Establishing the Basis for Rabies Control and Elimination in Liberia

Inauguraldissertation

Zur

Erlangung der Würde eines Doktors der Philosophie

vorgelegt der

Philosophisch-Naturwissenschaftlichen Fakultät

der Universität Basel

von

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Basel, 2024

Genehmigt von der Philosophisch-Naturwissenschaftlichen Fakultät
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Basel, den 21. Februar, 2023

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To my companions, the disturbing voices in my head.

Zogbo. Yamah. Seklau. Nathan

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Summary

Background: Rabies is a fatal zoonotic disease. Every year, an estimated 59'000 rabies-related deaths are reported globally, affecting four out of ten children. In endemic regions, domestic dogs are the main reservoir of rabies, transmitting the disease to humans through the bite or scratch of an infected animal. Although rabies in animals and humans is notifiable in Liberia, surveillance is primarily passive. There are no accurate estimates of the "true" rabies situation in the country. Based on these shortcomings and the global drive to end dog-related human rabies deaths by 2030, this Ph.D. work aimed to establish animal rabies diagnostics and gather relevant baseline information that is crucial for control programs for rabies elimination in Liberia.

Objectives: This PhD thesis achieved the following objectives, i) established animal rabies diagnostics at the Central Veterinary Laboratory, ii) surveyed the community's knowledge about rabies and vaccination scenarios, and iii) investigated molecular and phylogenetic characteristics of circulating rabies strains in Liberia.

Methods: We used a One Health approach, collaborating between key national and international rabies stakeholders, to successfully establish three animal rabies diagnostic tests. We used an opportunistic sampling approach to collect and diagnose the first animal rabies strain in post-war Liberia. A randomized cross-sectional knowledge, attitude, and practice (KAP) household survey related to dog rabies and was used to estimate the dog population survey in rural and urban households. Based on this data, we developed three scenarios for mass dog vaccination. In addition, we strengthened the rabies surveillance system to collect suspected animal rabies samples to perform a genome-based phylogenetic analysis of RABV isolates circulating in Liberia.

Results: Analyses of the first rabies samples revealed that all isolates belonged to the Africa 2 lineage; Subgroup H circulates in the domestic dog population. This finding subsequently flagged Liberia as an endemic rabies country. It contributed, among other factors, to the country's score of 1.5/5 on the Stepwise Approach towards Rabies Elimination (SARE) tool, indicating that Liberia is in the early stages of dog rabies control. Results from the cross-sectional survey conducted in rural and urban households estimated a mass vaccination cost of USD 1.5 – 1.6 million to vaccinate the total dog population for one vaccination round. Rabies knowledge among

participants was low, thus influencing risky practices. Results from strengthening rabies surveillance identified the disease hotspots. It further demonstrated that RABV isolates circulating in Liberia were clustered into the phylogroup H within the Africa 2 clade. This information is necessary for future rabies control and eradication programs.

Conclusion: This PhD work generated relevant information for rabies control in Liberia. Baseline information is crucial for effective rabies control. Our findings obtained from the household survey are important for developing a national rabies control strategy and intervention. At the same time, findings from the molecular and phylogenetic study are vital to develop appropriate vaccination strategies for dogs and allow their effectiveness to be assessed in Liberia.

List of Abbreviations

Anigen test:	Anigen Rapid Rabies Ag Test kit
CARI:	Central Agriculture Research Institute
CDC:	United States Centers for Disease Control
CSOs:	County Surveillance Officers
CU:	Cuttington University
CVL:	Central Veterinary Laboratory
DRIT:	Direct rapid immunohistochemical test
DIB:	Development Impact Bonds
EPT-2:	Emerging Pandemic Threat 2
ECTAD:	Emergency Centre for Transboundary Animal Diseases
FAO:	Food and Agriculture Organization of the United Nations
FAT:	Fluorescent Antibody Test
GARC:	Global Alliances for Rabies Control
GHSA:	Global Health Security Agenda
GMD:	Greater Monrovia District
IP-Paris:	Institut Pasteur-Paris
IZSVe:	Istituto Zooprofilattico Sperimentale delle Venezie
KAP:	Knowledge Attitudes Practices
LFD:	Lateral flow device
LISGIS:	Liberia Institute of Statistics and Geo Information System
MOA:	Ministry of Agriculture Liberia
MOH:	Ministry of Health
MDGs:	Millennium Development Goals
NGS:	Next Generation Sequencing

NPHIL:	National Public Health Institute of Liberia
ODK:	Open Data Kit
OH:	One Health
OHCP:	One Health Coordination Platform
OR:	Odds Ratio
WOAH:	World Organization for Animal Health (formerly OIE)
PARACON:	Pan African Rabies Control Network
PEP:	Post Exposure Prophylaxis
Prep:	Pre-Exposure Prophylaxis
PhD:	Doctor of Philosophy
PBS:	Phosphate Buffer Saline
PPS	Probability proportional to the size
RABV:	Classical rabies lyssavirus
REDISSE:	Regional Disease Surveillance Systems Enhancement
RIDT:	Rapid Immunodiagnostic Test
RIG:	Rabies Immunoglobulin
RNA:	Ribonucleic Acid
RT-PCR:	Reverse transcription polymerase chain reaction
SARs:	severe acute respiratory syndrome
SARE:	Stepwise Approach for Rabies Elimination
Swiss TPH:	Swiss Tropical and Public Health Institute
SDGs:	Sustainable Development Goals
SP:	Sampling Point
USAID:	United States Agency for International Development
UAR:	United Against Rabies

UN SDGs:	United States Sustainable Development Goals
UL:	University of Liberia
VP:	Vaccination Point
WRD:	World Rabies Day
WHO:	World Health Organization
GPS:	Global Positioning System

Acknowledgements

I sincerely thank Prof. Dr. Jakob Zinsstag, “I called him the Swiss father of One Health,” for agreeing to work with me during this challenging Ph.D. journey. I remember our first discussion about doing a Ph.D.: you said, “I am not promising you a thing, but I will send you a link to apply...” Here I am today with a Ph.D. I cannot thank you enough for allowing me to work in your research group, the Human and Animal Health Unit of the Swiss TPH.

I am truly grateful to you, Prof. Peter Odermatt, for accepting to serve as my second supervisor. It means a lot to me. Thanks

Many thanks, Mr. Roland Varkpeh, for offering the position of Animal Health Expert on the rabies project, which led to this PhD.

Thanks to Dr. Abdallah Traore for introducing me to rabies diagnosis (Fluorescent Antibody & Rapid Immunodiagnostic Tests). What an opportunity! Together, we diagnosed and reported the first dog case at the Central Veterinary Laboratory in post-war Liberia.

Thanks to Joseph R.N. Anderson for the administrative support during my entire study. You supported me in many ways.

Thank you, Dr. Stephanie Mauti, for the mentorship. I learned a lot from you! I mean a lot. You could not be more right by the saying, “We learn by doing.”

Thanks to Dr. Jan Hattendorf for his tireless support during the study design and data analysis.

Thank you, Dr. Celine Mbilo, for sharing your PhD experience and critical comments on documents. Although our encounter was short at the institute, your advice guided me in many ways.

Many thanks, Lisa Crump, for always being available for language proofreading. You are an expert in what you do, and it makes life easier for non-English native speakers like myself.

Thanks to Christine Mensch! An expert in communication. Christine, you are more than efficient in coordinating student activities. You are one of the best! Thanks for all the administrative support.

Many thanks to Laura Innocenti and Dagmar Batra for their administrative support during my study.

Profound thanks to my colleagues at the Central Veterinary Laboratory of Liberia for their support during sample collection and diagnostics at the lab. I thank you, Varney M. Kamara, Watta Anthony, Brenda Musa, Roosevelt Gweh, Linda Yini, and Morris Smith. You have been my backbone when I needed you the most.

I sincerely thank Laurent Dacheux and the team (Institut Pasteur-Paris) for the collaboration and diagnostic support. I almost gave up on the RNA extract until you restored my hopes. Thank you.

Many thanks to the field data collectors. With you, this work was a success. Thanks to you, Watta Anthony, Arthur S. Wenna, Amos Mulbah, William Kwei, Linda Yeni, Mercy Varkpah, Roosevelt Gweh, Rufus Monbo, Saymajukon Forkay, and the entire Epi Unit of the Ministry of Agriculture for the tireless effort during the data collection. Thanks to the village chiefs and interviewees.

Thanks to you, Frank M. Koikoi, my professional colleague. You perceive life from a unique point of view, and I owe you a lot just by listening to some of the suggestions you shared on various matters.

My deepest gratitude to Mrs. Seklau E. Wiles. You are a mother and mentor beyond every measure. May your soul rest in perpetual peace.

Lastly, I sincerely thank Zogbo, Nathan, Yammah, Josephine, Adam, James, Gbeme, Jerome, Willie, and Nancy for their guidance and support.

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Thesis Outline

This Ph.D. thesis is structured into three core parts. Part one details the implementation of animal rabies diagnostics through a One Health approach. Part two focuses on communities' knowledge about rabies and mass vaccination scenarios. Part three covers the molecular characterization of circulating rabies strains in Liberia. A general discussion and conclusion follow the three parts.

The PhD is composed of four scientific papers, of which three are published, and one manuscript is submitted for publication on the following topics:

- Rabies Diagnosis
    - Rabies control in Liberia: Joint efforts towards Zero by 30 (Chapter 2)
    - Complete genome sequences of five rabies virus strains obtained from domestic carnivores in Liberia (Microbiol Resour Announc. 2022 Jan 20;11(1): e0104721. doi: 10.1128/mra.01047-21. Epub 2022 Jan 20. PMID: 35049353) (Chapter 3).
- Rabies KAP study
    - Preparing Liberia for rabies control: Human-dog relationship and vaccination scenarios Acta Trop. 2022 May; 229:106331. doi: 10.1016/j.actatropica.2022.106331. Epub 2022 Feb 6. PMID: 35139326 (Chapter 4).
- Genome-based phylogenetic analysis of rabies virus circulating in Liberia (Manuscript submitted to PLOS Neglected Tropical Diseases) (Chapter 5).
- A policy brief toward dog rabies elimination in Liberia to the governmental authorities (Chapter 6).

1 Introduction

Zoonotic diseases persist in resource-limited countries, resulting in serious public health threats. Over 200 types of zoonotic diseases are transmitted between humans and animals. Of these different diseases, 60% are infectious to humans, and 75% of newly emerging diseases are zoonotic. The most common zoonoses that impact livestock workers globally are in low and middle-income countries and have caused an estimated 2.4 billion cases of illness and 2.7 million human deaths annually (Grace et al., 2012). Despite the negative impact of zoonotic diseases on poor and marginalized communities in Sub-Saharan Africa, they are often the least prioritized diseases for public health interventions. Maybe these illnesses lack the same potential to spread globally, like severe acute respiratory syndrome (SARS) or highly pathogenic avian influenza. Despite being neglected, some of these diseases are preventable.

Rabies, the family *Rhabdoviridae* Genus *Lyssavirus*, is a deadly, yet 99% vaccine-preventable, zoonotic disease. The disease is transmitted through the bite or scratch of an infected rabid animal, and incubation periods can last from a few days to several months, depending on the distance from the viral entry site to the brain. This incubation period can last from a few days to several months (Jackson, 2013a). In rabies-endemic regions, domestic dogs are the main transmitter of the disease, causing an estimated 59’000 deaths, mostly in Asia (60%) and Africa (36%) (Hampson et al., 2015). Despite the high burden, rabies is seriously underestimated, especially in rural areas with limited healthcare access. For instance, a study conducted in Tanzania (Sarah Cleaveland, Fèvre, Kaare, & Coleman, 2002) found up to 100 times higher incidence of human rabies cases when incidence was estimated from animal bite occurrence compared to the incidence estimated through passive surveillance data. Although there is no cure for clinical rabies, the disease can be prevented through timely post-exposure prophylaxis (PEP) administration.

Rabies elimination is possible in Sub-Saharan Africa. The disease can be prevented and controlled through disease awareness, post-exposure prophylaxis, and mass dog vaccination. For example, dog-mediated rabies has been successfully eliminated from most developed countries, including Western Europe, Canada, the USA, and Japan. There have as well been great strides made in Latin American countries, which have

reported no human rabies deaths from infected rabid animals. In addition, significant progress has been made in reducing the disease burden in countries like Bangladesh, Tanzania, South Africa, and Vietnam.

1.1 A One Health approach to rabies control

Over the years, the One Health concept has gained tremendous support in the fight against zoonotic diseases. Zinsstag et al., 2015 define One Health as “any added value in terms of human and animal health, financial savings or sustained environmental benefit from closer cooperation of human and animal health sectors at all levels of organization.” This closer cooperation between sectors is key in the fight against rabies.

Rabies is a typical example of One Health in practice. A collaborative approach can reduce disease transmission and human rabies cases substantially. In 2017, after implementing animal rabies diagnostics at the Central Veterinary Laboratory in Liberia, we witnessed an improved collaboration, through Integrated Bite Case Management, between the veterinary and public health sectors. For instance, a rabid dog bit three household members, and they could not afford the expensive post-exposure prophylaxis. However, with effective communication between technicians of the Central Veterinarian laboratory and the National Public Health Institute, the victims who were initially denied treatment at health facilities received a free full course of government-subsidized PEP (Voupawoe, Garmie “World Rabies Day: Early diagnosis and prompt treatment save lives” Impact Stories | Swiss TPH 28.09.2021). In this scenario, the benefit of closer cooperation goes beyond the unnecessary use of expensive PEP to saving victims with a 20% probability of death.

PEP treatment alone in humans cannot eliminate human rabies deaths. However, mass dog vaccination targeting 70% coverage is the WHO-recommended cost-effective and sustainable strategy for curbing the disease. This approach has been used in many developed counties, where rabies control has successfully targeted the disease in the host species, mostly dogs. A study conducted in Chad (Mindekem et al., 2017) compared the cost of PEP alone with dog mass vaccination, PEP, and communication between sectors; the study showed that the latter is more cost-efficient than administering PEP alone. Effective communication between sectors plays a crucial role in rabies control because One Health seeks to overcome the historical

boundaries between traditional disciplines and other human institutions to disseminate knowledge and positive action as widely as possible.

1.2 Current state of rabies elimination in West Africa.

Although rabies is a notifiable disease in humans and animals in most West African countries (Knobel et al., 2005), achieving the global goal of eliminating human rabies deaths by 2030 remains a huge challenge. Over the years, research has revealed shortcomings in reducing human rabies cases in rabies endemic countries. The challenges include ineffective surveillance methods due to a lack of laboratory diagnosis, poor disease awareness with bite victims not seeking medical attention, and disease misdiagnosis by clinical staff, resulting in massive underreporting of human and animal cases. Alternatively, mass dog vaccination is a core component and cost-effective measure for human control and spillover prevention. For example, North America, Western Europe, and Japan have shown that dog rabies can be eliminated through vaccination of dog populations (Zinsstag et al., 2017), accurate surveillance, and information on dog population demographics. In other countries with effective rabies control measures, modern rabies vaccines are imported or locally produced and administered, and are instrumental in reducing rabies cases in humans and animals.

The West African region comprises culturally and economically diverse countries and is often subject to political unrest. Knowledge about rabies within the region is often low (Mbilo et al., 2021). Rabies diagnoses that should strengthen surveillance are usually confined to the capital, whereas remote settings that are mostly affected are without diagnostic capacity. Unfortunately, most countries within the region hold a traditional habit of consuming dog meat as a protein supplement (Ameh, Dzikwi, & Umoh, 2014; Voupawoe et al., 2022), thus risking dog owners, hunters, and butchers of rabies infection. The extent of the disease's transboundary movement is another concern, whereas cooperation between member states could help prevent this translocation.

Overall, data on rabies in West African countries remains poor and inconsistent because of financial limitations and lack of government support. However, gathering reliable data to understand rabies epidemiology is an important first step in rabies control. So far, only two West African countries (Mali and Cote d’Ivoire) have reached

stage two on the so-called SARE ladder, with little or no literature on rabies for Guinea, Gambia, and Bissau Guinea (Rupprecht, Mani, Mshelbwala, Recuenco, & Ward, 2022). For Burkina Faso, despite the regular occurrence of rabies-related deaths, dog vaccination coverage is still low (Savadogo et al., 2021) while in Côte d'Ivoire, in official reports of rabies, incidence is underreported (Kallo et al., 2022). After three years of rabies intervention, Liberia is still at 1.5/5 of the so-called SARE ladder (Voupawoe et al., 2021). Rabies transmission is becoming a concern in the healthy dog population in Ghana (Tasiame et al., 2022). Despite the high infection rate from dog bites, few victims are completing the course of PEP administration in Senegal (Traore et al., 2020). With low vaccination coverage, the rabies burden is eminent throughout Nigeria (Audu, Mshelbwala, Jahun, Bouaddi, & Weese, 2019). In Benin, the death rate due to dog rabies is high. Despite the many challenges, rabies groups continue to push towards the global rabies drive. The Regional Disease Surveillance Systems Enhancement (REDISSE) program and other partners support programs throughout West Africa to improve data collection.

1.3 Challenges for rabies control in Liberia

Despite the available tools and knowledge to control rabies, there are several challenges in controlling rabies in Liberia. The veterinary sector lacks funding. It depends on outside funders such as FAO, the United States Agency for International Development (USAID), and the World Bank REDISSE program to sponsor most animal disease surveillance and interventional programs (Personal Information, Garmie Voupawoe, 2022). The implication is that with donor money, activities can continue. There is insufficient laboratory capacity for rabies diagnostics. Currently, there are no public health laboratories with the capacity to diagnose humans. As a result, diagnostics of animal rabies are used as a proxy to determine positive human exposure to the disease.

Similarly, there is only one animal rabies laboratory in the capital, with no other regional laboratories. This situation underrepresents the actual disease burden as not all suspected samples reach the laboratory in acceptable condition. The animal surveillance system needs to be better structured. Unlike the public health sector, which has a surveillance structure from the community to the national level, the veterinary domain needs more staff. There are about fifteen surveillance officers who are assigned at the district level. Although there are trained community Animal health

workers, they are not employed with the Ministry of Agriculture but assist when there are donor-sponsored projects. The surveillance officers at the district level can only cover some of the counties of assignment. There need to be more laws and regulations about rabies. There is no legal framework mandating dog registration, vaccination of dogs and cats against rabies, and responsible dog ownership. Veterinary officers are limited in their functions because they need more power to certain executive mandates, such as the power to confiscate or destroy samples. Human and animal rabies vaccines are almost impossible to obtain. Where they exist, they are often found within private health facilities and can be extremely expensive, especially for low-income families. These challenges illustrate the difficulties of achieving global dog-mediated human rabies death by 2030.

1.4 Opportunity for rabies elimination

In Liberia, there are strengths and opportunities for rabies control. Establishing the One Health Coordination Platform (OHCP) of Liberia allows for effective rabies control. One Health focuses on an integrated, collaborative, and multidisciplinary method to improve human, animal, and environmental Health. The One Health definition developed by the One Health High-Level Expert Panel (OHHLEP) states: "***One Health*** *is an integrated, unifying approach that aims to sustainably balance and optimize the health of people, animals, and ecosystems."* Therefore, to adequately confront emerging and remerging health challenges both now and in the future, we must shift our mindset from working and providing intervention within any single domain alone. Sectors' collaboration must be enabled to maintain pre- and post-rabies elimination effectively. Rabies is one of the priority diseases under the OH platform of Liberia and is the focus of a rabies technical working group, a multi-sectoral and multidisciplinary body. Heads of laboratory and surveillance of the Ministry of Agriculture, Ministry of Health, National Public Health Institute of Liberia, and international stakeholders, including the Food and Agriculture Organization of the United Nations, comprise the RTWG. Members meet periodically to discuss and share information about rabies situation and control strategies at the national level. In addition, the global drive to eliminate rabies and the MDGs (Millennium Development Goals) to end human suffering have brought rabies stakeholders together to control the disease.

1.5 Research Gaps

Despite nationwide reports of animal bite cases and clinically suspected rabies cases in humans and animals, little is known about the disease situation in Liberia. Until now, the rabies surveillance system was weak and dysfunctional in remote parts of the country, thus, contributing to underreporting. In addition, relevant information such as dog population estimate, formal KAP studies, molecular studies, and cost estimates of nationwide dog mass vaccination campaigns that are crucial for sustainable and effective rabies control and possible elimination were lacking for the country.

1.6 Aims and specific objectives

1.6.1 Aims

The principal goal of this PhD research was to set the basis for rabies control in Liberia by implementing animal rabies diagnosis and generating for the first-time baseline information about rabies knowledge and collection of dog population data and suspected rabies strains. Our findings established the basis for effective rabies prevention, control, and elimination strategies. The findings are expected to be used to develop a national rabies strategy for rabies control and to advocate and enforce rabies legislation.

1.6.2 Objectives

The objectives of the PhD work were to:

1. Establish rabies diagnostic tests in post-war Liberia for accurate rabies diagnosis
2. Conduct a cross-sectional household survey to gather information on people's Knowledge, Attitude, and Practice (KAP) relating to rabies and estimate the owned dog population size in urban and rural settings
3. Ascertain the molecular and phylogenetic characterization of circulating rabies strains in host species

1.7 Study design

1.7.1 Study site

The Republic of Liberia is located along the West African Coast, and Monrovia is the capital. Its terrain includes sandy coastal plains, rolling hills, and dissected plateaus further inland. The country borders the Republic of Guinea, the Atlantic Ocean, Cote d'Ivoire, and Sierra Leone to the north, south, east, and west. According to the 2021 population projection, the country had about 5'214'000 inhabitants, of which 48% live in rural areas. Liberia is divided into fifteen counties with a land boundary of 1’667 km. Christianity and Islam comprise 86% and 12%, respectively, of the total population (LISGIS 2008) (July 2021, estimate) (Central Intelligence Agency, USA, 2018).

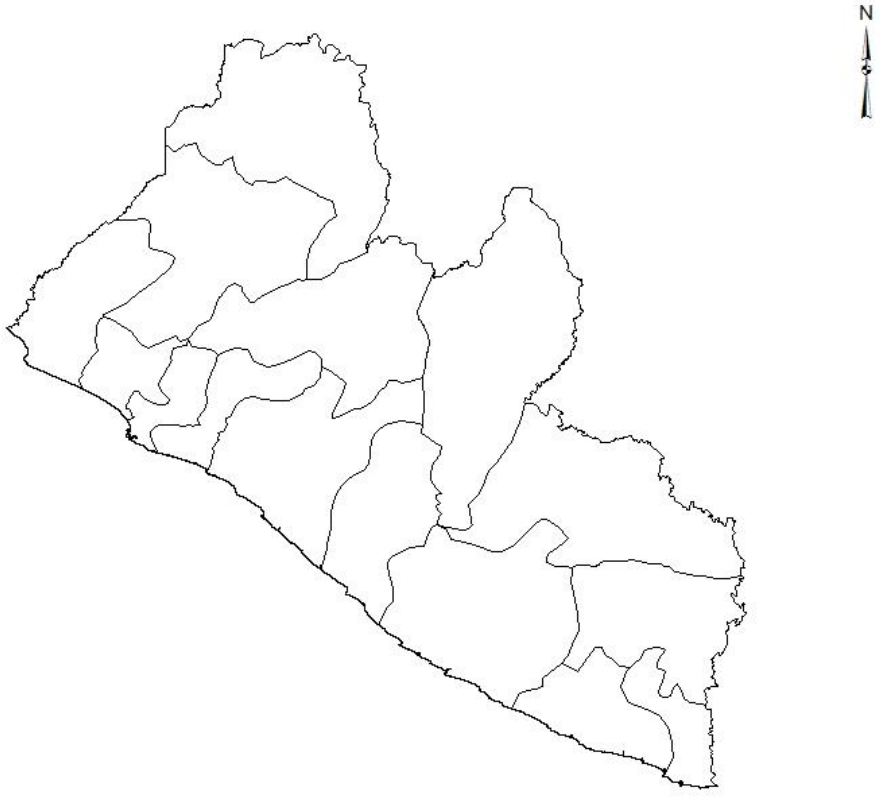

Figure 1: Map of the Republic of Liberia

2 Rabies control in Liberia: Joint efforts towards Zero by 30

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2.1 Abstract

Despite declaration as a national priority disease, dog rabies remains endemic in Liberia, with surveillance systems and disease control activities still developing. The objective of these initial efforts was to establish animal rabies diagnostics, foster collaboration between all rabies control stakeholders, and develop a short-term action plan with estimated costs for rabies control and elimination in Liberia. Four rabies diagnostic tests, the direct fluorescent antibody (DFA) test, the direct immunohistochemical test (dRIT), the reverse transcriptase polymerase chain reaction (RT-PCR) assay and the rapid immunochromatographic diagnostic test (RIDT), were implemented at the Central Veterinary Laboratory (CVL) in Monrovia between July 2017 and February 2018. Seven samples (n=7) out of eight suspected animals were confirmed positive for rabies lyssavirus, and molecular analyses revealed that all isolates belonged to the Africa 2 lineage, subgroup H. During a comprehensive in country One Health rabies stakeholder meeting in 2018, a practical workplan, a short-term action plan and an accurately costed mass dog vaccination strategy were developed. Liberia is currently at stage 1.5/5 of the Stepwise Approach towards Rabies Elimination (SARE) tool, which corresponds with countries that are scaling up local-level interventions (e.g., dog vaccination campaigns) to the national level. Overall, an estimated 5.3 – 8 million USD invested over 13 years is needed to eliminate rabies in Liberia by 2030. Liberia still has a long road to become free from dog-rabies. However, the dialogue between all relevant stakeholders took place, and disease surveillance considerably improved through implementing rabies diagnosis at the CVL. The joint efforts of diverse national and international stakeholders laid important foundations to achieve the goal of zero dog mediated human rabies deaths by 2030.

Keywords: Rabies Liberia Diagnostic Phylogenetics SARE tool GDREP tool

2.2 Introduction

Rabies elimination succeeded in much of the western world but remains a huge challenge in resource limited countries. Despite being a preventable viral disease, which can be controlled through effective vaccination and population management in the reservoir species, an estimated 59,000 people still die each year and roughly 3 million remain at risk. Of these deaths, the majority occur in children in rural communities in Asia (60%) and Africa (36%), where domestic dogs are the main reservoir species (Hampson et al., 2015; WHO, 2018a). In these regions, the disease is mainly caused through the canine associated classical rabies lyssavirus (RABV) in the genus Lyssavirus of the family Rhabdoviridae. It is a single strand negative-sense RNA virus with five genes (N-, P-, M-, G- and L-gene) (ICTV, 2018). In Africa, four different lineages (Africa 1-4) are known, with the Africa-2 clade the dominant lineage in West- and Central Africa (De Benedictis et al., 2010; Kissi, Tordo, & Bourhy, 1995; Talbi et al., 2009; Cécile Troupin et al., 2016).

Rabies surveillance methods are often ineffective in resource-limited countries due to lack of laboratory diagnosis, poor disease awareness with bite victims not seeking medical attention, and disease misdiagnosis by clinical staff resulting in massive underreporting of human and animal cases. These factors contribute to a cycle of neglect and lead to under-representation of the true disease burden, preventing decision makers from allocating funds for disease control measures (Sarah Cleaveland et al., 2002; Mallewa et al., 2007; Nel, 2013; WHO, 2018a).

Recently there has been a global drive to eliminate canine-mediated human rabies by 2030, in line with the United Nations Sustainable Development Goals (UN SDGs). The Food and Agriculture Organization (FAO), the World Organisation for Animal Health (OIE), the World Health Organization (WHO) and Global Alliance for Rabies Control (GARC) launched the United Against Rabies (UAR) initiative in 2018 (Lembo et al., 2011; Minghui, Stone, Semedo, & Nel, 2018). To reach zero dog-transmitted human deaths by 2030, the UAR is the leading global coordination for the implementation of rabies control programmes. In addition, FAO, GARC, US Centers for Disease Control and Prevention (US CDC) and other partners have joined efforts to develop tools, guidelines and initiatives to assist countries in achieving the global goal. Several rabies-dedicated regional networks were established in dog-rabies endemic regions, including the Pan-African Rabies Control Network (PARACON) in 2014 under the

secretariat of GARC. Its mission is to provide countries with a platform for sharing knowledge, the dissemination of tools, and advocacy to prioritise rabies and facilitate elimination of dog-mediated human rabies in sub-Saharan Africa by 2030 (Scott, Coetzer, de Balogh, Wright, & Nel, 2015). For effective disease control, it is fundamentally important that all rabies control activities are planned and carried out with close collaboration between the animal and human health sectors. This 'One Health' concept should be adapted to specific local settings, based on the human-animal relationship as governed by cultural and religious context. A One Health approach further facilitates better disease surveillance and communication and results in health benefits and financial savings. However, such collaboration often does not exist in low- and middle-income countries (LMICs) (L´echenne et al., 2015; Scott et al., 2015; WHO 2018a; Zinsstag et al., 2015, 2005). Within PARACON, collaboration amongst African countries is highly promoted. Representatives from the partner countries frequently meet for meetings and workshops. A widely used tool within rabies networks like PARACON is the 'Stepwise Approach towards Rabies Elimination' (SARE) tool. The SARE evaluates a country's current situation in relation to dog-mediated rabies control and elimination efforts and facilitates the development and implementation of an effective national control programme (A. Coetzer et al., 2016). The tool consists of two components, the SARE component and the Practical Workplan component. The SARE component evaluates the country's situation by generating a comprehensive list of accomplished and pending activities, resulting in a SARE score, ranging from 'endemic for dog-mediated rabies' to 'freedom from dog-mediated rabies', based on progression. While the accomplished activities are useful for advocacy and resource mobilisation, the pending activities help countries focus their efforts toward continued implementation of disease intervention initiatives. The Practical Workplan component uses the country's SARE assessment output to automatically create a workplan populated with existing objectives/priority actions, outcomes, responsible authorities, timeframes (including Gantt charts), and deliverables for each of the pending activities. Each activity in the workplan is automatically populated with content based on recommendations from a global panel of experts. Although pre-populated, all content can be modified by the users, customising the workplan into a detailed, country-centred technical document. Countries can easily develop effective, actionable workplans based on sound monitoring and evaluation approaches and principles in a relatively short timeframe

(Coetzer et al., 2018, 2016; FAO and GARC, 2012; Scott et al., 2017, 2015). The Global Dog Rabies Elimination Pathway (GDREP) was developed by the US CDC in 2017, to complement the SARE tool. GDREP is a user-friendly Microsoft® Excel-based budgeting tool to produce cost estimates for national mass dog vaccination programmes, based on data gathered from rabies vaccination campaigns in Haiti, Ethiopia, the United States (USA), Vietnam and Latin America (Undurraga et al., 2017; Wallace, Undurraga, Blanton, Cleaton, & Franka, 2017). The GDREP tool requires information on the size of the human and dog population in the country, current dog rabies vaccination coverage, available workforce and dog vaccination rate. With the user-provided information - gathered prior to and during the SARE workshop - the tool generates a phased framework specifying how many years remain for a specific country to progress to freedom from dog-mediated rabies, coupled with the estimated costs required as both annual and phased sums.

Evaluating the true burden of rabies in dogs is required to understand the current disease situation and to develop strong control strategies. Efficient surveillance programmes, where samples from suspect and biting animals are sent to Central Veterinary Laboratories (CVLs) for RABV detection, analysis and reporting, are crucial. Therefore, elimination in endemic countries also relies on availability of fully functioning and accurate diagnostic facilities. As of 2018, the direct fluorescent antibody (DFA) test and the direct immunohistochemical test (dRIT) - antibody-based protocols for detection of viral antigen - and conventional or real-time-polymerase chain reaction (RT-PCR) assays - molecular investigations for detection of viral RNA - are the diagnostic assays recommended for post-mortem rabies diagnosis by the OIE (OIE, 2018). Having different techniques ready to perform rabies diagnostics offers flexibility to overcome major limitations, for example, from lack of equipment, maintenance, or reagent supply. Although the DFA is an accurate and easy test, it is often challenging to implement in LMICs. The dRIT has several advantages over the DFA and is currently promoted through GARC in PARACON partner countries. The dRIT requires only a basic light microscope, whereas the DFA needs a fluorescence microscope. The dRIT is also easier to interpret in degraded or archived samples, and preserving samples in glycerol seems to influence DFA more than dRIT (Andre Coetzer et al., 2017; Durr, Meltzer, Mindekem, & Zinsstag, 2008; Scott et al., 2015). However, Lembo et al. (2006) reported that storage in glycerol did not seem to

influence the DFA, and they and Prabhu et al. (2018) demonstrated full corroboration in detection of virus from field samples between DFA and dRIT. Advantages of DFA over dRIT are the following: less chemicals are used, which is particularly important in countries where waste disposal is not well regulated; several commercialised rabies conjugated antibodies are marketed; and the test protocol is much simpler with fewer steps. The third OIE-recommended assay is the RT-PCR approach, which is the only recommended technique to detect rabies in decomposed samples (Markotter et al., 2015; McElhinney, Marston, Brookes, & Fooks, 2014; Prabhu et al., 2018). Molecular detection by RT-PCR is the only technique available in some veterinary laboratories in Africa, particularly those recently equipped for rapid diagnosis of avian influenza and other transboundary diseases. Nevertheless, implementing a molecular based technique to detect RABV requires great care to prevent sample cross-contamination, so a validated disinfection protocol and good laboratory practice is fundamental (Aiello, Zecchin, Caenazzo, Cattoli, & De Benedictis, 2016). Ideally, reliable efficient rabies diagnosis would be through the availability of an antibody-based protocol, either DFA or dRIT, and a molecular protocol for diagnostic confirmation. But proper application of the recommended tests in developing countries often remains limited, due to poorly equipped laboratories, challenges maintaining reagent cold chains, appropriate sample transportation, and lack of quality control systems. Existing surveillance data often reflects only the rabies situation of the urban areas near CVLs. In this context, recently developed rapid immunochromatographic diagnostic tests (RIDTs) based on the lateral flow principle, which do not rely on a functional laboratory or adequate cold chain, offer new opportunities for decentralised rabies diagnosis in remote areas where the majority of animal bites cases are reported to occur. Although the sensitivity of RIDTs has been under debate, it has been demonstrated that applying a modified protocol of the Bionote kit results in an increased sensitivity and specificity, ranging from 93% to 98% and from 95% to 99% when compared to DFA, respectively (L´echenne et al., 2016; Mauti et al., 2020; Yale et al., 2019). To reach a higher detection performance, it is necessary to omit the 1:10 dilution of the original sample in phosphate buffered saline (PBS), although the dilution is still recommended by the manufacturer. Nevertheless, RIDTs can now be considered as a practical field tool for initial surveillance purposes. However, confirmation of rabies cases can only be achived by means of one of the gold standard techniques (Duong et al. 2016; Eggerbauer et al., 2016; L´echenne et al., 2016; OIE, 2018; WHO, 2018b).

Interrupting virus transmission requires at least 70% vaccination coverage of the affected dog population (Coleman & Dye, 1996; WHO, 2018a). Several studies demonstrate that mass dog vaccination is the only cost-effective and sustainable control measure (Hampson et al., 2015; Mindekem et al., 2017; Zinsstag et al., 2009). But it is crucial to know more about the target dog population and existing human-dog relationship before planning and implementing dog rabies vaccination programmes (S. Mauti et al., 2017; WHO, 2018a). In N'Djamena, the capital city of Chad in Central Africa, canine rabies transmission was interrupted after two consecutive vaccination campaigns with sufficient vaccination coverages. However, rabies reappeared earlier than predicted. Based on phylogenetic and phylodynamic analysis, Zinsstag et al. (2017) hypothesised that reintroduction may have been due to influx of infected dogs from neighbouring areas, underlining the importance of including neighbouring settings for rabies control. Domestic dogs are tied to humans, so the role of humans and the precise mechanisms governing rabies diffusion should be further investigated. In some areas, long-distance transport of infected dogs is a known risk for rabies introduction or reintroduction. However, additional analysis of rabies genetics in combination with landscape features from new areas will further clarify disease spread (H. Bourhy et al., 2016; Brunker, Hampson, Horton, & Biek, 2012; Cori et al., 2018; Dellicour et al., 2017).

In Liberia, dog rabies is endemic and surveillance systems and disease control activities are still in the early phase. However, rabies vaccination in humans has been documented since 1949 (Poindexter, 1953), but following civil war from 1989-2003 and the devastating Ebola outbreak in 2014-2015, health care services and infrastructure were substantially weakened (National Transitional Government of Liberia, 2004; The Lancet, 2014). Large areas of the country still do not have electricity. A few studies have described rabies prevalence, the molecular characterisation of circulating rabies virus isolates and estimated post-exposure prophylaxis (PEP) demand based on dog bites (Jomah et al., 2013; Monson, 1985; Olarinmoye et al., 2019). During 2008-2012, 488 dog bite cases were registered at several county hospitals, with children under 10 years of age the most affected group (Jomah et al., 2013). In the 2018 annual report, the National Public Health Institute (NPHIL) registered 1,645 bite cases and 10 related deaths (Unpublished report from NPHIL in 2018). However, data on the biting animals is poorly captured on the

veterinary side. Olarinmoye et al. (2017) applied a decision tree model to human bite data for Monrovia, the capital city of Liberia, estimating 155 human rabies deaths annually and high demand for PEP. However, the actual burden of rabies in Liberia remains unknown. PEP in Liberia is based on wound washing and post-exposure vaccination of exposed persons, since rabies immunoglobulin (RIG) is not available. Rabies vaccine is limited to major cities, with remote and marginalised communities having no access to life-saving treatment. Usually, health facilities in these areas lack continuous power supply to store rabies vaccines, thus limiting possibilities for the adequate supply of vaccine to these remote areas. Collaboration between the public health and veterinary service is minimal, and functional rabies surveillance remains a substantial challenge. However, a One Health Coordination Platform was created in 2017 to coordinate zoonotic disease activities between sectors. The first rabies case was diagnosed by DFA at the CVL in Liberia. Rabies was subsequently declared a priority disease and is currently the focus of a working group which promotes joint disease surveillance systems between the veterinary and human sectors. Whereas dog owners normally have to pay for dog vaccination, free small scale dog vaccination campaigns were conducted between 2012 and 2018. About 1500 dogs, mostly from Monrovia, were vaccinated against rabies during the World Rabies Day (WRD) activities (personal communication). Within the Global Health Security Agenda (GHSA) programme, FAO is committed to improve Liberian national animal health services to assist country compliance with International Health Regulations (IHR, 2005). The technical reorientation of the USAID-funded FAO Emerging Pandemic Threats (EPT-2) led FAO Emergency Centre for Transboundary Animal Diseases (ECTAD) teams to develop work plans supporting implementation of the GHSA against four Action Packages, including Zoonotic Diseases (ZD), Biosafety and Biosecurity (BB), Laboratory Systems (LS) and Workforce Development (WD). Under the ZD Action Package, GHSA countries are expected to conduct a national zoonotic disease prioritisation process using the CDC One Health Zoonotic Disease Prioritisation Tool (OHZDPT). Rabies was deemed a top five priority zoonotic disease in all FAO GHSA countries, including Liberia. Lastly, Liberia was part of a larger study, led by the Swiss Tropical and Public Health Institute (Swiss TPH), to estimate the burden of rabies in Ivory Coast, Mali, Chad and Liberia. The aims for Liberia were to establish diagnostic capacity for animal rabies and collect laboratory data on rabies

cases. The research aim coincided with FAO and GARC plans and to avoid overlap of activities close collaboration was sought.

The aim of the present work was to establish animal rabies diagnostics at the CVL, to foster collaboration between all stakeholders involved in rabies control in Liberia inter alia through a comprehensive in-country rabies stakeholder workshop in Margibi County and to develop a short-term action plan for rabies control and elimination in Liberia.

2.3 Material and methods

2.3.1 Study area

Liberia is located in West Africa and has never been colonised. It is bordered by Guinea to the north, Cote ˆ d'Ivoire to the east, the Atlantic Ocean to the south and Sierra Leone to the west. The country is divided into 15 counties, covering 111,369 km2. The estimated population of Liberia was approximately 4.9 million inhabitants in 2018. The capital city Monrovia forms one district, which is a subunit of Montserrado County. Phylogenetic analyses (see point 2.4.) were performed on rabies strains originating from three counties, urban Monsterrado and Margibi and rural Lofa. Montserrado County is in the northwest of Liberia, with a population of around 1.1 million people. Margibi County borders Montserrado to the west and has around 199,689 inhabitants. Lofa is in the north, with 276,863 inhabitants (Central Intelligence Agency, USA, 2018) fig (2).

2.4 Implementation of rabies diagnostics at the Central Veterinary Laboratory

Implementation of rabies diagnostic tests was a joint effort by Swiss TPH, FAO, Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe – FAO Reference Center for rabies) and GARC. The FAO organised assessment missions for quality assurance (October 2016) and diagnostic techniques (January 2017). Subsequently, in 2017, the FAO renovated the Leon Quedlum Central Veterinary Diagnostic Laboratory (CVL) in Monrovia, supported by the GHSA programme. To design a functional and modern diagnostic laboratory according to biosafety/ biosecurity (B/B) and quality assurance (QA) standards (ISO 17025), the initial renovation over several months included full infrastructure restoration, including roof repair, city power grid connection and restructuring of the water supply system. A molecular unit was configured, including

an RNA extraction room, a PCR mix room and a “gel” room, in addition to reception and necropsy rooms. Key equipment and required reagents were provided mainly by FAO, with support from Swiss TPH. Between July 2017 and February 2018, the three OIE recommended rabies tests were implemented: the DFA test (by FAO and the FAO Reference Center (RC), the IZSVe, and Swiss TPH and their Malian study partner, the CVL Bamako), the dRIT (by Swiss TPH and GARC) and the molecular conventional RT-PCR protocol (by FAO and the IZSVe) (De Benedictis et al., 2011). Additionally, the RIDT (Anigen/Bionote Inc.) was introduced by Swiss TPH and the CVL Bamako. Staff were trained on standard protocols in sessions organised by FAO, IZSVe and Swiss TPH. Laboratory staff were instructed on B/B (May 2017), QA, Good Laboratory Practices (GLPs), and the most commonly used molecular methods for animal pathogen diagnosis (reverse transcription (RT), end-point and real time polymerase chain reaction (PCR)) (December 2017) by FAO. The FAO RC invited the CVL to carry out the rabies diagnosis proficiency testing (PT) in November 2018, which provided time to the laboratory staff to practice the implemented techniques. This was an effort to support the government in improving the sector through capacity building.

A PT panel of 10 blind samples including two controls for the exercise were provided. The PT samples consisted of lyophilized material prepared from healthy mammals’ brain homogenates, including 5 samples which were mixed with mice brain experimentally infected with RABV or rabies-related lyssaviruses. The PT panels were prepared according to the ISO 17043 and were shipped, using a dedicated courier, as dangerous goods. In order to be cost effective, a unique parcel containing the PT panel and the 2 controls (1 positive and 1 negative) along with extra control vials used for the training purposes were shipped on dry ice prior to the training course organized by FAO and IZSVe (on DFA and RT-PCR). The results of this PT programme which included 14 laboratories from Sub-Saharan African countries are presented in the work of Gourlaouen et al. (2019). A high concordance rate was achieved amongst the participants with 87.7% and 98.2% for the DFA and the RTPCR, respectively (Gourlaouen et al., 2019).

2.5 Sample collection and rabies diagnostics

Sample collection was event driven. After the implementation of rabies diagnostics at CVL, fifteen County Livestock Officers, Health Surveillance Officers and members of the OH platform were asked to contact staff of CVL via mobile phone following a suspected dog bite or identification of suspected animals. CVL staff, trained on proper animal handling, travelled to field sites and transported the animal or carcass by car to the CVL. If a field visit was not possible, the animal head was transported in an icebox to CVL using a local transport company. Sample collection was performed at the CVL and brain samples were tested with the DFA test and RIDT tests. A questionnaire was completed with information on the biting animal, date of examination and origin of the sample. However, the renovation work of the CVL in 2017 interrupted the sample collection process and analysis of suspected rabies samples as there was no electricity and laboratory space to store and analyse suspected samples.

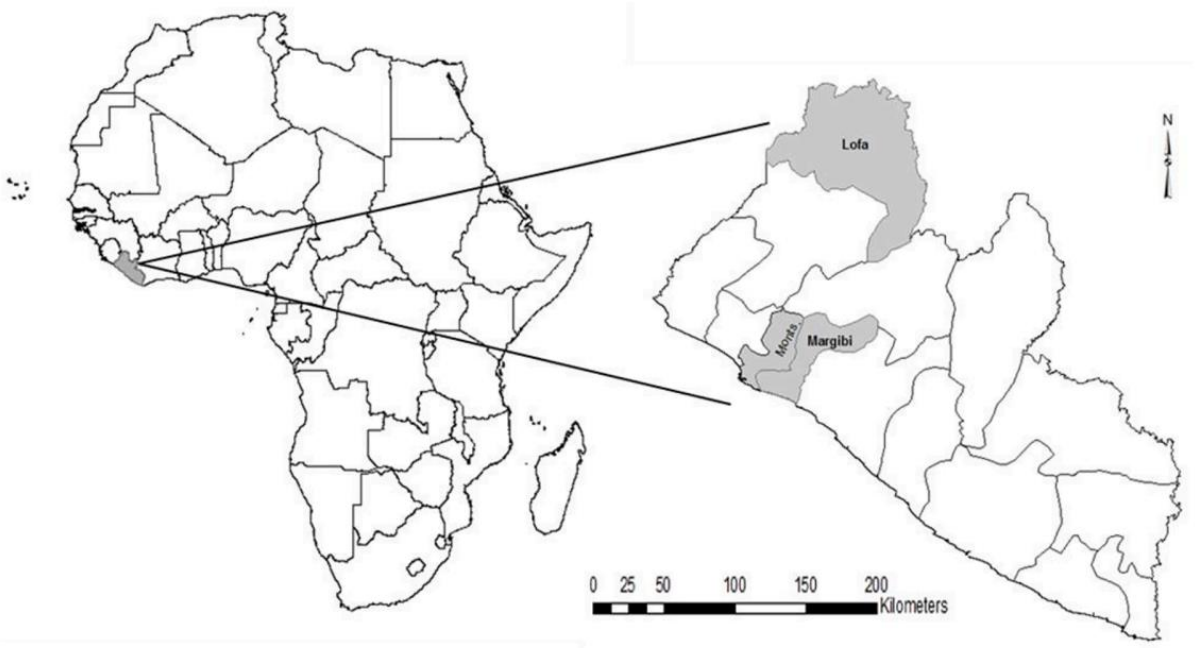

Figure 2: Map of Liberia (Study areas in grey)

2.6 Confirmatory testing and phylogenetic analysis

Following an agreement with Liberian veterinary services, aliquots of all rabies suspected samples (n=8) were shipped (some in parallel) to IZSVE (n=6) and Institut Pasteur Paris (n=7, RNA samples) between May 2017 and August 2018 for confirmatory testing and molecular characterisation. DFA test, cell culture test and/or molecular techniques (conventional and real-time RT-PCR) were used to re-test the samples in the international reference laboratories as described previously (Dacheux et al., 2016, 2008; De Benedictis et al., 2011; OIE, 2018). Sequencing of the complete N and G gene sequences was performed after amplification as previously described (Hervé Bourhy et al., 2008; Fusaro et al., 2013), with specific primers available upon request, or by next-generation sequencing (WHO, 2018c). Using jModelTest2 (Darriba, Taboada, Doallo, & Posada, 2012), the best-fit model of nucleotide substitution according to the Bayesian Information Criterion was the general time reversible model plus gamma-distributed rate heterogeneity (GTR+G), which was further confirmed using Smart Model Selection in PhyML (Lefort, Longueville, & Gascuel, 2017). A maximum-likelihood phylogenetic tree was constructed using subtree-pruning-regrafting branch-swapping and PhyML version 3.0 (Stéphane Guindon & Gascuel, 2003). The robustness of individual nodes on the phylogeny was estimated using 1000 bootstrap replicates with aBayes branch supports. In addition to the Liberian sequences, sequences from West and Central African countries were included in the analysis.

2.7 Development of a short-term action plan for rabies control and elimination

Following on from establishment of rabies surveillance in Liberia, and to advance rabies control and elimination efforts in general, representatives from all governmental stakeholders and line ministries involved in rabies control participated in a comprehensive in-country One Health rabies stakeholder meeting organized by FAO and GARC. The workshop took place in Liberia from May 28 to June 1, 2018. Three work-streams were completed: undertaking a SARE assessment, developing a practical workplan using the SARE tool's Practical Workplan component, and estimating costs of mass dog vaccination with the GDREP tool. The cost estimates generated were based on a cost of USD 2.60 per dog vaccinated, which is

representative of published values for the region (Kayali, Mindekem, Hutton, Ndoutamia, & Zinsstag, 2006).

2.8 Ethical considerations

Research approval was granted by national authorities in Liberia and the Ethics Committee of Northwest and Central Switzerland (EKNZ Basec Req-2017-00495) in July 2017. The research project fulfilled all ethical and scientific standards and posed no health hazards. All involved personnel were vaccinated against rabies following the instructions of the vaccine producer. All data were handled confidentially.

2.9 Results

2.9.1 Phylogenetic analysis of the first laboratory-confirmed animal rabies positive cases

Between February 2017 and April 2018, eight suspected animals were submitted to CVL. Seven samples tested by RIDT and DFA were confirmed positive for rabies virus. Details on positive animals are shown in (Table 1).

Full sequences of the genes encoding for viral glycoprotein and nucleoprotein from isolates 17013LIB_Liberia_2017,18005LIB_Liberia_2017,18007LIB_Liberia_2017, 18008LIB_Liberia_2017, 18009LIB_Liberia_2018, 18018LIB_Liberia_2017, IZSVe_18RD/ 666_4_Liberia_2017 were obtained and subsequently published in GenBank (accession numbers MN049979 – MN049984; MH481708 - MH481713). The phylogenetic analyses of the N genes revealed that the RABV detected in Liberia belonged to the Africa 2 lineage subgroup H, which circulates in central and western African canine populations. The Liberian viruses from this study clustered together with viruses circulating in neighbouring countries (Cote ˆ d'Ivoire, Mali, Mauritania and Burkina Faso). Isolate 18018LIB_Liberia_2017 had high similarity to isolate 01007CI_Cote_Ivoire_2001 from Cote ˆ d'Ivoire (Fig. 3).

2.9.2 Results from the Stepwise Approach toward Rabies Elimination (SARE) tool – SARE assessment and Practical Workplan component.

Based on the SARE assessment undertaken during the in-country workshop, Liberia achieved a nationally-endorsed SARE score of 1.5/5, indicating it is in the process of scaling up intervention campaigns based on existing data. To help Liberia progress up the SARE ladder to freedom from dog-mediated human rabies, national stakeholders

developed a practical workplan utilising the SARE tool. Based on consensual agreement amongst participants, only remaining content from workplan Stage 0 and Stage 1 activities (all relating to core, fundamental programmatic activities like small-scale vaccination campaigns and local-level dog population estimates) were finalised to ensure a solid foundation before scaling-up to nationwide control efforts. The workplan, focussing primarily on fundamental activities at the local level, was used to populate a short-term rabies action plan to be actioned by government personnel for the next three years (2019 – 2021). The short-term rabies action plan can be used to ensure programmatic implementation at the local-level and advocate for additional funding necessary for disease intervention initiatives to continue and expand in waves.

2.9.3 Results from the Global Dog Rabies Elimination Pathway (GDREP)

Based on the information provided for Liberia and information gathered prior to and during the workshop, the GDREP tool estimated that dog-mediated human rabies deaths could be eliminated by 2025 and dog rabies could be completely eliminated by 2028, followed by self-declaration of freedom from dog rabies by 2030. These estimates are based on a three-phase approach where total cost of the proposed elimination programme (through mass dog vaccination) will scale up over the three phases. During phase I (years 1-3), an additional 75,000 USD per year is required in addition to the estimated 1,000 USD now spent annually on in-country rabies control efforts. For phase 1, a total of 228,000 USD is needed over the initial three years to strengthen capacity for surveillance and vaccination and to implement demonstration projects (e.g., small-scale mass dog vaccination events at pre-selected local areas). These activities will generate data to support scale-up of activities and raise disease awareness. In phase II (years 4-6), additional funds needed increase from an estimated 75,000 USD per year to 662,000 USD per year. This three-year phase focusses on increasing the national dog vaccination coverage from <18% to the required 70% coverage. Phase III (years 7-13) is considered the maintenance phase and is the most critical phase with regards to mobilisation of funds and sustainable governmental commitment. Phase III focusses on maintenance of adequate vaccination coverage to ensure dog rabies elimination. To accomplish this, Liberia requires an estimated additional 746,000 USD per year to eliminate dog rabies and undertake the self-declaration process for freedom from dog-mediated rabies. In total, it is predicted that Liberia requires an investment of 5.3 – 8 million USD over the next

13 years to successfully implement and maintain dog vaccination coverage to end disease transmission and eliminate canine rabies in the country.

Table 1: Information on rabies-Positive tested animals

Nr.	Species	Known owners	Sex	Age	Symptoms	Vaccination status	Date of sample collection	Date of examination	Orgin of the sample	Genbank accession numbers
17013LIB_Liberia_2017	dog	yes	male	adult	change in behavior, no food intake	unvaccinated	2/27/2017	7/25/2017	Margibi County	MN049979
18005LIB_Liberia_2017	cat	yes, animal neighbor	male	subadult	change in behavior, no food intake	unknown	12/5/2017	2/14/2018	Montserrado County	MN049983
18007LIB_Liberia_2017	dog	yes, animal of the household	female	puppy	change in behavior, no food intake	unvaccinated	9/25/2017	2/16/2018	Montserrado County	MN049982/ MH481712
18008LIB_Liberia_2017	dog	yes, animal of the household	female	adult	change in behavior	unvaccinated	3/25/2018	4/25/2018	Montserrado County	MN049981/ MH481711
18009LIB_Liberia_2018	dog	yes	na	na	na	na	na	na	Lofa County	MN049980
18018LIB_Liberia_2017	dog	na	na	na	na	na	na	na	na	MN049984
IZSVe_18RD/ 666_4_Liberia_2017	na	na	na	na	na	na	na	na	na	MH481713

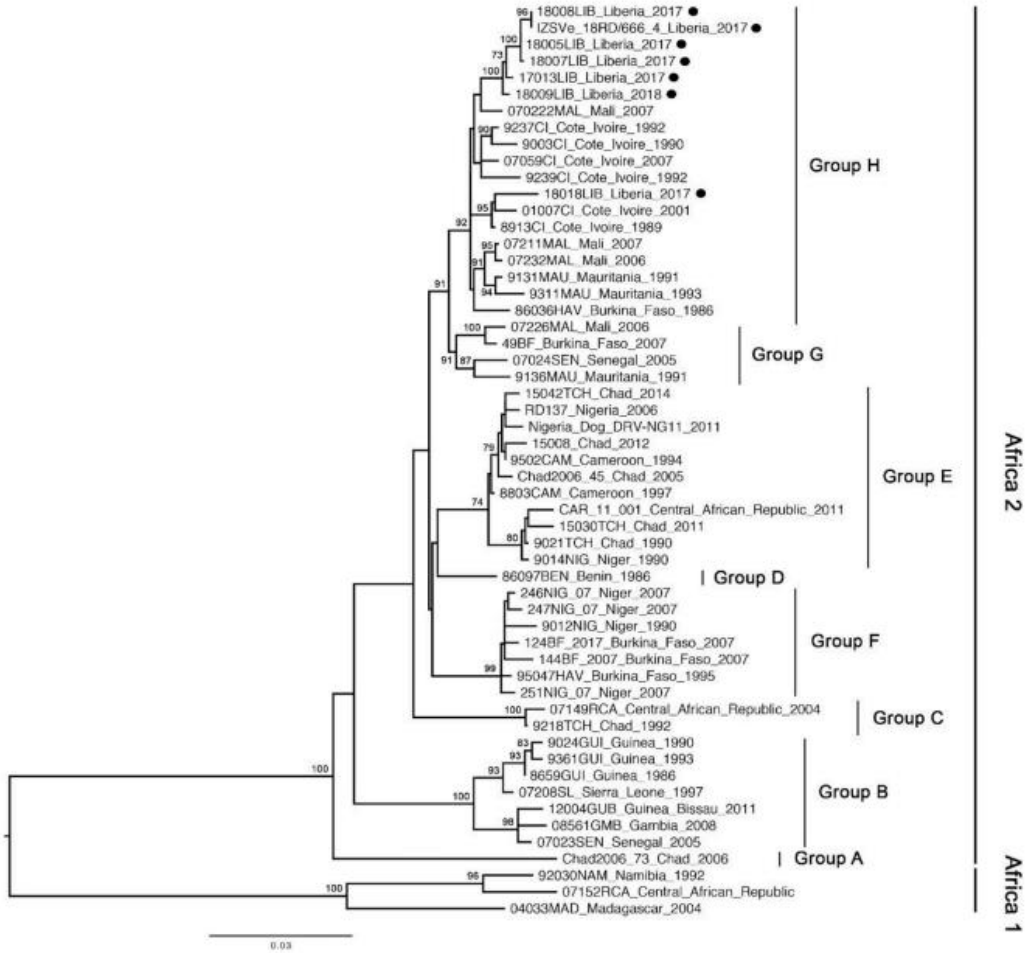

Figure 3: Maximum-likelihood phylogenetic tree based on 1350-nt nucleoprotein genes of seven rabies virus sequences from Liberia, 2017-2018 and representative sequences from Mali, Cote d'Ivoire, Mauritania, Burkina Faso, Senegal, Nigeria, Chad, Cameroon, Central African Republic, Niger, Benin, Guinea, Sierra Leone, Gambia, Guinea Bissau, Namibia and Madagascar. Subgroups A-H within the Africa-2 lineage are indicated, with Liberia sequences obtained during this study marked by black dots. Bootsrap values (1000 replicates) > 70% are shown next to nodes. Scale bar indicates nucleotide substitutions per site.

2.10 Discussion

Controlling and eliminating dog-mediated rabies in Liberia is a complex undertaking. However, a diverse set of stakeholders recently joined together to achieve this goal. A major initial hurdle was overcome through implementation of effective rabies diagnosis in the country, resulting in the first laboratory-confirmed animal rabies cases being diagnosed and reported. Documentation of the disease within the country enabled government authorities to begin planning for control and elimination of canine rabies in Liberia. A practical workplan and short-term action plan were developed using the SARE tool and an accurately costed mass dog vaccination strategy produced using the GDREP tool at a 2018 rabies stakeholder meeting. Liberia is currently at stage 1.5 of the SARE tool, signifying the country is preparing for local-level intervention dog vaccination campaigns, and needs investment of 5.3 – 8 million USD over the next 13 years for successful elimination of rabies by 2030.

There are still no accurate estimates of the 'true' national rabies burden in Liberia, so future research activities should focus on developing a well-functioning 'One Health' rabies surveillance system. This can be achieved through timely confirmation of suspect rabies samples at the Monrovia CVL using the recently established DFA test, and through decentralisation of rabies diagnosis using dRIT or RIDT. The latter test is especially useful in areas lacking electricity, as it does not require a microscope and can be stored at room temperature (Lechenne et al., 2016). Some challenges were experienced during implementation of the dRIT test. A break in cold chain seemingly influenced the viability of the diagnostic reagents, and it was difficult to locally source some required test reagents. However, these challenges should not influence implementation of the test in Liberia or other resource-limited countries, because the test has many advantages. Regarding molecular detection of rabies, further commitment is needed to ensure successful accurate diagnosis. In addition to laboratory confirmation of rabies cases, improvement of rabies awareness for the community and health care personnel is also crucial for effective disease surveillance. Exposed people need to have timely access to PEP, which consists of effective wound washing, rabies vaccination and, under certain circumstances, administration of RIG, to reach the goal of zero human deaths by 2025 as projected by the GDREP. Approval from the responsible authorities to use RIG throughout the country should be prioritised to ensure feasibility to reach the goal.

Molecular characterisation of the RABV-positive samples improved the resolution of the surveillance network and revealed that the seven laboratory-diagnosed and sequenced samples all belong to subgroup H of the lineage Africa-2. This result is not surprising, as the Africa 2 lineage is widely distributed in central and western Africa with subgroup H being prevalent in Côte d'Ivoire, Mauritania, Mali and Burkina Faso (Talbi et al., 2009). This demonstrates the transboundary nature of rabies (Andre Coetzer et al., 2017; Hayman et al., 2011) and has implications for its control in Liberia. One Liberian isolate was similar to an isolate from Côte d'Ivoire and suggests a wide distribution of this subgroup over a large area including at least Liberia and Côte d'Ivoire. The recently published study of Olarinmoye et al. (2019) detected the Africa-2 lineage as well as the China lineage 2 and Africa lineage 3 in Liberia, but these results are still being debated by the wider scientific community. Based on this discrepancy among studies, more information is required to better understand the current rabies situation in Liberia through improved molecular epidemiological studies – possible through well-established collaborations fostered during this study and through regional rabies networks such as PARACON. With such studies, it would be possible to identify rabies hotspots and areas of concern for rabies transmission, enabling more strategic targeted mass dog vaccinations with a more cost-effective approach. Dogs are inevitably linked to humans and thus to human-mediated transportation within the region, and future rabies control efforts must take this into account (Bourhy et al., 2016; Brunker et al., 2012; Dellicour et al., 2017; Talbi et al., 2010). For better resolution of virus circulation patterns in Liberia and the neighbouring countries, a broader range of samples originating from neighbouring areas should be included in future analyses.

While improved burden estimates are important for disease prioritisation and elimination, improving active and passive surveillance programmes are long-term activities that require considerable resources and time. In an effort to ensure short-term progress and maintain governmental support, the SARE tool was used to identify additional activities that need to be accomplished to contribute to rabies elimination. By accomplishing the activities, Liberia can advocate for further operational and financial support from both domestic and international donors and stakeholders to ensure that the national strategy for rabies elimination remains adequately resourced throughout the 13-year time period. As evidenced by the GDREP tool, an estimated

total cost of 7,436,000 USD is required over 13 years to achieve elimination through mass dog vaccination, re-emphasising the need for continued, long-term and stable investment. The estimates generated by the GDREP tool were based on a cost of USD 2.60 per dog vaccinated, which is representative of published values in the region (Kayali et al., 2006). However, as there is limited data available for Liberia due to the limited number of vaccination campaigns undertaken in the country, these estimates should be refined further with efforts made to reduce these costs. Additionally, detailed information on the size and structure of the dog population should be studied in future research projects. By obtaining a more accurate cost per dog vaccinated and reducing costs where feasible, the costs towards dog-rabies elimination by mass dog vaccination can be dramatically reduced. The full benefit of the GDREP tool not only accurately estimates costs of dog vaccination campaigns, but also generates realistic, evidence-based figures for stakeholders to create long-term resource mobilisation plans and implement effective strategies for timely resource mobilisation.

Important questions remain on who pays for rabies control and how necessary funds may be secured up front. One interesting possibility is development impact bonds (DIB) (Anyiam et al. 2017), a performance-based investment instrument, where costs of rabies control efforts are shared between the government, private investors and outcome funders. With such an approach, the investment risk is shared, securing resources over a longer-term and mobilising current resources to drive intervention campaigns. With clear objectives, deliverables and timelines and the short-term action plan for Liberia developed using the SARE tool's Practical Workplan component, the investment impact and outcomes are more easily measured and quantified. Other non-financial resources are also available to facilitate country efforts towards achieving elimination. Mass dog vaccination is key to achieving rabies elimination, so procurement and delivery of dog vaccine remains vital. Through utilisation of resources such as the OIE rabies vaccine bank, which provides high-quality vaccine at an affordable price in a timely manner, Liberia can reduce associated costs and immediately initiate planned local-level intervention strategies, as detailed in the short-term action plan. This, and the many other available resources, can help Liberia scale up efforts towards nationwide intervention programmes.

2.11 Conclusion

This study in Liberia illustrates the difficulties of rabies control and elimination in LMICs in Africa. Liberia still has a long road to become free of dog-rabies. However, the dialogue between all relevant stakeholders occurred and preparations for small-scale intervention campaigns began. Following implementation of rabies diagnosis at the CVL, which improved disease surveillance, improved communication between the animal and human health sector remains of utmost importance. RIG, which is not currently available in the country, should be made immediately available so adequate treatment of category III exposed individuals and category II immune-compromised persons is possible. Through implementation of accurate laboratory diagnosis, initiation of molecular epidemiological analyses, improved rabies surveillance, formation of a One Health taskforce and development and implementation of a detailed, accurate workplan and short-term action strategy, Liberia and its partners laid the foundation towards achieving the goal of zero dog-mediated human rabies deaths by 2030. The results obtained from the above-described project activities in Liberia pave the way to developing rabies control strategies in other African countries and beyond.

Acknowledgments

We thank the dog owners, the County Livestock Officers, the Health Surveillance Officers, the members of the OH platform and the laboratory staff for their great commitment. We also want to acknowledge Lisa Crump for the language editing.

3 Complete genome sequences of five rabies virus strains obtained from domestic carnivores in Liberia

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3.1 Abstract

As in other African countries, canine rabies is endemic in Liberia. However, data concerning the genetic diversity of rabies virus isolates circulating in this country remain limited. We report here the complete genome sequences of five rabies viruses obtained from domestic animals. All of them belonged to subgroup H within the Africa 2 clades.

Rabies virus (RABV) is the main etiological agent of rabies, an acute and always fatal form of encephalomyelitis which can potentially affect all mammalian species. This zoonotic virus belongs to the prototype species Rabies lyssavirus within the genus Lyssavirus, family Rhabdoviridae (order Mononegavirales) (Walker et al., 2018). Rabies viruses circulating in dogs are the main cause of human rabies, with an estimated 59,000 deaths worldwide each year, especially in Asia and Africa (Hampson et al., 2015). As in other sub-Saharan countries, canine rabies remains endemic in Liberia (Voupawoe et al., 2021). However, available data about the genetic diversity of RABV isolates circulating in this country remain limited.

Brain samples collected from four dogs and one cat suspected of rabies were collected from different regions of Liberia in 2017 and 2018, within the framework of a joint effort program to strengthen rabies surveillance in the country (Table 2) (Voupawoe et al., 2021). All the samples were confirmed positive for rabies by fluorescence antibody test (FAT) (WHO, 2018a) and by a modified version of a rapid immunochromatographic diagnostic test (RIDT) (S. Mauti et al., 2020). For four samples, RNA was extracted locally from brain biopsy specimens (approximatively 0.5 cm3 each) using the Direct-zol RNA miniprep kit (Zymo Research) and then purified using Agencourt RNAClean XP beads (Beckman Coulter) at a 1:1.8 ratio. The last sample was extracted at Institut Pasteur using TRIzol reagent (Invitrogen) from an FTA card (Whatman FTA card technology; Sigma-Aldrich) impregnated with ground brain material as previously described (Table 2) (Lechenne et al., 2016). The five RNA samples were processed for next-generation sequencing (NGS) as previously described (Dacheux et al., 2019; Luo, Li, et al., 2021; Pallandre et al., 2020). Briefly, an rRNA depletion step was first carried out using Terminator 59-phosphate-dependent exonuclease (Epicentre iotechnologies). After purification, depleted RNA was reversetranscribed into cDNA using Superscript III reverse transcriptase (Invitrogen), and double-stranded DNA (dsDNA) was synthesized as already described (Dacheux et al., 2019; Luo, Li, et al., 2021; Pallandre et al., 2020). Finally, dsDNA libraries were constructed using the Nextera XT DNA library preparation kit (Illumina) and sequenced using a 2 x 150-nucleotide (nt) paired-end strategy on the NextSeq 500 platform (Dacheux et al., 2019; Luo, Li, et al., 2021; Pallandre et al., 2020). NGS data were analyzed using de novo assembly and mapping (both using CLC Assembly Cell; Qiagen), with a dedicated workflow built on the Institut Pasteur Galaxy platform (Dacheux et al., 2019; Fabien Mareuil, 2017; Luo, Li, et al., 2021;

Pallandre et al., 2020). Contig sequences were assembled to produce the final consensus genome using Sequencher version 5.2.4 (Gene Codes Corporation).

Table 2: Description of the genome sequences of the five rabies virus strains obtained from Liberia domestic carnivores

Virus	Host	Animal status	Location	Yr of collection	Support[a]	Total no. of reads	No. of mapped reads (%)	Avg coverage (×)	Genome nucleotide length (bp)	GC content (%)	ORF nucleotide length (aa)[b]					GenBank accession no.	SRA accession no.
											N	P	M	G	L		
18005LIB	Cat	Owned	Margibi	2017	Beads	4,317,876	934,436 (21.6)	11,567.36	11,922	45	1,353 (450)	891[c] (296)	609 (202)	1,575 (524)	6,384 (2,127)	OK135144	SRX12176932
18007LIB	Dog	Owned	Montserrado	2017	Beads	2,228,096	4,593 (0.2)	56.95	11,923	45	1,353 (450)	891[c] (296)	609 (202)	1,575 (524)	6,384 (2,127)	OK135145	SRX12176933
18008LIB[d]	Dog	Owned	Montserrado	2018	Beads	5,132,556	12,554 (0.2)	155.23	11,885[e]	45	1,353 (450)	891[c] (296)	609 (202)	1,575 (524)	6,384 (2,127)	OK135146	SRX12176934
18009LIB	Dog	Owned	Lofa	NA[f]	FTA	1,916,466	25,596 (1.3)	316.18	11,923	45	1,353 (450)	894 (297)	609 (202)	1,575 (524)	6,384 (2,127)	OK135147	SRX12176935
18018LIB	Dog	NA	NA	NA	Beads	7,209,068	826,598 (11.5)	10,135.10	11,923	45	1,353 (450)	894 (297)	609 (202)	1,575 (524)	6,384 (2,127)	OK135148	SRX12176936

aTotal RNA was extracted in Liberia from strains 18005LIB, 18007LIB, 18008LIB, and 180185LIB (recovered from brain biopsy specimens [approximatively 0.5 cm3] from separate animals) and purified using Agencourt RNAClean XP
beads (Beckman Coulter) at a 1:1.8 ratio following the manufacturer's instructions, with the exception of the last resuspension step in nuclease-free water. The dried beads with RNA were shipped at a cold temperature with
icepacks to Institut Pasteur (Paris), where they were resuspended in 30mL nuclease-free water. Strain 18009LIB was sent to Institut Pasteur using an FTA card (Whatman FTA card technology; Sigma-Aldrich) impregnated with
ground brain material and then extracted using TRIzol reagent (Invitrogen).
bORF, open reading frame; aa, amino acid.
cP ORF with premature stop codon (missing the last amino acid C).
dStrain 18008LIB was also partially sequenced at Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe-FAO Reference Center for Rabies) in Italy, and the sequence was found to be identical to the one obtained by Institut
Pasteur Paris (IPP).
eGenome with incomplete 59 UTR (untranslated region) (leader sequence missing 38 nucleotides).
fNA, not available.

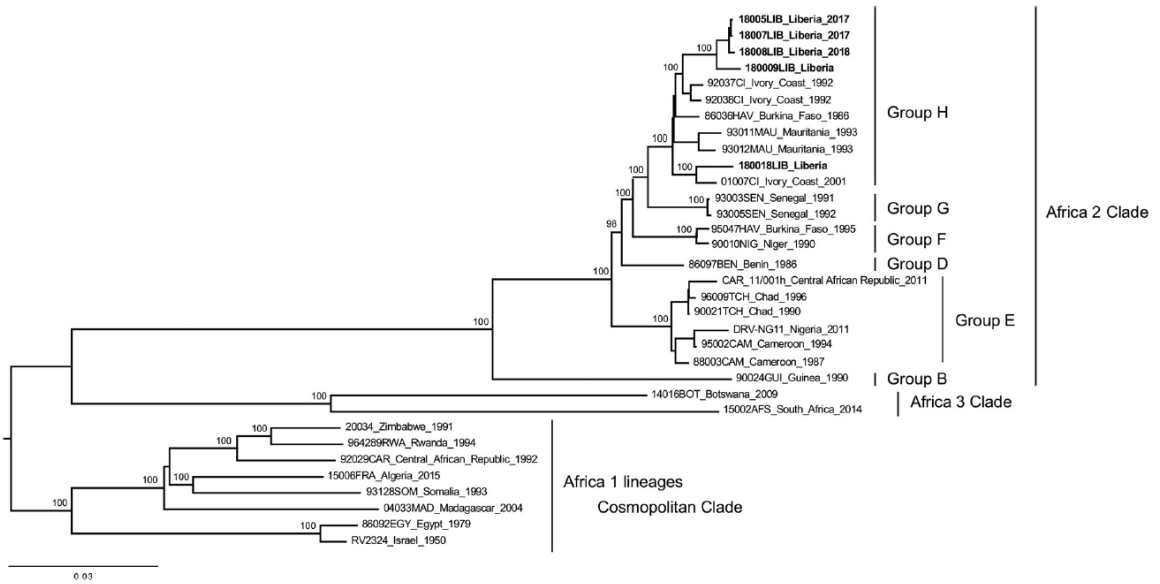

Figure 4: Phylogenetic analysis of the five RABV strains from Liberia and different representative African strains. The tree was based on the nearly complete genome sequences (11,800 to 11,804 nucleotides [nt]) and constructed using the maximum-likelihood approach, based on the generalized time-reversible model proportion of invariable sites plus the gamma-distributed rate heterogeneity (GTR1I1C4), utilizing subtree pruning and regrafting (SPR) branchswapping, as estimated in PhyML version 3.0 (13) with Smart Model Selection (http://www.atgc-montpellier.fr/phyml-sms/). The robustness of individual nodes was estimated using 100 bootstrap replicates. The different phylogenetic clades, lineages, and groups have been previously described (3, 15, 16). Groups A and C were missing from the Africa 2 clade, due to the lack of complete genome sequences available. Only bootstrap values of $90 are indicated. The scale bar indicates the number of nucleotide substitutions per site.

available complete genomes (Fig. 4; Table 2). Identification of the open reading frames was performed using SnapGene software version 5.3.2. The nucleotide identity was determined using Ident and Sim software implemented in the Sequence Manipulation Suite (https://www.bioinformatics.org/sms2/ident_sim.html) (Stothard, 2000). Maximum likelihood (ML) phylogenetic analysis was performed on the nearly complete genome sequences (11,800 to 11,804 nt) of the five RABV strains and different representative African strains using PhyML (S. Guindon et al., 2010), after multiple alignment performed using ClustalW version 2.1 (Larkin et al., 2007) implemented in the Institut Pasteur Galaxy platform (Fabien Mareuil, 2017). The ML phylogenetic tree was visualized using FigTree (http://tree.bio.ed.ac.uk/) (Fig. 4). All protocols were performed according to the manufacturer's instructions, and all tools were run with default parameters, unless otherwise specified.

The genome sequences presented the five canonical genes encoding the nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G), and RNA polymerase (L) (Table 2). The leader and trailer sequences were 58 and 70 nucleotides long, respectively. The transcription initiation (TI) signal AACA and the transcription termination (TTP) TGA7 was observed for all the genes, except for the G gene, which presented the AGA7 motif for TTP. Three sequences presented a premature stop codon in the P gene. The nucleotide identity between four of the genome sequences was high (>99.1%), whereas strain 18018LIB was slightly more divergent (>97.5%). Genetic analysis confirmed that they clustered together in group H within the Africa 2 clade (Talbi et al., 2009; Cécile Troupin et al., 2016; Voupawoe et al., 2021) (Fig. 4).

Data availability. The complete genome sequences of the five rabies viruses from Liberia were deposited at GenBank under the accession numbers OK135144, OK135145, OK135146, OK135147, and OK135148 and the BioProject accession number PRJNA763029.

ACKNOWLEDGMENTS

We thank the dog owners, the county livestock officers, the health surveillance officers, the members of the OH platform, and the laboratory staff for their great commitment. We are grateful to the sequencing facilities of the Plate-forme de microbiologie mutalisée (P2M) of Institut Pasteur (Paris, France) for technical assistance with NGS sequencing. We also acknowledge Lisa Crump for language editing. This work was

funded through the Global Alliance for Vaccines and Immunization (GAVI) (VISLACRV19122014, work stream 3), the Wolfermann Nägeli Foundation, the Swiss African Research Cooperation (SARECO), research funds from Stay on Track of the University of Basel, the Stiftung für wissenschaftliche Forschung (SWF), the Freiwillige Akademische Gesellschaft (FAG), and the Novartis Foundation for Biomedical Research. The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.

4 Preparing Liberia for Rabies control: Human-dog relationship and practices, and vaccination scenarios

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Acta Trop, 2022 May:229:106331. doi: 10.1016/j.actatropica.2022.106331. Epub 2022 Feb 6

4.1 Abstract

To reach zero dog-related human rabies deaths by 2030, Liberia must prioritize rabies as a public health threat. Understanding dog demography parameters are imperative and sets the basis for planning cost-effective and sustainable mass dog vaccination programs nationwide. We conducted a cross-sectional household survey in eleven rural districts of Bong and one urban district of Montserrado County to gather baseline information on the canine population, human-mediated dog movements, people's relationships and practices towards dogs, and further information to estimate costs for a nationwide campaign. In total, 1282 respondents were interviewed (612 rural and 670 urban). About 34% of the rural and 37% of the urban households owned at least one dog. The canine: human ratios were 1:6.1 in the rural and1:5.6 in the urban area and did not differ notably among both counties. The estimated canine population for Liberia is 594,640. The majority of respondents (55%) reported poor waste disposal. Muslims were less likely to own a dog than Christians (39% vs 19% OR: 0.4 95% CI: 0.2-0.6) ($p < 0.001$). Six percent of respondents mentioned that a family member was exposed to a dog bite in the past year, and most victims were adult males. Four of the victims reportedly died after showing rabies compatible symptoms. Twenty-seven percent of dog-owning households in rural areas reported that at least one dog originated from urban areas, and 2% of urban households brought in dogs from another country. In addition, 43% of respondents consumed dog meat at least once. Fifty percent of the respondents claimed knowledge of rabies but only 5.7% and 1.9% mentioned rabies transmission through rabies-infected saliva and rabies-infected mucus on broken skin. Forty percent of the respondents did not know whether rabies was incurable in humans once clinical signs appear. Assuming 30 vaccinators could vaccinate 50 dogs per day for eighteen months (371 working days), the total cost for the vaccination of the national Liberian canine population is estimated at 1.6 million (USD) for one vaccination round.

Our study reveals an overall poor disease knowledge and the potential for spread of rabies in the study areas. A nationwide rabies awareness is crucial to enhance rabies prevention and control through mass dog vaccination.

Key words: rabies, dog, Liberia

4.2 Introduction

Rabies is a deadly viral disease, which causes tens of thousands of deaths each year, mostly in Africa. Per annum, deaths (21'476) are underreported for the continent, and children below the age of 15 are at greater risk (Hampson et al., 2015). Rabies-related deaths are averted by timely administration of post-exposure prophylaxis to bite victims. Despite being 99.9% vaccine-preventable, rabies is still a neglected public health threat in many parts of Sub-Sahara African (Mbilo et al., 2020). The lack of robust rabies surveillance systems, limited diagnostic tools, poor disease awareness, and misdiagnosis contribute to sustained transmission (S. Mauti, Léchenne, M., Mbilo, C., Nel, L., Zinsstag, J., 2019). Dogs are the main reservoir of human rabies, and tackling the disease at its source population is the recommended control strategy (S. Cleaveland et al., 2014).

Rabies control is possible, as the tool needed to control the disease is available. In the past five years we have observed a remarkable international engagement on rabies control. Dr. Margaret Chan, the former director of the World Health Organization (WHO) stated in December 2015 in Geneva, Switzerland: “Let us make rabies history,” (witnessed by Jakob Zinsstag). Subsequently the so called Tripartite, the cooperation of the international organizations, WHO, World Organization for Animal Health (WOAH) and the Food and Agriculture Organization of the United Nations (FAO), joined forces to produce a global strategic plan to end human deaths from dog-mediated rabies by 2030 (World Health Organization, 2018). The Vaccine Alliance decided to include rabies and cholera in its Vaccine Investment Strategy, which was approved in 2018 (VIS 2018).

Although dog mass vaccination can eliminate rabies (Zinsstag et al., 2017) and the cumulative cost of dog mass vaccination with PEP is lower compared to the cumulative cost of PEP alone after ten years (Mindekem et al., 2017), there is yet no similar global engagement for a well-coordinated dog mass vaccination campaign to eliminate rabies in Africa. Despite the laudable efforts of the Pan African Network for Rabies Control (Scott et al., 2015), dog rabies control in Africa remains fragmented with very limited efforts by national governments, which are not to the scale and intensity required for effective elimination. Out of 22 West and Central African countries, only two have reached level two on the so-called Stepwise Approach towards Rabies Elimination ladder, indicating that national governments have truly prioritized rabies elimination.

Overall dog rabies elimination remains stuck due to lacking government commitment and financial constraints (Mbilo et al., 2020). For a country like Liberia, achieving this goal means that the government must prioritize rabies and engage in mass dog vaccination campaigns to interrupt the disease transmission cycle between humans and dogs.

In post-war Liberia, rabies circulates in domestic dogs, and small-scale activities involving the dog population are documented (Voupawoe et al., 2021). Between 2008 and 2012, 488 dog bite cases were registered at several county hospitals, with children below 10 years of age the most affected group (Jomah et al., 2013). In addition, in 2019 and 2020, the National Public Health Institute of Liberia recorded 1’729 and 1’407 animal bites cases including seven clinically confirmed rabies deaths. Of these, the majority of rabies confirmed biting animals were dogs (National rabies report, Ministry of Agriculture). Olarinmoye et al. (2017) applied a decision tree model to human bite data for Monrovia, estimating 155 human rabies deaths annually and high demand for PEP. Despite these alarming statistics, a dog population estimate, which is crucial for planning and implementing dog rabies vaccination programs, is unknow for Liberia (Mauti et al., 2017; Mindekem et al., 2005; WHO, 2018a). About 11’820 domestic animals, mostly dogs, were vaccinated between 2019 and 2021 in an attempt to reduce the burden of rabies in high-risk areas. However, such episodic vaccination campaigns have no lasting effect. Therefore, to prepare Liberia for a comprehensive rabies control strategy, the present study was undertaken to gather baseline information on dog demography, rabies knowledge and vaccination scenarios.

4.3 Material and methods

4.3.1 Study area

The Republic of Liberia is located along the West African Coast, and Monrovia is the capital. Its terrain includes sandy coastal plains, rolling hills, and dissected plateaus further inland. The country borders the Republic of Guinea, the Atlantic Ocean, Cote D'Ivoire, and Sierra Leone to the north, south, east, and west. According to the 2021 population projection, the country has about 5'214'000 inhabitants, of which 48% live in rural areas. Liberia is divided into fifteen counties with a land boundary of 1’667 km. Predominantly, Christianity and Islam comprise 86% and 12%, respectively, of the

total population (LISGIS 2008). (July 2021, estimate) (LISGIS 2008, Central Intelligence Agency, USA, 2018). (LISGIS 2008).

Between April and May of 2019, a survey was carried out in eleven rural districts of Bong County and one urban district of Montserrado County. Bong, mostly rural, is headed by a superintendent and is located in the north-central portion of Liberia. The county is predominately inhabited by the Kpelle ethnic group who are mostly subsistence farmers. Bong is divided into districts, clans, villages and covers an area of 8'700 km^2. Montserrado, on the other hand, is located in the northwest of Liberia and has four districts covering 1'900 km^2. Greater Monrovia District (GMD), which includes the capital, is the only urban setting in Montserrado. The district is densely populated and includes all the sixteen ethnic groups of Liberia. GMD is subdivided into zones, quarters, and communities.

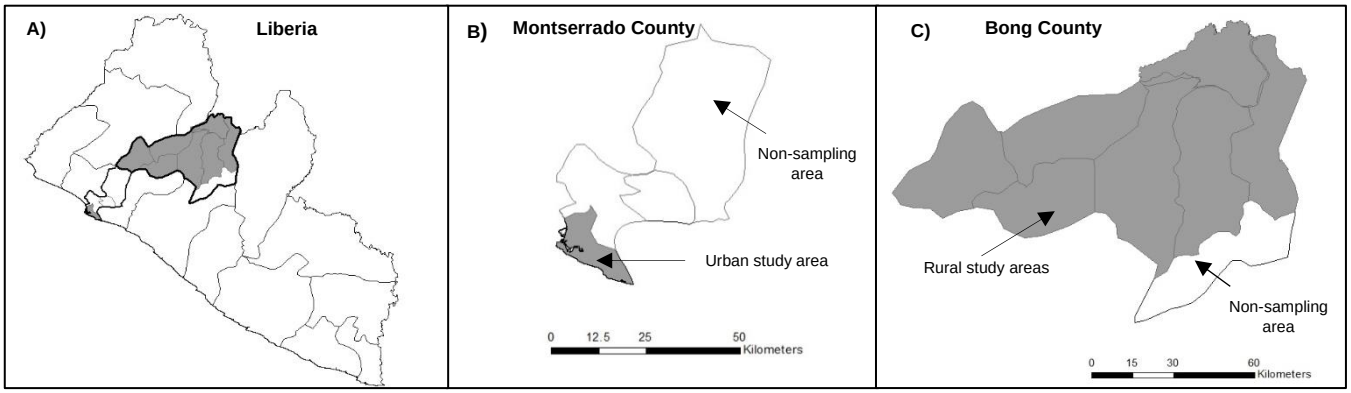

Figure 5: Maps showing Liberia A), urban study area, Montserrado County B), and rural study areas, Bong County

4.4 Sample size calculation and sampling procedure

Sample size determination was done separately for urban and rural settings. We assume an intra cluster correlation coefficient of 0.2 and a prevalence of rabies knowledge of 30% in rural areas. We aimed to enroll 65 clusters with 10 households each to estimate the population parameter with a precision – defined as one half length of the confidence interval – of 6 percentage-points. Because this sample size was believed to be too high to be realized in both settings, we aimed in the urban area for a minimum sample size of 20 clusters with 12 households each, which would roughly translate into a precision of 10 percentage-points given the same set of assumptions. Fortunately, the time allocated for the urban survey could be extended that almost the

same number of interviews could be realized in the urban area (56 clusters with 12 households).

Different sampling approaches were used to avoid sampling bias in the rural and urban areas. To avoid bad road conditions during the rainy season, we first carried out the study in the rural area.

In the rural area, a multi-stage sampling approach with sampling probability proportional to the size (PPS) was used. At stage one of sampling, eleven districts were randomly selected by PPS of the human population at stage two of the sampling, one clan per district (in total, 11 clans) was randomly selected by PPS of the human population. Because of the lack of data on population at the village level, a simple random sampling approach was used to select the interview households.

Because of the incomplete household registries in the urban setting, a random geo-coordinate sampling procedure, as recommended by Schelling and Hattendorf (2015), was utilized. Two hundred fifty random geo-coordinates, which served as interview starting points for the survey team, were generated. The points were created using R software version 3.3.1 and exported as a kml file to Google Earth software version 7.1.2.2041. Points lying outside the study area were discarded, and new points were generated to obtain the required geo-coordinates. The starting points were identified with the aid of the GPS. A fishbowl draw was carried out by listing the four nearest households directly north, south, east, and west of the starting point. One draw was made, and the selected household was chosen for the first interview. Then moving in a counter-clockwise direction, every third household was selected, until 12 households were interviewed.

4.5 Data collection

In May 2019, a cross-sectional household survey was conducted in eleven rural districts of Bong and one urban district of Montserrado. A structured questionnaire was developed together with expert rabies researchers. The questionnaire was conducted in English. The first section contained questions on household demographic characteristics, and the second section obtained information about the dog population, bite incidents in households and respondent's knowledge about rabies. A household was defined as an individual or group of related or unrelated persons sharing the same housing. The questionnaire was administered to household head or a member of the family above 18 years of age.

For the rural study area, eight interviewers (gender-balanced) were recruited from the Ministry of Agriculture, the University of Liberia, the Cuttington University, and the Central Agriculture Research Institute (CARI). Recruits residing in Montserrado were transported to join the rest of the team in Bong. A one-day training course including the scope of the study and about the questionnaire was held at the 101 Conference Center in CARI, Bong County. The questionnaire was pre-tested in households similar to the study population, and some questions were revised for better understanding. Four teams were drawn, with each team consisting of an interviewer and a supervisor. The farthest distance covered in one day was 52 km. Teams were randomly assigned sampling villages and a community entry approach where village authorities provided informed consent before the study was carried out. When household listing was not available, the village was divided into equal segments, and a quick census was conducted. A household was chosen as a starting point, then the next household was selected.

For the urban study area, four of the previous interviewers were replaced, and the replacement (same institution as for the rural area) were trained in a one-day course at the Ministry of Agriculture, Monrovia. The questionnaire was once again validated in the field and revised before the survey. Initially, the communities were identified by plotting the starting points into a Google software. In the community, the teams then located the starting points with the aid of the GPS. A community entry approach was used, and sampling houses were selected in a counter-clock movement until ten interviews were conducted.

We randomly selected eight Zones in GMD by probability proportional to the size of the human population in urban area. Two hundred fifty random geo-coordinates were then generated as starting points (SP) using R software and overlaid on a Google Earth map of the study areas containing the eight Zones. Coordinates that fell outside the selected study areas were discarded, and a new coordinate was generated. A total of 20 starting points were generated by the following proportion, Caldwell = 1 SP, Central Monrovia a = 2 SP, Central Monrovia b = 1 SP, Clara Town = 2 SP, Gardnerville = 4 SP, Logan Town = 1 SP, Paynesville = 7 SP, and Sinkor = 2 SP. We located each SP with a Global Positioning System (GPS). A fishbowl draw was carried out by listing the four nearby households directly in the north, south, east, and west directions of the SP. One draw was made, and the selected household was chosen

for the first interview. Then moving in a counter-clock direction, every third household, until 12 households were interviewed. If a person refused to participate in the survey or if there was nobody in the selected household, the next home in the sampling direction was sampled.

4.6 Preparation of country-wide vaccination campaign

Vaccination activities must be localized in the fifteen counties of Liberia. The established One Health collaboration between the public health and veterinary sector at the county level can provide the necessary logistics and human resources needed to conduct the campaign. Staff and vaccinators needed for the campaign can be recruited and trained from each county. Between 2019 and 2021, OIE provided 10’000 dosages of vaccines for a small-scale nationwide rabies vaccination campaign (Nation Rabies Report, MOA).

Information about the nationwide campaign can be disseminated through posters displayed in public places, flyers given to dog owners, radio broadcasts, and megaphone announcements in communities. Providing precise information to the public reduces unnecessary inquiry, thus reducing campaign costs. Radio announcements can be broadcast in local vernaculars for a better understanding of the campaign. Before a campaign begins in any county, a One Health stakeholder meeting should be held with superintendents of the fifteen (15) counties. The superintendents and members of the local government should further disseminate the information about the campaign to local authorities for ease of operation. Then an SMS can be sent to remind the public about the campaign dates.

When planning a mass dog vaccination, it is essential to consider seasonal variation. Because of bad road conditions, remote villages and most of the country's southeastern regions are inaccessible during the rainy season (May to October). Reports about mass dog vaccination (World Rabies Day celebration) in Monrovia show that a vaccinator can vaccinate between 50 and 60 dogs per day. As such, we assume that a vaccinator, on average, can vaccinate 50 dogs per day for a nationwide campaign.

Annually, an estimated (0.3%) of the total dog population is vaccinated in Liberia (Personal information, Garmie Voupawoe, 2021). We present three scenarios to assess how different cost items impact the overall costs of a countrywide campaign aiming at (70%) of the total dog population (**Table 3**). Scenario 1: 30 vaccinators, 50

dogs vaccinated per vaccinator per day for 18 months (371 working days), resulting in an estimated 556'500 dogs vaccinated for one round. Fifteen motorbikes and two cars with drivers and supervisors coordinate the team and supply the vaccination points with materials.

4.7 Costs analysis

The equipment, consumables, and personnel costs are referenced from the 2020 vaccination campaigns conducted in Monrovia. Analogous to (Anyiam et al., 2017), we break up the campaign costs into public and private costs. The cost analzses are based on the Global Dog Rabies Elimination Pathway (GDREP) tool.

Public costs = Marginal vaccination cost + equipment of vaccination post (VP) + Staff allowance + Information + Transportation.

Private costs = Lost work time + Transportation.

Societal costs = Public costs + private costs

Public cost: One syringe and needle, one vaccine dose, and one vaccination certificate are marginal vaccination costs per dog. Local importers provide quotes for items (equipment of VP) available in Liberia, unavailable items are imported. As required, staff handling dogs are protected from rabies exposure with pre-exposure prophylaxis. All persons working with the vaccination campaign receive a daily payment.

Information and transportation costs: The nationwide campaign includes participation in radio talk shows, electronic media, and print media coverage. Campaign announcements are aired twice a week on national and local radio stations. For the entire campaign, car and motorbike travel is estimated 6’587 with total fuel consumption of 4’012 litres.

Private costs include salary loss due to lost work time and transportation costs for taking an animal to the VP. These as the VP will be stationed within the communities and that non-working household members will be responsible for taking an animal for vaccination. The average per capita income in Liberia is 71’800 LRD (418 USD) per month, i.e., or 410 LRD (2.39 USD) per hour. If it takes 90 mins on average to vaccinate one dog, then a dog owner cost of the lost work time can be equivalent to the time used to inoculate a dog, i.e., 615 LRD.

Societal cost: The sum of the public cost plus the private cost will equal the societal cost of mass rabies vaccination. The only cost for a dog owner will be to bring the animal to the vaccination post.

Table 3: Cost of the national-wide campaign

Item	Unit	Working days	Average cost
Dog population	594’640	N/A	N/A
Vaccines (10% wastage)	654’104	N/A	$0.91
Syringes and needles (10% wastage)	654’104	N/A	$0.05
Vaccination certificates	594’640	N/A	$0.13
Dog marking	594’640	N/A	$0.05
Supervisor (training)	1	5	$55.00
Supervisors (Vaccination)	6	371	$45.00
Supervisor(information)	1	371	$42.50
Driver	2	371	$38.00
Vaccinators (training)	30	5	$12.50
Vaccinator (vaccination)	30	371	$22.50
Veterinarians	2	371	$45.00
Community health worker	30	371	$13.00
Delegate of the Ministry of agriculture	2	1	$92.50
Superintendent of counties	15	1	$55.00
Human vaccine	70	N/A	$85.00
Tables	0	N/A	$0.00
Chairs	0	N/A	$0.00
megaphones	15	N/A	$65.00
First-aid	20	N/A	$52.00
Cooling Element	100	N/A	$2.50
Cooling box	17	N/A	$5.00
Muzzles	30	N/A	$22.00
Gloves	100	N/A	$35.00
Radio talk show	15	3	$310.00
Electronic media coverage	5	3	$80.00
Print media coverage	3	1	$80.00
communication cards	50	1	$10.00
Documentary	15	15	$35.00
Banner	15	N/A	$155.00
Flyers	594’640	N/A	$0.10
T-shirt and caps	30	N/A	$18.00
Vehicle	2	1	$2’500.00
Gasoline	1’060	0	$3.00
Motorcycle	15	1	$3’000.00

All costs are in United States Dollars (Total average cost: $ 1’645’799)

4.8 Ethics

Table 4: Scenarios used for the cost analysis

	Scenario 1	Scenario 2	Scenario 3
Vaccinators available	30	20	20
Dogs vaccinated per vaccinator per day (rate)	50	50	50
Campaign vaccination in months	18	24	36

Authorization to carry out the study in Liberia was obtained from the University of Liberia review board (UL-PIRE IRB, protocol #: 19-02-157), and approval for the study was obtained from the Cantons of Basel's ethical review board in Switzerland (Ethikkommission Nordwest-und Zentralschweiz, EKNZ BASEC req-2017-00495). Verbal or written consent was obtained before interview was conducted.

4.9 Data entry and analysis

Data were stored on the ODK server during the survey and downloaded in Excel (Microsoft Office™) format and analyzed using STATA version 15.1. All maps were produced in ArcMap™ 10.6.1. Associations with independent variables (sex, age, religion, occupation, ethnic group, dog ownership, and rabies knowledge) were analyzed using univariable and multivariable analysis. Rabies knowledge was assessed in five categories: rabies symptoms in humans and dogs, transmission, prevention, human treatment and dog vaccination. The proportion of correctly answered questions was calculated separately in each category and summed up, resulting in a total score ranging between 0-5 (was created) during the data analysis. Afterwards, the knowledge score was categorized into low and high rabies knowledge based on the visual inspection of the distribution of the scores.

For binary outcome variables, rabies awareness and, dog ownership, generalized estimating equations for binomial distribution outcomes and logit link functions were used. Estimates are presented as odds ratios (Adjusted OR) with the corresponding 95% confidence interval.

GEE logistic regression models were employed to account for potential correlation within clusters. Predictors were included in the multivariable model based on plausibility. Two-sided p-values under 0.05 were considered statistically significant.

Costs-data of the vaccination campaign were obtained from government sources and the 2020 World Rabies Day small-scale vaccination campaign in Liberia.

Costs analysis was derived using the GDREP, which is a Microsoft® Excel-based tool – developed by the Centers for Disease Control and Prevention (US CDC).

4.10 Results

4.10.1 Sample size and participants demographics

Respondents were, in total, 1282, with 612 rural and 670 urban dwellers. Overall, the number of inhabitants in the interviewed households was 10240, with a median household size of 7 (IQR:5). Male (66%) and age groups (38 – 47) and (48 years and above) represented the highest proportion of respondents. Christianity (83%) and Islam (8%) were the predominant religious group.

4.10.2 Dog demography and ownership

Dogs totalled 809 dogs in the 209 rural households and 949 dogs in the 251 urban households. Overall, (36%) of households owned at least one dog. Table 5 shows the demography and ownership of the dog population in the rural and urban areas. When considering the 10240 individuals recorded during the survey, the overall dog: human ratio was 1:6 (95% CI: 0.140-0.197). An estimate of owned dogs for the rural and urban areas based on an extrapolation of the overall dog: human ratio predicted 594’640 owned dogs.

Dog ownership was associated with religion, with much fewer dogs owned by Muslims (39% vs 19% OR: 0.4 95% CI: 0.2-0.6) ($p< 0.001$). Table 6 indicates factors associated with dog ownership. About 7% of respondents reported seeing an ownerless dog in their neighborhood, while 30% fed them. Mostly, fathers were the owners of dogs, and dogs were kept as house guards and often fed with household leftovers.

When respondents were asked about their perception of dog meat consumption, (34%) stated that it was a good practice, and almost half of respondents (43%) said they consume dog meat at least once or twice. When further asked if they knew someone who consumes dog meat, the majority (71%) said yes.

4.10.3 Bite incidents and health-seeking

Six percent of rural and five percent of urban respondents had at least one family member exposed to an animal bite in the last 12 months. Out of 74 bite victims, male adults aged 16 years and above were the most affected in rural and urban settings. All biting animals were dogs, and the majority were neighbor-owned. Forty-three (23 rural and 20 urban) of the bitten victims washed the wound with water and soap, while the majority sought further

Treatment at a nearby hospital. Only 4% of rural dwellers received free post-exposure prophylaxis at a health facility. Four bite victims (3 rural and one urban) were reported dead, and one rural respondent mentioned that clinical rabies symptoms were diagnosed before death.

Table 5: Dog demography in rural and urban areas

	Rural (Bong)	Urban (Montserrado)
Dog-owning households	(209/612)	(251/670)
Number of owned dogs	809	949
Number of persons	4’743	5’497
Dog/person	0.166	0.179
Canine: human ratio	01:06.1	01:05.6
Estimated dog population	594’640	
Sex distribution		
Male	342	422
Female	467	527
Sex ratio	0.7:1	0.8:1
Age distribution		
(0-3) Months	155	285
(4-11) Months	300	247
(1-7) Years	283	291
(>7) Years	71	126

Table 6: Factors associated with dog ownership

Characteristics	N	Dog owner %(n)	OR(95%CI) p-value	adjOR(95%CI) p-value
Residence				
Rural	612	34% (209)	reference	reference
Urban	670	37% (251)	1.2 (0.9-1.6) 0.341	1.1(0.8-1.6) 0.572
Sex				
Female	434	36% (155)	reference	reference
Male	848	36% (305)	1.0 (0.8-1.3) 0.923	1.0(0.8-1.2) 0.873

Age				
18-27	64	28% (18)	reference	reference
28-37	247	35% (86)	1.4 (0.8-2.3) 0.248	1.3(0.7-2.2) 0.375
38-47	425	38% (161)	1.6 (0.9-2.6) 0.085	1.4(0.8-2.4) 0.187
> 48	546	38% (195)	1.4 (0.8-2.4) 0.196	1.3(0.8-2.2) 0.339
Religion				
Christian	1058	39% (410)	reference	reference
Muslim	101	19% (19)	0.4 (0.2-0.6) <**0.001**	0.4(0.2-0.6) **<0.001**
Others*	123	25% (31)	0.5 (0.3-09) 0.01	0.6(0.3-0.9) 0.025
Occupation				
Private sector	506	36% (181)	reference	reference
Public Sector	139	45% (63)	1.5 (1.1-2.1) 0.025	1.5(1.0-2.1) 0.044
Farmer	423	34% (142)	0.9 (0.7-1.2) 0.535	1.0(0.7-1.4) 0.892
Unemployed	214	35% (74)	0.9 (0.7-1.3) 0.74	0.9(0.7-1.2) 0.58

4.11 Bite incidents and health-seeking

Six percent of rural and five percent of urban respondents had at least one family member exposed to an animal bite in the last 12 months. Out of 74 bite victims, male adults aged 16 years and above were the most affected in rural and urban settings. All biting animals were dogs, and the majority were neighbor-owned. Forty-three (23 rural and 20 urban) of the bitten victims washed the wound with water and soap, while the majority sought further treatment at a nearby hospital. Only 4% of rural dwellers received free post-exposure prophylaxis at a health facility. Four bite victims (3 rural and one urban) were reported dead, and one rural respondent mentioned that clinical rabies symptoms were diagnosed before death.

4.12 Household waste management

We questioned respondents about household waste disposal. Overall, (55%) and (43%) disposed of waste in the backyard and public dumpsite, respectively. The majority of households never had alternative means of waste disposal, and a household was 20 meters (57%), 40 meters (27%), and more than 50 meters (16%) from a garbage site.

4.13 Human-related movement of dogs.

We classified the direction of human-mediated movement as immigration when dogs moved into a location of interest, and emigration when dogs left a location of interest. One hundred eighty-five urban dogs were imported to 75% of rural dog owning-households, and 325 rural dogs were imported to 16% of urban dog owning-households. Five hundred thirty-one dogs were locally obtained from villages within Bong County alone. Fewer dogs were imported from other rural areas to rural Bong County than from urban area. It was observed that no dog was brought from Nimba, RiverCess, Maryland, and RiverGee Counties to the rural study areas. Thus, the urban areas, Montserrado, was the important source of dogs to the rural study area.Only (2%) of households reported bringing dogs from another country.

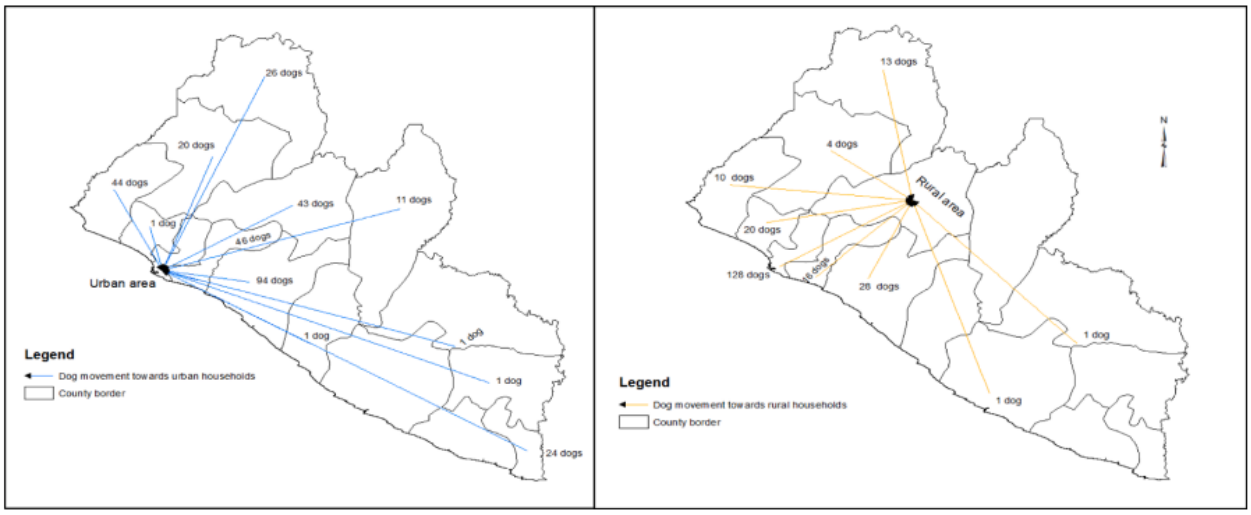

Figure 6: Diagram of human-mediated dog movements within the study areas

4.14 Rabies knowledge in rural and urban settings

In our study, about (45%) of respondents claimed to have prior knowledge of rabies. However, rabies knowledge was higher among urban respondents than rural respondents (57% vs 43%) respectively (Table 6). The source of rabies knowledge was acquired from five sources: A friend (32%), radio (30%), health worker (12%), tv (0.47%), and newspaper (2%).

Rural and urban respondents were assessed about their knowledge of rabies. More than half (53%) of the respondents mentioned dog bite as a mode of rabies

transmission, but only (5.7%) and (1.9%) mentioned rabies-infected saliva and rabies-infected mucus on broken skin, respectively. When asked about who is susceptible to rabies (all mammal, Human, Monkey, Cat, Bat, and dog), almost half (48%) of the respondents named Human as being susceptible, followed by all mammals (10%). 40% of the respondents did not know whether rabies was incurable in humans once clinical signs appear. When further questioned about dog vaccination, more than half (55%) responded yes, a dog could be vaccinated against rabies.

4.15 Factors associated with rabies knowledge

The mean score of assessed rabies knowledge was 0.7 (SD:0.5), and a minimum and maximum score of 0 and 2.9, respectively. Based on the range, a cut-off points of >=0.8 was used to delineate two categories of rabies knowledge (poor and good). Only 45% of respondents had good rabies knowledge, with roughly similar proportion of rabies knowledge in rural and urban settings. Overall, none of the observed variables were important predictor of rabies knowledge (47% vs 43%) and male and female respondents (47% vs 44%). Surprisingly, there was also no noteworthy difference between dog owners (47%) and non-dog households (44%). Farmers had with 42% a significantly lower proportion of good knowledge compared to the 47% from respondents of the private sector (adjusted OR: 0.6, 95% CI: 0.4-0.8, p = 0.002). In the self-reported bite history group, 54% persons had a high knowledge score compared to 44% in the no-bite history group. However, the number of bitten persons were relatively small; therefore, the differences were not statistically significant in the multivariable analysis (adjusted OR: 1.5, 95% CI: 0.9-2.5, p = 0.71). Christians had a higher proportion of respondents with good knowledge (47%) compared to Muslims 34%) or other religions (40%); although the differences were not statistically significant.

Table 7: Factors associated with rabies knowledge

Characteristics	N	Good knowledge % (n)	OR(95%CI) p-value	adjOR(95%CI) p-value
Residence				
Rural	612	47% (286)	reference	reference
Urban	670	43% (289)	0.9 (0.6-1.3) 0.492	0.7 (0.4-1.1) 0.165
Sex				
Female	434	47% (203)	reference	reference
Male	848	44% (372)	0.9 (0.7-1.1) 0.352	0.9 (0.7-1.2) 0.378
Age				

18-27	64	45% (29)	reference	reference
28-37	247	55% (132)	1.4 (0.8-2.3) 0.201	1.4 (0.8-2.4) 0.191
38-47	425	43% (181)	0.9 (0.6-1.4) 0.633	0.9 (0.5-1.4) 0.579
> 48	546	43% (233)	0.9 (0.6-1.5) 0.661	0.9 (0.5-1.5) 0.636
Religion				
Christian	1058	47% (492)	reference	reference
Muslim	101	34% (34)	0.6 (0.4-0.9) **0.030**	0.6 (0.4-1.0) 0.072
Others*	123	40% (49)	0.8 (0.4-1.3) 0.322	0.8 (0.5-1.4) 0.488
Ethnic group				
Kpelleh	647	48% (309)	reference	reference
Other tribe	635	42% (266)	0.8 (0.6-1.1) 0.173	0.8 (0.6-1.1) 0.234
Foreign	24	29% (7)	0.5 (0.2 - 1.0) **0.054**	0.6 (0.2-1.4) 0.219
Occupation				
Private sector	506	47% (240)	reference	reference
Public Sector	139	50% (69)	1.1 (0.8-1.6) 0.641	1.1 (0.7-1.6) 0.693
Farmer	423	42% (179)	0.8 (0.5-1.2) 0.314	0.6 (0.4-0.8) **0.002**
Unemployed	214	41% (87)	0.8 (0.6-1.0) 0.083	0.7 (0.5-1.0) 0.07
Dog ownership				
No	822	44% (361)	reference	reference
Yes	460	47% (214)	1.1 (0.9-1.4) 0.407	1.0 (0.8-1.4) 0.723
Bite history				
No	1208	44% (535)	reference	reference
Yes	74	54% (40)	1.5 (0.9-2.4) 0.125	1.5 (0.9-2.5) 0.713
Dog Consumption				
Yes	554	47% (261)	Reference	
No	703	44% (308)	0.9 (0.7-1.1) 0.265	1.0 (0.8-1.2) 0.841
No response	25	24% (6)	0.4 (0.2- 0.8) **0.012**	0.4 (0.2-0.9) 0.032

Others: Bassa, Kru, Gio, Lorma, Kissi, Gbandi, Grobo

4.16 Campaign and scenario costs

We calculated the cost of the nationwide vaccination campaign based on the estimated dog population of Liberia (594’640), the number of vaccinators, the number of dogs vaccinated per vaccinator per day, and the duration of the vaccination campaign in working days. The average cost of the three scenarios is between 1.6 and 2 million USD for one round of vaccination campaign, i.e., 371 and 743 working days of Liberia. Considering scenario 1 (371 working days): the proportion allocated to the Marginal cost = $ 732’002; equipment of VP cost = $ 11’912; Staff allowance = $ 503’454; information = $ 344’302; and transportation = $ 54’130 United States Dollars. **Table 3**. shows the breakdown of the vaccination costs.

4.17 Discussion

4.17.1 Dog population

Tackling rabies in the host species through mass dog vaccination is a cost-efficient and optimal strategy to prevent dog-related human rabies burden (S. Cleaveland et al., 2014; Hampson et al., 2015). Critically, for rabies intervention, knowledge of the dog population structure plays a key role for planned nationwide vaccination campaigns, and community rabies awareness. To the best of our knowledge, published literature on dog population structure for Liberia is lacking. In this study, an average of (36%) of all households owned dogs, where (33%) of rural households and (33%) of urban households kept dog. These findings are in a medium range for the proportion of households owning dogs, when compared with similar studies conducted in Africa. For example, in Chad (Mbilo et al., 2017) reported that 45% of all households own dogs. The proportion of households owning dogs were 81% in Kenya, (Kitala et al., 2001); 28% in N'Djamena, (Mindekem, Kayali, Yemadji, Ndoutamia, & Zinsstag, 2005); and 13% and in Tanzania, (Gsell, Knobel, Kazwala, Vounatsou, & Zinsstag, 2012). The observed dog: human ratio 1:6.1 in rural area and 1:5.6 in urban area is comparable to studies including, Nigeria 1:3.5-5.7 (Hambolu et al., 2014). Considering the dog: human ratio, we predicted the owned dog population of the rural and urban areas at 594'640 dogs. This finding suggests large financial investment, in terms of vaccine doses and human resources needed to achieve the 70% vaccination coverage, for implementing mass dog vaccination campaigns. However, these financial costs for Liberia are in a medium range when considering higher dog populations in other parts of Africa. Muslims were less likely to own a dog than Christian. This findings are consistent with findings in Mali (S. Mauti et al., 2017) and Chad (Mindekem et al., 2005) but not with Ethiopia (Tschopp, Bekele, & Aseffa, 2016). In our study, (55%) and (43%) of respondents disposed of waste in the backyard and public dumpsite, and the majority of households (57%) were 20 meters away from garbage sites. This implies that free-roaming dogs, the main contributor to spreading rabies (Morters et al., 2014), have access to people. The lack of proper waste disposal plays a key role in dog population growth (Wright, Subedi, Pantha, Acharya, & Nel, 2021). One of the most surprising findings of dog-keeping practices in our study was the self-reporting of dog meat consumption among participants in rural and urban settings. A fairly high proportion of respondents consumed dog meat, and dog

butchers reportedly were at risk of rabies exposure. Several plausible explanations exist for this finding. One factor could be the limited knowledge of dogs as the main transmitter of rabies. Also, dog meat consumption undermines the number of suspected rabies samples reaching the laboratory for analysis. Although this study did not gather information on dog slaughtering, it is reasonable to assume that the practice is privately done and exposes consumers to the disease risk.

4.17.2 Waste management

In our study, (55%) and (43%) of respondents disposed of waste in the backyard and public dumpsite, and the majority of households (57%) were 20 meters away from garbage sites. This implies that free-roaming dogs, the main contributor to spreading rabies (Morters et al., 2014), have access to people. The lack of proper waste disposal plays a key role in dog population growth (Wright et al., 2021).

4.17.3 Bite incidents and health-seeking

All victims in our study were bitten by dogs. A proportion of 6% bite victims were mostly male. This is far less than findings in(Ngugi, Maza, Omolo, & Obonyo, 2018; Tenzin et al., 2012) and slightly higher than other Africa countries, such as the 5% in Tanzania (Sambo et al., 2014) and 85% observed in Nigeria and Guinea, respectively. Contrary to many studies, most victims in our study were adults above 16 years of age. This finding highlights serious underreporting of bite cases among children in rabies endemic regions. Because of the shorter stature of children, they are at higher risk of rabies exposure, and it is likely that they do not report bite cases to caregivers. Dog bites represent a considerable problem, with the high death rate and cost of PEP, in rabies-endemic regions.

4.17.4 Human-related dog movements

This study found that human-related dog movement might have influenced the population dynamics of dogs in rural and urban settings (Morters et al., 2014; Villatoro, Sepúlveda, Stowhas, & Silva-Rodríguez, 2016). Although a high proportion (84%) of dog-owning households locally obtained dogs in urban study areas, the number of dogs moving from other rural parts of the country to the urban study areas were three times higher than dogs moving in the opposite direction. Maybe rural-urban migration or vice-versa could be a possible explanation. Dogs brought into a given location (human-related dog movements) could be a potential source of emerging or re-

emerging rabies. In the capital of Chad, N'Djamena, (Laager et al., 2019) reported that rabies was sooner reintroduced, import of new dogs, than predicted into the city after two consecutive mass dog vaccination campaigns. On the other hand, dogs were not reported to have come from the three neighbouring countries, i.e., Sierra Leone, Guinea, and Ivory Coast, although close to localities where dogs were reported to have come from (**Fig. 6**). This could suggest that the dog movement between the Mano River Union (MRU) Countries is infrequent, rendering the proportion of foreign dogs in Liberia negligible. Our findings are in line with (Villatoro et al., 2016).

4.17.5 Rabies knowledge and factor associated with rabies knowledge

Contrary to our rather low prior rabies knowledge (45%) among the study participants, (Mbilo et al., 2017; Sambo et al., 2014) reported that most respondents had heard about rabies. In addition to the low rabies knowledge, only a few respondents could mention other means of rabies transmission (rabies-infected saliva or mucus on broken skin). Most respondents could not state whether other species can transmit rabies, and only a few respondents mentioned that rabies was incurable once clinical signs appeared. Our findings suggest that more awareness about rabies is required in both study areas. This report is consistent with (Sambo et al., 2014). Health-seeking behaviour. (6%) of respondents reported that a family member was exposed to a dog bite in the past 12 months (6% reported dead) and (70%) of the biting dogs were neighbour-owned. In our study, most bite victims were male adults. This report is not in line with studies conducted in Chad (Frey et al., 2013; Mbilo et al., 2017), where children were reported as the most affected group. The majority of bite victims (58%) mentioned wound washing as first aid. (82%) and (4%) of the bite victim seek further treatment at a hospital/clinic and with a traditional healer, respectively. Respondents reported that vaccine (45%) was paid for, while (36%) mentioned that the vaccine was not available at the health facilities during their visit.

There was no factor influencing good rabies knowledge in all the study areas, implying that all population sectors are at higher risk of rabies exposure regardless of location. The results also suggest a crucial need to increase awareness about the disease in all sectors. These findings are inconsistent with (Ameh et al., 2014; Ntampaka, Nyaga, Niragire, Gathumbi, & Tukei, 2019).

4.17.6 Campaign and scenarios costs

This study derived the nationwide vaccination costs (three scenarios) based on the 2020 budget of the World Rabies Day (WRD) activities and the number of dogs vaccinated during the WRD campaign in Liberia. The economic costs of the campaign were broken down into worker per diem, transportation cost, awareness coverage, and equipment for the vaccination points. Our costs estimate is similar to (Anyiam et al., 2017). In our study, we estimated the total cost of the nationwide vaccination campaign to range between 1.6 and 2 million United States Dollars for one round of vaccination campaign. This amount is a minimum cost to vaccinate the targeted dog population in Liberia compared to reports of (Anyiam et al., 2017; Hatch et al., 2017). Considering that workers allowance, which is inflated by working days, consumes a greater proportion of the budget, suggests scenario 1 (**Table 4**) is less costly than scenario 3. Referring to the development impact bond rabies elimination study for Chad (Anyiam et al., 2017), which considers a total period of ten years and approximately 3.5 full rounds of dog vaccination, we can consider a total cost of 5.6 million USD sufficient to eliminate rabies from Liberia. This amount is relatively low and should be possible to mobilize within concurrent international development cooperation, of course with all the collateral organizational need as described in the above study by Anyam and colleagues. This paper does contribute to the planning of a well-coordinated pan African dog rabies elimination campaigns as envisioned by Mauti et al. (2019).

4.18 Conclusion

A canine mass vaccination campaign is a cost-effective strategy of achieving zero human-related rabies death. Our reports of dog population parameters (canine: human ratios and vaccination scenarios) establish the basis for a countrywide vaccination campaign in Liberia. In addition, our report shows that rabies knowledge is low among participants, and it is crucial to design awareness strategies to increase people’s understanding of rabies.

5 Genome-based phylogenetic analysis of rabies virus circulating in Liberia

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5.1 Abstract

Rabies remains a neglected and endemic disease in nearly all the African countries, including Liberia. Despite recent efforts conducted in this country, veterinary workforce is still understaffed, thus limits an effective response to rabies situation. More particularly, data regarding the rabies virus (RABV) epidemiology present in this country remain scarce. However, this is an essential element in the fight against this zoonosis, in order to be able to adapt surveillance and vaccination programs to the situation. In this context, the aim of this study was to perform a genome-based phylogenetic analysis of RABV isolates circulating in dog, the main reservoir of human rabies worldwide. Between September 2017 and January 2022 and under the One Health Platform of Liberia to coordinate joint rabies activities, a total of 104 samples were obtained from the 15 counties of Liberia and biologically confirmed for rabies by reference techniques locally and in an external laboratory partner. Based on the implementation of a combined next generation sequencing approach (Illumina and Nanopore technologies) developed in this study, 46 complete or nearly complete RABV genome sequences were obtained. Phylogenetic analysis performed on this dataset, in addition to other African representative sequences already available, demonstrated that RABV isolates circulating in Liberia were clustered together into the phylogroup H within the Africa 2 clade. This information represents key pieces of information in future rabies control and eradication programs in Liberia, which CVL demonstrated its monitoring and diagnostic capabilities through this study.

Keywords: Rabies, lyssavirus, Liberia, dog, diagnosis, complete genome, phylogeny, Africa 2 lineage, next generation sequencing, Nanopore sequencing, RIDT

5.2 Introduction

Rabies still claims thousands of lives each year in resource-limited countries despite the available knowledge and tools for its elimination. An estimated 3 million people are at risk of the disease worldwide, and about 59'000 deaths, mostly children in rural communities in Asian or African low- and middle-income countries, are estimated per annum (Hampson et al., 2015). Dog remain the main reservoir and vector of this zoonotic disease, being responsible for mostly all the cases of human rabies. However, other animal species among the Carnivora and the Chiroptera orders can also act as reservoir and vector of the disease, but with a lower public health impact. Rabies is a fatal form of acute viral encephalomyelitis which can affect all mammals (including human) after its transmission by the saliva of an infected animal through bites, scratches or licking of wounds or mucous (WHO, 2018a). Although the disease poses a serious public health burden, it is neglected and often a low priority due to fragile surveillance systems to report the actual disease burden.

Rabies virus (RABV) is the main etiological agent of the disease, and belongs to the *Rabies lyssavirus* species, one of the 17 officially recognized species within the *Lyssavirus* genus, *Rhabdoviridae* family (Walker et al., 2018) (https://ictv.global/report/chapter/rhabdoviridae/rhabdoviridae/lyssavirus). This virus is characterized by a single-strand negative-sense RNA genome encompassing five genes, with N, P, M, G, and L coding for the nucleoprotein, the phosphoprotein, the matrix protein, the glycoprotein and the polymerase, respectively (Fooks et al., 2017). The genetic diversity of RABV is large and mainly conditioned by the geographical origin and the associated animal reservoir. To date, 6 main phylogenetic clades of RABV are circuling worldwide, with 3 found in Africa, one of the most affected regions for rabies, with the Africa 2 and Africa 3 clades, as well as the Cosmopolitan clade with the Africa 4 and the Africa 1 lineages (and its numerous sublineages for the latter) (Talbi et al., 2009; Cécile Troupin et al., 2016). However, the precise genetic diversity of the RABV strains circulating in this continent, especially at the country level, is largely unknown, such as in Liberia, while it is vital to monitor rabies hotspots and distribution in domestic and wild hosts species

Mainly due to the lack of robust rabies surveillance and accurate diagnosis, the epidemiological situation of rabies in this West African country is largely unknown (Mbilo et al., 2021; Voupawoe et al., 2021). This means that current data on circulating

rabies strains are insufficient and do not provide a vivid picture of the disease situation in the country. Although rabies surveillance has improved over the years, Liberia's veterinary workforce is still understaffed, thus limits an effective response to rabies situation (Mbilo et al., 2021; Voupawoe et al., 2021). For instance, only one surveillance officer is assigned per county to respond to animal disease outbreaks, including rabies (personal information). Of course, the limited staff coverage throughout the country compounded by the porosity of borders with neighboring countries facilitates illegal human-mediated animal movements, a potential threat of transboundary transmission of animal diseases, including rabies (Olarinmoye et al., 2019). Like most developing countries, rabies diagnostic in Liberia is still confined to the capital. In contrast, remote and rabies-affected communities are without diagnostic capacities. Suspected rabies samples from distant counties are often shipped to the only veterinary diagnostic laboratory. These samples are sometimes delayed or damaged en route because of bad road conditions or lack of transportation. These challenges hindered the early detection of cases, thereby warranting a miss identification of rabies hotspots around the county.

Consequently, few studies have characterized circulating rabies strains in domestic dog population in Liberia. However, these findings were either compromised by limited geographical areas or discrepancies between studies. For instance, a study conducted in Liberia by Voupawoe et al. reported that the Africa-2 lineage subgroup H circulates in dogs (Voupawoe et al., 2021), which was subsequently confirmed on the first complete genome sequences of 5 Liberian strains (S. Mauti et al., 2022). Of these strains, an isolate corresponds to an isolate from neighboring Cote d'Ivoire. In addition, Olarinmoye et al. reported the co-circulation of the Africa-2 lineage, China lineage-2, and Africa lineage 3 in Liberia [9], which has been however challenged (Zhao et al., 2019). Therefore, a representative sample from the country is required better to understand the resolution of rabies virus in host species.

The aim of this study was to performed, for the first time, a comprehensive phylogenetic analysis of the rabies virus strains circulating in Liberia, based on the genome sequences of representative isolates collected all over the country between 2017 and 2022. In addition, the use of a mixed next generation sequencing approach (Illumina and Nanopore technologies) developed in this study provide key elements of

comparison and methodological optimization in the perspective of an on-site sequencing application.

5.3 Material and methods

5.3.1 Study area

Liberia is located in West Africa, and its terrain includes sandy coastal plains, rolling hills, and dissected plateaus further inland. Guinea borders it to the north, Côte d'Ivoire to the east, the Atlantic Ocean to the south, and Sierra Leone to the west. The country is divided into 15 counties, covering 111,369 km^2. The estimated population of Liberia was approximately 4.9 million inhabitants in 2018, with the majority of people spread between Montserrado, Nimba, and Bong. The climate is tropical, hot, and humid all year round, and the rainy season runs from May to October. Christianity and Islam are the predominant religions, comprising 86% and 12% of the total population (LISGIS 2008).

5.3.2 Training of animal surveillance officers in animal brain sampling and rapid diagnostic testing

Fifteen County Surveillance Officers (CSOs) of the Ministry of Agriculture were trained in a one-day course at the CVL. All participants were fully vaccinated against rabies before the training was held. The training, which was rolled out in two main parts, focused on sampling of animal brain tissue and rabies diagnosis using the rapid diagnostic test kit (RIDT) (Hampson et al., 2015; WHO, 2018a). Each participant had a hands-on experience during the training exercises and participants who needed more practice were again drilled through extra practice section.

5.3.3 Sample collection

Between September 2017 and January 2022, an integrated (Veterinary and Public health sectors) rabies surveillance systems were established under the One Health Platform of Liberia to coordinate joint rabies activities. Animal surveillance officers assigned in various counties worked closely with their public health surveillance officers, communities, and health facilities during animal bite incident and rabies outbreak. Animal brain tissues and decapitated heads of suspected animals were collected by CSOs and shipped to the CVL for analyses (Walker et al., 2018). Initially, brain tissues of suspected rabid animals, mostly dogs, were sampled in field and portion of the brain was shipped to the laboratory. However, due to limited supply of

sampling equipment and personal protective equipment, only decapitated animal heads along with a metadata of the biting animal, date of collection, and origin of the sample were submitted to the laboratory because of limited logistical problems in field among other things. Sample collection was mostly performed at the CVL, and brain samples were tested with the DFA and RIDT tests.

Between September 2017– January 2022, a total of 104 brain tissues and decapitated heads, obtained during rabies outbreaks, of suspected rabid animal were shipped from various parts of Liberia to the CVL (Figure 6).

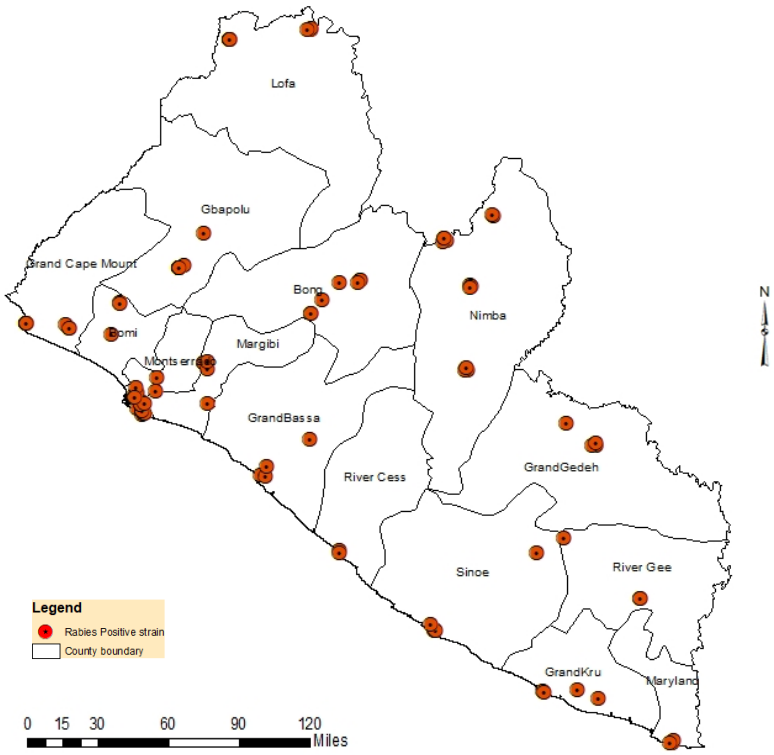


Figure 7: Collection sites of suspected animal rabies samples (2017 – 2022)

5.3.4 RNA extraction

For nearly all samples, total RNA extraction was performed on a brain biopsy (approximatively 0.5 cm^3) from each animal using the Direct-zol RNA Miniprep kit (ZymoResearch), following the manufacturer's instructions and performed at the Central Veterinary Laboratory (CVL) in Liberia. In the absence of the possibility of sending these RNAs in dry ice to Institut Pasteur, Paris, different strategies for their preservation and their shipment at room temperature or refrigerated were performed,

as previously described (Fooks et al., 2017; C. Troupin et al., 2016). First, total RNA was purified using Agencourt RNAClean XP beads (Beckman Coulter) at a ratio of 1:1.8, following the manufacturer's instructions, without the last resuspension step in nuclease-free water. In parallel, RNAs were also deposited in a 96-well plate (RNAstable; Biomatrica), before overnight air-drying in a laminar flow hood, following the manufacturer's instructions (Table 8). Dried RNAs in an RNAstable 96-well plate or on beads were shipped to Institut Pasteur, Paris, France, at ambient and cold temperature with ice packs, respectively, and resuspended in 30 µl of nuclease-free water. In addition, an aliquote of inactivated and crushed brain sample in TRIzol reagent (Invitrogen) of nearly all the specimens was also send at cold temperature (ice packs) and extracted at Institut Pasteur using the Direct-zol RNA Miniprep kit or the classical TRIzol protocol based on isopropanol RNA precipitation, as recommended by the manufacturer (Table 8). Lastly, and for a subset of the samples, impregnated FTA card (Whatman® FTA® card technology, Sigma-Aldrich) with ground brain material was shipped to Institut Pasteur, and then extracted using TRIzol reagent (Table 8). All RNA were quantified by spectrophotometric method using Nanodrop (Invitrogen) and the samples presenting the best integrity values (ratio at 260/280 nm) and the highest concentration were selected for further analysis.

5.3.5 Confirmatory molecular testing by RT-qPCR

The confirmation of the FAT results obtained at CVL was done by the detection of viral RNA using specific SYBR Green based-RT-qPCR targeting the nucleoprotein (N) gene [6]. Briefly, 5 µl of a 1:5 diluted RNA in nuclease-free water was used, in addition to 10 µl of a mix including 2X Universal SYBR Green reaction mix (BioRad), 0,25 µl of iTaq RT enzyme mix (BioRad), 1,2 µl of each primer JW12 (5'-ATGTAACACCYCTACAATG-3' and N165-146 (5'-GCAGGGTAYTTRTACTCATA-3') at 10 nM each, and 2,35 µl of nuclease-free water. Amplification was performed using a AB7500 thermocycler (Applied Biosystem) using the following sequential conditions: a reverse-transcription step at 50°C for 10 min, an initial denaturation at 95°C for 5 min, an amplification step of 40 cycles each including a denaturation step of at 95°C for 10 s and a hybridation/amplification step at 60°C for 32 s, and lastly a dissociation step including the following cycle: 15 s at 95°C, 1 min at 55°C, 15 s at 95°C with a temperature ramp of 1 s / °C and 15 s at 60°C with the same temperature ramp. Detection results for each sample were based on the quantitative threshold (Cq) value

as well as the melting temperature (Tm) value as well as the shape of the melting curve.

5.3.6 Full genome sequencing based on Illumina technology

Genome sequences were obtained using next generation sequencing (NGS) as previously described (Mbilo et al., 2021; Olarinmoye et al., 2019; Voupawoe et al., 2021). After RNA extraction and depending of the initial concentration of each sample, a ribosomal RNA depletion step was first carried out of a maximum of 2−4 µg of RNA and 1 µl of Terminator 5′-Phosphate-Dependent Exonuclease (Epicentre Biotechnologies), in addition to 2 µl of buffer A and 0.5 µl of RNAsin Ribonuclease inhibitor (Promega). After being adjusted to 20 µl with nuclease-free water, the mix was incubated for 1 h at 30°C. The depleted RNA was then purified using Agencourt RNAclean XP beads (Beckman Coulter) at a ratio of 1:1.8, following the manufacturer's instructions. Reverse transcription in complementary DNA (cDNA) of purified RNA was then done using the SuperScript III First-Strand Synthesis SuperMix kit (Invitrogen) according to the manufacturer's instructions. For this step, 8 µl of RNA was first incubated at 65°C for 5 min with 1 µl of Annealing Buffer (Invitrogen) and 1 µl of 50 µM of random hexamers (Invitrogen), then placed on ice. The complementary step was performed with the addition of 10 µl of 2X First-Strand Reaction Mix and 2 µl of Superscript III Reverse transcriptase / RNaseOUT Enzyme Mix for a final volume of 22 µl. The mix was incubated at 25°C for 10 min then at 50°C for 50 min. Finally, inactivation of the enzymes was performed after incubation at 85°C for 5 min. Afterward, double-stranded DNA (dsDNA) was synthesised in a reaction mix containing 20 µl of fresh cDNA, 10x Second-Strand Synthesis Reaction Buffer (New England Biolabs), 3 µl of 10 mM dNTP mix (Invitrogen), 1 µl (10 U) of Escherichia coli DNA ligase (New England Biolabs), 4 µl (40 U) of E. coli DNA polymerase I (New England Biolabs), 1 µl (5 U) of E. coli RNase H (New England Biolabs), and 43 µl of nuclease-free water, after incubation at 16°C for 2 h. The total volume (80 µl) of dsDNA was finally purified for each virus, using a ratio of 1:1.8 of AMPure XP beads (Beckman Coulter) following the manufacturer's instructions. Finally, dsDNA libraries were constructed using the Nextera XT kit (Illumina, Evry, France) and sequenced using a 2 × 150 nucleotide paired-end strategy on the NextSeq500 platform as previously described [7–9].

The NGS data were analyzed using de novo assembly and mapping (both using CLC Assembly Cell, Qiagen) with a dedicated workflow built on the Galaxy platform of Institut Pasteur (S. Mauti et al., 2022; Mbilo et al., 2021; Olarinmoye et al., 2019; Voupawoe et al., 2021). Contig sequences were assembled and manually edited to produce the final consensus genome using SnapGene software (version 5.3.2) (Dotmatics). The quality and the accuracy of the final genome sequences were checked after a final mapping step of the original cleaned reads and visualized using Tablet [11].

5.3.7 Full genome sequencing based on Nanopore technology

Multiplex primer sequences were designed using Primal Scheme website (https://primalscheme.com/) on a consensus sequence which was obtained based on a set of five available references RABV sequences from Liberia (GenBank reference numbers OK135144.- OK135148) (Fooks et al., 2017).

For the MinION library preparation, cDNA was generated in 10 µL mix reaction using 8 µL of RNA and 2 µL of LunaScript RT SuperMix (New England BioLabs) containing random hexamer, oligo-dT primers, dNTPs, Murine RNase Inhibitor and Luna Reverse Transcriptase. Reverse transcription was initiated at 25°C for 2 min and the performed at 55°C for 20 min, followed by heat inactivation for 1 min at 95°C. In a 25 µL mix reaction, 2.5 µL of cDNA was added to the multiplex PCR reaction mix containing 12.5 µL of Q5 Hot Start High-Fidelity 2X Master Mix (New England BioLabs), 4.3 µL of 10 µM primer pool 1 or 2 (final concentration of each primer at 0,045 µM) and 5.7 µL of nuclease-free water. To generate primers pools 1 and 2, a total 5 µl of each respective primer were mix (initial concentration at 100 µM) to constitute a final solution at 10 µM which was used for the next steps. The multiplex PCR reaction conditions were as following: initial denaturation at 98°C for 30 s; 35 amplification cycles including each a denaturation step at 98°C for 15 s and a hybridation/amplification step at 65°C for 5 min, and final amplification step at 65°C for 5 min. Amplicon pools 1 and 2 for each sample were combined into one tube and underwent clean-up using 1X volume of AMPure XP beads (Beckman Coulter). Samples were mixed with beads, incubated 5 min at room temperature and placed on magnetic stand to remove supernatant. After washing twice in 80% ethanol, DNA was eluted off the beads with 30 µL nuclease-free water. In a new PCR strip-tube, 50 ng of purified DNA in 12.5 µL were used for end-repair PCR reaction using Ultra™ II End Repair/dA-Tailing Module (New England

BioLabs). The PCR reaction conditions included an incubation at 20°C for 10 min followed by 10 min at 65°C. A volume of 7.5 µL of previous amplification was then used in the barcoding PCR reaction using 10 µL of TA Ligase (Blunt/TA Ligase Master Mix, New England BioLabs) and 2.5 µL of barcode per sample (EXP-NBD196, Oxford Nanopore Technologies). PCR reaction was conducted at 20°C for 20 min and following by 65°C for 10 min. After barcoding, samples were pooled and the library was cleaned-up with 0.6X volume of AMPure XP beads; incubated 10 min at room temperature and after 2 minutes on magnetic stand, the mix was washed twice with 250 µL of SFB (EXP-SFB001, Oxford Nanopore Technologies), followed by one wash in 80% ethanol. The library was eluted off the beads with 30 µL nuclease-free water and the totality was used for adapter ligation reaction has followed: 5 µL of Adapter Mix AMII (EXP-AMII001), 5 µL of Quick T4 DNA Ligase and 10 µL of Quick 5X Ligation Buffer. The reaction was then incubated 20 min at room temperature followed by new clean-up step with 1X volume of AMPure XP beads and only two SFB wash. Finally, the library was eluted in 15 µL of elution buffer (EB) and 15 ng was used for FlowCell 9.4 loading. Sequencing was performed 12 hours on MinION Mk1c sequencer.

Row data collection was performed using the MinKNOW software, and data analysis was conducted using an adapted bioinformatic protocol disposable on the ARTiC Network. The RAMPART tool was used to visualize data and to stop the run. in real-time. Finally, the pipeline using Guppy-minimap2 2.17-r941 and medaka 1.0.3 tools was used for basecalling, de-multiplexing, mapping and generation of the consensus sequence. Final gaps remaining in the MinION concensus sequences were fulfilled by Sanger sequencing, after obtaining the missing amplicons which were individually generated by PCR simplex from cDNA (and using the same protocol than the ampliseq-sequencing strategy).

5.3.8 Phylogenetic analysis

Phylogenetic analyses were performed on some of the complete or nearly complete genome sequences of RABV strains from Liberia and different representative African strains. All these sequences were aligned using ClustalW (version 2.0) (Pyana et al., 2022) or Clustal Omega (version 1.2.4, implemented in SnapGene version 5.3.2) (Fooks et al., 2017), and checked manually for accuracy. Phylogenetic trees were constructed using the maximum-likelihood approach based on the generalized time-reversible model proportion of invariable sites plus gamma-distributed rate

heterogeneity (GTR+I+Γ4) utilizing SPR branch-swapping, as estimated in PhyML 3.0 [14] with Smart Model Selection (http://www.atgc-montpellier.fr/phyml-sms/). The robustness of individual nodes was estimated using 100 bootstrap replicates. The ML phylogenetic tree visualized with FigTree (http://tree.bio.ed.ac.uk/). The different phylogenetic clades, lineages and groups have been previously described in (Dacheux et al., 2019; Fooks et al., 2017; Pallandre et al., 2020; Walker et al., 2018)

5.4 Results

5.4.1 Sample collection and antigen-based laboratory diagnosis

Between September 2017 and January 2022, based on the integrated rabies surveillance systems which was initially established under the One Health Platform of Liberia to coordinate joint rabies activities [3], a total of 104 animal samples were received and tested at the CVL (Table 8) (Fig. 7). These samples were collected from all over the country, with representative samples obtained from the 15 counties. The most represented one were Montserrado (n=20, 19.2%), Nimba (n=11, 12.5%) and Lofa (n=10, 10.6%). Nearly all of these samples were collected from dogs, excepted four of them which were obtained for one cat, one squirrel and two African civets (*Civettictis civetta*) (Table 8)

Sample ID IPP	Internal ID	Host	Location	Collection date	Latitude	Longitude
17013LIB	817/17	Dog	Montserrado	2017	6°14'16.58"N	10°41'49.06"W
18005LIB	Lib02_2017	Cat	Margibi	05/12/2017	6°17'57.31"N	10°19'50.62"W
18006LIB	Lib03_?	Dog	Montserrado	NA[a]	6°17'35.81"N	10°42'13.96"W
18007LIB	Lib04_2017	Dog	Montserrado	25/09/2017	6°22'15.75"N	10°37'10.32"W
18008LIB	Lib05_2018	Dog	Montserrado	25/03/2018	6°16'14.22"N	10°43'47.22"W
18009LIB	Lib6	Dog	Lofa	NA	8° 1'5.42"N	10°16'25.61"W
18018LIB	Lib01_?	Dog	Montserrado	NA	6°23'52.87"N	10°29'4.92"W
20006LIB	Lib01_2019	Dog	Bong	19/10/2019	6°53'8.80"N	9°40'35.48"W
20007LIB	Lib02_2020	Dog	Montserrado	12/03/2020	6°15'40.61"N	10°42'9.86"W
20008LIB	Lib03_2019	African civet	Sinoe	01/11/2019	5°27'26.89"N	8°27'57.40"W
20009LIB	Lib04_2019	Dog	Gbarpolu	03/11/2019	7° 4'5.14"N	10°29'26.16"W
20010LIB	Lib05_2019	Dog	Gbarpolu	15/11/2019	7° 4'5.14"N	10°29'26.16"W
20011LIB	Lib06_2019	Dog	Lofa	03/12/2019	8°25'20.99"N	9°45'8.60"W
20012LIB	Lib07_2020	Dog	Grand Cape Mount	04/02/2020	6°43'28.87"N	11° 6'39.79"W
20013LIB	Lib08_2020	Dog	Gbarpolu	12/03/2020	7° 4'5.14"N	10°29'26.16"W
20014LIB	Lib09_2019	Dog	Margibi	20/11/2019	6°32'1.75"N	10°21'3.59"W
20015LIB	Lib10_2019	Dog	Lofa	21/10/2019	8°21'45.17"N	10°12'21.38"W
20016LIB	Lib11_2019	Dog	Nimba	06/10/2019	6°58'31.64"N	8°50'29.03"W
20017LIB	Lib12_2020	Dog	Grand Gedeh	03/02/2020	6° 3'44.49"N	8° 7'52.01"W
20018LIB	Lib13_2019	Dog	Bong	05/11/2019	6°48'52.71"N	9°44'44.80"W
20019LIB	Lib14_2020	Dog	Gbarpolu	03/03/2020	7° 4'5.14"N	10°29'26.16"W
20020LIB	Lib15_2020	Dog	Gbarpolu	21/02/2020	7° 4'5.14"N	10°29'26.16"W
20021LIB	Lib16_2020	Dog	Gbarpolu	19/03/2020	7° 4'5.14"N	10°29'26.16"W
20022LIB	Lib17_2020	Dog	Grand Cape Mount	20/02/2020	6°45'20.91"N	11°21'18.60"W
20023LIB	Lib18_2020	Dog	Grand Gedeh	03/02/2020	6°11'26.54"N	8°17'42.12"W
20024LIB	Lib19_2020	Dog	Grand Gedeh	03/02/2020	6° 3'44.49"N	8° 7'52.01"W
20025LIB	Lib20_2020	Dog	Grand Cape Mount	25/03/2020	6°45'20.91"N	11°21'18.60"W
20026LIB	Lib21_2020	Dog	Grand Cape Mount	03/03/2020	6°45'7.39"N	11° 8'10.42"W
20027LIB	Lib22_2020	Dog	Grand Cape Mount	25/03/2020	6°45'20.91"N	11°21'18.60"W
20028LIB	Lib23_2020	Dog	Grand Cape Mount	14/03/2020	6°45'20.91"N	11°21'18.60"W
20029LIB	Lib24_2020	Dog	Grand Cape Mount	15/03/2020	6°45'20.91"N	11°21'18.60"W
20030LIB	Lib25_2020	Dog	Grand Cape Mount	14/03/2020	6°43'28.87"N	11° 6'39.79"W
20031LIB	Lib26_2020	Dog	Lofa	13/03/2020	8°21'45.17"N	10°12'21.38"W
20032LIB	Lib27_2020	Dog	Margibi	03/03/2020	6°32'1.75"N	10°21'3.59"W
20033LIB	Lib28_2020	Dog	Lofa	12/03/2020	8°25'20.99"N	9°45'8.60"W
20034LIB	Lib29_2020	Dog	Bong	03/03/2020	7° 0'6.74"N	9°27'46.17"W
20035LIB	Lib30_2020	Dog	Montserrado	12/03/2020	6°15'40.61"N	10°42'9.86"W
20036LIB	Lib31_2022	Dog	Nimba	10/11/2020	6°29'46.60"N	8°51'31.17"W
20037LIB	Lib32_2020	Dog	Maryland	12/03/2020	4°22'54.04"N	7°41'45.76"W
20038LIB	Lib33_2020	Dog	Bong	28/02/2020	6°48'52.71"N	9°44'44.80"W
20039LIB	Lib34_2020	Dog	Montserrado	23/02/2020	6°15'40.61"N	10°42'9.86"W
20040LIB	Lib35_2019	Dog	Bong	10/12/2019	7° 0'6.74"N	9°27'46.17"W
20041LIB	Lib36_2020	Dog	Margibi	07/01/2020	6°29'38.88"N	10°19'34.06"W
20042LIB	Lib37_2020	Dog	Grand Gedeh	03/02/2020	6° 3'44.49"N	8° 7'52.01"W
20043LIB	Lib38_2020	Dog	Lofa	10/09/2020	8°25'20.99"N	9°45'8.60"W
20044LIB	Lib39_2020	Dog	Sinoe	06/09/2020	5° 1'7.07"N	9° 2'32.67"W
20045LIB	Lib40_2020	Dog	Lofa	12/03/2020	8°25'20.99"N	9°45'8.60"W
20046LIB	Lib41_2020	Dog	Montserrado	11/04/2020	6°14'35.65"N	10°41'13.31"W
20047LIB	Lib42_2019	Squirrel	Lofa	12/08/2019	8°21'45.17"N	10°12'21.38"W
20048LIB	Lib43_2019	Dog	Montserrado	06/09/2019	6°19'1.92"N	10°42'36.50"W
20049LIB	Lib44_2019	Dog	Montserrado	19/09/2019	6°20'46.82"N	10°44'56.58"W
20050LIB	Lib45_2019	African civet	Sinoe	12/07/2019	5°32'20.79"N	8°18'46.10"W
20051LIB	Lib46_2019	Dog	Montserrado	19/12/2019	6°15'40.61"N	10°42'9.86"W
20052LIB	Lib47_2020	Dog	Lofa	28/01/2020	8°21'45.17"N	10°12'21.38"W
21001LIB	Lib48_2020	Dog	Gbarpolu	23/09/2020	7° 5'0.98"N	10°27'42.99"W
21002LIB	Lib49_2020	Dog	Margibi	28/10/2020	6°32'25.43"N	10°19'51.08"W
21003LIB	Lib50_2020	Dog	Grand Kru	12/10/2020	4°40'52.93"N	8°13'52.17"W
21004LIB	Lib51_2020	Dog	Nimba	23/03/2020	7°21'59.93"N	8°42'37.26"W
21005LIB	Lib52_2020	Dog	Grand Bassa	03/12/2020	5°53'50.93"N	10° 1'43.60"W
21006LIB	Lib53_2020	Dog	Gbarpolu	05/12/2020	7°16'9.89"N	10°21'2.36"W
21007LIB	Lib54_2020	Dog	River Gee	28/08/2020	5°11'49.81"N	7°52'44.26"W
21008LIB	Lib55_2020	Dog	Grand Kru	26/04/2020	4°37'50.90"N	8° 6'55.98"W
21009LIB	Lib56_2020	Dog	Montserrado	04/12/2020	6°17'47.87"N	10°42'32.19"W
21010LIB	Lib57_2020	Dog	Montserrado	14/03/2020	6°17'28.70"N	10°42'22.71"W
21011LIB	Lib58_2021	Dog	Grand Kru	19/11/2021	4°40'14.64"N	8°25'44.00"W
21012LIB	Lib59_2021	Dog	Grand Gedeh	23/11/2021	6° 3'43.92"N	8° 8'45.69"W
21013LIB	Lib60_2021	Dog	Nimba	25/11/2021	7°13'31.09"N	8°58'18.85"W
21014LIB	Lib61_2021	Dog	Nimba	26/11/2021	7°12'56.36"N	8°59'28.86"W
21015LIB	Lib62_2021	Dog	Lofa	27/11/2021	8°25'27.60"N	9°44'42.20"W
21016LIB	Lib63_2021	Dog	Montserrado	29/10/2021	6°20'2.45"N	10°44'18.99"W
21017LIB	Lib64_2021	Dog	Grand Kru	30/11/2021	4°40'3.21"N	8°25'9.02"W
21018LIB	Lib65_2021	Dog	Nimba	01/12/2021	6°29'13.31"N	8°51'26.56"W
21019LIB	Lib67_2021	Dog	Nimba	06/12/2021	6°29'22.46"N	8°52'10.76"W
21020LIB	Lib68_2021	Dog	Nimba	07/12/2021	6°30'28.15"N	8°51'50.15"W
21021LIB	Lib69_2021	Dog	River Cess	09/12/2021	5°28'1.87"N	9°34'46.51"W
21022LIB	Lib70_2021	Dog	Bong	11/12/2021	6°59'10.97"N	9°34'43.64"W
21023LIB	Lib71_2021	Dog	Bomi	11/12/2021	6°52'17.12"N	10°49'37.12"W
21024LIB	Lib72_2021	Dog	Grand Bassa	12/12/2021	6° 5'50.35"N	9°45'5.83"W
21025LIB	Lib73_2021	Dog	Nimba	15/12/2021	6°57'55.68"N	8°50'0.66"W
21026LIB	Lib74_2021	Dog	River Cess	16/12/2021	5°27'11.18"N	9°34'51.70"W
21027LIB	Lib75_2021	Dog	Grand Bassa	18/12/2021	5°53'27.50"N	10° 0'14.66"W
21028LIB	Lib76_2021	Dog	Grand Gedeh	19/12/2021	6° 4'30.95"N	8° 7'42.02"W
21029LIB	Lib77_2021	Dog	Nimba	19/12/2021	6°57'36.06"N	8°50'33.17"W
21030LIB	Lib78_2021	Dog	Montserrado	19/12/2021	6°26'51.23"N	10°36'54.93"W
21031LIB	Lib79_2021	Dog	River Gee	19/12/2021	5°11'46.73"N	7°52'46.18"W
21032LIB	Lib80_2021	Dog	Bomi	20/12/2021	6°41'43.82"N	10°52'23.10"W
21033LIB	Lib81_2021	Dog	Maryland	21/12/2021	4°23'43.82"N	7°41'24.85"W
21034LIB	Lib82_2021	Dog	Maryland	21/12/2021	4°22'46.10"N	7°42'41.85"W
21035LIB	Lib83_2021	Dog	Grand Bassa	22/12/2021	5°56'34.65"N	9°59'46.48"W
21036LIB	Lib84_2021	Dog	Nimba	23/12/2021	7°14'18.07"N	8°59'23.18"W
21037LIB	Lib85_1_2021	Dog	Sinoe	23/12/2021	5° 1'32.58"N	9° 2'38.53"W
21038LIB	Lib85_7_2021	Dog	Sinoe	23/12/2021	5° 0'46.58"N	9° 2'11.04"W
21039LIB	Lib86_2021	Dog	Nimba	23/12/2021	7°20'15.69"N	8°57'10.56"W
21040LIB	Lib87_2021	Dog	Bong	26/12/2021	6°59'12.32"N	9°28'29.62"W
21041LIB	Lib88_2021	Dog	Montserrado	26/12/2021	6°21'45.61"N	10°43'46.25"W
21042LIB	Lib89_2021	Dog	Maryland	26/12/2021	5° 3'0.85"N	9° 3'48.95"W
21043LIB	Lib90_2021	Dog	Nimba	28/12/2021	7°22'12.38"N	8°42'53.83"W
21044LIB	Lib91_2021	Dog	Lofa	28/12/2021	8°25'9.20"N	9°45'39.78"W
21045LIB	Lib92_2022	Dog	Gbarpolu	01/01/2022	7° 4'6.52"N	10°29'30.72"W
21046LIB	Lib93_2022	Dog	Grand Cape Mount	01/01/2022	6°45'22.62"N	11°21'13.75"W
21047LIB	Lib94_2022	Dog	Bomi	01/01/2022	6°52'14.62"N	10°49'38.58"W
21048LIB	Lib95_2022	Dog	Montserrado	02/01/2022	6°18'2.58"N	10°41'3.45"W
21049LIB	Lib96_2022	Dog	Montserrado	02/01/2022	6°23'34.80"N	10°43'59.80"W
21050LIB	Lib97_2022	Dog	Montserrado	02/01/2022	6°20'23.87"N	10°44'22.68"W

Table 8: Description of the samples collected from September 2017 to January 2022 in Liberia. Legend: [a] NA: non applicable.

The biological diagnosis of rabies was performed by FAT for all the brain samples received, and was found positive for 104 specimens (X%) (Table 9). In parallel, detection of rabies antigen by RIDT (Anigen Rapid Rabies Ag Test Kit, BioNote) was performed at the CVL on nearly all these samples (n=101, 97.1%), according to a modified protocol previously described (Hampson et al., 2015; WHO, 2018a). Among the 101 samples tested, only 15 specimens were found positive, leading to a sensitivity of 14.8% (95% CI: 7.9%-21.8%) (Table 9). As the comparison between the two methods (FAT as reference technique and RIDT as evaluated technique) was performed only on FAT positive samples, specificity and other comparative values were not possible.

5.4.2 Diagnosis confirmation based on viral RNA detection

All the 104 brain samples were extracted at CVL, before proceeding to the sequential transfer to Institut Pasteur, Paris, of the RNA (using either RNAstable or Agencourt bead support) and/or the remaining crushed brain tissue in TRIzol and/or on FTA card support. Due to the lack of dry ice and difficulties in sending infectious material, we have been forced to multiply, for each specimen, the shipments and the format/support of shipped non-infectious samples at ambient temperature and/or with ice-pack. Thus, in order to multiply the chance to receive good quality RNA or samples, nearly four different formats (RNA alone, RNA on Agencourt beads, RNA in RNAstable plate, crushed brain tissue in TRIzol and/or on FTA card) per specimen, and only the best preserved were subsequantially tested for molecular confirmation (Table 9). The quality of the RNA was found higher when the extraction was performed at Institut Pasteur, Paris from the suspension of crushed brain in TRIzol (n=63, 60.6%) compared to the quality of RNA shipped from Liberia. For nearly half of the samples (n=56, 53.8%), two different formats were selected for further molecular analysis.

Table 9: Results of the laboratory diagnosis (rabies antigen and rabies virus RNA based detection) for for the samples collected from September 2017 to January 2022 in Liberia.

Legend: [a] ND: Not done, [b] Trizol c: extraction performed at IPP on the remaining TRIzol brain pellet of samples initially extracted at CVL, [c] Trizol nb: extraction performed at

IPP on new crushed brain samples prepared at CVL, [d] Doubtful: only one of the duplicate positive, [e] NA: non applicable.

Table 9: Results of the laboratory diagnosis (rabies antigen and rabies virus RNA based detection) for for the samples collected from September 2017 to January 2022 in Liberia.

Sample ID IPP	Internal ID	Rabies antigen-based detection		Rabies virus RNA-based detection						
		FAT results	RIDT results	Selected format	RT-qPCR results	Cp value	Alternative selected format	RT-qPCR results	Cp value	Final results
17013LIB	817/17	Positive	ND[a]	FTA card	ND	ND	NA[e]	ND	ND	NA
18005LIB	Lib02_2017	Positive	Positive	Agencourt beads	ND	ND	NA	ND	ND	NA
18006LIB	Lib03_?	Positive	Positive	Agencourt beads	ND	ND	NA	ND	ND	NA
18007LIB	Lib04_2017	Positive	Positive	Agencourt beads	ND	ND	NA	ND	ND	NA
18008LIB	Lib05_2018	Positive	Positive	Agencourt beads	ND	ND	NA	ND	ND	NA
18009LIB	Lib6	Positive	ND	FTA card	ND	ND	NA	ND	ND	NA
18018LIB	Lib01_?	Positive	ND	Agencourt beads	ND	ND	NA	ND	ND	NA
20006LIB	Lib01_2019	Positive	Positive	RNAstable	Doubtful[d]	36.94	NA	ND	ND	Doubtful
20007LIB	Lib02_2020	Positive	Negative	TRIzol c[b]	Positive	30.53	NA	ND	ND	Positive
20008LIB	Lib03_2019	Positive	Negative	TRIzol c	Positive	34.12	NA	ND	ND	Positive
20009LIB	Lib04_2019	Positive	Negative	TRIzol c	Positive	35.01	NA	ND	ND	Positive
20010LIB	Lib05_2019	Positive	Negative	TRIzol c	Positive	33.59	NA	ND	ND	Positive
20011LIB	Lib06_2019	Positive	Negative	TRIzol c	Positive	28.25	RNA without stabilisant	Positive	29.99	Positive
20012LIB	Lib07_2020	Positive	Negative	Agencourt beads	Positive	23.16	TRIzol c	Positive	25.41	Positive
20013LIB	Lib08_2020	Positive	Negative	TRIzol c	Positive	29.2	NA	ND	ND	Positive
20014LIB	Lib09_2019	Positive	Negative	TRIzol c	Positive	28.88	NA	ND	ND	Positive
20015LIB	Lib10_2019	Positive	Negative	TRIzol c	Positive	31.16	NA	ND	ND	Positive
20016LIB	Lib11_2019	Positive	Negative	TRIzol c	Positive	30.81	NA	ND	ND	Positive
20017LIB	Lib12_2020	Positive	Negative	TRIzol nb[c]	Negative	Negative	TRIzol c	Negative	Negative	Negative
20018LIB	Lib13_2019	Positive	Negative	TRIzol c	Positive	32.11	TRIzol nb	Positive	33.4	Positive
20019LIB	Lib14_2020	Positive	Negative	TRIzol c	Positive	32.36	NA	ND	ND	Positive
20020LIB	Lib15_2020	Positive	Negative	TRIzol c	Positive	30.02	NA	ND	ND	Positive
20021LIB	Lib16_2020	Positive	Negative	TRIzol c	Positive	31.01	NA	ND	ND	Positive
20022LIB	Lib17_2020	Positive	Negative	TRIzol c	Positive	31.68	NA	ND	ND	Positive
20023LIB	Lib18_2020	Positive	Negative	TRIzol c	Doubtful	34.88	TRIzol nb	Negative	Negative	Doubtful
20024LIB	Lib19_2020	Positive	Negative	Agencourt beads	Doubtful	32.79	TRIzol c	Doubtful	35.42	Doubtful
20025LIB	Lib20_2020	Positive	Negative	TRIzol c	Positive	29.74	NA	ND	ND	Positive
20026LIB	Lib21_2020	Positive	Negative	TRIzol c	Positive	30.6	NA	ND	ND	Positive
20027LIB	Lib22_2020	Positive	Negative	TRIzol c	Positive	31.67	NA	ND	ND	Positive
20028LIB	Lib23_2020	Positive	Negative	TRIzol c	Positive	33.75	RNA without stabilisant	Positive	24.72	Positive
20029LIB	Lib24_2020	Positive	Negative	TRIzol c	Positive	34.02	RNA without stabilisant	Positive	26.45	Positive
20030LIB	Lib25_2020	Positive	Positive	TRIzol c	Positive	20.86	RNAstable	Positive	24.51	Positive
20031LIB	Lib26_2020	Positive	Positive	Agencourt beads	Positive	16.65	TRIzol c	Positive	21.71	Positive
20032LIB	Lib27_2020	Positive	Negative	TRIzol c	Positive	31.42	TRIzol nb	Doubtful	36.85	Positive
20033LIB	Lib28_2020	Positive	Negative	TRIzol nb	Doubtful	36.7	TRIzol c	ND	ND	Doubtful
20034LIB	Lib29_2020	Positive	Negative	TRIzol c	Positive	32.06	NA	ND	ND	Positive
20035LIB	Lib30_2020	Positive	Negative	TRIzol nb	Positive	23.18	TRIzol c	Positive	26.88	Positive
20036LIB	Lib31_2022	Positive	Negative	TRIzol c	Positive	31.89	NA	ND	ND	Positive
20037LIB	Lib32_2020	Positive	Negative	TRIzol nb	Positive	33.48	TRIzol c	ND	ND	Positive
20038LIB	Lib33_2020	Positive	Negative	TRIzol nb	Positive	23.82	RNA without stabilisant	Positive	27.91	Positive
20039LIB	Lib34_2020	Positive	Positive	TRIzol nb	Positive	27.96	TRIzol c	Positive	21.04	Positive
20040LIB	Lib35_2019	Positive	Positive	TRIzol nb	Positive	19.57	TRIzol c	Positive	15.06	Positive
20041LIB	Lib36_2020	Positive	Negative	TRIzol c	Positive	31.33	RNA without stabilisant	Positive	26.12	Positive
20042LIB	Lib37_2020	Positive	Negative	TRIzol c	Positive	30.55	NA	ND	ND	Positive
20043LIB	Lib38_2020	Positive	Positive	TRIzol nb	Positive	11.68	TRIzol c	Positive	14.49	Positive
20044LIB	Lib39_2020	Positive	Negative	TRIzol c	Positive	30.99	RNAstable	Positive	20.12	Positive
20045LIB	Lib40_2020	Positive	Negative	TRIzol c	Positive	32.03	RNA without stabilisant	Positive	36.67	Positive
20046LIB	Lib41_2020	Positive	Negative	TRIzol c	Positive	30.58	TRIzol nb	Positive	29.25	Positive
20047LIB	Lib42_2019	Positive	Negative	Agencourt beads	Doubtful	35.65	TRIzol c	Positive	29.71	Positive
20048LIB	Lib43_2019	Positive	Negative	TRIzol c	Positive	11.65	Agencourt beads	Positive	10.05	Positive
20049LIB	Lib44_2019	Positive	Positive	TRIzol c	Positive	33.6	RNA without stabilisant	Positive	27.57	Positive
20050LIB	Lib45_2019	Positive	Negative	TRIzol c	Positive	33.38	RNA without stabilisant	Positive	34.22	Positive
20051LIB	Lib46_2019	Positive	Positive	TRIzol c	Positive	15.77	TRIzol nb	Positive	17.55	Positive
20052LIB	Lib47_2020	Positive	Negative	TRIzol c	Positive	24.19	RNAstable	Positive	30.08	Positive
21001LIB	Lib48_2020	Positive	Negative	TRIzol c	Positive	33.23	NA	ND	ND	Positive
21002LIB	Lib49_2020	Positive	Positive	TRIzol c	Positive	16.22	TRIzol nb	Positive	21.22	Positive
21003LIB	Lib50_2020	Positive	Negative	TRIzol c	Positive	29.97	TRIzol nb	Positive	30.96	Positive
21004LIB	Lib51_2020	Positive	Negative	TRIzol c	Positive	29.55	NA	ND	ND	Positive
21005LIB	Lib52_2020	Positive	Negative	TRIzol c	Positive	30.6	NA	ND	ND	Positive
21006LIB	Lib53_2020	Positive	Positive	TRIzol c	Positive	18.95	RNAstable	Positive	24.82	Positive
21007LIB	Lib54_2020	Positive	Positive	TRIzol c	Positive	24.94	RNAstable	Positive	33.03	Positive
21008LIB	Lib55_2020	Positive	Negative	TRIzol c	Positive	22.3	TRIzol nb	Positive	25.29	Positive
21009LIB	Lib56_2020	Positive	Negative	TRIzol c	Positive	32.06	TRIzol nb	ND	ND	Positive
21010LIB	Lib57_2020	Positive	Negative	TRIzol c	Positive	31.41	RNAstable	Doubtful	36.05	Positive
21011LIB	Lib58_2021	Positive	Negative	RNAstable	Positive	13.39	TRIzol c	Positive	15.71	Positive
21012LIB	Lib59_2021	Positive	Negative	Agencourt beads	ND	ND	TRIzol nb	Positive	11.63	Positive
21013LIB	Lib60_2021	Positive	Negative	TRIzol nb	Positive	11.8	NA	ND	ND	Positive
21014LIB	Lib61_2021	Positive	Negative	TRIzol c	ND	ND	TRIzol nb	Positive	32.57	Positive
21015LIB	Lib62_2021	Positive	Negative	TRIzol nb	ND	ND	Agencourt beads	Positive	12.31	Positive
21016LIB	Lib63_2021	Positive	Negative	TRIzol nb	ND	ND	Agencourt beads	Positive	11.69	Positive
21017LIB	Lib64_2021	Positive	Negative	TRIzol c	Positive	15.49	TRIzol nb	Positive	15.91	Positive
21018LIB	Lib65_2021	Positive	Negative	TRIzol c	Positive	28.13	Agencourt beads	Positive	30.76	Positive
21019LIB	Lib67_2021	Positive	Negative	TRIzol nb	Positive	11.66	TRIzol c	ND	ND	Positive
21020LIB	Lib68_2021	Positive	Negative	TRIzol c	Positive	13.33	TRIzol nb	Positive	16.37	Positive
21021LIB	Lib69_2021	Positive	Negative	TRIzol c	Positive	24.13	TRIzol nb	Positive	34.83	Positive
21022LIB	Lib70_2021	Positive	Negative	TRIzol nb	ND	ND	Agencourt beads	Positive	11.76	Positive
21023LIB	Lib71_2021	Positive	Negative	Agencourt beads	Positive	35.51	TRIzol c	ND	ND	Positive
21024LIB	Lib72_2021	Positive	Negative	Agencourt beads	Positive	34.85	TRIzol nb	ND	ND	Positive
21025LIB	Lib73_2021	Positive	Negative	TRIzol nb	ND	ND	Agencourt beads	Doubtful	36.87	Doubtful
21026LIB	Lib74_2021	Positive	Negative	TRIzol nb	ND	ND	Agencourt beads	Positive	12.26	Positive
21027LIB	Lib75_2021	Positive	Negative	TRIzol c	Positive	26.05	Agencourt beads	Positive	34.2	Positive
21028LIB	Lib76_2021	Positive	Negative	TRIzol nb	Positive	12.39	Agencourt beads	Positive	12.34	Positive
21029LIB	Lib77_2021	Positive	Negative	TRIzol nb	Positive	11.09	Agencourt beads	Positive	12.43	Positive
21030LIB	Lib78_2021	Positive	Negative	TRIzol nb	Positive	11.05	Agencourt beads	Positive	12.72	Positive
21031LIB	Lib79_2021	Positive	Negative	TRIzol c	Positive	29.63	Agencourt beads	Positive	27.79	Positive
21032LIB	Lib80_2021	Positive	Negative	TRIzol nb	Positive	11.24	Agencourt beads	Positive	21.2	Positive
21033LIB	Lib81_2021	Positive	Negative	TRIzol nb	Positive	10.93	Agencourt beads	Positive	11.56	Positive
21034LIB	Lib82_2021	Positive	Negative	TRIzol nb	ND	ND	Agencourt beads	Positive	<3	Positive
21035LIB	Lib83_2021	Positive	Negative	Agencourt beads	Positive	29.78	NA	ND	ND	Positive
21036LIB	Lib84_2021	Positive	Negative	TRIzol c	Positive	19.3	Agencourt beads	Positive	32.44	Positive
21037LIB	Lib85_1_2021	Positive	Negative	Agencourt beads	Positive	32.44	NA	ND	ND	Positive
21038LIB	Lib85_7_2021	Positive	Negative	TRIzol c	Positive	29.3	NA	ND	ND	Positive
21039LIB	Lib86_2021	Positive	Negative	TRIzol nb	Positive	12.5	Agencourt beads	Positive	<3	Positive
21040LIB	Lib87_2021	Positive	Negative	TRIzol nb	Positive	19.65	NA	ND	ND	Positive
21041LIB	Lib88_2021	Positive	Negative	TRIzol nb	ND	ND	Agencourt beads	Positive	12.68	Positive
21042LIB	Lib89_2021	Positive	Negative	Agencourt beads	Positive	29.62	NA	ND	ND	Positive
21043LIB	Lib90_2021	Positive	Negative	TRIzol nb	ND	ND	TRIzol c	Doubtful	33.27	Doubtful
21044LIB	Lib91_2021	Positive	Negative	TRIzol c	Positive	17.61	NA	ND	ND	Positive
21045LIB	Lib92_2022	Positive	Negative	TRIzol nb	ND	ND	Agencourt beads	Positive	12.73	Positive
21046LIB	Lib93_2022	Positive	Negative	Agencourt beads	Positive	12.37	TRIzol nb	Positive	11.92	Positive
21047LIB	Lib94_2022	Positive	Negative	TRIzol nb	Positive	12.42	Agencourt beads	Positive	12.81	Positive
21048LIB	Lib95_2022	Positive	Negative	TRIzol nb	Positive	11.1	Agencourt beads	Positive	12.45	Positive
21049LIB	Lib96_2022	Positive	Negative	Agencourt beads	Positive	14.57	NA	ND	ND	Positive
21050LIB	Lib97_2022	Positive	Negative	TRIzol nb	Positive	11.58	Agencourt beads	Positive	11.35	Positive

The detection of rabies virus RNA by RT-qPCR was performed for 90 out of the 104 samples (86.5%) collected and tested positive by FAT. Among them, 30 samples (30.9%) were tested under one single format, whereas the detection of viral two different formats were evaluated in two different formats for the others (Table 9). For the latter case, no discordant results (negative vs positive) were observed. Viral RNA was detected in 83 samples (92.2%), and the results were considered doubtful (only one technical duplicate positive) for 6 samples (6.7%). Only one sample was found negative. When grouping the doubtful and the negative samples together (n=7), the sensitivity of the RT-qPCR was 92.2% (95 CI: 86.7% - 97.8%) compared to the FAT method. Similarly, to the evaluation of the RIDT technique, specificity and other comparative values were not possible due to the lack of FAT negative samples. Among the RT-qPCR positive samples, the Cq values were highly variable, ranging from <3 to 36.85 (mean = 23.94, mediane = 26.67).

5.4.3 Full genome sequencing

An Illumnia sequencing strategy previously validated (Mbilo et al., 2021; Olarinmoye et al., 2019; Voupawoe et al., 2021) was implemented on a selected panel of samples. This selection was mainly based on the quality (260/280 absorbance ratio value) and the concentration of the extracted RNA. A total of 84 samples were submitted to Illumina NGS sequencing, with 5 samples (18005LIB, 18007LIB, 18008LIB, 18009LIB and 18018LIB) already described (Fooks et al., 2017) (Table S8). Among them 84 samples, only 36 (42.9%) exhibited a coverage higher than 18x (min = 18.6x, max = 11567.4x) with a mean and median coverage values of 1179.3x and 304.9x, respectively (Table 10). For these 36 samples, the percentage and the total number of mapped reads ranged from 0.14% to 27.4% (mean = 3.8%, median = 1.2%) and from 1550 to 934436 (mean = 95643, median = 27768), respectively. Among these 36 samples, the nearly complete genome sequence was obtained by Illumina sequencing for 33 samples (91.7% and 39.3% of the total number of samples sequenced). A total of 17 samples were found negative (no read mapped), despite a total number of reads generated ranged from 115554 to 3030382 (mean = 1158298, median = 958422) (Table S1). Among them, 16 were tested by RT-qPCR and only one was also found negative (20023LIB), whereas the others exhibited Cq values ranging for 29.2 to 35.65 (mean = 32.14, median = 32.06) (Table S8).

Table 10. Description of the sequencing results obtained by Illumina and Nanopore approaches on selected Liberian samples.

Legend: [a] ND: Not done, [b] Trizol c: extraction performed at IPP on the remaining TRIzol brain pellet of samples initially extracted at CVL, [c] NA: non applicable, [d] Trizol nb: extraction performed at IPP on new crushed brain samples prepared at CVL.

Table 10: Description of the sequencing results obtained by Illumina and Nanopore approaches on selected Liberian samples.

Sample ID IPP	NGS Illumina	Selected format	Total no. cleaned reads	Total no. of mapped reads	% of mapped reads	Coverage (x)	NGS Nanopore	Selected format	No. positive amplicons / Total no. amplicons)	Final consensus sequence (nt)
18005LIB	Done	Agencourt beads	3410906	934,436	27.4	11567.36	ND	NA	NA	11922
18007LIB	Done	Agencourt beads	1768746	4593	0.26	56.95	ND	NA	NA	11923
18008LIB	Done	Agencourt beads	4539882	12,554	0.28	155.23	ND	NA	NA	11864
18009LIB	Done	Agencourt beads	1611950	25,596	1.59	316.18	ND	NA	NA	11923
18018LIB	Done	Agencourt beads	5670536	826,598	14.58	10135.1	ND	NA	NA	11923
20012LIB	Done	Agencourt beads	657326	10	0.0015	0.12	Done*	Agencourt beads	36/38	11830
20012LIB	Done	TRIzol c[b]	3139760	6	0.00019	0.08	ND	NA	NA	11830
20030LIB	ND[a]	NA[c]	NA	NA	NA	NA	Done	TRIzol c	38/38	11830
20031LIB	Done	Agencourt beads	378482	1832	0.48	22.42	Done	Agencourt beads	38/38	11824
20031LIB	Done	TRIzol c	951572	514	0.054	6.27	ND	NA	NA	11824
20035LIB	Done	TRIzol c	1140642	6	0.0005	0.07	Done*	TRIzol nb	37/38	11830
20039LIB	Done	TRIzol nb[d]	99794	964	0.97	11.65	Done	TRIzol nb	38/38	11837
20039LIB	Done	TRIzol c	280510	455	0.16	5.49	ND	NA	NA	11837
20040LIB	Done	TRIzol nb	4548	770	16.93	9.27	Done	TRIzol nb	38/38	11900
20040LIB	Done	TRIzol c	151158	4063	2.69	49.16	ND	NA	NA	11900
20043LIB	Done	TRIzol nb	377678	35,322	9.35	425.41	Done	TRIzol nb	38/38	11923
20043LIB	Done	TRIzol c	89234	2519	2.82	30.23	ND	NA	NA	11923
20048LIB	Done	TRIzol c	1498562	303,033	20.22	3676.56	Done	TRIzol c	38/38	11923
20051LIB	Done	TRIzol c	483294	6496	1.34	77.96	Done	TRIzol c	38/38	11869
20052LIB	Done	TRIzol c	111838	10	0.0089	0.12	Done	TRIzol c	38/38	11830
21002LIB	Done	TRIzol c	648500	7917	1.22	96.38	Done	TRIzol c	38/38	11902
21004LIB	Done (Negative)	TRIzol c	1102170	0	0	0	Done*	TRIzol c	37/38	11830
21005LIB	ND	NA	NA	NA	NA	NA	Done*	TRIzol c	37/38	11830
21012LIB	Done	Agencourt beads	5485292	27575	0.5	340.8	ND	Agencourt beads	38/38	11895
21013LIB	Done	TRIzol nb	5079130	24170	0.48	298.97	Done	TRIzol nb	38/38	11897
21015LIB	Done	TRIzol nb	5415426	67360	1.24	832.52	Done*	TRIzol nb	37/38	11884
21016LIB	Done	TRIzol nb	4627158	71296	1.54	881.78	Done*	TRIzol nb	37/38	11883
21017LIB	Done	TRIzol c	1586268	7904	0.5	97.87	Done	TRIzol c	38/38	11849
21018LIB	Done	TRIzol c	3586084	28	0.00078	0.35	Done*	TRIzol c	36/38	11830
21019LIB	Done	TRIzol c	4456624	440491	9.88	5482.36	Done*	TRIzol nb	37/38	11898
21020LIB	Done	TRIzol c	933952	7898	0.846	98	Done	TRIzol c	38/38	11880
21021LIB	Done	TRIzol c	644466	26	0.004	0.33	Done*	TRIzol c	37/38	11830
21022LIB	Done	TRIzol nb	4908814	39827	0.811	492.34	Done	TRIzol nb	38/38	11902
21026LIB	Done	TRIzol nb	4153822	152247	3.67	1889.69	Done	TRIzol nb	38/38	11907
21027LIB	Done	TRIzol c	3334190	244	0.0073	2.99	Done*	TRIzol c	37/38	11815
21028LIB	Done	TRIzol nb	4516922	20312	0.45	251.32	Done	TRIzol nb	38/38	11879
21029LIB	Done	TRIzol nb	4235010	39433	0.93	488.13	Done	TRIzol nb	38/38	11897
21030LIB	Done	TRIzol nb	3869940	45,166	1.167	559.07	Done	TRIzol nb	38/38	11889
21032LIB	Done	TRIzol nb	1466648	35,949	2.45	442.86	Done*	TRIzol nb	36/38	11889
21033LIB	Done	TRIzol nb	3166728	26928	0.85	333.19	Done	TRIzol nb	34/38	11879
21034LIB	Done	TRIzol nb	3752000	49660	1.32	614.17	Done	TRIzol nb	38/38	11899
21039LIB	Done	TRIzol nb	2173372	3012	0.14	37.29	Done	TRIzol nb	28/38	11910
21040LIB	Done	TRIzol nb	8442	1550	18.36	18.57	Done*	TRIzol nb	33/38	11873
21041LIB	Done	TRIzol nb	2689416	54196	2.015	671.45	Done	TRIzol nb	37/38	11889
21044LIB	ND	TRIzol c	NA	NA	NA	NA	Done	TRIzol c	38/38	11830
21045LIB	Done	TRIzol nb	1062334	27961	2.63	340.94	ND	NA	NA	11901
21046LIB	Done	Agencourt beads	4030806	28575	0.709	353.5	Done	Agencourt beads	35/38	11896
21047LIB	Done	TRIzol nb	4361210	24499	0.56	302.96	Done	TRIzol nb	31/38	11916
21048LIB	Done	TRIzol nb	4022044	37539	0.93	464.25	Done*	TRIzol nb	30/38	11903
21049LIB	Done	Agencourt beads	1640462	5568	0.34	68.74	Done*	Agencourt beads	33/38	11899
21050LIB	Done	TRIzol nb	3085245	39086	1.27	483.58	Done*	TRIzol nb	30/38	11923

Next, we developed an Nanopore sequencing strategy based on multiplex primer sequences design and overlapping amplicon sequencing, corresponding to 38 amplicons of 400 bp covering 11830 nt of the genome sequence (out of 11923 nt), in order to increase the number of nearly complete genome sequences and/or to fulfil and confirm the sequences already obtained with the Ilumina approach. A total of 87 samples were submitted for sequencing (Table S9). Among them, 46 consensus sequences were kept, based on the number of amplicons sequenced (≥36) and or/ to fulfil gaps or confirm under covered regions on the Illumina genome sequences (Table 10). For some of these sequences (n=15), a final step of Sanger sequencing were

necessary to complete the missing amplicons (Table 10). Finally, we were able to obtain 46 complete (11922-11923 nt) or nearly complete (11815-11916 nt) (Table 10).

5.4.4 Phylogenetic analysis

The 46 genome sequences obtained after Illumina and Nanopore sequencing were used for phylogenetic analysis. Similarly, to the preliminary study conducted on 5 out of the 46 Liberian sequences (Fooks et al., 2017), a dataset of 28 other genome sequences obtained from GenBank and representative of the major clades/lineages in Africa (especially from West Africa) were included (Table S10). After alignment, a maximum likelihood phylogenetic analysis was performed on sequences of 11795-11797 nt length (Figure 8). As expected, all isolates belonged to the Group H of the Africa 2 Clade, where they strongly clustered together, forming a clear distinct Liberian cluster. Among this cluster, two groups were observed: one with two sequences (18009LIB and 20052LIB) and the second with 43 sequences. No specific relationships between location, year or animal host were associated with these two subclusters. Only one sequence (18018LIB) was found outside of this Liberian cluster, and closely related to a sequence from Ivory Coast (01007CI), suggestive of putative exchange between these two neighbouring countries (Figure 8).

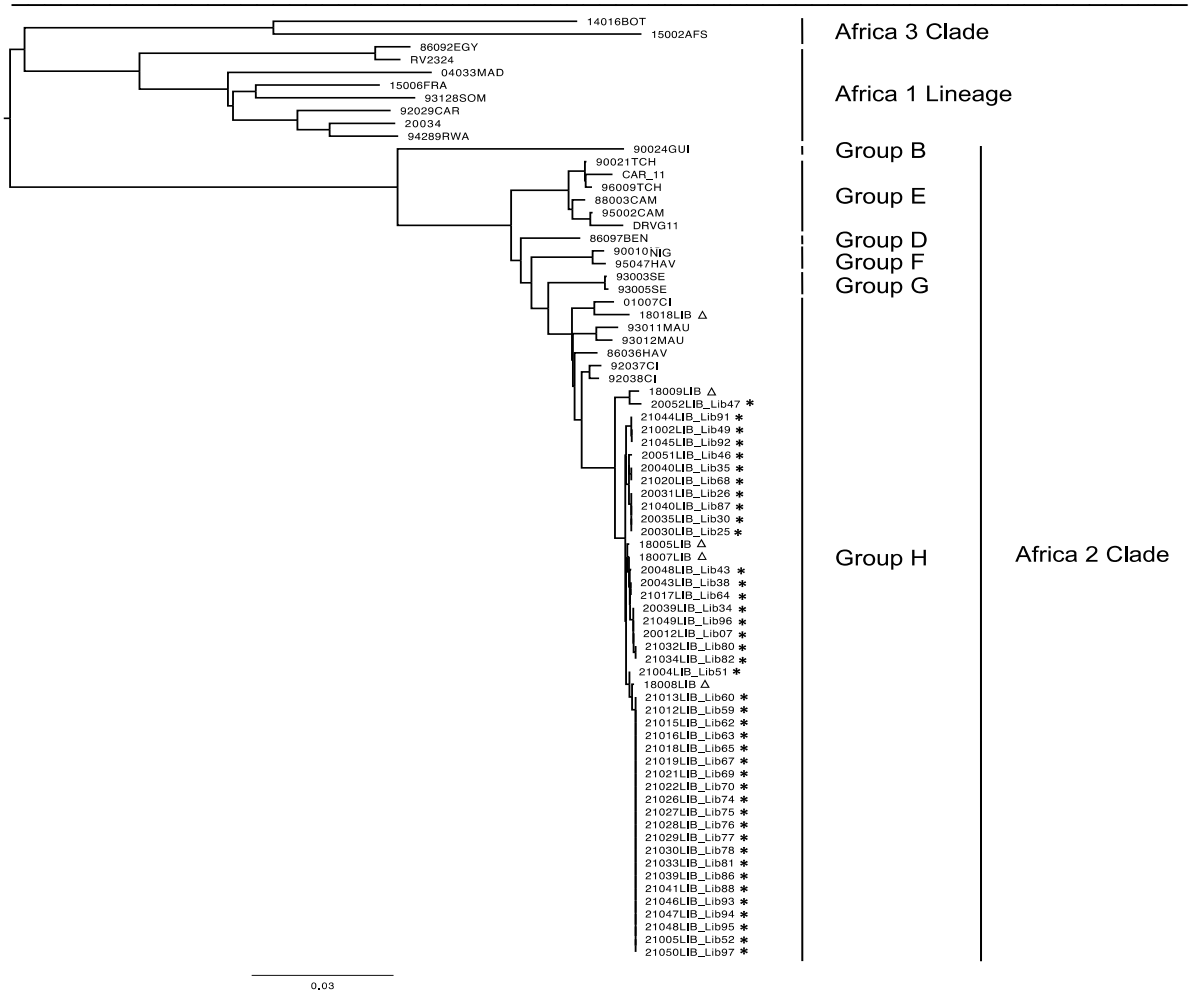


Figure 8: Phylogenetic analysis of the 46 RABV strains from Liberia and different representative African strains.

Figure 8. Phylogenetic analysis of the 46 RABV strains from Liberia and different representative African strains. The tree was based on the nearly complete genome sequences (11795-11797 nt) and constructed using the maximum-likelihood approach based on the generalized time-reversible model proportion of invariable sites plus gamma-distributed rate heterogeneity (GTR+I+Γ4) utilizing SPR branch-swapping, as estimated in PhyML 3.0 (Luo, Zhou, et al., 2021) with Smart Model Selection (http://www.atgc-montpellier.fr/phyml-sms/). The robustness of individual nodes was estimated using 100 bootstrap replicates. The different phylogenetic clades, lineages and groups have been previously described in (Dacheux et al., 2019; Fooks et al., 2017; Pallandre et al., 2020; Walker et al., 2018). The two groups A and C within the clade Africa 2 were missing, due to lack of complete genomes available. Only bootstrap values ≥ 90 are indicated. Scale bar indicates nucleotide substitutions per site. Liberian sequences labelled with a triangle were described in (Fooks et al., 2017),

whereas the other Liberian sequences indicated with an asterisk were obtained from this study.

5.5 Discussion

Any surveillance and control programs of canine rabies required an operational laboratory diagnosis process, to identify, confirm and then report on a regular basis the positive cases to the public health authorities. This laboratory confirmation allows the implement of the relevant post-exposure prophylaxis to the people exposed to the rabid animal, or on the contrary interrupt it in case of negative result. These results also guide mass vaccination strategies for dogs and allow their effectiveness to be assessed. Lastly, the collection of positive samples give the opportunity to decipher the genetic diversity of the viral strains circulating in the region of interest, based on the sequencing of their complete or partial genome.

However, the implementation of a robust laboratory system, associated to the collection and the analysis of the positive cases at the level of the country remains challenging, especially in enzootic regions in Africa. It was the case for Liberia, where this surveillance system was until recently limited. Indeed, joint efforts of diverse national and international stakeholders were conducted to establish animal rabies diagnostics, foster collaboration between all rabies control stakeholders, and develop a short-term action plan with estimated costs for rabies control and elimination in this country (Voupawoe et al., 2021). Based on this implementation, an integrated (Veterinary and Public health sectors) rabies surveillance system was established between September 2017 and January 2022 and under the One Health Platform of Liberia to coordinate joint rabies activities. One of the main objectives of this system was to decipher the genetic diversity of the canine RABV strains circulating at the level of the country Liberia, through the positive samples from all over the country and the genome sequencing of the associated RABV strains.

During the nearly 4 and half year's study period, a total of 104 samples, mostly dog, were obtained from the 15 counties of Liberia and tested positive by FAT at the CVL. This result demonstrates a great advance in canine rabies surveillance, which was previously limited to sporadic case reporting.

In parallel to FAT, most of these samples (n=101) were tested by a modify protocol of RIDT (Anigen, BioNote) (S. Mauti et al., 2020), but the sensitivity was unexpectedly

found very low with a sensitivity of 14.8% (95% CI: 7.9%-21.8%). This result is puzzling and inconsistent with the sensitivity obtained in other similar studies conducted in Africa, such as in Chad, Ivory Coast or Mali (Lechenne et al., 2016; S. Mauti et al., 2020), or even in other continents like India or Asia (Kimitsuki et al., 2020; Mananggit et al., 2021; Yale et al., 2019). Based on these studies, the sensitivity obtained with RIDT ranging from 94.3% to 100%. Unfortunately, it was not possible to repeat the RIDT on site (at CVL) or in another laboratory such as IPP to confirm these results. Different hypotheses can be made to explain this low sensitivity. First, the samples were tested retrospectively at CVL after storage at -80°C from a few weeks to several months or years, without any reconfirmation by FAT conducted in parallel. This storage could impact the results of RIDT, although the impact of this temperature was not observed in other studies (Lechenne et al., 2016; S. Mauti et al., 2020). Another hypothesis could rely on the quality of the batch of the RIDT used, because some batch-to-batch variations were previously observed, depending of the manufacturers (Eggerbauer et al., 2016). In our case, only one batch of RIDT was used and we were not able to perform the technique using a different batch. Lasty, we can make the hypothesis of a low viral load in the samples tested, leading to a lower sensitivity compared to the one obtained with FAT. However, this hypothesis can be excluded because the Cq values obtained for the 11 positive RIDT samples which were tested by RT-qPCR ranging from 11.68 to 36.94, whereas the Cq valuers of the 75 negative RIDT also tested by RT-qPCR ranging from 10.93 to 36.7 (expected for samples 20017LIB and 20023LIB which were negative and doubtful, respectively) (Table 8).

Despite the difficulties to ship the samples to IPP without dry-ice and in non-infectious form, all of them were finally received in this laboratory for further molecular analysis. Various formats were received and analysis, from total RNA extracts without preservative to crushed brain samples in TRIzol suspension. After checking the quality of the RNA extracted at CVL or IPP, the best-preserved samples were tested by RT-qPCR to: 1/ perform a comparison with the results obtained with FAT and 2/ estimate the viral load, based on the Cq value, for further sequencing analysis. In total, 86 samples were tested by RT-qPCR and only 1 sample was found negative (20017LIB) whereas 6 samples were considered as doubtful (20006LIB, 20023LIB, 20024LIB, 20033LIB, 21025LIB and 21043LIB) (Table 9). At the end, the sensitivity of RT-qPCR compared to FAT, two reference techniques for the diagnosis of rabies [13], was high,

with 92.2% (95% CI: 86.7% - 97.8%) when included the doubtful samples as negative, or 98.9% (95% IC: 96.8% - 101%) when excluded these samples. Thus, these results demonstrate the success in the implementation of the biological diagnosis of animal rabies in Liberia, within the framework of the strengthening of capacities for the control and surveillance of this disease in this country (Voupawoe et al., 2021).

For the first time, we applied for this study a mixed approach of NGS for RABV genome sequencing, combining a metagenomic strategy based on Ilumina technology to an ampliseq Nanopore technique. So far, Nanopore sequencing has been rarely used for RABV, and only two studies reported its application for genome sequencing (Brunker et al., 2020; Gigante et al., 2020; Kumar et al., 2022). This combination was first motivated by the limited initial results obtained with the metagenomic approach, which had however proved effective in similar contexts (S. Mauti et al., 2022; Pyana et al., 2022), which thus required to use specific amplification strategies. The difficulties to obtain complete or nearly complete genome sequences (36 samples out of 84 - 42.9% - exhibiting a coverage higher than 18x) with the Illumina approach were most probably in link to the quality of the samples (RNA or TRIzol suspension) received and/or tested. This hypothesis is all the more reinforced as the number of nearly complete genome sequences obtained with the Nanopore ampliseq strategy was also limited (38 samples out of 88 - 43.2% - with ≥36 amplicons sequenced out of 38). When considering samples (n=65) tested by both sequencing approach with a same format (*e.g.,* TRIzol c or TRIzol nb), 12 samples negative with Illumina (no read mapped on the reference sequence) were however positive with the Nanopore sequencing, with sequenced amplicons ranging from 3 to 34. This discordant result is largely explained by the difficulty of obtaining libraries of sufficient quality with the Illumina approach when the initial RNAs are of low or limited quality. In this case, the total number of reads generated is generally too low to obtain a consensus sequence, even partial, or even any single reads mapped. Another advantage of the Nanopore strategy compared to the Illumina approach, relies on its easy implementation in local setting, such as the CVL for an on-site sequencing of the positive samples, for genetic characterization, which will represent the future step.

In total, the mixed sequencing strategy allows to obtained 46 complete or nearly complete RABV genome sequences from Liberia, including 41 newly sequences (S. Mauti et al., 2022). Phylogenetic analysis conducted on these sequences with other

available representative RABV sequences from Africa, especially West Africa, demonstrated that the RABV isolates circulating in Liberia were clustered together into the phylogroup H within the Africa 2 clade. Interestingly, only one strain (18018LIB) was phylogenetically distinct for the others, clustering with a strain from Ivory Coast (01007CI). This result suggests that, even limited, RABV circulation between neighbouring countries (such as Ivory Coast) could exist. Lastly, all the RABV sequences obtained from Liberia, originated from dog for the majority of them but also from wild fauna (2 African civet and 1 squirrel), were associated to canine rabies virus. Even limited in terms of sampling, we were not able to observe other epidemiological rabies cycle in our dataset, except the classical dog reservoir. However, these phylogenetic data need to be confirmed on additional samples collected from the wildlife, as well as to include more representative isolates from neighbouring countries to decipher the potential exchanges between these countries.

5.6 Conclusion

This study describes for the first time the RABV isolates circulating in Liberia, at the country level, based on genome sequences phylogenetic analysis. Our data demonstrated that nearly all these isolates, collected from September 2017 and January 2022, were genetically closely related. More specifically, and although these data need to be confirmed on a larger population, we have not observed any circulating sylvatic rabies in dogs, nor any significant mechanisms of circulation between neighbouring countries. This information represents key pieces of information in future rabies control and eradication programs in Liberia, which CVL demonstrated its monitoring and diagnostic capabilities through this study.

This work was supported through the Wolfermann Nägeli Foundation

6 A policy brief towards dog rabies elimination in Liberia

This policy brief is adapted to Liberia from a generic rabies elimination policy brief by (Helle et al., 2021)

Key policy messages

- Establishing and implementing Animal Disease Law and Veterinary Law is fundamental for rabies control in Liberia.
- Dog mass vaccination, with ≥ 70% coverage, against rabies can eliminate rabies in humans.
- Robust surveillance and diagnostic capacity are essential for monitoring control and post-elimination.
- Closer cooperation between national stakeholders, Mano River Union (MRU) countries, and the West African region is critical for effective and sustainable control.
- Development Impact Bonds can complement national funding efforts for financing and risk-sharing during elimination campaigns.

Rabies is deadly and claims thousands of lives in endemic regions in 150 countries worldwide. In Sub-Saharan Africa, about 20’000 deaths are reported, which is the highest per capita mortality globally (Hampson et al., 2015). Of these deaths, children (40%) and their families living in rural settings are the most affected. *Rabies* is a zoonotic disease that is transmitted between humans and animals. All mammals can contract and transmit the disease, but domestic dogs in endemic countries are the main transmitter of the disease to humans through the bite or scratch of a rabid dog. The fatality rate in humans can be 100% if a bite victim does not seek timely administration of post-exposure prophylaxis. Despite the 100% fatality rate, rabies control is possible. Closer collaboration between the public health and veterinary sector, the One Health approach, has proven effective in rabies control. In addition, rabies prevention is effective through awareness, post-exposure prophylaxis, and mass dog vaccination with at least 70% coverage. Cost-effectiveness analyses demonstrate that canine vaccination with post-exposure prophylaxis (PEP) is also financially the best option for human rabies prevention **(Fig 9)** (Mindekem et al., 2017).

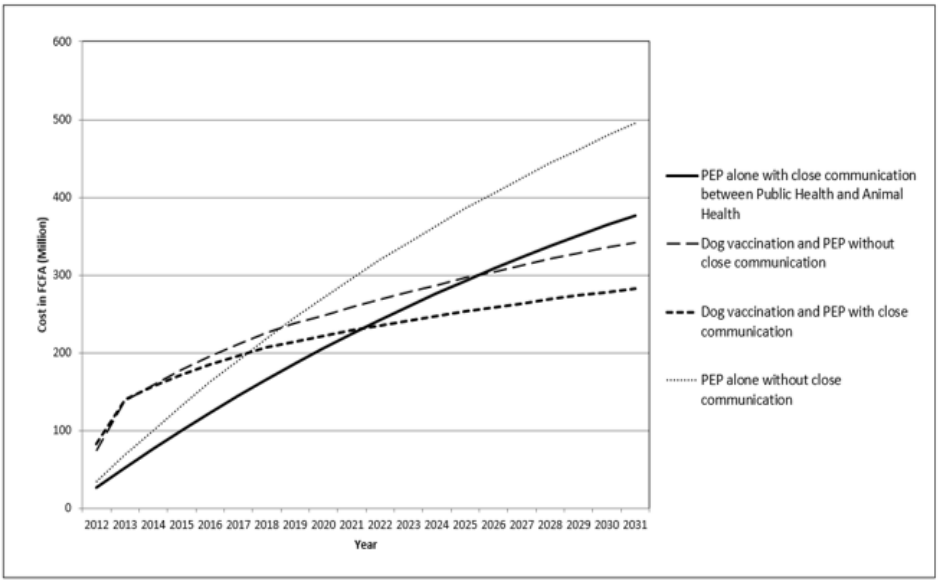


Figure 9: Cumulative cost of different rabies control intervention

6.1 Political and institutional engagement

A legislation framework is one of the essential tools that may be used to implement effective rabies control. Such regulation provides the veterinary domain the full authority to implement laws that are crucial for rabies control, such as responsible dog ownership, regulations on dog breeding and trade to control dog population movements, waste disposal management to enhance stray dog control, and declaration of rabies as a notifiable disease to improve surveillance efforts. The Animal Disease Law in force in Liberia is an old draft that pre-dates the post-war era and requires updating. Furthermore, the current update of the Animal Disease Law contains numerous shortcomings and is of poor quality, rendering it unable to facilitate effective animal disease surveillance, prevention, and control. These are serious concerns that must be addressed to ensure effective rabies control. Rabies has been significantly reduced in countries such as Bangladesh, Tanzania, South Africa, and Vietnam due to tackling the disease through mass vaccination in the host species and implementing relevant legislation. On the other hand, dog culling to reduce the dog population is counterproductive and against animal welfare policy. When used in conjunction with the Canine Rabies Blueprint, the SARE tool provides practical guidance and examples of how to implement rabies control activities, as well as

guidance on institutional responsibilities concerning each activity and who might carry out the work.

6.2 Funding

Most of the logistics and resources used by the National Livestock Bureau for disease investigation and intervention are provided by donors, such as the Africa-Union Interafrican Bureau for Animal Resources (AU-IBAR), the Food and Agriculture Organization (FAO), the United States Agency for International Development (USAID), and the World Bank Regional Disease Surveillance Systems Enhance (REDISSE) Project. This means that activities such as mass dog vaccination campaigns can be interrupted when donor funding stops. The situation is further challenging as dog owners in West and Central Africa often cannot afford to pay to vaccinate their dogs. However, elimination of rabies is a public good. Vaccination of the host species was shown to be less expensive than human treatment alone by 30% over time (Mindekem et al., 2017), so dog vaccination should be provided free of charge. As a remedy, Development Impact Bonds (DIBs) present a further funding mechanism where the investment risk is shared between the government, private investors, and institutional donors. In this model, the necessary investment comes at the beginning when it is most needed.

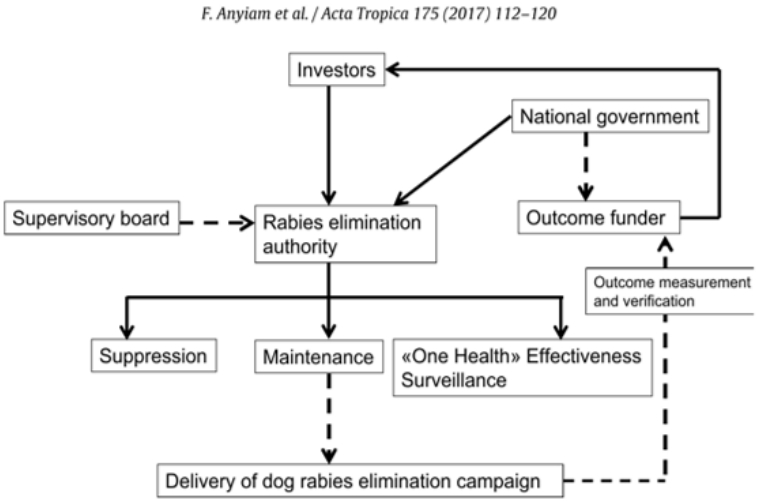


Figure 10: Flowchart of a Development Impact Bond for rabies elimination.

6.3 Surveillance and diagnostics

Because of disease underreporting, budget planning must prioritize disease surveillance. In Liberia, rabies surveillance is weak at the community level and the animal diagnostic capacity is confined to the suburb of the capital, Mount Barclay. The veterinary domain is understaffed due to funding. An estimated additional 106 veterinary para-professionals are needed for public service alone. Also, regional diagnostic laboratories with rabies capacity are lacking throughout the country. These problems contribute to serious underreporting of cases, which underestimates the actual disease burden. As such, political decision-makers are handicapped to take a decisive stand.

Instead of depending on the expensive direct fluorescent antibody (DFA) test, the direct rapid immunohistochemical test (dRIT), which is comparable in results with the gold standard fluorescence antibody test, is economical, easy to use, and requires only a basic light microscope. Therefore, dRIT can be used to set up regional laboratories. Additionally, the rapid immunodiagnostic test (RIDT) can be used by para-vets and surveillance officer, although cases tested with RIDT must be subsequently confirmed with either the DFA or dRIT at the Central Veterinary laboratory.

The established integrated Animal Disease Surveillance and Response System (ADSR) is currently in force in Liberia but requires strengthening, especially from the side of the veterinary services. This will improve the systematic data collection and management and prompt response to animal and public health outbreaks. Steps to improve surveillance are available at https://rabiessurveillanceblueprint.org/.

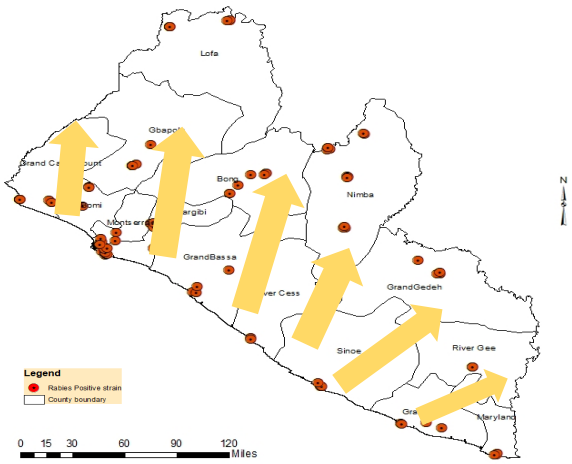
1. Joint effort towards mass vaccination

Scientific and non-scientific knowledge is essential for effective and successful mass dog vaccination. However, local inhabitants, who better understand the needs and conditions of their regions, are often left out during the planning phase of projects targeting their communities. Therefore, funding and implementing organizations must work equally with local stakeholders to ensure that activities are sustained after the project's lifespan. Rabies control is effective with joint efforts between human and animal health authorities. Community health needs can be combined into an integrated program where treatment targeting various illnesses is treated simultaneously. For

instance, vaccines for dog rabies and distemper and medication against tapeworm infection (echinococcosis) and leishmaniosis could be applied simultaneously. Joint delivery platforms for health interventions have proven to be timesaving, well accepted by the population, and cost-effective, especially for hard-to-reach rural communities (Lankester et al., 2019).

6.4 Regional coordinated mechanisms

Rabies is a transboundary disease requiring regional efforts to prevent and control it successfully. No single country can maintain an isolated rabies-free status in West and Central Africa because of porous borders and unregulated human-mediated dog movements of dogs between countries. Further investigation is required to better understand human involvement in long-distance dog movements, dog trade, sociocultural factors, virus natural barriers, viral genetics, and host ecology. However, pancontinental strategies like the Pan Africa Rabies Network (PARACON) are important for effective and sustained control. The global strategic plan by the WHO and partners to end human deaths from dog-mediated rabies by 2030 is too ambitious and should readjust at a regional level. For example, Mano River Union countries, Liberia, Guinea, Ivory Coast, and Sierra Leone, should agree on integrative measures to curb the disease within the region. Natural barriers like the Atlantic Ocean could be taken into account and utilized to block further viral spread.



Mauti et al. 2019, concept adapted with

Figure 11: Scenario of a possible internationally coordinated elimination strategy for West and Central Africa

6.5 Education and awareness

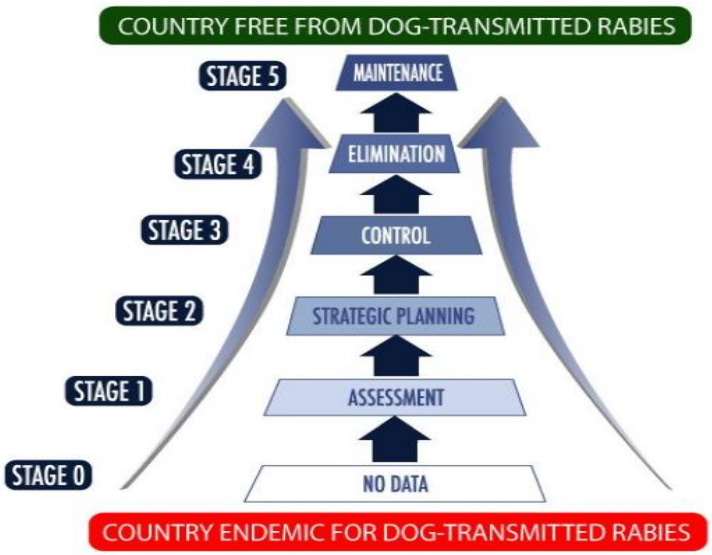
Rabies education plays an important role in the prevention and control of the disease, such that it reduces the financial burden and unnecessary deaths. However, knowledge among affected communities is often poor, and even health workers lack adequate understanding of the disease (Mbaipago et al., 2020; Mbilo et al., 2020). Therefore, rabies education must be precise and target the most vulnerable groups and communities. Educational campaigns must critically consider children, illiteracy, country boundaries, and the different socio-cultural contexts. Because children are mostly affected by dog bites, they must be adequately educated on how to interact with aggressive dogs and on the signs and symptoms of rabies. Schools should set up health clubs, and rabies education must be prioritized. Also, educational courses should target health workers to improve prevention and treatment in the country. Mass dog vaccination campaigns should be well planned and organized to achieve a maximum turnout. Campaign information should be disseminated via posters, radio, and loudspeaker announcements (local vernaculars). Through extensive information campaigns, the costs are reduced per animal due to the higher reach and number of vaccinated animals.

6.6 Community access to PEP

Prompt administration of post-exposure prophylaxis prevents 99.9% of rabies-related deaths. However, this lifesaving drug is often not available in resource-limited countries. In rural settings, which are mostly affected, the situation is far worse because of limited professional healthcare workers and cold chains to store the already expensive vaccines. As a result, bite victims are challenged by bad roads resulting in long distances to access treatments that are either too expensive to afford or unavailable. Government must improve and increase community access to PEP treatment. Rabies hotlines and mobile teams with community health surveillance officers should be set up in referral facilities within districts to respond to animal bite victims rapidly. Investments should prioritize vaccines for PEP rather than relying on expensive dog culling and rabies immunoglobulin, whose health benefits are controversial (Consortium, 2019). In addition, health authorities must closely monitor subsidized vaccines for the intended purpose.

6.7 Post elimination surveillance and prevention of reintroduction

Dog-related rabies elimination is possible through significant transdisciplinary effort, and maintaining zero human rabies-related deaths requires sustaining pre-elimination measures. Measures including mass dog vaccination (70%), dog population management, and rabies awareness have proven effective in rabies elimination. Sectors need to collaborate and combine strategies that are effective in curbing the disease, as rabies is a transboundary spillover disease which can be reintroduced. Government and local authorities must maintain active and robust rabies surveillance, especially in remote communities, which are often left inaccessible, with systems for early detection of cases in both humans and animals for rapid response. By this means, unnecessary deaths and treatments are avoided, and the public health sector benefits with financial savings from reduced costs of human PEP (Hampson et al., 2009).



A widely used instrument within networks like the Pan-African Rabies Control Network is the 'Stepwise Approach towards Rabies Elimination' (SARE) tool.

Figure 12: The 'Stepwise Approach towards Rabies Elimination' tool.

7 General Discussion

7.1 Overall Significance

With a One Health and quantitative approach, this PhD thesis sought to establish baseline information and provide new understanding and knowledge of the epidemiology of rabies in post-war Liberia for the first time. We implemented animal rabies diagnosis through our intervention by promoting intersectoral collaboration, cross-sectional surveys in rural and urban households, and laboratory analysis of suspected rabies strains. This work supports the United Nations Sustainable Development Goals (SDGs) and the rabies Tripartite leadership efforts to eliminate dog-mediated rabies death by 2030. Our findings are important for national and regional rabies control strategies.

7.2 Objectives and research output

7.2.1 Rabies control in Liberia: Joint efforts toward Zero by 30

7.2.2 Securing PEP

Our findings, the first laboratory-confirmed case, are consistent with and support other studies that the Africa 2 lineage is widely distributed in central and Western Africa, with subgroup H prevalent in the region (Talbi et al., 2009). This shows that regional collaboration is important in the control of the disease. Although the disease is endemic to Liberia, securing a lifesaving vaccine for high-risk groups remains challenging. PEP is often limited in rural settings and unaffordable for many people (Dodet et al., 2008). Even if PEP were subsidized, out-of-pocket expenses remain a huge cost burden to marginalized people. In Liberia, the costs to complete a PEP course in government hospitals and private health facilities are 0 and 125 USD, respectively (GARC, Rabies bulletin). Under such conditions, it is difficult to progress or maintain rabies control. There is no provision for a vaccine stockpile for humans and animals. As such, PEP in the country is based on wound washing and post-exposure vaccination of exposed persons since rabies immunoglobulin (RIG) is unavailable (G. Voupawoe et al., 2021). In 2019, during a small-scale mass dog vaccination in high-risk areas, vaccinators did not receive pre-exposure prophylaxis. This is counterproductive and reveals how vulnerable healthcare practitioners are at risk of contracting the disease. PEP is indispensable for preventing rabies after potential exposures.

Implementing four animal rabies diagnostic tests was a milestone in strengthening rabies surveillance because confirmatory laboratory surveillance plays an important role in improving disease reporting. Promoting the establishment of regional laboratories to enhance disease surveillance necessitates having tests available that are accurate and inexpensive, as resource-limited countries cannot afford to maintain the expensive fluorescence microscope. There were three WOAH (formerly OIE) commended tests and one lateral flow test implemented for animal rabies diagnosis. The DFA is an accurate and easy test but requires an expensive fluorescence microscope. The second recommended test, dRIT, is also easier to interpret in degraded or archived samples. The third test WOAH-recommended assay is the RT-PCR approach, which is the only recommended technique to detect rabies in decomposed samples (Markotter et al., 2015; McElhinney et al., 2014; Prabhu et al., 2018). When comparing the DFA and dRIT, they have demonstrated full compliance in laboratory performance. However, using a basic light microscope is one advantage of the dRIT over the DFA. On the hand, the DFA uses fewer chemicals. In addition to the three recommended tests, recently developed rapid immunochromatographic diagnostic tests (RIDTs), based on the lateral flow principle, offer a new opportunity for decentralized rabies diagnosis in rural settings. Ideally, reliable, efficient rabies diagnosis would be through the availability of an antibody-based protocol, either DFA or dRIT, and a molecular protocol for diagnostic confirmation. But the proper application of the recommended tests in developing countries often remains limited due to poorly equipped laboratories, challenges maintaining reagent cold chains, appropriate sample transportation, and lack of quality control systems.

Although timely administration of expensive PEP can prevent human rabies in 100% of exposures, tackling the disease through mass vaccination in the host species can end dog-mediated human rabies death. Nevertheless, these lifesaving vaccines are often unavailable and too expensive in endemic regions. PEP administration alone cannot interrupt human exposure. For example, a study in Chad revealed that it is more cost-effective when canine vaccination and PEP are used simultaneously than PEP alone. Like most Sub-Saharan countries, domestic dogs are Liberia's main source of rabies, resulting in 92% of all cases. The country has one of the lowest coverages (1%) of dog vaccinations in Africa, where small-scale mass dog vaccinations have been conducted during the World Rabies Day celebration since

2017. This coverage is far below countries such as Mali, and the World Health Organization's (WHO) recommended vaccination coverage of 70%.

Mass dog vaccination has been key in eliminating rabies in Western Europe and North America. A nationwide vaccination campaign must be properly coordinated to achieve success and sustainability. A stockpile of high-quality animal rabies vaccines must be procured and available in the country to avoid vaccine challenges. Information about the nationwide campaign is crucial, which can be disseminated through posters displayed in public places, flyers given to dog owners, radio broadcasts, and megaphone announcements in communities. Providing precise information to the public reduces unnecessary inquiry, thus reducing campaign costs. Radio announcements can be broadcast in local vernaculars. Before a campaign begins in any county, seasonal meteorological dynamics must be considered, and a One Health stakeholder meeting should be held with authorities of all fifteen counties. The superintendents and local government members should further disseminate the information about the campaign to local authorities for ease of operation. To carry out a nationwide vaccination campaign in rabies-affected areas in Liberia, operations and activities should be decentralized in the fifteen counties of Liberia. With the necessary arrangements, vaccines can be stored at a referral hospital and health facilities through the One Health Platform, which will provide the necessary logistics and human resources. From each county, staff and vaccinators needed for the campaign can be trained or recruited from a previous campaign. For example, an integrated and co-delivery strategy for rabies and peste des petits ruminants (PPR) vaccination was implemented. The two vaccination campaigns were organized over 13 days and dispensed 30,000 doses of rabies vaccine and 400,000 doses of PPR vaccine. Lessons learned, vaccinators, and logistics used in a previous campaign can be utilized for a nationwide vaccination campaign.

7.3 Integrated surveillance and response of rabies

Rabies is zoonotic, thus requiring cross-cutting intervention for effective control. However, the disease is under-reported due to weak surveillance systems and collaboration barriers between sectors. Currently, rabies surveillance is conducted in two main ministries, the Ministry of Agriculture and the Ministry of Health. In the Ministry of Agriculture, rabies is notifiable and a priority disease under the Animal

Disease Surveillance and Response (ADSR). Despite being a priority disease, surveillance is mostly passive. The veterinary sector is underfunded and number of animal surveillance officers are insufficient. This demonstrates the lack of proper surveillance mechanisms for animal diseases. In the Ministry of Health, rabies is a priority under the Integrated Disease Surveillance and Response (IDSR) system. Animal bites are a proxy for suspected rabies and are reported through the weekly standard. However, under the One Health framework, interconnections concerning Integrated Bite Case Management (IBCM) are recognized between the health and animal sectors, which aim at enhancing surveillance to guide human treatment and reduce unnecessary use of expensive PEP. However, such coordination of sharing information to prevent rabies infection and detect and respond to cases and outbreaks of the disease remains a challenge. Rarely do animal surveillance officers and health facilities update each other for joint investigations, although reporting bite victims at health facilities should trigger an investigation of the biting animal. If the animal's rabies status is established through laboratory diagnosis, such information should be shared with the public health sector to regulate the use of expensive PEP.

7.4 Enabling environment

In Liberia, numerous factors provide an opportunity to undertake a successful and sustainable rabies elimination. Rabies is a notifiable disease for humans and animals in the country, and several laws regulate disease control. These acts include the draft Animal Disease Law, Agriculture Law, Chapter 1 "Plant and quarantine act," draft Liberian Animal Welfare Law, and National Wildlife Law 2017, a legal framework establishing the basis for rabies control. Other factors include establishing a One Health framework in the country and increasing interest in rabies elimination by developmental partners. Through the support of the Regional Disease Surveillance Systems Enhance (REDISSE) program, a national One Health framework was developed in 2017 to facilitate cross-sectoral collaboration to address public health issues, including rabies elimination. Components of the platform include a One Health technical committee and several workgroups focusing on epidemiological surveillance, laboratory surveillance, preparedness and response, and human resource. The One Health Technical Committee (OHTC), a multi-sectoral multidisciplinary body, is made up of a technical working group where expertise and technical support are provided for implementing the activities of the One Health Coordination Platform. Rabies is a

focus and priority of the One Health Technical Working Groups. In the event of an outbreak of a One Health priority zoonotic disease, the OHTC assumes its responsibilities. The next and most important factor is donors' and partners' increased commitment and interest in rabies elimination. There is a global rabies elimination drive due to the disease's high burden on the health sector. Each year, about USD 1.3 billion is lost on expensive PEP, despite that the tools, vaccines, and know-how to eliminate the virus are available. These have resulted in organizations working together towards its control. Institutions such as REDISSE, FAO, WHO, WOAH, and PREDICT have committed themselves to the fight against rabies control in Liberia. Data shows an estimated 500'000 dogs in Liberia, and vaccinating them with 70% coverage is important. It is also important that groups like ECOWAS and MRU countries are working closely on controlling transboundary diseases.

7.5 Preparing Liberia for rabies control: Human dog relationship and practices and vaccination scenarios

Despite established rabies surveillance and small-scale mass dog vaccination, Liberia is still far from achieving the ambitious global drive to eliminate dog-mediated human rabies death by 2030. Currently, there is a limited legal framework for animal disease control, and the veterinary workforce needs to be more adequately funded and staffed to undertake nationwide rabies vaccination campaigns. PEP, which is 99.9% efficacious when promptly administered to rabies-exposed victims, is either too expensive or often unavailable in health facilities in remote communities that are mostly affected by the disease. Our findings are similar to many studies conducted in most rabies-endemic regions of Sub-Saharan Africa (Mbilo et al., 2017; Mauti et al., 2017; Mindekem et al., 2005; Wright et al., 2021). However, one surprising observation was the self-reporting of dog-meat consumption among respondents. Almost half of the respondents admitted to consuming dog meat at least once. When further asked if they knew someone who consumed dog meat, 71% of respondents reported knowing at least one person. In this light, it is logical to assume that more people than previously self-reported are exposed to the disease, especially dog-meat butchers. This study did not investigate rabies exposure concerning occupational risk. In addition, we estimated the dog population to be under 600’000, and a nationwide mass dog vaccination cost of USD 1.6 million for one round of nationwide vaccination activities. These findings of people’s knowledge, dog population, and dog vaccination

cost scenarios are essential for designing effective rabies intervention and control programs. Despite the vaccines, medicines, tools, and technologies to prevent human rabies deaths, Liberia must first prioritize the disease as a public health threat, and seek investment opportunities, for example, DIBs, which shares investment risk between the investor and government.

Future operational research is needed to:

- Conduct further research to understand occupational group, dog meat butchers, risk exposure to rabies virus
- Studies on stray dog population, identifying factors and sources, estimating numbers, distribution, and ecology
- Impact assessment surveys (determine reduction in rabies incidence, PEP usage and cost analysis)
- Assessment of best approaches to increase awareness about rabies and to improve healthcare-seeking behaviour for PEP.

Recommendations:

- Strengthen rabies surveillance in rural areas by establishing regional diagnostic capacity (RIDT and dRIT).
- Create and enforce legislation that are exclusively focused on sustainable and effective rabies control (For example: mandatory dog vaccination)
- Promote the use of the field rapid diagnostic test in settings without laboratories
- Establish regional laboratories with rabies diagnostic capacities
- Improve intersectoral collaboration at local and national levels
- Liberia should seek funding schemes that will share risk burden such as DIBs

7.6 Conclusion

Gathering reliable baseline information is a first step in rabies control. This PhD work established the basis for rabies control in post-war Liberia. Our intervention also strengthened rabies surveillance in the country by establishing rabies animal rabies diagnostic capacity in the capital. Our findings on people's knowledge regarding rabies and vaccination scenarios and molecular characterization of circulation rabies strains will contribute to policy development and rabies control strategy. Future research should focus on the economic benefits of rabies control, dog demographics and ecology, and alternative dog population management. What is further critically needed is to identify and secure resources that will enable sustainable and systematic planning at the national and regional levels.

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Appendix 1: Supplementary information for Chapter 6

	Bong	Montserrado	Total
	%(n) | (N = 612)	%(n) | (N = 670)	%(n) | (N = 1,282)
Resp. sex			
Female	29% (178)	38% (256)	34% (434)
Male	71% (434)	62% (414)	66% (848)
Resp. age			
18 - 27	4% (25)	6% (39)	5% (64)
28 - 37	18% (107)	21% (140)	19% (247)
38 - 47	34% (205)	33% (220)	33% (425)
> 48	45% (275)	41% (271)	43% (546)
Resp. religion			
Christian	78% (479)	86% (579)	83% (1058)
Muslim	4% (26)	11% (75)	8% (101)
Others	16% (107)	2% (16)	10% (123)
Ethnicity			
Kpelleh	83% (508)	21% (139)	51% (647)
Other ethnic group	17% (104)	76% (507)	48% (611)
Foreigner	0% (0)	4% (24)	2% (24)
Household mean	Mean: 7.5 sd: .18	Mean: 8.2 SD: .21	Mean 7.98 SD: .138
Resp. Occupation			
Private sector	19% (116)	58% (390)	40% (506)
Public sector	7% (44)	14% (95)	11% (139)
Farmer	68% (417)	1% (6)	33% (423)
Unemployed	6% (35)	27% (179)	17% (214)
Dog consumption			
Yes	52% (317)	35% (237)	43% (554)
No	45% (273)	64% (430)	55% (703)
No response	88% (22)	0% (3)	2% (25)

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1. Sex of the respondent?

Male	☐
Female	☐

2. Age of household head?

18-27	☐
28-37	☐
38-47	☐
48-57	☐
58-Abv	☐

3. Religion of household head?

Christianity	☐
Islam	☐
Traditional beliefs	☐
Bahai	☐
No religion	☐

4. To which ethnic group (Liberian) does the head of household belong?

Bassa	☐	Krahn	☐
Bella	☐	Kru	☐
Gio	☐	Lorma	☐
Dei	☐	Mano	☐
Gbandi	☐	Mandinga	☐
Gola	☐	Mende	☐
Grebo	☐	Vai	☐
Kissi	☐	Another country	☐
Kpelleh	☐		

5. What is the occupation of the head of household?

Housewife	☐	Private employed	☐
Student	☐	Unemployed	☐
Farmer	☐	No response	☐
Petite trader	☐	Others	☐
Govt. employed	☐		

6. Number of persons in the household.

Number below <15 yrs	☐
Number below >15 yrs	☐

WASTE MANAGEMENT

7. Where do you throw your dirt?

Trench	☐	No response	☐
Public dump site	☐	Don't know	☐

8. What other ways do you throw your waste?

Yes	☐	No response	☐
No	☐	Don't know	☐

9. How far is the household from the nearest garbage site?

Within 20 meters	☐
Within 40 meters	☐
>50 meters	☐

10. What is done with leftover food?

Food is used	☐	No response	☐
Disposed in garbage	☐	Don't know	☐
Feed dog	☐	No response	☐

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DOG DEMOGRAPHICS

11. Are there ownerless dogs in your neighborhood?
    - Yes ☐ No response ☐
    - No ☐ Don't know ☐

12. If yes, how may ownerless dogs have you seen? _____

13. Do you feed stray dogs?
    - Yes ☐ No response ☐
    - No ☐ Don't know ☐

14. Does the household own a dog?
    - Yes ☐
    - No ☐

15. Who is the primary owner of the dog?
    - Head of the family ☐ Other relatives ☐
    - Spouse of the head of household ☒ No response ☐
    - Son/Daughter (household) ☐
    - Food is used ☐

16. How many dogs? _____

Female: puppies (0-3months)	☐	Female: puppies (0-3months)	☐
Female: sub-adults (4-11months)	☐	Female: sub-adults (4-11months)	☐
Female: adults (1-7yrs)	☐	Female: adults (1-7yrs)	☐
Female: >7 years	☐	Female: >7 years	☐

17. Which county/ry did you acquire the dog(s)?

Bong	☐	RiverCess	☐
Nimba	☐	RiverGee	☐
Margibi	☐	Maryland	☐
Gbarpolu	☐	Sinoe	☐
Lofa	☐	Grand Bassa	☐
Montserrado	☐	Another country	☐

18. Why was the dog acquired?

Hunting	☐	Pet for Sale	☐
Companionship	☐	Don't know	☐
House guard	☐	No response	☐
Breeding for sale	☐		

19. How many dogs have you acquired in the last 12 months? _____

20. What is the reason, if the household doesn't own dog?

Do not like dog as pet	☐	To avoid contamination with dog feces	☐
Too expensive	☐	Dirty	☐
Religion	☐	Other	☐
No space	☐	No response	☐
No need for dogs	☐	Don't know	☐

BITE INCIDENCE

21. Has any member of the family been bitten by dog?
    - Yes ☐
    - No ☐

22. What is age of the bit victim?
    - 1-15 ☐
    - 16-38 ☐

23. Sex of the bite victim?

Male ☐
Female ☐

24. Was first aid applied to the bite wound?

Yes ☐ Don't know ☐
No ☐ No response ☐

25. After first aid, was the victim taken right away for further treatment?

Yes ☐ Don't know ☐
No ☐ No response ☐

26. If yes, what was done?

	Wait for the answer of the interviewee (Spontaneous response)	When the person has not said the spontaneous answer, cite all the possibilities (Probed response)
Went to a government health center		
Went to private clinic		
Went to pharmacy		
Went to traditional healer		
Went to animal health worker		
Nothing		
Don't know		
No response		

27. If the victim went to government health center did he get a rabies vaccination?

The vaccine was administered free of charge ☐
The vaccine was paid for at the facility ☐
Vaccine was free, but not available ☐

28. Did the health worker(s) provide further information about rabies?

Yes ☐ Don't know ☐
No ☐ No response ☐

29. If victim didn't seek further treatment, what is the reason?_____

30. What happened to the bite victim?

Yes ☐
No ☐
No response ☐

31. If died, reasons for the victim's death?

Death was not related to the bite ☐
The victim died after having shown rabies symptoms and rabies was diagnosed form a doctor ☐
The victim died after having shown rabies symptoms but rabies was not diagnosed from a doctor ☐
The victim died after the bite without showing rabies symptoms, don't know ☐

32. Who owned the biting dog?

Household ☐
Neighbor ☐
Ownerless dog ☐
Don't know ☐
No response ☐

33. What happened to the biting dog?

Alive ☐
Died ☐
No response ☐
Don't know ☐

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34. What were the symptoms in the biting animal?

Drool	☐	Not eating	☐
Change of crying	☐	Not drinking	☐
Agitated, Aggressive	☐	No symptoms	☐
Paralysis	☐	No response	☐

KNOWLEDGE ATTITUDE AND PRACTICE

35. How did you know about rabies?

Newspaper	☐	Health workers	☐
Radio	☐	Animal health workers	☐
Relatives	☐	TV	☐
Friend	☐	No response	☐

36. How does a person get rabies?

	Wait for the answer of the interviewee (Spontaneous response)	When the person has not said the spontaneous answer, cite all the possibilities (Probed response)
Dog bite		
Eating contaminated dog meat		
Infected saliva on broken skin		
Infected saliva on mucous membrane		
Scavenging garbage		
Don't know		
No response		

37. Who can be infected with rabies?

Humans	☐	Bats	☐
Cats	☐	All mammals	☐
Dogs	☐	Don't know	☐
Monkeys	☐	No response	☐

38. What are the signs of rabies in dogs?

	Wait for the answer of the interviewee (Spontaneous response)	When the person has not said the spontaneous answer, cite all the possibilities (Probed response)
Aggressive		
Agitation		
Change of voice		
Paralysis		
Convulsion		
Restlessness		
Salivation		
Loss of appetite		
Don't know		
No response		

39. If a patient shows sign of rabies can the disease be treated?

Yes	☐
No	☐
No response	☐
Don't know	☐

40. How to prevent rabies?

Vaccinate dogs	☐	Castration of dogs	☐
Dog killing	☐	Don't know	☐
Don't allow the dog to roam freely	☐	No response	☐
Waste control	☐		

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41. Do you know someone who has died of rabies?
- Yes ☐
- No ☐
- No response ☐
- Don't know ☐

42. In which institution can we find rabies vaccine for animals?
- Ministry of Health ☐
- Ministry of Agriculture ☐
- Don't know ☐
- No response ☐

43. Can dogs be vaccinated against rabies?
- Yes ☐
- No ☐
- No response ☐
- Don't know ☐

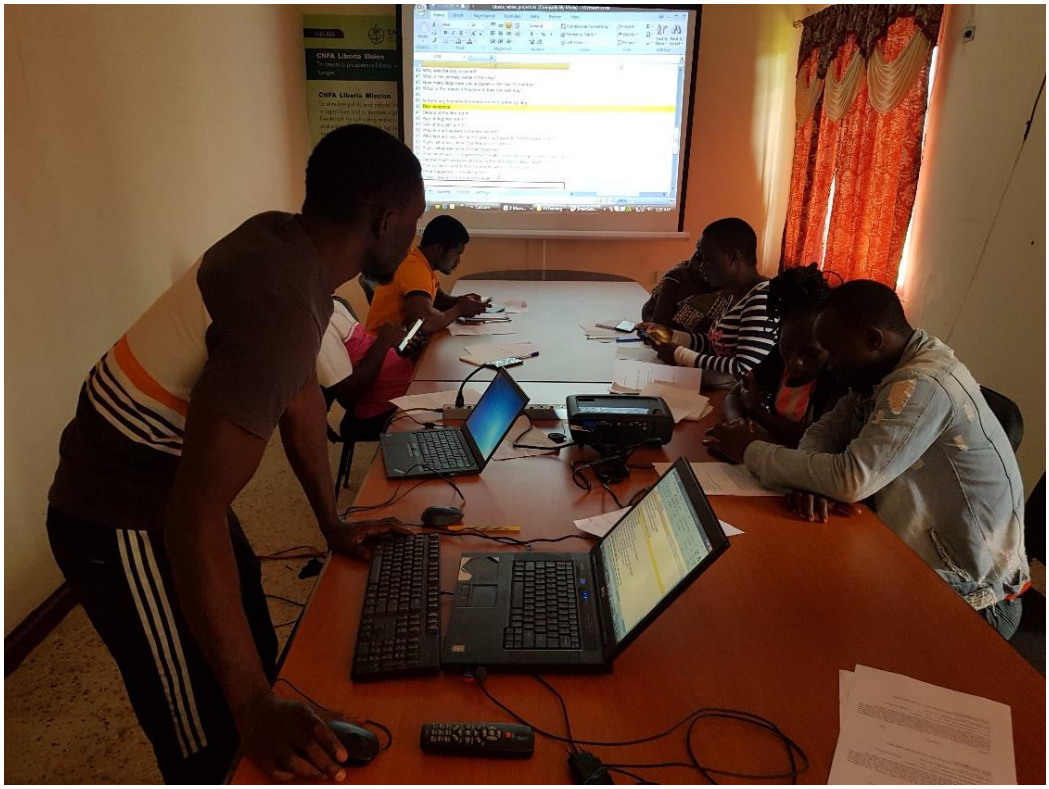
44. What to do when one sees a rabid dog?
- Immediately kill the ☐
- Report to an animal health worker ☐
- Capture and observe the dog for 14 days ☐
- Escape ☐
- Nothing ☐
- Don't know ☐
- No response ☐

45. Do you think dog meat is good for consumption?
- Yes ☐
- No ☐
- No response ☐
- Don't know ☐

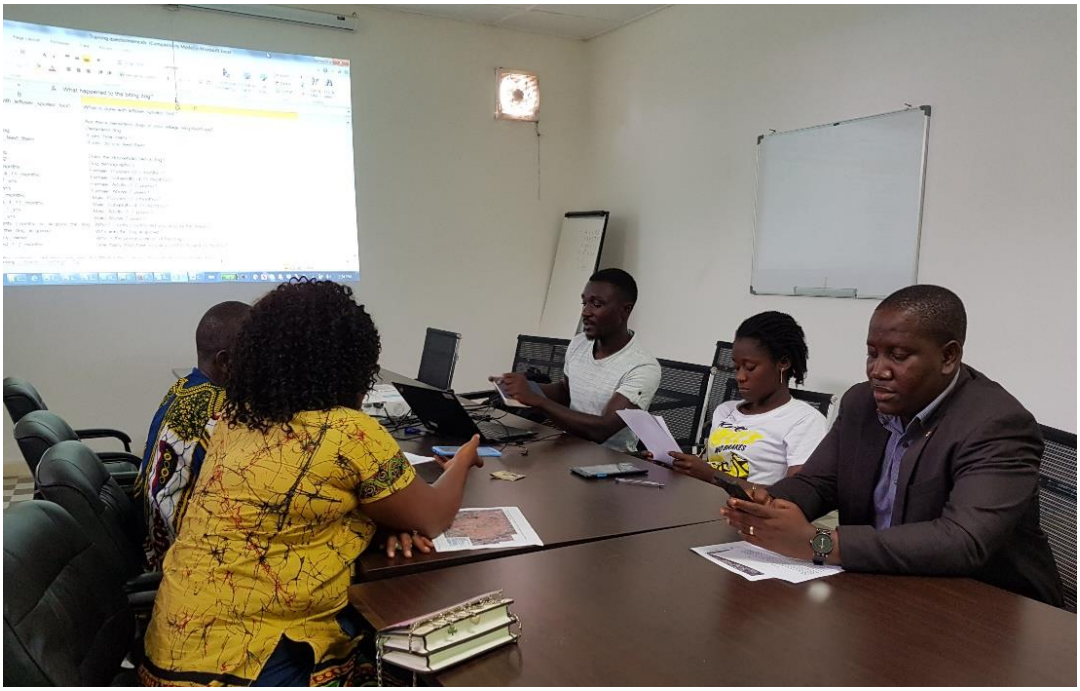
46. Investigator to smile: Have you tasted dog meat?
- Yes ☐
- No ☐
- No response ☐
- Don't know ☐

47. Do you know someone wo eats dog meat?
- Yes ☐
- No ☐
- No response ☐
- Don't know ☐
- Yes ☐

Appendix 2: Pictures from the field, Liberia



Picture 1: Training of interviewers on the survey questionnaire, 101 Building in SKT Bong County, 2019



Picture 2: Training of interviewers on the survey questionnaire, Epi. Center, Montserrado County, 2019

Picture 3: Survey team gathered for fieldwork, rural Bong County, Liberia, 2019

Picture 4: Survey team gathered for fieldwork, urban Montserrado County, Liberia, 2019

Picture 5: Conducting interview in rural Bong County, 2019

Picture 6: Conducting interview in rural Bong County, 2019

Picture 7: Helping a study participant after interview, Bong, 2019

Picture 8: Conducting interview in urban Montserrado County, 2019 (slum area

Picture 9: Had a breakdown while traveling for interview, Bong, 2019

Picture 10: Some obstacles encounter during the fieldwork in, rural Bong County, Liberia, 2019

Picture 11: Training of Surveillance Officers on animal brain sampling, Central Vet. Lab, Montserrado, Liberia, 2020

Picture 12: Training of Surveillance Officers on RIDT testing, Central Vet. Lab, Montserrado, Liberia, 2020