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Control of Rabies in Malawi – with a Focus on Diagnostics and the Role of the Paraveterinarian

Kontroll av Rabies i Malawi – med fokus på diagnostikk og paraveterinærens rolle

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Kull 2018

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Preface and acknowledgements

The opportunity to participate in a One Health project and spend two months in Malawi, exploring rabies control could not be missed. We still cannot believe our luck, and we hope that this marks the start of an exciting career in veterinary medicine working with zoonotic diseases.

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Elizabeth Akinsanmi-Guren and Charlotte Eikeskog Ravnås

Summary

Title: Control of Rabies in Malawi – with a Focus on Diagnostics and the Role of the Paraveterinarian

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Rabies is a neglected viral disease that kills 59,000 people worldwide each year. Most of these deaths occur in Africa and Asia and are almost always due to dog bites. Rabies is vaccine preventable and mass dog-vaccination can effectively block the transmission of rabies to humans. Malawi is a South-Eastern African country where rabies is endemic. Due to inadequate testing of suspected rabid animals and poor surveillance systems, the true burden of rabies is unknown in Malawi. In this study, barriers to rabies control and sampling were explored. Different transport media for submission of samples to laboratories were assessed, as well as the suitability of RT-qPCR and LFT testing methods in Malawi's Central Veterinary Laboratory. The current situation in rabies control and surveillance was evaluated through individual interviews and focus group discussions with Malawian Assistant Veterinary Officers. The elimination of rabies in Malawi is hindered by poor resource allocation to awareness campaigns, dog vaccination, rabies diagnostics and inefficient rabies surveillance systems. A 'One Health' approach is required with true collaboration between

animal and human healthcare sectors, with more resource allocation to rabies control, so that Malawi can progress towards eradicating dog-mediated rabies cases in humans.

Norsk Sammendrag

Rabies er en neglisjert zoonose som årlig dreper 59 000 mennesker. De fleste dødsfallene rammer i Afrika og Asia, og de aller fleste tilfellene skyldes hundebitt. Rabies kan forhindres med vaksinasjon. Vaksinasjon av hundepopulasjonen vil effektivt forhindre rabiessmitte til mennesker. Malawi er et land i sør-østlige Afrika, med endemisk forekomst av rabies. Mangelfull testing og dårlig overvåkning gjør det vanskelig å vurdere omfanget av sykdommen i Malawi. I denne studien undersøkes barrierer for kontroll og prøvetakning av rabies i Malawi. Ulike transportmedier for prøvemateriale til laboratorieanalyser ble vurdert. Det ble også gjort en sammenlikning av de diagnostiske metodene RT-qPCR og LFT ved CVL. Fokusgruppe-diskusjoner og individuelle intervjuer med malawiske paraveterinærer ble gjort for å undersøke hva som gjøres for å kontrollere og overvåke rabies i felt. Rabiesbekjempelse i Malawi hindres av ressursmangel i alle ledd, fra holdningskampanjer til vaksinering, diagnostikk og overvåkning. Et «Én Helse»-perspektiv med tverrfaglig samarbeid mellom sektorer for folkehelse og -dyrehelse, i kombinasjon med økt bevilgning av økonomiske midler til kontrolltiltak er essensielt for fremgang i bekjempelse av hundemediert rabies hos mennesker i Malawi.

Abbreviations and Definitions

AVO	Assistant Veterinary Officer
BSC	Blood spot card
BSL3	Bio-Security Level 3
cDNA	Copy Deoxyribonucleic acid
CNS	Central Nervous System
CVL	Central Veterinary Laboratory
Cq	Quantification cycle
DAHLD	Department of Animal Health and Livestock Development
DAHLD-O	District Animal Health and Livestock Development Officer
dFAT	Direct fluorescent antibody testing
DNA	Deoxyribonucleic acid
EBC	Elution buffer control
EPC	Elution positive control
FAO	Food and Agriculture Organization
FGD	Focus group discussion
FITV	Fluorescein isothiocyanate
FTA	Flinders Technology Associates
GARC	Global Alliance for Rabies Control
ILRI	International Livestock Research Institute
LFT	Lateral Flow Test

LSPCA	Lilongwe Society for the Protection and Care of Animals
NGO	Non-governmental organisation
LUANAR	Lilongwe University of Agriculture and National Resources
NVI	Norwegian Veterinary Institute
PBS	Phosphate buffer saline
PCR	Polymerase Chain Reaction
PEP	Post-exposure prophylaxis
PPE	Personal protective equipment
PrEP	Pre-exposure prophylaxis
RIG	Rabies immune globulin
RNA	Ribonucleic Acid
RT-qPCR	Reverse Transcriptase-qualitative Polymerase Chain Reaction
SARE	Stepwise Approach towards Rabies Elimination
SD	Standard deviation
Se	Sensitivity
Sp	Specificity
UV	Ultraviolet
WHO	World Health Organization
WOAH	World Organization for Animal Health

1. Introduction

1.1 Rabies

Rabies is a long-feared zoonotic disease and has been recognised for at least 4,000 years (Tarantola, 2017). The virus targets the central nervous system of most mammals, including humans. Once clinical signs appear, infection with the disease is fatal in almost all cases.

1.1.1 The virus

The classical rabies virus is enveloped, bullet-shaped and measures approximately 180 nm × 75 nm (Madhusudana & Sukumaran, 2008). The external surface glycoprotein (G), one of five proteins encoded by the virus genome, forms spikes that surround the virus envelope and is necessary for viral attachment to cell receptors. It is also the chief antigen that can induce the production of rabies virus neutralizing antibodies (Madhusudana & Sukumaran, 2008).

Fifteen other lyssaviruses have been identified that all share structural characteristics with the rabies virus. While rabies can have multiple host reservoirs, these 15 lyssaviruses have bats as their sole reservoir (Wunner & Conzelmann, 2020). Currently, only six of the 16 lyssaviruses in the genus have caused a rabies-like encephalomyelitis in humans (Wunner & Conzelmann, 2020).

The rabies virus itself is fragile, and can be destroyed by sunlight, desiccation, soap and water and other agents such as 40-70% alcohol or iodine solution (Pounder, 2005). In polar conditions infectious rabies virus has been recovered from a frozen fox carcass months after the death of the animal, while viral inactivation could occur within hours in the decomposing tissues of an animal under warm summer temperature (Wunner, 2007).

1.1.2 Pathogenesis

Transmission of rabies most commonly occurs via a bite from an infected animal when saliva containing the virus encounters pierced skin (James F. Zachary, 2017). Other routes of infection have been described, such as contact with mucous membranes or via aerosol in bat caves (Dietzschold et al., 2008). The virus first replicates in tissues, enters nerve cells through unmyelinated axon terminals and is retrogradely transported to the cell soma. It is estimated that the virion travels along axons at a rate of 3mm/h, but the exact mechanism that permits trans-synaptic travel is still unknown (Dietzschold et al., 2008). After travelling along the spinal cord and reaching the brain, viral replication within cerebral neurons leads to cell damage, inflammation and encephalitis (James F. Zachary, 2017). In the late stages of infection, rabies migrates to the salivary glands, where it replicates in mucogenic acinar cells (Dietzschold et al., 2008) and is released into the host's saliva, thus allowing infection of the next host through a bite.

1.2 Epidemiology, occurrence, and reservoirs

The rabies virus has a variety of hosts and can be transmitted to all mammals, but to varying degrees. There are for example, no known rodents that are reservoirs for the disease (Wunner, 2007). Bats are the ultimate reservoir for all 16 known lyssaviruses, with rabies being the sole virus in the genus that has numerous host reservoirs (Wunner & Conzelmann, 2020). Rabies has two transmission cycles, sylvatic and non-sylvatic.

While infected dogs are the cause of 99% of human rabies cases, wild carnivores such as jackals in Africa can function as maintenance hosts (Wunner, 2007). Outbreaks of bat-mediated rabies are likely to be linked to habitat disturbance (Del Médico Zajac et al., 2020).

Rabies kills approximately 59,000 individuals annually. Of these deaths, over 95% occur in Africa and Asia and four in ten of the victims are children aged under 15 years (John

et al., 2021). Due to extensive vaccination efforts, human rabies cases in the developed world are now extremely rare (Algeo et al., 2017)

1.2.1 Rabies in animals

A bite from another infected animal is the most common route of rabies transmission for animals. Infection of carnivores through eating rabies-infected carcasses is possible, but uncommon (Wunner, 2007). In animals, the incubation period is from ten days to several months (Wunner, 2007), and is followed by a short prodrome period, encephalopathy, and death.

Initial signs of rabies vary and can include fever, anorexia, vomiting and lethargy. Behavioural abnormalities can also be observed, such as aggression. After the prodromal period, an acute neurologic period follows within one to two days. This period involves hyperactivity, caused by the replication of the rabies virus in the brain. The two different clinical manifestations of the virus, encephalitic and paralytic rabies, are thought to be a result of viral replication and damage at specific CNS sites (Wunner, 2007). Death within one week to ten days of the onset of clinical signs usually occurs (Wunner, 2007).

1.2.2 Rabies in humans

In humans five stages of rabies are observed: incubation, prodrome, an acute neurologic stage followed by coma, and death (Rupprecht, 1996). There are only 15 documented cases of human survival after clinical signs have emerged (Subramaniam Mani, 2016). The incubation period is most commonly between one and three months, but less than ten days and over two years have been recorded (Shankar et al., 2012). The Incubation period in children or individuals bitten close to the CNS, for example in the face, may be shorter. (Rupprecht, 1996). Once symptoms occur, they are generally non-specific (fever and tiredness); however, affliction of the respiratory, gastrointestinal and CNS systems can occur

(coughing, dyspnoea, vomiting, diarrhoea, anxiety and nervousness). Humans can also experience pain or paraesthesia at the site of the bite wound (Rupprecht, 1996). The acute neurologic stage is reached when a person displays CNS dysfunction and manifests as the encephalitic, (furious) form of rabies with hyperactivity or the paralytic (dumb) form of rabies if paralysis is the main clinical sign (Ghosh et al., 2009). In both forms, individuals can suffer from fever, convulsions, hyperventilation, and increased salivation (Rupprecht, 1996).

The three standard features of furious rabies include 1) evidence of autonomic dysfunction, 2) wavering consciousness, and 3) phobic spasms (Madhusudana & Sukumaran, 2008). Hydrophobia and aerophobia are phobic spasms manifested by almost all patients with furious rabies, however, they may not be observed in the later stages of sickness when sleepiness and coma are present. Hydrophobia is almost pathognomonic for rabies (Madhusudana & Sukumaran, 2008). Patients with furious rabies ultimately become confused and aggressive, before falling into a coma and dying. Individuals with paralytic rabies can be harder to diagnose due to there being a number of differential diagnoses. Approximately 20% of people develop this form of rabies where the clinical signs associated with encephalitic rabies never emerge or emerge late in course of illness. Progressive paralysis dominates, with impairment typically starting around the bite site, progressing to all limbs and the pharyngeal and laryngeal muscles, and patients usually always present with a fever (Madhusudana & Sukumaran, 2008). Hydrophobia is uncommon and only manifests shortly before coma and death. Patients with the paralytic form of rabies usually survive longer than those with the encephalitic form (Madhusudana & Sukumaran, 2008).

1.3 Prevention of rabies

Human rabies cases persist due to the failure to implement tried and tested tools and procedures. Indeed, many countries have succeeded in controlling dog mediated rabies in humans, and effective measures are available. In 2015, WHO, WOAH, Food and Agriculture

Organization (FAO) and the Global Alliance for Rabies Control (GARC) set a goal of 'Zero by 30' the aim being to stop human deaths caused by dog-mediated rabies by 2030. Dog vaccination, disease surveillance, public awareness and post-exposure prophylaxis (PEP) in humans are examples of such measures available. To reach this goal, STOP-R, a five-pillar framework was developed. This action plan encompasses 1: Socio-cultural, 2: Technical, 3: Operational, 4: Political and 5: Resource-related elements to support countries hit by rabies in eliminating the disease (Abela-Ridder et al., 2018).

At the socio-cultural level (**S**TOP-R), awareness of the disease amongst the communities where rabid dogs are found is vital. Awareness encompasses responsible dog ownership and care of dogs, knowing how to act after a dog bite and where to access PEP. Awareness of rabies should also extend to travellers visiting countries where rabies is found, so that they can consider Pre-exposure prophylaxis (PrEP) and avoid free-roaming dogs (Pounder, 2005). At the technical level (S**T**OP-R), effective animal and human healthcare systems are key, and this includes vaccination, rabies diagnostics and surveillance. Vaccination is probably the most central measure all together. PrEP or PEP vaccination of humans can prevent rabies in exposed humans, but large-scale canine vaccination is the most important and most cost-effective control measure as it can break transmission of the virus from dogs to humans. In many parts of Central and South America, dog-mediated rabies has been successfully brought under control through dog vaccination programs (Lembo et al., 2010).

With regards to operation (ST**O**P-R), the One Health approach is at the heart of rabies elimination efforts. This approach can be defined as a collaborative, multisectoral, and transdisciplinary approach, that draws on the expertise of animal, human and environmental health specialists. (Haselbeck et al., 2021). Rabies is a zoonosis, and a One Health approach against rabies is essential at the organisational level so that these three sectors truly

collaborate, align their work plans, and measure their outcomes. Through controlling dog-mediated rabies, the cost of rabies control in humans is reduced (by lowering the number of PEP treatments required, for example) (Acharya et al., 2020).

Rabies has the highest case fatality ratio of all infectious human diseases, yet is still a neglected disease (Haselbeck et al., 2021), demonstrating that elimination of the disease cannot be achieved without the political prioritisation (STO**P**-R) of rabies control and investment of resources (STOP-**R**).

1.3.1 Vaccination

It was first in 1885 that a human patient was administered Louis Pasteur's live attenuated rabies virus vaccine and PEP was achieved. (Tarantola, 2017). No matter the source of viral exposure, human rabies cases are preventable with the correct wound care immediately after the bite, speedy administration of the rabies vaccine and rabies immune globulin (RIG) (Franka et al., 2017). Individuals vaccinated before exposure (PrEP) still require treatment after exposure to the virus. The WHO provides guidelines for wound care and the type of PEP to be provided depending on an individual's level of exposure to the virus (WHO, 2023).

It is also recommended that on an annual basis, at least 70% of the dog population must be vaccinated to reach herd immunity and block the transmission cycle (Mbilo et al., 2021). Oral vaccinations of animals can be used to interrupt the sylvatic transmission cycle of rabies, and these have been successfully employed in Western Europe from 1977 onwards to reduce rabies cases amongst the fox population (Wunner, 2007). In many developed countries, including North America, Western Europe, Japan and South Korea and parts of Latina America, mass vaccination of dogs has successfully controlled or eliminated canine rabies and thus also dog mediated human rabies (WOAH, 2018). Rabies control, such as dog vaccinations and oral vaccines for sylvatic carriers is costly, however. The 2017 cost of

annual rabies control in the United States was estimated to be equivalent to $646 million (Algeo et al., 2017).

1.3.2 Rabies in developing countries

In developing countries of Asia and Africa, a dog bite can still evoke fear, as 95% of the world's human rabies cases occur there. The disease is endemic in Africa, and no African country has yet been reported free from rabies to date (Algeo et al., 2017). It is estimated that more than a third of the annual 59,000 human rabies deaths occur on the continent (Hampson et al., 2015). However, inadequate rabies surveillance systems and irregular reporting mean it is difficult to assess the true impact of the disease and move towards the 'Zero by 30' goal of elimination (Mbilo et al., 2021).

1.4 Surveillance and detection of rabid animals

Disease surveillance is necessary to determine the prevalence of a disease, its geographic spread and to uncover outbreaks (Franka and Wallace, 2018). Surveillance programs help enable control, prioritisation of eradication efforts, establish prevention influence policy and allocation of resources (Taylor et al., 2015). A well-functioning diagnostics and surveillance program combined with a national disease notification system is essential to achieve the "Zero by 30" goal (Mbilo et al., 2021).

Surveillance of rabies is essential in rabies-endemic countries to control and eradicate the disease (WOAH, 2018). Rabies is notifiable in both humans and animals in Malawi, and the country has a passive surveillance for rabies through testing of potentially rabid animals at the Central Veterinary Laboratory (CVL), the national reference laboratory for rabies. However, relatively few samples are being analysed compared to presumed occurrence in the dog population, and underreporting and misdiagnosis is a problem (Kainga et al. 2023, Mastala et al., unpublished)

Confirmation of rabies in animals is done post-mortem by laboratory analysis (WOAH, 2018). Therefore, in rabies endemic countries, animals with clinical signs of rabies, or animals dead after displaying clinical signs of rabies should be tested (WOAH, 2018).

Euthanasia of suspected rabid animals and rabies testing might also be indicated to protect human and livestock health, for example in bite-cases.

1.4.1 Sampling and testing for rabies

Rabies is deadly, and safety measures must be taken when handling and sampling suspected rabid animals. Samples should be collected by trained, vaccinated personnel, with personal protective equipment (PPE), such as gloves, face masks and face shields. Sharp equipment should be handled carefully, to avoid self-contaminating injuries.

WOAH recommends that brain samples from suspected rabid animals are collected during necropsy in an appropriate laboratory facility from the brain stem, Ammon's horn, thalamus, cerebral cortex, cerebellum, and medulla oblongata (WOAH, 2018).

If sampling of brain tissue is done in the field, it is recommended to avoid opening the cranium. One method is the occipital foramen route for brain sampling which involves inserting a hollow plastic tube (pipette with tip removed, straw, truncated syringe, etc), with a diameter of approximately five mm, from one lateral end of the occipital foramen towards the eye of the opposite side. This sample will contain brain tissue from the mentioned areas of the brain (WOAH 2018). The second method for field use is the retro-orbital route. The sampled brain tissue is the same as for the occipital foramen route, but it is collected in the opposite direction through a hole in the posterior wall of the eye socket using a biopsy needle (WOAH 2018, Montaño Hirose et al., 1991).

Diagnostic specimens from animals with suspected rabies should be sent chilled (0-4°C), as fast as possible, to a diagnostic laboratory (WOAH, 2018). The specimen should be double-packed in leakage-proof containers with absorbent material. Samples should be

transported according to regulations given in the International Carriage of Dangerous Goods by Road and/or International Air Transportation Association.

Specimens can be fixed in formalin, glycerol/phosphate buffer saline (PBS), or by other molecular techniques, if it is not possible to send the sample refrigerated or frozen (WOAH, 2018). In countries with high temperatures, frequent power-outages, and poor infrastructure this might be the best way to preserve the virus in the sample during transport (WOAH, 2018).

1.5 Sampling for rabies testing

For many years, direct fluorescent antibody testing (dFAT) was the "Gold-standard" for post-mortem testing for rabies (WOAH, 2018, WHO, 2018). The test has high sensitivity and specificity, and results can be ready in hours. Slides are prepared by touch impression smears from the brainstem and cerebellum, fixed with acetone and stained with antibodies conjugated to fluorescein isothiocyanate (FITC). The antibodies attach to the nucleocapsid proteins of the lyssavirus, and the FITC emits a characteristic apple-green fluorescens with fluorescent microscopy. The disadvantage of this test is that the sensitivity is reduced if the sample is of poor quality, i.e., autolysis due to decomposition.

Reverse Transcriptase-qualitative Polymerase Chain Reaction **(**RT-qPCR) has recently been listed as a primary diagnostic test for lyssavirus (WHO, 2018, Cappelari et al., 2022, WOAH, 2018). The method is highly sensitive and able to detect RNA in samples unfit for testing by dFAT due to decomposition (Prabhu et al., 2018). Risk of cross-contamination and the higher cost is a disadvantage compared to dFAT.

The principle of RT-PCR is that a segment of viral RNA is amplified and detected. RNA is extracted from samples, converted to cDNA by the enzyme reverse transcriptase, before a target sequence is amplified by specific primers in a closed system (WHO, 2018).

The PCR-product is measured after every PCR-cycle (real time) using a TaqMan-probe, which binds the segment between the two primers. In a rabies-positive sample the measured fluorescent signal is higher than the background signal (baseline). The number of cycles needed to surpass the baseline is the Cq-value.

The lateral flow test (LFT) is a rapid immunochromatography based test. Liquid sample material is deposited in a sample slot, and if the sample contains the target analyte, in this case rabies virus, it binds to the conjugates that will bind antigens and resulting in a colour change. Despite the convenience of rapid field testing, LFTs are not approved as a standard diagnostic tool by WOAH. Studies have shown none of the six commercially available LFT perform satisfactorily (Eggerbauer et al., 2016). The tests can be used as an initial screening tool but should be supported by laboratory testing.

1.6 Malawi

Malawi is a landlocked, south-eastern African country, bordered by Tanzania to the north, Mozambique to the east, south and west and Zambia to the north-west. It is divided into three geographical regions: North, Central, and South (Figure 1) and 28 administrative districts (Kainga et al., 2023). Of particular relevance to this study are the four districts Mzimba, Dedza, Lilongwe and Thyolo. The last population and housing census (2018), set the human population at approximately 17.6 million, and of these individuals, 84% reside in rural areas (NSO, 2022).

Figure 1 - Map showing the three regions and mainland districts of Malawi (Maduekwe and De Vries, 2019)

1.6.1 Rabies in Malawi

Rabies is endemic in Malawi (Edelsten, 1995). Estimates suggest that at least 500 people die of rabies annually, costing the country $13 million (Hampson et al., 2015). Domestic canines serve as principal maintenance hosts and can also transmit the virus to wildlife, thus boosting the sylvatic cycle (Kainga et al., 2023). Using a pan-African human to dog ratio of 21.20:1 and the 2018 census, the dog population in the country can be estimated to be 1.2 million, the majority of which are believed to be owned (Mazeri et al., 2021), although also most likely free-roaming (Lembo et al., 2010).

In 2019, Malawi was assessed to be at stage 1.5 by the Stepwise Approach towards Rabies Elimination (SARE) by the GARC. The SARE was developed as a One Health

evaluation tool to guide countries in their work against rabies. The 1.5 ranking, out of five stages, held by Malawi indicates that only small-scale rabies control initiatives are in place (GARC, 2023).

The Animal Health and Livestock Development Policy in Malawi acknowledges rabies as an endemic disease and recommends that 80% of the dog population in the country be vaccinated (Mazeri et al., 2021). However, vaccination coverage is estimated to be 0.5%, and only small areas of satisfactory dog vaccination coverage has been achieved due to the efforts of local non-governmental organisations (NGOs) (Mazeri et al., 2021). Furthermore, there is little data available on the occurrence of rabies in dogs and humans or on vaccination coverage of dogs in the country.

The Department of Animal Health and Livestock Development (DAHLD) forms part of the Ministry of Agriculture in Malawi (Kainga et al., 2023) and is responsible for the control of rabies in Malawi. DAHLD activities include for example, the procuration of canine rabies vaccinations and their distribution to the districts (Kainga et al., 2023). The administration of government procured vaccinations is carried out by Assistant Veterinary Officers (AVOs). AVOs are key animal health personnel in Malawi, where there are few qualified veterinarians in the country (Åsbjer, 2010). They complete a three-year diploma in Animal Health and Production and are allocated to the administrative districts in Malawi. AVOs are intended to be the first point of contact for animal bite-victims in Malawi and provide a referral letter to the victim if rabies exposure is suspected. This letter allows the bite victim to access PEP at the hospital (Åsbjer, 2010). AVOs are also responsible for rabies awareness campaigns and taking samples from suspected rabid animals for diagnosis. Rabies diagnostics occurs at the Central Veterinary Laboratory (CVL) in Lilongwe City. This the veterinary reference laboratory for diseases in Malawi.

2. Study Aims

Surveillance of rabies is necessary for disease control. Sampling, laboratory analyses and reporting of results are fundamental to disease surveillance. In Malawi, rabies kills humans and animals every year, but the national surveillance is not capturing this information.

Our overall aim was to assess the existing barriers for rabies surveillance and control in animals in Malawi, focusing specifically on sampling and testing. A secondary aim was to investigate how AVOs work to control rabies in the field and what barriers they face in their work. We approached our aim through the following objectives:

1. Identifying the most cost-efficient and reliable transport media for rabies samples relevant to conditions in Malawi.
2. Testing agreement between different diagnostic methods for rabies detection, available in Malawi.
3. Describing how rabies surveillance and control is practiced at field level and identifying the barriers.
4. Investigating the potential of increasing and improving sampling by providing training for AVOs.

3. Materials and Methods

3.1. Study design

The study comprised of several parts answering to the objectives of the study and is illustrated in Figure 2.

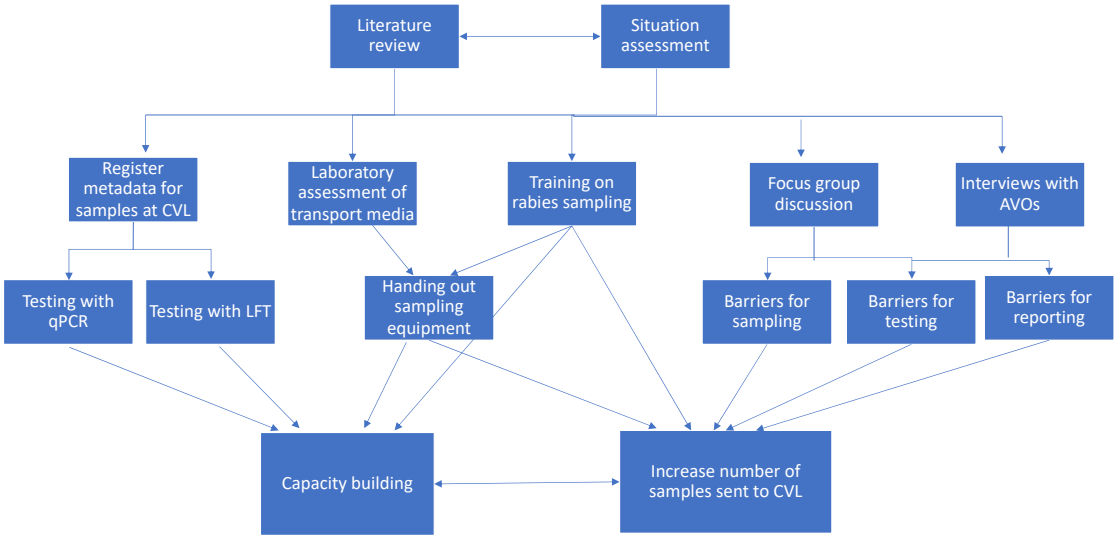

Figure 2- Illustrates how the objectives of the study were approached to answer our aims, assess existing barriers for rabies surveillance and control and to investigate how Assistant Veterinary Officers work to control rabies and the barriers they face.

In brief, this study included the following activities, with the numbers in parenthesis referring to the corresponding objective:

- A literature and preliminary situation review to understand the current rabies situation in Malawi, and prepare for field work in Malawi (2, 4).
- A laboratory assessment of five different transport media to determine what transport media to hand out to the AVOs in Malawi (2, 4).
- Preparations for building capacity in AVOs, for rabies samples collection, including development of training material and provision of sampling equipment (2, 4).
- Create an electronic record of all rabies samples received at CVL including available metadata and previous results from dFAT analysis (1, 3).
- Supporting implementation of RT-qPCR as a diagnostic method for rabies analysis at CVL and test available brain samples from potentially rabid animals (3).
- Test selected brain samples with a rapid Lateral Flow Test (3).

- Perform an agreement analysis between rabies detection methods used in Malawi and compare Lateral Flow Test with RT-qPCR, and dFAT with RT-qPCR (3).
- Provide training for AVOs on collection of rabies samples. Measure whether this training resulted in an increased number of samples at CVL (2).
- Focus group discussions and interviews to investigate how surveillance and control of rabies is happening at field level in Malawi and identify barriers faced by AVOs in performing this work (1, 4).

3.2 Testing different media for preservation of brain tissue spiked with rabies virus

The purpose of this laboratory experiment was to meet objective 2), namely "to identify the most cost-efficient and reliable transport media for rabies samples relevant to conditions in Malawi". Two different lysis buffers, DNA/RNA shield and two types of biosample collection cards were assessed for their ability to preserve rabies virus RNA in brain tissue over different temperatures and time. The presence of rabies virus in the brain was assessed by RT-qPCR.

The experiment required brain tissue with an appropriate concentration of rabies virus. For safety and practical reasons (availability), it was decided to spike brain tissue from a dead cat (*Felis gatus*) with inactivated rabies virus from a commercial rabies vaccine. This would allow us to work outside of the BSL3 laboratory.

The experiment was divided in three parts; Part 1; determining how to spike brain tissue with sufficient concentrations of rabies virus. Part 2; using rabies virus-spiked brain tissue to assess performance of different lysis buffers, DNA/RNA Shield and biosample collection cards for storage at different temperatures and time periods. Part 3; RNA isolation and RT-qPCR analysis of samples from parts 1 and 2. The work was conducted at the NVI.

3.2.1 Determining spiking level of brain tissue (Part 1)

For brain samples to be positive for rabies virus by RT-qPCR with a sufficient viral load, while minimizing risk of cross-contamination due to high viral loads, it was decided that the virus spiked brain tissue ideally should yield a Cq-value between 25 and 30 in the RT-qPCR. This Cq-range coincides with values observed for rabies positive samples from infected foxes and reindeer from Svalbard (Dr Torfinn Moldal, NVI, personal communication). This experiment was performed to determine what volume of vaccine to add per mg of brain tissue to achieve a Cq-value within this range.

Rabisin vet. ® vaccine (Boehringer Ingleheim Lot 497924 – 02.06.2024, Copenhagen, Denmark) supplied inactivated rabies virus strain G52 ≥2,09 $\log_{10}$ OD_{50} (equalling ≥ 320 virus/µl). Brain tissue was aseptically collected from a cat delivered for necropsy at the NVI on the 01.02.2023. Since mainland Norway is rabies free, the cat was considered rabies free.

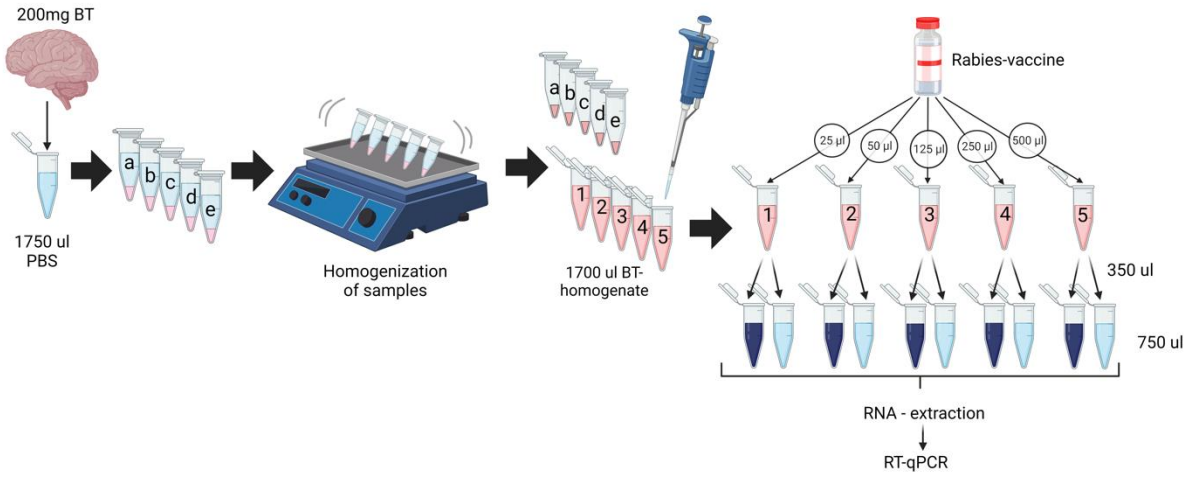

Figure 3 - Experimental set up showing brain tissue (BT) added to PBS in five Eppendorf-tubes (a-e) and homogenized, before 1700µl BT-homogenate were transferred to new Eppendorf-tubes (1-5). Rabisin vet. ® were added, in different volume to tubes 1-5 to achieve increasing concentrations of virus in the samples. From each rabies-spiked BT-homogenate a volume of 350µl were added to two parallel series of Eppendorf tubes, one containing Nuclisens easyMAG Lysis buffer (light blue) and the other containing MagNA Pure External Lysis Buffer (dark blue). Figure made with BioRender.com

Five parallels of homogenized brain tissue were prepared: 200 mg fresh brain tissue were mixed with 1750 µl phosphate-buffered saline solution in a Tungsten Carbide Bead 3

mm tube (Qiagen, Köping, Sweden), (Table 1, Figure 3). The samples were homogenised using Retsch Mixer Mill (MM301, Retsch, Haan-Gruiten, Germany) for 10 minutes at 25 hz. The homogenate was inspected visually to ensure that the brain tissue was completely fluid, and 1700 µl of each aliquot was transferred to new 2 ml Eppendorf tubes. Rabies vaccine was added in increasing volumes to each tube (Table 1).

Table 1 - Increasing volumes of Rabisin vet. ® vaccine was added to five different tubes of a 1700µl of brain tissue homogenate each prepared by homogenization of 200 mg brain tissue from a cat and 1750µl of phosphate-buffered saline.

Tube	Rabisin vet. ® (µl)	Est. rabies virus particles/µl
1	25	4.0
2	50	8.0
3	125	20.0
4	250	37.0
5	500	67.0

Rabies virus in brain tissue from potentially rabid animals are usually inactivated using lysis buffer. In this study, despite using inactivated virus, the real situation was emulated by adding the vaccine-spiked brain homogenate to two different lysis buffers for comparison. These were Nuclisens easyMAG Lysis Buffer (Biomerieux, Hampshire, United Kingdom) and MagNA Pure External Lysis Buffer (Roche, Mannheim, Germany).

Four 350µl aliquots from each concentration of rabies vaccine-spiked brain tissue homogenate was added to 1.5 ml Eppendorf tubes containing 750 µl Nuclisens easyMAG Lysis Buffer or MagNA Pure External Lysis Buffer (Figure 3). This resulted in a total of ten tubes with vaccine-spiked brain tissue homogenates. Nucleic acid was extracted from the samples and RT-qPCR was run to assess Cq-values with respect to rabies vaccine volume in the sample, as described for Step 3, below.

3.2.2 Assessing transport media for storing rabies-spiked brain tissue (Part 2)

This experiment was performed to determine which of the lysis buffers, DNA/RNA Shield or biosample collection cards would best preserve rabies virus RNA in brain samples over time and at different temperatures. Five commercially available media were chosen based on previous experience with use at the NVI, as well as the cost and availability. They included: Nuclisens easyMAG Lysis Buffer, MagNA Pure External Lysis Buffer, DNA/RNA Shield (Zymo Research, Irvine, USA), QIAcard FTA Micro (FTA) (Qiagen, Hilden, Germany) and Blood Spot Cards (BSC) (LipiDx, Oslo, Norway).

Vaccine-spiked brain tissue was prepared as described above for Part 1, using 200mg of feline brain, 1750µl of PBS buffer in a total of 15 two millilitre Eppendorf tubes before homogenisation. The reason for spreading the material in 15 tubes, is that the weight distribution in the homogeniser is more stable with the smaller Eppendorf-tubes. After homogenisation, one millilitre from each of the 15 tubes were combined in a 50 ml Falcon tube to make a final volume of 15 ml of brain homogenate, before 800 µl Rabisin vet. ® (Lot 497924), equalling an estimated total amount of 256,117 rabies virus particles, were added to the brain homogenate. The brain-vaccine homogenate was mixed by turning the tube carefully.

A volume of 700 µl vaccine-spiked brain homogenate was then added to nine 2 ml Eppendorf tubes; three of which contained 1500 µl Nuclisens easyMAG Lysis Buffer, three with 1500 µl MagNA Pure External Lysis buffer and three with 1500 µl DNA/RNA Shield. In addition, 150 µl vaccine-spiked brain homogenate was added to the sample field of 15 FTA- and 15 BSC-cards, marked by a square (A) or circle (B), respectively (Figure 4). The BSC have two fields for samples, only one was used per card in this experiment. The cards were left to dry for 10 minutes before they were folded.

Figure 4 - BSC (A) with square sample fields and FTA-cards (B) with round sample field, used as a transport media for rabies spiked samples.

RNA was extracted from an aliquot from each of the buffer types and one of each biosample card immediately after preparation (Day 0 samples) and presence of rabies virus was determined by RT-qPCR. Subsequently, one tube with each of the lysis buffers, the DNA/RNA Shield and five of each of the biosample cards were stored at three different temperatures: 4°C in a refrigerator, at 21°C in the laboratory (no direct sunlight) and at 36°C in a heating cabinet.

An aliquot from each lysis buffer, DNA/RNA Shield and one of each biosample card were taken aseptically from each storage temperature on days 3, 7, 10, 14 and 21. Together with the day 0-samples, these were all subject to RNA extraction and RT-qPCR analyses as described for Part 3 below.

3.2.3 RNA isolation and RT-qPCR analysis (Part 3)

For lysis buffers and DNA/RNA Shield samples, a 350 µl aliquot was transferred to new 1.5 ml Eppendorf tubes. For BSC- and FTA cards, the whole sample field containing sample material were cut out, aseptically, and folded using two sterile scalpels. The sample field was placed in 2 ml Eppendorf tubes, containing 1.5ml Nuclisens easyMAG Lysis Buffer, for 10 minutes to dissolve the sample and inactivate rabies virus.

Extraction of viral RNA for samples in Part 1 and 2 was performed using RNeasy Mini Kit (Qiagen, Hilden, Germany) on the QIAcube (Qiagen, Hilden, Germany) instrument

as this most resembles the method used in the laboratory in Malawi. In short, samples were transferred to spin columns and balanced in the column slots in the QIAcube. 70% etanol, Buffer RW1, Buffer RW2 and RNase-free water were added in the appropriate shelf in the QIAcube. RNA isolations were done using the "Two elution step" protocol. In each extraction round, negative and positive controls were included; Nuclisens easyMAG Lysis Buffer were used as a negative control, while brain tissue from a rabies-positive arctic fox was used as positive control. The positive eluation control is expected to have a Cq-value of 25 +/- 2.5.

The presence of rabies virus was tested using an established RT-qPCR method (Nadin-Davis SA, 2009), on a AriaMx Real-time PCR system (Agilent, Santa Clara, USA). Primers and probes are depicted in Table 2 and cycling conditions in Table 3. One reaction contained 8.6 µl of nuclease-free water, 12.5 µl of reaction mix (Thermo Fisher Scientific, Oslo, Norway), 1.25 µl of Rabies qPCR-assay, 0.1 µl of RNaseOut 40 U/µl) (Thermo Fisher Scientific, Oslo, Norway), 0.5 µl of Superscript™ III RT/Platinum ® Taq Mix (Thermo Fischer Scientific, Oslo, Norway), 0.2 µM of each primer (Table 3), 0.1 µM probe and 2 µl sample/control. The Master mix was added to flat-domed PCR strip tubes on 96 well plates, and 2 µl volume of each RNA isolate was added to individual wells.

Samples with Cq-value of 35 or less were considered positive. Samples with Cq-value between 35 and 40 were considered inconclusive and should be verified with new RNA-extraction from sample material before a new RT-qPCR run. Samples with a Cq-value over 40 were considered negative.

When testing different transport media stored at various temperatures and time, each sample was run in triplicates, negative and positive reaction control was also included in each run. The negative reaction control was nuclease free water (2 µl), and the positive test control was RNA (2 µl) extracted from a rabies positive brain from an arctic fox.

Table 2 - Primers and probe used for Reverse Transcriptase-qualitative Polymerase Chain Reaction of rabies virus RNA. F: Forward, R: Reverse, P: Probe

Primer/Probe	Gene sequence	Type of probe
139-RABVD1-F	ATG TAA CAC CYC TAC AAT G	
140-RABVD1-R	GCM GGR TAY TTR TAY TCA TA	
141-RABVD1-P	CCG AYA AGA TTG TAT TYA ARG TCA AKA ATC AGG T	5'-FAM, BHQ-3'

Table 3 - Table illustrating the temperature cycles for the Reverse Transcriptase-qualitative Polymerase Chain Reaction used for amplification and detection of rabies virus.

Temperature	Time	
50°C	30 min	
95°C	2 min	
95°C	15 sek	X45
50°C	60 sek	

3.2.4 Statistics and data handling

Cq-values of the spiked brain samples were plotted against volume of rabies vaccine per mg brain tissue and visualised in a line plot using Excel v. 16.77 (Microsoft Corp., California, USA). From triplicate analyses of different transport media from part 2, the average Cq-value and the SD, were visualised graphically using Excel, and compared against time stored and temperature.

3.3 Registration and rabies analysis of brain samples at CVL

During the first month of our stay in Malawi, a laboratory technician from the NVI was working at CVL to build capacity for rabies testing and set up the PCR methods. Prior to this the only available method at CVL for rabies analysis was the dFAT method. Our thesis work was embedded in the capacity building where all available rabies samples were tested by PCR and LFT by ourselves, local CVL staff and the laboratory technician from Norway.

3.3.1 Electronic registration of rabies samples

All samples or animals submitted to CVL for analysis or necropsy are registered manually in a registration book, dating back to 1994. All dogs and wildlife delivered for necropsy are routinely tested for rabies. To create an electronic registry of all rabies samples available for testing and the dFAT results, entries in the book were systematically checked for samples received for rabies testing from 1st January 2021 to 8th May 2023. The entries in the registration-book include date of arrival, species, name and/or organisation of the sender, responsible section for further analysis at CVL, specimen and date out. Information regarding the contact information of the sender and clinical signs of the animals are kept on paper in a separate filing system, and results from dFAT testing are registered manually in another notebook. Access was given at CVL to all mentioned files and all available data was registered in an Excel spread sheet. Samples received during our stay in Malawi were also registered. To get further metadata for the samples, The Lilongwe Society for the Protection and Care of Animals (LSPCA) provided access to their electronic registrations on samples delivered to CVL from them.

Subsequently, the biobank (freezer) of the CVL was checked for each of the samples and it was noted in the spread sheet if the sample was available or not. All available samples in the biobank were tested with RT-qPCR and LFT, as described below.

3.3.2 RNA isolation of samples stored at CVL

RNA isolation took place in a designated extraction area in one of two molecular laboratories at CVL. Briefly, brain tissue (< 30 mg) was placed into a 2 ml tube with screw cap with an o-ring (Simport Scientific, Beloeil, Canada) and 3mm carbide bead (Qiagen, Köping, Sweden). Nuclisens easyMAG lysis buffer (1000 µl) was added and samples were left at room temperature, for at least 10 minutes to inactivate rabies virus. The exterior of the

tube was disinfected with a Virkon solution 1% (Antec International Ltd, Sudbury, UK). Samples were stored at -20°C, until extraction.

On the day of extraction, the samples were thawed at room temperature for 10 minutes before homogenisation until smooth with a VELP Scientifica classic vortex machine (VELP Scientifica, Usmate, Italy). The homogenates were centrifuged with a MiniSpin centrifuge (Eppendorf, Hamburg, Germany) for 10 seconds at maximum speed to spin down remaining tissue.

Subsequent isolation of RNA was completed using the RNeasy Mini Kit. First, 350 µl homogenate was transferred to a 2 ml Eppendorf tube before adding 350 µl 70% ethanol and mixed gently, but thoroughly, by hand. An elution buffer control (EBC) was made by adding 350 µl of nuclease free water to 350 µl of 70% ethanol.

Samples and controls of 700 µl were transferred into an RNeasy mini spin column and placed in a 2 ml collection tube and centrifuged for 15 seconds at 10,000 rpm twice to ensure that the solution passed through the spin column filter. The flow-through was discarded, and the column was placed into a new 2 ml collection tube. Buffer RW1 (700 µl) was added to this spin column the column was centrifuged for 15 seconds at 10,000 rpm.

The procedure was repeated twice with discarding of flow-through and addition of buffer RPE (500 µl) and spinning. To dry the membrane, the spin column was centrifuged for 60 seconds at full speed, and the flow through was discarded. Finally, to eluate the RNA, the spin column was placed into a 1.5 ml collection tube, 50 µl RNase free water was added and the column was left to stand for 1 minute and then centrifuged for 1 minute at 10,000 rpm. The flow-through with RNA was kept and kept at -20°C until analysis within 12 hours.

An elution positive control (EPC) was prepared by adding 350 µl of pre-made positive control to 350 µl of 70% ethanol. For the first five RT-qPCR runs the EPC was made from a

positive artic fox sample made at NVI. For subsequent runs the EPC was made at CVL from a positive brain sample from a Hyena, diluted with at 1:1000 with lysis buffer.

3.3.3 RT-qPCR at CVL

The same rabies virus RT-qPCR protocol from NVI was used at CVL, as described above (3.2.3). The PCR machine was the QuantStudio™ 1 Real-Time PCR machine (Thermo Fischer Scientific, Massachusetts, USA).

Certain adaptions were made to suit the working conditions at CVL. The master mix was made in a designated master mix preparation area of the pre-PCR room. RNA isolates were added to wells in a designated sample preparation area, which was a safety cabinet. Finally, the wells were transported to the second working room, used only as a post-PCR room where the PCR machine was housed, with no entry permitted back into the pre-PCR room once samples had been moved to the post-PCR room.

3.3.4 LFT for detection of rabies

Samples at CVL with sufficient brain tissue remaining after dFAT and RT-qPCR were selected for testing using the BIONOTE ONE STEP Anigen Rapid *Rabies* Ag *Test Kit* (BIONOTE, Gyeonggi-do, Republic of Korea). The test is marketed as a 'chromatographic immunoassay for qualitative detection of rabies virus antigen in fresh brain tissue of canines, bovines or raccoon dog' (Bionote, 2016). According to the manufacturer, the sensitivity and specificity of the test is 94% and 100%, respectively, compared to qPCR.

Brain samples had not been pre-treated. Therefore, for safety reasons, one researcher had the role of assistant and the other as lead technician, working more actively with the samples. The assistant passed materials to the lead technician and kept time throughout the process. Both made use of protective gear (safety goggles, hair nets, laboratory coats and 3M-

Aura disposable respirator, FFP3 face masks), however, the lead technician had an extra pair of short length gloves placed over long gloves.

Brain tissue samples were taken from the freezer, placed into zip-lock bags, and carried to the bacteriology laboratory with a Sterilgard biological safety cabinet. The bags with samples were placed in the cabinet. An additional zip-lock bag was also opened and placed in the safety cabinet for waste generated under the procedure. The sample testing was performed according to manufacturer's instructions. Briefly, the lead technician used the disposable swab included in the BIONOTE ONE STEP Rabies Antigen Test Kit to obtain a small amount of brain tissue from the bag and introduced the swab to the assay diluent tube, mixing carefully for 19 seconds. With the plastic pipette, the brain homogenate was extracted, and four drops applied to the sample hole of the Anigen rapid rabies test device.

The test results were interpreted after a minimum of 5 minutes and a maximum of 10 minutes. Control (C) line had to be visible for the test to be valid. The test (T) line indicates that rabies virus antigen was present in the device and gives a positive reading.

During testing, care was taken to avoid contaminating the gloves with brain tissue. If the gloves were contaminated between samples, the outer gloves were removed and replaced. Once sampling was complete, all the zip-lock bags containing brain tissue samples were closed. All the zip-lock bags in the Sterilgard biological safety cabinet were sprayed with a Virkon solution, 1% and the solution was left to work for 10 minutes. Finally, the Sterilgard safety cabinet was wiped dry, and the UV program was run for additional disinfection. Waste was disposed of in bins for hazardous material.

3.3.5 Statistics and data handling

Information on each sample delivered between January 2021 and May 2023, all available metadata and all laboratory results, were collected, digitized and plotted in an Excel

spread sheet. Results from RT-qPCR and LFT were plotted in an Excel spread sheet with the dFAT results, species and the dates of arrival. Two agreement analyses and Kappa statistic (https://epitools.ausvet.com.au/comparetwotests) were performed to compare RT-qPCR with LTF and dFAT results, respectively. Uncertain samples were treated as negative. The level of agreement was interpreted according to the Kappa value cut-offs as advised in Dettori and Norvell, 2020.

3.4 Qualitative data collection from AVOs

To answer objective 3 and 4, FGDs were selected as an ideal form of data collection to reduce the importance of the researcher-interviewer and allow conversation to flow more freely between participants (Leung & Savithiri, 2009). This method allowed AVOs themselves to highlight what was important in their work and to achieve a greater depth of information.

To ensure that some essential data was collected, the FGD was followed by individual interviews with AVOs. The fixed structure of interviews facilitated the extraction of specific information points that the researchers were interested in. This would also allow for direct comparisons of the data across the different regions and ensure that knowledge could be gained from AVOs that may have been less talkative during the focus group discussions. The interviews also provide an opportunity for the more silent FGD-participant to voice their concerns, and to speak more freely about topics they might not feel comfortable discussing in a group setting.

3.4.1 Structure of the day when performing FGDs and interviews

Each session with the AVOs followed the same structure (Table 8). It was decided that the FGD should precede the one-to-one structured interviews, so that interview questions would not influence the FGD. A short break with refreshments followed, after which

individual interviews were conducted. For the success of the sampling element of the project, it was necessary to ensure that each AVO had been provided with the knowledge and materials required to return rabies samples to CVL. The last part of the day was dedicated to capacity building for rabies sampling. Participants joined a PowerPoint presentation on rabies and rabies sampling and were given equipment to take rabies samples. After the presentation AVOs were offered vaccination against rabies at a local clinic. The plan for the day is included in Appendix I.

3.5 Ethical Considerations

A study proposal for the full project "Implementation and operation of a One Health platform to combat rabies in Malawi" in which the FGDs and individual interviews were embedded, was reviewed and approved by the Malawi University of Science and Technology Research Ethics Committee (Reference number: P.05/2023/052).

The data collection was conducted after obtaining informed consent from the participants. Before each FGD all participating AVOs were provided with oral and written information on the purpose of the FGD and interviews, and that their identity, would remain confidential. They were encouraged to ask questions if anything was unclear, and Dr. Joseph Nkhoma, Deputy Officer in Charge at CVL provided information in Chichewa, for those who needed a translation.

Each AVO signed a consent form (see Appendix II), which they had received some days in advance of the FGD via WhatsApp, in two copies; one for the AVO to keep and one for the researchers keeping. The AVOs was compensated for transportation costs related to the meeting and were served snacks.

3.6 Study area and Study Population

AVOs from four districts (Mzimba, Dedza, Lilongwe and Thyolo) distributed in all three regions of Malawi were invited to participate.

Mzimba district is in the Northern region and with an estimated population of 940,184 people, the district makes up 5.4% of the population in Malawi (NSO, 2018e). Mzimba district hosts the regional Mzuzu Veterinary Laboratory (MVL).

Dedza district is found in Central Malawi and has a population of 830,512 people, 4.7% of the population in Malawi (NSO, 2018a).

Lilongwe district is found in Malawi's Central region, and includes Lilongwe City district (where Malawi's capital, Lilongwe, is found). The 2018 census estimated that 1,637,583 people reside in Lilongwe district and 989,318 persons in Lilongwe City district, meaning that together the districts make up 14.9% of the total population in Malawi (NSO, 2018b; NSO, 2018c). Thyolo district is in the Southern region and 721,456 people reside in Thyolo, comprising 4.1% of the population in Malawi (NSO, 2018d). The NGO, The LSPCA is based in Lilongwe district and through community outreach work, providing veterinary care and rabies vaccination campaigns they work to improve animal health and welfare in the district.

The aim was to recruit five AVOs working in the four chosen districts for voluntary participation in the FGDs. Participants for each FGD were identified and invited through a convenience sample. Dr Joseph Nkoma liaised with the DAHLD-O (District Animal Health and Livestock Development Officer) so that the DAHLD-O could then select and contact AVOs. Requirements for the selection of participants included that they were willing to participate and that they were located within a reasonable travel distance to where the FGDs would take place. It was also necessary that each AVO had a sufficient level of English that would enable them to understand and engage with the questions.

3.7 Focus group discussions

Given that the researchers had not facilitated a FGD previously, they received some training on how to conduct FGDs and how topic guides could be designed by Dr Katelyn

Mills of the University of British Columbia and Dr Dinah Seligsohn from the National Veterinary Institute, Sweden.

The topic guide and FGD questions were elaborated from a list of key points of interest based on the project aims. Five main research questions were included, each with between two-six probing questions in case participants did not touch on the points of interest. Two questions had a greater focus on animal welfare (for the thesis of Ragnhild Kvisle Abildsnes), and three on the awareness of rabies in Malawi and the role of the AVO. The topic guide, which is presented in Appendix III, also included a warm-up question as well as a question providing the opportunity for participants to raise any additional points that may have been overlooked. The FDG was intended to take between 1 and 1.5 hours.

At every FDG, one person (the same for all FDGs) was facilitator and two people were note-takers. The role of the facilitator, aside from reading out the questions, was to ensure that all points of interest were covered and to encourage quieter members of the group to share their thoughts and limit individuals that dominated the conversation. Extra clarification around questions was also provided by the facilitator if required.

Note-takers registered specific time-points in the discussion to assist with the verbatim transcription of each FGD. They also registered body-language – nodding for example, participant actions such as laughter and movement in and out of the room. Note-takers also assisted the facilitator in keeping time, by signalling when there were two minutes remaining for each section.

Prior to the FGD sessions, a full FGD pilot was run on the 13th of March 2023. Participants were selected from CVL staff members based on their previous employment as field based AVOs. Five persons participated: three males and two females. After the pilot, some clarifying modifications were made to the topic guide, based on the participants' feedback. A one-on-one session also took place on 16th March 2023 with one participant from

the FGD pilot. This enabled the authors to discuss each FGD question in more detail, and gain suggestions on how they could be changed.

The information read out to the participants before starting the FGD was slightly modified. For example, an instruction to please try and not speak over each other for the sake of the recording was removed because the participants explained that this request made them a lot more controlled in their responses and stilted the discussion. The final version of the information read out to participants is included in Appendix IV.

FGDs followed the same structure, briefly described now: Information on the FGD was read out to participants. The AVOs would then sign a written consent form and be presented with two copies to sign, one for their own keeping. Following the collection of the consent form, the audio recording of the FGD using two mobile phones. Participants were given a number to replace the use of their names. Each FGD started with the warm-up questions, progressed into the research questions, and then closed by the facilitator asking the participants if they had any further information to add.

3.7.1 Data analysis

Supervision for thematic analysis was provided by Dr Ana Lorena Ruano of the University of Bergen on analysis of FGDs. The analysis started with careful reading of each FGD transcript and transforming the text into codes. A code is, in short, a short sentence, without punctuation, that is formulated based on a direct quote from the text or is a summary of a point discussed in the text. One code can then be considered a unit of analysis. Codes that emerge numerous times from the transcript can be presented as weighted by marking the frequency of which this certain code appears in the text. This frequency could help indicate how important participants believe a point is. Codes are then grouped into categories and categories are subsequently grouped into themes (see Figure 5).

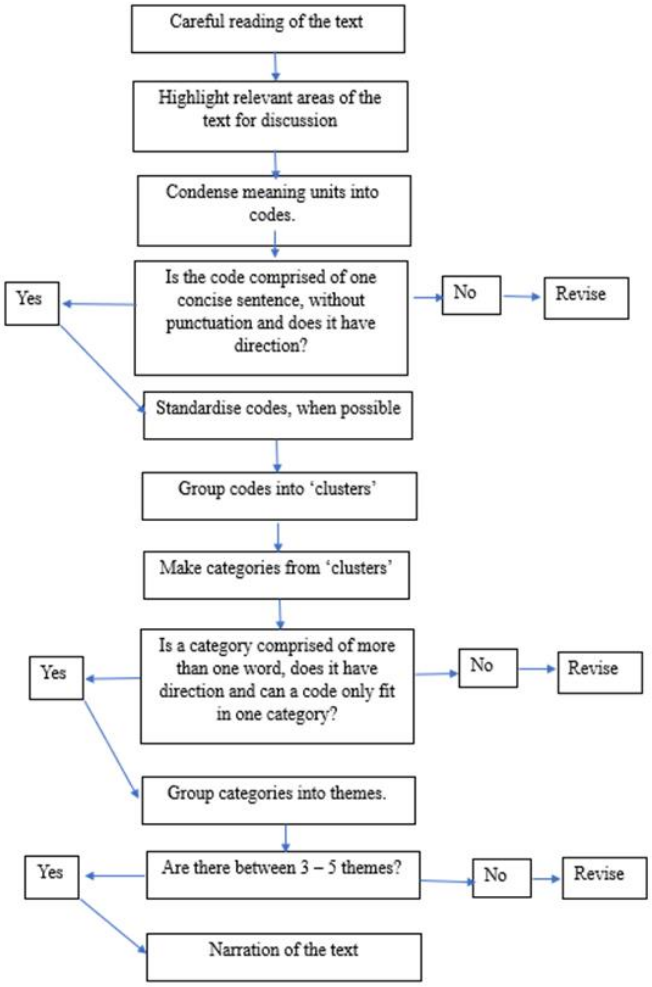

Figure 5 - Step-by-step description of the process used for thematic data analysis of focus group discussions.

3.8 Structured interviews with AVOs

Interview questions were formulated based on the project aims. As with the FGD, certain interview questions were included to support data extraction for Ragnhild Kvisle Abildsnes' thesis on dog-welfare and euthanasia in Malawi.

The remaining questions would explore the role of the AVO, rabies reporting and surveillance and the sampling of suspected rabid animals.

A total of 29 questions were included. Of these questions, 12 were specifically intended to extract information on dog welfare, and 12 focused on the role of the AVO and diagnostics of rabies in Malawi. It was desired that the interview would take 30 minutes.

Interview questions were piloted in a one-on-one session on the 10th of March 2023 with a CVL member of staff, who had previously worked as an AVO. Following the pilot, questions were edited slightly for clarity. For example, a question on surveillance was expanded so that instead of multiple options under the one question, each option was presented as a separate question with follow-up questions asking for more information on each point, for example: 'Do you register reports on suspected rabid animals?', with follow up questions to be asked if the participant answered yes. The questionnaire is presented in Appendix V.

Each interview followed a similar structure. The researchers plus fellow veterinary undergraduate veterinary student from NMBU conducted interviews. To limit the variation in delivery of questions, those conducting the interviews practiced together. For 'yes, no, I don't know' questions, the interviewer would inform the interviewee that this was a 'yes/ no' question and that they could also answer 'I don't know'. For open-ended questions, interviewees were told that they could answer as they please, and for categorical questions interviewees were told to select all that apply.

The questionnaire was printed on paper, and the answers were recorded by hand. However, participants were also able to have their own copy of the interview form so they could read the questions if they felt more comfortable this way.

3.7.1 Data analysis

Completed interview documents were reviewed in plenum by the researchers. The responses were then recorded in Excel documents for each district. The same participant numbers used during the FGDs were used to identify interviewees. A master database was

then made in Excel with all AVO responses. Similar answers in free text questions were categorised and the frequency of responses analysed.

3.9 Building capacity of AVOs to perform rabies sampling

The last part of the meetings with the AVOs for each FGD session was dedicated to capacity building for rabies sampling. The presentation material included a PowerPoint presentation, an instruction video on how to collect samples, a poster about rabies, and a package for each AVO containing sampling equipment.

3.9.1 Rabies sampling instruction video

A short instruction video was created to support AVOs with rabies sampling in the field and to compliment the presentation on sampling that would be delivered to AVOs in Malawi. Furthermore, by sharing the video with the AVOs to their smartphones, the hope was that they could review the sampling steps at a pace that suited them and as often as required. For this video, the authors participated both as writers and actors. Research Professor Bjørnar Ytrehus, veterinarian and pathologist at the Norwegian Veterinary Institute, provided an outline for the script and following this, the authors added to the dialogue. The World Organisation for Animal Health's Terrestrial Manual (WOAH, 2023) was used to select the three sampling methods that were demonstrated in the video, to ensure that the teachings the AVOs would receive were in line with recommended sampling techniques. The script was crafted in January 2023 and then submitted to the NVI communications team for review. Shane Colvin, graphic and digital designer, also assisted in editing the script.

Filming took place on the 8th and 9th of February 2023 at the NVI and a forest location. In the video, a fox (*Vulpes vulpes*) carcass was used to demonstrate the three sampling methods. Shane Colvin filmed and directed the three veterinary students who had the

following roles as presenter (Akinsanmi-Guren), bystander who found the fox (Ravnås) and rabies diagnostics sampler (Kvisle Abildsnes).

Following filming, Shane Colvin edited the film. Voiceover was provided by Akinsanmi-Guren and text and graphics were subsequently added to the video for clarity.

3.9.2 AVO presentation on rabies sampling and biosecurity

A PowerPoint presentation was also devised to provide AVOs with information and guidance on rabies sampling methods. The importance of personal safety was also discussed.

The presentation was developed by the authors with input and guidance from Bjørnar Ytrehus and Malin Rokseth Reiten, veterinarians and pathologists at the NVI, and supervisors. Information on rabies sampling and safety was based on WOAH guidelines specified in the terrestrial manual.

During the presentation a general information video about rabies, created by the International Livestock Research Institute (ILRI) of Kenya, was also shown. This video targets the general public to educate them on the dangers of rabies and how to prevent infection. This video was kindly translated by ILRI into Chichewa, the main indigenous language spoken in Malawi, in time for our project, and the hope was that the AVOs would share this video with their communities.

3.9.3 Distribution of equipment to AVOs for rabies sampling

Participants were provided PPE and materials required for taking up to five rabies samples in the field. The PPE included was based on the WOAH's recommendation for rabies sampling (WOAH, 2023). However, goggles and a 3M-Aura disposable respirator, FFP3 face mask replaced the face shield suggested by WOAH, because a face shield presents more of a challenge to disinfect completely in the field as compared to safety goggles. AVOs were also given a list of equipment distributed and sampling instruction sheet, a requisition form and an

A4 educational poster in Chichewa that the AVOs could share with members of the public, also designed by ILRI and translated to Chichewa.

4. Results

4.1 Determining inoculation dose for brain tissue with inactivated rabies virus.

For Part 1 of the laboratory experiment, the purpose was to determine the vaccine inoculation dose for brain tissue homogenate with the Rabisin vet ® vaccine. Results from RT-qPCR are presented in Figure 6A with the Cq-values of the samples plotted against increasing volumes of Rabisin vet ®. The lowest concentration of added vaccine (25 µl) yielded a Cq-value in the RT-qPCR of 21.4 and 22.1, respectively.

Beyond an addition of 125 µl to the 1700 µl of brain tissue homogenate, the Cq-value increased as more vaccine was added. Initially, the aim was to determine a vaccine inoculation volume resulting in a Cq-value of 25-30 in the RT-qPCR. However, because spiked samples with a lower Cq-value gave more predictable RT-qPCR results, it was decided in Part 2 to proceed with a Rabisin vet ® concentration equivalent to a volume around 100 µl of Rabisin vet. ® to a 1700 µl of brain tissue homogenate (corresponding to a concentration of approximately 5 virus/µl). This would most likely give a Cq-value of approximately 25.

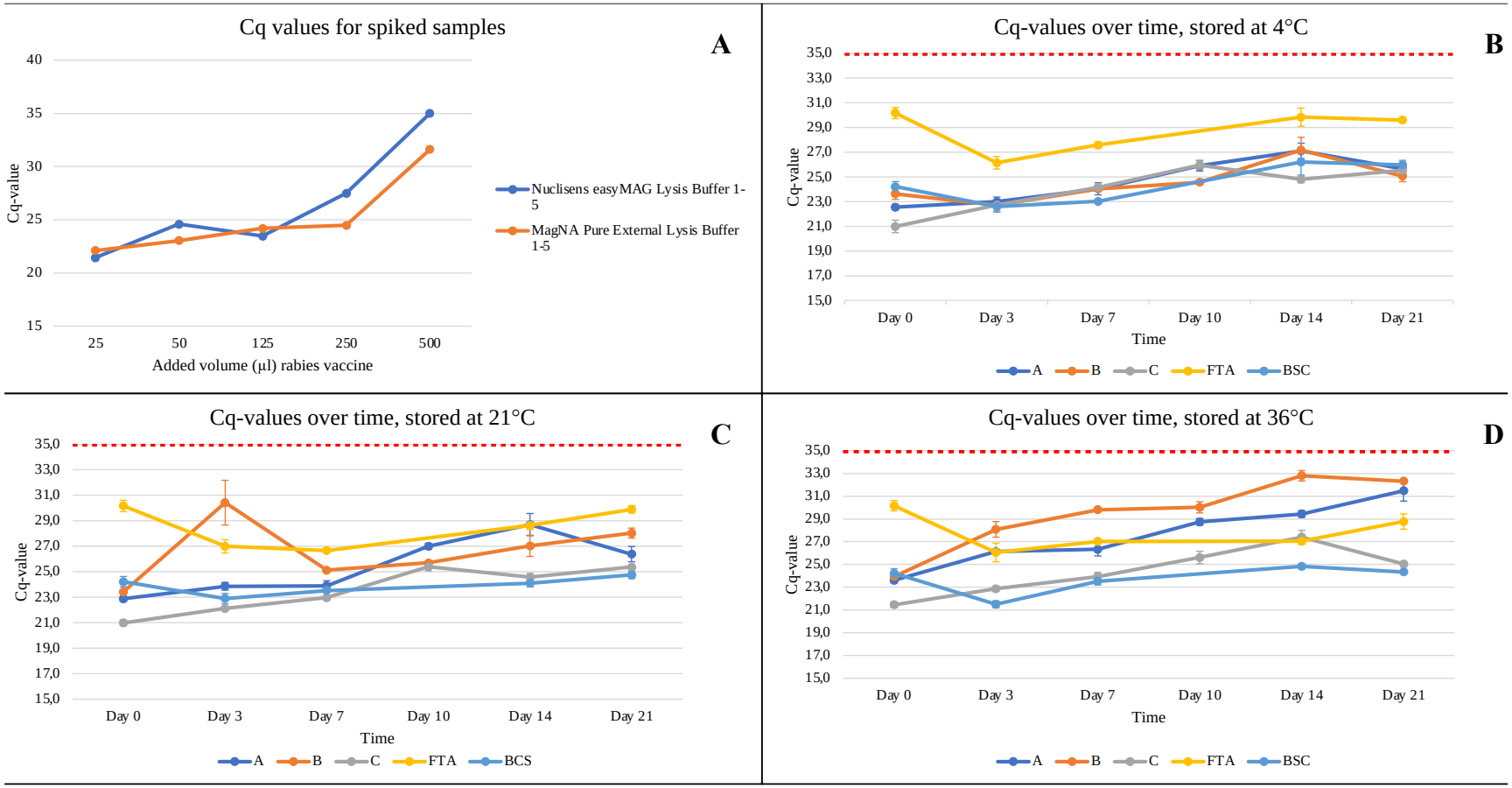

Figure 6 - The graph illustrate the Cq-values of brain tissue spiked with different volume of Rabisin vet. ® . **B**. Cq-values of samples stored over time by different transport media at 4˚C. C. Cq-values of samples stored over time by different transport media at 4˚C**. C**. Cq-values of samples stored over time by different transport media at 21 ˚C. **D** Cq-values of samples stored over time by different transport media at 36 ˚C. B-D: The red-dottet line marks the maximum Cq-value for positive samples. Dark blue line marked "A" = Nuclisens easyMAG Lysis Buffer, orange line marked "B" = MagNA Pure External Lysis Buffer, grey line marked "C" = DNA/RNA Shield, yellow line marked "FTA" = FTA-cards and light blue line marked "BCS" = Blood spot cards.

4.1.1 The transport media's ability to preserve rabies virus RNA over time and temperatures.

In Part 2 of the laboratory experiment, brain tissue samples from Nuclisens EasyMAG Lysis Buffer, MagNA Pure External Lysis Buffer, DNA/RNA Shield and biosample cards were analysed for the presence of rabies virus after 0, 3, 7, 10 (except for FTA/BSC cards), 14 and 21 days of storage at three different temperatures. Each sample was analysed using RT-qPCR and in triplicates. Results are visualized in Figure 6B, C and D.

With respect to temperature and time, the Cq-values of the samples kept at 4°C, show a slight increase in Cq over the 21-day time period for all media, samples in DNA/RNA Shield showing the greatest increase of Cq-value from 21.0 on day 0 to 25.9 on day 14. Samples on FTA had a higher Cq-value at 30.3 on day 0 and dropped to 26.1 on day 3, before increasing to 29.6 on day 21.

Samples stored at 21°C, gave similar results to those observed at 4°C, with a slight increase in Cq-values over time for all media (Figure 4C). However, in contrast to results from 4°C the largest increase in Cq for samples at 21°C was observed for MagNA Pure Lysis Buffer. These rose from an average Cq-value of 23.4 on day 0 to 30.4 on day 3, then dropped again to 25.1, before rising gradually, ending at a Cq-value of 28.0 on day 21. At this temperature, samples on FTA-cards had the most stable, but higher, Cq values over time, starting with an average of 30.1 on day 0 and ending with a Cq-value of 30.2 on day 21. The BCS had the lowest Cq-values of 24.1 and 24.8 on day 14 and day 21.

For samples stored at 36°C, the Cq-values increase of the Cq-value over time was more prominent for the lysis-buffers EasyMAG and MagNA Pure, starting with Cq-values averaging 23.6 and 23.9, respectively, and rising to Cq-values of 31.5 for EasyMAG on day 21 and 32.8 for MagNA Pure on day 14. The samples stored in DNA/RNA Shield and BSC increased slightly over time, the Cq-values of the BSC were the lowest of all transport media

kept at 36 as of day 3 (21.3). The samples stored on the FTA-cards in average dropped from 30.2 to 28.8.

4.2 Electronic registration of rabies samples at CVL

A total of 72 brain samples registered for rabies testing were identified in the registry book between the 1st of January 2021 and 8th of May 2023, 11 of these arrived between 1st of January 2023 and 30th of April of 2023 (average 2.75/month). An additional 14 samples were delivered between 1st of May and 31st of August 2023 (3.5/month), following the capacity building among AVOs for sampling. Of these 14 samples, three were from an AVO that had participated in FGDs and training activities.

All 72 samples were previously tested with dFAT, 43 (59.7%) were positive, 27 (37.5%) were negative, and two were not suitable for testing. Of the 72 samples, 26 were identified in the CVL biobank. Most of the samples in the biobank were from dogs (n=21). The rest of the samples were from sheep (n=2), bovine (n=1), hippo (n=1), and hyena (n=1).

The majority of the 72 samples were delivered to CVL from the Central Region (n=42), with the LSPCA delivering most of these samples (86%, n=36). The LSPCA delivered 50% of all samples tested for rabies at CVL in the time-period between January 2021 and May 2023. From the Northern region, the Mzuzu Regional Veterinary Laboratory submitted 12 of the samples. Only two samples were from the Southern Region. It was not possible to trace the origin of the last 10 samples. The samples and dFAT results from prior testing at CVL are presented in Table 4.

Table 4 - Illustrating the direct Fluorescent Antibody Testing results for samples (n=72) delivered to Central Veterinary Laboratory, between the 1st of January 2021 and 8th of May 2023, for rabies analysis. Samples are organized by region and illustrating the distribution of samples delivered by different organization and of different species.

Region	Organisation	Number of samples	Species								dFAT		
			Dog	Cat	Hyena	Jackal	Bovine	Sheep	Donkey	Hippo	Positive	Negative	NA
Central	LSPCA	36	35	1							22	14	
	Various	6	3		1		2				5		1
	CVL Animal Unit	2						2				2	
	Lilongwe Wildlife Centre	1				1					1		
	Lilongwe Police	1	1									1	
	Carnivor Research Malawi	1			1							1	
	AVO	1	1								1		
Northern	Mzuzu Regional Veterinary Laboratory	12	9				3				8	3	1
Southern	Liwonde National Park	1								1		1	
	Various	1					1					1	
Unknown	Various	10	7		2				1		6	4	

4.3 RT-qPCR and LFT of rabies samples at CVL

All available (n=26, 16 positive and 10 negative on dFAT) brain samples in the biobank at CVL were tested for rabies virus using RT-qPCR (Table 5). Sixteen of the dFAT positive samples were also positive with RT-qPCR. One of the samples that tested negative with dFAT tested positive with RT-qPCR (Cq 34.9). Seventeen of the 26 (65%) samples were positive by RT-qPCR. Four of the negative dFAT samples were inconclusive with RT-qPCR (15%), and five (19%) were negative with both methods.

Of the 26 samples run by RT-qPCR, material from 18 was available for analysis by LFT. Of these, 11 (61%) were positive with LFT, dFAT and RT-PCR. One (5%) was negative with LFT and dFAT, but positive with RT-qPCR and six were negative with both dFAT and LFT.

Table 5 - Illustrates the previously completed, direct Fluorescent Aantibody Test results and Reverse Transcriptase-quantitative Polymerase Chain Reaction (RT-qPCR) and Lateral Flow Test (LFT)-results and species of 26 rabies samples stored at the Central Veterinary Laboratory biobank. ND: Not done.

Date recieved	Animal species	Results			
		dFAT	RT-qPCR	Cq-value	LFT
26.01.2022	Dog	Positive	Positive	17.2	ND
24.03.2022	Dog	Negative	Uncertain	37.7	Negative
29.03.2022	Dog	Negative	Uncertain	35.5	Negative
06.06.2022	Dog	Positive	Positive	13.3	ND
23.06.2022	Dog	Positive	Positive	15.3	Positive
22.08.2022	Dog	Positive	Positive	16.8	Positive
23.08.2022	Dog	Positive	Positive	16.5	Positive
07.09.2022	Dog	Positive	Positive	14.1	Positive
27.09.2022	Dog	Negative	Uncertain	36.2	Negative
27.10.2022	Dog	Positive	Positive	15.0	Positive
07.11.2022	Dog	Positive	Positive	15.1	Positive
13.12.2022	Sheep	Negative	Negative	>40	ND
14.12.2022	Sheep	Negative	Negative	>40	ND
31.12.2022	Hippo	Negative	Positive	34.9	Negative

05.01.2023	Dog	Positive	Positive	24.8	ND
05.01.2023	Dog	Positive	Positive	19.6	ND
20.01.2023	Hyena	Positive	Positive	15.8	Positive
27.01.2023	Dog	Negative	Uncertain	38.2	Negative
28.01.2023	Dog	Negative	Negative	>40	ND
30.01.2023	Bovine	Positive	Positive	19.2	Positive
27.02.2023	Dog	Negative	Negative	>40	ND
22.03.2023	Dog	Positive	Positive	18.6	Positive
21.04.2023	Dog	Negative	Negative	>40	Negative
03.05.2023	Dog	Positive	Positive	16.2	Negative
03.05.2023	Dog	Positive	Positive	17.6	Positive
08.05.2023	Dog	Positive	Positive	17.2	Positive

4.3.1 Agreement analysis

The agreement analyses comparing results from dFAT and RT-qPCR (n=26) (Table 6) gave a Kappa-value of 0.92 and the agreement between the methods is interpreted as very good. If the four samples with an uncertain result in RT-qPCR were counted as positive, instead of negative, the Kappa-value was 0.55, which is interpreted as moderate agreement.

Table 6 - Comparing results of samples tested for rabies virus with Reverse Transcriptase-qualitative Polymerase Chain Reaction and direct Fluorescent Antibody Testing. Uncertain results from RT-qPCR were interpreted as negative.

	RT-qPCR positive	**RT-qPCR negative**	**RT-qPCR Total**
dFAT positive	16	0	16
dFAT negative	1	9	10
dFAT Total	17	9	26

A 2x2 table comparing results from RT-qPCR and LFT is shown in Table 7, and the agreement analysis resulting in a Kappa-value of 0.88, defined as a very good agreement.

Table 7 - Comparing results of samples tested for rabies virus with Reverse Transcriptase-qualitative Polymerase Chain Reaction and Lateral Flow Test.

	PCR positive	**PCR negative**	**PCR Total**

LFT positive	11	0	11
LFT negative	1	6	7
LFT Total	12	6	18

4.4 Focus group discussions

A total of four focus group discussions (FGDs) were completed in Malawi (Table 8). Five AVOs consented to join each FGD in the Dedza, Lilongwe and Mzimba districts, while four AVOs joined in Thyolo (because there were no more than four AVOS in Thyolo). The age of participants ranged from 23 years – 56 years, and time spent in the AVO role ran from 1 year to 27 years.

A total of 588 codes were defined from the quotes in the FDGs, and these were divided into 52 clusters and eventually into five themes. See Appendix VI for the codes and categories that compose the themes. In the following section a narrative of the information provided from the AVOs during the FGDs is presented reflecting the insights shared by the AVOS. Direct quotes illustrating the themes are presented for each theme in italics.

Table 8 – Dates, locations and participant information for focus group discussions carried out in Malawi.

Date	**Location (by district)**	**Number of participants**	**Gender distribution of participants**
March 22nd 2023	District D	5	6 females 13 males
March 30th 2023	District C	5	
April 5th 2023	District B	5	
April 18th 2023	District A	4	

Theme 1: Rabies is a big issue in Malawi, and more can be done to control and document the disease

AVOs in all focus groups stated that they have seen rabid dogs, and that rabies is common in Malawi. They further stated that it is a dangerous disease, and that people are dying from rabies in their communities. AVOs believe that most community members have heard about rabies deaths. They think rabies is killing too many people and feel sad when they hear of people dying of rabies.

Controlling rabies is the AVOs responsibility, and one participant described AVOs as frontline workers in the communities taking their responsibility to protect their communities from rabies seriously. All AVOs confirmed that they play a role in rabies control but stated that lack of resources and existing knowledge gaps in the communities are bottlenecks in their work. Even though rabies is a life-threatening disease they lack funding to control it. The small monthly funding they receive is not enough to cover expenses. Some believed that the government was more serious about control of rabies in the past, and that more can be done to control rabies.

> *"I think there's a lot of work to be done, … we have the rules, we have the regulations … we just have to put them into good practice."*
>
> *(Participant 3, FG C).*

In one FGD, it was stated that there are too few AVOs in Malawi to cover the work, and AVOs in all FGDs mentioned that they lack updated training to control rabies. Some AVOs want to include influential community leaders to help control rabies. Some areas receive help from NGOs, and one AVO said that Malawi needs help to reduce the number of rabies cases.

"We lose a lot of people. So, we need help. …You can see that we lack the knowledge, the proper knowledge."

(Participant 1, FG A).

Although AVOs in all FGD said they carry out some surveillance, for example by writing monthly reports on bite-cases and suspected rabies in animals, AVOs in two FGD stated that there is poor surveillance of rabies in Malawi. Several AVOs seemed a little unsure on what rabies surveillance entails.

*"There is poor rabies surveillance. We are just waiting for somebody who's going to come to say, "I have been bitten by a dog"**.** We may not even trace the dog. If it's a stray dog, it's difficult … and the dog has gone. We give the document to receive the vaccine. It ends there. So, I don't think we are doing it (surveillance). Like the monitoring we do in the other diseases."*

(Participant 1, FG A).

The AVOs lack information on the occurrence of rabies in their communities, and do not get information on human rabies cases from the hospitals or the government. The hospital has statistics on human rabies deaths, but the Health Department does not share this information with the Veterinary Department. AVOs in one FGD described it as a one-way system of reporting suspected rabies cases.

Theme 2: Creating awareness on rabies is important for rabies control

The topic of awareness was consistently and elaborately covered in all FGDs, engaging most participants. With few exceptions, AVOs agreed that people in the

communities lack awareness when it comes to rabies, especially in rural areas. They defined awareness- and knowledge building as their responsibility and said they work to create awareness in the communities, for example on the importance of vaccinating dogs. AVOs in several FGD stated the need for more awareness campaigns.

> *"We need to reach out to the people, we should not just assume everybody knows, because there is a large number of people who doesn't know how dangerous rabies is."*
> *(Participant 2, FG C)*

AVOs in one FGD stated that there is also a lack of awareness within the health sector and said that doctors do not always suspect that their patients suffer from rabies. AVOs in another FGD stated that hospitals often don't confirm the diagnosis in patients.

> *"…Even doctors, human doctors, they were not maybe in support of my decision that it could be rabies. After they take some tests, two days before death they confirmed that this is rabies."*
> *(Participant 2, FG D).*

AVOs in all FGDs stated that people don't know how to protect themselves against the disease, that they underestimate the danger of the disease, and lack knowledge on less common transmission routes.

> *"...Rabies is dangerous, I saw it with my naked eyes, my friend died. But people they don't know. And they don't even take care once they have been bitten by a dog, even a scratch, they just say, ahh this is just a mere wound…"*

(Participant 2, FG D).

AVOs in several FGDs said that unless the dog displays classical aggressive symptoms, most people cannot recognise a rabid dog. They also said that most people struggle to differentiate dogs with rabies from dogs that are vicious for other reasons.

"They say those dogs are vicious, they have rabies. But that's what I'm saying, they don't understand the difference between a rabid dog and some dog which is just vicious."
(Participant 2, FG B).

The AVOs described various strategies to spread information on rabies. Some contribute to educating children in schools and use government meetings to spread rabies awareness and educate dog owners on vaccination. They also use WhatsApp groups and the annual dog vaccination campaigns to raise awareness. AVOs in one FGD said that they try to involve local leaders, and AVOs in two FGDs take advantage of natural gatherings for example in churches and funerals. AVOs in two separate FGDs said that some people do not take the awareness campaigns seriously and that few people show up when they spread information. AVOs in one FGD sometimes feel ridiculed by people when they try to talk about rabies.

AVOs in all FGDs stated that the lack of resources is a barrier when they organize awareness campaigns, and that without resources they stay idle. Many people live in hard-to-reach areas, and it is difficult for the AVOs to travel there. Some mentioned that that awareness campaigns carried out by hospitals are allocated more resources and are very effective.

"…I admire the people from the hospital, when they are doing campaigns, their vaccinations, polio, for example, it's everywhere! But, in rabies we don't see much of that."

(Participant 2, FG B).

AVOs in most FGDs had ideas on how to reach more people and improve rabies awareness, for example, to hand out posters in villages. AVOs in one FGD said they want to spread awareness trough entertainment, for example using movies to show rabies symptoms.

Theme 3: AVOs vaccinate dogs and cats against rabies in their communities

AVOs in all FGDs stated that they work to control rabies through vaccination of dogs, and that they do yearly vaccination campaigns for dogs and cats in their communities.

" ...we only vaccinate. There is no other control (of rabies)."

(Participant 4, FG B).

The government provide AVOs with the rabies vaccines, but AVOs in all FGDs said they often receive too few doses compared to the number of dogs. AVOs in one district said that sometimes they are not provided with equipment like syringes. Because of this, they must prioritize where to vaccinate, and some stated that the inadequate coverage results in new "rabies hot spots".

The AVOs often question the quality of the vaccines they use, because of cold-chain failure and long-distance transport. They fear that vaccinated dogs might not be protected and contract rabies due to mishandling of the vaccines. AVOs do not have access to refrigerators or cooler boxes and cannot maintain the cold chain during transport and storage. Some said they ask hospitals to store the vaccines for them.

"We don't have refrigerators, we bring the vaccine from the office. And, you have to beg for, to ask for the hospital to keep the vaccine. But also ... blackouts (are common), ... so sometimes you start questioning the validity of this cold chain."
(Participant 5, FG B).

AVOs in three FGDs stated that they lack resources, such as fuel, to conduct vaccination campaigns and can't reach all areas. Some said they have to use their own resources when vaccinating dogs.

"It's really a burden for us. As for me initially, I used to use my own resources, I used this year… I became tired, no. I can no longer."
(Participant 5, FG D).

Most AVOs have not received PrEP, and AVOs in one FGD stated that people vaccinating dogs should be protected and provided with vaccines, muzzles and other PPE. They said that if they are bitten during campaigns, they have to source for PEP themselves.

AVOs in all FGDs said they launch campaigns to publicize vaccination campaigns, but most AVOs lack promotional material such as posters, so it is difficult to reach out to the communities. With poor promotion of vaccination days, many dog owners miss the vaccination day, and dogs have been let out to roam when the AVOs come to vaccinate.

Often it is children who bring the dogs for vaccination, and one AVO explained that because dogs are not accustomed to handling it is difficult for the children to restrain them, causing some dogs to escape before vaccination. They also mentioned the problem of stray- and roaming dogs in Malawi, and that vaccinating non-owned, stray dogs is not feasible. AVOs in one FGD would like oral vaccines for stray dogs.

AVOs in all FGDs explained how some dog owners do not bring their dogs for vaccination due to misconceptions. Examples provided include that people think their dog might die due to the vaccine, or that female dogs will become infertile. Some people also believe their guarding and hunting dogs will become more docile and less aggressive.

AVOs stated that a minority of people are willing to pay for vaccines for their dogs. People in more urban areas in Malawi are more likely to take their dogs to a vet, while people living in more rural areas will wait for the free vaccination campaign. One AVO said that people with good knowledge of rabies are more likely to pay for the vaccine.

AVOs in most FGDs stated that vaccinated dogs should be registered. Vaccinated animals are supposed to receive a card, proving their vaccine status, but AVOs often lack these cards. It was also mentioned that owners lose the certificates. AVOs in one FGD said they report how many dogs they vaccinate to the district.

Theme 4: Not all human bite-case victims receive PEP.

AVOs are responsible for following up dog bite victims. They provide advice on wound care and refer the victims that might need further treatment to the hospital. One AVO said they can get 90 bite-cases each month, another estimated that they receive 15-20 bite-cases a month.

AVOs in all FGDs said they investigate each bite case to assess if the dog is likely to be rabid. They interview bite case victims to understand the circumstances around the bite, as well as the dog's behaviour and appearance. They try to identify the dog owner to inquire if the dog has been vaccinated. AVOs in two FGDs said that they try to uncover if the dog was provoked to bite. Others said they try to gain insight into how the dog is kept and if it might roam into rabies hot spots. If it is feasible, some AVOs ask the dog owner to bring the dog so they can observe it themselves, or they travel to the field to observe the dog.

AVOs in two different FGDs stated that owned dogs could be confined and kept for observation after biting someone. The dog is considered rabid if it develops symptoms or dies. Usually, the dog owner reports back to the AVOs, but not always. It is not feasible to observe unowned dogs after they bite.

AVOs in all FGDs think it is difficult to assess whether biting dogs are likely to have rabies or not. It can be problematic to identify the owner, the dog may have run away, was a stray, or has already been killed. Free roaming dogs can often not be traced, and one AVO said that dog owners might deny ownership if their dog bites someone. Dog owner often can't produce proof of vaccination, or they may say that the dog is vaccinated but they have lost the certificate. As a result of these difficulties, AVOs may record the dogs as stray or unvaccinated, even though it might have an owner and is vaccinated. Many of the AVOs said they define most biting dogs as potentially rabid when assessing bite cases.

AVOs in all FGDs confirmed that they write a referral letter for bite-case victim to take to the hospital, and that this letter influences whether the victim receives PEP. In the letter they provide information about the bite-event, and their own judgement of the rabies status of the dog. Bite-victims sometimes go directly to the hospital, but the hospital may refuse to treat them and may send them back to the AVOs to get the referral. AVOs in one district had experienced that the hospitals refuse to provide PEP because the referral letter was not detailed enough.

AVOs in all FGDs said that some bite-victims want the referral letter regardless of whether the dog has rabies or not. Some had experienced that the bite-victims lie, withhold information, or tell the AVOs what they think they want to hear to receive a referral letter. For example, they might tell the AVO that they don't know the dog or the dog owner. AVOs in one FGD stated they might suffer repercussions from the bite-victim and the community if

they don't write the referral letters. The AVOs also worry that they will be held responsible for human deaths if they don't write the referral letters.

> *"You are afraid when he comes back, he'll say, you didn't help me, look at my child now he's dying"*
> *(Participant 5, FG D).*

Even if the dog-bite victims get the referral letter they might not get or complete the PEP-treatment. AVOs in all FGDs stated the supply of human rabies vaccine in Malawi is poor, and that many health facilities lack the human rabies vaccine. Bite-victims may have to approach several health facilities to get treatment, and sometimes the victim is told to come back later. Sometimes the health facility runs out of vaccines before all doses are completed. One AVO said the vaccines are more likely to be available in private hospitals. The difficulty in receiving PEP leads to people giving up searching for treatment. One AVO described an instance where someone died of rabies because none of the hospitals they visited had vaccines available.

> *"She found me, I wrote her, she was bitten by a suspected rabid dog, she went to Central, there was no vaccine, they said vaccine was in Nkhata Bay, she went to Nkhata Bay, there was no vaccine, she had to go to Mzimba to vaccinate, but she died."*
> *(Participant 4, FG B).*

AVOs in one FGD stated that the PEP is expensive so some people cannot afford it, and since transportation is expensive, some people do not finish the course of treatment.

Difficulties in procuring treatment and misconceptions on how to prevent rabies may cause bite victims to try traditional medicine rather than PEP.

> *"He just pulled some hair from the dog and applied on his wounds. That's a common belief that most people have."*
> *(Participant 4, FG C)*

AVOs from all FGDs had heard about people who did not seek help after dogs-bites or waited until symptoms developed. Many of the people who get rabies did not seek help in time.

Theme 5: Taking rabies samples is a challenge

AVOs in several FGD stated they are responsible for taking rabies samples and delivering them to the laboratory for testing. Except for one AVO, AVOs in all FGD said that they do not take samples. One said they could send the head of the dog for testing if they had the resources and if the dog had been killed. Some said they know how to take rabies samples but have never done it, and one said they lack a protocol for rabies sampling. None of the AVOs in any of the FGDs had received training on sample collection, and some believed that training may lead to more sampling.

> *"But we can do (take rabies samples), we can do as long as we have the proper equipment, training, and everything. We can easily do that."*
> *(Participant 2, FG D)*

Many of the AVOs stated that the general public usually try to kill and bury suspected rabid animals quickly, and that the AVOs are only informed about potential rabies cases later.

It is challenging to locate and exhume animals that have been buried. The animal might also have decomposed before the AVO can take a sample.

> *"On handling the cases, most of the times we hear the finished story, where some just say I've been bitten by a dog, it's either I've killed the dog, or I don't know where the dog is. So, we mostly handle the person not the dog."*
> *(Participant 2, FG B)*

Lack of resources is a barrier for sample collection and shipment to the laboratory. The AVO's office might not have the resources to send the sample to CVL, and some AVOs said they use their own private resources to collect and send samples.

> *"We, as frontline workers within the community, we don't have the necessary resources to take samples and to send to the laboratory."*
> *(Participant 3, FG C)*

None of the AVOs had personal protective equipment for sample collection, such as gloves. Because of this, they fear contracting rabies during sampling. They also lack equipment for sampling and may use axes or machetes to take the head of the animal off. AVOs in one district said they lack containers to ensure safe transport and to keep samples cool.

AVOs in all FGDs said lack of transport is a barrier for the collection and shipment of samples. This includes poor road conditions, and lack of vehicles and fuel. One AVO said they walk many kilometres to reach some villages. Some ask the farmers to pay for their transport, but the farmers are reluctant to cover expenses for sampling and testing of rabid

animals. The AVOs also believe that long distances, and the poor conditions of the roads cause samples to decompose before reaching the laboratory.

AVOs in one FGD said people might get suspicious of the AVOs intentions when taking the dead animal from the village, making it difficult to send samples to the laboratory.

> *"I mean, the people in the village cannot easily believe that you are going with that head to the lab…*
> *… (People think) you want to do with the head something magical."*
> *(Participants 1 & 5, FG D)*

AVOs in several FGDs said that the laboratory charges for analyses, and that the government used to cover this cost, but now expect the AVOs to pay. Farmers or dog-owners are not willing to pay. AVOs in one FGD stated they do not have the resources to pay the laboratory fee and are not willing to spend their own money unless it is a high-profile case.

4.5 Structured interviews with AVOs

Nineteen individual structured interviews were carried out with AVOs (Table 11). Each interview took approximately 30 minutes.

All participants confirmed in questions 1 – 3 said that they deal with animals with suspected rabies, as well as humans bitten by dogs, and that they contribute to rabies vaccination campaigns. Responses to the question on how one recognises a dog with rabies (Q 4) are presented in Figure 7. All participants were able to describe relevant clinical signs for dogs suffering from rabies. The most frequently selected clinical signs were 'salivation' (13/19), 'biting people' (11/19) and 'behavioural changes' (8/19). Some erroneous answers were included, for example, two participants specified that dogs develop red eyes when infected with rabies.

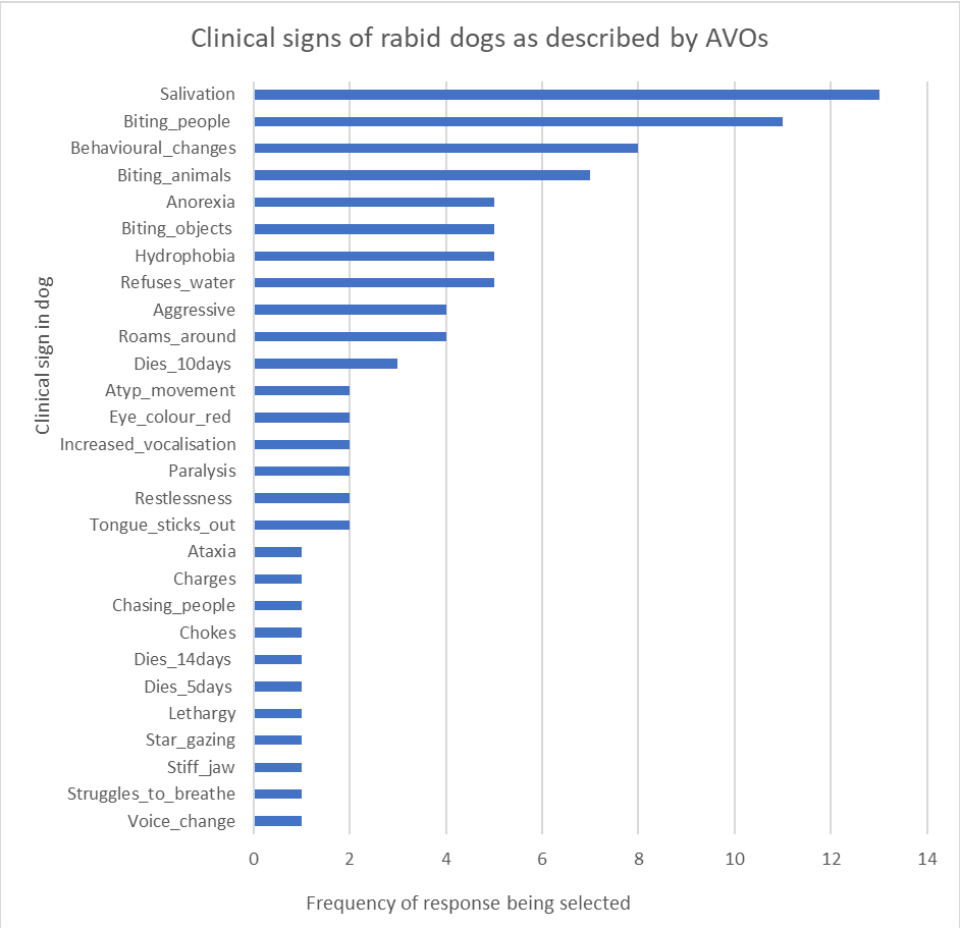

Figure 7- Assistant Veterinary Officers describe the clinical signs they recognise in suspected rabid dogs.

When asked how to prevent a dog from getting rabies (Q 5), all 19 AVOs selected 'vaccination'. 'Confinement' was the second most frequent response (13/19), followed by 'spaying' (3/19) and 'healthy diet' (2/19). One AVO answered that restraining the dog could prevent the dog from getting rabies, and another AVO from the same district suggested both 'control and supervision of the dog' and 'avoiding contact with other dogs'. Both responses are close to the 'confinement' option in the question. One AVO suggested that providing a breeding partner within the compound would help prevent a dog from getting rabies.

To the question, 'what treatment should be advised for humans bitten by dogs?' (Q 6), all participants answered that they would advise 'PEP' (post-exposure prophylaxis), and 14 AVOs also said that they would advise washing the wound with soap and water. Two AVOs, from one district, answered that they would also recommend 'prayer'. Two AVOs from

another district advised 'surgery to stitch the wounds' and 'referral to hospital with a dog bite referral letter'.

Regarding how many times the AVO had been contacted about a suspected rabid animal in the last 14 days (Q 7), answers varied from 0 to over 20 cases. Three AVOs from three different districts estimated that they had been contacted 20, 15 and 14 times, respectively. Nine AVOs stated that they have not been contacted about any cases.

When asked to estimate the number of times the AVO had been contacted about a human bitten by a dog in the last 14 days (Q 8), the AVOs answered from 0 to over 20 times. The four AVOs who estimated the highest numbers were from three different districts and said they had been contacted by more than 20, about 20, 16 and 14 human bite case victims, respectively in the last 14 days. The remaining 15 AVOS s estimated that they had been contacted about 0 to 9 bite case victims, and of these, one AVO said they had not been contacted about any bite case victims.

In the follow up question, (8-i) asking how many of these humans were referred for PEP, 11 of the 18 AVOs who had encountered human bite case victims reported that 100% of their bite case victim had been referred for PEP. In the second follow up (Q 8-ii), AVOs were asked 'If some were not referred for PEP, please give examples of reasons why'. An AVO from District D who had been contacted about three bite case victims, said that only two of these were referred for PEP. One person was not referred for treatment because the dog was 'vaccinated, and the dog owner was known'. One AVO from the same district (D) answered that 8/9 individuals were referred for PEP and that one person was not referred for treatment because the dog was 'vaccinated, still alive and not showing signs of rabies and has been provoked'. Another AVO, also from district D stated that they do not know how many of the two human bite case victims were referred for PEP. One AVO from District A answered that eight of approximately 20 individuals were referred for PEP and that not all bite case victims

were referred for treatment because they had been ‘bitten by own dog’. In addition, this AVO specified, that the dog would be 'observed for behavioural changes and PEP would be sought if required’. Another AVO from the same district (A) responded that 7/16 dog-bite victims were referred for PEP and that ‘some were only referred for wound dressing. Another AVO from District A answered that 1/5 individuals had been referred for PEP and that not all were referred for treatment as they had been ‘bitten by own dog’. The remaining AVO from district A stated that s/he had encountered two human bite case victims, but the interviewer failed to register how many of these had been referred for PEP.

When asked whether they register reports on humans bitten by dogs (Q 10), all but two AVOs, who said they register humans bitten by dogs ‘sometimes’, the remaining 17 responded that they always register report on humans bitten by dogs. In the follow up question (Q 10-i), the majority (17/19) indicate that reports are registered as handwritten documents. One AVO in District D registers reports on humans bitten by dogs in person, as well as by handwritten documents. An AVO from District C answered that s/he registers reports by phone call and also in the monthly report. The remaining AVO from District A said that s/he uses a computer to register these reports.

There was variation amongst AVOs regarding the reporting of suspected rabies cases in humans (Q 11). Twelve AVOs responded ‘yes, always’, five AVOs responded ‘yes, sometimes’ and the remaining two from districts D and C said ‘No’. In the follow up question (Q 11-i), the majority of AVOs (18/19) indicated that reports are registered as handwritten documents. One AVO from District A said that the information is also registered in a digital document as well as in a handwritten document. The remaining AVO from District A answered that s/he uses a computer.

In the second follow up question (Q 11-ii) asking whether the AVOs report data on suspected rabies cases in humans to anyone, ten of the 17 AVOs who were asked this

question, answered ‘yes, always’ and 5/17 responded ‘yes, sometimes’. Two AVOs in two different districts answered that they do not report the data.

Regarding who these data were reported to (Q 11-ii-a), answers from the 15 qualifying AVOs varied between those in the same district. One AVO from District D stated that s/he report the data to the ‘Senior Veterinary Officer’, another AVO also from District D answered that s/he report to the District Animal Health and Livestock Development Officer (DAHLD-O), while another AVO from District D said that s/he reports the data to the ‘Head of District’, who is also a direct manager.

Reporting to the DAHLD-O was the most common response to question 11-ii-a, with 6/15 participants spread across all four districts specifying ‘DAHLD-O. In addition to reporting to the DAHLD-O, one AVO in District A also reports the data to a local NGO. However, of the remaining participants from three different districts who said they report the data, two AVOs report to their ‘direct manager’, another said s/he reports to the hospital and yet another said s/he reports both the regional veterinary laboratory and to the hospital. One AVO said s/he reports to both the regional veterinary laboratory and the district health officer while the remaining AVO said s/he reports to the district veterinary office.

All participants responded that they register dog vaccinations (Q 12), with 15 of the 19 participants answering ‘yes, always’ and four answering ‘yes, sometimes’. In the follow up question (Q 12-i), the majority (15/19) replied that reports are registered as handwritten documents. Two AVOs from District D and District A, specified that they use vaccination cards to register the data. One AVO in District D said that the information is also registered in a monthly report and the remaining AVO from District A answered that s/he uses a district template to register the data.

When asked if they had received training on the sampling of suspected rabid animals (Q 13), 11 AVOs answered ‘No’ and the remaining eight, ‘Yes’. All AVOs from District B

(n=5) had been trained in sampling. When asked if they ever collect samples from animals with suspected rabies (Q 14), 13 participants responded 'no', whilst six participants answered 'yes, but rarely'.

Figure 8 of question 14-sub presents reasons given by the AVOs for not taking samples of suspected rabid animals. 'Lack of resources' was the most frequent response (9/19), followed by 'lack of equipment', 'lack of knowledge' and 'lack of training' (3/19).

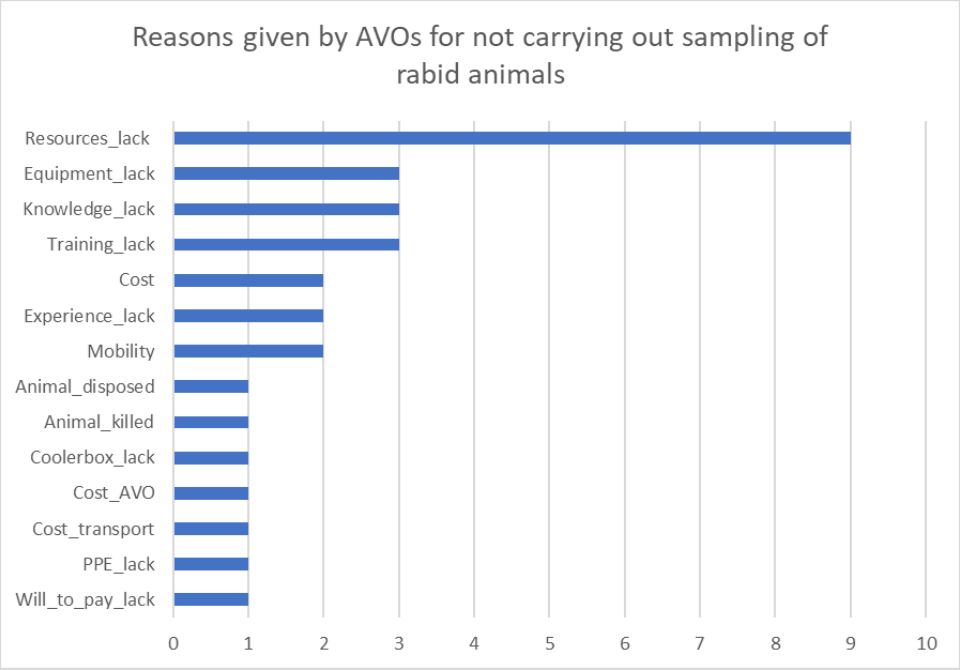

Figure 8 – Reasons given by Assistant Veterinary Officers for not taking samples of suspected rabid animals.

Amongst the six AVOs that answered that they do take samples of rabid animals, but rarely, the answers to follow up question on how they send the samples (Q 14 ii), show that they organise a form of transport (motorcycle, minibus, or local transport) to send the samples to the laboratory. Three of these six AVOs specified that they deliver the samples themselves. Table 9 shows how the selection of six AVOs submit samples to the laboratory.

Table 9 - Methods of transport used by Assistant Veterinary Officers to submit samples from suspected rabid animals to the laboratory.

AVO's District	Method(s) of transport used for submission of samples to the laboratory.		
C	Motorcycle	Car	
C	Vehicle		
B	Motorcycle	Local_transport	Delivers_self
B	Glass_slides	Delivers_self	
B	Delivers_self		
A	Minibus		

When asked to list the type of equipment used for sampling potentially rabid animals (Q 14-iv), use of a knife was the most common answer given (see Table 10). None of the AVOs made use of the full set of PPE as recommended by the WHO. However, all the AVOs (n=6) that said they sample suspected rabid animals, were vaccinated against rabies. In total, 11 of the 19 interviewees responded in question 15 that they have been vaccinated against rabies prior to the interview day.

Table 10 - Equipment used by Assistant Veterinary Officers when taking samples from suspected rabid animals.

AVO's District	Equipment used by AVOs when taking out rabies samples			
C	Knife	Facemask	Goggles	Gloves
C	Knife	Facemask	Gloves	
B	Knife	Facemask	Gloves	
B	Knife	Facemask (when available)	Gloves	
B	Axe	Gloves (when available)		
A	Knife	Gloves		Other: blood sampling glass

In the question on how they pack samples before shipping (Q14-v), all six AVOs who had answered that they submit potential rabies samples, said they use a bag to pack the samples, with 5/6 specifying that they used a plastic bag. Three mentioned using a cooler box.

When asked about vaccination campaigns for dogs in their district in the last two years (Q 16), all participants (19/19) answered 'yes' that there had been campaigns. In the follow up question (16-i) about who organised the campaign(s), 16/19 AVOs responded 'Government' and 9/19 specified 'Civil society organisation'. One AVO from District D responded, 'I don't know'.

In question 16-ii 'Did you participate in this/these campaign(s)?', 17/19 AVOS confirmed that they did participate, while 2/19 stated 'No'.

4.6. Building capacity of AVOs to perform rabies sampling

4.6.1 PowerPoint presentation for AVOs

A PowerPoint presentation on rabies sampling was presented to 19 AVOs. The presentation included:

- An ILRI Kenya video in Chichewa that AVOs could share with the public on the importance of vaccinating dogs and how to act after being bitten by an animal https://www.youtube.com/watch?v=qqic71k91fQ
- Detail on PrEP and PEP treatment
- Detail on rabies pathogenesis
- A step-by-step guide on how to safely take rabies samples
- Use of PPE
- Disposal of biohazardous elements after sampling
- The importance of safety when taking rabies samples
- How to submit samples to CVL
- Distribution of materials to AVOs and information posters in Chichewa

The PowerPoint contained 24 slides in total and took approximately 45 minutes to deliver (Appendix VII) AVOs in all districts showed interest in the presentation and asked for the ILRI video be sent to them so they could share it with their communities.

4.6.2 Video showing how to collect rabies samples

The final instruction video prepared in Norway before field work in Malawi is available here: https://www.youtube.com/watch?v=Jbx9Fowrkkc. It was shown to the AVOs during the presentation. This video was sent to all AVOs with Smart phones.

4.6.3 AVO presentation on rabies sampling and biosecurity

During the PowerPoint presentation, the sampling instruction video was played to all 19 AVOs. In the video a fox (*Vulpes vulpes*) carcass was used to demonstrate the three sampling methods, submission of the fox's head, extraction of brain tissue and application of brain tissue to blood spot cards for transport to the laboratory. The final version of the film is seven minutes and four seconds long. The film evoked several practical questions during all the sessions, and several AVOs expressed that they had not seen these techniques performed before.

4.6.4 Distribution of equipment to AVOs for rabies sampling

After the presentation, all AVOs were given PPE and sampling equipment. AVOs would be able to take samples using the three sampling methods presented in the video and equipment was included to help them do this. AVOs stated that they were pleased to receive PPE and sampling equipment.

The list of equipment distributed and sampling instruction sheet and requisition form is found in Appendix IX.

4.6.5 Provision of poster

The poster in Chichewa was developed by ILRI and provides information on how to behave around dogs and potentially rabid dogs, as well as how to respond to dog bites in areas where rabies was endemic. The AVOs were grateful for the poster and expressed interest in using it in their work. Some of them said they would have liked more to distribute in the communities. The poster in both Chichewa and English is in Appendix VIII.

5. Discussion

5.1 Assessing the ability of different transport media to preserve rabies virus RNA over time and different temperatures

When determining how to spike samples with inactivated rabies virus from a commercial vaccine, it was unexpected that the Cq-values increased with increasing volumes of added vaccine. These results would normally suggest reduced amount of virus in the sample. Possible reasons include that a high concentration of the vaccine saturated the PCR reagents and made the amplification process less efficient (Schrader et. Al, 2012), or that the presence of aluminium hydroxide adjuvant in the Rabisin vet. ® vaccine inhibited the PCR reaction (Kuffel et. Al, 2020). Because of this, it was decided in the subsequent phase of the laboratory experiment not to add the volume of vaccine that had resulted in the desired Cq-value, but to accept a slightly higher concentration of virus and thus a lower Cq-value than initially planned. In retrospect, it may have been relevant to test even lower concentrations of vaccine, and perhaps to dilute the vaccine and add the dilutions to the brain tissue homogenate. This could have revealed if inhibition could be counteracted, and if lower concentrations of vaccine could stably provide slightly higher Cq-values. Lower concentrations of virus in the tissue may also have been more relevant to naturally infected samples collected in the field in Malawi, where decomposition of the animal and the samples brain tissue is likely to begin before analyses.

To identify a cost effective and reliable transport media for rabies samples in Malawi, we compared the ability of different media to preserve viral RNA over time and at different temperatures. Overall, the BSC performed the best with low and stable Cq-values over time, for all temperatures tested. Of the lysis buffers, the DNA/RNA Shield had the most stable Cq-values over time, especially at the highest temperatures while EasyMAG, MagNA Pure and

the FTA-cards had some variable results. The FTA-cards resulted in Cq-values closest to the cut-off value for positive samples at 35.0, at 4˚C and 21˚C but produced stable Cq-values at 36˚C.

Because all the transport media performed adequately in the experiment, other factors such as cost, and availability were considered to make our selection on which media to bring for use in Malawi. The DNA/RNA-shield performed best of the lysis buffers, but it is slightly more expensive than Nuclisens EasyMAG Lysis Buffer. The latter also has a supply-vendor in South Africa. The MagNA Pure lysis buffer performed poorer under high temperatures and is also the most expensive and was therefore disregarded for further use. The biosample cards performed well, and the BSC was best overall, especially when stored at higher temperatures. It is also significantly cheaper than the FTA-cards. It was, therefore, decided that we would bring both the Nuclisens Lysis buffer, the DNA/RNA-shield and both biosample cards to Malawi. Even though we showed an overall preference for the BSC cards, it was decided to bring both types of biosample collection cards to test how they perform under field conditions. The laboratory experiment provided useful information to help decide on what sample transport media might work in Malawi.

WOAHs Terrestrial Code advises that samples are preserved when it is not feasible to send refrigerated samples. For example, samples preserved in formalin, or a glycerol/PBS mix can be used to preserve samples for dFAT and other diagnostic methods (WOAH, 2018). Using FTA-cards to preserve rabies samples in low-access areas has previously been tested (Picard-Meyer et. Al, 2007) and shown high specificity and sensitivity when tested with dFAT and RT-qPCR (Rasolonjatovo et al., 2020). However, we could not find any information on the use of the other transport media to store and preserve rabies samples over time.

5.2 Registration of samples from potentially rabid animals at CVL

Between January 2021 and May 2023 altogether 72 samples had been received at CVL from potentially rabid animals. Because of missing data, it is difficult to say with certainty how many samples comes from each district and region.

The registration book at CVL does not include metadata (e.g., symptoms of animal, date of death, reasons for sampling, whether it has bitten anyone, location etc) or information about the sender or sampling date. Hence, it was not possible to match result of the RT-qPCR with symptoms of the animal or infer the potential sample quality and decomposition of the sample. For efficient surveillance, routines should be put in place for registration of necessary metadata at CVL.

For the 29-month period in question, 12 rabies samples were sent from the Mzuzu Regional Veterinary Laboratory in the Northern region of Malawi, which is a high number considering the distance from Lilongwe and the complicated logistics of sample shipment. However, it was not possible to confirm the identity of the individuals submitting the samples to triangulate information with data from interviews and FGDs in the district. The AVOs from the FGDs in Mzuzu were the only ones that had received training in handling rabies cases after they started working as AVOs. They were also the only AVOs to state that some EPAs in their region had equipment for sampling. One AVO in the Northern region said in the FGD that they sent “a lot of samples” to the CVL for rabies testing, but in the subsequent interview the same AVO stated that they sent samples rarely. With respect to the reported lack of sampling by AVOs in Thyolo, it is possible the NGO Mission Rabies operated in this area and that they perform sampling and testing independently.

5.3 Rabies testing of samples at CVL

Of the 26 samples available for testing by RT-qPCR, 17 were positive and four were uncertain, with a Cq-value between 35.1-40, compared to 16 positives by dFAT. Even in samples stored for as long as 14 months, were clearly positive with RT-qPCR. The sensitivity of the RT-qPCR is less hindered by sample autolysis than dFAT (WOAH, 2018), enabling detection in more decomposed samples. This might explain the sample that came out positive by RT-qPCR (Cq-value, 34.2), but negative with dFAT, and the high Cq values (35.5-38.2) in the four samples deemed uncertain by RT-qPCR and negative by dFAT. The possibility of cross-contamination, false positive results during RT-qPCR, or the need to evaluate the limit for the threshold values for positive and negative samples must be considered. False positives should have been detected by the controls in each run. The uncertain samples should ideally be tested again, using both dFAT and RT-qPCR (WOAH, 2018).

The fact that 65% of tested samples at CVL were positive by RT-qPCR, indicates that the threshold for sampling and testing might be too high in Malawi, where rabies is endemic. Considering there are 500 human rabies deaths annually (Hampson et al., 2015) in humans, the number of samples from animals analysed at CVL should be much higher. Ideally, surveillance for rabies in animals in Malawi would include passive surveillance with testing of all potentially rabid animals, and an active surveillance component with testing of for example selected roadkill or other diseased and dead animals with unexplainable or unknown symptoms. Then the number of negative samples should be greater than the number of positive samples, and the annual number of tested animals should far surpass the number of human rabies deaths.

Establishing efficient diagnostic method (RT-qPCR) in Malawi in 2023 was a step in the right direction for strengthening diagnostics and surveillance of rabies. Some AVOs mentioned that samples they sent often were too decomposed to be analysed by dFAT when

they reached the laboratory. The potential for RT-qPCR to detect rabies virus in samples kept in less ideal conditions or stored over a longer time, may thus increase testing. However, rabies virus is an RNA virus, and RNA is vulnerable to degradation (Fleige and Pfaffl, 2005). Therefore, samples for RT-PCR-analyses must also be transported in appropriate media and under appropriate conditions. In the future, transport of whole carcasses to CVL should be encouraged. In areas where this is not realistic, use of transport media such as BSC as suggested in this study, can be encouraged further.

A weakness in this study is that samples with brain tissue at CVL are homogenised using a vortex, not a homogeniser, making the process less effective and RNA extraction possibly less reliable. CVL also lacks a freezer cold enough to store RNA-samples and positive controls for the RT-qPCR. The freezer at CVL holds -20°C, while RNA should be stored at -80°C. In addition, occasional power cuts also mean that sample material and controls may degrade.

5.4 Agreement between LFT and RT-qPCR

Eighteen of the samples were tested by LFT and results showed a very good agreement with the RT-qPCR results. The rabies LFT clearly has a lower sensitivity than RT-qPCR although the manufacturer claims that specificity and sensitivity are 100% and 94.1%, respectively, (https://www.woodleyequipment.com/product/792/Anigen-Rapid-Rabies-Ag). LFT is not recommended as a standalone diagnostic method in the WOAH terrestrial guide. With an acceptable sensitivity and specificity, the LFT could be used for field screening when sample transport and analyses at the reference laboratory is expected to take time. In such cases, samples could be tested by LFT in the field and then sent to the reference laboratory for final confirmation with PCR and/or dFAT.

A high-test specificity and a high positive predictive value of LFTs could provide bite victims a rapid and reliable result regarding the animal's rabies status. In the FGDs, AVOs

said they are often informed about suspected rabid animals late in the process, which results in delays for sampling and testing of the animal, if it is sampled at all. A positive LFT test in the field might also encourage PEP treatment of bite victims more rapidly and enhance efforts to identify other bite-victims. It might also encourage implementation of control measures in the area, such as vaccination of local dogs and cats and quarantine other suspected animals.

Because rabies has a near 100% mortality rate when symptoms occur, a high sensitivity and high negative predicative value is also essential. A false negative LFT-test from a dog that has bitten, could potentially lead to no PEP in an exposed human bite case victim. Hence, even with a negative LFT-result, PEP-treatment should be initiated in bite case-victims of non-vaccinated dogs. If, upon testing with RT-PCR and/or dFAT, the negative result is confirmed, further PEP treatments can be cancelled saving the bite-victim further costs for treatment and transportation. Considerations would still need to be taken to ensure the person working with the LFT in the field have sufficient funds to travel and sample the animal, PPE and training on how to use the LFT.

Unfortunately, a relatively low number of samples were tested by LFT (n=18). Ideally the dataset would have been greater in order to evaluate the test more robustly. The samples were not fresh either, which is recommended for this test, and may have influenced results. Further investigation should be done before considering if the LFT could be used for screening rabies samples in the field.

5.5 Control and surveillance of rabies in Malawi

5.5.1 Rabies Awareness

This study has shown that AVOs are important contributors to several pillars of the STOP-R framework. Firstly, they act as sources of knowledge of rabies in their communities, by recognising the disease, understanding transmission and the control measures. In the

interviews, all AVOs selected 'vaccination' as a manner of preventing rabies in a dog, with the majority (13/19) also choosing 'confinement'. Most dogs in Malawi are owned but free roaming, (Mazeri et al., 2021) and many of the AVOs acknowledged that free roaming dogs increase the risk of rabies transmission.

Through their rabies awareness activities, AVOs spread knowledge about rabies and help operationalise the socio-cultural pillar of the STOP-R global framework. Awareness work was a significant topic in all the FGDs, and created a lot of engagement among the AVOs, who stated that the communities are often ignorant to the dangers of rabies, and do not take potential infection seriously. They had many ideas on how awareness work should be done and how it can be improved and mentioned that they use natural gatherings to create awareness. This type of awareness work can be less resource demanding and might be an area where the AVOs feel that they can have a significant impact. Nilsson (2014) found that school children in Lilongwe who had participated in rabies education programs had a better understanding of rabies than children who had not participated, and knew that it was a zoonosis, transmitted through bites. This is vital knowledge that can increase the likelihood that dog bite victims seek treatment (Abela-Ridder et al., 2018).

5.5.2 Control of rabies by AVOs and the barriers to performing this work

AVOs also support the technical pillar in the STOP-R framework by vaccinating dogs and referring dog bite victims for PEP and wound care. It is important that the AVOs, who are often the first line of contact, can correctly advise dog bite victims on wound-care guidelines and PEP as specified by the WHO (WHO, 2018). In FGDs, and interviews AVOs explained that people come to them for help after a dog-bite, showing that community members recognise AVOs as rabies healthcare workers. Nevertheless, although AVOs are ideally positioned to contribute to rabies prevention work in Malawi, there are a number of barriers

preventing them from completing their work to its full potential. The findings in this study suggest that AVOs are poorly resourced in all elements of their work.

AVOs stated in FGDs that there are too few AVOs to cover the workload. This is supported by the veterinary authorities at CVL where it was estimated that only one of the four study areas is at 53% AVO capacity, with the remaining three under 50% capacity (Dr. Joseph Nkhoma, Deputy Officer in Charge at CVL, personal communication). It is easy to understand the frustration expressed by many of the AVOs who described encountering preventable human deaths, potentially being blamed for deaths, knowing that they are responsible for control of the disease, however not being given the necessary means for effective rabies prevention. AVOs stated that this has an emotional impact on them and amongst veterinary technicians based in different countries, Kogan et al. (2020) found that high emotional exhaustion and low professional efficacy can lead to burnout.

Due to inadequate monthly funding, AVOs in all districts struggle with mobility and end up vaccinating fewer dogs. Furthermore, too few vaccine doses and syringes are provided for the annual vaccination campaigns, and AVOs said they had used their own resources to vaccinate dogs. They also lack cooler boxes to maintain vaccine quality. AVO fear that vaccinated dogs contract rabies is justified, as the reduced effect on immunisation that inadequate transport and storage of vaccines have is well documented and has prompted calls for the development of thermotolerant rabies vaccines (Lugelo et al., 2021).

Inadequate funding is also a reason why AVOs say they do not collect samples of rabies-suspected animals. Some mentioned that they use their own money or time to transport rabies samples to the laboratory and several also mentioned that they must pay a fee to the laboratory. However, testing of notifiable diseases in Malawi is free (Dr. Joseph Nkhoma, Deputy Officer in Charge at CVL, personal communication), but this may not have been

communicated efficiently to the front-line workers. As a result, there is a failure of sampling and therefore diagnostics impacts rabies surveillance systems (Haselbeck et al., 2021).

AVOs state clearly in all FGDs that they desire more rabies sampling training and feel that the lack of this is a barrier against sampling. If AVOs cannot take samples, even when the resources are available, the local capacity in rabies surveillance in Malawi is further reduced.

Contrary to WHO vaccine recommendations (Rupprecht et al., 2018), not all AVOs were vaccinated against rabies. Eleven of the 19 AVOs from the FGDs had been vaccinated against rabies at some point. However, we lack information on when they last were vaccinated, or whether the booster was long overdue. Additionally, PPE is not available for sampling of suspected rabid animals. This means AVOs put their own health at risk in the field when vaccinating dogs and sampling rabies suspected animals, and places them in the dilemma of having to compromise their own health and finances (to transport samples) to fulfil their job. The underfunding of AVO activities exists within a wider context of poor resource allocation to rabies in Malawi, which is typically seen in high burden rabies countries (Haselbeck et al., 2021).

5.5.3 Responding to dog bites and rabies in humans

In FGDs the AVOs describe the difficulties they face when they follow up human bite cases to assess whether the dog was likely to be rabid or not. The responsibility of AVOs to write a recommendation letter for bite victims to receive PEP in health care facilities seems like an unreasonable burden, because it is often impossible to conclude that a patient has not been exposed to rabies.

AVOs described that not all dog-bite victims referred to the hospital for PEP are able to access and complete treatment often due to poor availability, mobility, and cost. This is problematic as rabies awareness is only of benefit if PEP is accessible (Hasanov et al., 2018). It should be administered rapidly after exposure and delays caused by treatment unavailability

and time spent gathering funds, complicates access to PEP for the poorest (Lembo et al., 2010).

Poor funding for rabies control, plus a lack of awareness on the true burden of the disease (exacerbated by inadequate surveillance), means that other diseases are prioritised before rabies (Kaare et al., 2009). Malaria is also endemic in Malawi with an estimated 4.4 million cases reported in the country in 2020 (Mangani et al., 2022), making malaria more of a pressing issue for the health authorities compared to rabies.

AVOs mentioned in FGDs that doctors in Malawi do not always suspect that patients are suffering with rabies, suggesting that other more common diseases, like malaria, may be better recognized amongst doctors. Mallewa et al. (2007) found that 11.5% of child deaths in a Malawian hospital were initially attributed to cerebral malaria, but rabies was the cause.

Many people do not recognize the clinical signs of rabies and many deaths occur at home (Taylor et al., 2015). A post-mortem diagnosis by detection of antigens in brain samples using dFAT is recommended in humans who have died with symptoms of acute, progressive encephalitis resulting in coma and death within 7-10 days of onset of symptoms (Rupprecht et al., 2018). As of now, there is no public health laboratory in Malawi that analyses rabies samples from humans. Very few samples from humans are sent to the CVL for testing, suggesting that rabies is not a priority for the Public Health Department. Another challenge when it comes to diagnosing rabies in humans is that the next of kin in rabies endemic countries are often reluctant to consent to autopsies due to various cultural, religious, or other beliefs (Lawrence *et al.*, 2021, Rupprecht et al., 2018). In humans, two or more ante-mortem tests, such as antibody testing in serum, CSF or nuchal skin biopsies, or RNA detection in saliva, skin biopsies or CSF can be used to confirm rabies symptoms in unvaccinated individuals (Jackson, 2016, Rupprecht et al., 2018).

5.5.4. Surveillance of rabies

The AVOs lack information on rabies diagnosis and case metadata recorded in hospitals and health facilities. The study found a lack of cooperation at the local level between public health facilities and animal health workers, which most likely reflects a similar weakness in the whole length of the system. A One-health approach, ensuring cooperation between the Veterinary branch of government and Public Health branch of Malawi is necessary to build a robust surveillance program (Franka and Wallace, 2018, WOAH, 2018). Including public health programs in a more active role against rabies could also be beneficial because public health-campaigns have more resources and reach a broader audience.

An aim of the study was to find out how the AVOs contribute to rabies surveillance and what the bottle necks are to this work, and in the FGDs we asked how the AVOs contribute to surveillance and to describe the challenges they face in this work. It became evident that the AVOs, who are responsible for reporting cases and collecting samples in the field, do not seem to understand the proper meaning of surveillance. For example, one AVO suggested that they record dog vaccination data in vaccination certificates provided to dog owners. In every FGD the conversation quickly drifted to sample collection, vaccination, and awareness, when we asked about surveillance.

It was established that the AVOs carry out some activities necessary for rabies surveillance by collecting rabies-related data and submitting monthly reports. The information recorded by AVOs in the monthly reports, if combined with test results and location of suspected cases, could be used as data points in a surveillance program. However, during the interview there were large discrepancies in the monthly reports made by the AVOs. They send their reports to different offices and made contradicting descriptions of correct procedure. This suggests that there is no well communicated system or that the AVOs lack the training. Because most AVOs treat all bite-cases as rabies cases when the investigation is

challenging, the reported numbers might not be reliable either. Ideally, reports should be founded in a confirmed diagnosis, with locations and species and supplied with reports from the hospital.

Establishing a good surveillance program, which can be used to detect outbreaks, prioritize funding, and control programs is costly and requires competence. At CVL there are currently around 30 employees. In comparison, the sister institution in Norway, NVI, has approximately 300 employees, even though Norway has few infectious and zoonotic diseases affecting humans and livestock. There is clearly a need for increased funding and a competent workforce in Malawi. The establishment of a veterinary education at LUANAR in 2013 is a step in the right direction to provide competence and veterinary personnel.

There are currently a number of barriers to effective rabies control and surveillance in Malawi, both within the AVO role and beyond due to the allocation of resources to this work in the country. Malawi is a low-income country, and it can be challenging to request greater investment in controlling rabies. However, Lembo et al. (2010) argue that spending more money on dog vaccination saves not just human lives, but money in government and household budgets, that ultimately would have been spent on PEP. Where canine rabies exists, people will continue to be infected, and both Ministries of Health and of Agriculture should share investment in dog vaccinations to eliminate dog-mediated rabies in humans. Ineffective campaigns with low vaccination coverage are a waste of resources and can be highly demoralising for veterinary staff and communities (Lembo et al., 2010) and this sentiment was clearly expressed by the AVOs in the FGDs.

Attitudes towards dogs is a barrier when establishing lasting efforts against rabies. Dogs are not a valued, so there is less incentive to spend resources on sick dogs. People do not prioritize getting them vaccinated and because dogs don’t provide any income or source of food, people don’t want to spend time or money on vaccines or treatments. Many people

also fear dogs, partly due to rabies, and mistreat them. Responsible dog ownership is recognized as an important aspect of rabies control in the STOP-R framework (WOAH, 2022). Similar attitudes towards dogs were also registered in Malawi (Kvisle Abildsnes, 2023).

To define rabies as a priority, greater surveillance is required, so that the true burden of the disease is documented (Lembo et al., 2010). There is no structured rabies surveillance in Malawi at this moment, and no regular reporting of rabies in humans or animals. They lack a digital recording system, and all samples and metadata are registered on paper. Results from laboratory analyses are reported back to the sender, but it is rare that they influence the outcome of the case.

Malawi is fortunate that it has AVOs motivated to work with both animal and human health, who are available for training in sampling of rabies-suspected animals. Providing AVOs with training would boost local capacity in rabies surveillance, as AVOs clearly express that more training would result in more sampling. During the fieldwork period, 19 AVOs received training in rabies sampling, by video and PowerPoint. These training sessions were well received by the AVOs, and it is possible that such endeavours could be upscaled to reach a wider number of AVOs in a cost-effective manner. Contributing to AVOs professional development may also improve their job satisfaction (Kogan et al. 2020).

However, upscaled training must be matched with funding for transport and shipping. In the case of this study, although well received, the capacity building and provided sampling equipment did not give the result we hoped for. There was a small increase in samples sent to the CVL in the four-month period after our meetings with the AVOs, but this might be a coincidence. It is possible that reminders and encouragement of the participating AVOs to collect samples, for example by WhatsApp messages could have increased the number of samples submitted to CVL during the project period.

It was uplifting, nevertheless, that one AVO had gone from taking zero samples in the nine years they had been an AVO, to three samples in the four months after training. The 18 remaining AVOs had still not sent samples to the laboratory for testing per 20.09.2023 (Dr. Joseph Nkhoma, Deputy Officer in Charge at CVL, personal communication).

Based on results from FGDs, rabies sampling requires time and resources from the AVOs and is often not feasible. Following our visit, two of the AVOs from the FGDs requesting funding to go and collect a rabies sample (Dr. Joseph Nkhoma, Deputy Officer in Charge at CVL, personal communication), indicating a will to sample but lack of funds. Despite provision of training, equipment and rabies vaccination, the reality is that the AVOs must locate the suspected rabid animal, possibly exhume the animal and cover transportation costs to retrieve and ship the sample. This speaks to the importance of funding to facilitate sampling and surveillance, which clearly needs to be a priority for all stakeholders involved in rabies control in a country.

Although the saturation point was being approached after four FGDs with little new information being offered at the final FDG, it may have been advantageous to conduct a few more FGDs to confirm this. However, time was a limiting factor in this project.

Initially, the aim of this thesis project was to focus on improving sampling and detection of rabies in animals in Malawi. The idea was first to perform the laboratory experiment in Oslo, and then do field work in Malawi providing training and sampling equipment to AVOs. We would provide them with three different transport media and ask them to collect samples in triplicates in the different media. Then we would analyse these by RT-qPCR at CVL and assess performance of the media under field conditions. However, during our preparations we became aware of the significant challenges that exist for AVOs to submit samples to CVL, and were warned that we may not receive sufficient samples. We therefore decided to adapt the study, adding a component of interviews and FGDs to hear

from AVOs themselves how they work to control rabies, and what the barriers are to their work.

Indeed, the number of submitted samples during our visit in Malawi was low. In addition, we received feedback from AVOs who had attended FGDs that community members react negatively when they try and remove the head of a dead animal making sampling difficult. The FGDs and interviews however, provided a truly rich dataset on how the AVOs work, how they are committed to their duties despite lack of resources and what the main barriers are in their work for surveillance and control of rabies in Malawi.

This study points to a high capacity and motivation among AVOs, but also a number of bottlenecks that are hindering them from delivering work to their full potential. We believe that results from this study, might be relevant to inform policy in Malawi that could potentially support rabies surveillance and control.

6. Conclusion

This study shows promising results for the use of various transport media and biosample cards to store rabies samples in field conditions where immediate testing is not feasible. Implementation of RT-qPCR for rabies testing in Malawi by a bioengineer from NVI facilitates the use of these transport media, which could lead to more reliable diagnosis, especially in samples sent from rural areas or from more autolytic brain tissue.

Unfortunately, sampling of suspected rabid animal in the field is a neglected task, and failure to increase sample collection is likely a combination of lack of resources, lack of training and lack of manpower. As of today, the AVOs mainly work with rabies by creating awareness in their community, vaccination of dogs and cats and by investigating bite cases and referring the victims to the hospital for further treatment.

This study points to the high capacity and motivation among AVOs to contribute to controlling rabies, but also the bottlenecks that are hindering them in their work. Laboratory investigations of media for sample transport indicate that it can be possible to preserve samples adequately in Malawi for some time, even without proper cold chain. It is our hope that results from this study, and studies like it, could influence policies and political prioritisations contributing to improving surveillance and control of rabies in Malawi.

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Appendices

Appendix I – Plan for focus group discussions, interviews and presentations

Plan for meeting and focus groups

Focus group

- Information for participants – by facilitator (see separate document)
- Participants sign consent sheet
- Start audio recording – facilitator will ask participants to present themselves and give each a number
- Warm up
- Focus group discussion:
    - Facilitator will read questions in English
        - Facilitator will encourage discussion by using the following type of comments: "Could you expand on that please?", "Could you tell me more about…", and to people who are not talking: "Do you have something to add?", or "Are your experiences the same? Could you tell me a bit about what you do/see/think"
        - If the group discussion doesn't cover the desired topics, the facilitator will ask sub-questions to guide the conversation.
    - Notetakers will make notes of what is said, body language, tone, non-verbal cues, group dynamic etc.
    - The facilitator will make sure everyone participates by inviting them into the conversation using follow up questions
    - Facilitator will guide the discussion onwards to the next questions when the time is up
        - See separate document with questions, theme and approximate schedule
    - Notetaker 1 will keep track of time and signal to facilitator when it's time to move on
- Before we finish, we ask if anyone have anything else they would like to add
- Thank the participants and tell them we will move on to the interviews after a short break

Break (15 minutes)

Interview

- Give the participants information on upcoming interview
- Take the participants and interview them one by one
    - Interviews will be executed by all three students (and Joseph)
- Start the interview by filling out the information at the top of the sheet
- A copy of the interview notes form can be handed out to allow the participants to read the questions themselves
- Tell the participants that you will start asking them some questions - if they don't know the answer(s), assure them that it is okay to answer "I don't know". If there are any questions they do not wish to answer, that's okay.
    - If they answer something other than the given alternatives, select "other" and ask them to specify. Write down the answer in your copy of the questionnaire.
    - Yes/No/I don't know questions – Start by saying "This is a yes or no question" and continue reading the question as it is written in the questionnaire and note answer on your copy.

- If the question is a multiple-choice question – read the question as it is written in the questionnaire and tell them you will give them multiple alternatives and that they can select all answers that apply to them
  - Read the alternatives slowly, so the participants can answer as you read them out loud
  - Take note of all the answers in your copy of the interview notes form
  - Ask the participants if they have anything to add for "other"
- If it is an open-ended question – start by saying "this is an open-ended question, please answer in your own words" and read the question as written in the interview notes form. Write down the answer as precisely as possible in your copy of the interview notes form.
  - After they have answered, let them think for a while and ask if there is anything else they would like to add
- For the questions (7 and 8) under caseload, let the participant state an approximate number and write the number in the following box using digits
- For the question under surveillance, specify that the first questions (9) are regarding suspected rabid animals
  - Only ask the follow up-questions if the answer to the first question is "yes" (9 "Do you register reports on suspected rabid animals?")
  - After finishing the questions for potential rabid animals tell them that we will ask the same questions regarding human bite case victims, proceed to read the following question (10. Do you register reports on humans bitten by dogs?) as it is written in the interview notes form
    - Only ask the follow up-questions if the answer to the first question is "yes" (10. "Do you register reports on human bitten by dogs?").
  - After finishing the questions for human bite case victims tell them that we will ask the same questions regarding suspected rabid cases in humans, proceed to read the following question (11. "Do you register reports on suspected rabies cases in humans?") as it is written in the questionnaire.
    - Only ask the follow up-questions if the answer to the first question is "yes" (11 "Do you register reports on suspected rabies cases in humans?")
  - After finishing the questions for suspected rabies cases in humans tell them that we will ask the same questions regarding dog vaccinations proceed to read the following question (12. "Do you register reports on dog vaccination?") as it is written in the interview form
    - Only ask the follow up-questions if the answer to the first question is "yes" (12. "Do you register reports on dog vaccination?")
- For the questions under sampling, remember to ask the follow-up questions if they answer "yes" to question 13. ("Do you ever collect samples from animals with suspected rabies?")
- For the question under rabies prevention, remember to ask the follow-up questions if they answer yes to question 16. ("In the last two years, have there been any rabies vaccination campaigns for dogs in your district?")
- For the questions under dog welfare and euthanasia, remember to ask follow up questions for question 27 "By law, who is authorised to kill a rabid dog" and if they:
  - Answer "Yes" to question 18. ("Have you ever witnessed cruelty towards dogs?")
  - Answer "Yes", "some of them" or "No", to question" 29. ("If given the opportunity, would dog owners in your community want to have their dog(s) vaccinated against rabies?")

- Thank the participants for taking part in the interview

Break (5 minutes)

Presentation

- Show ILRI video – tell them we want to send it to them so they can share it.

- Presentation – focus on collection and safety, go through equipment.
  - Sampling technique
  - Use of tubes, BCS and FTA cards
    - Amount of sample needed in the tube
    - BCS and FTA cards must be dried before they are folded
  - Store in refrigerator, or at least in the shadow
  - Requisition form must be complete before sending sample
  - Mark EVERYTHING
  - Send WhatsApp message to Joseph every time they take a sample
- Show Norwegian Veterinary Institute rabies sampling video
- Ask the participants what they think about this film and how it compared to the field situation in Malawi
- Hand out sampling equipment and ask them to collect samples of suspected rabid animals and send them to us
- Information on booster vaccination and sample submission – by Joseph
- Take any questions?

Thank the participants for helping us.

Vaccinate participants – Joseph takes the participants to a health centre where they can administer the vaccines.

Appendix II – Participant information and consent form

Participant Information Sheet and Consent Form

Study title
Procedures for dealing with potentially rabid dogs and human bite case victims in Malawi – sampling, surveillance and animal welfare.

Invitation
You are being invited to take part in a research project. Please read this information and ask questions if anything is unclear. If you decide to participate, please sign the consent form.

Who are we and what is the project's purpose?
The researchers are Dr. Joseph Nkhoma, veterinarian at the Central Veterinary Laboratory (CVL) and PhD student at Lilongwe University of Agriculture and Natural Resources (LUANAR), Charlotte Eikeskog Ravnås, Elizabeth Akinsanmi-Guren and Ragnhild Kvisle Abildsnes, veterinary students, from the Norwegian University of Life Sciences (NMBU). The study is part of the project "Improving implementation and operation of a One Health platform to combat rabies in Malawi", led by the Norwegian Veterinary Institute (NVI) in collaboration with CVL.

The purpose of the study is to gather information on procedures for responding to rabies in the field setting in Malawi to inform interventions to strengthen control of rabies in Malawi.

What will you do and what are your rights?
You will be invited to participate in a focus group and an interview. You will be asked about your professional experiences related to dog welfare, rabies management and surveillance. Altogether, it will take about 2 hours. The first 1-1,5 hours will be spent in a focus group with 3-5 other colleagues where the group will discuss 4-6 questions. An interview lasting about 30 minutes will be conducted with each participant individually. If any field visits are possible, the study group will accompany the participant to observe how rabies cases are managed.

Participation is voluntary. If you decline to participate this will be kept confidential. You may refuse to answer any question and can withdraw at any time during the focus group or interview. Withdrawal within two weeks of participating gives you the right to have the data you have supplied deleted.

What happens to the information and who has access to it?
The information will be used in our final thesis write-up and related presentations. It may also be used in reports or journal publications. All the information you provide is confidential and you will remain anonymous. The audio recordings will be stored securely and deleted once it has been anonymously transcribed within four weeks. Data access will only be shared with the researcher group at LUANAR, NMBU and NVI. All identifiable data will be deleted within one year, remaining data will be deleted within five years.

Contact for further information
If you have any concerns or questions, feel free to contact the researchers through email: (Email addresses of Dr. Joseph Nkhoma,Ragnhild Kvisle Abildsnes, Elizabeth Akinsanmi-Guren and Charlotte Eikeskog Ravnås)

Consent Form

- I confirm that I have read and understood the information above.
- I voluntarily consent to participate in this project.
- I consent to the focus group being audio recorded.
- I understand that notes will be taken during the interview and the focus group.
- I understand that I can refuse to answer questions without any consequences.
- I understand that I can withdraw from the project up until two weeks after the interview, in which case the data will be deleted.
- I understand that the data from my interview and any observations will be handled confidentially.
- I understand that my contributions may be anonymously quoted in the report, final thesis write-up or subsequent related presentations or journal publications.
- I understand that I am entitled to access the information I have provided at any time while it is stored as specified above.
- I understand that I can contact any of the people involved in the research to seek further information.

Focus group number: _____

_____ _____ _____

Name of participant Date Signature

_____ _____ _____

Name of person taking consent Date Signature
(if different from researcher)

_____ _____ _____

Researcher Date Signature

Appendix III – Topic guide Focus group discussions

Topic Guide Focus groups
Brief warm up: how many of you have dogs? Why do people keep dogs in your communities? We are curious, where we come from people often describe their dogs as family members - what about in Malawi, how do people feel about their dogs here?

We would like to start with a question on animal welfare – or welfare for dogs. Could you describe what you think gives good welfare for dogs? 10 mins

- What sort of welfare do dogs in your communities have?
- Do you think dogs ever struggle or suffer in your communities? If so, what causes these challenges?

How would you describe the awareness of rabies in your communities? (10 minutes)

- What do people in your communities know about protection against rabies?
- How common is rabies in animals, for example have most people seen a rabid dog?
    - Do humans get rabies in your communities?
- How do people react around potentially rabid animals?
- Do you think that fear of rabies affects people's attitudes to dogs? If so, how?

How do you work to control rabies in your communities? 10 mins

- Have you received any training on how to work with control of rabies?
    - What sort of training?
- What are your responsibilities in your work within control of rabies?
- How would you describe rabies surveillance in your work? With surveillance we mean reporting and monitoring.
- What barriers exist for surveillance/reporting rabies in animals, or dog bites in humans to central authorities?

Please describe your routines when you are contacted about a potential rabid animal? (10 mins)

- Who will contact you about a rabid animal?
- How does the animal generally get killed?
    - What are your thoughts about this type of killing?
    - What prevents the use of shooting or drug induced killing/euthanasia?
- What challenges do you face sampling rabid animals?
    - What challenges do you face when submitting samples to the laboratory?

How do you respond when you are contacted by a person who has been bitten by a dog, what are your routines? (10 mins)

- What are the challenges when responding to a dog bite case?
- Are there any barriers preventing people from getting PEP, and completing the whole course of treatment?

Is there anything else you would like to say about control of rabies in Malawi or about looking after dogs? Or anything we have not talked about?

Appendix IV – Participant information for focus group discussions

Information at Focus Groups

Welcome! Thank you so much for coming. This is Dr. Joseph Nkhoma, (job title), and we are three veterinary students from Norway; Charlotte, Elizabeth and Ragnhild. This meeting is part of a study to find out how control of rabies is done in Malawi, and how people perceive dogs and dog welfare.

You are the experts and we want to learn from you. With the information we get from you, our ultimate goal is to help improve control of rabies in Malawi. That is what Joseph is doing for his PhD work.

Should we start with a prayer? (Let one of them lead).

Today's meeting will be split in three parts, the first part is a Focus group, the second is individual interviews and the third is training on rabies sampling. Everything that happens here today is confidential, so please speak freely. None of the information you tell us today can be traced back to you if published.

A focus group is a group discussion. We will ask you five questions, and then you will discuss each question as a group. Don't hold back, we are trying to learn, and everything you think of as relevant is useful to us.

We wish to do an audio recording of the focus group, so that when we transcribe the data no important points will be left out. The recording will be deleted within a month. When we transcribe the information, we will use a number instead of your names.

Now we would ask you to please read the information sheet provided, and to sign the consent form before we proceed. If you have any questions, please let us know and we will try to clarify. Do you have any questions? SAMLE INN ALLE SKJEMA.

Thank you – then we are ready to begin and we will start the recording shortly. Once I have started the recording, I will say welcome and introduce myself, then I'll ask that you introduce yourself. Tell us your name, your job title and your workplace, how long you have worked as an AVO, and then, as mentioned, I will give you each a number. The number is just so that we use the number instead of your name when we use the data.

Turn on audio and introduce yourself, («I'm Elizabeth, I'm a 5th year veterinary student at the Norwegian university of environmental science») point to the person who will introduce themselves first, say 'number one' after the name and then point to the next person.

After focus group:

Thank you so much for participating. Let's take a short break before we move on to the interviews.

Following the break:

Welcome back! Now we will interview each of you using a questionnaire. This is to get some more answers to questions we have on control of rabies and attitudes to dogs in Malawi. This will not be recorded, but we will register your answers on paper.

Finally, we have some videos and information to share about sampling of animals for rabies and will give you some equipment as well.

Appendix V – Individual interview questions



Veterinærinstituttet
Norwegian Veterinary Institute


Norwegian University of Life Sciences

Interview Notes Form

Date: **Time:** **Location:**
Interviewer:

Personal data

Name	
Date of Birth	
Gender	
Education incl. field of study	
Profession	

AVO Duties

1. Do you deal with animals with suspected rabies in your work?

- ☐ Yes
- ☐ No
- ☐ I don't know

2. Do you refer or advise humans bitten by dogs in your work?

- ☐ Yes
- ☐ No
- ☐ I don't know

3. Do you contribute to vaccination campaigns in dogs in your work?

- ☐ Yes
- ☐ No
- ☐ I don't know

Rabies Knowledge

4. How do you recognise a dog with rabies?

5. How can you prevent a dog from getting rabies?
Select all that apply:
- ☐ Healthy diet
- ☐ Confinement
- ☐ Vaccination
- ☐ Spay/neuter
- ☐ I don't know
- ☐ Other

If other, please specify

6. What treatment should be advised for humans bitten by dogs?
Select all that apply:
- ☐ Herbal medicine
- ☐ Post-exposure prophylaxis (vaccination)
- ☐ Prayer
- ☐ Washing the wound with soap and water
- ☐ Other

If other, please specify

Caseload

7. In the last 14 days, estimate how many times you have been contacted about a suspected rabid animal?

8. In the last 14 days, estimate how many times you have been contacted about a human bitten by a dog?

i. Please estimate how many of these human bite case victims were referred for treatment with post-exposure prophylaxis (vaccination)?

ii. If some were not referred for PEP, please give examples of reasons why

Surveillance (registration and reporting)

9. Do you register reports on suspected rabid animals?

- Yes, always
- Yes, sometimes
- No

If yes, ask the following questions:

i. How are these reports registered?

ii. Do you report these data to anyone?

- Yes, always
- Yes, sometimes
- No

If yes, ask the following questions:

a. To whom do you report the data?

b. How do you report the data?

c. How often do you report the data? i.e. immediately, weekly, monthly…

10. Do you register reports on humans bitten by dogs?

- Yes, always
- Yes, sometimes
- No

If yes, ask the following questions:

i. How are these reports registered?

ii. Do you report these data to anyone?

- Yes, always
- Yes, sometimes
- No

If yes, ask the following questions:

a. To whom do you report the data?

b. How do you report the data?

c. How often do you report the data? i.e. immediately, weekly, monthly…

11. Do you register reports on suspected rabies cases in humans?

- Yes, always
- Yes, sometimes
- No

If yes, ask the following questions:

i. How are these reports registered?

ii. Do you report these data to anyone?

- Yes, always
- Yes, sometimes
- No

If yes, ask the following questions:

a. To whom do you report the data?

b. How do you report the data?

c. How often do you report the data? i.e. immediately, weekly, monthly…

12. Do you register dog vaccinations?

- Yes, always
- Yes, sometimes
- No

If yes, ask the following questions:

i. How are these data registered?

ii. Do you report these data to anyone?

- Yes, always
- Yes, sometimes
- No

If yes, ask the following questions:

a. To whom do you report the data?

b. How do you report the data?

c. How often do you report the data? i.e. immediately, weekly, monthly…

Sampling

13. Have you ever received training on how to collect samples from suspected rabid animals?

- ☐ Yes
- ☐ No
- ☐ I can't remember

14. Do you ever collect samples from animals with suspected rabies?

- ☐ Yes, regularly
- ☐ Yes, but rarely
- ☐ No

If no, or rarely, why not? _____

If yes, ask the following questions:

i. What types of samples do you submit to the laboratory?

Select all that apply:

- ☐ Brain tissue
- ☐ Blood
- ☐ Head
- ☐ Carcass
- ☐ Other

If other, please specify _____

ii. Where do you send the samples?

_____

iii. How do you send the samples?

_____

iv. For sampling, do you use any of the following equipment?

Select all that apply:

- ☐ Knife
- ☐ Goggles
- ☐ Face mask
- ☐ Gloves
- ☐ Protective clothing
- ☐ Other

If other, please specify _____

v. How do you pack the samples before they are sent to the laboratory?

_____

15. Prior to today, have you ever been vaccinated against rabies yourself?

- Yes
- No
- I don't remember

Rabies prevention

16. In the last two years, have there been any rabies vaccination campaigns for dogs in your district?

- Yes
- No
- I don't know

If yes, answer the following questions.

i. Who organised the campaign(s)?

- Civil society organisation
- Government
- I don't know
- Other

If other, please specify

ii. Did you participate in this/these campaign(s)?

- Yes
- No

Dog welfare and euthanasia

17. Have you ever witnessed cruelty towards dogs?

- Yes
- No

i. If yes, please give examples of what you have witnessed:

18. To your knowledge, what do people in your communities usually do if <u>their own dog</u> is sick or injured?

Select all that apply:

- ☐ Contact a veterinarian
- ☐ Contact an AVO
- ☐ Capture the dog
- ☐ Give medicine
- ☐ Give herbal medicine
- ☐ Kill the dog
- ☐ Nothing
- ☐ Other

If other, please specify _____

19. To your knowledge, what do people in your communities usually do if they discover a sick or injured <u>unowned dog</u>?

Select all that apply:

- ☐ Contact a veterinarian
- ☐ Contact an AVO
- ☐ Contact the police
- ☐ Capture the dog
- ☐ Give medicine
- ☐ Give herbal medicine
- ☐ Kill the dog
- ☐ Nothing
- ☐ Other

If other, please specify _____

20. To your knowledge, what do people in your communities usually do when discovering a suspected rabid dog?

Select all that apply:

- ☐ Contact a veterinarian
- ☐ Contact an AVO
- ☐ Contact the police
- ☐ Capture the dog
- ☐ Give medicine
- ☐ Give herbal medicine
- ☐ Kill the dog
- ☐ Nothing
- ☐ Other

If other, please specify _____

21. To your knowledge, when people seek help regarding a suspected rabid dog, when does help typically arrive?

- ☐ Within a day
- ☐ Within 2-3 days
- ☐ Within a week
- ☐ Help rarely arrives

22. To your knowledge, what are the typical symptoms described when people report suspected rabid dogs?

Select all that apply:

- □ General signs of illness i.e. depressed, lethargic, fever, vomiting, no appetite
- □ Behavioural changes
- □ Aggression
- □ Drooling (hypersalivation)
- □ Abnormal vocalisation (different bark)
- □ Problems moving (paralysis)
- □ Seizures (spasms or cramps)
- □ Other

If other, please specify

23. To your knowledge, which of the following methods are used to kill rabid dogs in your communities?

Select all that apply:

- □ Killed by strangling
- □ Killed by beating
- □ Euthanasia (drug induced killing)
- □ Killed by stoning
- □ Killed by drowning
- □ Killed by shooting
- □ Other

If other, please specify

24. In your opinion, what is the best way to capture or restrain a suspected rabid dog?

25. In your opinion, which of the following methods of killing of rabid dogs provides acceptable animal welfare?

Select all that apply:

- □ Beating
- □ Shooting
- □ Drug induced
- □ Drowning
- □ Stoning
- □ Other

If other, please specify

26. To your knowledge, who are most rabid dogs killed by?

Select all that apply:

- ☐ Veterinarians
- ☐ AVOs
- ☐ The police
- ☐ Their owners
- ☐ Members of the general public
- ☐ Other

If other, please specify

27. By law, who is authorised to kill a rabid dog?

i. Are they usually available when a potentially rabid dog should be killed?

- ☐ Yes
- ☐ No
- ☐ I don't know
- ☐ Other

If other, please specify

28. Have you ever used or considered quarantine or observation as an alternative to immediate killing when handling a suspected canine rabies case?

29. If given the opportunity, would dog owners in your community want to have their dog(s) vaccinated against rabies?

- ☐ Yes, most of them
- ☐ Yes, some of them
- ☐ No
- ☐ I don't know

i. If not, or just some, why not?

Appendix VI – Focus group discussion themes, categories and frequencies

Theme 1 – Rabies is a big issue in Malawi.

Categories in theme 1:
Too many people die from rabies in Malawi
AVOs are trying to control rabies
AVOs lack funding to do their work
The AVOs feel more could be done against rabies
There are too few AVOs in Malawi
AVOs carry out some surveillance of rabies
There is a one-way system of reporting suspected rabies cases
The AVOs would like more open communication with health facilities regarding rabies

Too many people die from rabies in Malawi:

Rabid dogs are common in Malawi.	1
Many of the AVOs have seen rabid dogs.	2
AVOs know that rabies is a zoonotic disease.	1
AVOs are aware that rabies is a dangerous disease.	1
People get rabies in Malawi.	4
AVOs suspect that some people in their communities die from rabies.	1
AVOs hear of people dying of rabies.	6
The AVOs have heard about kids dying from rabies.	1
Some AVOs know people who have died from rabies.	1
The AVOs are saddened when they hear about people that have died of rabies.	1
The AVOs think too many people die due to rabies.	1
AVOs think rabies is a big issue in Malawi.	2

AVOs are trying to control rabies:

Control of rabies is part of the AVOs job.	1
AVOs work to control rabies.	1
AVOs are trying to control rabies.	1
AVOs are responsible for control of rabies.	1
AVOs take their responsibilities seriously.	1
AVOs are in the field working with infectious diseases.	1
AVOs are the frontline workers in the community against rabies.	1
AVOs want to protect their communities.	1
AVOs do the best to control rabies with the resources they have.	1
The knowledge gap is a big challenge in the AVOs work against rabies.	1
It's challenging because many of the people the AVOs work with have low literacy levels.	1
An AVO thinks that the government was serious about rabies control in the past.	2

AVOs lack funding to do their work:

The AVOs don't have resources even though rabies is a life-threatening issue.	1
The monthly funding received is not large enough to cover their expenses.	1
AVOs receive monthly funding to cover their costs.	1
AVOs' monthly funding is minimal.	1
AVOs monthly funding does not cover all the fuel they need to move around.	1
Funding for fuel is a problem.	1
It's difficult to reach remote villages due to lack of fuel.	1
It's difficult for AVOs to cover all the areas that they are responsible for.	1

The AVOs feel more could be done against rabies:

The AVOs feel more could be done against rabies.	1
AVOs receive some help to control rabies from NGOs.	1

AVOs would like to include local leaders in their work against rabies.	2
Local leaders are influential in the community.	1
AVOs would like to include other influential people in their work against rabies.	2
The AVOs think Malawi needs help in reducing the number of rabies-cases.	1

There are too few AVOs in Malawi:

Controlling rabies requires a lot of effort from the AVOs.	1
There are not enough AVOs to cover the workload.	1
There are too few AVOs in Malawi.	1
There are too few AVOs to cover the EPAs.	1
EPAs where there are supposed to be 3 AVOs only have 1.	1
It is challenging for the AVOs to cover the workload.	1

AVOs carry out some surveillance of rabies:

The AVOs write reports for bite-cases cases.	1
AVOs report rabies cases to the authorities.	2
The AVOs record how many bite-case victims they receive a month.	2
The AVOs record how many suspected rabies cases they have each month.	1
AVOs send monthly reports on suspected rabid dogs.	1
AVOs send annual reports on suspected rabid dogs.	1
AVOs carry out some surveillance through written reports.	1
Rabies surveillance is not properly done.	1
There is poor rabies surveillance.	1
There is better surveillance of other zoonotic infections.	1
The lack of mobility is a challenge for the AVOs when it comes to surveillance.	2
Lack of resources is a barrier when it comes to rabies surveillance.	3

There is a one-way system of reporting suspected rabies cases:

It is difficult for AVOs to say whether humans get rabies or not.	1
AVOs don't have the figures on human rabies deaths.	1
The health department might record information on human rabies cases.	1
AVOs do not get feedback on rabies cases from the Ministry of Health.	1
Health centres with human rabies cases do not report these to the veterinary department.	1
The veterinary department and the Ministry of Health do not collaborate on rabies cases.	1

The AVOs would like more open communication with health facilities regarding rabies:

The hospitals have figures on human deaths caused by rabies.	1
The AVOs do not get numbers from the hospitals on how many people in their area die from rabies.	1
In 2022 an NGO recorded 12-13 deaths in one region due to rabies.	1
Hospitals are not good at communicating with AVOs.	1
Hospitals do not report back to AVOs what happens to dog-bite victims.	1
Hospitals do not inform AVOs whether dog-bite victims contracted rabies or not.	1
The AVOs would like more open communication with health facilities regarding rabies.	1

Theme 2 – Creating awareness on rabies is important for rabies control.

Categories in theme 2:
People lack awareness of rabies
AVOs want to do more rabies awareness campaigns
People do not have enough knowledge about rabies to protect themselves from the disease
Very few people can recognize a rabid dog
AVOs spread information on rabies in different ways
AVOs take advantage of natural gatherings to inform about rabies
It's challenging that people do not take rabies awareness seriously
Lack of resources makes it harder for AVOs to spread awareness on rabies
AVOs have ideas on how to improve rabies awareness

People lack awareness of rabies:

People lack awareness of rabies.	3
People's awareness of rabies is limited to vaccination.	1
Rabies awareness is better in the Southern regions.	2
AVOs are aware that rabies is a dangerous disease	1
Rabies awareness is important to control rabies.	1
People should be made aware of the dangers of rabies.	1
AVOs are responsible for informing the local communities about rabies.	1
AVOs carry out awareness campaigns to prevent rabies.	1
AVOs are responsible for awareness campaigns on rabies.	1
AVOs need to reach out to people to educate them on rabies.	1
AVOs are responsible for educating the community on the importance of canine vaccination against rabies.	1
Doctors do not always suspect that patients are suffering from rabies.	1
Hospitals do not always confirm if people actually have rabies.	1

AVOs want to do more rabies awareness campaigns:

AVOs need to raise rabies awareness amongst dog owners	1
AVOs need to reach out to people to educate them on rabies.	1
Awareness campaigns include information on the benefits of dog vaccination.	1
AVOs feel there are few rabies awareness campaigns.	1
AVOs want to do more rabies awareness campaigns.	1
Some AVOs do not think the current awareness campaigns are good enough.	1

People do not have enough knowledge about rabies to protect themselves from the disease:

People have no knowledge of rabies.	1
The community lacks knowledge on the dangers of rabies.	1
People don't know how dangerous rabies is.	3
People don't know what happens when you contract rabies.	1
People do not know that rabies is deadly.	1
People do not have enough knowledge about rabies to protect themselves from the disease.	2
Some people do not take the dangers of rabies seriously.	3
Some people underestimate the dangers of rabid dogs.	1
People lack knowledge on the transmission of rabies.	1
People know that rabies can be transmitted through bites.	3
People are not aware of less common routes of transmission.	1
People view wounds from potentially rabid dogs as trivial.	1
People eat livestock bitten by stray dogs.	1
AVOs should not assume that everyone knows about the dangers of rabies.	1

Very few people can recognize a rabid dog:

Very few people can recognize a rabid dog.	3
Very few people can describe a rabid dog.	1
People might only recognize severe signs of rabies.	1
People do not recognise rabies in dogs before the dog starts biting.	1
People think that all vicious dogs are rabid.	2
Some people cannot differentiate between a rabid dog and a vicious dog.	1
Most people have seen a rabid dog.	1
Most people have seen rabid dogs without realizing it.	1

AVOs spread information on rabies in different ways:

AVOs conduct awareness campaigns in primary schools.	1
AVOs conduct awareness campaigns in secondary schools.	1
Sometimes AVOs send information on rabies to teachers.	1
AVOs spread information on rabies through government meetings.	1
AVOs attend VDC meetings to educate people on dog vaccination.	1
AVOs attend VDC meetings to educate people on keeping dogs.	1
AVOs educate farmers on the importance of rabies.	1
AVOs educate farmers on the importance of vaccinating their dogs against rabies	1
AVOs educate people on the benefits of dog vaccination.	1
AVOs spread information on rabies through farmer training.	3
AVOs spread information on rabies through WhatsApp groups.	3
Yearly vaccination campaigns help raise awareness of rabies.	1
AVOs use local leaders to spread awareness on rabies.	1
Working with local leaders would help the AVOs to educate the community on rabies.	1

AVOs take advantage of natural gatherings to inform about rabies:

AVOs take advantage of social gatherings to spread information on rabies.	3
AVOs spread information on rabies in churches.	2
AVOs use church gatherings to spread awareness on rabies.	1
AVOs use funerals to spread awareness on rabies.	1
AVOs spread information on rabies in funerals.	2

It's challenging that people do not take rabies awareness seriously:

Some people do not take the AVOs awareness campaigns seriously.	1
People laugh at AVOs if they are talking about rabies awareness.	2
AVOs may want to inform 100 people about rabies but only 20 turn up.	2
Farmers would rather farm than come to rabies information meetings.	1
Some farmers do not prioritize rabies information meetings.	1
It's challenging that people do not take rabies awareness seriously.	2

Lack of resources makes it harder for AVOs to spread awareness on rabies:

AVOs wish they had the resources to reach as many people as possible.	2
AVOs lack resources to do awareness campaigns.	1
Lack of resources makes it harder for AVOs to spread awareness on rabies.	1
The lack of resources makes it difficult for the AVOs to reach many people with their awareness campaigns.	1
It's hard for AVOs to sensitise people about rabies when they live in hard-to-reach areas.	1
Sometimes AVOs need to leave their motor bike and walk to people who live in remote areas.	1
Rabies does not get the same attention as human health campaigns.	1
There are more resources being allocated towards health campaigns initiated by hospitals.	1
Information campaigns on human health issues are very effective.	1
AVOs need to organise their resources well	1
AVOs stay idle when there are no resources.	1

AVOs have ideas on how to improve rabies awareness:

AVOs lack posters to inform people about rabies.	1
Animal vaccinators could reach more people with posters instead of word-of mouth.	1
Animal vaccinators should be given booklets on the dangers of rabies to distribute.	1
Animal vaccinators should travel from village to village to spread awareness on rabies.	1
Cooperating with cinemas could be useful in awareness campaigns.	1
Movie showings could inform people on rabies.	1
Awareness campaigns should include videos showing rabies symptoms in humans.	2
Awareness campaigns should include information on people dying from rabies.	1
Videos of people dying from rabies could help educate others.	2

Theme 3 – AVOs work with rabies vaccination.

Categories in theme 3:
There are no other means of controlling rabies aside from vaccinations
The government supplies AVOs with rabies vaccines
It's a challenge that the cold chain is not kept properly
AVOs cannot meet the targets when vaccinating dogs
The people who vaccinate dogs should be protected against rabies
There is poor promotion of dog vaccination dates
Dogs can run away without being vaccinated because a young person is handling them:
Roaming dogs are difficult to vaccinate
It's challenging to convince people with certain beliefs to vaccinate their dogs
Some dog owners buy rabies vaccinations for their pets
AVOs lack vaccination cards for dogs that are vaccinated against rabies

There are no other means of controlling rabies aside from vaccinations:

There are no other means of controlling rabies aside from vaccinations.	3
Vaccination campaigns help control rabies in communities.	1
AVOs carry out vaccinations to prevent rabies.	1
AVOs are responsible for dog vaccinations.	1
AVOs are responsible for vaccinating animals.	1
AVO responsibilities include vaccination campaigns against rabies.	1
AVOs vaccinate dogs against rabies.	1
The AVOs vaccinate dogs in their area at no cost to the owner.	1

The government supplies AVOs with rabies vaccines:

AVOs do yearly vaccination campaigns to control rabies.	3
AVOs have vaccination campaigns almost every year.	1
AVOs do vaccination campaigns yearly if they are provided with vaccines.	1
The government supplies the AVOs with rabies vaccines.	2
The AVOs receive some vaccine doses to distribute in their area.	1
AVOs don't get enough vaccine doses during the vaccination campaigns to cover the dog population in their areas.	1
Sometimes the number of doses provided does not meet the number of dogs in the population.	1
Vaccine doses can be provided without all the necessary resources.	1
Rabies vaccines are provided without syringes.	1
When AVOs are given too few doses they have to choose which areas to prioritize.	1
AVOs target areas that are most affected when they lack enough vaccine doses.	2
AVOs risk outbreaks in areas not vaccinated when they prioritize other areas.	2
One AVO only managed to vaccinate less than one third of the population in his area.	1
AVOs in District B once went three years without vaccinating dogs because the government lacked vaccines.	1
AVOs in District B have been provided with plenty of rabies vaccinations these past years.	1
The government wanted all dogs to be vaccinated against rabies in the past.	1
The government should try and match the number of vaccines with the number of dogs and cats in the population.	1
AVOs could control rabies more effectively if they had more resources.	1

It's a challenge that the cold chain is not kept properly:

Keeping the cold chain is a real challenge.	3
The AVOs fear that the cold chain is not kept for the vaccines.	1
It's a challenge for AVOs to store vaccines.	3
AVOs must find storage for rabies vaccines themselves.	1
AVOs are not given means to keep the vaccines cold.	1
AVOs do not have access to refrigerators.	2
AVOs beg hospitals to store vaccines because they lack refrigerators.	1

It's hard to keep rabies vaccines cool if you don't have a fridge.	1
AVOs must find their own cooler box for rabies vaccines.	1
AVOs borrow coolers from health workers at the district hospital when they vaccinate dogs.	1
Power-cuts are common in Malawi.	3
The AVOs think the frequent blackouts affects the vaccines.	3
The vaccines are often transported in hot weather.	1
The vaccines are often transported long distances.	1
AVOs fear long transportation times affect the quality of the vaccines.	1
The AVOs fear that they are giving vaccines that are not effective due to mishandling.	4
Vaccinated dogs get rabies due to vaccine mishandling.	1

AVOs cannot meet the targets when vaccinating dogs:

AVOs should be equipped with the resources they need to vaccinate dogs.	1
AVOs must sort their own transport when vaccinating dogs.	1
Lack of transport makes it difficult for AVOs to vaccinate dogs.	1
AVOs end up vaccinating less dogs because they lack fuel.	1
An NGO gave AVOs in District A vaccines and they failed to vaccinate dogs because they lacked fuel.	1
AVOs must use their own resources to vaccinate dogs.	1
Moving around is difficult for AVOs because the terrain is not so good.	2
AVOs fail to vaccinate all dogs during vaccination campaigns.	1
Some AVOs have been able to provide vaccines even in hard-to-reach areas.	1
Sometimes AVOs only target rabies hot spots due to lack of resources.	1

The people who vaccinate dogs should be protected against rabies:

The people who vaccinate dogs should be protected against rabies.	1
Animal vaccinators should be given PrEP treatment.	1
Most AVOs have not received PrEP	1
Animal vaccinators should be given PPE.	1
Animal vaccinators should be given muzzles.	1
AVOs do not receive PEP if bitten during vaccination campaigns.	1

There is poor promotion of dog vaccination dates:

AVOs must launch awareness campaigns themselves to share rabies vaccination dates.	1
In District D promotion-vehicles might publicise rabies vaccination the day before the event.	1
There is poor promotion of dog vaccination dates.	1
Vaccination day posters are not provided.	2
It is difficult for AVOs to promote dog vaccination dates because they lack posters.	1
The dogs are often out when the AVOs do vaccination campaigns.	1
AVOs notice that not all dog-owners bring their dogs for vaccination.	1
Only a few dogs come to be vaccinated against rabies.	1
Not all dog owners attend but AVOs still do yearly vaccinations.	1
Dog owners cannot vaccinate their dogs if they miss vaccination day.	1
People should know to get their dogs vaccinated against rabies.	1

Dogs can run away without being vaccinated because a young person is handling them:

Parents are not involved in bringing dogs for vaccination.	1
It is often children who bring dogs to vaccination campaigns.	1
Children might bring their dogs for vaccination because they see other people doing it.	1
Children bring their dogs for vaccination because it seems like fun.	1
Dogs are often not used to being handled.	1
The dogs that are brought to vaccination campaigns are often hard to handle.	1
Dogs can become wild when they are being vaccinated.	2
It's difficult for the AVOs to vaccinate dogs if a young person is handling the dog.	2
Some dogs run away before they can be vaccinated.	2
Dogs can run away without being vaccinated because a young person is handling them.	2
AVOs as vaccinators cannot handle dogs that not even their owners can manage.	1

An AVO estimates thirty of a hundred dogs run away because they can't be handled when vaccinating them.	1

Roaming dogs are difficult to vaccinate:

There are a lot of stray dogs in Malawi.	1
Roaming dogs are difficult to vaccinate.	1
Stray dogs living in the tea fields are not vaccinated.	1
AVOs only have injectable rabies vaccines.	1
AVOs need oral rabies vaccines for stray dogs.	1
AVOs do not have access to oral vaccines for stray dogs.	1

It's challenging to convince people with certain beliefs to vaccinate their dogs:

In some villages people refuse to vaccinate their dogs.	1
Some people avoid vaccinating their hunting dogs due to urban myths.	1
Some people think that dogs die after vaccination.	1
Some people avoid vaccinating their dogs in fear that they will become less aggressive.	2
Some people are afraid hunting-dogs might become docile due after vaccination.	2
Some people avoid vaccinating their hunting dogs because they think they will become lazy.	2
Some people avoid vaccinating their hunting dogs because they think they will become weak.	2
Some people think female dogs can't have puppies after vaccination.	2
People avoid vaccinating female dogs because they fear she will abort puppies.	3
People fear that puppies will die once you vaccinate them.	1
There are a lot of misconceptions about vaccination even in areas where NGOs are active.	2
It's challenging to convince people with certain beliefs to vaccinate their dogs.	1
The government should help people understand that AVOs are well-equipped to vaccinate dogs.	1

Some dog owners buy rabies vaccinations for their pets:

AVOs think that people in urban areas are more likely to take their pet to a vet.	1
People in rural areas are less likely to pay for rabies vaccines.	1
People in rural areas are more likely to wait for a free rabies vaccination campaign.	1
It's an issue that owners wait for free vaccination campaigns to vaccinate their dogs.	1
Some dog owners buy rabies vaccinations for their pets.	1
Some dog owners buy rabies vaccinations because they know the dangers of rabies.	1
Some dog owners buy rabies vaccinations and AVOs vaccinate their dogs for them.	1
People with good knowledge of rabies are more likely to buy rabies vaccines for their pets.	1

AVOs lack vaccination cards for dogs that are vaccinated against rabies:

Every dog should have a record of vaccination.	1
The government should register vaccinated dogs.	1
It is difficult to keep track of vaccination dates without records.	1
It is difficult to know if a dog is vaccinated when there are no records.	1
Dog vaccination cards would help AVOs plan the second phase of vaccinations.	1
Not everyone who vaccinates a dog documents it.	1
Lack of vaccination records is a challenge for the AVOs.	1
Some NGOs provide dog owners with vaccination cards.	1
AVOs are given vaccination doses without vaccination cards in rural areas.	1
The government does not provide dog owners with vaccination cards.	1
AVOs lack vaccination cards for dogs that are vaccinated against rabies.	1
The AVOs write the vaccine record on whatever is available.	1
AVOs provide owners of vaccinated animals with certificates of vaccination.	1
AVOs give people receipts when they vaccinate dogs to inform owners of the next vaccination date.	1
Most people in the community do not keep dog vaccination certificates.	1
Many dog owners do not see the need for vaccine certificates.	2
It's challenging that people do not keep dog vaccination certificates.	2
AVOs keep records on the dogs that they vaccinate.	1
The information on the dog vaccinated is reported to the district.	1

Theme 4 - People who have been bitten by potentially rabid dogs do not always receive PEP.

Categories in theme 4:
AVOs are responsible for handling dog-bite cases
AVOs investigate the likelihood of a dog being rabid in each bite-case.
The AVO tries to figure out who owns the dog when investigating bite-cases
AVOs usually recommend observing owned dogs for 10 days
It is difficult for AVOs to conclude that a dog is free from rabies
Lack of vaccination records makes it hard for the AVO to evaluate the risk of rabies after bite-cases
AVOs write a referral letter for the dog-bite victim if they suspect the dog might be rabid
Hospitals refuse to provide PEP without a referral letter.
Dog-bite victims just want a referral letter for the hospital
Dog-bite victims with referral letters may still not have access to vaccines
Some dog-bite victims cannot afford PEP treatment
Lack of transportation stops people from getting vaccines
People try to treat dog-bites using traditional methods
Some people do not seek the help of an AVO after being bitten by a dog

AVOs are responsible for handling dog-bite cases:

AVOs are responsible for handling dog-bite cases.	1
People call the AVOs to tell they've been bitten by a dog.	1
AVOs are usually visited (not called) by the dog-bite victim after a bite-case.	1
The AVOs are often contacted by guardians (parents) of the bite-case victim.	1
People who are suspected of having come in contact with rabid animals are asked to meet at the AVOs office.	1
People show AVOs their dog-bite wounds.	1
AVOs give advice to dog-bite victims.	1
AVOs give advice on how to treat bite wounds.	2
The AVOs help bite-case victims to treat their wounds.	1
AVOs are responsible for directing bite-case victims to the hospital.	6
Deciding on treatment is not the AVOs duty	1
One AVO estimates that each month around 15-20 people report that they are bitten by a rabid dog or a cat.	1
Some EPAs record 90 bite-case victims a month.	1
Some EPAs record 40-50 bite cases from suspected rabid dogs a month.	1

AVOs investigate the likelihood of a dog being rabid in each bite-case:

AVOs investigate the likelihood of a dog being rabid in each bite-case.	1
AVOs investigate the circumstances around the bite in dog-bite cases.	1
AVOs ask the dog-bite victim simple questions.	1
AVOs ask dog-bite victims what happened when they got bitten.	2
The dog's behaviour can help determine if it is rabid or not.	2
The AVO asks the bite-case victims to describe the behaviour of the dog.	1
AVOs ask dog-bite victims to describe the dog's appearance.	1
The AVO evaluates the situation surrounding the dog-bite.	1

The AVO tries to figure out who owns the dog when investigating bite-cases:

The AVO tries to figure out who owns the dog when investigating bite-cases.	2
AVOs try to find out if a dog has been vaccinated after it bites someone.	1
Dog owners should produce a dog vaccination certificate after their dog bites someone.	1
The date in the vaccination record is helpful when evaluating if the rabies vaccine is still valid.	1
The dog's history can help determine if it is rabid or not.	1
AVOs request the dog's history when faced with a suspected rabid dog.	1
Some people are bitten because they provoked the dog.	1
Dogs can bite in response to a person attacking them.	1

Sometimes dog-bite victims were stoning the dog before it bit them.	1
The AVO investigates the possibility of the dog being provoked in the investigation surrounding a dog bite case.	1
AVOs often ask the owner of the dog that has bitten someone to bring the dog to their office.	1
AVOs observe the dog's behaviour when faced with a suspected rabid dog.	1
It is rare for dog-owners to bring their dogs to the AVOs office when requested.	1

AVOs usually recommend observing owned dogs for 10 days:

AVOs usually recommend observing owned dogs for 10 days.	2
A dog is considered rabid if it dies during monitoring.	1
AVOs tell dog-bite victims to come back to them if the dog dies during the observation period.	1
The dog owners report back to the AVOs on the status of the observed dog.	1
The AVOs usually get feedback on the dog's status if it is owned.	1
Some people call the AVOs to let them know that the dog is still alive.	1
Dog owners do not report back to AVOs if their dog is displaying rabies signs.	1
AVOs might travel to the field to observe dogs suspected of being rabid.	1
Unowned dogs are not observed after biting people.	1

It is difficult for AVOs to conclude that a dog is free from rabies:

It is difficult for AVOs to conclude that a dog is free from rabies.	1
The dog has often run away when people report potential rabies cases.	1
Dog owners can only be identified in a few bite cases.	1
Dog owners can deny ownership if their dog bites someone.	1
Often the AVOs record bite-cases as stray dogs because it is difficult to track down the owner.	2
Tracing suspected rabid dogs is difficult.	1
AVOs may not even trace the dog because it's a stray dog.	1
It's hard to conclude that a dog is rabid if it is a stray dog.	1
Most dogs in Malawi roam around freely.	1
It is difficult to trace free-roaming dogs.	1
Bite-case investigation is often difficult for the AVO due to the dog being killed.	1

Lack of vaccination records makes it hard for the AVO to evaluate the risk of rabies after bite-cases:

The dog owner will often tell the AVO that the dog is vaccinated against rabies.	1
Dogs owners don't remember when their dog was vaccinated.	1
Dog owners say their dog was vaccinated by a different AVO.	1
Dog owners will often tell the AVOs that they have lost the vaccination certificate.	1
Dog owners do not keep vaccination documents for their dogs.	2
Dog owners often have no proof of vaccination.	1
AVOs cannot depend on owners to remember when they vaccinated their dogs.	1
AVOs cannot always conclude that the dog has been vaccinated when investigating bite cases.	1
AVOs can rarely trace vaccination certificates for dogs.	1
It's a challenge for the AVOs that dog owners do not keep vaccination records for their dogs.	1
Lack of vaccination records makes it hard for the AVO to evaluate the risk of rabies after bite-cases.	1
AVOs have to treat dogs as unvaccinated because people do not keep dog vaccination certificates.	1
AVOs register dogs as unvaccinated if there is no evidence of vaccination.	1
Every case is treated as a rabies case due to the lack of dog vaccination records.	1
AVOs in one district say they write that owned-dogs are stray dogs when reporting bite-cases.	1
AVOs often treat the patient as if they have been bitten by a stray dog.	1

AVOs write a referral letter for the dog-bite victim if they suspect the dog might be rabid:

AVOs write a referral letter for the dog-bite victim if they suspect the dog might be rabid.	3
AVOs write referral letters for dog bite victims that require treatment.	1
People with referral letters get treated with rabies vaccination.	1

The AVO writes a referral letter for people who have been bitten by stray dogs.	1
AVOs usually always write that they suspect the dog to be rabid in their referral letters.	1
The AVOs write referral letters for people who have come in contact with the rabies virus.	1
AVOs write referral letters for care takers of rabies victims.	1
Family members taking care of humans with rabies receive vaccines.	1
AVOs can write referral letters to family members of people with rabies infection.	1
AVOs provide information on a dog's vaccination status in the letter.	1
The letter includes information on a dog's presumed rabies status.	1
AVOs document a suspected dog as rabid so that bite victims can be vaccinated.	1
The information in the letter influences the treatment people receive at the hospital.	1
Dog-bite victims go to the hospital to receive vaccination.	1
Dog-bite victims need a referral letter from an AVO before the health facility will provide them with rabies vaccines.	1

Hospitals refuse to provide PEP without a referral letter:

Some people go directly to the hospital after being bitten by dogs.	2
Hospitals refuse to provide PEP without a referral letter.	2
Hospitals send dog-bite victims back to AVOs for a referral letter.	1
Dog-bite victims usually come to AVOs after visiting the hospital.	1
Guardians come to AVOs for referral letters while the dog-bite victim is at the hospital.	1
Sometimes dog-bite victims are refused treatment at health facilities because the referral letter is not detailed enough.	2

Dog-bite victims just want a referral letter for the hospital:

People bitten by dogs want documents to get vaccinated regardless of whether it's likely the dog has rabies.	1
People want the AVOs to give them a referral letter no matter what.	3
AVOs don't always hear the full story in a dog bite case.	1
Dog-bite victims can lie to get a letter from an AVO.	1
Dog-bite victims change their story to receive a referral letter from the AVO.	2
Dog-bite victims deny that they know the dog that bit them to get a referral letter.	2
Dog-bite victims deny that they know the owner of the dog to get a referral letter.	2
Dog-bite victims deny that they know the owner to stop AVOs investigating further.	2
Dog-bite victims look disappointed if they don't get a referral letter.	1
Dog-bite victims who provoked the dog are disappointed if AVOs don't write a referral letter.	1
AVOs can face disappointment from the bite victim if they don't write referral letters.	1
If AVOs do not write the referral letter they get backlash from the community.	1
AVOs often write referral letters even though the dog was provoked.	1
AVOs worry that they will be held responsible for human rabies deaths.	2

Dog-bite victims with referral letters may still not have access to vaccines:

AVOs do not know if all dog-bite victims get PEP.	1
Dog-bite victims with referral letters may still not have access to vaccines.	1
The supply of human rabies vaccine in Malawi is poor.	1
People do not always complete the full vaccination course after a dog bite.	2
PEP treatment is rarely available at government hospitals.	1
Sometimes the hospital lacks PEP treatment.	3
AVOs sometimes hear that there is no PEP at the hospital.	1
Bite-case victims can be sent around to various hospitals to find PEP.	3
Lack of vaccines in the hospital prevents bite case victims from getting treatment.	1
Sometimes the hospital runs out of vaccines before the treatment is done.	1
Sometimes rabies treatment is delayed because the health facility runs out of vaccines.	1
Lack of vaccines sometimes keeps people from finishing the whole course of treatment.	1
Sometimes the hospital tells dog-bite victims to come back another time for treatment.	1
Lack of vaccines in hospitals may cause people to give up looking for health facilities that have the vaccine.	2
Lack of vaccines may cause misunderstandings on the need to finish the treatments.	1
PEP treatment is available at private hospitals.	1

People die because hospitals lack rabies vaccines.	2
The rabies vaccine should be available in health centres.	1

Some dog-bite victims cannot afford PEP treatment:

AVOs have heard that PEP treatment is expensive.	1
Some dog-bite victims cannot afford PEP treatment.	4
Some people fail to go to the hospital due to financial issues.	1
Some people cannot afford the transportation costs to the hospital.	1
Lack of funds may cause people to give up looking for the vaccine.	1
Bite-case victims are often burdened with large costs to receive treatment.	1
Some owners of unvaccinated dogs pay for the treatment of dog-bite victims.	1
It is costly for dog owners to pay for the treatment of dog-bite victims.	1

Lack of transportation stops people from getting vaccines:

The cost of travel in Malawi is high.	1
Some people fail to go to the hospital due to transport issues.	1
Lack of transportation stops people from getting vaccines.	1
People in remote areas lack transport.	1
Long distances prevent people from getting PEP.	2
Cost of transport may cause people to give up looking for health facilities that have the vaccine.	1
Dog-bite victims sometimes ask AVOs for transport to the hospital to get PEP.	1

People try to treat dog-bites using traditional methods:

Difficulties getting vaccines may cause people to try traditional medicine as treatment.	2
People try to treat dog-bites using traditional methods.	1
People burn hair from the dogs and use it as an ointment for the dog-bite wound.	1
One AVO have met a dog-bite victim that pulled some hair from the dog to treat the wound.	1
It's a common belief that rabies can be avoided by applying the dog's hair to the wound	1

Some people do not seek the help of an AVO after being bitten by a dog:

Not everyone takes necessary precautions when bitten by dogs.	1
Some people do not seek the help of an AVO after being bitten by a dog.	1
Some people do not seek treatment when they are bitten by dogs.	2
AVOs have met a dog-bite victim that didn't go to the hospital for treatment.	1
Some people do not seek help when they are scratched by dogs	1
Some people do not prioritize going to the AVOs for referral letters.	1
Not all bite-case victims come to get the referral letter.	1
People who survived dog bites tell other dog-bite victims that they will be fine.	3
Some bite-case victims take their chances on not getting treatment.	3
Some people do not seek help before the clinical signs of rabies emerge.	1
AVOs try to educate dog bite victims who did not make contact after being bitten by a dog.	1
Sometimes the AVO has to contact the dog-bite victim themselves.	1
Sometimes, the information about suspected rabies cases never reaches the AVOs.	1
People die because they do not seek help after being bitten by dogs.	1
Many of the people who die from rabies do not seek help in time.	1

Theme 5 – Taking rabies samples is a challenge for the AVOs.

Categories in theme 5:
Most AVOs do not take rabies samples in their work
Animals are often already dead and decomposed when the AVOs arrive to sample them
AVOs lack resources for sample collection
Most AVOs lack protective equipment when taking rabies samples
AVOs lack material to take rabies samples
AVOs lack material to store and send the samples properly
Long distances are a challenge when AVOs collect rabies samples
Fuel is a challenge when collecting rabies samples
Transporting the sample from the field to the laboratory is a challenge
It is costly to send rabies samples to CVL

Most AVOs do not take rabies samples in their work:

AVOs are responsible for collecting rabies samples.	2
AVOs are responsible for transporting samples to the laboratory for testing.	2
Samples sent to CVL can confirm if a dog is rabid or not.	1
The AVOs are not taking samples in their work.	1
One AVO states that he sends a lot of samples.	1
Sometimes the AVOs send the head of the suspected rabid dog to CVL.	1
AVOs can send samples to CVL if the suspected rabid dog has been killed.	1
AVOs send the dog's head to CVL for testing when they have resources.	1
Some AVO know in theory how to take a rabies sample.	1
Some of the AVOs have knowledge but lack resources when it comes to taking samples.	1
Some AVOs have only observed other stakeholders taking rabies samples.	1
AVOs in District B say they do not collect rabies samples on their own.	1
AVOs do not have a chance to collect samples from rabid dogs.	1
AVOs cannot take rabies samples.	1
AVOs say that none of them can take rabies samples.	1
AVOs have not received training on taking rabies samples.	1
None of the AVOs got trained on how to take rabies samples.	1
AVOs do not collect samples from suspected rabid animals.	1
Some AVOs have never taken rabies samples.	1
Some AVOs have never done a brain crush smear.	1
Some AVOs have never taken out brain samples.	1
AVOs know they should bring the dog for sampling to CVL.	1
AVOs don't send samples for rabies testing even though they're obliged to.	1
The AVOs lack a protocol when they take rabies samples.	1
The AVOs think a refresher course could lead to more samples being taken.	1

Animals are often already dead and decomposed when the AVOs arrive to sample them:

The AVOs sample animals after they are dead.	1
The dog needs to be killed before taking the rabies sample.	2
AVOs usually hear about potentially rabid dogs after they are killed.	1
Suspected rabid dogs are usually killed by the general public.	3
Dogs that bite humans are often killed.	1
Suspected rabid animals are disposed of by the public.	1
Suspected rabid animals are often buried after death.	1
AVOs are often informed about potential rabies-cases late in the event.	1
It is often hard to collect samples of suspected rabid animals.	1
Locating the dead animal is often a challenge when sampling.	1
Often the AVO has to exhume the animal to take samples.	2
It's challenging to dig up buried animals for sampling.	1
A suspected rabid animal might already be decomposed when the AVO arrives.	1

AVOs lack resources for sample collection:

AVOs lack the resources to collect rabies samples.	1
AVOs lack resources to move around when taking samples.	1
Samples may not be delivered due to lack of resources.	1
The AVO's office might not have enough funds to send the rabies sample to the laboratory.	1
AVOs have to spend their own money to send rabies samples to the laboratory.	2
AVOs do not have the necessary resources to send samples to the laboratory.	1
AVO question whether they should take rabies samples because they lack the means to deliver the samples to the lab.	3
The lack of resources is a challenge when sampling suspected rabid animals.	2
Lack of resources is a barrier when sending samples to the laboratory.	1

Most AVOs lack protective equipment when taking rabies samples:

Some areas have equipment for taking rabies samples.	1
Some areas have personal protection equipment for taking rabies samples.	1
AVOs lack equipment to sample suspected rabid animals.	3
AVOs lack proper attire for sampling suspected rabies cases.	1
The lack of equipment is a challenge when sampling suspected rabid animals.	1
The AVOs lack gloves when they take rabies samples.	2
AVOs have to take out rabies samples with their bare hands.	1
AVOs lack protective gear when sampling suspected rabid animals.	1
AVOs fear contracting rabies in their work.	1
AVOs must work at their own risk when controlling rabies.	2

AVOs lack material to take rabies samples:

AVOs lack materials to collect samples from rabid dogs.	1
The AVOs have to use whatever they have available when sampling potential rabid animals.	1
AVOs do not have glass slides for taking samples.	1
Some AVOs use plastic bags when they take rabies samples.	1
Some AVOs know they can't submit rabies samples in plastic bags.	1
The AVOs use pangas (machetes) when sampling potential rabid animals.	1
The AVOs use axes when sampling potential rabid animals.	1

AVOs lack material to store and send the samples properly:

Lack of materials is a challenge when sending samples to the laboratory.	1
The AVOs lack sampling containers.	1
The AVOs lack cooler boxes to store samples in.	1
AVOs lack containers to transport specimens.	1
The AVOs lack fridges to store the samples in.	1
AVOs don't know how to store rabies samples before they are sent to the laboratory.	1
Storing the samples is a challenge.	1
One AVO says they cannot submit a whole head to the laboratory.	1

Long distances are a challenge when AVOs collect rabies samples:

Mobility is a challenge for the AVOs when taking samples.	1
AVOs often have to travel long distances to collect samples.	1
It is not easy for the AVOs to reach areas with suspected rabies cases.	1
The poor quality of the roads is a challenge when sampling suspected rabid animals.	1
AVOs often need a vehicle to reach the animal in order to take a rabies sample.	1
AVOs have to park their vehicles and walk 7-8 km to reach remote villages.	1
It's a challenge to travel to certain areas because not all AVOs have motor bikes.	1

Fuel is a challenge when collecting rabies samples:

AVOs lack fuel to travel out and sample suspected rabid animals.	1
The AVOs office can lack the fuel to send rabies samples.	1
The cost of fuel is a significant barrier when sending rabies samples to the laboratory.	1
The lack of fuel is a barrier for AVOs when taking rabies samples.	1

AVOs do not have resources to pay for transportation to collect samples for suspected rabid animals.	1
It's usually farmers that pay for the fuel to get the AVO to their farm.	1
AVOs ask farmers to give them a certain amount to cover the cost for fuel.	1
Farmers suspect the AVOs are stealing from them when they ask farmers to cover transport costs.	1
The transport is the main challenge when it comes to sampling suspected rabid animals.	1

Transporting the sample from the field to the laboratory is a challenge:

Transporting the sample from the field to the laboratory is a challenge.	4
Sending a rabies sample requires transportation.	1
The AVO's office can lack the vehicle to send rabies samples.	1
One AVO says the main challenge is transport when it comes to submitting samples to the lab.	1
AVOs have to transport rabies samples themselves.	1
The government expects AVOs to pay for transport of samples to the laboratory.	1
AVOs have to pay for the transportation of rabies samples to the lab.	1
Samples may decompose before they reach the laboratory.	1
AVOs in District B state the poor quality of the roads mean that samples can decompose before reaching the laboratory.	1
AVOs in District B state the poor quality of the roads mean that samples can be contaminated before they reach the laboratory.	1
The charge of sending samples to the laboratory is a challenge for the AVOs.	1
AVOs can't remove the head of the dog from the village.	1
People in the village suspect that AVOs are using dog heads for magical purposes.	2

It is costly to send rabies samples to CVL:

It is costly to send rabies samples to CVL.	3
The labs charge for the equipment used in the labs to test sample.	1
AVOs are charged for the submission of rabies samples to the lab.	1
The government used to pay for the submission of samples to the laboratory.	1
AVOs got used to the government paying for submission of samples to the laboratory.	1
The AVOs charge the farmers for the fee they receive from the laboratory.	1
Farmers are not willing to pay for the testing of rabies samples.	1
Farmers suspect the AVOs are stealing from them when they ask farmers to cover laboratory costs.	1
Dog owners are not willing to pay for testing of samples in the lab.	1
No one wants to take the cost of sending rabies samples to the lab.	1
People need to know that they are expected to pay for the submission of samples to the laboratory.	1
It can be a challenge when farmers can refuse to pay for lab samples.	2
It's a challenge to find out who will pay for the submission of lab samples.	1
AVOs do not want to pay for the submission of samples to the laboratory.	1
AVOs would only be willing to use their own money to test samples in the lab if it was a high-profile case.	1

Appendix VII – Power point presentation for AVOs

Veterinærinstituttet
Norwegian Veterinary Institute

Norwegian University of Life Sciences

Sampling for rabies in the field

Focus Group Session
April 18th 2023, District A, Malawi

Charlotte Eikeskog Ravnås, Ragnhild Kvisle Abildsnes & Elizabeth Akinsanmi-Guren

1

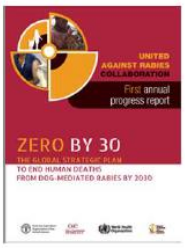
Aim for today

- Learn how to safely collect rabies brain samples from carcasses

2

Overarching goal

- Prevent rabies among people
- WHO/WOAH/FAO/GARP zero vision by 2030



https://apps.who.int/iris/handle/10665/328053

3

Introduction

- Dr. Joseph Nkhoma - Deputy Officer in Charge, CVL
  - Working with control rabies as a part of his ph.D project
- Elizabeth & Charlotte
  - Writing about rabies sampling and testing in our thesis
- Ragnhild
  - Writing about dog welfare and attitudes towards dogs in Malawi

4

Video

ILRI | CGIAR

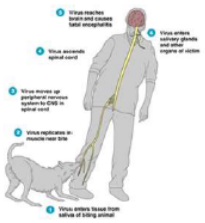
https://www.ilri.org/

- ILRI (International Livestock Research Institute, Nairobi) video about the basics of rabies prevention and what to do if you are bitten
- We will send this film to you on WhatsApp
- Please share in your community

5

PrEP vs PEP

- Pre-exposure prophylaxis is the best way to prevent rabies in humans
  - Effective after 2-3 weeks after vaccination
- Post-exposure prophylaxis can still be effective - if done soon after the bite
- If you are bitten you should still get post-exposure prophylaxis, even though you already have been vaccinated with the pre-exposure prophylaxis



https://www.metrohealthhmo.com/rabies/

6

Why is it important to test animals for rabies?

- To ensure correct diagnosis and treatment of cases in humans and animals
- To detect outbreaks
- For surveillance - important to prioritize vaccines, disease control, influence government policy etc

ILRI - https://www.ilri.org/

7

How to take the sample

- The person taking the sample must be vaccinated against rabies.
- If it is not already dead, humanely euthanise the animal
- Sampling, submit:
  - Whole carcass
  - Head
  - Brain sample

8

Sampling equipment

Safety

- Gloves
- Face masks
- Apron
- Goggles

Sampling

- Knife
- Plastic pipettes
- Blood spot cards
- FTA cards
- Collection tube with lysis-buffer
- Collection tube with shield-buffer

9

Sapmling equipment

Packaging

- Marker
- Zip-lock bag
- Requisition form - must be completly filled out!

Disposals / Desinfection

- Waste-disposal bags
- Plastic bottle + vircon
- Disinfectant

10

Brain sample using the pipette method

Remove the head

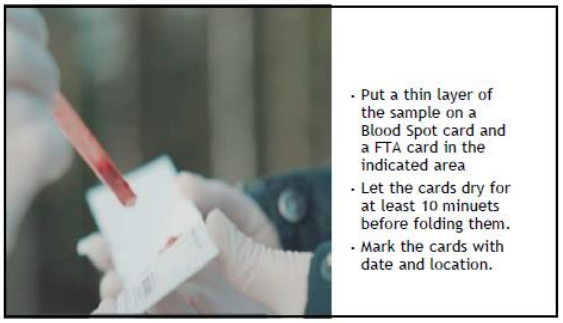
11

- Use a pipette or firm straw with the end cut off diagonally
- Squeeze the balloon on the pipette
- Insert at one side of the foramen magnum, ensure that the pipette does not slip under the dural meninges

12

- First go horizontally on the cranial floor, before you aim for opposite eye, releasing the pressure on the balloon gradually
- Remove the pipette carefully

13



- Put a thin layer of the sample on a Blood Spot card and a FTA card in the indicated area
- Let the cards dry for at least 10 minuets before folding them.
- Mark the cards with date and location.

14

- Put a small piece of the sample in a collecting tube with buffer solution
- It is important that the solution covers the entire piece sample
- After the lid is on, turn the sample tube a couple of times.

15

- Mark all samples with the date of the sample
- Put all samples in a zip-lock bag and close it.
- Marke the bag with your name, date and location

16

Biosafety

- Dispose of used equipment safely!
- Reusable equipment (goggles, knife) should be disinfected with 80% ethanol or vircon (if neither are available use hot water and soap)

17

Disposables

- Put all used disposables (pipette, gloves, apron) in a plastic bag (burn)
- The animal must be buried deep, so no scavengers can get hold of the carcass

18

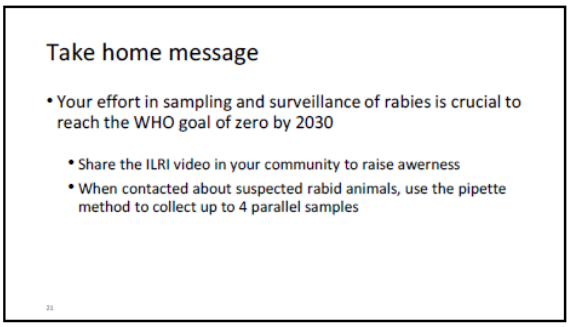
Video

19

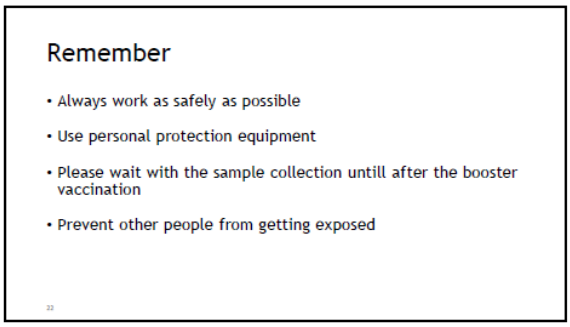
Storage and shipment

- Mark all samples with date and location taken
- Fill out the entire requisition form
- Contact Dr. Nkhoma on whatsapp
- The best is to send samples cooled immediately
- If it must be stored, optimally store it in a refrigerator or freezer
- If this not is possible, keep it in a cool, dark space.
- Send to lab within three weeks

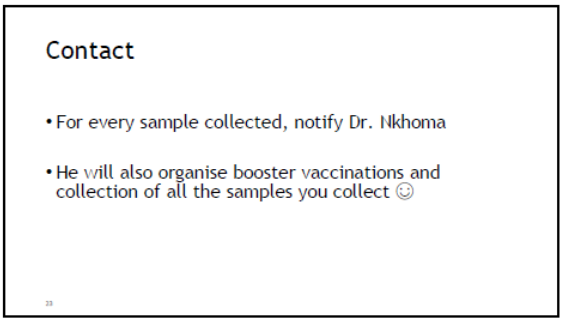
20


Take home message
• Your effort in sampling and surveillance of rabies is crucial to reach the WHO goal of zero by 2030
• Share the ILRI video in your community to raise awerness
• When contacted about suspected rabid animals, use the pipette method to collect up to 4 parallel samples

21


Remember
• Always work as safely as possible
• Use personal protection equipment
• Please wait with the sample collection untill after the booster vaccination
• Prevent other people from getting exposed

22


Contact
• For every sample collected, notify Dr. Nkhoma
• He will also organise booster vaccinations and collection of all the samples you collect ☺

23


Veterinærinstituttet
Norwegian Veterinary Institute
Norwegian University of Life Sciences
Thank you!
• Please go out and take lots of samples
• Let's work together against rabies

24

Appendix VIII – Rabies awareness posters in Chichewa and English


10 things you should know about rabies
Rabies KILLS
REST IN PEACE
You can catch rabies from...
...a scratch...
...or bite...
...from an infected dog
Vaccinate your dog
DOG VACCINATION CERTIFICATE
every year!
Don't let your dog roam
Give your dog a good home
FOOD
WATER
Keep away from stray animals
Don't kick dogs...
...or throw stones
Wash bites...
...with soap and running water
If bitten or scratched...
...see a doctor immediately!
Report dogs acting strangely to veterinary authorities
OHRECA
ILRI
CGIAR
This leaflet was authored and developed by Annabel Slater, Geoffrey Njenga, Lian Thomas and Nicholas Bor for rabies control in dogs in Machakos County, Kenya.

Appendix IX – Equipment list with rabies sampling instructions and requisition form

Rabies Sampling Information Sheet

Sampling and testing for rabies is vital in the fight to eradicate dog-mediated rabies in humans! Therefore, you are making a difference when you collect and submit samples from suspect rabid animals!

Included in this sampling kit for five samples:

- 1 pair of safety goggles - reusable
- 5 pairs of gloves
- 5 facemasks
- 5 plastic aprons
- 5 zip-lock plastic bags
- 5 medium-sized plastic bags
- 1 knife - reusable
- 5 plastic pipettes
- 5 sampling tubes with lysis buffer marked “lysis”
- 5 sampling tubes with lysis buffer marked “shield”
- 5 Blood spot cards
- 4 FTA-cards
- 2 rapid lateral flow tests
- 1 marker pen - reusable
- 1 pen - reusable
- 1 bottle of disinfectant
- 5 Virkon tablets

Collecting samples:

1. Kill the animal humanely.

Sampling procedure:

2. Put on protective gear: gloves, face mask, goggles and apron.
3. Make sure you are working with the carcass in a place and in a way that protects humans and other animals from exposure.
4. Remove the head from the body of the carcass.
5. Turn the head so the lower jaw faces upwards, and the skull downward, and locate the foramen magnum.
6. Squeeze the balloon on the pipette, insert it at one side of the foramen magnum. Be careful not to insert the pipette underneath the dural meninges.
7. First, screw the pipette along the floor of the skull, then turn it slightly upward and angle it towards the opposite eye while carefully releasing the vacuum in the pipette.
8. Extract the pipette carefully.

Adding sample to collection tubes and cards:

9. Each sample should be added to 2 different sampling tubes with buffer, and two sample collection cards (only 4 FTA cards available).

10. Add tiny parts of the sample in the sample area (circle) on the Blood Spot Card and FTA card. Let the cards dry for at least 10 minutes before closing and folding them.
11. Add a small sample to each of the two different types of sample collection tubes with buffer in them (one is marked "lysis", the other is marked "shield"). Turn the tubes to ensure that the entire sample is covered by buffer-solution.
12. Mark all tubes and cards with the date and location of the sample.
13. Put all samples from the one animal in a zip-lock bag and close it.
14. Write the sampling date, location and your name on the bag.

Clear up, sample storage and submission:

15. Throw single use equipment in the red biohazard bag.
16. Disinfect reusable equipment with alcohol and/or Vircon. If using Vircon, mix it 1 tablet with 5 dl water. The equipment must lie in the Vircon solution for at least 10 minutes, then discard the solution.
17. Make sure the carcass is buried deep to ensure that scavengers can't get hold of it.
18. Burn the red biohazard bag with its contents.
19. Store the samples in a refrigerator, or, if that's not possible, keep them a dark shady place.
20. Fill out a requisition form completly for every sample.
21. Make arrangements for transport of samples to CVL. **Please contact Dr. Joseph Nkhoma on +265 888 555 777 to inform him each time you take a sample.**

Procedure for Lateral flow test (2 tests provided):

The lateral flow test provides PRELIMINARY results only and should not be used as the only test. Samples from the animal tested must be submitted to CVL.

22. Add approximately 1 g of brain sample to 9 ml of PBS, mix the solution.
23. Use the swab to collect a sample from the brain tissue homogenate.
24. Insert the swab into the assay diluents tube.
25. Mix until the sample is dissolved.
26. Remove the test device from the foil pouch and place it on a flat and dry surface.
27. Using a disposable dropper, take the supernatant sample in the tube.
28. Add four (4) drops of mixed sample into the sample hole, drop by drop, vertically.
29. Start the timer. If the sample Is not visible in the result window after 1 minuet, add one more drop to the sample hole.
30. Interpret the results after 5-10 minutes. Do not read after 20 minutes.
31. Add lateral flow test to the zip-lock back with the other samples.

If you take a suspected rabies sample using the rapid lateral flow tests: Please contact Dr Joseph Nkhoma immediately after receiving a positive or negative result.
When you have taken five samples, please request more sampling equipment. If you have any questions, please contact Dr. Joseph Nkhoma.

Useful links

- Norwegian Veterinary Institute video for guidance on rabies sampling in the field: https://m.youtube.com/watch?v=Jbx9Fowrkkc&feature=youtu.be
- International life stock research institute video, to share with the community: https://www.youtube.com/watch?v=-qVgkUkUEWw
- Mission rabies, for further information on the signs of rabies in dog to share with the community: https://www.youtube.com/watch?v=keeigDzDf34

These links will also be available digitally via WhatsApp.

Requisition Form for Rabies Samples

Please fill out this form when submitting a rabies sample to CVL.

Your details

Name:
Position:
Address:
E-mail:
Phone number:

Case details

Animal species: Sex: F / M
Location found, address/GPS (as exact as possible):

Date animal was found (ddmmyy):

Found: Dead ☐ Alive ☐
If found dead, had the animal been buried? Yes No
How was the animal killed:
Any observed symptoms:

Did the animal bite someone?	Yes	No
Was PEP recommended?	Yes	No
Did they receive PEP?	Yes	No

Sample details

Date of sampling:
Date of shipping:

Norges miljø- og
biovitenskapelige
universitet

Postboks 5003
NO-1432 Ås
67 23 00 00
www.nmbu.no