storage, preparation of homogenates for laboratory analysis (virus detection)	Joel Lutomiah
1300–1400	Lunch	
1400–1600	Field work	All
3 December 2019		
0700–0800	Collect catches and set traps	All
0900–1030	Taxonomy 2: species identification	Joel Lutomiah
1030–1100	Break	
1100–1300	Sample processing: pooling, labelling the tubes, linking pools to metadata, storage, preparation of homogenates for laboratory analysis (virus detection)	Joel Lutomiah
1300–1400	Lunch	
1400–1600	Data management and reporting	All
4 December 2019		
0900–1300	Biosafety procedures for both field and laboratory workers	
1300–1400	Lunch	
1400–1600	Reporting	
5 December 2019		
0900–1200	Recap and post-training assessment	Dan Tumusiime and Patrick Abila
1200–1300	Closing the workshop	All

Training materials

The training materials are accessible via this Dropbox link:
https://www.dropbox.com/sh/cxo7pywyqoax12h/AADl_U_l-56jC3fc_GGv7HAqa?dl=0

List of participants

Name	Organization	Sex
Naboth Ngambe	Sheena District Local Government	Male
Arinaitwe Eugene	MAAIF	Male
Alinga Richard	NARO/NALIRRI	Male
Patrick Abila[a]	NARO/NaLIRRI	Male
Ahimbisibwe Emmanuel	Mbarara District Local Government	Male
Namanya Mercy	Isingiro District Local Government	Female
Tumuheirwe Honest	Kabale District Local Government	Female
Tukacungurwa Julius	Kabale District Local Government	Male
Bahati Milton	MAAIF, NADDEC	Male
Mugoza Julius	MAAIF	Male
Higenyi Siraji	Budaka District Local Government	Male
Mulabbi Emmanuel	Butebo District Local Government	Male
Kaima Peter	Mbarara District Local Government	Male
Awula Daniel	Sembabule District Local Government	Male
Mutabazi Maurice	Ibanda District Local Government	Male
Twinamatsiko Robert	Kiruhura District Local Government	Male
Bayo John Paddy	Isingiro District Local Government	Male
Osujo Job	Soroti District Local Government	Male
Cema Philliam	Arua District Local Government	Male
Esau Martin	MAAIF, NADECC	Male
Kiwanuka Sperito	MAAIF	Male
Sheila Ayoo	ILRI	Female
Francis Njoroge	ILRI	Male
John Gachoya*	KEMRI	Male
Dunstone Beti*	KEMRI	Male
Bernard Bett*	ILRI	Male
Dan Tumusiime*	MAAIF	Male
Sheila Bitoh Agha*	ILRI	Female
Joel Lutomiah*	KEMRI	Male

*Trainer