DEVELOPMENT AND EVALUATION OF A NOVEL VACCINE STORAGE DEVICE FOR EFFECTIVE VACCINATION STRATEGY AGAINST RABIES

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A THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY OF SOKOINE UNIVERSITY OF AGRICULTURE. MOROGORO, TANZANIA.

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EXTENDED ABSTRACT

Canine-mediated human rabies has the highest case fatality rate of any known infectious disease and kills approx. 59,000 people annually, with most deaths being children under 15 years. The vast majority (99%) of these fatalities occur in Africa and Asia, where access to appropriate Post Exposure Prophylaxis (PEP) is limited and where canine vaccination is either inconsistently applied or, commonly, non-existent. Epidemiological studies have shown that rabies has epidemiological features that make elimination a realistic and feasible goal, with success demonstrated by comprehensive mass dog vaccination programmes in the Americas, Western Europe and Japan. In rabies endemic countries, dogs are often vaccinated using annually delivered central point clinics at the village level on an annual basis. For this method to be effective at least 70% of the dog population must be vaccinated to maintain herd immunity between campaigns. However, studies have shown that only a few programmes utilising this approach have attained this level of coverage, highlighting the need for alternative approaches to address this challenge.

The current study was carried out to develop an alternative strategy for delivering and maintaining herd immunity over time in low-resource field settings. Through an iterative process of improvement and testing with local communities in northern Tanzania, a passive cooling device “Zeepot” for storage of thermotolerant rabies vaccines was developed at a cost of $11 per unit. The results from a non-inferiority randomised controlled trial showed that the serological response of dogs vaccinated using Nobivac® Rabies vaccine stored within the Zeepot was not inferior to the response of dogs vaccinated using cold-chain stored vaccine ($z = 1.1$, $df = 313$, p-value = 0.25). Indeed, the 28-day post vaccination group geometric mean titre was 1.8 IU/ml and 2.0 IU/ml for dogs vaccinated with vaccines stored under cold-chain versus non-cold-chain respectively. Moreover, the percentage of

dogs that seroconverted in each arm was almost identical (85%). There was a positive linear trend between Body Condition Score (O.R. 2.2, 95% CI: 1.1 – 5.1) and seroconversion, suggesting that dogs of poor condition may not respond as expected to vaccination. Our study demonstrated that the potency of Nobivac® Rabies vaccine is not impacted following storage in Zeepot Clay locally designed Passive Cooling Device.

A total of 17,571 dogs were vaccinated in the field study that was conducted to evaluate the effectiveness of the standard delivery method also known as Central Point Vaccination (CPV) and a novel strategy, Decentralized Continuous Vaccination (DCV), 2654 dogs were vaccinated using CPV and 14,917 dogs were vaccinated through three sub-strategies of DCV. At time point 1 (1 month after the initial vaccination campaign), the vaccination coverage achieved by DCV coverage was higher (64.10%; 95CI 62.12 – 66.04) than for CPV (35.86%; 95CI 32.59 – 39.51). Similarly, at time point 2 (11 months after the vaccination campaign) the coverage in DCV (60.67%; 95CI 58.47 – 62.84) remained high while the coverage in pulse vaccination was lower (32.10%; 95CI 28.82-35.57). Overall, > 84% of the respondents were satisfied with the vaccination services they received. DCV has the potential to consistently maintain population level immunity.

Overall, this thesis showed that Zeepot Clay has a utility for storing thermotolerant vaccines at sub-ambient temperatures. Moreover, DCV has the potential to consistently maintain population level immunity making it a useful strategy to further investigate for scaling up of mass dog vaccination in East Africa. Together the findings of this work have potential application for rabies elimination programmes, particularly in achieving the global target of zero human deaths by 2030 and potentially for use in other vaccination programmes aiming to reach remote or inaccessible communities.

DECLARATION

I, **Ahmed Kambinda Lugelo**, do hereby declare to the Senate of Sokoine University of Agriculture that this thesis is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution for a higher degree award.

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DEDICATION

I dedicate this thesis to my parents, Hamidu Mohamed Lugelo (who passed away during the write up of this thesis) and Anna Augustino Haule, my wife Judith Frank Msomba, my children Jax, Edgar and Sarah for their patience, encouragement, and moral support throughout the time of my study.

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LIST OF PAPERS

This PhD Thesis is organized in a Publishable Manuscript format. It is composed of three Journal Papers written and formatted in line with Publishers' guidelines. Summarized hereunder are details of these documents:

PAPER 1

Lugelo, A.; Hampson, K.; Bigambo, M.; Kazwala, R.; Lankester, F. Controlling Human Rabies: The Development of an Effective, Inexpensive and Locally Made Passive Cooling Device for Storing Thermotolerant Animal Rabies Vaccines. *Trop. Med. Infect. Dis.* **2020**, *5*, 130. https://doi.org/10.3390/tropicalmed5030130

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PAPER 3

Lugelo, A.; Hampson, K.; Ferguson, E.A.; Czupryna, A.; Bigambo, M.; Duamor, C.T.; Kazwala, R.; Johnson, P.C.D.; Lankester, F. Development of Dog Vaccination Strategies to Maintain Herd Immunity against Rabies. *Viruses* **2022**, *14*, 830. https://doi.org/10.3390/v14040830

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LIST OF ABBREVIATIONS AND SYMBOLS

AAS	African Academy of Science
AESA	Alliance for Accelerating Excellence in Science in Africa
ANOVA	Analysis of variance
AOA	Afrique One ASPIRE
APHA	Animal and Plant Health Agency, UK
ASPIRE	African Science Partnership for Intervention Research Excellence
BCS	Body condition score
BHK	Baby Hamster Kidney
CI	Confidence interval
CNS	Central nervous system
COSTECH	Tanzania Commission for Science and Technology
CPC	Central Point Clinics
CSV	Challenge virus standard
DCV	Decentralized continuous vaccination
dFAT	direct Fluorescent antibody test
DVO	District Veterinary Officer
FAVN	Fluorescent Antibody Virus Neutralization
FITC	Fluorescein isothiocyanate
GARC	Global Alliance for Rabies Control
GAVI	Global Alliance for Vaccines and Immunisation
HHQ	Household questionnaire
IHI	Ifakara Health Institute
IRB	Institutional Review Board

IU	International units
LFO	Livestock Field Officer
LGA	Local Government Authority
LMIC	Low-and-Middle Income Countries
MDV	Mass Dog Vaccination
NARCES	National Rabies Control and Elimination Strategy
NEPAD	The New Partnership for Africa's Development Planning and Coordinating
NIMR	National Institute for Medical Research Institute
OHC	One Health Champion
OIE	World Organisation for Animal Health
PCD	Passive Cooling Device
PEP	Post-exposure prophylaxis
RC	Rabies Coordinator
RVSNA	Rabies virus-specific neutralising antibodies
SD	Standard deviation
SDD	Solar Direct-Drive
SUA	Sokoine University of Agriculture
TAWIRI	Tanzania wildlife research
$TCID_{50}$	Median Tissue Culture Infectious Dose
™	Trademark
TPP	Thematic Training Program
UK	United Kingdom
USA	United States of America
WHO	World Health Organisation
WOAH	World Organisation for Animal Health

CHAPTER ONE

1.0 INTRODUCTION

Canine rabies causes approximately 60 000 human deaths every year worldwide, with most of these victims being children under 15 years of age (Hampson *et al.,* 2015). More than 95% of these fatalities occur in LMICs, where the disease is prevalent due to lack or failure of disease control programs or limited access to post-exposure prophylaxis (PEP) (Hampson *et al.,* 2009b). Canine rabies is among the oldest known infectious diseases, and hence has been extensively studied by scientists over the centuries. Considerable effort has been invested in understanding various aspects of canine rabies, including aetiology, pathogenesis, epidemiology, diagnosis, and control (Singh *et al.,* 2017; Fooks *et al.,* 2014). Recent studies have focused on developing tools and demonstrating evidence-based interventions that could be used to eliminate the disease globally (Fooks *et al.*, 2014).

Epidemiological studies have revealed key features that make rabies elimination a realistic goal. These include demonstrating that i) domestic dogs are the principal reservoir host in Africa and Asia, and evidence to suggests that vaccinating domestic dogs prevents transmission of the disease both within the dog populations and thus to other secondary hosts, including wildlife and humans (Haydon *et al*., 2002; Lushasi *et al*., 2021) ii) for rabies, the basic reproductive number, R_0, which is defined as the number of secondary cases resulting from a single case in a fully susceptible population (Anderson and May, 1992), across different ecological settings is consistently low (~1.2), also supporting the possibility of controlling rabies through vaccination (Hampson *et al.,* 2009b) iii) vaccination coverage of 70% is adequate to disrupt cycles of infections within the host population and other secondary hosts (Townsend *et al.,* 2013) iv) most of the domestic

dogs in sub-Saharan Africa are owned and therefore accessible for vaccination (Kayali *et al.,* 2003).

In demonstration of the feasibility of elimination of canine rabies, comprehensive mass dog vaccination campaigns carried out in the United States (US), Western Europe, Japan and other developed countries have successfully brought canine rabies under control (Rupprecht *et al.,* 2002) . Similar efforts carried out in Latin America have achieved good progress (Belotto *et al.,* 2005). This evidence has led the tripartite of WOAH, OIE and WHO uniting in their efforts and working together with the aim to global eliminating canine-mediated human rabies by 2030.

A successful immunization program typically depends on the presence of a well-functioning cold chain system, availability of vaccines and vaccine delivery systems. However, in many rabies endemic countries these infrastructures are lacking or not fully developed due to high cost of installation, maintenance, or unreliable electric power, especially in remote areas (McCarney *et al.*, 2013). Thus, it can be difficult for immunization programs to deliver vaccination campaigns in those areas. The constraints of storing and transporting vaccines in areas without access to or with unreliable power have inspired the development of electric-free vaccines storage devices also called *off-grid refrigerators* (S.-I. Chen *et al.*, 2015; Robertson *et al.*, 2017). Despite their usefulness, the batteries used to run off-grid refrigerators have limited capacity and are costly to maintain (Lloyd and Cheyne, 2017; McCarney *et al.*, 2013). As a response to this challenge, another generation of off-grid refrigerators “Solar direct- drive refrigerators” which do not rely on batteries to operate have been developed (Dawoud, 2007). These Solar direct- drive refrigerators use the solar power to freeze the cooling media into ice banks, which are then used to keep the inside of the equipment cool. Like the predecessor generation, the Solar

direct- drive refrigerators are costly to purchase and maintain, limiting their use (Haidari *et al.,* 2017). As a consequence, manufacturers of cold chain equipment have directed their efforts to develop another generation of vaccine storage devices, Passive Cooling Devices (PCDs) (Gasperino and Yildirim, 2011; Norman *et al*., 2013), that do not require power (electric, solar or gas) to keep vaccines cool. Rather, PCDs use cooling media such as ice packs or water, and insulation to maintain a cool storage environment (Chen *et al.,* 2015). PCDs can maintain the vaccines at the correct temperature for over 10 days without the need of electric power. A variety of PCDs are on the market with the ability to keep vaccines cool even in very hot environments, but price remains an obstacle. For example, the ARKTEK™ PCD can keep vaccines between 2-8˚C for up to 4 weeks, but a single carrier cost $1200-$2400. Furthermore, most of the commercially available PCDs require regular replacement of ice, which may not be readily available in the rural areas *(Kahn et al.,* 2017). Therefore, there is a pressing need for the development of simple and affordable PCDs to address these challenges.

A parallel approach to addressing the challenges of the cold chain system has been the development of thermotolerant vaccines. These are vaccines that can be stored outside the traditional refrigeration temperatures of 2˚C to 8˚C for extended periods without losing their potency (D. Chen and Kristensen, 2009; Kumar *et al.*, 2022). As a result, several of thermotolerant vaccines have been produced and are available on the market (Sutanto *et al.*, 1999; Zipursky *et al.,* 2012; Mariner *et al.,* 2013; Zhang *et al*., 2020). A study conducted in 2015 demonstrated that the commonly used Nobivac® rabies vaccine was thermotolerant following storage at fixed experimental conditions at 25˚C for 6 months and 30˚C for 3 months (Lankester *et al.,* 2016). This is a promising development as thermotolerant vaccines played a major role in successful eradication of both smallpox and rinderpest (Henderson and Klepac, 2013; Mariner *et al.,* 2013). The application of findings

from the Lankester *et al*. (2016) study could bring an equally transformative effect on the global elimination of canine-mediated rabies; however, it remains unknown whether the potency of the rabies vaccine will not be impacted following storage in a locally made PCD.

The strategy for delivering vaccines is another important factor that determines the success of an immunization program. In most of rabies endemic countries, mass dog vaccination campaigns are conducted on the yearly at central-point vaccinations (CPV) (Cleaveland *et al.,* 2003; Durr *et al.*, 2009; Mazeri *et al.*, 2018; Evans *et al.*, 2019). Empirical studies and mathematical modelling have shown that for this approach to be effective at least 70% of the dog population must be vaccinated annually to maintain herd immunity above the critical level of 20-45 per cent between campaigns (Coleman and Dye, 1996; Hampson *et al.*, 2009). This level of coverage is needed due to the fast turnover in the dog population, and loss of vaccine-induced immunity (Arief *et al*., 2016; Beran, 1982; Kitala *et al*., 2002). However for various reasons, only a few programs utilizing this strategy have managed to attain the required 70% coverage (Cleaveland *et al.*, 2003; Kitala *et al.*, 2002; Mulipukwa *et al.*, 2017). This underscores the need to develop alternative strategies capable of maintaining adequate coverage. Decentralized continuous vaccination (DCV) of new dogs entering the population may be able to maintain higher level of coverage between annual campaigns.

1.2 Problem Statement and Justification

Rabies remains a major public health problem in developing countries. Neglected and ineffectively controlled in many parts of the world, rabies is responsible for an estimated 55 000 to 70 000 deaths globally each year (WHO, 2013). Besides human mortality, the economic burden of rabies in developing countries is significant. The total costs to

complete PEP according to WHO guidelines in Tanzania is estimated to be $100 per person (Sambo *et al.*, 2013), which is higher than previously estimated US$ 39.57 (WHO, 2013). Globally, the economic losses caused by rabies are estimated to be ~ US$8.6 billion annually, with the highest proportion of this being the cost of seeking PEP (Hampson *et al.*, 2015). The burden of rabies in Africa is estimated to be around US$20 million, while the cost of PEP to patients is typically around US$40 per patient in Africa and US$ 49 in Asia, accounting for 6% and 4% of annual per capita gross national income, respectively. Rabies also impacts on livestock and other domestic animals such as donkeys, camels and horses (Knobel *et al.*, 2005; Shwiff *et al.*, 2013; Jibat *et al.*, 2016).

Rabies is endemic in Tanzania, causing more than 1 500 human deaths annually (Cleaveland *et al.*, 2002). The disease was first reported in 1930s in the southern regions and has since then been reported in other parts of the country (Sambo *et al.*, 2013). Like in many other rabies endemic countries (Kayali *et al.*, 2006), in Tanzania there is no official or regular government intervention strategy against rabies. Epidemiological modelling and historical data collected as part of the ongoing rabies project in Mara region over the period of two decades indicate that on average around 2 500 people are exposed to animal bites each year, and between 20 - 40 of them died of rabies (*unpublished*). However government authorities have conducted sporadic campaigns when there are outbreaks (Cleaveland *et al.*, 2003; Hampson *et al.*, 2009). An effective and sustainable rabies control strategy able to achieve comprehensive vaccination coverage, including in hard-to-reach areas, is needed to control rabies in Tanzania and countries where rabies remains stubbornly endemic.

The findings from this study are likely to be integrated in the National Rabies Control and Elimination Strategy, which in turn will immediately or later influence the scaling up of

rabies vaccination campaigns across the Mara region and potentially throughout the whole country.

1.3 Research Questions

The following key research questions underpin the objectives of this study

1. What is the cost of manufacturing a PCD that can keep the vaccine temperatures below 30^0C even under fluctuating field conditions?
2. Is the immunological response of dogs immunized with Nobivac® Rabies vaccine kept in a locally-made PCDs inferior to those of dogs immunized with the same vaccine kept under cold chain, at 2^0C -8^0C?
3. What is the vaccination coverage achieved by CPV and DCV at one month and nine months post vaccination?

1.4 Objectives of the Study

1.4.1 Main objective

The main objective of the study was to develop and pilot alternative strategies for dog vaccination for rabies control and elimination in a low-resource field setting.

1.4.2 Specific objectives

i. To develop and evaluate the performance of PCDs for storage of Nobivac vaccine in rural conditions in Tanzania.
ii. To determine if Nobivac® Rabies vaccine remains efficacious following storage in PCDs under fluctuating ambient temperatures.
iii. To evaluate vaccination coverage achieved through the standard delivery method “CPV”, and through a novel method, decentralized continuous vaccination (DCV), at one month and nine months post-vaccination.

1.5 The Study Location

The study was carried out across five administrative districts of the Mara region (34°-35°E, 1°30´-2°10´S) in northern Tanzania. These districts are Bunda, Butiama, Rorya, Serengeti and Tarime. The districts are composed of multi-ethnic, agro-pastoralist communities. According to the 2022 national population and housing census (NBS, 2022), approximately 1 941 178 people live in the study area, i.e., in the 5 selected districts. The dog population in these districts was estimated to be 323 530 dogs based on the human to dog ratio of 6:1. The study area was chosen purposely to take advantage of the robust rabies research platforms that have been active in the areas for the past three decades. Such platforms provided easy access to field infrastructure and interactions with experienced rabies researchers.

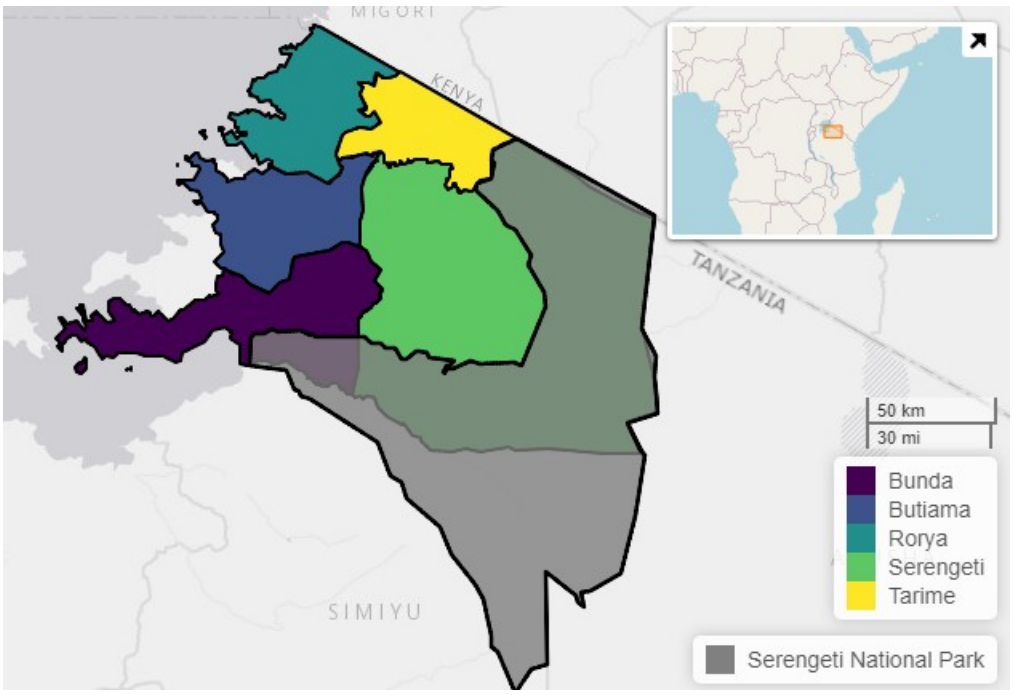

Figure 1.1: Map of the Mara region showing the location of the study district

Study Design

The study was undertaken in three phases, with the outputs from the first phase (Objective 1) forming the foundation for the activities in the next phase (Objective 2). Firstly, the study commenced with the development and field testing of a range of PCDs. A one-health-based collaborative, multisectoral & trans-disciplinary approach was employed in the development of PCDs. This component of the study was conducted in the Serengeti district, and further field testing was carried out in Tarime and Bunda districts to validate the performance of the selected prototype. Following the completion of the validation process, the manufactured device was deployed for storage of the vaccine in the immunogenicity study (Objective 2). Secondly, a randomized controlled trial was conducted to assess the impact of vaccine storage conditions on serological response. The study involved a population of owned-free roaming dogs in the Serengeti district. Blood samples were collected from dogs, and the Fluorescent Antibody Virus Neutralization assay was used to measure the antibody titres. Lastly, a field vaccination trial was implemented to assess the effectiveness of vaccination strategies. This part of the study capitalised on the results obtained from Objectives 1 and 2. Household surveys were conducted to collect data on the vaccination of dogs in the study villages. Mixed method approaches were used to analyse the data.

1.6 Organization of the Thesis

This thesis describes the development and assessment of i) PCDs for storage of a thermotolerant rabies vaccine and ii) vaccination strategies to maintain herd immunity against rabies.

Chapter 1 Lay the foundation of this thesis by giving an overview of the development of the cold chain industry and methods of vaccination against rabies. Focusing on the cold

chain industry, it discusses the strengths and limitations of various cold chain equipment and how these limitations have led to the evolution of a new generation of cold chain equipment called PCDs. It also highlights the usefulness and challenges of the methods used for the control of rabies in Africa and Asia and proposes alternative strategies to overcome the identified challenges.

Chapter 2 describes the development of the passive cooling device and subsequent field testing. In brief, I used a participatory community engagement approach to gather ideas on the feasibility of manufacturing locally made low-tech cooling devices for storage of thermotolerant rabies vaccine. In consultation with local manufacturers, we developed range of prototypes of PCD and carried out a series of field tests, before selecting the final product for storage of vaccine.

In chapter 3 I describe an investigation to determine the efficacy of the Nobivac vaccine following storage in the PCD developed in chapter 2. I ran serological analyses to compare immunological responses of dogs vaccinated with vaccine stored in the novel device and those vaccinated with vaccine stored in the refrigerator. The aim of the investigation was to determine the impact of storage conditions, and dog body condition, sex and age on seroconversion.

Chapter 4 describes the development, implementation, and evaluation of dog rabies vaccination strategies. I built on results from chapter 3 to develop novel strategies for maintaining herd immunity against rabies. Specifically, I deployed a developed PCD in all wards selected in the study and used them to store batches of vaccine. I trained rabies vaccinators on several aspects of the study including how to vaccinate dogs and cats

according to their designated strategy, collect and submit vaccination data, and to store vaccines and take care of the developed PCD.

In chapter 5 I summarize the key results of the research and draw general conclusions to guide policy on the best way to conduct rabies control programs in Tanzania and other countries facing similar challenges.

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CHAPTER TWO

2.0 CONTROLLING HUMAN RABIES: THE DEVELOPMENT OF AN EFFECTIVE, INEXPENSIVE AND LOCALLY MADE PASSIVE COOLING DEVICE FOR STORING THERMOTOLERANT ANIMAL RABIES VACCINES

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2.1 Abstract

Thermotolerant vaccines greatly improved the reach and impact of large-scale vaccination programmes to eliminate diseases such as Smallpox, Polio and Rinderpest. A recent study demonstrated that the potency of the Nobivac® Canine Rabies vaccine was not impacted following experimental storage at 30˚C for three months. We conducted a study to develop a Passive Cooling Device (PCD) that could store thermotolerant vaccines under fluctuating sub-ambient temperatures. Through a participatory process with local communities in northern Tanzania, we developed innovative PCD designs for local manufacture. A series of field experiments were then carried out to evaluate the effectiveness of five PCDs for vaccine storage under varying climatic conditions. Through an iterative process of improvement and testing, a final prototype "Zeepot Clay" was developed at the cost of $11 per unit. Following a further field-testing phase over a 12-month period, the internal temperature of the device remained below 26^0C, despite ambient temperatures exceeding 42^0C**.** Our study thus demonstrated that locally designed PCDs have utility for storing thermotolerant rabies vaccines at sub-ambient temperatures. These results have application for the scaling up of mass dog vaccination programmes in low-and-middle income countries, particularly for hard-to-reach populations with limited access to power and cold-chain vaccine storage.

Keywords: Rabies; Thermotolerant; Passive Cooling Devices; vaccines

2.3 Introduction

Infectious diseases remain a major public health problem in low-and-middle income countries (LMICs) with approximately 43% of total deaths from these diseases occurring in such countries (Mathers *et al.,* 2009; Rashid *et al.,* 2004). In recent years considerable efforts have been invested to tackle these health problems leading to the production of many safe, effective and high-quality vaccines and the implementation of large-scale immunization programs (Chan, 2014; Ozawa *et al.,* 2017). Indeed, immunization has been shown to be a cost-effective way of controlling and even eradicating infectious diseases (Andre *et al.,* 2008; Dowdle, 1998). For example, in 1966 the World Health Organization (WHO) launched the global campaign to eradicate smallpox which led to the disease being declared eradicated in 1980 (Breman *et al.,* 1980).

The cold-chain system is a crucial component of successful immunization programs and describes the environment in which vaccines are stored and transported within a recommended temperature range (4-8°C) from the manufacturer to the point of immunization, with the aim of maintaining the potency of vaccines. The system consists of cold chain equipment, such as cold rooms, refrigerators, freezers and vaccine carriers, as well as temperature monitoring devices. In LMICs, public health care and veterinary resources are often limited resulting in difficulties maintaining cold chain conditions and unreliable electric power and poor roads often hinder installation of cold chain equipment (McCarney *et al.,* 2013). Technological innovations have begun to tackle these challenges. For example, electric-free vaccine storage devices (*off-grid refrigerators*) have been developed to store vaccines in areas without access to or with unreliable power (Chen *et al.,* 2015; Robertson *et al.,* 2017). However, the batteries used in off-grid refrigerators often have limited capacity and are costly to maintain (McCarney *et al.,* 2013; Lloyd & Cheyne, 2017). Solar direct-drive refrigerators have been developed to replace battery

powered refrigerators (Dawoud, 2007). ‘Direct-drive’ technology uses solar energy to freeze cooling media into ice banks, which are then used to keep refrigerators cold. However, these refrigerators are costly to purchase and maintain, limiting their use (Haidari *et al.,* 2017). Another generation of vaccine storage devices, Passive Cooling Devices (PCDs) (Gasperino & Yildirim, 2011; Norman *et al.,* 2013), do not require power (electric, solar or gas) to keep vaccine cool. Rather, PCDs use cooling media such as ice packs or water, and insulation to maintain a cool storage environment (Chen *et al.,* 2015). A variety of PCDs are on the market with the ability to keep vaccines cool even in very hot environments, but price remains an obstacle. For example, the ARKTEK™ PCD is able to keep vaccines between 2-8^{0}C for up to 4 weeks, but a single carrier cost $1200-$2400. - Cheaper alternatives, affordable in LMICs, are required.

Canine-mediated human rabies is almost vaccine preventable through both provision of post-exposure prophylaxis for people bitten by rabid dogs and large-scale mass dog vaccination programs to interrupt transmission in domestic dog populations. Despite this fact, rabies is responsible for causing over 59 000 deaths every year worldwide, with most occurring in LMICs (Knobel, 2005). Mass dog vaccination (MDV) is the control measure that offers the prospect of eliminating rabies at source. However, to implement MDV programs at the scale required to control the disease requires functioning cold chain systems, availability of vaccines and vaccine delivery systems. Given the challenges many rabies endemic countries face with lack of provision of power, especially in remote areas, benefits could be achieved through innovations in the development of thermotolerant vaccines and effective and affordable vaccine storage systems that allow vaccines to be stored below ambient temperatures.

In recent years, efforts have been directed to addressing the challenges of storing rabies vaccine in remote areas without cold chain infrastructure. A study conducted in 2015 determined that the Nobivac™ Canine Rabies vaccine (MSD Animal Health, Boxmeer, Netherlands) remained potent following storage at 25˚C for six months and at 30˚C for three months (Lankester *et al.,* 2016). These findings opened up the possibility of storing vaccines outside of refrigeration units, an outcome that could benefit rabies control in remote areas where electricity provision is poor. Keeping vaccines in such locations would still require some form of storage units and given that temperatures over 30˚C were shown to impact vaccine potency (Lankester *et al.,* 2016), the temperature inside such storage units should be as cool as possible. This study describes the development and subsequent testing of a PCD to store rabies vaccines for extended periods in advance of MDV campaigns. Key objectives were that the PCD be designed and developed using local knowledge and affordable materials. Given that a commonly used canine rabies vaccine is thermotolerant, the development of an effective PCD, that allows storage of vaccines at sub-ambient temperatures, could greatly benefit the scaling up of national rabies control and elimination strategies, which are currently being developed across East Africa.

2.4 Materials and Methods

The study, which was conducted in the Mara region in northern Tanzania, comprised four phases: PCD design and development, field testing, refinement and final testing.

2.4.1 Phase I: PCD design and development

Initially a one-day workshop was held that involved forty-five stakeholders from the Ministry of Livestock and Fisheries Development, representatives from the Mara region community, public health professionals and researchers. The objectives of the workshop were two folds i) to communicate research findings from (Lankester *et al.*, 2016) study

which demonstrated the thermotolerance of Nobivac rabies vaccines ii) to allow workshop delegates to use these findings to formulate design of PCDs which could be locally made and used to store rabies vaccine for extended periods. Participants were divided into groups and were asked to discuss among themselves potential designs that can be made from locally available materials and keep inside temperature below 30°C. After formulating the designs in the paper, participants were asked to present and illustrate them. A general group discussion was employed to rank each suggested design in term of effectiveness at keeping the temperature low. At the end of this activity, five PCD designs were selected for prototype development and testing. Following the workshop, local product designers, with the required skills to manufacture the suggested PCDs, were recruited and given illustrations to build a prototype of each design.

2.4.2 Phase II: Field testing

Field testing of the five prototype PCDs, shown in Figure 1, was carried out in Bonchugu village in the Serengeti district. A locally typical house was selected as the venue for testing. The house was divided into two equal compartments, the first a closed area with walls that completely sealed the room (indoor), and the second a porous compartment with open walls (outdoor) as shown in Figure 1. An identical set of prototypes was placed in the indoor and outdoor areas. Each prototype was equipped with a digital temperature data logger (Sensormetrix, www.sensormetrix.co.uk) configured to record daily temperatures at intervals of three hours over a two-week trial period. In addition, the peak ambient temperature was recorded each day. The performance of each PCD was evaluated by comparing the means and temperature ranges of the prototypes. Similarly, the performance of each prototype in the indoor and outdoor areas was compared. Selection criteria for the next phase of the study were the ability of the prototype to maintain storage temperatures below 30^0C, with minimal

variation, the cost of PCD production, its storage capacity and cooling media replenishment frequency.

(a) (b)

Figure 2.1: PCD designs stored indoors (A) and outdoors (B). The different PCDs are labelled as follows: i) Zeepot cementsand, ii) Zeepot Clay, iii) Coolbox sand, iv) Coolbox sawdust and v) Coolgardie.

2.4.3 Phase III: Refinement

The two prototype designs that were selected during Phase II (field testing) were improved using feedback provided by the field-testing team and then were subjected to a further two-month period of testing. Data analysis and performance appraisal of each prototype was undertaken according to the previously described criteria. At the end of Phase III, a single prototype was selected to proceed to Phase IV (final testing).

2.4.4 Phase IV: Final testing

The performance of the selected prototype was evaluated over a 12-month period in a single location. The selected PCD was placed within a typical building with temperature loggers inside the PCD to record internal temperatures and on an internal building wall to record ambient temperatures at 3-hour intervals. Further to the 12-month test, the performance of the selected prototype was also tested in three separate sites with different weather conditions for two months, during the warm season from 4th March to 3rd May 2019. The selected sites represented a range of temperature environments. Sirari village in Tarime district typically experiences relatively low temperatures with an average temperature during the final testing

period of 21^{0}C; Mugumu town in Serengeti district experiences more moderate temperatures (average temperature 24^{0}C) and Bokore village in Bunda district experiences higher ambient temperatures (average temperature 26^{0}C) based on recent average ambient temperature data from the Tanzania Meteorological Authority website (http://www.meteo.go.tz/). Descriptive statistics were used to analyze the logged temperature data. The null hypothesis that the means of the temperature across prototypes were equal was tested using ANOVA. Timeseries and boxplots were used to examine temperature trends and distributions. All data were analyzed using the R statistical computing language (R Development Core Team, 2019).

2.5 Results

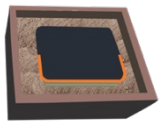
2.5.1 Phase I: PCD design and development

The five novel designs of PCDs that were selected for prototype development during the workshop and the ranking they received is shown in Table 2.1. Pot-in-Pot systems: The system that delegates ranked the highest was a ‘pot-in-pot’ system (consisting of an inner pot nested within a larger outer pot) that exploited the evaporation of water as a cooling mechanism. The pot-in-pot system included a middle compartment filled with absorbent materials (multi-layer) providing an effective barrier against heat exchange between the external surroundings and the cooling compartment. The design was named a ‘Zeepot’ after a traditional food storage pot commonly used in Africa to keep vegetables fresh. Two different Zeepot designs were suggested for development, i) Zeepot Clay and ii) Zeepot Cementsand. The two Zeepot prototypes were similar in design, however the Zeepot Clay was made from clay soil whereas the Zeepot Cementsand was made from cement and sand (Figure 2.2).

Cool Box systems: Two cool box system prototypes (Coolbox-sand and Coolbox-sawdust) were selected for development. The cool box systems were constructed using ready-made cool boxes (*Princeware*) sold in local shops placed inside a rectangular box made from wooden materials. Sand or sawdust was added into the compartment between the constructed box and the cool box to form Coolbox-sand and Coolbox-sawdust, respectively (Figure 2.1).

Coolgardie system: This system was manufactured using a combination of the constructed wooden box with its insides lined with cloth. A five-litre plastic bottle was placed on top with two drainage pipes connecting the bottle and the cloth (Figure 2.1). The Coolgardie system required replenishment of water every 3 hours.

Table 2.1: PCD models developed by local designers and used in the first round of testing.

Design name	Zeepot Clay	Zeepot Cementsand	Coolbox Sand	Coolbox Sawdust	Coolgardie
Shape					
Materials	Clay	Cement, sand	Wood, coolbox (*Princeware*), 6ltrs	Wood, coolbox (*Princeware*), 6ltrs	Wood, fabric, plastic
Manufacturing time (Days)	7	7	4	4	5
Production Cost (US$)	11	30	17	17	26
Absorbent	Sand	Sand	Sand	Sawdust	Fabric
Cooling media	Water	Water	Water	Water	Water
Estimated lifespan (years)	7	>10	2	2	1
Ranking	1	4	2	3	5

2.5.2 Phase II: Field testing and selection

The temperatures recorded within the candidate prototypes during the field testing and selection phase are given in Table 2.2. The internal temperature range of all five prototypes that were stored indoors ranged from 15.5°C to 21.6°C, whilst the internal temperature range of the same prototypes that were stored in the outdoor area was wider, 15.1^{0}C to 24.3^{0}C. This suggests that storing the PCDs inside a building with solid walls was preferable. Across the five PCD prototypes, the Coolgardie PCD stored in the indoor compartment had the lowest mean internal temperature (18.2°C). However, because the Coolgardie required a continuous supply of water to achieve this result, its use was

determined to be too costly and this design was not considered for the next phase. Excluding the Coolgardie, the designs with the lowest mean internal temperatures were the two Zeepot PCDs (Clay and Cement Sand) with 18.6^{0}C and 19.3^{0}C, respectively. Relative to the other prototypes investigated, the Zeepot Clay also had the narrowest temperature variation (standard deviation of 0.8 vs 1.2 of the Coolgardie Indoor). As a result, the two Zeepot PCD prototypes were both selected to proceed to Phase III (refinement).

Table 2.2: Summary statistics of the temperatures recorded within each PCD prototype during the initial 2-week testing period

Prototype	**Location**	**Temperature range in ˚C**	**Mean temperature**
Zeepot Clay	Indoor	16.6 – 20.5	18.6 (0.8)
	Outdoor	16.5 – 22.0	19.1 (1.1)
Zeepot Cementsand	Indoor	16.9 – 21.6	19.3 (0.9)
	Outdoor	16.4 – 22.0	19.6 (1.2)
Coolbox sand	Indoor	16.5 – 21.4	18.9 (1.0)
	Outdoor	16.4 – 24.3	20.1 (2.1)
Coolbox sawdust	Indoor	16.8 – 21.5	19.3 (1.0)
	Outdoor	16.5 – 23.4	20.0 (1.8)
Coolgardie	Indoor	15.5 – 20.7	18.2 (1.2)
	Outdoor	15.1 – 22.7	18.9 (1.9)

(a) (b)

Figure 2.2: Second generation Zeepot (a) Clay and (b) Cementsand prototypes manufactured and tested during the refinement phase

2.5.3 Phase III: Refinement

The Zeepot PCD design improvements from Phase II (field-testing) included increasing the volume from a storage capacity from 3.7 litres (which would allow storage of 40 vials of vaccine) to 23.4 litres (which would allow storage of 550 vials), strengthening the external clay wall and re-designing the lid seals to fit more tightly (Figure 2.2). Following these improvements, the second generation of Zeepot Clay and Zeepot Cementsand prototypes were manufactured and subjected to a two-month period of testing. The results from the testing are shown in Figure 2.3. Both Zeepot prototypes maintained internal temperatures below 24°C, however, because the temperatures were consistently lower within the Zeepot Clay, this PCD prototype was selected to proceed to Phase IV (final testing).

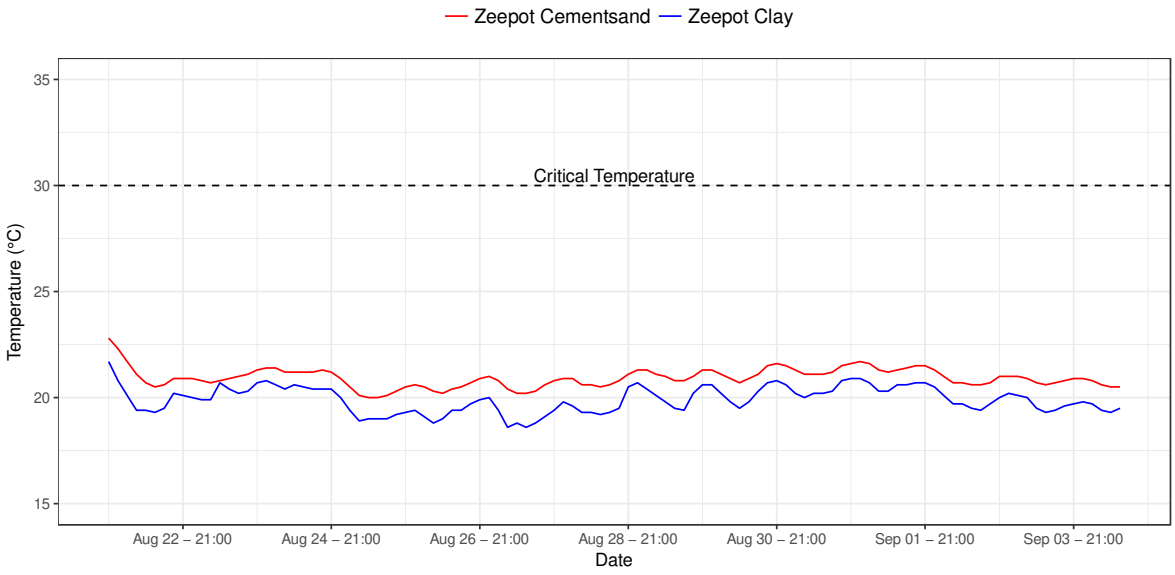


Figure 2.3: Time series showing the performance of the improved Zeepot Clay (red line) vs Zeepot Cementsand (blue line) designs. The critical temperature above which the potency of thermotolerant rabies vaccines decrease is shown with a broken line.

2.5.4 Phase VI - Final testing

The results from the 12-month trial conducted in Bonchugu village, Serengeti district are shown in Figure 2.4. The mean ambient temperature was 25.5˚C (19.1˚C - 32.1˚C, standard deviation, SD = 2.6), whilst the mean temperature within the Zeepot Clay was 21.0˚C (18.3˚C - 25.2˚C, SD = 1.5). Thus, despite ambient temperatures fluctuating by more than 10˚C during the year and maximum temperature exceeding 32˚C, the temperature inside the Zeepot Clay remained relatively constant and the maximum temperature remained well below the critical temperature of 30˚C.

Summary statistics on the internal temperatures of each Zeepot Clay and the ambient temperatures across the three locations over the two-month testing period are given in Table 2.3 and their performance is compared in Figure 2.5. The highest ambient temperature was recorded in Bunda (43.5˚C), whilst the lowest was recorded in Tarime 17.2˚C. At the time of the highest ambient temperature, the temperature inside the Zeepot Clay was 22.4˚C (Figure 2.5). The mean ambient temperature across the three sites was 26.0˚C, with a range of 17.2˚C to 43.5˚C, while the mean temperature inside the Zeepot Clay across the three sites was 21.4˚C, with a range of 20.4˚C to 22.8˚C. The logger used in Bunda district stopped recording the temperature inside the Zeepot Clay just before the end of the trial period (Figure 2.5a).

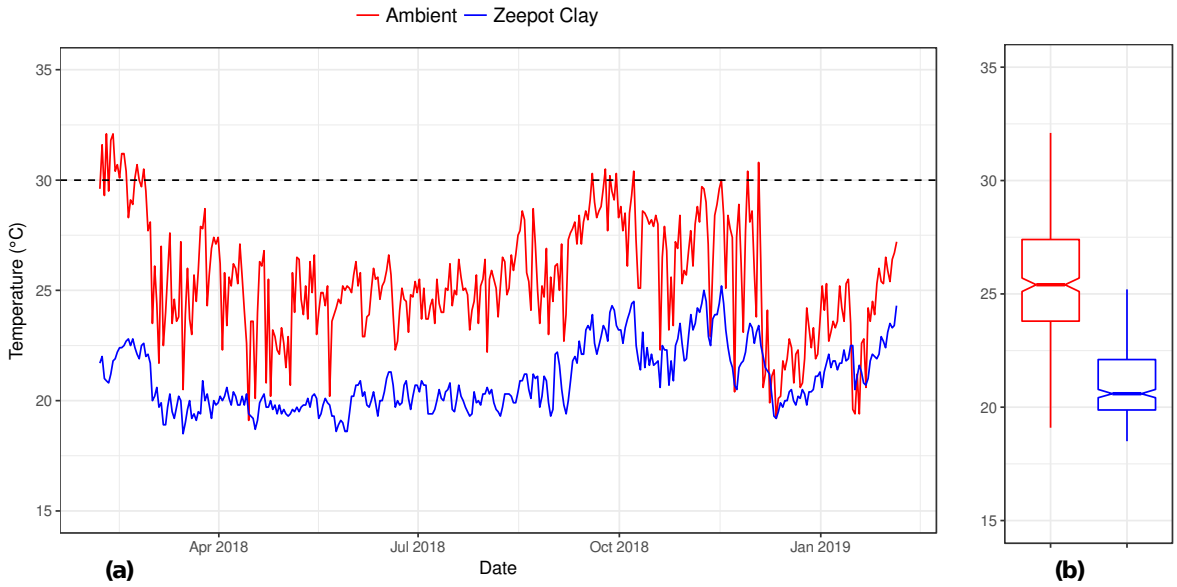


Figure 2.4: (a) Daily timeseries of ambient (red) and internal temperatures within the Zeepot Clay (blue) and (b) median and interquartile range ambient temperatures (red) and Zeepot Clay internal temperatures (blue) over the 12 months. The maximum storage temperature above which the Nobivac™ Canine Rabies vaccine loses potency is indicated by the dashed line.

Table 2.3: The mean ambient temperature and internal temperature of the Zeepot Clay across three sites in Mara region

Site (village, district)	Prototype	Temperature Range in ˚C	Mean temperature	95% CI
Bokore, Bunda	Zeepot Clay	20.7 - 24.5	22.8 (0.9)	22.7-22.9
	Ambient temperature	20.8 - 43.5	28.7 (5.0)	28.2-29.1
Sirari, Tarime	Zeepot Clay	18.9 -23.3	21.0 (0.8)	20.9-21.0
	Ambient temperature	17.2 - 35.2	24.3 (4.9)	23.9-24.7
Mugumu, Serengeti	Zeepot Clay	18.5 - 23.4	20.4 (0.9)	20.4-20.5
	Ambient temperature	18.9 - 32.4	24.9 (3.3)	24.6-25.2

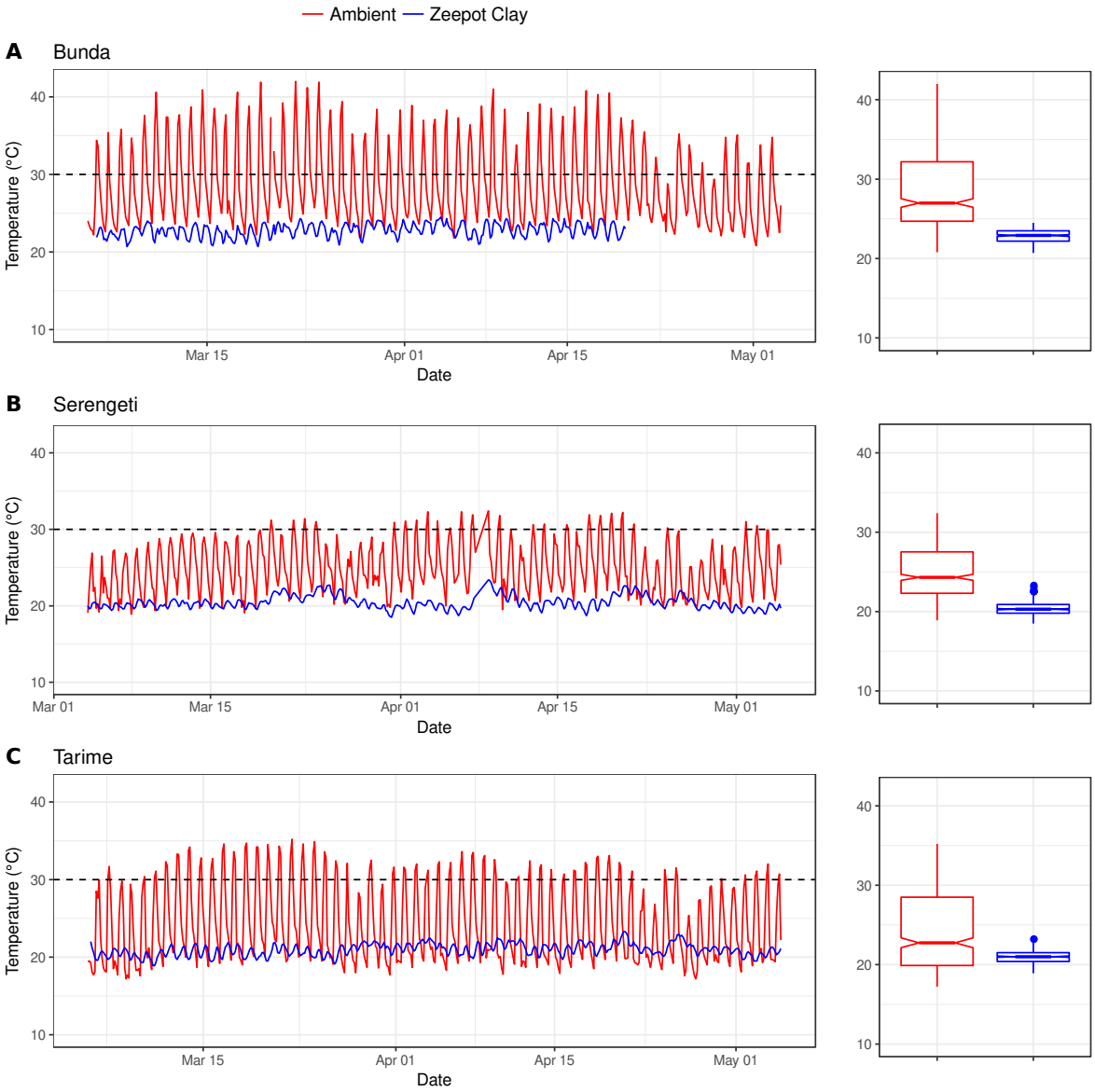


Figure 2.5: Ambient vs storage temperatures within the Zeepot Clay over a 2-month period in three sites (A – C). Timeseries and boxplots indicating the median and range of temperatures in the three districts. The maximum storage temperature above which the Nobivac™ Canine Rabies vaccine loses potency is indicated by the dashed line.

2.6 Discussion

A global target to eliminate human deaths from canine-mediated rabies has been set for 2030 (WHO, 2018). For this goal to be reached, systems for the delivery of mass dog vaccination programmes need to be operational in LMICs with endemic rabies. To address this challenge, we embarked on a study to develop a practical and affordable PCD for

storage of a thermotolerant rabies vaccine in areas with limited electricity. In total, five designs were investigated as potential prototypes for vaccine storage of which one, the Zeepot Clay, was selected as the best performing design. The internal temperature of the Zeepot Clay design remained below 26^0C over a 12-month testing period (maximum temperature of 25.2˚C), despite ambient temperatures exceeding 42^0C, with seasonal and geographical variation having minimal impact on performance. Temperature fluctuations can also have a detrimental effect on vaccine potency (Keja *et al.,* 1988), and so it was encouraging that the Zeepot Clay had the narrowest temperature variation of the prototypes. Our results therefore suggest that the Zeepot Clay could be used for thermotolerant vaccine storage in places where refrigeration is not available. With respect to rabies specifically, we find that the Zeepot Clay is an affordable and effective locally produced PCD that can support the scaling-up of mass dog rabies vaccination campaigns in areas with limited electricity across Tanzania and potentially elsewhere with similar environmental conditions where cold-chain storage of dog vaccines is challenging.

Commercial PCDs have been used in the public sector to store vaccines for tuberculosis, polio, influenza, whooping cough, tetanus, hepatitis B and diphtheria. For example, the Arkteks® PCD was used to deliver vaccines during the 2014-2015 Ebola outbreak in West Africa and in Nepal to assist in the immunization campaign after the 2015 earthquake (Widdowson, 2016; Olsen & Elliott, 2017). However, despite their utility, the price of commercially manufactured PCDs is often very high, ranging from $700-$3000 per unit (Norman *et al.,* 2013). Other designs require substantial maintenance, for example the Arkteks® and the Nano-Q® PCDs which, although both able to keep temperatures inside the vaccine compartment at refrigeration temperature for many days, requires recharging with ice. Whilst the Zeepot Clay is not designed to maintain refrigeration temperatures, it is capable of maintaining temperatures below levels at which thermotolerant vaccines such as

the Nobivac™ Canine Rabies vaccine begin to lose potency (30^0C). And at $11 per unit, the Zeepot Clay is considerably cheaper than commercial PCDs and being made of a material that is readily available in most areas across Sub-Saharan Africa and Asia (clay soil), makes the Zeepot Clay feasible to manufacture locally at a low cost.

Other factors for consideration in the design and use of PCDs are their maintenance demands. All the commercially available PCDs require prior cooling through use of ice or placement inside refrigeration units, and as such none are able to keep vaccines cool for extended periods without power when ambient temperatures are high. For example, the Apex™ Vaccine Carrier Box AIVC 44 that is available in the market at $9.16 and has a storage capacity of 1.67 litres, uses ice packs to keep the vaccine compartment at refrigeration temperature for up to 35.7 hours. The protocol for maintenance of the Zeepot Clay is cheap and simple, requiring only 1.5 litre of water to be added as a coolant once every other day, making it very simple to use with minimal training required. In our study livestock field officers received 1-hour on-site training and were considered proficient to take care of the device. These features suggest that the Zeepot Clay might be an affordable and logistically attractive option for storing thermotolerant rabies vaccines for extended periods in remote settings.

PCDs have been used in public health intervention programmes that have protected millions of people against vaccine preventable lethal diseases (Friend *et al.*, 2015) and, through its ability to store thermotolerant vaccines for extended periods, we consider that the Zeepot Clay will extend this utility of PCDs by enabling penetration of mass dog vaccination campaigns to hard-to reach communities. This will bring clear benefits to national governments planning to scale-up rabies elimination programs. While the original intention for the Zeepot Clay was to store thermotolerant rabies vaccines in areas with no

existing cold chain system, we anticipate that this tool could be used to store other thermotolerant vaccines, however more work is required to confirm this. The Zeepot Clay has only been used to store Nobivac® Canine Rabies vaccine which has been shown to be thermotolerant. If evidence of thermotolerant properties can be demonstrated in other canine rabies vaccines (as expected) or in vaccines used for other vaccine preventable diseases that affect communities living in areas of limited resources, the utility of the Zeepot Clay could be extended.

2.7 Conclusions

Ongoing innovations in the development of PCDs have great potential to address the challenge of vaccine storage for last-mile immunization. Our PCD prototype, the Zeepot Clay, consistently maintained temperatures below 26°C even when the ambient temperature exceeded 40°C. This suggests that, under proper care and monitoring, the tool can be used to store rabies vaccine in areas without electric grid for up to three months without compromising vaccine potency. Storing small batches of vaccines (300-500 vials) in PCDs such as the Zeepot Clay in a 'decentralized' manner across remote landscapes could potentially allow vaccination efforts to reach many more dogs than is currently achieved through the traditional 'centralized' approach. Given that sustaining high levels of vaccination coverage is a critical component of any successful vaccination campaign, this could be of great importance as we strive to eliminate human rabies globally by 2030.

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CHAPTER THREE

3.0 INVESTIGATING THE EFFICIENCY OF A CANINE RABIES VACCINE FOLLOWING STORAGE OUTSIDE OF THE COLD-CHAIN IN A PASSIVE COOLING DEVICE

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3.1 Abstract

Thermostable vaccines greatly improved the reach and impact of large-scale programmes to eliminate infectious diseases such as smallpox, polio and rinderpest. A study from 2015 demonstrated that the potency of the Nobivac® Rabies vaccine was not impacted following experimental storage fixed at 30˚C for three months (Lankester et al., 2016) . Whether Nobivac® Rabies vaccine would remain efficacious following storage under more natural, fluctuating temperature conditions remains unknown. We carried out a randomised controlled non-inferiority trial to compare serological responses in dogs following vaccination with doses stored under cold chain conditions with those stored within a locally made Passive Cooling Device under fluctuating temperature conditions. Our hypothesis was that storage within the PCD would not impact potency. Nobivac® Rabies vaccine was stored under either cold-chain conditions or within the PCD for two months. Daily ambient temperatures and temperatures within the PCD were recorded every three hours. Following storage, 412 domestic dogs were randomly assigned to receive either cold-chain or PCD stored Nobivac® Rabies vaccine. Baseline and day 28-post vaccination blood samples were collected. Serological analysis (Fluorescent Antibody Virus Neutralization assay (FAVN)) was carried out. A threshold of 0.5 IU/ml was used to determine seroconversion. In addition, the impact of dog body condition score, gender and age on the percentage of dogs that seroconverted was carried out. The serological response of dogs vaccinated using Nobivac® Rabies vaccine stored within the PCD was not inferior to the response of dogs vaccinated using cold-chain stored vaccine ($z = 1.1$, $df = 313$, p-value = 0.25). Indeed, the 28-day post vaccination group geometric mean titre was 1.8 IU/ml and 2.0 IU/ml for cold-chain versus non-cold-chain storage, respectively. Moreover, the percentage of dogs that seroconverted in each arm was almost identical (85%). There was a positive linear trend between Body Condition Score (BCS) (O.R. 2.2, 95% CI: 1.1 – 5.1) and seroconversion, suggesting dogs of poor condition may not respond as expected to

vaccination. Our study demonstrated the potency of Nobivac® Rabies vaccine is not impacted following storage under elevated fluctuating temperatures within a PCD. These results have potentially exciting applications for scaling up mass dog vaccination programmes in low-and-middle income countries, particularly for hard-to-reach populations with limited access to power and cold-chain vaccine storage.

Keywords: Dog-mediated rabies; Mass Vaccination; Thermotolerance; Passive Cooling Device, Non-inferiority trial

3.2 Introduction

Canine rabies causes approx. 60,000 human deaths every year worldwide, most of the victims being children under 15 years of age (Hampson *et al.,* 2015). More than 95 per cent of these fatalities occur in developing countries. Since the first rabies vaccine was developed in the 1880s by Louis Pasteur, considerable efforts have been invested in the development of high-quality rabies vaccines for humans and animals (Abraham *et al.*, 2017; Yang *et al.*, 2013). Epidemiological and operational research has shown that domestic dogs are the reservoir for rabies in countries where human rabies remains a concern, and that mass dog vaccination is a cost-effective way of controlling and ultimately eliminating rabies from dog and human populations. However, implementing vaccination programmes at scale requires functioning cold chain systems which are often not available in the countries where rabies is still endemic. Indeed, the high cost of installation, training of personnel and unreliable electricity have been identified as major challenges that hinder establishment of cold chain system in developing countries *(Chen et al., 2015; Norman et al.,* 2013).

Challenges of transporting and storing vaccines hinder the last mile of immunization, and as such considerable efforts have been invested to tackle these challenges on many fronts (Kartoğlu *et al.*, 2010; Kristensen *et al.,* 2016; Norman *et al.,* 2013). For example, in recent decades stakeholders in the field of public health have encouraged vaccine manufacturers to produce thermotolerant vaccines as one way of addressing the problem. As a result, a number of thermotolerant vaccines have been developed and are already available (Fisher, n.d.; Zipursky *et al.,* 2014; Mariner *et al.,* 2017; Zhang *et al.,* 2020;), whilst several others are at different stages of development and approval [13]. For example, thermotolerant vaccines have been developed and used to effectively control small pox, meningitis A, Newcastle disease and rinderpest (Mgomezulu *et al.,* 2021; Sutanto *et al.,* 1999; Zipursky *et al.,* 2014). In addition to these well-known examples, a study conducted in 2015 demonstrated that the potency of Nobivac® Rabies canine vaccine was not impacted following storage under *fixed* experimental conditions at 25˚C for six months and 30˚C for three months (Lankester *et al.,* 2016). This later example provides hope that this rabies vaccine can be used following local storage outside of cold chain conditions in remote places where rabies remains endemic.

In addition to efforts to develop thermotolerant vaccines, innovations to develop equipment suitable for storage of vaccines in isolated areas is also ongoing (Kahn *et al.,* 2017; Robertson *et al.,* 2017). These innovations have led to the production of a variety of off-grid equipment ranging from simple cold boxes, used to transport vaccines from the storage facility to the immunization site, to solar-powered refrigerators (Robertson *et al.,* 2017). Further, the development of off-grid refrigerators, such as solar refrigerators, have helped to bring life-saving vaccinations to hard-to-reach populations who would otherwise be lost to vaccine preventable diseases. Despite their benefits, however, solar refrigerators are costly to establish and maintain. Additionally, solar refrigerators are sophisticated

pieces of equipment and require specialist technicians, who are typically not available in remote regions of Sub-Saharan Africa and Asia, to repair problems that occur (McCarney *et al*., 2013; WHO, 2015). Thus, the use of this equipment has been constrained to a limited number of places. In response to these issues, manufacturers have directed their efforts to develop another generation of cold chain equipment known as passive cold devices (PCDs). These devices do not need electricity to keep the internal compartments cool, instead they rely on effective insulation and cooling media to create a cool environment. For example, the Arktek® passive vaccine storage device, which was used to transport and store vaccines during the 2014 Ebola outbreak in west Africa, uses dry ice for cooling and has the ability to keep the vaccine compartment at 2 to 10 $^{\circ}$C for 35 days. However, the high purchase price of the Arktek® device which ranges from US$1,200 to US$2,400 and the lack of availability of ice in remote areas has limited the widespread utility of this tool in developing countries (Lugelo *et al.*, 2020). These limitations have encouraged designers to focus their efforts into developing inexpensive and simple PCDs. One promising outcome of this challenge has been the development of the very low cost PCD called a Zeepot which is built from local materials such as clay and wood. A single Zeepot costs approximately $11 and is able to keep, on average, the internal temperature at 20°C below ambient temperature (Lugelo *et al.*, 2020).

Availability of thermotolerant vaccines and affordable PCDs such as the Zeepot have the potential to transform ongoing rabies control efforts in resource-poor-settings. The primary aim of this study, therefore, was to investigate whether the potency of the thermotolerant Nobivac® Rabies vaccine was impacted by long term storage inside the locally made Zeepot under field conditions where temperatures will naturally fluctuate. A secondary aim of the study was to investigate the impact that body condition score and age of domestic dogs have on immunogenicity. It is expected that the outcomes from this study will inform

alternative cost-effective means of delivering rabies vaccination to dogs at scale in remote regions which frequently suffer from cold chain constraints.

3.3 Materials and Methods

A controlled and randomised non-inferiority study was carried out to compare the serological response in dogs following vaccination with doses of Nobivac® Rabies vaccine (MSD, Boxmeer, Netherlands) stored under either cold-chain conditions (cold-chain storage) or within the Zeepot PCD (non-cold-chain storage).

3.3.1 Preparation of vaccines

Prior to the commencement of the field trial, Nobivac® Rabies vaccines were stored under cold-chain or non-cold chain (Zeepot) conditions for the period of two months. The daily temperature inside the Zeepot PCD was collected at three hourly intervals using a digital temperature data logger (Sensormetrix®). Similarly, the ambient temperature of the storage room in which the Zeepot was kept was recorded using the same device.

3.3.2 Study location and enrolment of dogs

The field trial was carried out in eight villages within the Serengeti district of northern Tanzania. Two teams of researchers accompanied by a local community leader walked on foot to locate dog keeping households within each study village. Upon arriving at a household, the researcher with the help of the community leader explained the purpose of the study and asked the head of the household whether they were happy for their dog to participate in the study. Following the signing of Informed Consent by the owner, all unvaccinated dogs present at the household, irrespective of their BCS and ages, were enrolled in the trial. Previously vaccinated dogs were not enrolled in the study, instead they received a standard-cold chain stored vaccine.

3.3.3 Sample size calculations

Sample size calculations were the same as those performed in the prior controlled and randomised non-inferiority themotolerance study carried out by Lankester *et al.* (2015) (Lankester *et al.*, 2016). In brief, the sample size for this study was estimated to be 50 dogs per group. This would give at least 80% power at the 5% significance level to detect non-inferiority of serological responses (the primary measurable output) to non-cold chain relative to cold-chain-stored vaccine, assuming a non-inferiority margin of -1.2 $\log^2$ titre units and a standard deviation of 1.8 $\log^2$ titre units. We also planned to carry out an exploratory analysis to assess the impact of Body Condition Score (BCS) and age as determinants of immunogenicity. Given this exploratory analysis, the aim of increasing confidence in the results and the availability of large numbers local dogs, the sample size was increased to approximately 200 dogs per group.

3.3.4 Sample collection and vaccination

Prior to sample collection, each enrolled dog was registered, had key biodata collected (e.g., age, BCS, sex), and a microchip inserted into the scuff of the neck. Whole blood samples were collected from the cephalic vein in S-Monovette ® tubes (Sarsted AG, Nümbrecht, Germany). Collected blood samples were placed in a cool box for transportation to the laboratory.

3.3.5 Vaccination

The vaccines were prepared in the following manner: equal quantities of Group 1 (cold-chain) and Group 2 (PCD) stored vaccine were loaded in the syringes each day prior to commencing field activities. Dogs were randomly assigned to receive vaccine from either group. The randomization was achieved through the random assortment of syringes loaded with vaccine and subsequent inoculation to dog being enrolled. Vaccination was carried

out through subcutaneous injection of 1ml of Nobivac® Rabies vaccine. The vaccines prepared on each day were all used to ensure the number of dogs recruited in each group were the same. This process was repeated on the following day until the number of 200 dogs per group was reached. The vaccines were coded so that dog owners and research staff knew the treatment given to each individual dog. Dog owners were told to contact the research team veterinarian via the telephone number written on the consent form if they observed any adverse effects that were likely attributable to the inoculation.

3.3.6 Follow up visit

Post-vaccination blood sampling was carried out at day 28 (4 weeks) after the inoculation. The field team visited the same households, study dogs were identified via the microchip reader and blood samples were collected and stored in the same manner as at day zero. All dogs were then vaccinated with a cold-chain stored vaccine to ensure effective immunogenicity.

3.3.7 Sample processing

Following storage in the cold box and transfer to the laboratory, all blood samples were kept at room temperature for 12 hours before being centrifuged to separate the serum. Aliquots of serum (1ml) were taken from each sample and were kept at -20^{0}C prior to shipment to the Animal and Plant Health Agency (APHA, UK) for serological analyses.

3.3.8 Serological analyses

The Fluorescent Antibody Virus Neutralization assay (FAVN), as described previously (WHO, 2018). was used to determine levels of antibody titres against rabies. In brief, Serial threefold dilutions of the positive and negative serum controls as well as of the test serum was prepared in the microplates. Each serum was added to four adjacent wells and

serially diluted four times. A 50-µL of a dilution of challenge virus standard (CSV-11) containing 50-200 TCID50/ml was then added to each serum dilution well. The microplates were then incubated for 1-hour at 37°C in a humidified incubator with 5% CO_2. Following incubation, 50-µL of the cell suspension with concentration of $4x10^5$ BHK-21 cells/ml was added to each well and further incubated for 48-hours at 37°C in a humidified incubator with 5% CO_2. After incubation, the plates were fixed in 80% acetone, dried and stained with fluorescein isothiocyanate (FITC) anti-rabies monoclonal globulin to each well. For each serum dilution, four wells were examined for the presence or absent of virus-infected cells. The 50% endpoint of the antibody (D50) content of test sera and virus titrations (TCID50) were calculated according to the Spearman- Kärber method. By international convention, this titre will be converted to a value in IU/mL by comparison of results obtained with those of the positive standard. The level of rabies neutralizing antibodies was expressed in International Unit per millilitre (IU/ml). A cut off value of 0.5 IU/ml was used to determine seroconversion as recommended by (WHO, 2018).

3.3.9 Statistical analysis

Titre measurements were statistically analysed using Two-Sample T-test. Post-hoc estimates of the differences between elevated storage conditions and the cold-chain stored vaccine were calculated with one-sided 95% confidence intervals. The null hypothesis, of elevated storage condition being inferior to the cold-chain storage, was rejected if the difference between the groups did not exceed < 1.2 $\log_2$ titre units. Multivariate logistic regression analysis was used to determine whether storage conditions, BCS or age of dog impacted the likelihood of seroconversion (titres ≥0.5 IU/ml).

3.4 Results

3.4.1 Study population

The enrolment and treatments of dogs for immunogenicity is shown in the study flow chart (Figure 3.1). In total, 412 domestic dogs with no previous history of vaccination to rabies vaccine were included in the study. Of 412 dogs, 205 received cold-chain and 207 received PCD stored vaccine.

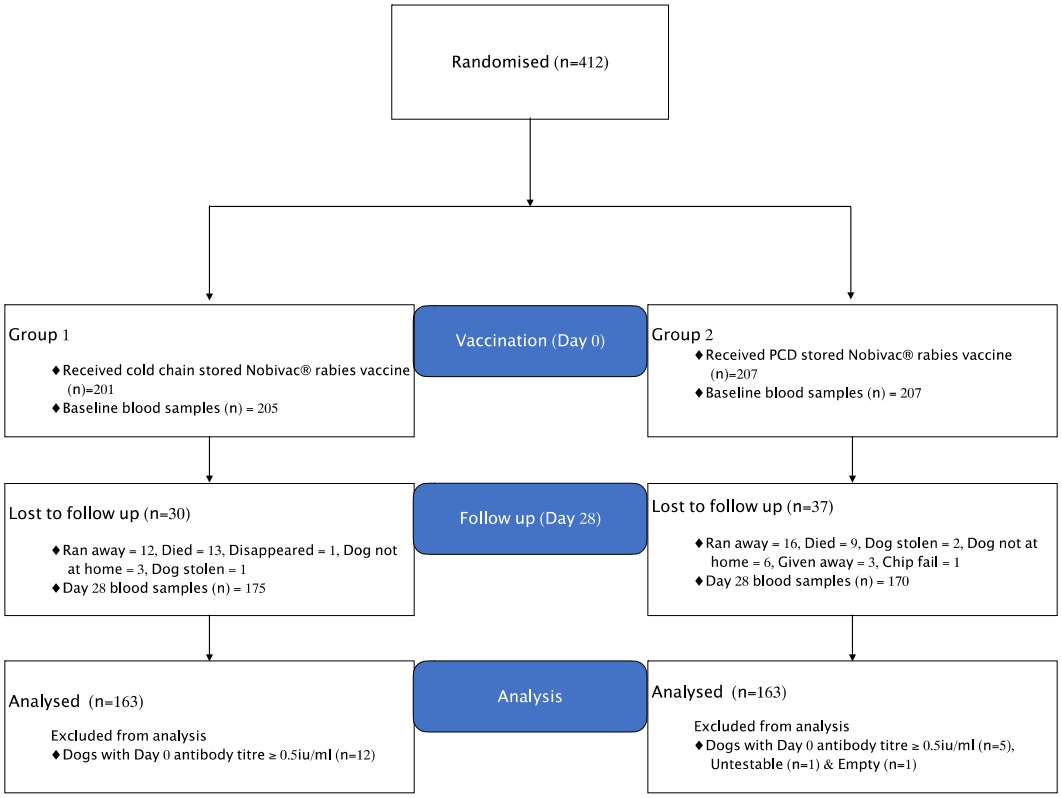

Figure 3.1: Flowchart showing enrolment, treatments and losses of dogs involved in the vaccine trial. Empty means vial without sample and Untestable mean sample inappropriate conditions to be tested.

3.4.2 Vaccine-related adverse events

During follow up visit at day 28, a total of 22 dogs, thirteen dogs in Group 1 and nine dogs in Group 2 were reported to have died. Of the 22 dogs, one dog died suddenly without showing any clinical signs and four other dogs died due to fatal injuries caused by severe

bites from other dogs (n=1), bites from hyena (n=1), bitten by the owner (n=1) and trampled to death by cows (n=1). The remaining 17 dogs died as a result of diseases. These dogs showed signs of illness approximately two weeks following the vaccination. The clinical signs included vomiting, diarrhoea, emaciation, coughing, anorexia, nasal discharges, dullness and incoordination. Dogs in both groups presented similar clinical signs. Further investigation revealed that dogs from households not participating in the study within and outside the study villages exhibited similar clinical signs and died. This suggest that the cause of death among the study animals was not related to immunization and, although the symptoms were suggestive of an infectious aetiology, a definitive diagnosis was not determined.

3.4.3 Lost to follow up and post-inclusion removal

Out of 412 dogs enrolled into the study at day zero, only 341 dogs were available at day 28 for re-sampling, with 67 dogs lost to follow-up for the reasons shown in (Figure 3.1). Fourteen out of 412 dogs sampled at day zero were found to have pre-vaccination antibody titres ≥0.5 IU/ml and were thus excluded from the analysis. As a result, a total of 326 dogs were included in the final analyses, with 163 dogs in each treatment group.

3.4.4 Assessment of vaccine storage in the PCD

The room temperatures and the vaccine storage temperatures inside the PCD recorded over the two-month storage period are shown in the Figure 3.7. The mean room temperature was 22.84^0C ranging from 17.00^0C to 32.2^0C whereas the average temperature inside the Zeepot was 19.81^0C fluctuating between 17.7^0C and 22^0C. Despite ambient temperatures exceeding 30˚C, the internal PCD temperature did not exceed 22˚C, which was below the storage temperature at which the vaccines have been shown to remain potent (30˚C) (Lankester *et al.*, 2016).

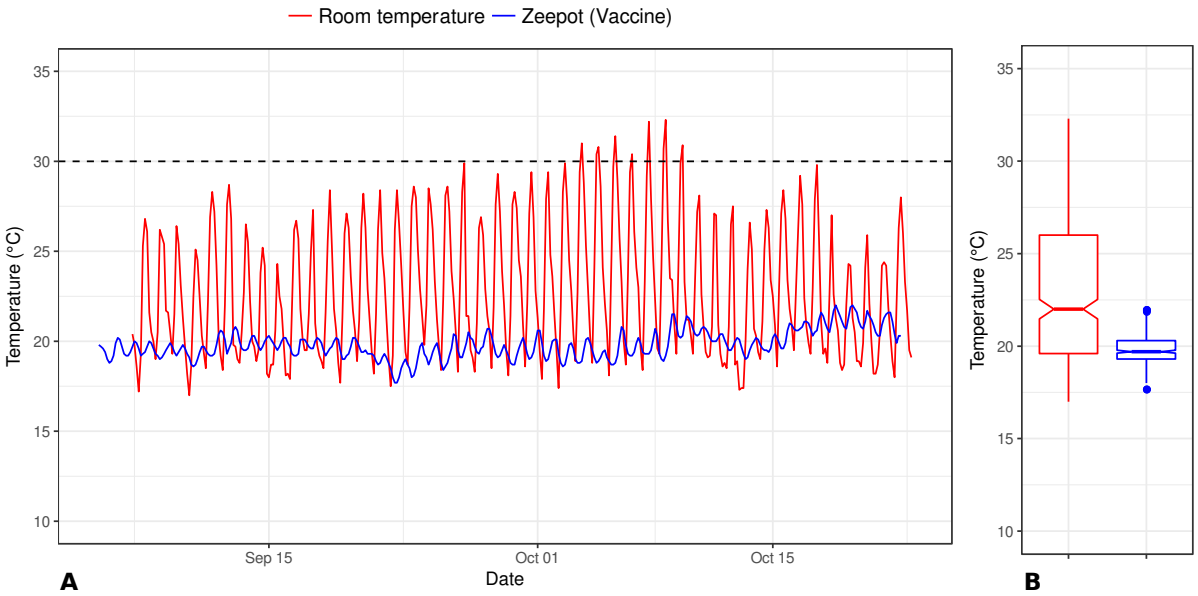

Figure 3.2: Time series and boxplot showing the temperatures of vaccines stored within the Passive Cooling Device (blue line) and the ambient room temperature (red line); dashed line indicates the maximum storage temperature below which Nobivac® Rabies vaccines have been shown to remain potent.

3.4.5 Day-28 Antibody titre comparison

The primary outcome variable was serological titre at day 28, and the predictor variable was the treatment group to which each dog belonged. The range of 28-day titres recorded for each group are shown in Figure 3 and the 28-day $\log_2$ and geometric mean titres are shown in Table 3.2.

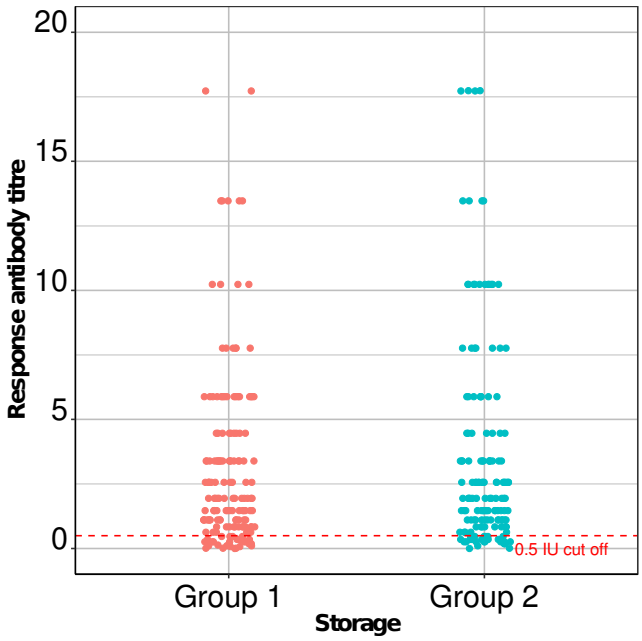

Figure 3.3: A dot-plot showing the titres (IU/ml) of all the dogs in the two groups of the trial. The dashed red line indicates the 0.5 IU / ml cut off point above which dogs are considered to have seroconverted.

Table 3.1: Group summary data – the number of dogs in each group and the day-28 log mean, standard deviation and geometric mean values are shown; day-28 log_2 mean and day-28 SD represent the average and standard deviation of the log (base 2) transformed data for each group.

Group	No. of dogs	Day-28 mean (log_2)	Day-28 SD log_2	Day-28 Geometric mean (IU/ml) log_2
1	163	0.9	2.0	1.8
2	163	1.0	1.9	2.0

Table 3.2: Estimate and one-sided confidence interval for the difference of titre levels between the cold-chain stored vaccine group (Group 1) and the elevated-temperature stored vaccine group (Group 2) as derived from T-test results.

Comparison	Estimate	Standard error	Lower 95% confidence limit (one-sided)
Group 2 Group 1	0.18	0.21	-0.17

The 28-day post vaccination group geometric mean titre was 1.8 IU/ml and 2.0 IU/ml for cold-chain storage (Group 1) and non-cold-chain storage (Group 2), respectively. Further, according to the pre-set criteria (i.e. the lower 95% confidence limit was not > -1.2 $\log_2$ titre units), the results indicate that Group 1 was not inferior to Group 2 (Table 3.3). As such the null hypothesis that non-cold-chain stored vaccine are less potent than cold-chain stored vaccine was rejected.

A secondary outcome variable was the percentage of dogs that seroconverted in each group (Table 3.4), which were almost identical (approximately 85%).

Table 3.3: Group seroconversion data – the number of dogs in each group, the number that seroconverted (≥ 0.5 IU/ml) and the seroconversion percentage are shown.

Group	Total no. of dogs tested	No. of dogs seroconverted	% seroconversion
1	163	139	85.3
2	163	140	85.7

3.4.6 Exploratory analysis: Impact of BCS and age on seroconversion at day-28

Logistic regression analyses were performed to examine the impact that body condition score, sex and age might have on the likelihood of seroconversion. The results (Table 3.6) indicated that, in corroboration of the T-test results, storage conditions did not affect the proportion of dogs that seroconverted (z = 1.1, df = 313, p-value = 0.25). There was a positive linear trend between Body Condition Score (BCS) (O.R. 2.2, 95% CI: 1.1 – 5.1) and seroconversion (Figure 3.4), implying that as BCS increased so did the likelihood of seroconversion. Neither sex nor age impacted seroconversion.

Table 3.4: Multivariable logistic regression of a range of explanatory variables on the probability of seroconversion; STORAGE_PCD compares non-cold-chain storage with the baseline cold-chain storage; AGE = age of dog; SEX_MALE compares serological responses of male dogs with the baseline female dogs; BCS = body condition score.

Variable	Odds ratio	95% CI	Z value	P-value
(intercept)	4.4	2.1-9.3	3.9	< 0.001
Storage_PCD	1.5	0.7-2.9	1.1	0.25
Age	1.1	0.9-1.6	0.9	0.35
Sex_Male	0.9	0.5-1.8	-0.2	0.81
BCS	2.2	1.1-5.1	2.0	0.04*

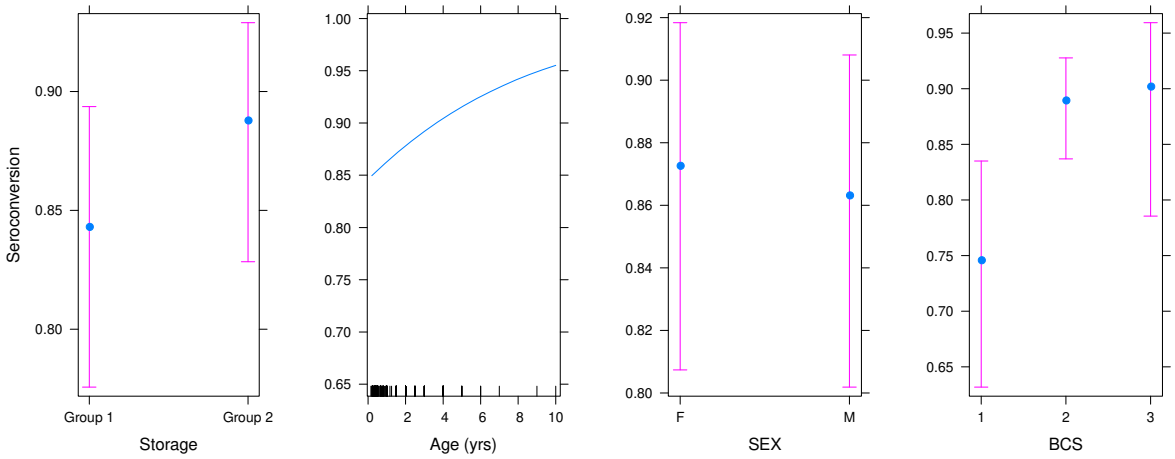

Figure 3.4: Plot showing the effect of storage condition, age, sex and body condition on seroconversions

3.5 Discussion

This randomized controlled field trial demonstrates that the Nobivac® Rabies vaccine stored within the PCD under fluctuating temperature conditions for up to three months elicited a neutralizing antibody response equivalent to vaccine kept under stable cold-chain conditions. This conclusion is supported by the results from the logistic regression which showed storage condition had no effect on the seroconversion.

Following vaccination against rabies, dogs are considered protected when the immune system has elicited sufficient amount of rabies virus-specific neutralising antibodies (RVSNA). RVSNA are a crucial component of adaptive immunity and contribute to the clearance of the viral infection (Dietzschold, 1993). Globally, the antibody titre level of 0.5 IU/ml is recognised as the cut off antibody titre above which humans, dogs and cats are classified as having developed a protective antibody response against against rabies infection (Kennedy *et al.*, 2007; WHO, 1992), although the true minimum protective level is likely well below this. In this study, ~85% of the dogs both in the cold chain and non-chain group passed this threshold (Table 3.2). This proportion is slightly less than reported

in the previous Lankester *et al.* (2016) study in which 92% of vaccinated dogs successfully seroconverted (Lankester *et al.*, 2016). A possible explanation for this reduction could be the health status of enrolled dogs. In the (Lankester *et al.*, 2016) study the experimental conditions of the trial were carefully controlled with storage temperature of the different treatment groups kept stable, the age of enrolled dogs kept between 6 months and 5 years and the BCS between 2 and 4. In contrast, the conditions in this field study were purposively less stringent so that the trial reflected natural conditions more closely. As a result, storage temperatures were allowed to fluctuate and dogs were enrolled regardless of their body condition score and age. This allowed investigation of the impact that age and BCS might have on sero-conversion and resulted in the finding that there was a statistically significant effect of BCS on the likelihood of seroconversion, with dogs of good body condition being more likely to seroconvert than dogs with poor BCS. This supports a previous study that suggested that loss of body condition was associated with immuno-suppression *(Morters et al.*, 2014). As such, it is possible that the reduction in the percentage of dogs that seroconverted was due to dogs of low BCS being included.

The other factor that could have resulted in the lower seroconversion percentage as compared to the Lankester *et al.* (2016) study was the fluctuating storage temperatures. However, as the percentage that seroconverted was the same for both treatment groups (cold-chain and non-cold-chain stored vaccine) the difference could not reflect either an impact of non-cold-chain storage or temperature fluctuations. This finding is important as it indicates that the thermotolerance of the Nobivac Rabies vaccine is not dependent upon constant storage temperature conditions, as were used in the (Lankester *et al.*, 2016) study, but rather is sustained despite the fluctuating storage temperatures as shown in Figure 3.2.

While the current study was not able to generate data on the longevity of the immune response in the non-cold chain group previous studies have showed that when RVSNA are produced the immune memory B-cell immunity persists even after RVSNA drop below 0.1 IU/ml, (Dodds *et al.,* 2020). Since both treatment groups elicited similar levels of antibody response, it seems unlikely that dogs vaccinated with the non-cold chain stored vaccine will have significantly shorter duration of immunity compared to those vaccinated with cold chain stored vaccine.

The multivariate analysis demonstrated that gender had no effect on the seroconversion or titre. This result is consistently with findings from previous studies Berndtsson et al (Berndtsson *et al.,* 2011) and (Mansfield *et al.,* 2004).

The finding that the potency of the Nobivac vaccine was not impacted following storage in the PCD for the duration of three months has important implications for developing new immunization strategies in remote or hard to reach areas facing logistical challenges in the transportation and storage of vaccine. As a result, decentralized storage strategies, involving vaccines being stored outside of cold-chain conditions for up to three months can be designed, implemented and tested for cost-effectiveness with the knowledge that such storage will not impact potency. Thermotolerance was a key factor that contributed to the successful campaigns to eradicate smallpox and rinderpest (Henderson & Klepac, 2013; Mariner *et al.,* 2013). For example, with a shelf life of 8 months when kept at 37^0C, it was possible to keep the rinderpest vaccine outside the cold chain for 30 days at a time, which gave sufficient time for vaccinators to travel on foot to implement the vaccination campaign in extremely remote areas. This would not have been possible if conventional vaccine storage of 2-8^0C was required. Likewise, the vaccination campaign against menginitis using the thermotolerant MenAfriVac's vaccine delivered in a controlled

temperature device resulted in very high population coverage levels being achieved (Zipursky *et al.,* 2014). The use of thermotolerant rabies vaccines stored without the need for electrical power in locally made PCDs outside of cold chain conditions could bring similar benefits, in coverage and cost-effectiveness, to the control of dog mediated human rabies.

3.6 Conclusion

The results obtained in this study clearly demonstrated that the Nobivac® Rabies vaccine kept in the locally made Zeepot PCD elicited protective neutralizing antibody response equivalent to vaccine stored under cold chain conditions. These findings will help in the design of cost-effective vaccine delivery strategies for use in resource-poor settings and will be of benefit to nations designing national elimination plans in an attempt to hit the international commitment of zero human deaths by 2030 (‘Zero by 30’) (WHO, 2018).

Conflict of interest

The authors and the funders have no conflict of interest to declare. The majority of animals in Sub-Saharan Africa are vaccinated with either Rabisin (Merial Animal Health Ltd, UK) or Nobivac Rabies (Intervet UK, Ltd). This study chooses to use Nobivac® vaccine because it is the widespread vaccine used for vaccination of pets in Tanzania, thus the findings from this study would have direct application on the ongoing efforts to control rabies in the country.

Research and ethical clearance

The study was approved by the Institutional Animal Care and Use Committee, Washington State University [Approval No. 04577 – 001], the Tanzania Medical Research Institute [NIMR/HQ/R.8a/Vol.IX/2788], Ifakara Health Institute [IHI/IRB/No:024-2018] and

Ethical Committee of the Sokoine University of Agriculture permit no. SUA/CVMBS/R.1/2019/6.

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CHAPTER FOUR

4.0 DEVELOPMENT OF DOG VACCINATION STRATEGIES TO MAINTAIN HERD IMMUNITY AGAINST RABIES

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4.1 Abstract

Human rabies can be prevented through annual mass dog vaccination campaigns that achieve 70% coverage. However, in rabies endemic countries, pulsed central point campaigns do not always attain this coverage. This study describes the development of a novel approach to dog vaccination aiming to sustain high coverage over time based on decentralized and continuous vaccination delivery within communities. A rabies vaccination campaign was conducted across 12 wards in the Mara region of northwest Tanzania to test this approach and refine delivery strategies. Household surveys were used to obtain data on vaccination coverage based on microchipping, certificate presentation and owner recall, as well as factors influencing dog vaccination and owner's satisfaction of dog vaccination services. A total 17,571 dogs (and 2 877 cats) were vaccinated, with 2654 dogs vaccinated using routine central point delivery and 14,917 dogs using one of three strategies of decentralized continuous vaccination. One month after the first vaccination campaigns, coverage in areas receiving decentralized vaccinations was higher (64.1, 95% Confidence Intervals 62.1 – 66%) than in areas receiving pulsed vaccinations (35.9%, 95% CIs 32.6 – 39.5%). Follow-up surveys 10 months later showed that vaccination coverage in areas receiving decentralized vaccinations remained on average over 60% (60.7%, 95% CIs 58.5 – 62.8%) and much higher than in villages receiving pulsed vaccinations where coverage was on average 32.1% (95% CIs 28.8-35.6%). Overall, >84% of the respondents were satisfied with the vaccination services they received. We conclude that decentralized continuous dog vaccinations have the potential to improve vaccination coverage and maintain herd immunity against rabies.

Keywords: rabies 1; vaccination strategy 2; herd immunity 3; decentralized continuous vaccination 4; mass dog vaccination 5

4.2 Introduction

Rabies remains a major public health problem causing over 60,000 human deaths every year worldwide. More than 95% of these fatalities occur in Africa and Asia, where canine rabies is endemic (Knobel *et al.,* 2005). Beside causing human mortality and anxiety and economic burden to those who are exposed, rabies further impacts livelihoods through losses of livestock (Jibat *et al.,* 2016). Domestic dogs are the principle reservoir of the virus in Africa and are responsible for causing more than 99% of human rabies deaths (Fooks *et al.,* 2014). Humans become infected by the rabies virus via the bite of a rabid animal. Prompt provision of post-exposure prophylaxis (PEP) is essential to prevent the development of disease in bite victims. However, in many rabies endemic countries, PEP is expensive and often unavailable (Knobel *et al.,* 2005). Mass vaccination of domestic dogs has been shown to significantly reduce the incidence of rabies, both in human and dog populations (Cleaveland *et al.,* 2003), and several studies have demonstrated that targeting the source of rabies infection through dog vaccination is the most cost-effective approach to human rabies prevention (Briggs, 2012; Lembo *et al.,* 2010; Zinsstag *et al.,* 2009).

Centralized pulsed vaccination is the standard delivery method for dog vaccination across much of Africa (Gsell *et al.,* 2012; Conan *et al.,* 2015; Arief *et al.,* 2016; Cleaton *et al.,* 2018; Kaneko *et al.,* 2021). This method involves teams of vaccinators setting up temporary static-point clinics in a convenient central location that typically last for one day (Minyoo *et al.,* 2015a; Lechenne *et al.,* 2016). At least 70% of the dog population needs to be vaccinated in each annual campaign (Coleman & Dye, 1996a; WHO, 2018), to prevent herd immunity dropping below the critical threshold in the 12 month interval before the next campaign (Coleman & Dye, 1996a; Hampson *et al.,* 2009; Morters, McKinley, Restif, *et al.,* 2014). But studies have shown that annual campaigns sometimes fail to attain this

recommended coverage (Kitala *et al.,* 2002; Cleaveland *et al.,* 2003; Kaare *et al.,* 2009; Mulipukwa *et al.,* 2017; Barbosa Costa *et al.,* 2020).

Herd immunity can decline by 30-50% per year in rabies endemic countries (Beran, 1982; Kitala *et al.*, 2002) due to high dog population turnover (Kitala *et al.,* 2002; Arief *et al.*, 2016; Hampson *et al.,* 2009). With the elimination of rabies dependent on continuously high vaccination coverage, alternative approaches to pulse vaccination may help to maintain herd immunity over time (Davlin & VonVille, 2012). Availability of thermotolerant rabies vaccines (Lankester *et al.*, 2016), and affordable devices for storage of vaccines in settings with limited cold chain (Lugelo *et al.,* 2020), open up avenues for the development of improved strategies for sustaining vaccination coverage across populations.

In this article, we report results from a feasibility study that was carried out to generate data to inform the design of a large-scale dog vaccination trial in northwest Tanzania. Specifically, the feasibility study was designed to: a) investigate approaches to implement community-based mass dog vaccination; b) compare three methods of assessing the vaccination status of individual dogs and the level of vaccination coverage in the community (at the ward level); and c) assess travelling and waiting times at central point clinics and dog owner's perceptions regarding the quality of services offered under three models of community-based dog vaccination delivery.

4.3 Materials and Methods

The aim of the study was to compare dog vaccination delivery approaches, specifically routine centralized pulsed vaccinations (CPV) normally led by a government team of vaccinators vs novel decentralized continuous approaches to vaccination (DCV), which

involve a Rabies Coordinator (RC) designated for this role by the district veterinary office (typically a livestock field officer) and a village-based worker, that we refer to as a One Health Champion (OHC). A potential advantage of a decentralized approach is that vaccines can be stored locally where they are needed rather than at the district veterinary office headquarters. Since decentralized continuous vaccination is novel, and the best methods for implementation were not clear, an important objective was therefore to compare the following alternative delivery strategies of this approach:

Village-level vaccination strategy: RCs and OHCs worked together to deliver a static-point clinic in month 1 at a designated central point within each village. After the vaccination activities in month 1 were completed, the OHC continued to compile a list of dogs in their village that either missed the central point vaccination in month 1 or were brought into the village or were born after month 1. These dogs were vaccinated when the RC returned to the village in months 3, 6 or 9. The RC together with the OHC were assigned to work together to decide whether the dogs requiring vaccination in months 3, 6 and 9 were to be brought to a central point for vaccination or whether these dogs would be better reached through a house-to-house approach. On demand vaccination services were also provided throughout the year, whereby dog owners called the RCs to come and vaccinate their dogs at their home.

Sub-village vaccination strategy: The RCs and OHC hosted a static-point clinic in month 1 at a designated central point within each sub-village of each village (there are typically 3 to 9 sub-villages per village in Tanzania). The subsequent vaccination activities were carried out as explained in the Village-level strategy detailed above.

Discretionary vaccination strategy: Prior to the beginning of the campaign, the RC, OHC and village leader held a consultative meeting and agreed the vaccination strategy they considered to be the best for their village based on their local setting. That is, static-point clinics at either the village or the sub-village level, house-to-house or a mixture of the two strategies were agreed upon. Like in the previous strategies, on demand vaccination was also to be provided throughout the year.

All vaccination approaches were provided free of charge

4.4 Logistics and dissemination of equipment

Global Animal Health Tanzania purchased vaccination equipment (syringes, needles, certificates, smartphones, cool boxes, vaccine storage devices etc) and MSD Animal Health donated vaccines (Nobivac® Rabies). RCs were loaned smartphones for data collection and communication. Supplies were delivered to the district veterinary office in the quantities required, while the vaccine storage devices "Zeepots" (Lugelo *et al.*, 2020) were sent directly to the wards where RCs were based.

4.5 Study Sites

This study was conducted between July 2019 and July 2020 in 12 wards of Butiama, Tarime and Rorya districts of the Mara region (34°-35°E, 1°30´-2°10´S) in northwest Tanzania. Four wards were selected randomly from all the wards in these districts that had a livestock field officer in each of these three districts. Wards in Tanzania typically comprise 4 villages but can have as many as 6, whereas the number of sub-villages within the village varies from 3 to 9. According to the 2012 national population and housing census, approximately 142 886 people live in the study area, i.e. in the 12 selected wards (Tanzania National Bureau of Statistics, 2012). The dog population in these wards was

estimated to be 23 814 dogs based on the human to dog ratio of 6:1 (Czupryna *et al.*, 2016; Minyoo *et al.*, 2015b).

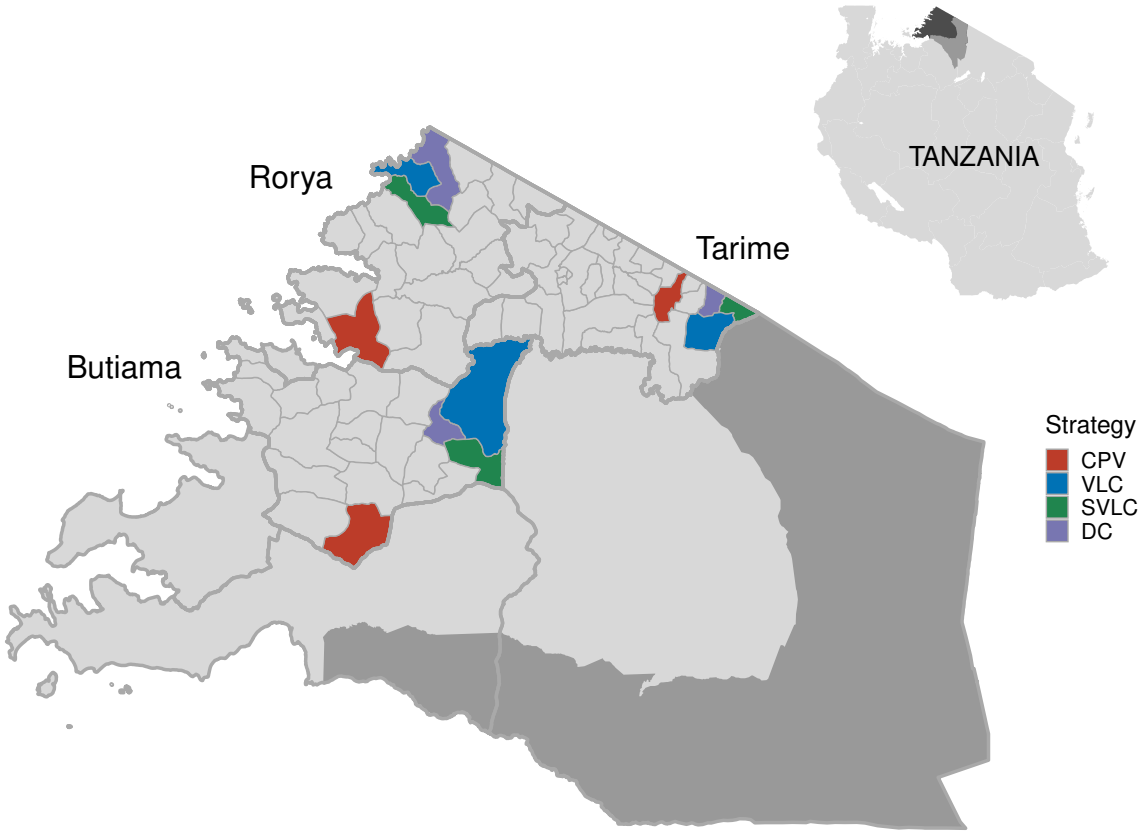

Figure 3.5: Map of the Mara region in Tanzania showing study wards and the assigned vaccination delivery approaches. Approaches comprised centralized pulsed vaccination (CPV), and three strategies of decentralized vaccination, specifically village-level continuous (VLC), sub-village level continuous (SVLC), and discretion continuous (DC).

4.6 Dog Vaccination Campaigns

4.6.1 Centralized pulsed vaccination

One of the four selected wards in each district was randomly assigned to receive pulse vaccination, with all villages within this ward receiving the same vaccination delivery

treatment. The district veterinary officer in each respective district managed the vaccination team, comprising of three people, the vaccinator, certificate writer and data collector. The team held a one-day central point dog vaccination clinic in each village, after which these villages did not receive any further dog vaccinations for the remainder of the year. Prior to the campaign, communities were sensitized as previously described (Kaare et al., 2009). Briefly, dog owners were informed through posters placed in prominent places, mainly markets, schools and village notice boards, approximately 7 days before the campaign. One day before the vaccination campaign, a member of the vaccination team delivered announcements using megaphones to invite people to bring their dogs for vaccination the following day. The advertiser used a motorbike to reach different parts of the village. Dogs that were presented at the vaccination clinic were registered in a vaccination register, recording the owner's name and the dog's name, sex, and age. The same information were also entered into the Mission Rabies App (Gibson *et al.*, 2018). Following registration, all dogs were inoculated subcutaneously with 1ml of Nobivac Rabies vaccine, a microchip was then inserted subcutaneously into the scruff of the neck and a vaccination certificate was issued to the person who brought the dog to the central point.

4.6.2 Decentralised continuous vaccination

Under the decentralized continuous vaccination approach, a batch of vaccine as well as other vaccination equipment were stored and managed by the livestock field officer assigned to be the RC for the ward. The RC in collaboration with a community representative (the OHC) planned and executed vaccination activities throughout the year as needs arose. Like in the pulsed vaccination method, the district veterinary officer provided overall supervision of the vaccination.

4.6.3 Recruitment and training

A local villager was recruited from each participating village to take responsibility as the OHC. Their appointment was performed by community leaders. To qualify for this position, the person had to meet specific criteria that included being influential and respected in the local community and basic literacy. The RC and assigned OHC were then invited to attend a three-day training program aiming to provide specific skills and knowledge needed to conduct their assigned strategy for decentralized continuous vaccination. During the training the RCs were taught how to request and store vaccine in the Zeepot. In addition to the strategy specific training, the RCs and OHCs were taught other aspects of the vaccination programme including: a) their roles and responsibilities; b) planning and delivering vaccination campaigns according to their designated strategy; and c) data collection and microchipping vaccinated dogs. Implementation manuals were provided to both RCs and OHCs for referral.

4.6.4 Pre-vaccination preparations

Within a week of the training, the RCs and OHCs developed vaccination planning timetables and shared these with local community leaders. The RCs submitted requests of their needs to the district veterinary officer based on the estimated dog population in their ward. In contrast to CPV, advertising was undertaken by OHCs who used loudspeakers and word-of-mouth to sensitize the community about the forthcoming dog vaccination campaign. Posters detailing the date and locations of the clinics were placed in the village notice board and in marketplaces. The RCs travelled by motorbike or public transport to the district veterinary office to collect vaccines in a coolbox as well as other consumables required. Upon returning to their home base, RCs stored vaccines in their Zeepot, as previously described (Lugelo *et al.,* 2020). Each batch of vaccines that was issued was estimated to be sufficient to allow for six months of vaccination activities. To ensure dogs

were vaccinated with high quality vaccine, any vaccines remaining at the end of the six-month period were returned to the district veterinary office and destroyed, and a new batch of vaccines was delivered. Once set, the OHC delivered another advisement by loudspeaker the day before the vaccination campaign inviting people to bring their dogs and cats the following day. Each RC conducted the campaign as per the strategy assigned during the training.

4.7 Household Surveys

Surveys to assess vaccination coverage were conducted at two timepoints, from September to October 2019 and June to July 2020 respectively, corresponding to one month and 11 months after the initial vaccination campaigns. Different households were visited at each timepoint. The survey team was composed of the interviewer and the sub-village leader who provided guidance on the movements and introductions to households. The survey involved sampling in all villages in the twelve study wards. In each village three sub-villages were randomly selected and 10 households per sub-village sampled. With the help of the sub-village leader, a household (located at the far end of the sub-village) was chosen as the index household were the survey commenced. From this household the interviewer walked in a zigzag pattern, towards the centre of the sub-village to the opposite side of the sub-village, sampling every fifth household. If no adult was present in the selected household or no dog was owned in the household, that household was skipped. Interviews continued until 10 households were completed in each sub-village. Prior to the administration of the questionnaire, permission was sought from the household head, or other household member of at least 18 years of age in the absence of the household head. Interviewers explained the study background to each respondent and obtained written informed consent to carry out the questionnaire. The questionnaire captured details regarding the quality of the dog vaccination services provided by the RCs and OHCs, the

accessibility and availability of these services, the total number of dogs living in the household, and their vaccination status, according to whether either a microchip was found, a certificate was shown, and the owner statement (recall). At the second time point, data were also collected on the number of dogs that were present and available for microchip checking (this number may be lower than the total number of household dogs if dogs were roaming at the time of the visit). Reasons for not vaccinating dogs, if there were any unvaccinated dogs found at the household, were also recorded.

4.8 Statistical Analyses

The accuracy of assessing a dog's vaccination status using certification and owner recall were assessed against the assumed gold standard of microchipping. Sensitivity and specificity were estimated with 95% confidence intervals calculated using the binom.test function in R. In this context, the sensitivity of the certification and owner recall tests were regarded as the ability to correctly identify dogs that have been vaccinated whereas the specificity meant the ability of a test to correctly identify dogs that have not been vaccinated. Because test results were recorded at the household level and not for individual dogs, only single-dog households visited at timepoint 1 were included in this analysis.

Vaccination coverage at each of the two survey time points was estimated for each of the four vaccination strategies as the proportion of dogs with microchips among the surveyed dog population. The denominator used to produce these estimates – the total number of dogs living in surveyed households, which was the only denominator option that was collected at both time points – means that they are slight underestimates. However, the microchip assessment when using the number of dogs seen at the household (available for the second time point) as a denominator can be used as a gold standard from which to evaluate the accuracy of using vaccination certificates and owner recall to estimate

coverage. This evaluation of coverage estimation methods was achieved by fitting generalised linear mixed models (GLMMs) with binomial error distributions to three different household-level response variables: 1) the number of microchipped dogs out of all dogs seen in surveyed households at time point two; 2) the number of vaccination certificates seen out of all dogs recorded as living at surveyed households surveyed at time point two; and 3) the number of dogs that owners reported to be vaccinated out of all dogs recorded as living at surveyed households surveyed at time point two. District and vaccination strategy were included as categorical fixed effects in all three GLMMs, along with random intercepts forward, village, sub-village, and household (the observation level). The DHARMa package (Hartig, 2020) was used to simulate residuals and check model assumptions were met. Estimates of the time point two vaccination coverage for each study ward were extracted from each model, with confidence intervals estimated from 1000 bootstrap samples using the bootMer function from the lme4 package (Bates *et al.*, 2015).

Variation in duration of time travelling to and waiting at the central point clinics was modelled using a linear mixed model (LMM) fitted using REML, where $\log_e$(duration) was the response, intervention and district were categorical fixed effects, and nested random intercepts were fitted at the ward, village and subvillage levels. The response was log-transformed to meet the assumptions of homoscedasticity and normality of residuals, which were checked visually by plotting the residuals against the fitted values. Differences between districts and intervention arms in mean of $\log_e$ duration of travel times and wait times at the central point clinics were tested using an F-test. Wald 95% confidence intervals for mean duration within each intervention arm were calculated in the lmerTest package (Kuznetsova *et al.*, 2017) ("lmerTest") using Satterthwaite's approximation to the t-distribution degrees of freedom. Back-transformed mean durations of travel and wait

times at the central point clinics and their 95% confidence limits from the log scale were adjusted for bias due to Jensen's inequality (Nakagawa *et al.*, 2017). Data analyses were performed using the statistical programming software, R version 3.5.3 (R. Development Core Team, 2019).

4.9 Ethics Statement

The study was approved by the Institutional Animal Care and Use Committee, Washington State University [Approval No. 04577 – 001], the Tanzania Medical Research Institute [NIMR/HQ/R.8a/Vol.IX/2788], Ifakara Health Institute [IHI/IRB/No:024-2018] and Ethical Committee of the Sokoine University of Agriculture permit no. SUA/CVMBS/R.1/2019/6. Prior to the administration of the questionnaires written informed consent was obtained from the head of each household or, if not available, an adult family member. All data collection was conducted in Swahili.

4.10 Results

4.10.1 Vaccinations

In total 17 571 dogs were vaccinated in the study areas, as summarized in Table 4.1. Of these animals, 2 654 were vaccinated through centralized pulsed vaccination; 5 109 through decentralized continuous vaccination at village level; 5 172 through decentralized continuous vaccination at sub-village level and 4 636 through the discretionary continuous vaccination strategy. Over the same period 2 877 cats were vaccinated, with 178 vaccinated through centralized pulsed vaccination, and 595, 939 and 1 185 vaccinated from the three decentralized continuous vaccination strategies (Table 4.1).

Table 4.1: Numbers of dogs and cats vaccinated across the different strategies

Sn	District	Ward	Strategy	Dogs	Cats
1	Butiama	Masaba	Pulse	524	50
2	Butiama	Buswahili	Village-level	2 198	192
3	Butiama	Sirorisimba	Sub-village-level	2 403	435
4	Butiama	Nyamimange	Discretionary	1 594	332
5	Rorya	Komuge	Pulse	1 326	80
6	Rorya	Tai	Village-level	1 418	212
7	Rorya	Mkoma	Sub-village-level	1 280	58
8	Rorya	Bukura	Discretionary	1 606	531
9	Tarime	Nyamwaga	Pulse	804	48
10	Tarime	Gorong'a	Village-level	1 493	191
11	Tarime	Nyanungu	Sub-village-level	1 489	476
12	Tarime	Itiryo	Discretionary	1 436	322
	Total			**17 571**	**2 877**

Table 4.2: Average number of dogs and cats vaccinated per day during the main campaign in each strategy

Strategy	Dogs	Cats
Pulse	223	16
Village-level	275	24
Sub-village	72	13
Discretionary	71	17

During the main campaign, the vaccinators are undertaking the centralized pulsed vaccination and the village-level decentralized continuous vaccination strategies vaccinated the most dogs per day compared to those implementing the decentralized sub-village level and discretionary continuous strategies (Table 4.2). Subsequent campaigns in the decentralized continuous approach resulted in further dogs being vaccinated that would

have been left unvaccinated if centralized pulsed vaccination was used. About 1 642 more dogs (32.14%) were vaccinated in the follow-up campaigns under the village-level strategy, while follow up campaigns in the sub-village level and discretionary strategies led to 1 456 dogs (28.15%) and 1 139 dogs (24.57%) being vaccinated (Figure 4.1). Of the 14 917 dogs vaccinated under the continuous vaccination approach, 14 196 (95%) were vaccinated using central point clinics held at village and sub-village levels, and only 2.74% and 2.09% of dogs were vaccinated using house-to-house and on-demand methods respectively.

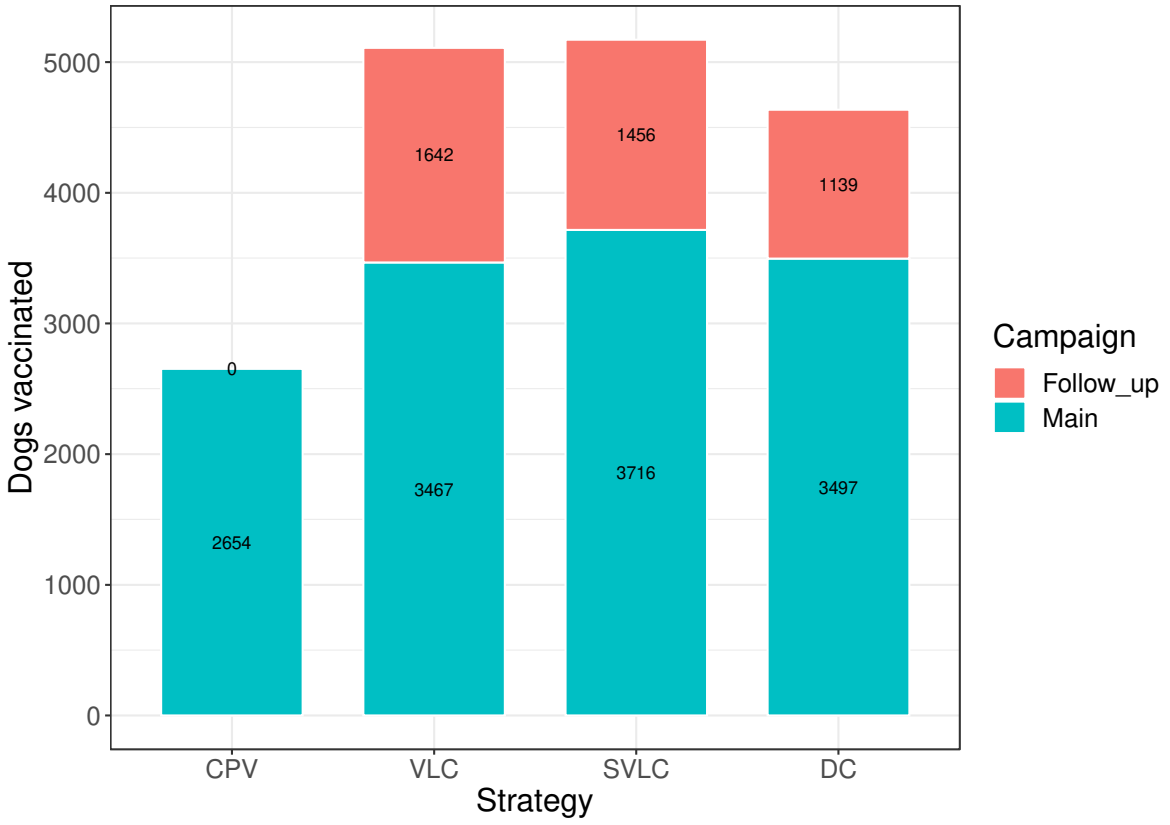

Figure 4.1: Dogs vaccinated in each strategy in the main and follow up campaigns. CPV = centralized pulsed vaccination, VLC = village-level continuous decentralized vaccination, SVLC = sub-village level continuous decentralized vaccination, DC = discretion continuous decentralized vaccination.

4.10.2 Accuracy of dog vaccination status assessment

The accuracy of certification and owner recall was assessed using data from 598 single-dog households. The sensitivity and specificity of certification of dog vaccination status were 91.9% (95% CI 88.3 – 94.6) and 83.5% (95% CI 78.6 – 87.6) respectively. Owner recall of dog vaccination status had sensitivity of 98.1% (95% CI 96.0 – 99.3) and specificity of 79.5% (95% CI 74.3 – 84.1).

4.10.3 Vaccination coverage

The vaccination coverage achieved varied under the different delivery strategies (Figure 4.2). At time point 1 (1-month post-vaccination campaign), the overall vaccination coverage achieved using the decentralized continuous method was higher at 64.10% (95% CI 62.12 – 66.04) than under the centralized pulsed vaccination approach at 35.86% (95% CI 32.59 – 39.51). Similarly, at time point 2 the continuous method had a higher level of coverage of 60.67% (95% CI 58.47 – 62.84) compared to centralized vaccination with 32.10% (95% CI 28.82-35.57). Between the two time points, the coverage dropped by 3.76% and 3.43% in the centralized vaccination approach and continuous methods respectively. There was also variation in coverage amongst the decentralized continuous vaccination sub-strategies. The discretionary strategy attained high initial coverage of 68% but declined to 58.91% toward the end of the study. In contrast, there was an increase in coverage in sub-village-level from 60.97% to 64.88% (Figure 4.2).

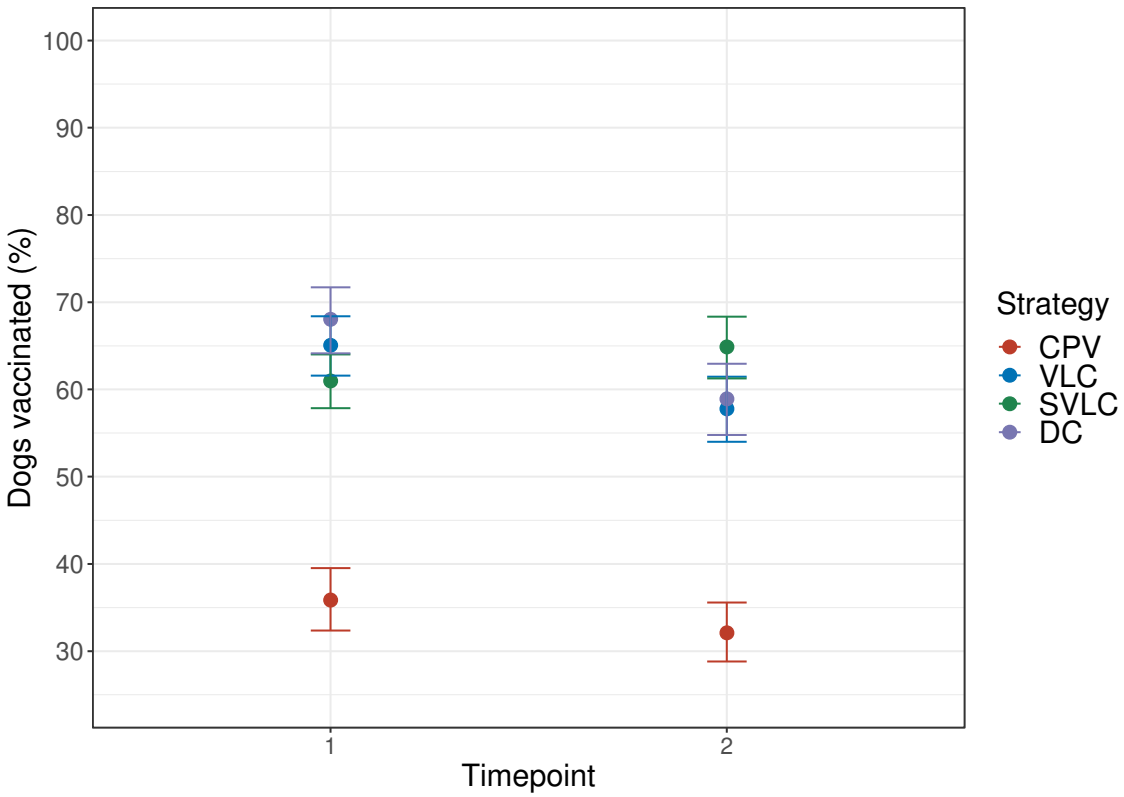

Figure 4.2: Vaccination coverage attained in each strategy at month 1 and month 11 post vaccination.

Coverage was estimated from the number of dogs with microchips out of all dogs recorded as living at surveyed households. CPV = centralized pulsed vaccination, VLC = village-level continuous decentralized vaccination, SVLC = sub-village level continuous decentralized vaccination, DC = discretion continuous decentralized vaccination.

Estimation of vaccination coverage using owner recall overestimates coverage (relative to the gold standard approach using microchips) by an average of 2.5%, though this difference between the two estimation methods varies between wards and strategies (Figure 4.3). Overestimation by the owner recall approach is most substantial under the centralised pulse vaccination strategy and is less evident under the continuous strategies, where underestimation sometimes occurs. Coverage estimates from certificates, however, underestimate coverage on average by 3.6%, though this again varies by ward and strategy, with underestimation being most substantial for the continuous strategies (certificates tended to overestimate vaccination under centralised pulsed vaccination).

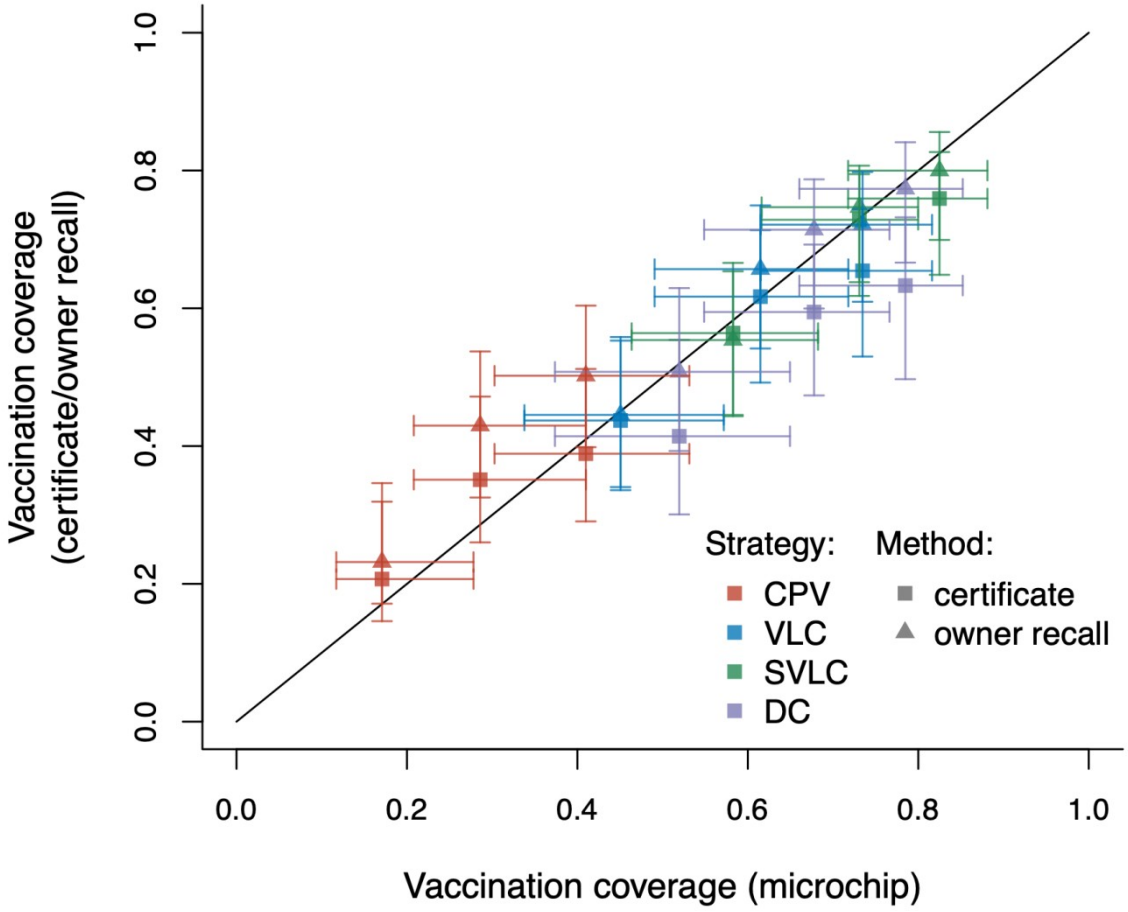

Figure 4.3: Coverage estimates according to different methods of measuring coverage. Generalised linear mixed model (GLMM) estimates in each of the twelve study districts under each of the three coverage estimation methods at 11 months after the main vaccination campaign. CPV = centralized pulsed vaccination, VLC = village-level continuous decentralized vaccination, SVLC = sub-village level continuous decentralized vaccination, DC = discretion continuous decentralized vaccination.

Table 4.3: Respondent perceptions regarding the dog vaccination services

Variable	Pulse	Village-level	Sub-village	Discretionary
When taking your dog for vaccination, were there sufficient people to vaccinate your dog?	140/186 (75.27%)	294/294 (100%)	326/326 (100%)	244/245 (99.60%)
When taking your dog for vaccination, was the equipment available to vaccinate your dog?	156/185 (84.32%)	276/292 (94.52%)	325/325 (100%)	244/245 (99.60%)
Is the respondent aware there is a RC in their ward?	NA	301/361 (83.37%)	233/348 (66.95%)	237/296 (80.06%)
If yes, do they know how to contact the RC?		256/301 (85.05%)	200/233 (85.84%)	235/237 (99.16%)
Is the respondent aware there is a OHC in their village?		330/361 (91.41%)	315/388 (81.19%)	150/296 (50.68%)
If yes, do they know how to contact the OHC?		303/330 (91.81%)	302/315 (95.87%)	144/153 (94.12%)
Are you satisfied with the vaccination services provided by the RC and OHC in your area?		324/355 (91.27%)	354/388 (91.24%)	249/294 (84.70%)

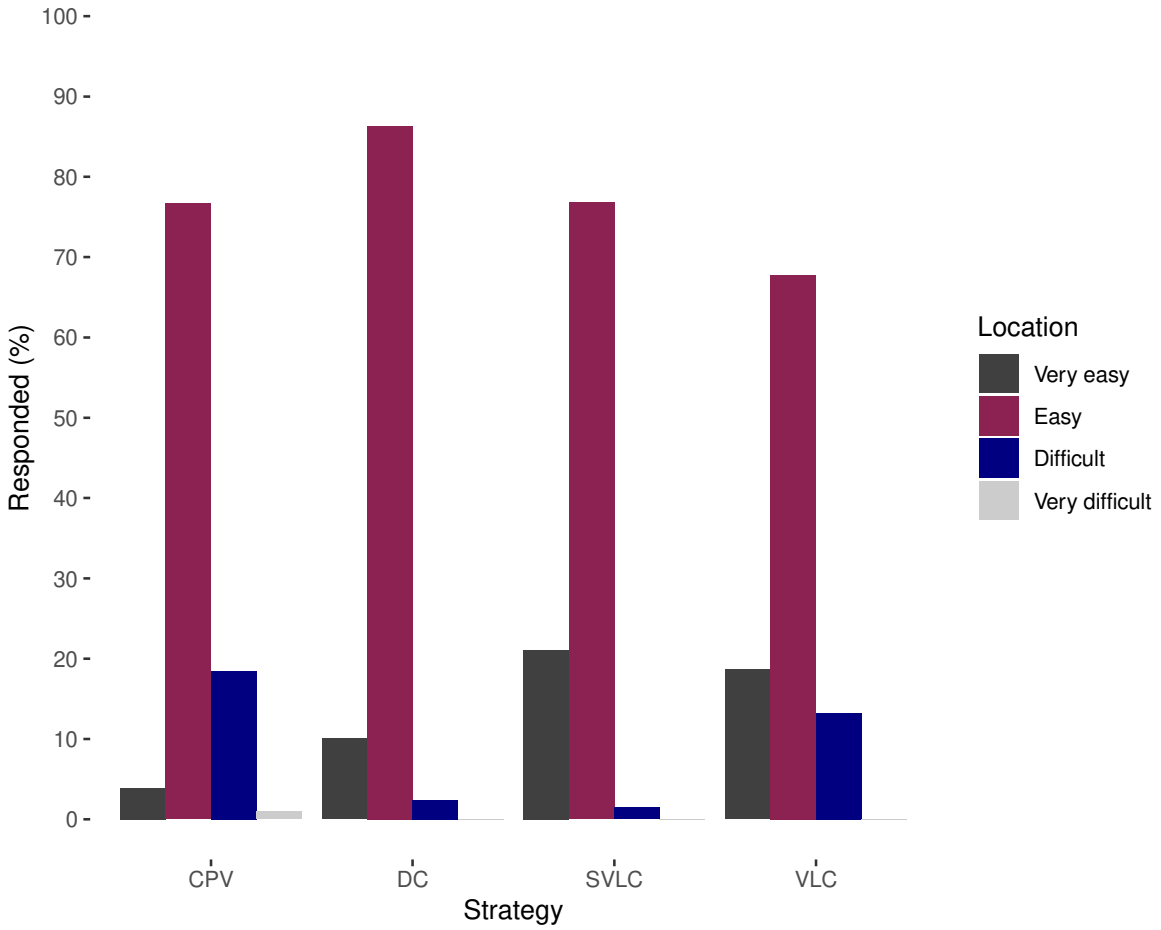

Figure 4.4: Respondent's perceptions regarding the location of the household relative to the central point clinic.

CPV = centralized pulsed vaccination, VLC = village-level continuous decentralized vaccination, SVLC = sub-village level continuous decentralized vaccination, DC = discretion continuous decentralized vaccination.

4.10.4 Vaccination services

Over 70% of all respondents across the strategies found it easy to travel from their household to the vaccination point (Figure 4.4). Almost all respondents in the areas with continuous vaccination delivery said the number of staff at the central point was sufficient. On the contrary ~25% of the respondents in the pulse strategy considered the number to be insufficient. Similarly, over 94% of respondents in the continuous method said there was sufficient vaccination equipment at the vaccination point, whilst only 84% reported this in

the pulse strategy. The proportion of respondents who were aware of the presence of the RC in their ward was lower in areas vaccinated at Sub-village-level (67%) compared to Village-level (83%). More than 85% of people who were aware of the RC knew how to contact them if they needed the service. About half of the respondents in Discretionary decentralized vaccination arm knew there was a OHC in their village, whilst the majority of respondents (>80%) in the other decentralized continuous vaccination strategies were aware of the presence of the OHC and >90% of them knew how to contact the OHC. Overall villagers were satisfied (>84%) with the services they received from the RC and OHC (Table 4.3).

4.10.5 Reasons for not vaccinating dogs

There was a significant difference between vaccination strategies in the reason reported by respondents for not vaccinating their dogs. In those areas under the centralized pulsed vaccination approach 22% of respondents cited lack of awareness about the campaign as the main reason for not vaccinating their dogs, whereas only 7% of the respondents in the decentralized continuous vaccination sub-strategies indicated lack of information regarding the vaccination campaign as the reason for not vaccinating dogs.

Table 4.4: Reasons for not vaccinating dogs under pulse and continuous vaccination strategies

Reason	Pulse (n)	%	Continuous (n)	%
1. Dog had puppies	10	5.13	4	1.04
2. Dog sick	2	1.03	3	0.78
3. Dog aggressive	9	4.62	13	3.39
4. Acquired/born after vaccination	28	14.36	159	41.51
5. Didn't hear the campaign	44	22.56	7	1.83
6. Difficult to restrain	14	7.18	48	12.53
7. Long distance	1	0.51	1	0.26
8. Dog not at home	7	3.59	23	6.01
9. Owner sick	7	3.59	3	0.78
10. Dog too young	2	1.03	12	3.13
11. Ran away	28	14.36	84	21.93
13. Owner unavailable	36	18.46	19	4.96
14. Not enough time	6	3.08	0	0.00
15. Mistrust	1	0.51	0	0.00
16. Vaccine finished	0	0.00	3	0.78
17. Too many dogs at home	0	0.00	4	1.04
Total	**195**	**100.0 0**	**383**	**100.0 0**

Note: CPV = centralized pulsed vaccination and DCV = decentralized continuous vaccination.

The vast majority of respondents with unvaccinated dogs in the areas under the decentralized vaccination approach reported that their dogs were not vaccinated because they were acquired or born after the campaign was conducted (41.51%, Table 4.4). Although the continuous vaccination was supposed to be available throughout the year, the results suggest that there were fewer vaccination campaigns conducted than originally planned in the implementation manual. In both approaches, distance to the central point clinic was rarely mentioned as a reason for not vaccinating the dogs (0.51% and 0.26% in

the pulse and continuous strategies respectively). Similarly, dogs running away comprised a large proportion of dogs that were not vaccinated in both approaches.

Differences between districts and intervention arms in mean of $\log_e$ duration of travel times and wait times at the central point clinics are shown in Table 4.5. There was not a statistically significant difference in travel and wait time at the central point clinic between districts and interventions ($F(2, 3) = 0.6$, $p = 0.56$) and ($F(2, 3) = 3.4$, $p = 0.09$) respectively. Thus, district was dropped from the model.

Table 4.5: Results of F tests on the fixed effects using Satterthwaite approximation in wait and travel time to the central point

	Fixed effect	**Sum of Squares**	**Mean Squares**	**Df (num,den.)**	**F value**	**P value**
Travel time	Intervention	1.6	0.5	3,6.1	1.3	0.37
	District	0.5	0.3	2,6.2	0.6	0.56
Wait time	Intervention	3.1	1.0	3,6.1	1.9	0.22
	District	3.7	1.9	2,6.1	3.4	0.09

Table 4.6 summarizes the time that dog owners reported traveling from their house to the central point, and the time spent at the central point clinic waiting for their dog(s) to be vaccinated. There was a significant difference in mean travel time to the central point vaccination clinics (df = 3, F = 28.9, $p < 0.0001$) between the strategies. There was a significant difference in mean travel time to the central point clinics between the SVLC and the VLC ($p < 0.0001$), whereby people in the VLC spent 11 min more to locate the

central point clinic compared to people under SVLC strategy. Also, people under DC strategy spent 8 min less to locate the central point clinic compared to people under VLC ($p < 0.0001$). Similarly, there was a significant difference in mean wait time at the central point vaccination clinics (df = 3, F = 13.1, $p < 0.0001$) between the strategies. There was a significant difference between the SVLC strategy and the CPV ($p < 0.0001$) with people receiving CPV strategy waiting on average 25 min more than those under the SVLC strategy. There was also a significant difference between DC and CPV ($p < 0.0001$) with people under CPV strategy waiting on average 23 min more than those under the DC strategy.

Table 4.6: Average time spent by dog owners at the central point and time taken to reach the central point.

Strategy	Mean Travel Time (min)	95% CI	Mean Wait Time (min)	95% CI
CPV	22.4	14.8–33.9	58.5	23.7-144.0
VLC	28.1	18.8-42.2	81.3	33.2-199.5
SVLC	17.3	11.5-25.9	34.0	13.8-83.3
DC	20.2	13.4-30.6	35.3	14.3-87.3

4.11 Discussion

This study describes the development and implementation of a novel approach to the delivery of mass dog vaccination, taking advantage of a rabies vaccine that can be stored in a passive cooling device. Overall, the use of decentralised continuous vaccination attained higher initial vaccination coverage than the standard method “pulse vaccination” and sustained greater coverage over time following measurement at 11 months.

Our findings are consistent with previous studies in rural Sub-Saharan Africa, which show that few pulsed vaccination campaigns manage to reach the recommended coverage of

70% (Cleaveland *et al.*, 2003; Kaare *et al.,* 2009; Touihri *et al.*, 2011; Jibat *et al.*, 2015; Mulipukwa *et al.,* 2017; Kaneko *et al.,* 2021). In this study, overall coverage in the decentralised continuous vaccination varied from 64.10% to 60.67% at time point 1 and 2 respectively. Hampson *et al.* (2009) reported that vaccinating 60% of the dog population should be sufficient to prevent coverage declines to below the critical threshold, with only very few and small outbreaks ever observed in areas with coverage of 60% or more. A previous study in northwest Tanzania attained a coverage of 64% which resulted in significant reductions of the incidence of rabies in both humans and dogs (Cleaveland *et al.,* 2003). Indeed, 70% coverage is recommended by WHO for campaigns conducted annually. In our study, the overall coverage in decentralised continuous vaccination was slightly lower than the WHO recommendation, however it remained well above the lower critical bound of 55% (Coleman & Dye, 1996b) between the two time points at which coverage was measured (months 1 and 11).

Vaccination coverage is frequently assessed at only one timepoint, usually within a few days of campaigns, however herd immunity wanes over time due to population turnover (Butler & Bingham, 2000; Kitala *et al.,* 2002; Townsend *et al.,* 2013). Thus, high birth and death rates may result in declines in coverage even if the campaign attains high initial coverage (Conan *et al.,* 2015; Gsell *et al.,* 2012; Morters, McKinley, Restif *et al.,* 2014). One way to maintain effective vaccination coverage is to ensure vaccination services are provided free of charge and continuously as the need arise. Continuous vaccination as applied in this study helped to stabilize the coverage at relatively constant levels throughout the study period, with 4 237 dogs (28.40%) vaccinated via follow up campaigns conducted in the decentralised continuous vaccination, however the declines in coverage between timepoints generally appeared to be less that expectations.

The combination of central point vaccination clinics and house-to-house strategies have proven to be effective for achieving 70% coverage across a variety of dog populations (Kaare *et al.,* 2009; Gibson *et al.,* 2016; Taylor *et al.,* 2017). For example, (Morters, McKinley *et al.,* 2014) reported a coverage of up to 82% through house-to-house visits and Gibson et al report 33.43% vaccinated through door-to-door (Gibson *et al.,* 2016). In our study, only 2.74% and 2.09% dogs were vaccinated using house-to-house and on-demand respectively, with 95% of dogs vaccinated at central point clinics. There are several possible reasons to explain this from the perspective of supply (service providers) and demand (community). The household surveys revealed that most respondents were not aware of the availability of house-to-house and on-demand services, while some owners feared that calling a RC to vaccinate their dogs would incur a charge. During follow up campaigns the RCs and OHCs were instructed to vaccinate dogs using any method they considered the best, however in practice they might have preferred central point clinics because they are logistically more simple and less labour intensive compared to house-to-house and on-demand (Mazeri *et al.,* 2018; Barbosa Costa *et al.,* 2020). Also, as government employees RCs have other administrative tasks that might compete with their time to conduct intensive house-to-house and on-demand campaigns. Lack of self-motivation to go out and vaccinate dogs could be another reason to consider (Bardosh *et al.,* 2014).

Data from this study suggested that owner recall tended to overestimate coverage at the ward level, particularly under pulse vaccination, while vaccination certificates tend to underestimate coverage. These results agreed with our data on accuracy of assessment at the individual dog level, with owner recall being more sensitive than certification, while both methods had similar specificities. Underestimation by certificates is likely due to poor storage or loss of certificates (Conan *et al.,* 2015; Mezeri *et al.,* 2018; Kaneko *et al.,* 2021).

There were also some cases of overestimation through certificates, however, possibly due to presentation of invalid or expired certificates, or certificates being shared between dogs (Minyoo *et al.,* 2015). On average, however, the differences between coverage estimates via these alternative methods and the gold standard microchipping method were relatively small, suggesting that they may all be reliably used as alternative methods to assess coverage.

Lack of awareness regarding vaccination campaigns appears to be a major obstacle for people to vaccinate their dogs. Despite the efforts made to sensitize the community about vaccination campaigns, 22.56% of respondents in the areas receiving pulse vaccination who did not vaccinate their dogs reported being unaware of the campaign as the reason for not participating. Lock of awareness of vaccination campaigns has been mentioned in other studies as a leading reason for failure to attend vaccination clinics (Durr *et al.,* 2009; Muthiani *et al.,* 2015; Minyoo *et al.,* 2015b; Castillo-Neyra *et al.,* 2017; Mazeri *et al.,* 2018). In contrast, only 1.83% of people who received decentralised continuous vaccination reported lack of awareness as a reason for not vaccinating dogs. Integrating OHCs in the decentralised continuous vaccination could underlie the improved awareness of the campaigns in this method. Among other duties, OHCs played a role in sensitizing the community about rabies campaigns and communicating vaccination schedules.

Introduction of new dogs from outside the study area and the birth of new puppies was mentioned (41.51%) as the main reasons for dogs not being vaccinated in the decentralized continuous vaccination method. Although the vaccination campaigns were supposed to be continuous, the household surveys indicated that the RCs performed few subsequent campaigns after the major campaigns in month one.

In contrast to other studies which have shown that coverage declines with increased distance (Kaare *et al.,* 2009) and the likelihood of people attending central point vaccinations decreases with increasing distance (Kayali *et al.,* 2003; Mazeri *et al.,* 2018; Sánchez-Soriano *et al.,* 2019, 2020), very few people in this study perceived distance as an obstacle to their participation (Table 4.4 and Figure 4.5). This finding is similar to a study in Uganda which also showed that people were willing to travel large distances to reach vaccination points (Evans *et al.,* 2019). Another study in Zambia reported that dog owners were willing to travel up to 5 km (Kaneko *et al.,* 2021) and in Chad owners did not perceive distance as a major obstacle (Lechenne *et al.,* 2016). One study that investigated the relationship between being unaware of the campaign and distance found that people who did not attend the vaccination campaigns were located far from the central point (Mazeri *et al.,* 2018). This suggests that being unaware of the campaign rather than distance could play a major role in influencing central point attendance. Also, the nature of the topography or location of the central point relative to the household may influence participation. For example, people from difficult terrain (steep hills, rivers) may find it harder to travel with their dogs to vaccination clinics as opposed to people living in urban areas with good road infrastructure (Castillo-Neyra *et al.,* 2017).

Assessing service delivery can provide understanding of how the quality of the services offered matches owners' perceptions and expectations. Experience from other studies shows that, feedback provided through such dialogue helps to improve service and maintain owners interest in the programme (Turkson, 2009). Our data analysis showed that, owners who received the village-level vaccination strategy on average spent more time to reach central points compared to those under pulse administration. The main campaign in the Village-level strategy was conducted at the village level just like the pulse delivery. Therefore, it is likely that the difference in travel time between strategies is

caused by household locations relative to central points. Dog owners under the sub-village-level strategy spent less time to reach vaccination points and less time (<30 min) to complete registration and vaccination at the central point (for the discretionary strategy also). This makes sense, given that a village-level campaign involves the entire village attending in a single day. During this study, some central point clinics at village-level experienced large turnouts. For example, in Baranga village in Buswahili ward, 557 dogs were vaccinated at one central point clinic in a single day. Registration, certificate writing and vaccination of such large numbers of dogs likely contributed to longer waiting times. But vaccinators at the Village-level also vaccinated more animals per day compared to vaccinators under the sub-village and discretionary strategies (Table 4.2). Nonetheless, results indicate that dog owner's satisfaction with RCs and OHCs was high across all the sub-strategies of the decentralised continuous vaccinations.

A limitation of this study is that the cost-effectiveness of these vaccine delivery strategies has not been compared and is an important consideration for large-scale interventions. All the delivery strategies were effectively government-led interventions utilizing existing animal health delivery infrastructure and government employees, and therefore do provide a model for scaling up mass dog vaccination to other parts of the country. Other studies of animal health or public health interventions suggest that decentralized government-directed interventions are more cost-effective (Cleaveland *et al.,* 2014) and sustainable (Taylor & Partners for Rabies Prevention, 2013; Wallace *et al.,* 2017). In addition, integration of community members into the program improved participation and may have helped bring a greater sense of ownership which is vital for the success of such a programme. Overall, this feasibility study provided clear evidence to support the use of a decentralized continuous approach to dog vaccination which will be fully evaluated through a randomized controlled trial implemented across the entire Mara region.

4.12 Conclusion

This study is the first to report the implementation of mass dog vaccination through a continuous vaccination approach using thermotolerant rabies vaccines stored locally in passive cooling devices (Zeepots). It also provides an estimate of the vaccination coverages achieved and maintained at critical timepoints post-vaccination. Our results suggest that a decentralized continuous vaccination approach is feasible to implement and can achieve and maintain sufficiently high coverage of the dog population. The deployment of the Zeepot and engagement of One health champions improved access to vaccines as well as community awareness and participation in the campaign. Combining various vaccination strategies in a continuous delivery approach can support the goal of maintaining 70% vaccination coverage. We hope that the results from this study will support the design and implementation of more effective dog vaccination campaigns to achieve the goal of dog-mediated human rabies elimination by 2030.

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CHAPTER FIVE

5.0 GENERAL DISCUSSIONS AND RECOMMENDATIONS

5.1 General Discussions and Conclusions

The key objective of this thesis was to develop rabies vaccine delivery strategies that can help to sustain the levels of coverage needed to maintain herd immunity. To achieve this objective, I started by developing an inexpensive and affordable Passive Cooling Device "Zeepot Clay", for storage of thermotolerant vaccines for extended periods in settings where power and refrigeration are limited (Chapter 2). Analysis of temperature data collected over the period of 12 months (representing a complete annual cycle) indicated that the device was robust against environmental changes in temperatures. Despite ambient temperatures at times exceeding 42^0C, the internal temperature of the device remained below 25^0C. The performance of the device will likely vary from place to place; however, I expect that the general performance of the device will remain similar Tanzania given that the field tests were conducted in the lake zone area, which is known to be one of the hottest areas in the country. The Zeepot Clay as shown in this study can store small batches of vaccines up to 500 vaccine vials, often sufficient to vaccinate most of dogs in a community

and thereby reducing the travel cost needed to restock vaccines from the district headquarters. The cost of manufacturing a single unit of Zeepot Clay is cheaper ($11) compared to the price of commercially manufactured PCDs ($700-$3000). Moreover, the Zeepot Clay is made of a material (clay soil) that is readily available in most areas across Sub-Saharan Africa and Asia (clay soil), making it feasible to manufacture locally in most areas at low cost. In this study we didn't measure the lifespan of the Zeepot, however based on the materials used to construct it, it we estimate that its service period can vary from 7 to 10 years if treated with care, although further work is required to investigate this.

In Chapter 3 I built on the results from Chapter 2 to investigate whether the potency of the Nobivac® Rabies vaccine would be impacted following storage in the Zeepot Clay. The findings from this study demonstrated that the neutralizing antibody response of dogs vaccinated with the Nobivac® Rabies vaccine stored within the Zeepot Clay was not inferior to that of dogs vaccinated with the same vaccine kept under stable cold-chain conditions. Similarly, the results of the logistic regression supported that the vaccine storage condition (cold-chain vs Zeepot Clay) did not impact seroconversion ($z = 1.1$, $df = 313$, p-value = 0.25). A secondary analysis indicated that there was a positive linear trend between Body Condition Score (BCS) (O.R. 2.2, 95% CI: 1.1 – 5.1) and seroconversion, with dogs of good body condition being more likely to seroconvert than dogs with poor BCS, however neither sex nor age impacted seroconversion. The findings of this study have demonstrated that the Zeepot Clay has a utility for storing thermotolerant rabies vaccines at sub-ambient temperatures for extended periods. Whilst the Zeepot Clay is not designed to replace the refrigerators we consider that its ability to maintain temperatures below levels at which thermotolerant vaccines can be stored could enable penetration of mass dog vaccination campaigns in hard-to reach communities. This will bring clear benefits to national governments planning to scale-up rabies elimination programs. While

the original intention for the Zeepot Clay was to store thermotolerant rabies vaccines in areas without existing cold chain systems, we anticipate that this tool could be used to store other thermotolerant vaccines, however more work is required to confirm this.

The successful development of a vaccine storage device formed a foundation upon which I was able to design and evaluate the effectiveness of novel ‘decentralized continuous vaccination (DCV)’ delivery strategies as well as assessing the perception of the dog owners regarding the quality of the services offered under the novel delivery strategies (Chapter 4). The results of the household surveys showed that the vaccination coverage achieved in areas that were targeted using the novel strategy the novel strategies was much higher (at both time points i.e., 1-month and 11-month post vaccination) than in areas receiving standard vaccinations. These results suggest that a DCV approach is feasible to implement and can achieve and maintain sufficiently high vaccination coverage across of dog population. The deployment of the Zeepot and engagement of One health champions appeared to improve access to vaccines as well as community awareness and participation in the campaign. Combining various vaccination strategies in a continuous delivery approach helped to increase the number of dogs being vaccinated, thus maintaining the herd immunity with minimal variation throughout the yearly cycle. I conclude that decentralized continuous dog vaccination strategies have the potential to improve vaccination coverage and maintain herd immunity against rabies, which is of great importance as we strive to eliminate human rabies globally by 2030.

5.2 Recommendations

Based on the findings from the study the following recommendations are made for effective control of rabies.

i. In many parts of LMICs including Tanzania, the cold chain system at the lower administrative levels, such as wards and villages, is not well developed thus making it difficult for the immunization programs to target out rural communities. In this study I have demonstrated that under proper care and monitoring the Zeepot Clay is able to store thermotolerant rabies vaccine. I advise the Government of Tanzania, through the Ministry of Livestock and Fisheries, to consider integrating this tool to store vaccines during their campaigns in rural areas. In doing this, the utilization of the Zeepot Clay could improve equity in health services and extend the reach of vaccination programmes to many more dogs in a decentralized manner.

ii. In order to bring a sense of ownership and sustainability, the communities should be engaged in the planning and implementation of rabies vaccination programmes. In this study integration of community members into the vaccination team improved community participation in the campaign, helped to disseminate campaign information, and aided identification of dogs that required vaccination.

iii. Sustaining high levels of vaccination coverage is a critical component of any successful vaccination programme. The decentralized continuous vaccination as applied in this study resulted in high levels of coverage (i.e., achieved approximate twice the coverage of centralized pulsed vaccination), as needed to maintain herd immunity. The confidence intervals of the centralized pulsed vaccination did not overlap with that of decentralized continuous vaccination indicating that the difference was significant. I therefore recommend decision makers and planners of rabies vaccination campaigns to consider adopting this approach

iv. During implementation of a large vaccination program, implementers will likely need to alter some of the components of the tested protocol, thus close supervision of the implementers is necessary to guarantee the success of the program.

v. Findings indicated that age had no impact on seroconversion following vaccination. However, dogs under dogs under 3 months are often considered too young for vaccination and remain unvaccinated as a result and pose a risk for transmission of rabies, including to humans. I therefore recommend that dogs of all ages should be vaccinated to maintain herd immunity.

vi. Dogs with poor body condition had less likelihood of seroconversion than dogs with good body condition. For the former, a booster vaccination administered at 4 weeks following the primary vaccination could help to boost immunity against rabies and overcome the impact of the low BCS. However, further studies are required to determine whether vaccinated dogs that did not seroconvert are nonetheless protected against rabies infection.

vii. Data from this study suggested that owner recall tended to overestimate coverage at the ward level, particularly under CPV, while vaccination certificates tended to underestimate coverage. However, the differences between coverage estimates via the two alternative methods and the gold standard microchipping method were non-significant, suggesting that certificates and owner recall may both be reliably used as cheap alternative methods to assess coverage.

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APPENDICES

Appendix 1: Animal owner informed consent

Animal Owner Informed Consent Form ***Fomu ya Makubaliano na mmiliki wa mnyama***	
Title of the study ***Jina la Utafiti***	Improving the Delivery of Mass Dog Vaccinations to Support the Elimination of Dog-Mediated Rabies in Tanzania *Tathimini ya athari ya uhifadhi wa chanjo za kuzuia kichaa cha mbwa kwa mbwa*
Study No.. ***Namba ya Utafiti***	301620-01 Wellcome Trust – University of Glasgow Afrique One ASPIRE – Ifakara Health Institute
With my signature I, the animal owner, agree to participate in the study as indicated above ***Kwa kuweka sahihi mimi mwakilishi wa mnyama, nakubali kushiriki katika utafiti tajwa hapo juu***	
I confirm that the Investigator has comprehensively informed me about: ***Nathibitisha kuwa mtafiti amenijulisha vema vya kutosha juu ya yafuatayo:***	
▪ **the objective(s) of the study** ***Malengo juu ya utafiti***	
▪ **the voluntary participation in the study** ***Ushirikiano wa hiari katika utafiti***	
▪ **the time schedule of the study and the need to present my animal(s) on the planned visit days** ***Ratiba ya utafiti na umuhimu wa uwepo wa mnyama katika siku maalumu za mahudhurio***	
▪ **the procedures involved in the study, in particular the blood sampling procedure** ***Mbinu zitakazotumika katika utafiti, hasa utoaji wa damu kwa mnyama***	
▪ **the possibility of removing my animal(s) from the study at any time** ***Uwezekano wa kumtoa/ kuwatoa wanyama katika utafiti huu wakati wowote***	
▪ **the need to observe my animal(s) and report any adverse event to the Investigator** ***Haja ya kuangalia mnyama/ wanyama wangu na kutoa taarifa yoyote ile kwa mtafiti***	
▪ **the need to inform the Investigator about all vaccinations given to my animal(s) throughout their life** ***Haja ya kumkulisha mtafiti kuhusu chanjo zote walipata wanyama wangu katika maisha yao yote***	
▪ **that I will not be charged for examinations, treatments, and medication as foreseen in the study** ***Kwamba sitatozwa hela kwa ajili ya uchunguzi, matibabu na dawa inavyyonyesha katika utafiti***	

Animal Owner Informed Consent Form ***Fomu ya Makubaliano na mmiliki wa mnyama***	
Title of the study ***Jina la Utafiti***	Improving the Delivery of Mass Dog Vaccinations to Support the Elimination of Dog-Mediated Rabies in Tanzania *Tathimini ya athari ya uhifadhi wa chanjo za kuzuia kichaa cha mbwa kwa mbwa*
Study No.. ***Namba ya Utafiti***	301620-01 Wellcome Trust – University of Glasgow Afrique One ASPIRE – Ifakara Health Institute
▪ **the confidentiality of the study** ***Usiri katika utafiti***	
Animal name(s) / chip ID ***Jina la mnyama/ wanyama/ Chip ID***	
Name and Address of Animal Owner ***Jina na anuani ya mwakilishi wa mnyama***	
Name Investigator ***Jina la Mtafiti***	
Date ***Tarehe***	____/____/_____
Signature Animal Owner / Agent ***Sahihi ya mwakilishi wa mnyama***	
Signature Investigator ***Sahihi ya Mtafiti***	
24 hour emergency contact details:	**Dr. Ahmed Lugelo tel: +255 764 782 703**

Appendix 2: Community Informed Consent Form for Animal Owners

English version
Researcher: Ahmed Lugelo – College of Veterinary Medicine and Biomedical Sciences
24- hour Emergency number: +255 764 782 703
Community member _____
Community member _____
Community member _____
Community member _____
Community member _____

Purpose of this form
I would like to include dogs in your community in an on-going research study designed to vaccinate dogs against rabies. This form is to provide information to help you decide whether you want your dogs to be in the study or not. **Please read this form carefully.**
You can ask questions about the purpose of the study, the possible risks and benefits, and anything else about the research or this form that is not clear. When we have answered all your questions, you can decide if you want your (type of animal) to be in the study or not.
Purpose of this Study
This study is being done to vaccinate dogs against rabies and to investigate how such activities can be done as efficiently as possible. As many dogs as possible will receive a vaccination in the scruff of the neck. The vaccines used will have been either stored in refrigeration units at 4 degrees Celsius or stored in cooling devices designed to keep the vaccines colder than ambient temperatures. The rabies vaccine used in this program has been shown to be equally potent following storage at 4 degrees Celsius or temperatures up to 30 degrees Celsius for three months. Consequently your dogs are equally likely to be protected against rabies following vaccination with vaccines stored either at 4 degrees Celsius or in temperatures less than 30 degrees Celsius for three months. I will monitor the temperature that the vaccines are stored at and any vaccines stored at temperatures greater than 30 degrees Celsius will not be used.
The risk to the health of the dogs is minimal although the vaccinations can sometimes cause minor irritation. In addition we would like to collect a 10 ml blood sample from a sample of dogs. These samples will be used to investigate whether the vaccine is working, and also whether your dogs have been exposed to certain viruses and bacteria. I will sometimes mark the dogs with water-soluble spray paint or collars. There will be no fee charged, nor will there be any compensation for taking part in the study.
You will receive an information sheet regarding the rabies vaccination program.
Enrolling dogs in this study is voluntary and you can withdraw permission and your dogs from the study at any time. You also understand that your dogs can be withdrawn from the study if the investigators find it necessary. If your dogs are withdrawn from the study for any reason, data already collected may continue to be used for research purposes.
Your dogs will not be treated differently if you decline to participate in the study. Your decision to participate, not participate, or withdraw dogs from the study will not affect your relationship with your local and national veterinary authorities or the research organizations conducting this study or any other treatment your dogs are receiving.

Name of researcher _Ahmed Lugelo _____Signature _____Date:_____

Subject's Statement

This study has been explained to me. I agree that our community dogs can take part in this research. I have had a chance to ask general questions about the research, with the researcher listed above. This study has been reviewed and approved by the Ifakara Health Institute of Health [IHI / IRB / No: 024-2018] and Sokoine Agricultural University [SUA / CVMBS / R.1 / 2019/6]. If I have additional concerns, I can call the secretary of the Ifakara Health Institute Research Ethics Committee Dr. Mwifadhi Mrisho +255 788 766 676, email: mmrisho@ihi.or.tz. I will receive a copy of this consent form.

I certify that I am a member of the target community and have the authority to consent medical treatment for the community dogs.

Name of the subjects Signature Date:

1.
2.
3.
4.
5.

Appendix 3: Community Informed Consent Form for Animal Owners

Swahili version
Fomu ya makubaliano na Jamii

Jina la Utafiti: Tathimini ya athari ya uhifadhi wa chanjo za kuzuia kichaa cha mbwa kwa mbwa

Mtafiti: Dr. Ahmed Lugelo
Namba ya dharura: +255 7864 703

Mjumbe wa jumuiya _____
Mjumbe wa jumuiya _____
Mjumbe wa jumuiya _____
Mjumbe wa jumuiya _____
Mjumbe wa jumuiya _____

Lengo la fomu hii

Tungependa kuwasajiri mbwa kutoka katika jumuiya yako kushiriki katika utafiti wenye lengo la kuwakinga mbwa dhidi ya ugonjwa wa kichaa cha mbwa. Lengo la fomu hii ni kutoa taarifa ambayo itakusaidia kuamua kama mbwa kutoka katika jumuia yako watashiriki katika utafiti huu au la. **Tafadhari soma fomu hii kwa makini**.

Unaweza kuuliza maswali kuhusu madhumuni ya utafiti, hatari na faida au kitu chochote ambacho hakijajulikana katika utafiti huu. Baada ya kujibu maswali yako yote, utaweza kuamua kama unataka (wanyama-mbwa) wawe katika utafiti au la.

Malengo ya Utafiti huu
Utafiti huu unafanyika ili kuzuia mbwa dhidi ya ugonjwa wa kichaa cha mbwa na kuchunguza jinsi gani shughuli hizo zinaweza kufanywa kwa ufanisi iwezekanavyo. Mbwa wengi kama iwezekanavyo watapata chanjo katika scruff ya shingo. Chanjo zitakazotumika zitakuwa zimehifadhiwa katika friji kwa digrii 4 za Celsius au zitakuwa zimehifadhiwa katika vifaa vya baridi ambavyo vinatengenezwa ili kuzuia chanjo isipate joto kubwa kuliko joto la kawaida. Chanjo ya kichaa cha mbwa itakayotumika katika jaribio hili imeonesha kuwa na uwezo kukinga wanyama dhidi ya ugonjwa wa kichaa cha mbwa ikihifadhiwa kwenye digrii 4 za Celsius au joto hadi digrii 30 Celsius kwa miezi mitatu. Hivyo tunategemea mbwa watakaochanjwa na chanjo iliyohifadhiwa kwenye digrii 4 za celsius watakuwa na kinga sawa na wale waliochanjwa na chanjo iliyohifadhiwa katika joto chini ya digrii 30 kwa muda wa miezi mitatu. Tutafuatilia joto ambayo chanjo imehifadhiwa, chanjo yoyote iliyohifadhiwa katika joto kubwa zaidi ya digrii 30 Celsius haitatumiwa.

Hatari kwa afya ya mbwa ni ndogo ingawa chanjo zinaweza wakati mwingine kusababisha muwasho kidogo. Pia tutatuchukua kiwango kidogo cha sampuli ya damu 10 ml. Sampuli

hizi zitatumika kuchunguza ikiwa chanjo inafanya kazi, na pia kama mbwa wako wamelipata magonjwa ya virusi au bakteria. Wakati mwingine tutawekea mbwa alama za utambulisho kwa kutumia kemikali isiyo na madhara au kola. Hakutakuwa na gharama za kushiriki, wala hakutakuwa na fidia yoyote kwa kushiriki katika utafiti.

Utapokea karatasi yenye taarifa na ratiba ya chanjo ya kichaa cha mbwa.

Hatukulazimishi kuwaandikisha mbwa katika utafiti huu bali tunakuomba ushiriki kwa hiari yako, una ruhusa ya kuwaondoa mbwa wako wasiendele kushiriki kwenye utafiti huu muda wowote. Pia tungependa uelewe kwamba mbwa wako wanaweza kuondolewa kutoka kwenye utafiti ikiwa wachunguzi wataona kuwa ni lazima. Ikiwa mbwa wako wataondolewa kwenye utafiti kwa sababu yoyote ile, taarifa zilizokusanywa tayari zinaweza kuendelea kutumika kwa madhumuni ya utafiti.

Mbwa wako hazitashughulikiwa tofauti ikiwa utakataa kushiriki kwenye utafiti. Uamuzi wako wa kushiriki, kutokushiriki, au kuondoa mbwa wako kutoka kwenye utafiti hautaathiri uhusiano wako na WSU au matibabu yoyote ambayo mbwa wako wanapokea.

Jina la Mtafiti Mkuu_____ Sahihi_____ Tarehe_____

Tamko la mmiliki wa mnyama.
Utafiti huu umeelezwa kwangu. Nakubali kwamba mbwa kutoka katika jamii yetu wanaweza kushiriki katika utafiti huu. Nimepata nafasi ya kuuliza maswali ya jumla kuhusu utafiti, na mtafiti aliyeorodheshwa hapo juu. Utafiti huu umepitiwa na kuidhinishwa na Taasisi ya Afya Ifakara [IHI/IRB/No:024-2018] na Chuo Kikuu cha Kilimo cha Sokoine [SUA/CVMBS/R.1/2019/6]. Na ili kuwasilisha malalamiko yeyote, tafadhari wasiliana na katibu wa Kamati ya Miiko ya Utafiti ya Taasisi ya Afya Ifakara Dk. Mwifadhi Mrisho +255 788 766 676, email:mmrisho@ihi.or.tz. Nitapokea nakala ya fomu hii ya idhini.

Ninathibitisha kuwa mimi ni mwanachama wa jumuiya husika na nina mamlaka ya kukubali na kutoa idhini ya matibabu ya mbwa wa jamii.

Jina la Mshiriki Sahihi Tarehe

1.
2.
3.
4.
5.

Appendix 4: Adverse event form

Adverse Event (AE) Reporting Form

1. CASE / PROBLEM TYPE

Check all that apply

Safety	
in animals	☐
in humans	☐
Post-Authorisation Study	☐
Lack of expected efficacy	☐
Literature	☐
Environmental problems /Ecotoxicity	☐
Residue violation / Withdrawal period issues	☐
Extra label use	☐

2. HOW RECEIVED

Phone	☐
Personal	☒
Email	☐
Letter	☐
Fax	☐
Other	

Date adverse event received by sender (dd/mm/yyyy): 22/11/2017

Local reference No. (if any):

Date first valid (dd/mm/yyyy): 20/11/2017

Type of report: Initial☐ Follow-up☒ (date, case number) 06/12/2017, 01

Person who reported the AE: veterinarian☐ veterinary nurse/technician☐ owner☒ other:

3. VETERINARIAN / NURSE /TECHNICIAN / OTHER

Name: AHMED LUGELO

Address: P.O Box 12266 ARUSHA TANZANIA

Telephone No. +255764782703

4. ANIMAL OWNER / HUMAN PATIENT

Name *(according to confidentiality legislation)*: JOSEPH KINYWANEHIRI

Address: Gesarya-Getarungu

Telephone No. NA

5. ANIMAL DATA

Animal characteristics (animal(s) showing signs)

Species: Canine **Breed/production type**: Mix/domestic dog

Sex/physiological status: female☐ male☒ pregnant☐ neutered☐ lactating☐ unknown☐

Weight (kilos): unknown☒ **Age**: 2yr unknown☐

State of health at time of treatment: good☒ fair☐ poor☐ critical☐ unknown☐

Concomitant medical conditions relevant to the case: Death due to injuries after being trampled by cow

Previous reaction to other products? yes☐ no☒ unknown☐ **If yes, describe**:

No. of animals exposed: 1

No. of animals treated:

No. of animals showing signs: 1

Outcome of Reaction to date:

	Recovered	Euthanized	Died	Under treatment	Alive with sequelae	Unknown
No. of animals						
Date when (01/11/2017):			1			

6. DETAILS OF SUSPECTED ADVERSE REACTION(S) IN HUMANS

Sex: Age/date of birth (dd/mm/yyyy): Occupation (with relevance to exposure):

Date of exposure (dd/mm/yyyy): Pregnant/nursing:

7. PRODUCT DATA # 1

~~**Trade name** (include dosage~~ form and strength): Nobivac Rabies **M.A. number:**

Version 1

Active substance(s) (INN):

Batch No.: **Expiry date** (dd/mm/yyyy): 01/01/2020 **Was product stored correctly?:** yes x☐ no x ☐ unknown☐

If no, explain:50% of vaccines were stored outside of cold chain for the purposes of a thermotolerance study

Treatment details: 1ml subcutaneous

Dose/frequency: once **Route of administration:** sub cutaneous

Site of administration: scruff of neck

Start date of treatment (dd/mm/yyyy): Give date **End date or duration** (dd/mm/yyyy) : Give date **Who administered the product?**: Veterinarian x☐ Vet nurse/tech☐ Owner/farmer ☐ Other

Use according to label: yes ☐ no x☐ unknown ☐ if no, explain: part of thermotolerance study where vaccines stored outside of cold chain

Action taken after reaction: drug withdrawn ☐ dose reduced ☐ other x ☐

Did reaction abate after stopping drug ? yes ☐ no ☐ not applicable x ☐

Did reaction reappear after reintroduction ? yes ☐ no ☐ not applicable x ☐

Reason(s) for use or initial diagnosis:

List all other relevant medications given to animal(s):
Give the list of the other veterinary medicinal products used concurrently and go to special field for completion of details (page 3)

8. PREVIOUS EXPOSURE AND REACTION(S) TO PRODUCT #1

Previous exposure to this product? yes ☐ no☐ unknown x ☐ **Date(s)** (dd/mm/yyyy):

Previous reaction to this product? yes ☐ no ☐ unknown x ☐ **If yes, describe:**

Has vet seen a similar reaction before? yes☐ no ☐ unknown☐ **If yes, describe:**

9. ATTENDING VETERINARIAN'S LEVEL OF SUSPICION THAT PRODUCT #1 CAUSED REACTION

Probable/high ☐ Possible/medium ☐ Unlikely/low x ☐ No attending vet ☐ Unknown ☐

10. REACTION DATA (*applicable for all types of adverse reaction(s) reported following administration of veterinary product(s)*

Date of onset of signs (dd/mm/yyyy):

End date of signs (dd/mm/yyyy) :

Describe the sequence of events including administration of product(s), all clinical signs, site of reaction, severity, pertinent lab tests, necropsy results, possible contributing factors (if necessary use extra sheet): Include details of treatment given to address this adverse reaction.

. Vaccination given as part of rabies vaccine thermotolerance study. Dog reported to have died by owner on 28 day follow up. No suspicion that death was related to vaccination.

Were the signs treated? Yes ☐ No x ☐ Unknown ☐ **If yes, describe:**

11. CAUSALITY ASSESSMENT RELATED TO PRODUCT (for internal use only)

Assessment: **A**-Probable☐ **B**-Possible☐ **O**-Unclassified ☐ **N**-Unlikely N ☐

Reason for Assessment:

12. NAME AND ADDRESS OF SENDER	Follow-up information expected: yes☐ no x ☐
Ahmed Lugelo	Signature: Date (dd/mm/yyyy):

To be replicated for each product used concurrently Local reference no:

DATA FOR PRODUCTS ADMINISTERED CONCURRENTLY – PRODUCT # <Enter sequential number; 2 or higher>

Trade name (include dosage form and strength): **M.A. number:**

Active substance(s) (INN)::

Batch No.: **Expiry date** (dd/mm/yyyy):: **Was product stored correctly?:** yes☐ no☐ unknown☐

If no, explain:

Treatment details:

Dose/frequency: **Route of administration:**

Site of administration:

Start date of treatment (dd/mm/yyyy): **End date or duration** (dd/mm/yyyy): **Who administered the product?**: Veterinarian ☐ Vet nurse ☐ Owner/farmer ☐ Other

Use according to label: yes ☐ no ☐ unknown ☐ if no, explain:

Action taken after reaction: drug withdrawn ☐ dose reduced ☐ other ☐

Did reaction abate after stopping drug? yes ☐ no ☐ not applicable ☐

Did reaction reappear after reintroduction? yes ☐ no ☐ not applicable ☐

PREVIOUS EXPOSURE AND REACTION(S) TO PRODUCT #

Previous exposure to this product? yes☐ no ☐ Unknown ☐ **Date(s)** (dd/mm/yyyy)::

Previous reaction to this product? yes☐ no ☐ Unknown ☐ **If yes, describe**:

Has vet seen a similar reaction before? yes☐ no ☐ unknown☐ **If yes, describe:**

ATTENDING VETERINARIAN'S LEVEL OF SUSPICION THAT REACTION WAS CAUSED BY PRODUCT #

Probable/high ☐ Possible/medium ☐ Unlikely/low ☐ No attending vet ☐ Unknown ☐

Appendix 5: Community Informed Consent Form for Animal Owners

ISO 9001: 2015 certified

1. **Jina la mradi**: "**Sayansi ya utokomezaji wa ugonjwa wa kichaa cha mbwa.**

2. **Taarifa za awali kuhusu kichaa cha mbwa**
   Kichaa cha mbwa ni ugonjwa hatari unaoua takribani watu 59,000 kila mwaka dunia nzima. Kwa kiwango kikubwa vifo hivi vinatokana na kung'atwa na mbwa, uambukizaji wa kichaa cha mbwa kutoka kwa mbwa kuja kwa binadamu unaweza kudhibitiwa kama mbwa watachanjwa dhidi yake. Ingawa chanjo ya mbwa ni njia Madhubuti ya kutokomeza kichaa cha mbwa, uendeshaji wa zoezi la chanjo ya mbwa maeneo ya vijijini ambako kichaa cha mbwa bado ni tatizo kunaambatana na changamoto ya utekelezaji wake, pia ni gharama kubwa.

   Lengo la mradi:

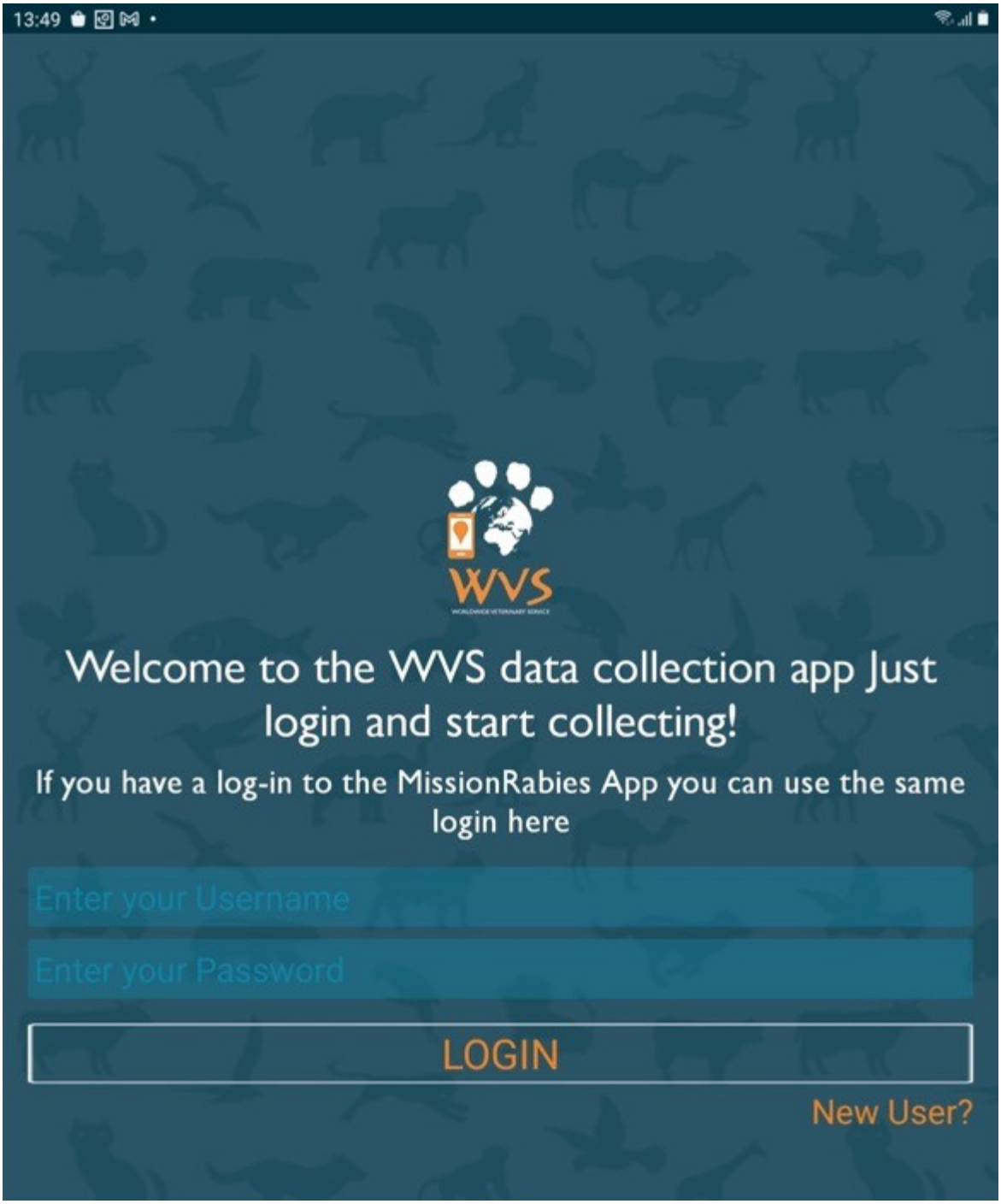
   - Kuamua mkakati bora wa utoaji wa chanjo ya mbwa na ufanisi zaidi kwa utekelezaji wao
   - Kuchunguza mbinu za kuongeza mikakati ya ufuatiliaji wa matukio ili kuongoza vizuri mikakati ya kutokomeza ugonjwa wa kichaa cha mbwa

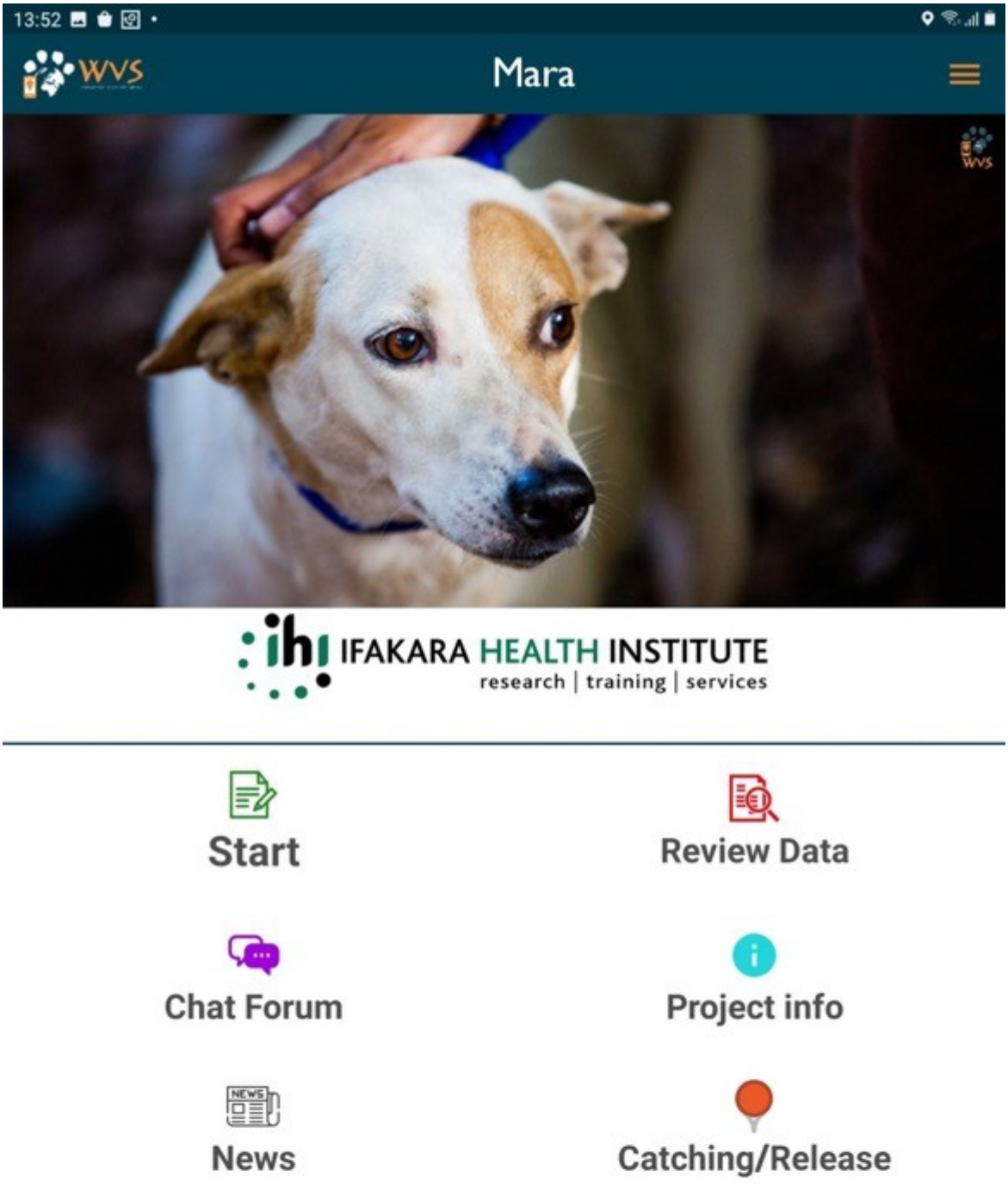
3. **Nini kitatokea kama nitaamua kushiriki?**
   Kama utaamua kushiriki katika utafiti huu, nitaulizwa maswali machache kuhusiana na ushiriki wangu katika zoezi la chanjo yam bwa lililopita. Hakuna madhara yeyote utayopata kwa kushiriki mbali na kuchukua muda wako kiasi (kama dk 20) kutujibu maswali tutakayokuuliza. Kwa kushiriki katika utafiti huu utakuwa umechangia katika elimu ya kudhibiti kichaa cha mbwa.

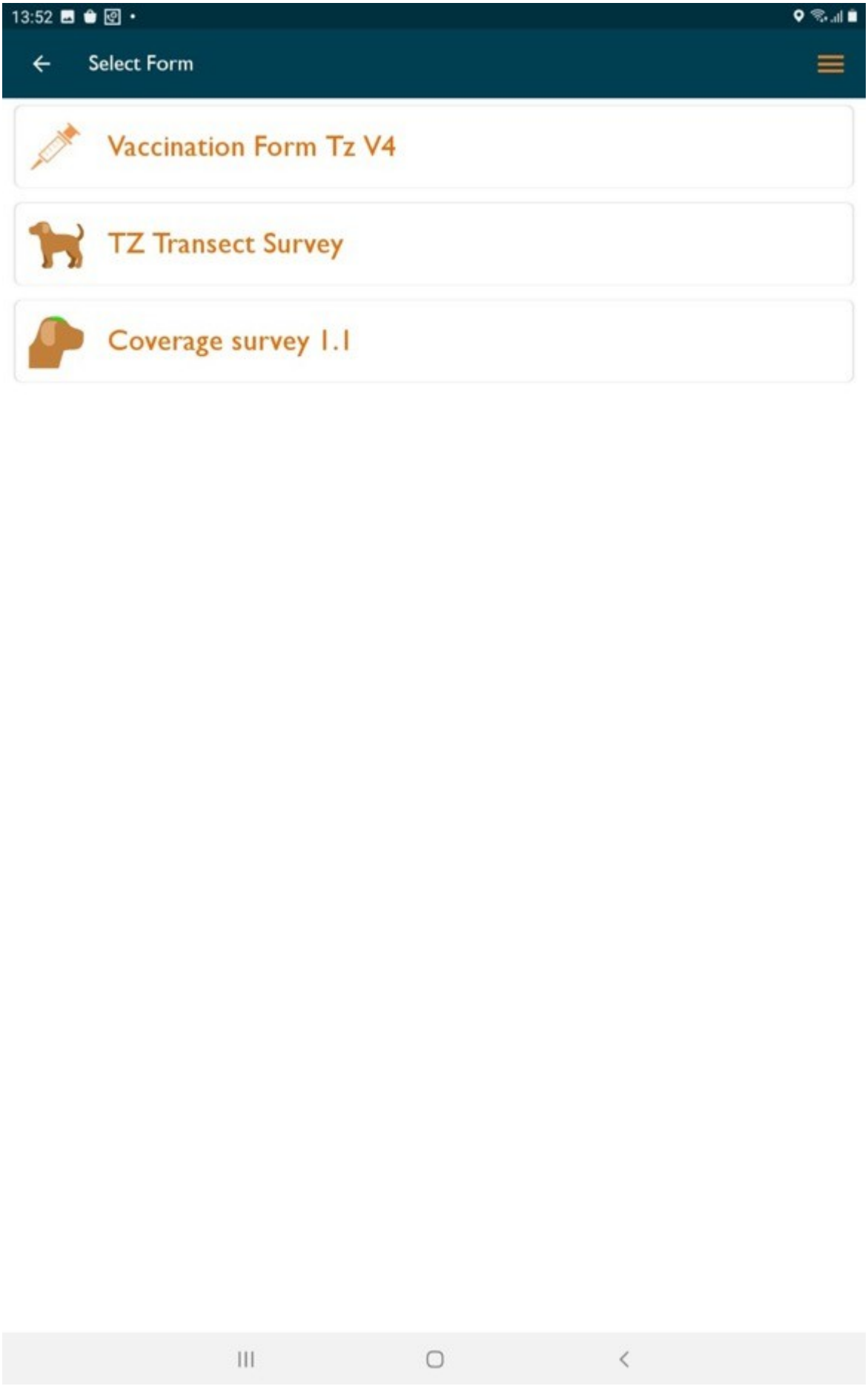
Na ili kuwasilisha malalamiko yeyote, tafadhari wasiliana na Katibu wa Kamati ya Miiko ya Utafiti ya Taasisi ya Afya Ifakara **Dk. Mwifadhi Mrisho +255 788 766 676, email: mmrisho@ihi.or.tz,**

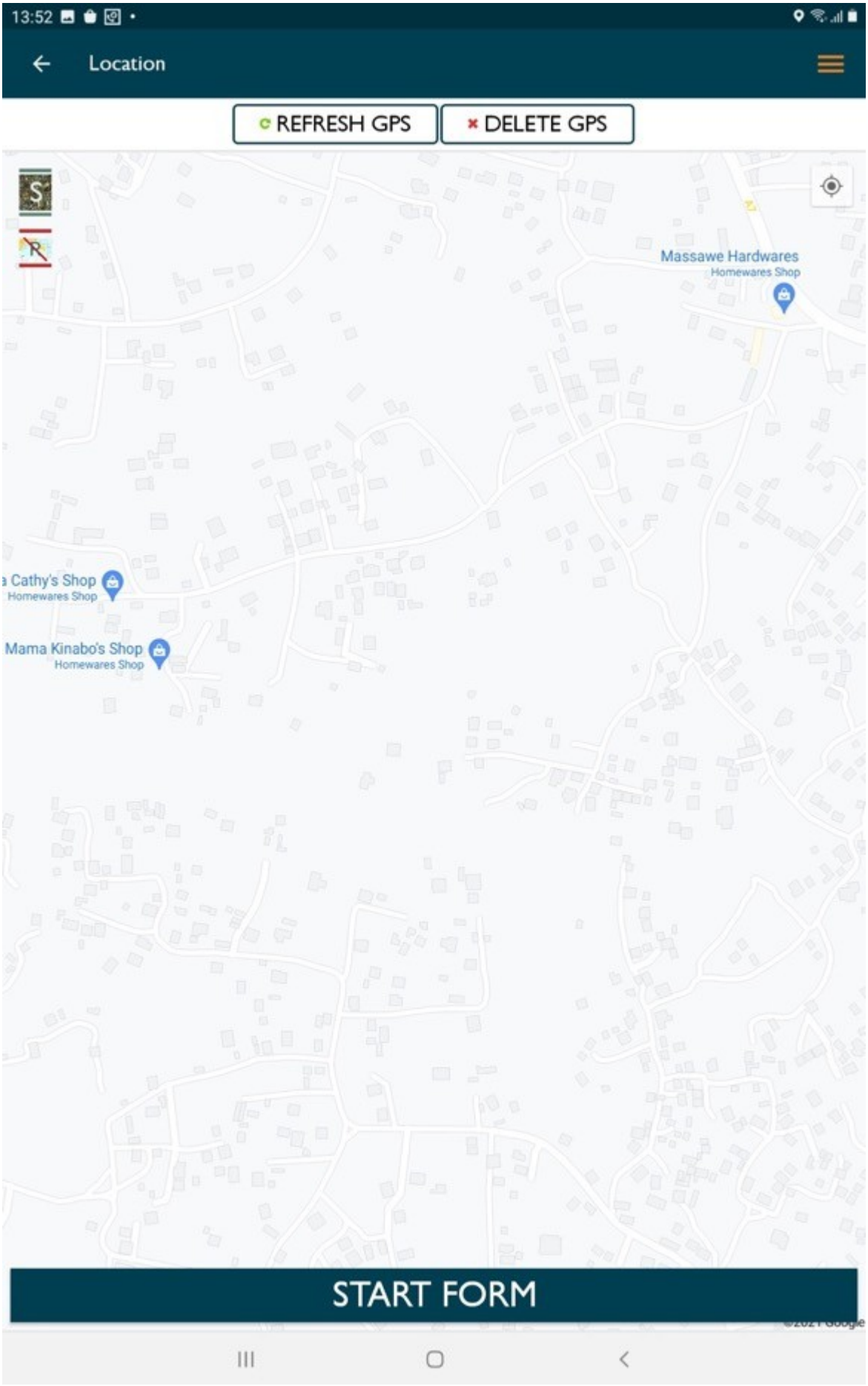
.....	.....	.....
Mshiriki	Mtafiti	Shahidi
.....	.....	.....
Sahihi/Dole gumba	Sahihi/Dole gumba	Sahihi/Dole gumba
.....	.....	.....
Tarehe	Tarehe	Tarehe

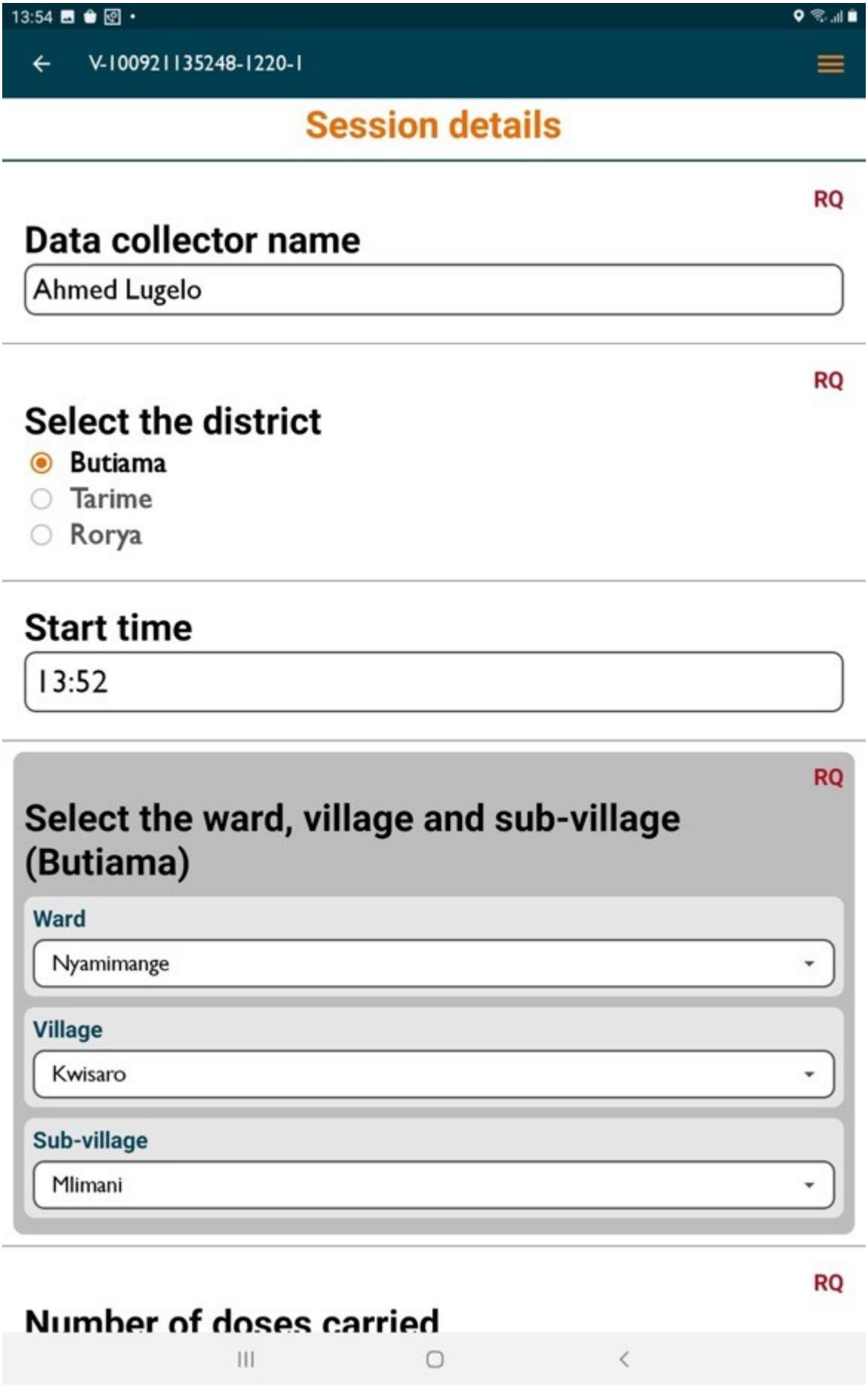
Appendix 6: Vaccination form: Rabies Mission app

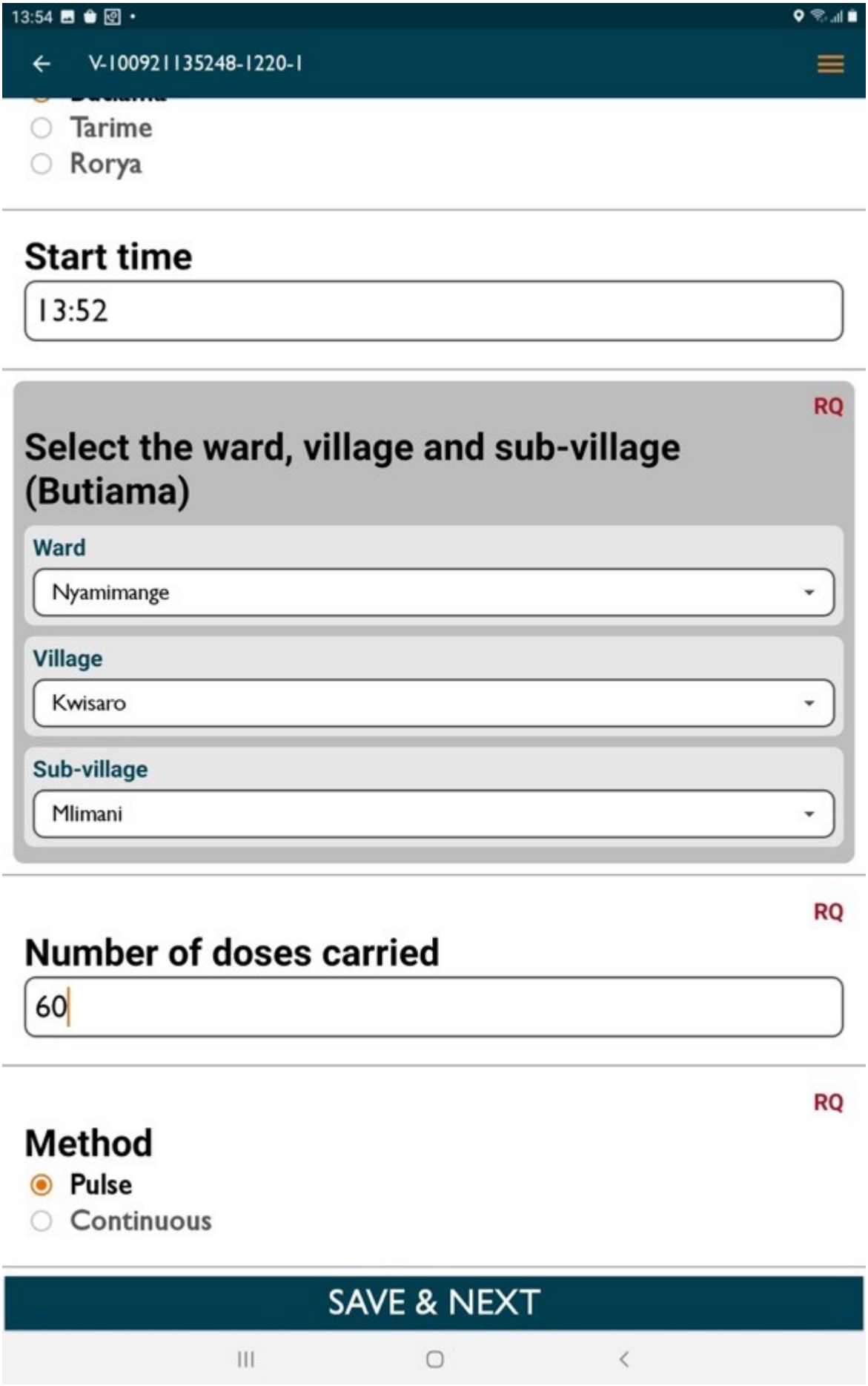


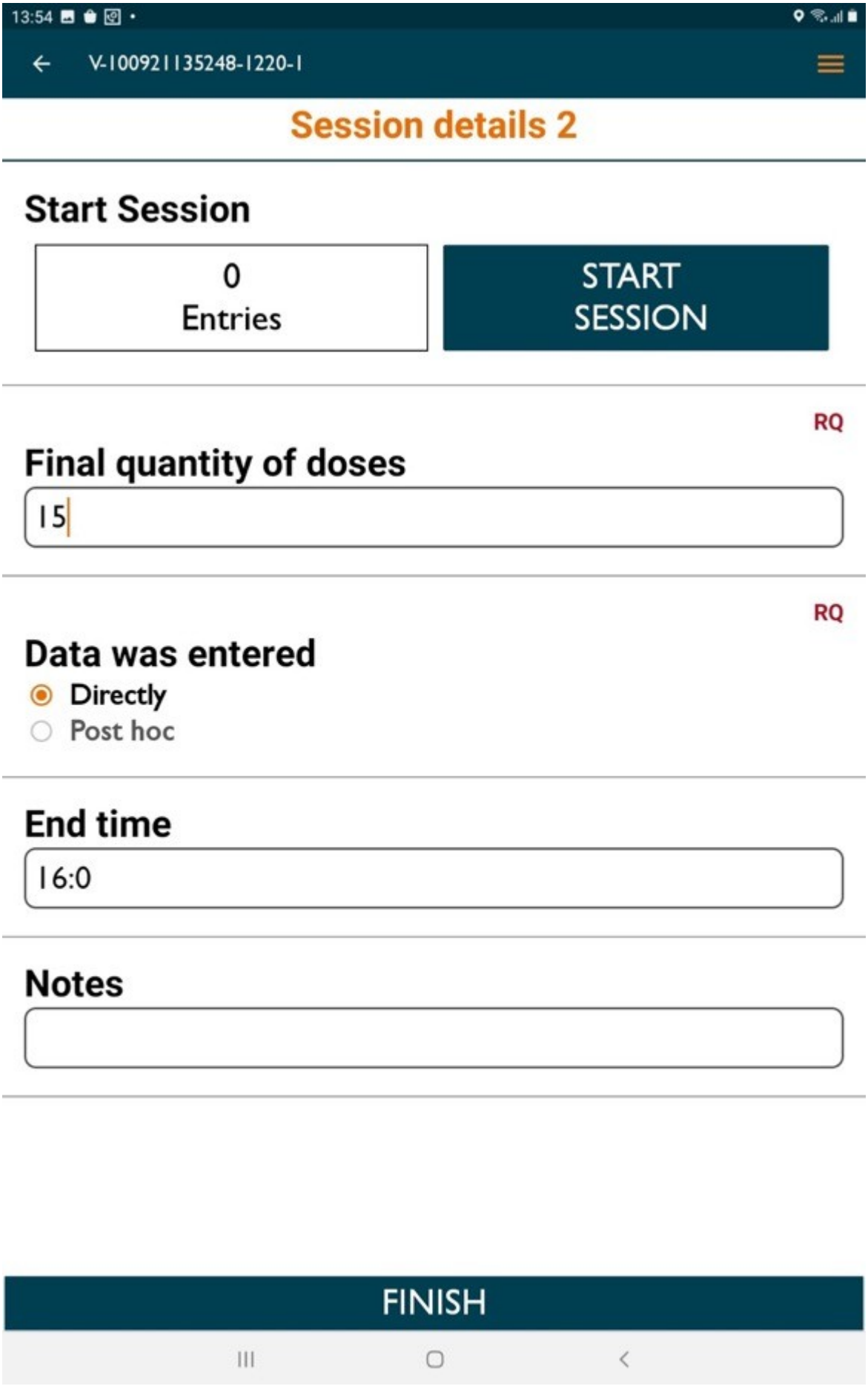

13:52
WVS
Mara
IFAKARA HEALTH INSTITUTE
research | training | services
Start
Review Data
Chat Forum
Project info
News
Catching/Release

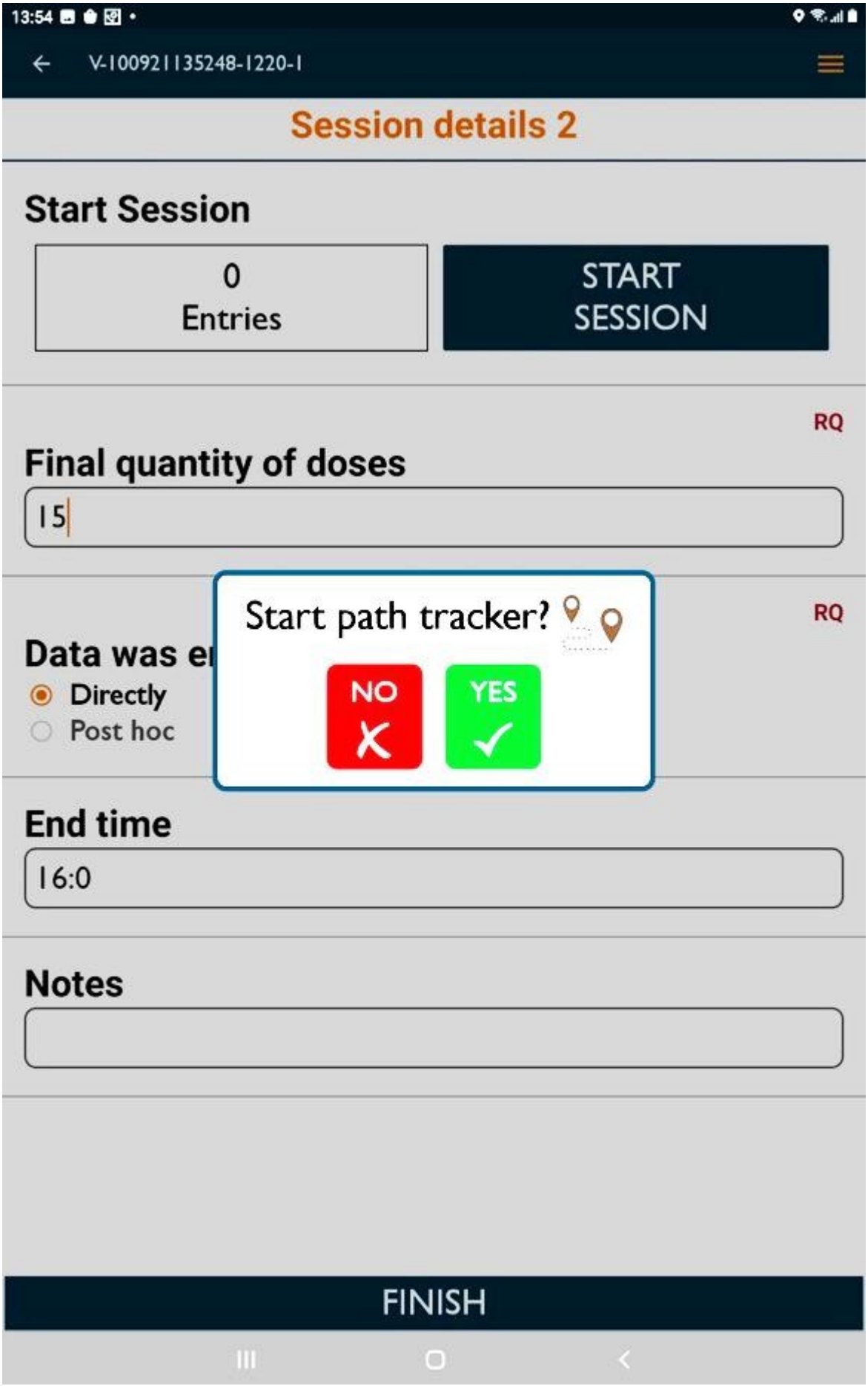

13:52
Select Form
Vaccination Form Tz V4
TZ Transect Survey
Coverage survey 1.1

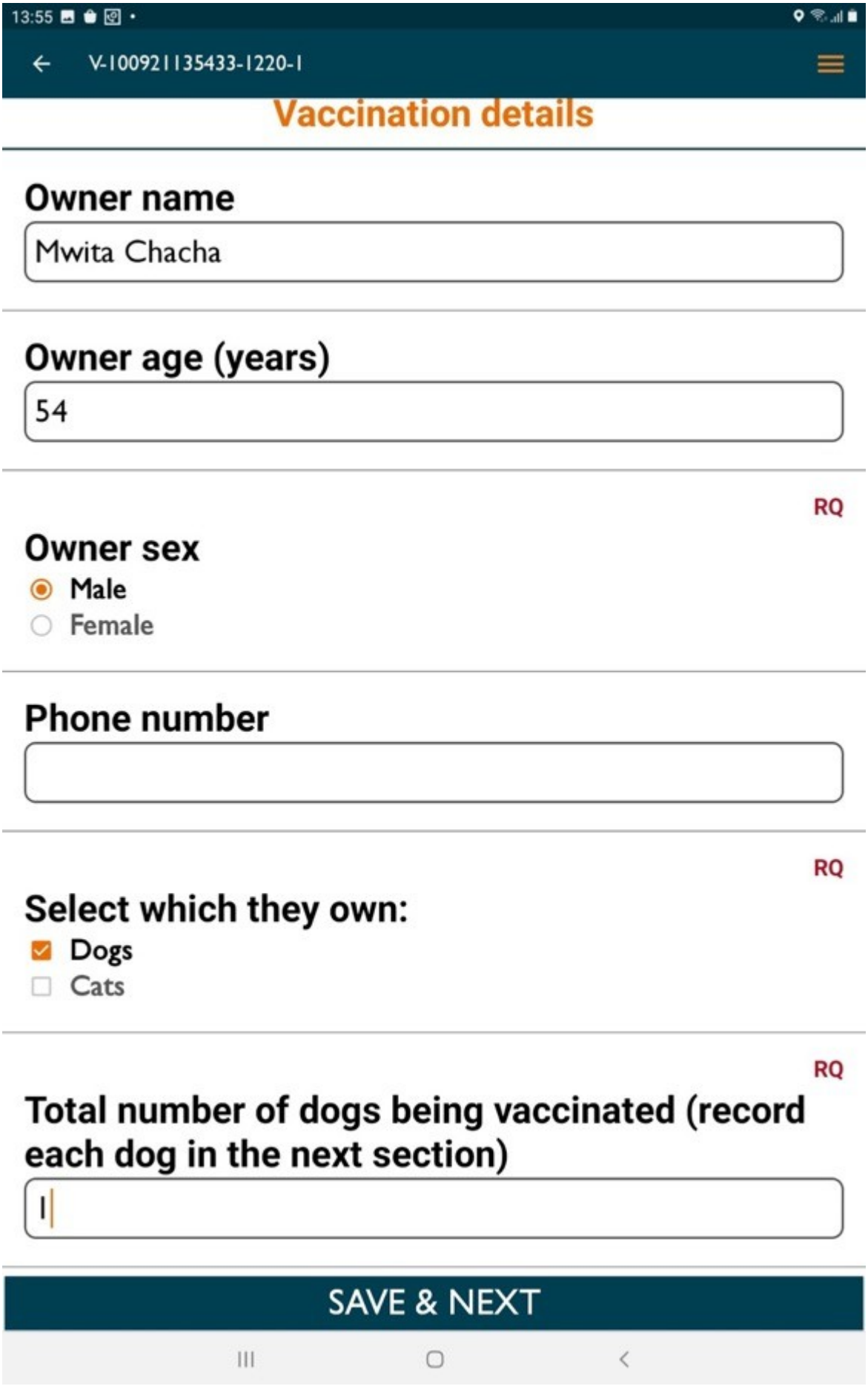

13:52
Location
REFRESH GPS
DELETE GPS
Massawe Hardwares
Homewares Shop
Cathy's Shop
Homewares Shop
Mama Kinabo's Shop
Homewares Shop
START FORM

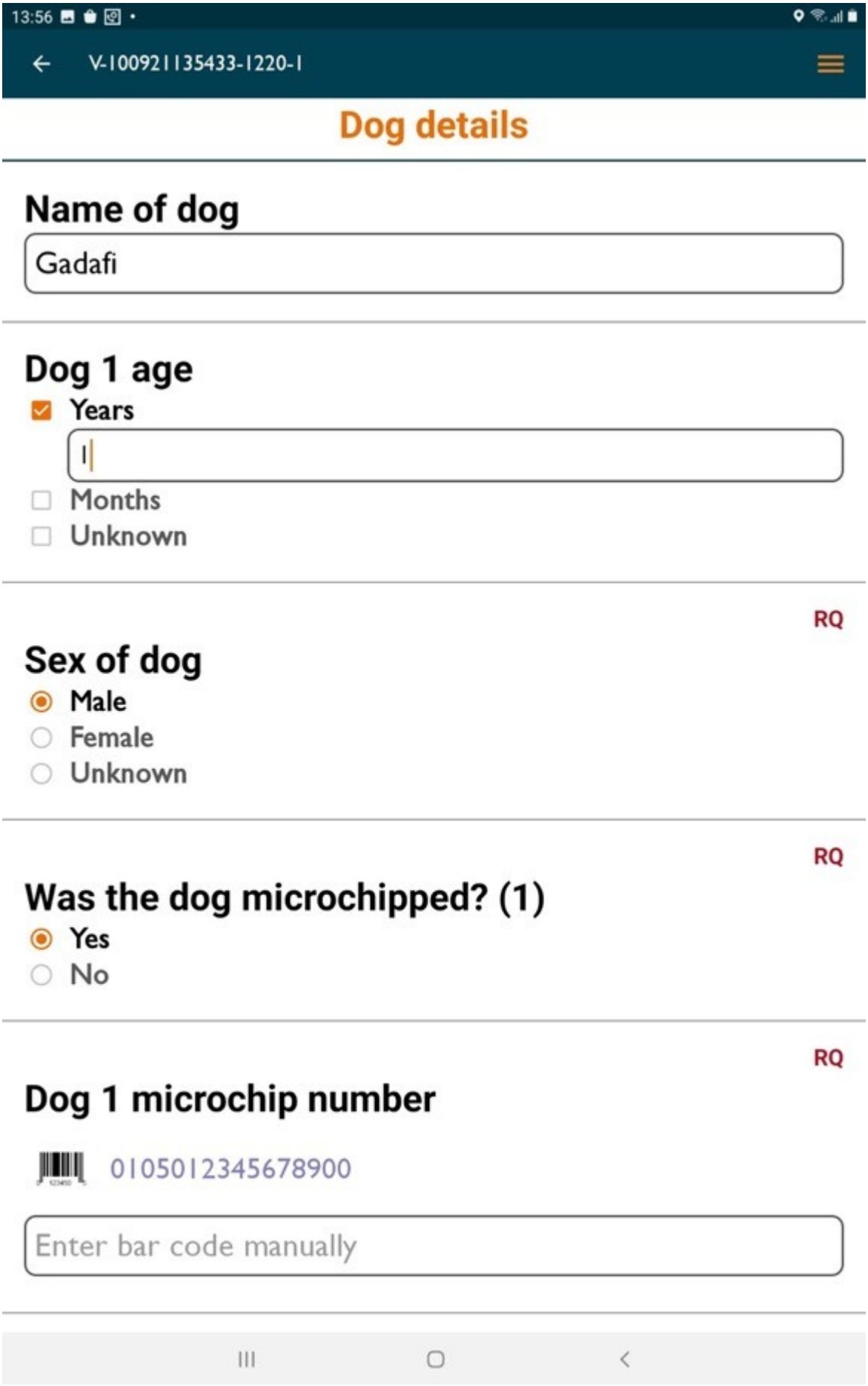

13:54
V-1009211135248-1220-1
Session details
RQ
Data collector name
Ahmed Lugelo
RQ
Select the district
Butiama
Tarime
Rorya
Start time
13:52
RQ
Select the ward, village and sub-village (Butiama)
Ward
Nyamimange
Village
Kwisaro
Sub-village
Mlimani
RQ
Number of doses carried

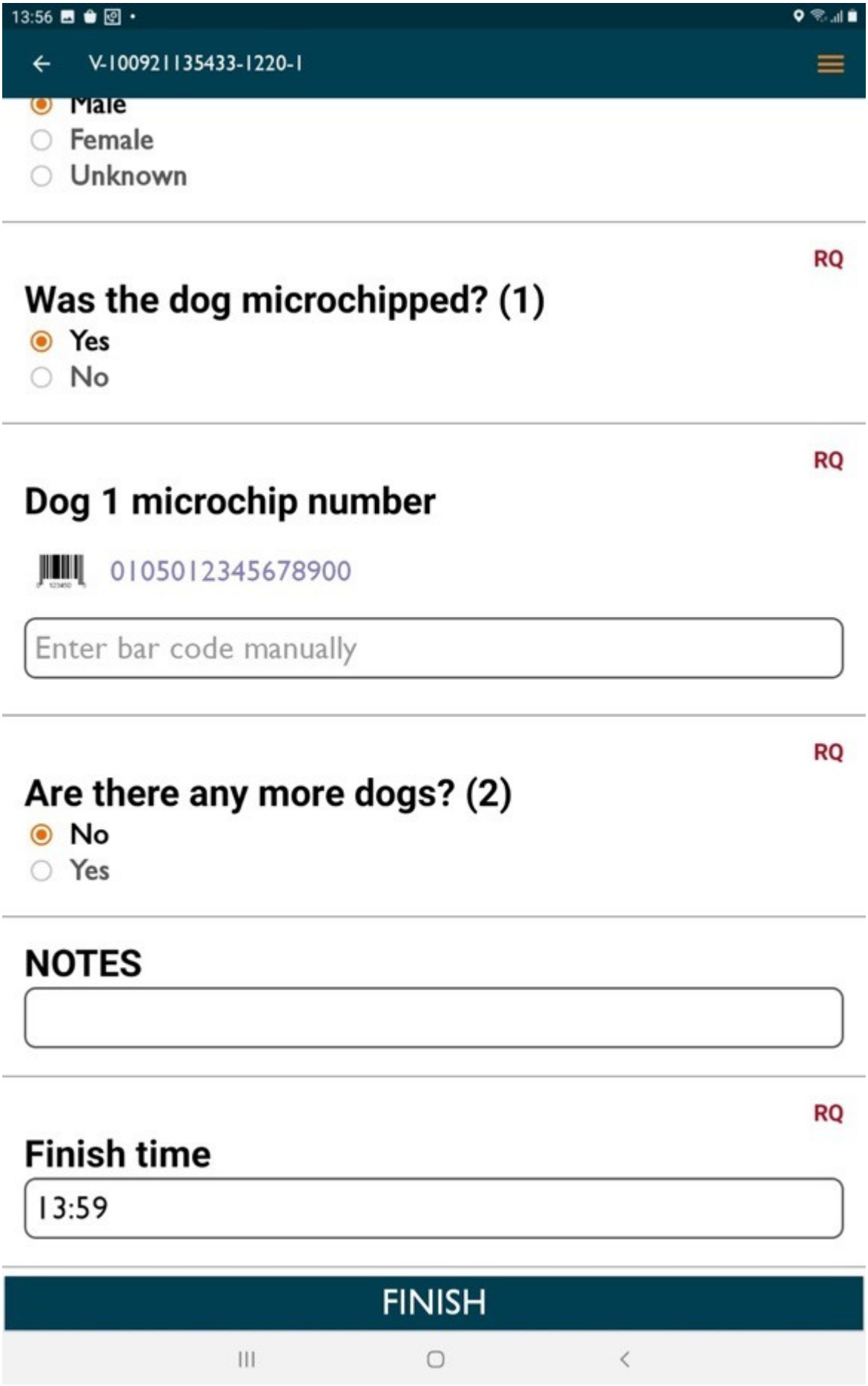

13:54
V-100921135248-1220-1
Tarime
Rorya
Start time
13:52
RQ
Select the ward, village and sub-village (Butiama)
Ward
Nyamimange
Village
Kwisaro
Sub-village
Mlimani
RQ
Number of doses carried
60
RQ
Method
Pulse
Continuous
SAVE & NEXT

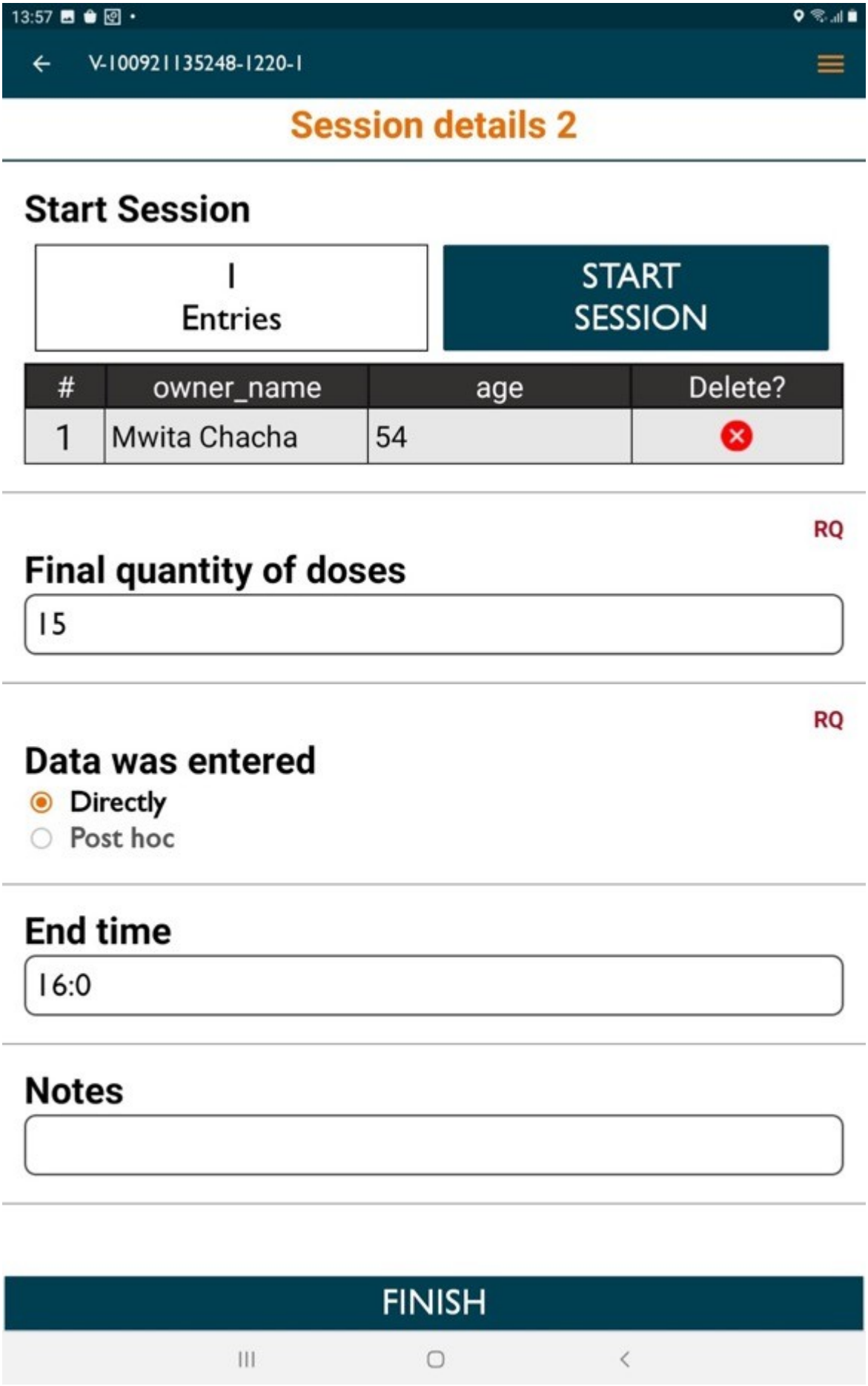

13:54
V-100921135248-1220-1
Session details 2
Start Session
0
Entries
START
SESSION
RQ
Final quantity of doses
15
RQ
Data was entered
Directly
Post hoc
End time
16:0
Notes
FINISH

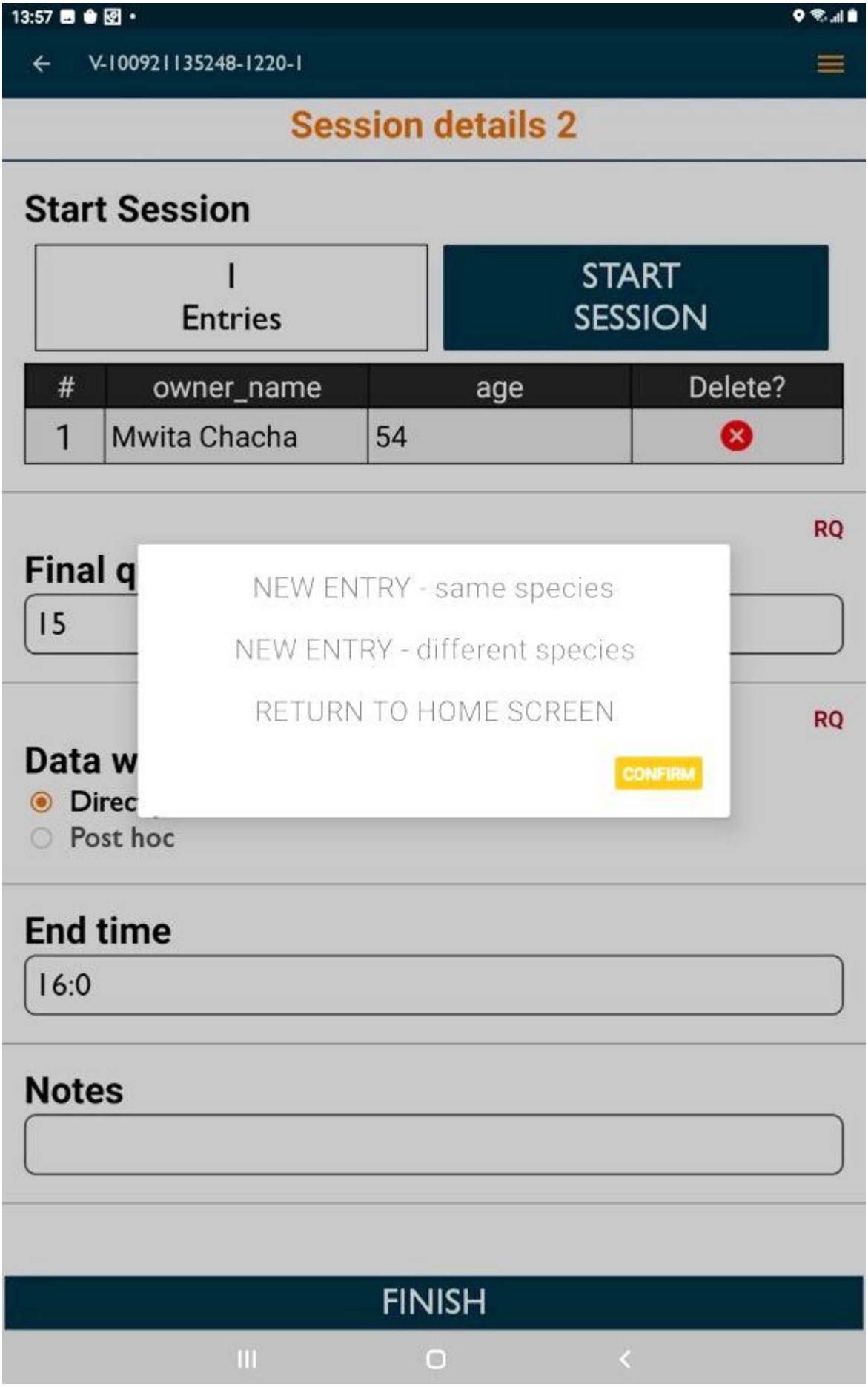

13:54
V-100921135248-1220-1
Session details 2
Start Session
0
Entries
START
SESSION
RQ
Final quantity of doses
15
Start path tracker?
NO
YES
RQ
Data was e
Directly
Post hoc
End time
16:0
Notes
FINISH


13:55
V-100921135433-1220-1
Vaccination details
Owner name
Mwita Chacha
Owner age (years)
54
RQ
Owner sex
Male
Female
Phone number
RQ
Select which they own:
Dogs
Cats
RQ
Total number of dogs being vaccinated (record each dog in the next section)
1
SAVE & NEXT


13:56
V-100921135433-1220-1
Dog details
Name of dog
Gadafi
Dog 1 age
Years
1
Months
Unknown
RQ
Sex of dog
Male
Female
Unknown
RQ
Was the dog microchipped? (1)
Yes
No
RQ
Dog 1 microchip number
0105012345678900
Enter bar code manually


13:56
V-100921135433-1220-1
Male
Female
Unknown
RQ
Was the dog microchipped? (1)
Yes
No
RQ
Dog 1 microchip number
0105012345678900
Enter bar code manually
RQ
Are there any more dogs? (2)
No
Yes
NOTES
RQ
Finish time
13:59
FINISH


13:57
V-100921135248-1220-1
Session details 2
Start Session
1
Entries
START
SESSION
owner_name age Delete?
1 Mwita Chacha 54
RQ
Final quantity of doses
15
RQ
Data was entered
Directly
Post hoc
End time
16:0
Notes
FINISH


13:57
V-100921135248-1220-1
Session details 2
Start Session
1
Entries
START
SESSION
owner_name age Delete?
1 Mwita Chacha 54
RQ
Final q
15
NEW ENTRY - same species
NEW ENTRY - different species
RETURN TO HOME SCREEN
CONFIRM
RQ
Data w
Direc
Post hoc
End time
16:0
Notes
FINISH

Appendix 7: Randomized Household Survey questionnaire

Question	Answer
1. Arm	Pulsed or continuous
2. Household ID code	
3. District name	
4. Ward name	
5. Village name	
6. Sub village name	
7. GPS	
8. Number of dogs owner says are living at the household	____dogs
9. Number of dogs that owner says have been vaccinated by this study?	____dogs
10. Number of dogs that owner says have not been vaccinated by this study?	____dogs
11. Number of dogs with a rabies vaccination certificate given by the study living at the household	____certificates
12. Date(s) and unique registration numbers of all available vaccination card(s) [from this study or otherwise]	Date 1 / unique reg. no.1 Date 2 / / unique reg. no.1
13. Number of dogs without a rabies vaccination certificate given by the study living at the household	____dogs
14. Number of dogs present at the household seen by the enumerator	____dogs
15. If dogs have been vaccinated, were they vaccinated by taking their dogs to a central point clinic or by a vaccinator coming to their house?	Central point_____dogs Vaccinated at home_____dogs
16. Is the person who took the dogs for the central point present?	Yes/No
17. If some dogs were vaccinated at home, how many dogs were vaccinated as a result of the owner asking the rabies coordinator to come to their house?	On demand_____dogs
18. If some dogs were vaccinated at home, how many dogs were vaccinated as a result of the rabies coordinator coming to the house during his routine rounds?	Routine visit_____dogs
19. If some dogs were vaccinated at home, how much time did it take in total to complete all of the registration and vaccination activities for all of the household dogs that were vaccinated?	_____Mins
20. If some dogs were vaccinated at a central point, how much time did it take to travel from home to the central point?	_____Mins
21. Once at the central point, how much time did it take in total to complete all of the registration and vaccination activities for all of the household dogs that were vaccinated at the central point?	_____Mins

22. After vaccination at the central point, how much time did it take to travel from the central point clinic to home?	_____Mins
23. If central point, how many family members brought the dogs for vaccination?	------- people
24. If central point, which family members brought the dogs for vaccination (mark all that apply and give number of each)	Husband no. _____ Wife no. _____ Adult male no. _____ Adult female no. _____ Child. No. _____
25. What would the primary registrant have been doing if they had not been having their dogs vaccinated?	
26. If some owned dogs were not vaccinated this year, please explain why: (*can give multiple answers)*	Too many to bring all of the dogs to the vaccination clinic Ran away Didn't think I needed to bring them all to the vaccination clinic Dog was sick Dog was not at home Dog was working (hunting, herding) Not aware of the campaign Dog had puppies Difficult to restrain/dog aggressive Other (explain)
27. If none of the dogs have ever been vaccinated, why not?	
28. (Continuous vaccination only) Is the respondent aware there is a Rabies Coordinator in their ward?	Yes / No
29. If yes, do they know how to contact the RC?	Yes / No
30. Please mention how?	____open answer
31. (Continuous vaccination only) Is the respondent aware there is a One Health Champion in their village?	Yes / No
32. If yes, do they know how to contact the OHC?	Yes / No
33. Please mention how?	____open answer
34. Did they pay the RC for the vaccination of their dogs?	Yes / no
35. If yes, how much	_____TZS / dog
36. (Pulse vaccination only) Does the respondent know who to contact if they needed to have à dog vaccinated?	Yes / no
37. If yes, who would they contact?	
38. Have you requested vaccination service from the RC/program and been refused?	Yes/No
39. If Yes, reason for refusal?	

Appendix 8: Ethical clearance

SOKOINE UNIVERSITY OF AGRICULTURE
College of Veterinary Medicine and Biomedical Sciences
P.O BOX 3015 CHUO KIKUU MOROGORO TANZANIA
TEL. 255 23 2 603511/4; DIR. 255 23 2 604542
FAX. 255 23 2 604647. TELEX 55308 UNIVMOG-TZ
TELEGRAMS "UNIAGRIC" MOROGORO
E-Mail: deanfvm@suanet.ac.tz

Our Ref: SUA/CVMBS//R.1/2019/6 **You Ref:** **Date:** 27/03 2019

Ahmed Lugelo
Department of Veterinary Medicine & Public Health
CVMBS

Dear Ahmed Lugelo,

RE: ETHICAL CLEARANCE CERTIFICATE FOR CONDUCTING ANIMAL RELATED RESEARCH IN TANZANIA

Kindly refer to the above heading.

We are delighted to inform you that your proposal submitted for ethical Review was evaluated and approved.

We therefore certify the approval of your research titled "**INVESTIGATING THE POTENTIAL OF A RABIES VACCINE TO IMPROVE STRATEGIES FOR RABIES CONTROL"**, in which you are the "Researcher".

Reference number for your ethical approval is **SUA/CVMBS//R.1/2019/6**

The committee reminds you and or your supervisors that you must ensure the following conditions are fulfilled:

i. MUST observe that the approval is valid for the period: **15/01/2019** to **07/05/2021**
ii. MUST observe and adhere to the approved protocols and timeline.
iii. In cases where you study takes longer time than previously anticipated or you are compelled to make changes during the implementation of your project, please make sure that you submit an amendment for review and approval
iv. Submit progress report by 31st December of each calendar year of your research

The committee wishes you all the best for a successful research.

Yours sincerely,

A. M. Lupindu

Chairperson – College Research, Innovations and Publication Committee

cc The Principal CVMBS

Appendix 9: Plagiarism test

PaperRater

1.0 Introduction Canine rabies causes approx. 60,000 human deaths every year worldwide, with most of these victims being children under 15 years of age (Hampson et al., 2015). More than 95% of these fatalities occur in LMICs, where the disease is prevalent due to lack or failure of disease control programs or limited access to PEP (Hampson et al., 2009b). Canine rabies is among the oldest known infectious diseases, and hence has been extensively studied by scientists over the centuries. Considerable effort has been invested in understanding various aspects of canine rabies, including aetiology, pathogenesis, epidemiology, diagnosis, and control (Singh et al., 2017; Fooks et al., 2017). Recent studies have focused on developing tools and demonstrating... (only first 800 chars shown)

Analysis complete. Our feedback is listed below in printable form. Some of the items have been truncated or removed to provide better print compatibility.

Plagiarism Detection

Original Work

Originality: 95%

No sign of plagiarism was found. That's what we like to see!

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https://www.bartleby.com/essay/The-Communicable...

More info on our originality scoring process (http://www.PaperRater.com/page/plagiarism-detection) .

Upgrade to premium (http://www.PaperRater.com/pricing) to see which phrases were found to be un-original

Spelling

Spelling Suggestions

Error	Suggestion
Hampson	Hampton, Sampson
LMICs	Mics
Hampson	Hampton, Sampson
	Books,