A BAT SAMPLING PLAN FOR STOP SPILLOVER, IN BUNDIBUGYO DISTRICT, UGANDA

A report for STOP Spillover

March 2023

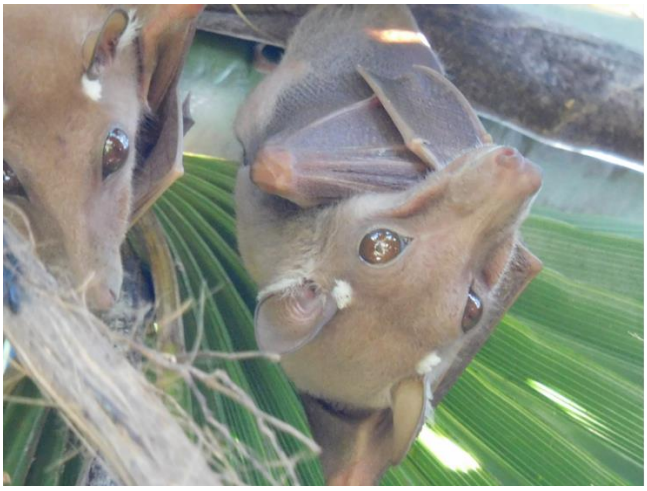


Photo credits: Uganda country team

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STOP SPILLOVER

Strategies to Prevent (STOP) Spillover, a USAID-funded project led by Tufts University, is a global consortium of experts in human, animal, and environmental health who will take the next step in understanding and addressing the risks posed by known zoonotic viruses that have the potential to spill over and cause pandemic crises.

This document is made possible by the generous support of the American people through USAID. The contents are the responsibility of STOP Spillover and do not necessarily reflect the views of USAID or the United States Government.

ACRONYMS

AFROHUN:	Africa One Health University Network
EID:	Emerging infectious diseases
IACUC:	Institutional Animal Care and Use Committees
PIT:	Passive Integrated Transponders
PPE:	Personal Protective Equipment
SOP:	Standard Operating Procedure
STOP:	Strategies to Prevent
USAID:	U.S. Agency for International Development
VHF:	Viral haemorrhagic fevers

INTRODUCTION

Surveying for bats and their roosts is an important aspect of bat work for both research and conservation purposes (JNCC, 2004). Part of the reason for the recent focus on bats in biological research; is linked to bats having been implicated in numerous emerging infectious diseases (EID) events, and being recognized as important reservoir hosts for viruses that can cross species barriers to infect humans and other domestic and wild mammals (Calisher et al., 2006). Before then bats were probably one of the most under-recorded groups of vertebrates. However, rising interest and the accumulation of data have led to a great increase in the number of records and a better idea of the distribution and relative abundance of the various bat species (JNCC, 2006). Advances in equipment, notably bat detectors, have increased the potential for surveying bats away from roosts and created a doorway to understanding the foraging and habitat needs of bats.

In terms of the number of living species and genera, bats are second only to rodents, which constitute the largest order (Rodentia) of mammals in overall species richness. The evolutionary trait for active flight has given bats unique vagility (potential for long-distance travel), and they often aggregate in very large colonies (Turmelle and Olival, 2009). However, despite their abundance and uniqueness, relatively little is known about the species from which zoonotic viruses emerge to cause human disease (Calisher et al., 2006). But the role of bats in viral disease is well known (Sulkin and Allen, 1974), particularly their role as hosts for paramyxoviruses, alphaviruses, flaviviruses, rhabdoviruses, and arenaviruses. In effect a lot of the information gathered on the role of bats in the maintenance and spread of viruses has been from species of Microchiroptera (insectivorous bats), and there is relatively little information available for members of the suborder Megachiroptera (flying foxes and fruit bats) (Mackenzie, Field and Guyatt, 2003).

ACTIVITY OBJECTIVES

To both improve understanding of the risk for spillover to support the development and implementation of effective interventions to reduce human exposure to bats, which will reduce spillover risk.

This document thus provides a detailed description of the procedures that will be followed to trap and collect specimen from bats in Bundibugyo district. It should be noted that this document could be used in other regions of the country implementing a similar program.

The specific objectives of the bat sampling framework are indicated in Table 1 below.

Table 1: Specific objectives

Objective 1: 1. To document the procedures that will be used to trap bats at the bat-human interface.
Objective 2: 2. To document the procedures that will be used to collect specimen from trapped bats.

ABOUT BUNDIBUGYO DISTRICT

The STOP spillover project team used an outcome mapping process from which national stakeholders selected the bat-human interface as a priority spillover risk in the country, and Bundibugyo district as a starting point. Bundibugyo is located in Western Uganda approximately 378 kms by road, from the capital city, Kampala. It is located along the Rwenzori Mountain ranges and in close proximity to the Democratic Republic of Congo. Predominant ethnicities include the Bamba-Babwisi and the Bakonjo however, other ethnicities coexist with them including the Batooro. The district lies between two conservation areas, the Semliki National Park and the Rwenzori National Park, which has led to a high level of human-wildlife-forest ecosystem interactions. The area has plenty of rock shelters and caves that are habitats for wildlife, including bats. The people of Bundibugyo are largely farmers who depend on cocoa, coffee, and vanilla to earn a living. Farming activities evolve around the two major rainy seasons of March-May and July-November.

Study sites

Within Bundibugyo district, three (3) regions were selected by stakeholders during a 3-day outcome mapping process for research and intervention activities around the bat-human interface. The regions considered and reasons for consideration are indicated below and in the map in Figure 1.

1. Burondo subcounty (neighbours Semuliki National Park)
2. Harugale subcounty (neighbours Rwenzori Mountains National Park)
3. Ntandi town council (represents areas with bats in homesteads, schools, churches and also has areas where bat hunting is known to occur).

Bats will be captured from several different sites and roosts within the selected sub-counties above. A series of roosting sites will be identified which include: caves, coffee/banana/cocoa plantations, and buildings like churches, hospitals, schools, and households. These roosting sites will provide an ideal opportunity to study the ecology of bats in the context of human disturbance and pathogen exposure risks and will be the primary study locations for the bat-human interface.

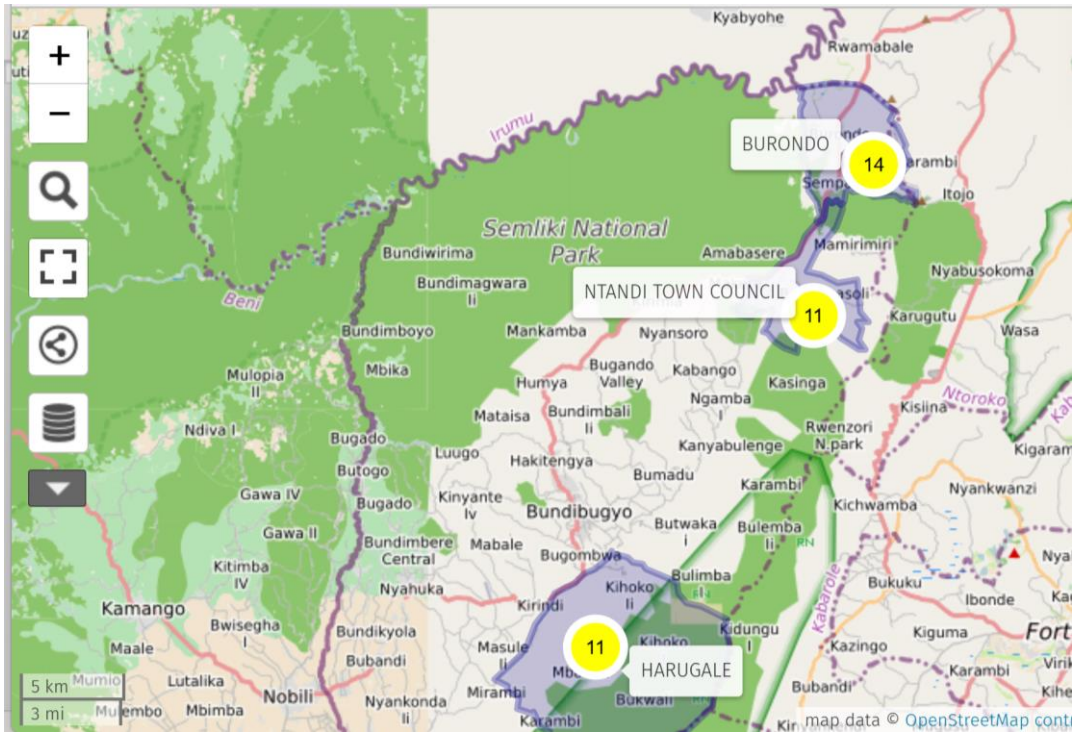

Figure 1. Map of Bundibugyo showing the 3 bat-human interface project areas and already mapped bat roosts numbers (in yellow).

Study species

Like many areas of Uganda, the Bundibugyo district has got a considerable diversity and abundance of both insectivorous (Microchiripotera) and fruit-eating bats (Megachiroptera). At present we estimate the Bundibugyo area to contain over 18 species of bats. But this estimate is largely dependent on previous studies by Kityo & kerbis (1996), and general personal observations by bat ecologists in the region. Although all these species may be preferred for capture and virus detection sampling, priority will be given to those bat species that have been reported or suspected to participate in Zoonotic diseases transmission; especially those related to Viral hemorrhagic fevers (VHF), such as Marburg and Ebola.

Examples of bats that may be chosen for sampling will include fruit bats such as: *Egyptian rousette* (*Rousettus aegyptiacus*), Least epauletted fruit bat (*Epomophorus minimus*), Franquet's Epauletted fruit bat (*Epomophorus franqueti*), Angolan soft-furred fruit bat (*Lyssonycteris angolensis*), Gambian epauletted fruit bat (*Epomophorus gambianus*), etc, and indecorous bats in the family *Hipposideridae*, *Rhinolophidae*, *Molossidae* and *Vespertilionidae*.

METHODS OF CAPTURE AND PROCESSING OF BATS

Bat capture

- **Harp Traps.** The trap consists of a frame supporting two banks of vertical strung nylon or wire lines. The trapping area is generally much smaller in size (2 m x 2 m) than a mist net (see below). Bats fly into the lines, slide down them and land in the collecting bag underneath. The lines are carefully arranged and tensioned to minimise damage to the bat. The traps are especially suitable for catching bats that weigh less than 30 g. The harp traps will be employed inside caves, at roost entry/exit points and major fly-ways. Harp traps are recommended by the American Society of Mammologists for use where larger concentrations of bats are expected, to minimize the time bats might remain entangled in a mist net.
- **Mist nets.** These usually consist of fine nylon or terylene netting, which is usually held in tension between two poles. Mist nets will also be used around roosts as bats emerge at dusk or return at dawn, but only when use of harp traps is not practical as the only means of capture. For example, a mist nets may be strung across a long narrow cavern opening when only a few bats are present. Mist nets will always be monitored by study personnel, and bats extracted immediately upon capture. Use of mist nets is a procedure recommended for capturing bats by the American Society of Mammologists. Upon capture, bats will be placed individually in a porous cloth bag and tied on a string to prepare for the sampling process. Bats will be gently handled and restrained using leather, puncture-proof gloves.
- **Hand-nets.** These are usually round or kite-shaped frames with handles and are usually available from entomological suppliers. These nets are more useful, particularly when bats are in corners, and free flying. The mechanism of bat capture here is similar to capturing butterflies and other free flying animals, using a sweep motion.

Handling, Processing and Sampling of captured bats

Handling Procedures

1. Each captured bat will be placed into a porous cotton bag (with a draw-string mouth), hung from a sturdy line over a polyethylene sheet (to catch urine), and kept in a cool, dry dark area until sampling time.
    - Bags will be marked with information on the individual bat and time of capture/collection.
    - Ensure that bags are spaced sufficiently to avoid injury.
    - Bats will be checked every hour and fruit juice or sugar water should be offered to frugivorous or nectivorous bats at each interval.
    - Any bats presenting signs of distress will be assessed by the veterinarian and treated accordingly or immediately released. Signs of stress may include excessive vocalization or movement within the bag, or reduced responsiveness.
2. Bats will be weighed (in grams) in bags using a Pesola hanging scale or a tabletop scale

with or without a container (such as a cup).

- The bag (or container) should be torn and both bat and bag should be weighed together. Once the bat is removed from the bag for sampling, the bag should be re-weighed and subtracted from the previous total.

3. The bat should be removed from the bag and the samples below collected. The order of sampling may vary. For example, urine may be expelled on initial handling and urine would then be the first sample collected.
   Note: check bag for fresh faeces before continuing. If fresh faeces are available, these may be used as a sample and then a rectal swab is not necessary. The sampler must be certain that the faeces belong to the bat being sampled. Bags should be either discarded after first use or washed/disinfected between uses.
4. Bats will not be held longer than 6 hours. Frugivorous and nectivorous bats will be given 100% fruit juice or sugar water prior to release. Animals will be released at the site of capture, taking care to avoid releases near deployed capture equipment.

Processing of bats

Before samples are collected from each bat, identification and biometric measurements may be collected at the discretion of the sampling experts, although they are not mandatory (unless they are needed for species identification).

These will include:

- Whole body photograph (that should include the animal ID, site and date of collection)
- Identifying characteristic photographs (that should include the animal ID, site and date of collection)
- Age class
- Sex
- Body weight
- Body condition
- Biometric measurements (see Biometrics section below for details)
- Additional morphometric measurements
- Reproductive status

Contingency plans

The team will have a veterinarian to help bat ecologists during handling, processing and sample collection on bats. Should unintentional severe injury occur to the bats or they become moribund, bats will be humanely euthanized in line with the approved IACUC protocol (Annex 1). Bat carcasses will be tagged and placed in formaldehyde for deposition in the Makerere University Museum collection.

PIT tag marking

Passive Integrated Transponders (PIT) tags (Avid Inc., Norco, California, USA) will be implanted in bats for the purposes of identifying individual bats over the course of the study. The 0.06 g, 12 3 2.1 mm PIT tags emit an instantaneous (0.04 sec) 125 kHz signal with a unique nine-digit code when they pass within about 15 cm of an activating reader. Through the use of this marking technique, we will have the power to identify individual bats recaptured in their original or neighbouring caves or other roosts, and determine recapture rates for population size estimation, and association of pathogen infection status over time. We will follow the established methods in accordance with the IACUC protocol.

SAMPLE COLLECTION PROCEDURES

A suite of samples can be collected, and the specific samples and preservation methods will vary according to the goals of each specific study. Here we provide guidance on collection of samples aimed at virus surveillance. The following basic set of samples should be collected from each animal where possible (If only one sample can be collected, then placed into Lysis/binding solution) The lysis/binding solution mentioned in this protocol is part of the MagMax Pathogen nucleic acid extraction kit. If you will be using a different kit/solution please refer to the manual recommendations regarding sample to lysis proportions.

1. Two oral swabs - each in 500 µL Lysis/binding solution
2. Two faecal samples - each with max of 300 µL/0.3cc faeces in 1 mL Lysis/binding solution

Or

Two rectal swabs - each in 500 µL Lysis/binding solution

3. Two whole blood samples - 2 x 200 µL aliquots, each in 500 µL Lysis/binding solution
4. Two serum samples - 2 x 500 µL aliquots (only if more than 2ml of blood available), frozen without media. A minimum of 100 µL serum (single aliquot) should be collected to be useful for STOP SPILLOVER diagnostic testing

   Note: If animals are too small to collect two blood tubes (for whole blood and serum), collect serum and save remaining clot in Lysis/binding solution after serum separation

   Two urogenital swabs/urine samples - each with max of 300 µL of urine in 500 µL Lysis/binding solution

All collected samples will immediately be Frozen in liquid nitrogen in the field and later, be transferred to -80°C lab freezer.

Sample of bats and conduct pathogen testing

This task will address viral shedding and spill-over risk to humans. Sub-tasks will also assess the seasonal shedding of pathogens by bats. Bats captured or re-captured will be non-destructively sampled (blood, oral and faecal swabs) and molecularly screened for a panel of pathogens. Upon capture of bats, the bat will be scanned for identification purposes, using PIT tag reader. If not yet

marked, a PIT tag will be implanted. Bats will be bled using a 27-gauge needle to lance the interfemoral (saphenous) vein directly into multiple heparinized haematocrit tubes in accordance with the IACUC protocol. Less than 0.6-1% of body weight in blood volume will be taken from individual bats, and each bat will be blood sampled no more than once every 3 months. Saliva, faecal, and blood samples will be collected in duplicate, with one aliquot directly into MagMax Lysis buffer to ensure inactivation of infectious agents and preserve samples for RNA extraction.

PPE guidelines

When capturing and handling bats, project participants will wear either Tyvek suits or close-front gowns, rubber boots, eye protection, and half-face respirators (see Annex II). Inside caves, hard hats, and head-lamps will also be used. For hand protection, double nitrile gloves will be donned in addition to leather puncture-proof gloves. When bats are being extracted from nets and manipulated, it will be necessary to remove the leather glove from one hand in order to maintain dexterity to safely remove the bat from the mist net or collect samples. The bat will always be held with a leather glove to protect the handler from being bitten. Half-face respirators will be chemically disinfected and reused, changing the filters every sampling day. Gowns, suits, nitrile gloves, and face shields will be chemically disinfected in the field using spray bottles of 5% Microchem Plus, and placed in biohazard bags for incineration.

Study timeline and endpoints

Seasonal field trips will be planned in order to obtain data from this population of bats during the multiple wet (Sept – Nov; Mar—May) and dry (Dec—Feb; Jun—Aug) seasons for this year of sampling. This will enable the monitoring of seasonal variations in species diversity and virus prevalence.

Team composition

The entire team shall constitute the following personnel as described in the Table 2 below.

Table 2. List of required personnel

SN	Personnel	Number	Description
01	Bat ecologist/biologist	02	Will be the main focal persons involved in Bat capture and processing
02	Wildlife veterinarian	01	To assist in sample collection and ensuring welfare of the bats under study
03	Research assistants	05	These will assist in casual work, such as carrying equipment and materials, assist in setting trap for bats. These might be some of the bat monitoring agents, already involved in bat monitoring
04	Laboratory technicians	01	Also to assist in sample collection from bats during bat handling and processing

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1. **Kityo Robert and Kerbis C. Julian (1996):** *Observations on the Distribution and Ecology of Bats in Uganda. Journal of East African Natural History, 85(1):49-63*. Nature Kenya/East African Natural History Society. http://www.bioone.org/doi/full/10.2982/0012-8317%281996%2985%5B49%3AOOTDAE%5D2.0.CO%3B2

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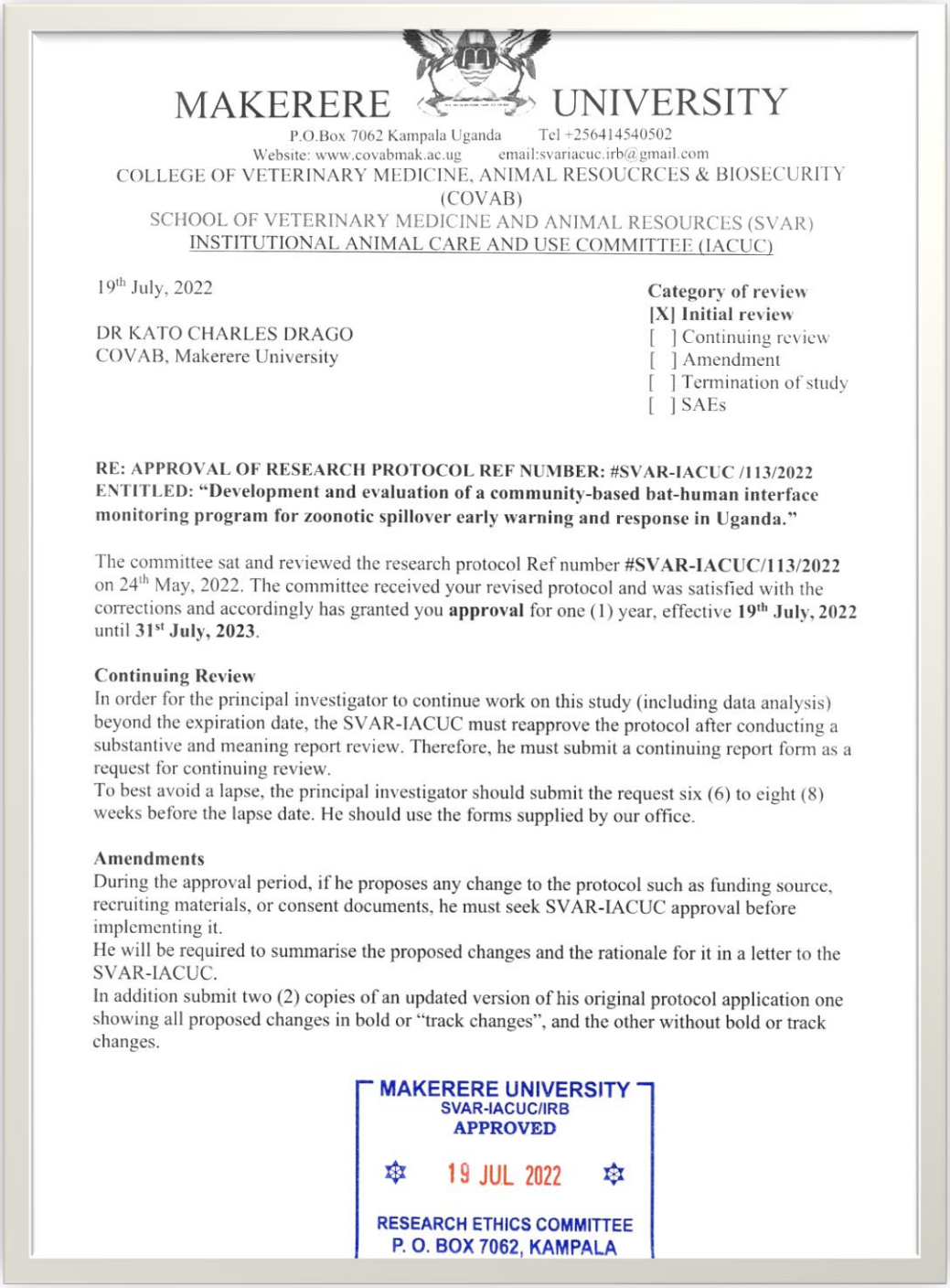
ANNEX

ANNEX ONE: IACUC PROTOCOL APPROVAL LETTER.



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SCHOOL OF VETERINARY MEDICINE AND ANIMAL RESOURCES (SVAR)
INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)

19th July, 2022

DR KATO CHARLES DRAGO
COVAB, Makerere University

Category of review
[X] Initial review
[ ] Continuing review
[ ] Amendment
[ ] Termination of study
[ ] SAEs

RE: APPROVAL OF RESEARCH PROTOCOL REF NUMBER: #SVAR-IACUC /113/2022 ENTITLED: "Development and evaluation of a community-based bat-human interface monitoring program for zoonotic spillover early warning and response in Uganda."

The committee sat and reviewed the research protocol Ref number **#SVAR-IACUC/113/2022** on 24th May, 2022. The committee received your revised protocol and was satisfied with the corrections and accordingly has granted you **approval** for one (1) year, effective **19th July, 2022** until **31st July, 2023**.

Continuing Review
In order for the principal investigator to continue work on this study (including data analysis) beyond the expiration date, the SVAR-IACUC must reapprove the protocol after conducting a substantive and meaning report review. Therefore, he must submit a continuing report form as a request for continuing review.
To best avoid a lapse, the principal investigator should submit the request six (6) to eight (8) weeks before the lapse date. He should use the forms supplied by our office.

Amendments
During the approval period, if he proposes any change to the protocol such as funding source, recruiting materials, or consent documents, he must seek SVAR-IACUC approval before implementing it.
He will be required to summarise the proposed changes and the rationale for it in a letter to the SVAR-IACUC.
In addition submit two (2) copies of an updated version of his original protocol application one showing all proposed changes in bold or "track changes", and the other without bold or track changes.

MAKERERE UNIVERSITY
SVAR-IACUC/IRB
APPROVED
19 JUL 2022
RESEARCH ETHICS COMMITTEE
P. O. BOX 7062, KAMPALA

ANNEX TWO: SUPPLY AND EQUIPMENT LIST

PPE

- ☐ Tyvek-like suits
- ☐ Flexible face shield or other eye
- ☐ protection N95 or P100 respirator
- ☐ Nitrile examination gloves
- ☐ Washable shoes

First Aid

- ☐ Betadine or benzalkonium chloride
- ☐ First aid kit (with post-exposure prophylactic vaccine if working in remote areas where vaccine is not rapidly accessible)

Data Collection

- ☐ Datasheets (or tablet for direct data entry) Pencils
- ☐ GPS

Capture and Handling

- ☐ Mist nets, poles and ropes
- ☐ Flagging tape
- ☐ Leather gloves
- ☐ Holding bags
- ☐ Spring/electronic balance
- ☐ Dial/digital caliper
- ☐ Stainless steel wing rulers
- ☐ Large ziplock bag
- ☐ Chemical restraint requirements
- ☐ Camera
- ☐ Identification guides

Sampling

- ☐ Processing trays
- ☐ Permanent lab markers for tube labeling
- ☐ Cryovials
- ☐ Needles 25G, 27G
- ☐ Needles and syringes for blood draws
- ☐ Sterile swabs (dacron/polyester)
- ☐ Cryo resistant tube labels
- ☐ Cryovial rack
- ☐ Cryoboxes and dividers

- [ ] 75 µL glass hematocrit tubes (heparinized)
- [ ] Plastic vacutainers (EDTA and dry)
- [ ] Pipettors and disposable tips
- [ ] Portable centrifuge for vacutainers
- [ ] Portable centrifuge for hematocrit tubes
- [ ] Cryo gloves
- [ ] Fine point forceps Scissors
- [ ] Dissection kit Lysis/binding solution reagent
- [ ] Viral Transport Medium (VTM)
- [ ] RNAlater reagent
- [ ] Buffered formalin
- [ ] 95% ethanol Lighter
- [ ] Liquid nitrogen shipper/liquid nitrogen

Waste Disposal and Decontamination

- [ ] Paper towel
- [ ] Sharps containers
- [ ] Bleach
- [ ] 95% ethanol
- [ ] Biohazard bags
- [ ] Sprayer