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Enhanced one Health surveillance approaches to guide the elimination of dog-mediated rabies in Tanzania

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ENHANCED ONE HEALTH SURVEILLANCE APPROACHES TO GUIDE THE ELIMINATION OF DOG-MEDIATED RABIES IN TANZANIA

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A Thesis Submitted in Fulfilment of the Requirements for the Degree of Doctor of Philosophy in Life Sciences of the Nelson Mandela African Institution of Science and Technology

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ABSTRACT

Rabies causes approximately 59 000 human deaths worldwide annually. A global target of zero human deaths from dog-mediated rabies has been set for 2030, and large-scale control programs are now advocated. However, there is limited surveillance and guidance on how rabies surveillance can be improved to increase the detection of rabid animals or to guide the management of rabies control programmes once elimination has been achieved or when its approached. Challenges to rabies elimination were investigated by undertaking detailed epidemiological studies collecting data from 2010/2011 to 2022; and enhancing surveillance using Integrated Bite Case Management (IBCM) across different settings in Tanzania from 2018 to 2022. In 24 districts, local government health and veterinary workers were trained to collect data through implementing IBCM, comprising risk assessments of bite patients by health workers and investigations of suspected rabid animals by livestock field officers. In addition, contact tracing was used to identify rabid animals, human rabies exposures and deaths, with additional whole-genome sequencing of viruses from rabies positive samples in 13 districts of Lindi and Mtwara region, including Pemba Island. From these data, transmission chains were probabilistically inferred, estimated case detection, quantified the public health burden in terms of numbers of rabies exposures, animal rabies cases, human rabies deaths and evaluated the impact and cost-effectiveness of a One Health approach to rabies surveillance and control. Reporting of bite patients at high risk of rabies exposure increased following the introduction of IBCM. Between 2011 and 2019, 688 probable exposures were identified in Southeast Tanzania, including 47 rabies deaths. Of 549 probable animal rabies cases identified: 303 were domestic dogs (55.2%) and 221 jackals (40.3%). Dog-to-dog transmission accounted for 40.1% of inferred transmission events, and wildlife-to-wildlife transmission accounted for approximately 32.6%, with the remainder from cross-species transmission. On Pemba Island, five transmission chains circulated from 2010. Rabid dogs, human exposures and deaths declined following the introduction and improved implementation of dog vaccination campaigns, and these transmission chains were eliminated by May 2014. In 2016 two introductions of dog rabies cases to the island that seeded re-emergence were identified. The ensuing outbreak was eliminated by October 2018 through reinstated island-wide dog vaccination. While post-exposure vaccines were highly cost-effective ($256 per death averted), their accessibility was limited and only dog vaccination interrupted transmission. A combined One Health approach rapidly eliminated rabies, was highly cost-effective ($1657 per death averted) and saved 20-130 families from rabid dog bites annually. Overall, IBCM greatly improved rabies detection and can be used to monitor the impact of mass dog vaccinations. In Tanzania domestic dogs appear to be the critical reservoir host of rabies, even in settings with evidence of wildlife transmission. Dog vaccination suppressed rabies in both dog and wildlife populations and reduced both public health and conservation risks. A One Health approach underpinned by dog vaccination and post exposure prophylaxes to animal bite patients is an efficient, cost-effective, equitable and feasible approach to rabies elimination, but needs scaling up across connected populations to sustain the benefits of elimination, as seen on Pemba, and for similar progress to be achieved elsewhere.

DECLARATION

I, Kennedy Lushasi, do hereby declare to the Senate of the Nelson Mandela African Institution of Science and Technology that this dissertation is my own original work and that it has neither been submitted nor being concurrently submitted for degree award in any other institution.

Kennedy Lushasi

Name and Signature of the Candidate   Date

The above declaration is confirmed by:

Dr. Emmanuel Mpolya   Date

Prof. Katie Hampson   Date

Prof. Daniel Haydon   Date

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CERTIFICATION

The undersigned certify that they have read and hereby recommend for acceptance by the Senate of the Nelson Mandela African Institution of Science and Technology a thesis titled "*One Health Surveillance Approaches to Guide the Elimination of Dog-Mediated Rabies*" in Fulfilment of the Requirements for the Degree of Doctor of Philosophy in Life Sciences of the Nelson Mandela African Institution of Science and Technology.

Dr. Emmanuel Mpolya Date

Prof. Katie Hampson Date

Prof. Daniel Haydon Date

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DEDICATION

I dedicate this thesis to my beautiful daughters (Catherine and Daniella Kennedy), my beautiful wife Niwaely Geofrey Mduma, and to my lovely parents for their patience, encouragement and moral support throughout the time of my study.

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LIST OF ABBREVIATIONS AND SYMBOLS

AIC	Akaike's Information Criteria
APHA	Animal & Plant Health Agency, UK
App	Application
BMGF	Bill and Melinda Gates Foundation
CAHW	Community Animal Health Worker
DFA	Direct Fluorescent Antibody
DMO	District Medical Offices
DoLD	Department of Livestock Development
DRIT	Direct Rapid Immunohistochemical Test
DVO	District Veterinary Offices
FAO	Food and Agricultural Organisation of the United Nations
FAT	Fluorescent Antibody Test
GARC	Global Alliance for Rabies Control
GLMM	Generalised Linear Mixed Model
HDR	Human-to-dog ratio
HMIS	Health Management Information System
IBCM	Integrated Bite Case Management
IDSR	Integrated Disease Surveillance and Response system
IDWE	Integrated Disease Weekly Ending
IHI	Ifakara Health Institute
Km2	Square Kilometre
LTRA	Land transport regulatory authority;
LFOs	Livestock field officers
LFT	Lateral Flow Test
LGAs	Local Government Authorities
LMIC	Low- and Middle-Income Countries
LMIS	Logistic Management Information System
MoLDF	Ministry of Livestock Development and Fisheries, Tanzania
MLF	Ministry of Livestock and Fisheries
MoH	Ministry of Health
MSD	Medical Store Department
NGO	Non-governmental organisations
NMAIST	Nelson Mandela African Institution of Science and Technology

OH	One Health
WOAH	World Organisation for Animal Health
OPD	Outpatient Department
PCR	Polymerase Chain Reaction
PEP	Post-Exposure Prophylaxis
PVLD	Pemba Veterinary Laboratory Department
RABV	Rabies virus
RDT	Rapid Diagnostic Tests
RNA	Ribonucleic acid
SUA	Sokoine University of Agriculture
TVLA	Tanzania Veterinary Laboratory Agency
UK	United Kingdom
VIC	Veterinary Investigation Centres
WGS	Whole Genome Sequence
WHO	World Health Organisation

CHAPTER ONE

INTRODUCTION

1.1 Background of the Problem

One Health (OH) is an approach that aims to manage multifaceted and interlinked health risks amongst humans, animals and the environment (Häsler *et al.*, 2014). Surveillance involves the systematic collection of data on disease incidence; analysis of these data and timely dissemination of results to guide interventions aimed at either preventing or controlling diseases in human and animal populations (Halliday *et al.*, 2012). Rabies, a viral disease with an almost 100% case fatality rate, is responsible for an estimated 59 000 human deaths, tens of millions of human exposures, and substantial livestock losses annually (Hampson *et al.*, 2015). Rabies is transmitted within animal populations (primarily domestic dogs) and transmitted to humans by bites from dogs. Therefore, prevention of human rabies deaths involves managing risks to humans i.e. prompt administration of Post Exposure Prophylaxes (PEP) and interrupting transmission in the dog population through regular mass dog vaccinations, hence a OH approach (Rushton *et al.*, 2012) supported by surveillance that links both the human and animal health sectors.

Rabies is one of the most feared zoonoses. Approximately 99% of human deaths from rabies are reported in low- and middle-income countries (LMICs), of which 56% occur in Asia and 44% in Africa (Hampson *et al.*, 2015). Despite the high burden of canine rabies in LMICs, the disease can be successfully eliminated through vaccination of animal reservoirs. For example, successful elimination programmes have been documented in high-income countries such as from Western Europe, North America, Most of Latin America and Caribbean (Lapiz *et al.*, 2012; World Health Organization, 2018) and these were achieved through mass dog vaccination programs coupled with strong political and financial commitments of the animal and human health sectors to their large-scale implementation (Tiziana *et al.*, 2010). In addition, the success of these elimination programs depended on effective surveillance to monitor the impacts of interventions and initiate timely outbreak responses where necessary (Townsend *et al.*, 2013). However, in most African and Asian countries, there has been little investment in dog vaccination and rabies has continued to circulate in domestic dogs with a high incidence of human deaths (Hampson *et al.*, 2015). As a result, a global goal to eliminate human deaths resulting from dog-mediated rabies by 2030 is now advocated (Minghui *et al.*, 2018) and large-scale dog vaccination programs are being initiated with this goal in mind.

An effective surveillance system that detects a sufficiently high proportion of cases to enable effective decision-making is needed during elimination programmes, for example, to establish disease absence and to promptly detect incursions (Townsend *et al.,* 2013). Commonly used approaches for rabies surveillance that aim to sample a proportion of the population are less sensitive because of the low incidence of rabies and short period when an animal shows signs of infection and therefore can be tested. Integrated bite case management (IBCM) which involves investigation of suspicious biting incidents has been shown to increase case detection (Wallace *et al.,* 2015) and has been proposed as a potential surveillance strategy for verifying freedom from rabies (Hampson *et al.,* 2016; Wallace *et al.,* 2015).

Moreover, genomics-informed surveillance as part of a One Health platform has enormous potential to inform and improve responses to pathogen outbreaks (Gardy & Loman, 2018). Pathogen sequence data can be used to distinguish pathogen strains, identify the origin of imported disease case clusters, uncover cryptic transmission, quantify the frequency of cross-border transmission and monitor the effectiveness of control efforts (Diop *et al.*, 2017; Dudas *et al.*, 2016; Gardy & Loman, 2018; Trewby *et al.*, 2017). The deployment of such technology has been crucial in the identification of rabies virus variants (Marston *et al.,* 2012), in quantifying emerging antimicrobial resistance (Didelot *et al.,* 2012), and strain typing to detect outbreaks and support surveillance (Mollentze *et al.,* 2014). In particular, portable real-time sequencing technology has enabled genomic surveillance capacity in low-resource areas and provided critical and timely insights into pathogen dynamics and spread. For example, real-time genetic data produced during the Ebola outbreak in Sierra Leone informed border closures that limited the epidemic, demonstrating that genomic information, acquired in real time, could help to contain future epidemics (Dudas *et al.,* 2016).

Surveillance systems designed to monitor and control rabies have been found to be effective models for other infectious diseases. For example, the World Health Organization (WHO) has emphasized the importance of integrating rabies surveillance into broader disease surveillance systems as part of their zoonotic disease control programs (World Health Organization, 2018). Furthermore, studies have highlighted the value of cross-sectoral collaboration and the One Health approach, which emphasizes the interconnectedness of human, animal, and environmental health, in addressing not only rabies but also diseases like avian influenza and Ebola (Gibbs *et al.,* 2016; Jakob *et al.,* 2017). The lessons learned from rabies control, including the implementation of vaccination campaigns and rapid response strategies, have been instrumental in combating other infectious diseases, such as polio and measles (Gastañaduy *et al.,* 2021; Grassly, 2013; World Health Organization, 2020). Therefore, it is evident that the principles and strategies applied in the context of rabies surveillance and elimination hold broader relevance and applicability in the fight against multiple infectious diseases.

1.2 Statement of the Problem

In LMICs such as Tanzania surveillance capacity is limited in both the animal and human health sectors. Disease detection is hampered by the absence of adequate laboratory facilities, and there are difficulties submitting samples from remote areas to laboratories for confirmation (Karimuribo *et al.,* 2012). Additionally, inadequate collaboration and communication between the veterinary and health sectors hinder the exchange of crucial information necessary for effectively responding to health-related threats specific to each sector (Nel, 2013). Yet, the World Health Organisation (WHO), the Food and Agricultural Organisation of the United Nations (FAO), the World Organisation for Animal Health (WOAH) and the Global Alliance for Rabies Control (GARC) have recognized canine rabies as a global health priority and have united to achieve zero human deaths by 2030 (Minghui *et al.,* 2018). Large-scale rabies control programmes are now being rolled out in countries around the world including Tanzania. However, there is very limited guidance on rabies surveillance to improve case detection as elimination is approached, or that can guide the management of these control programmes. The challenges of rabies case detection are that the period during which infection can be detected is short, the infection circulates at low prevalence, and recovering samples from suspected rabid animals is not always feasible (Hampson *et al.,* 2016). For these reasons, improved approaches to rabies surveillance to increase case detection and guide rabies control programmes aimed at elimination are necessary during the end game processes.

Domestic dogs are considered the maintenance hosts for rabies virus in Africa and Asia (World Health Organization, 2018), however, the diversity of wild carnivores across Africa has also led to ongoing debate regarding a role for wildlife in maintaining rabies in this region (Hikufe *et al.,* 2019; Sabeta *et al.,* 2007). Jackal species frequently represent a large proportion of reported wildlife rabies cases in Southern Africa (Moagabo *et al.,* 2009; Pfukenyi *et al.,* 2009) and in parts of Namibia, South Africa and Zimbabwe black-backed jackals (*Canis mesomelas*) appear to play a role in maintaining transmission (Bingham *et al.,* 1999; Courtin *et al.,* 2000; Hikufe *et al.,* 2019). Evidence from northern Tanzania suggests that domestic dogs are the only species necessary for maintenance of rabies virus (RABV) in this area, although other carnivores contribute to the reservoir as non-maintenance populations (Tiziana *et al.,* 2008). In contrast, very little is known about the transmission dynamics of rabies virus in southeast Tanzania and the role of wildlife areas as buffers against infection and /or the potential of wildlife to impede elimination efforts is still unknown.

Although mass dog vaccination can eliminate rabies, there are several challenges to achieving this goal. In most rabies endemic countries in sub-Saharan Africa mass dog vaccination campaigns have been sparse and typically very localised (World Health Organization, 2018). Moreover, the high reproductive rates and short life span of dogs in many LMICs, quickly leads to drops in vaccination coverage which need to be maintained through repeat campaigns (Davlin & VonVille, 2012). The virus can easily spread in dog populations that have low and heterogenous vaccination coverage (Mancy *et al.,* 2022) and incursions leading to outbreaks have been commonly reported (Bourhy *et al.,* 2016; Kristyna *et al.,* 2020; Jakob *et al.,* 2017), often facilitated by human-mediated movement of incubating dogs (Tohma *et al.,* 2016). This situation is compounded by weak surveillance which prevents effective monitoring of progress towards rabies elimination and limits the ability to determine disease freedom (Nel, 2013).

1.3 Rationale of the Study

The passive rabies surveillance that is employed by Livestock Field Officers (LFOs) in Tanzania has limited effectiveness. This is not only attributed to the high cost of developing and implementing a comprehensive surveillance system, but also the lack of trained field epidemiological investigators and inadequate laboratory infrastructures (Kitala *et al.*, 2010). Samples have to be transferred to laboratories at research institutions such as Sokoine University (SUA) or Tanzania Veterinary Laboratory Agency (TVLA); or shipped overseas for genetic sequencing, which is costly, and often the diagnostic results are too delayed to be of any use in informing control efforts.

New technologies such as Bionote rapid diagnostic tests kits (RDT) and MinION sequencing can potentially be used to overcome these resource challenges by providing real-time insights into pathogen spread. Sequencing can quickly determine the strain and likely origin of a pathogen and analysis of pathogen genomes can help us to understand the route of transmission of a new outbreak (Brunker *et al.*, 2020).

Active case finding through the implementation of IBCM has the potential to increase case detection in Tanzania (Wallace *et al.,* 2015). This potentially affordable approach can be used to improve provision of PEP and strengthen intersectoral partnerships and capacity needed for control of emerging zoonoses. The combination of both active and genomic surveillance could therefore improve the management of rabies control programmes. Through increased case detection, persistence foci of infections could be revealed which would enable more effective and targeted control (Hampson *et al.,* 2016). Timely detection of cases would enable immediate and effective response to outbreaks; and the knowledge of virus lineages could inform progress towards elimination whereas, the identification of sources of infection could guide targeting resources in response to incursions (Brunker *et al.*, 2020; Campbell *et al.*, 2022).

"Zero by Thirty" is an initiative backed by the United Against Rabies Coalition aiming to achieve zero human deaths worldwide from dog-mediated rabies by 2030 (Minghui *et al.*, 2018). Vaccination of domestic dogs and disease surveillance are key components of this initiative. Surveillance needs to include specific approaches for detecting cases in all species, including wildlife and to assess whether and how wildlife infections impact the effectiveness of dog vaccinations. More generally, there is a critical need to holistically link surveillance practices and animal disease control measures to cost-effectively reduce the burden of zoonotic pathogens.

1.4 Research Objectives

1.4.1 General Objective

The general objective of this study is to enhance rabies surveillance, using Integrated Bite Case Management (IBCM) and genomic surveillance, to inform the development of One health surveillance guidelines for the elimination of dog-mediated rabies in Tanzania.

1.4.2 Specific Objectives

The specific objectives include:

(i) To determine the extent to which rabies surveillance can be improved to detect more cases and more accurately monitor the epidemiological situation of rabies across the study sites.

(ii) To evaluate the role of wildlife in the transmission and maintenance of rabies in unstudied regions of Lindi and Mtwara, southeast Tanzania.

(iii) To determine how genomic surveillance can inform rabies elimination programmes and be incorporated into routine management of the health and veterinary systems on Pemba Island.

(iv) To estimate the impact and cost-effectiveness of a One Health approach to rabies elimination on Pemba Island.

1.5 Hypotheses

This study has two underlying hypotheses:

(i) The implementation of active IBCM and genomic surveillance approaches have the potential to guide the elimination process and verify rabies freedom in endemic areas.

(ii) If domestic dogs are the sole maintenance hosts of RABV, then control strategies directed at domestic dogs alone should reduce transmission of rabies and if sustained lead to elimination.

1.6 Research Questions

The following questions were addressed:

(i) To what extent can rabies surveillance be improved to detect more cases and more accurately monitor the epidemiological situation of rabies?

(ii) What is the role of wildlife in the transmission and maintenance of rabies in southeast regions of Tanzania, Lindi and Mtwara?

(iii) How can genomic surveillance inform rabies elimination programmes and be incorporated into routine management of health and veterinary systems?

(iv) What is the impact and cost-effectiveness of a One Health approach to rabies elimination on Pemba Island?

1.7 Significance of the Study

Lessons learnt from this study will be valuable as further efforts are made to eliminate the disease in other regions of the continent. More generally, the documentation of successful rabies surveillance strategies will build confidence in the feasibility and practicality of these strategies for eliminating rabies elsewhere in Africa, given appropriate sustained investment and commitment.

The combination of IBCM and genomic surveillance could improve the management of rabies control programmes. Increased case detection could reveal undetected persistent foci of infections and prevent premature discontinuation of control efforts. Timely detection should enable faster, more effective outbreak responses. Knowledge of variants could inform progress towards elimination and identification of sources of infection could guide targeting of resources. Investigating the implementation of IBCM will help guide decision-making, for example to verify freedom from rabies, and apply cautious PEP administration protocols, evaluating cost and risk implications for rabies among exposed individuals in communities. Improved border control may help reduce introductions, but scaling up mass dog vaccination in bordering and nearby populations could have even greater impacts on eliminating dog-related human rabies deaths.

1.8 Delineation of the Study

With target dates for regional and global elimination of canine rabies set, there is an urgent need for case studies of rabies elimination in practice. This thesis examines how these different approaches to rabies surveillance can therefore support progress toward the "Zero by 30" goal. The data utilized in this study encompasses mass dog vaccination campaigns conducted from 2011 to 2016 as part of the government-led rabies elimination and demonstration project in southeastern Tanzania (Lindi and Mtwara regions) (Mpolya *et al.*, 2017). Furthermore, the collection of surveillance data included both human bite and animal rabies cases, which were obtained from medical and veterinary records. Additionally, the previously established e-surveillance system for rabies in the study districts was utilized to gather this information. The data was then extracted and meticulously investigated, tracing the cases reported up until 2019. The study was also conducted in Mtwara, Lindi, Morogoro, and the Mara regions, piloting an IBCM surveillance system between 2018 and 2022 that aimed to link health and veterinary workers in controlling rabies while collecting data on both human and animal rabies related cases. The study further explored whether wildlife areas, such as the Selous game reserves, act as buffers against rabies infection in Lindi and Mtwara regions. The potential contribution of wildlife, specifically jackals, to reported animal bite cases in these regions as a barrier to rabies elimination is also investigated. Wildlife case data from hospital and veterinary records, along with reported animal rabies cases from other species, collected from 2011 to 2019, were traced and included in the analysis. Additionally, the study compares the dynamics of rabies between Pemba Island and non-island settings of the Tanzanian mainland. Genomic surveillance approaches were employed to verify rabies elimination on Pemba Island. Contact tracing data from 2010 to 2022, as well as brain samples collected from rabid dogs, were used to explore the dynamics of rabies between these two settings, and genomic surveillance was used to confirm the presence or absence of rabies on Pemba Island.

CHAPTER TWO

LITERATURE REVIEW

2.1 Rabies Control and the One Health Approach

Rabies is a zoonotic disease caused by a virus transmitted through the bite of an infected animal (Jackson, 2013). Around 59 000 people die of rabies each year, with over 99% of these deaths occurring in Low- and Middle-Income Countries (LMICs) (Hampson *et al.*, 2015). Yet the disease is entirely preventable through vaccination of dogs to eliminate infection in the reservoir population and by prompt administration of post-exposure prophylaxis (PEP) to people exposed to the virus (Fooks *et al.*, 2014; Jackson, 2013). Control of rabies requires collaboration between public health and veterinary sectors ('One Health' approach) to manage risks in humans and interrupt transmission in dogs (Rushton *et al.*, 2012). An example of One Health is the quadripartite (World Health Organisation [WHO], Food and Agricultural Organisation of the United Nations [FAO], World Organisation for Animal Health [WOAH] and the Global Alliance for Rabies Control [GARC] uniting to form a United Against Rabies Coalition [UAR] to confront the problem of rabies (WHO, 2018). Nonetheless the practical coordination of One Health activities by frontline public health and animal health workers remains challenging and this is exemplified by the implementation of rabies surveillance.

2.2 Rabies Surveillance

Surveillance is essential to control and ultimately eliminate infectious diseases (Townsend *et al.*, 2013). Effective disease surveillance involves the systematic collection and analysis of disease data and timely dissemination of results to guide planning and implementation of control strategies (Halliday *et al.*, 2012; Karimuribo *et al.*, 2012; Tambo *et al.*, 2014). Routine analysis of surveillance data can identify changes in disease incidence, including disease outbreaks and should inform public health professionals so as to improve the implementation of interventions and evaluate their impact (Zhang *et al.*, 2013). For rabies, surveillance could include data on persons bitten by rabid animals that are seeking PEP, human rabies deaths, diagnosed animal rabies cases and data that needs to be shared between sectors to inform control measures like dog vaccination campaigns and prevention through provisioning of PEP. Yet, in many rabies endemic countries there are no formal systems used for reporting bite patients, and even if bite patients are reported, information on the risk of rabies is not reported (Wambura *et al.*, 2019). Moreover, limited operationalization of One Health means that the veterinary or public health sectors rarely ever receive information from the other sector to guide their control and prevention activities.

2.2.1 Integrated Surveillance Programmes for Rabies Control

Rabies reservoirs encompass various species worldwide, with the virus variant primarily found in domesticated dogs posing the greatest risk and accounting for the majority of human rabies fatalities (World Health Organization, 2018). Controlling the spread of rabies requires managing the disease within dog populations through mass vaccination campaigns (World Health Organization, 2018). However, conducting such campaigns can have significant financial implications (World Health Organization, 2018). In Tanzania, like other developing countries (Hampson *et al.,* 2015; Hatch *et al.,* 2017), many human and dog rabies cases go unnoticed and unreported, hindering awareness, funding, and prevention efforts (Wallace *et al.,* 2015). Limited recognition and reporting, along with inadequate resources and infrastructure, restrict active rabies surveillance and lead to all dog bites being treated as suspected rabies exposures (Lushasi *et al.,* 2020; Millien *et al.,* 2015; Wallace *et al.,* 2015).

To address these challenges, an integrated bite case management (IBCM)for rabies control is advocated. The IBCM is an approach for rabies surveillance that directly and formally links workers in public health and veterinary sectors to assess the risk of rabies among animal bite patients and biting animals, respectively (Wallace *et al.*, 2015). The IBCM has been promoted to increase rabies case detection (Wallace *et al.,* 2015), improve the administration and cost-effectiveness of PEP (Undurraga *et al.,* 2017), and as a potential surveillance strategy for verifying freedom from rabies (Hampson *et al.,* 2016). This approach combines active community investigations of dog bites with passive surveillance of rabies in animals to provide personalized risk assessments for individuals potentially exposed to the virus. The approach involves risk assessments conducted by health workers, notification of high risk cases to animal health workers, and investigations conducted by trained livestock field workers (Lushasi *et al.,* 2020).

Upon a bite victim seeking medical treatment or community reports of suspect animals, investigations are initiated to locate the biting dogs (Lechenne *et al.*, 2017; Lushasi *et al.*, 2020; Wallace *et al.,* 2015). Rabid dogs are either euthanized or placed under observation, and potential bite victims identified, advised to seek PEP, and referred to appropriate healthcare facilities. While comprehensive dog vaccination is the optimal approach for rabies prevention and control (World Health Organization, 2018), IBCM programs offer an efficient solution for countries with a high risk of rabies transmission and inadequate vaccination coverage (Coetzer *et al.,* 2019; Perry *et al.,* 2007; Swedberg *et al.,* 2022). These programs play a crucial role in removing rabid dogs from the community, reducing the risk of further exposures, and ensuring timely medical care for bite victims. They also establish reliable case definitions, allowing individuals bitten by non-rabid dogs to avoid unnecessary PEP. Additionally, IBCM programs enhance surveillance data, enabling the assessment of disease burden, evaluation of intervention programs and strategies, informing policymakers, and supporting disease elimination efforts (Lechenne *et al.,* 2017; Lushasi *et al.,* 2020; Millien *et al.,* 2015; Swedberg *et al.,* 2022; Wallace *et al.,* 2015).

To further improve the management of rabies control programs, a combination of active and genomic surveillance can be employed. This approach increases case detection and helps reveal persistence foci of infections, leading to more effective and targeted control measures (Hampson *et al.,* 2016). Timely detection of cases enables immediate and effective responses to outbreaks, while knowledge of virus lineages informs progress towards elimination. Identifying sources of infection guides resource allocation in response to incursions (Brunker *et al.,* 2020; Campbell *et al.,* 2022).

2.2.2 Enhancing Surveillance: Challenges and Opportunities in Low- and Middle-Income Countries

Surveillance programmes should be well-managed and encouraged to maintain and sustain existing interventions. The experiences gained from the eradication of smallpox, rinderpest and elimination of polio (Henderson & Klepac, 2013; Jain *et al.,* 2014; Roeder *et al.,* 2013), shows that surveillance and response systems were critical to their success. However, surveillance and response systems for zoonotic diseases have not been developed and evaluated in many LMICs, such as Tanzania. But in resource limited countries, most surveillance systems inadequately respond to emerging threats due to scarce resources (Huntington, 2012). These challenges can be addressed by implementing a One Health approach, which involves collaborative efforts between the public health and veterinary sectors to jointly address a shared threat to both sectors (Lechenne *et al.,* 2017; Zhou, 2012). Another way of enhancing surveillance is through pathogen sequencing. Genomic surveillance can be used to detect cases and determine how related they are to one another. With the advent of portable real-time sequencing technology, sources of outbreaks and strains of viruses could be identified rapidly in the field (Quick *et al.,* 2016) demonstrating the potential to transform infection control practices. Applying this sequencing technology to rabies virus could support the identification of outbreaks and the genetic relatedness of cases, including highlighting cryptic transmission versus imported cases (Kuzmin *et al.,* 2012). Correct and rapid diagnosis of rabies is not only important in the management of human exposures, and of the in-contact animals, but also required for confirmatory testing to guide control efforts (Léchenne *et al.,* 2016) . With policy efforts now directed towards achieving a global goal of zero dog-mediated human rabies deaths by 2030 (World Health Organization, 2018), establishing effective surveillance tools is critical (Broban *et al.,* 2018). Genomic data can provide important and unique insights into rabies spread and persistence that can direct control efforts (Klepac *et al.,* 2013).

The capacity for genomic surveillance in low- and middle-income countries is hampered by limited laboratory infrastructures, cost, supply chains and other logistical challenges (Brunker *et al.,* 2020; Tiziana *et al.,* 2010). The high costs associated with the purchase and maintenance of laboratory equipment, such as fluorescence microscopes and acquisition of reagents/consumables, has made establishing well equipped laboratories extremely difficult in low income countries, including Tanzania (Tiziana *et al.,* 2006). Poor infrastructures such as roads, and insufficient storage and transportation facilities limit transport of samples from remote areas to laboratories for confirmatory testing (Hampson *et al.,* 2016). However, laboratory infrastructures with good diagnostic capacities can potentially break the underreporting of cases that have also contributed to the neglected nature of rabies (Tiziana *et al.,* 2006). The development of cutting-edge portable sequencing technology and lab-in-a-suitcase platforms have been used to build the level of genomic surveillance capacity in low-resource settings, and this can help to address the neglected endemic zoonoses which impose a significant burden on the disadvantaged communities (Brunker *et al.,* 2020; Quick *et al.,* 2016).

In addition, the use of field-based diagnostic alternatives such as the RDTs may provide a quick diagnostic solution for rabies viruses to guide control measures (Léchenne *et al.,* 2016). Furthermore, using a combination of both active investigations of suspicious cases including the risk assessments of biting animals (IBCM) and passive animal rabies surveillance may facilitate sample collection for quick laboratory diagnosis and genomic surveillance to identify the viral strains. This may also increase case detection and reporting of suspicious cases that may eventually break the chain of underreporting of rabies in endemic settings and ascertain the true burden of rabies, including verifying freedom from rabies where it has been controlled (Lushasi *et al.,* 2020; Wallace *et al.,* 2015).

2.3 Reservoir Dynamics of Rabies Infection in a Multi Host Population

The RABV is a true multi-host pathogen. Although typically maintained in distinct species-specific transmission cycles (Rupprecht *et al.,* 2002) the virus is capable of infecting any mammal. Rabies is spread primarily through bites from infected animals and cross-species transmission causes disease in humans, livestock, and wildlife (Hikufe *et al.,* 2019). The economic burden of rabies due to livestock losses is high, and rabies outbreaks within wildlife can threaten endangered species (Randall *et al.*, 2004). When planning control and elimination strategies for multi-host pathogens, it is important to identify the populations that are essential for their persistence (Haydon *et al.,* 2002). The terminology defined by Haydon *et al.* (2002) is used throughout this thesis. That is a single population capable of independently maintaining the pathogen of interest is termed a maintenance population. Where multiple interconnected host populations collectively maintain the pathogen, this is termed a maintenance community. A reservoir is made up of one or more epidemiologically connected populations capable of permanently maintaining the pathogen and from which infection is transmitted to a population of concern (the target population). If a single maintenance population exists, control measures targeted at this population should lead to elimination of infection from all populations. In the presence of a maintenance community, interventions may need to be targeted at multiple populations (Fig. 1).

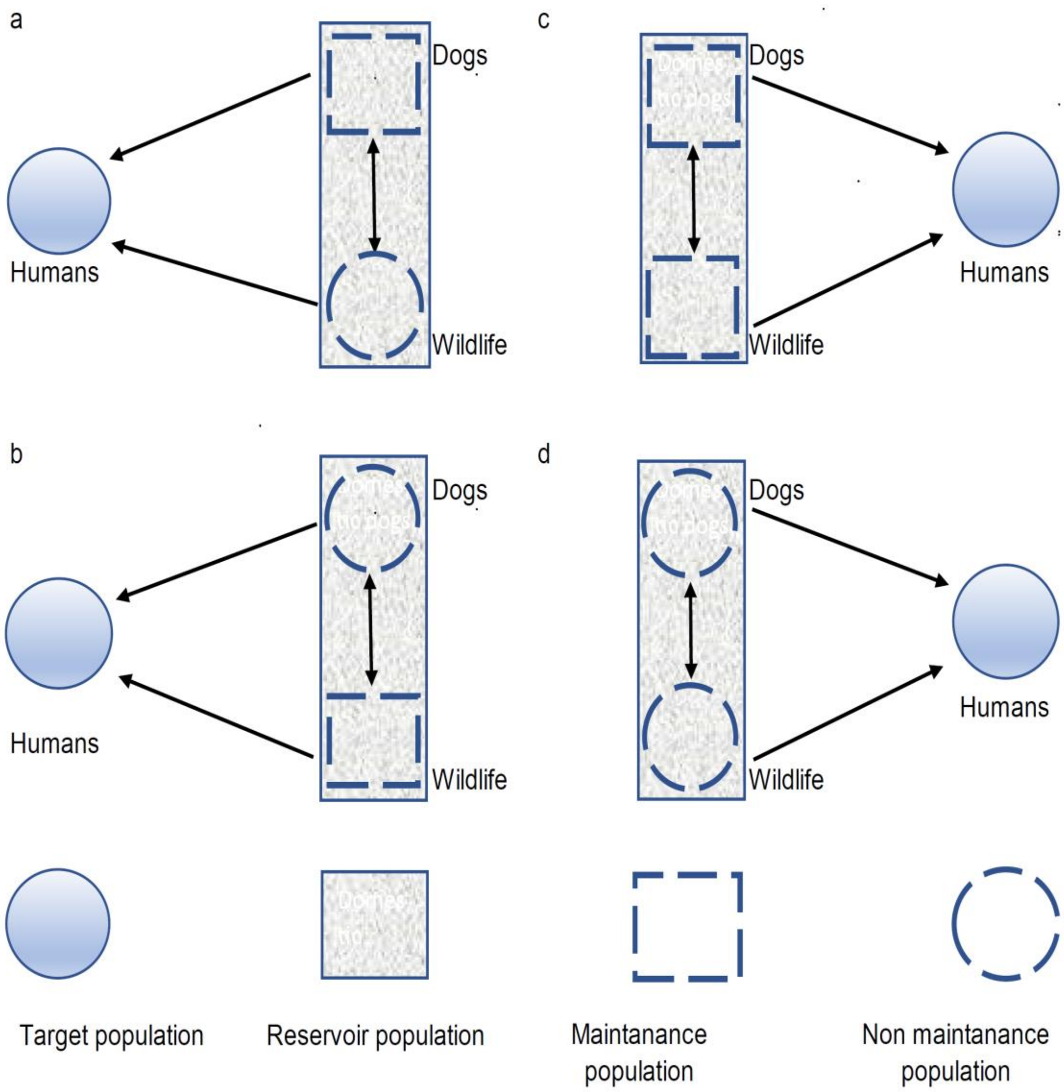

Figure 1: Potential rabies reservoir systems in Lindi and Mtwara regions, southeast Tanzania

Here humans are indicated as the target population, but the target may include livestock or endangered wildlife e.g. African Wild Dogs (*Lycaon pictus*). I investigated whether the reservoir consists of both maintenance and non-maintenance populations (a & b) transmitting infection to the non-maintenance target (humans); or either two maintenance (c) or non-maintenance (d) populations which are capable of transmitting infection to the target.

The global eradication of rinderpest is one example of the success of interventions targeted at a maintenance population. Although capable of infecting more than 40 domestic and wild artiodactyl species, cattle (*Bos taurus*) vaccination alone eradicated rinderpest virus (Youde, 2013). More recently the importance of understanding reservoir dynamics has been highlighted by the global Guinea Worm Eradication Programme where the recent discovery of dogs as a potential maintenance host for this parasitic infection has complicated eradication efforts (Molyneux & Sankara, 2017).

Although many RABV variants exist, each variant tends to associate closely with a particular mammalian species which serves as the maintenance host for that variant. Spillover of infection to other species does occur, but sustained transmission outside of the maintenance population is uncommon (Brunker *et al.*, 2015). Interventions targeted at the maintenance population should therefore be effective in controlling that variant. However, there are reported instances of multiple species maintaining a single RABV variant, either separately as distinct maintenance populations or together as a combined maintenance community. On some Caribbean islands, dogs and mongooses (family *Herpestidae*) maintain the same dog-derived RABV variant and act as a combined maintenance community (Nadin-Davis *et al.*, 2008; Velasco-Villa *et al.,* 2017). In such situations interventions may need to target both populations to achieve elimination. The presence of multiple maintenance populations can also have implications for disease re-emergence. In north-eastern Mexico, a dog/coyote RABV variant was believed to have been eliminated following widespread dog vaccination. However, the variant continued to circulate in the coyote (*Canis latrans*) population and was subsequently reintroduced to dogs via dog-coyote contact. Sustained transmission was possible given the waning herd immunity from inadequate vaccination coverage (Velasco-Villa *et al.,* 2017). Genetic sequence data from detected cases may be important in determining the species' role in maintaining circulation and transmitting the infection, for example: Distinguishing variants of dog rabies from other variants in wildlife species (Lembo *et al.,* 2007); distinguishing ongoing endemic transmission from incursions (Bourhy *et al.*, 2016; Mollentze *et al.*, 2014; Jakob *et al.*, 2017); and identifying sources of incursions (Tohma *et al.*, 2016). The capacity for an informed rapid response to newly detected cases will be important for the success of the control programmes (Henderson, 2011).

2.4 Cost-Effectiveness Approaches to Rabies Control

The cost-effectiveness of rabies control interventions becomes even more impactful when approached through a One Health perspective (Hampson *et al.,* 2011; Kaare *et al.,* 2009; Zinsstag *et al.,* 2009). By considering the interconnectedness of human, animal, and environmental health, a One Health approach can lead to comprehensive and sustainable strategies for rabies control. Fitzpatrick *et al.* (2016) conducted a study in India, a country burdened by high rabies incidence, to assess the cost-effectiveness of rabies control measures using a One Health approach. This study evaluated interventions such as dog vaccination, human post-exposure prophylaxis (PEP), and dog population management. By considering the costs and benefits of these interventions across human and animal health sectors, the researchers demonstrated the economic efficiency of integrated efforts (Fitzpatrick *et al.,* 2016). They emphasized that a coordinated approach to rabies control, addressing both human and animal aspects of the disease, can yield cost-effective outcomes and enhance overall public health.

Furthermore, the economic evaluation conducted by Kessels *et al.* (2019) in rural Africa focused on dog vaccination strategies as part of a One Health approach to rabies control. The study assessed the cost-effectiveness of different vaccination methods in resource-limited settings. By accounting for the costs involved in dog vaccination campaigns and estimating their impact on reducing human rabies cases, the researchers highlighted the value of integrating veterinary and public health efforts. Such integrated approaches not only yield better health outcomes but also optimize resource allocation, making them economically advantageous for sustainable rabies control (Kessels *et al.*, 2019).

By adopting a One Health approach, countries can identify synergies and optimize resources between human and animal health sectors, leading to cost-effective strategies for rabies control. These strategies encompass comprehensive interventions such as coordinated surveillance, improved diagnostics, public awareness campaigns, and targeted vaccination programs. Taking into account the interconnected nature of disease transmission, a One Health approach offers an opportunity to optimize investments, increase efficiency, and achieve effective and sustainable rabies control while maximizing the health benefits for both humans and animals.

2.5 Rabies Control in Tanzania

Rabies is endemic in East Africa. In Tanzania, the disease dates back since 1930s,with estimates of human deaths from rabies range between 172 to 1958 per year (Hampson *et al.,* 2015). However, this number may be an underestimate of the true burden of rabies due to the incapacity of the existing rabies surveillance systems to detect a few cases, where the majority go undetected (Mallewa *et al.,* 2007; Mazigo, 2011). This has resulted to low a priority being given to the control of rabies by the government. In 2010, the Tanzania government through the WHO country office, the Ministry of Health and the Ministry of Livestock Development started a rabies elimination demonstration programme which ran from 2010 to 2016. The programme was based on annual mass dog vaccinations campaigns, free supply of PEP to health facilities for animal bite victims and the establishment of an improved rabies surveillance system. These three steps are crucial in controlling canine rabies and eliminating human rabies deaths. The programme was implemented in six regions of Tanzania i.e Lindi, Mtwara, Morogoro, Dar es Salaam, Pwani and Pemba Island, off the coast of Tanzania's mainland. The programme's aim was to demonstrate that human deaths due to dog-mediated rabies can be eliminated through mass dog vaccination campaigns (Mpolya *et al.,* 2017).

Pemba is a small island (988 km^2) situated fifty kilometres from the Tanzanian mainland with a relatively isolated dog population that maintains rabies endemically. Fishermen occasionally transport dogs between Pemba and the mainland, and this was likely how rabies was introduced to Pemba in the late 1990s. Dog vaccinations on Pemba first began in 2010, with a small-scale campaign conducted by the animal welfare organisation, World Animal Protection. Over the following five years a rabies elimination demonstration project, funded by the Bill and Melinda Gates Foundation, coordinated by the World Health Organization, and led by the Tanzanian government, was implemented across southeast Tanzania, including Pemba (Mpolya *et al.,* 2017). In late 2016, an outbreak was detected and the initial response involved conducting mass dog vaccination campaigns in villages reporting cases. However, these efforts were not effective in preventing further spread of rabies across the island. An evaluation of the vaccination campaign was conducted immediately by the district veterinary officers and remedial campaigns followed. A door-to-door vaccination strategy was adopted in some villages where dog owners could not bring their dogs to allocated central points. This strategy increased the number of villages where vaccination campaigns were conducted to cover the whole island from 2017 onwards and since then, annual mass dog vaccination campaigns have been made a routine. In other parts of the country such as in Mara region, rabies control programmes are implemented through non-governmental organisations (NGOs) in collaboration with the Local Government Authorities (LGAs).

Currently, rabies surveillance in Tanzania is conducted through two separate systems: Animal Disease Surveillance by the Ministry of Livestock and Integrated Disease Surveillance Response (IDSR) by the Ministry of Health. While rabies in animals is a notifiable disease, the surveillance approach is mainly passive. Suspected rabies cases in districts are reported to the District Veterinary Officers (DVO), who then notify the Director of Veterinary Services (DVS). Additionally, rabies is considered a priority disease within the IDSR system, with health facilities reporting dog bite cases to the districts, which are subsequently reported at the regional and national levels. However, there is limited information sharing regarding rabies between the health and veterinary sectors. Effective collaboration between public health and animal health stakeholders is crucial for successful multisectoral efforts in combating priority zoonotic diseases and implementing comprehensive national strategies for prevention, detection, and control of zoonotic pathogens. In Tanzania, a One-Health Unit has been established, and initiatives are underway to develop an integrated zoonotic diseases surveillance guideline that prioritizes rabies for enhanced surveillance and control. An IBCM surveillance strategy which has been proposed by the United against Rabies Coalition, if integrated into the Tanzania national rabies control strategy will give hope towards the zero by 30 goal.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The study was undertaken across 24 districts in 4 regions of south-eastern (Lindi, Mtwara and Mororogo), and northern Tanzania (Mara region) and on Pemba Island. The study areas were chosen based on the presence of previous rabies control initiatives, such as mass dog vaccination, rabies surveillance, and provision of post-exposure prophylaxis (PEP) to individuals bitten by dogs (Mpolya *et al.,* 2017). Additionally, ongoing rabies intervention projects since 2010, were taking place in these areas. For Lindi and Mtwara regions, rabies incidence in both humans and animals had been reduced during this period and it was close to elimination. Pemba Island was selected to compare the dynamics of infection between island settings and on mainland Tanzania all subjected to rabies control using the same strategies. Primary data were collected for this study, while secondary data on dog vaccination, population, and transect surveys were utilized to estimate the dog population and vaccination coverage in the selected study sites. The total human population within these regions was estimated at a total of 8 107 187 in 2021, projected from the 2012 Population and Housing census survey (Tanzania National Bureau of Statistics, 2013). The average human: Dog ratio (HDR) in these settings was estimated at 30:1, but varied across districts, giving a dog population in 2019 of around 254 000. The areas comprise a range of cultural settings that vary from one site to another. For example, the northern study site is mainly agro-pastoralists and pastoralists, while farming and commercial activities dominate in the south; and fishing is the main economic activity for the people on Pemba Island. The location of the study area is indicated by Fig. 2. The human population density per square kilometre is indicated in colours as shown in the legend. The darker the shading, the higher the human population density.

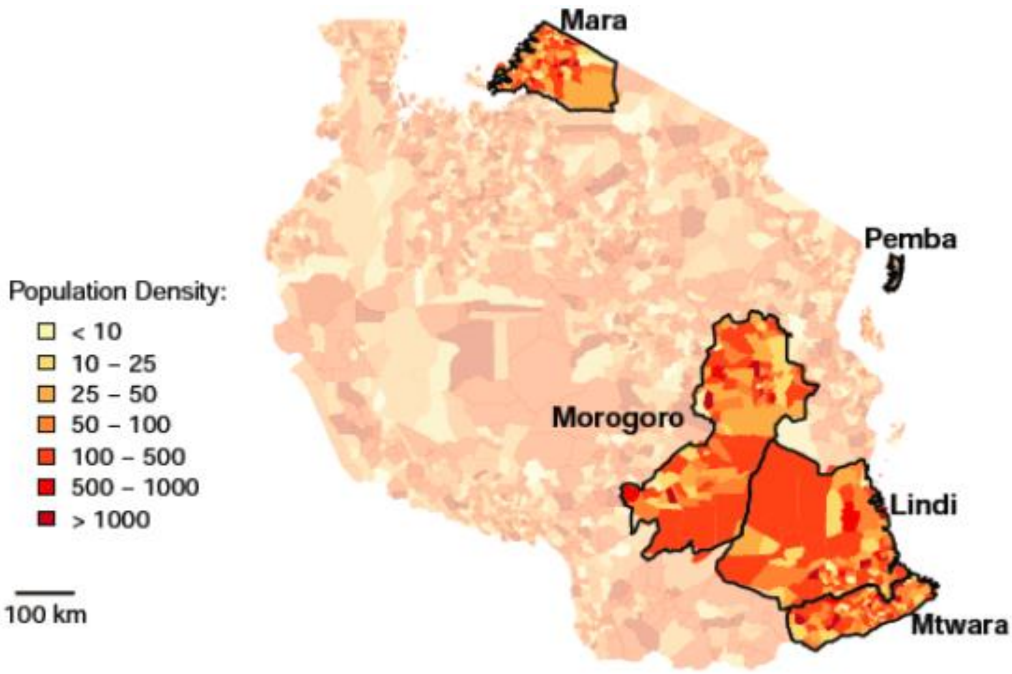

Figure 2: Map of Tanzania showing location of the study areas with bold black boarder line

The human population density of the study area is indicated in shadings. The darker the shading, the higher the human population density.

3.2 Study Design and Data Management

This study employed a prospective study design, building upon the successful rabies elimination proof of concept project conducted in southeast Tanzania from 2011 to 2015, which also included Pemba Island (Mpolya *et al.*, 2017). In addition, the study took advantage of the establishment of an enhanced rabies surveillance system from 2018 to 2022. The sample size for the study included all humans and animals within the designated study sites.

To ensure efficient data management, electronic methods were employed for data collection, storage, and backup. The data were meticulously collected through electronic means, allowing for systematic and organized records. These records were stored on local servers maintained by the Ifakara Health Institute (IHI), which served as a secure and centralized repository for the study's data. Additionally, regular backups were performed to prevent data loss and to maintain the integrity and availability of the collected information.

3.3 Establishment of an Integrated Bite Case Management

An IBCM approach was developed for integration within the existing health and veterinary sectors across the study sites (Fig. 3). The introduction of IBCM in 2018 involved training health workers to undertake risk assessments and LFOs, a paraprofessional cadre working within the Ministry of Livestock and Fisheries, to undertake animal investigations. The IBCM involves undertaking risk assessments for all patients who present to health facilities with animal bites to determine whether they were bitten by potentially rabid animals or normal healthy animals and to ensure that PEP is correctly administered to exposed individuals to prevent the onset of rabies. Epidemiological investigations were conducted for animals that bite people to diagnose animal rabies cases. Through these investigations, other exposed individuals were identified and referred to health facilities that offer PEP.

People bitten by suspect rabid animals attending hospitals for PEP were assessed for the possibility of being exposed to the rabies virus. Health workers delivered a rabies risk assessment to the bite patient to determine whether PEP is required or not based on the World Health Organisation classification guidelines for high versus low risk cases (World Health Organization, 2018). If the involved animal was suspicious for rabies, the health worker provided PEP to the patient and informed the designated rabies focal persons in the animal health sector (LFO) at the district level. The LFOs investigate an animal whenever they were informed by a health worker or directly by members of the community where the suspected case had been reported from. The LFOs investigating a case sought the owner of the suspected animal to find out its health history, vaccination status and, or to check for any signs consistent with rabies. If the animal was vaccinated against rabies and did not appear sick, no further investigation was done, however, the owner would be instructed to home quarantine the animal until 10 days have passed from the date of the biting incident. In a situation, where the biting animal was owned (dog/cat) and found healthy at the time of the investigation, the LFO would inform the health worker that no further PEP was required. During the home quarantine period, the animal would be under observation for any health or behavioural changes and when it fell sick, the owner would immediately inform the LFO to euthanize the animal and to continue with PEP.

If the animal had not been vaccinated against rabies or its vaccination status unknown, the LFO would evaluate the status of the animal according to whether it showed any clinical signs of rabies. If the investigation revealed the animal was suspicious for rabies, the LFO would also check within the community to determine there were no other persons or animals that were also exposed to rabies-suspected animals. All bite victims identified during the investigation were immediately referred to the nearest treatment centre for PEP. If during the investigation the LFO was required to euthanize the animal or found the animal had already been killed or died, the LFO would then collect samples from the animal. The LFO would immediately use the Lateral Flow test (LFT) to obtain the first diagnosis and ensure that triplicate samples from the animal were collected and sent to the Tanzania Veterinary Laboratory Agency (TVLA) for subsequent MinION sequencing and genetic characterization. The information collected during both the risk assessment by the health worker and the animal investigation by the LFO was captured on forms within the IBCM mobile phone application (Appendix 1).

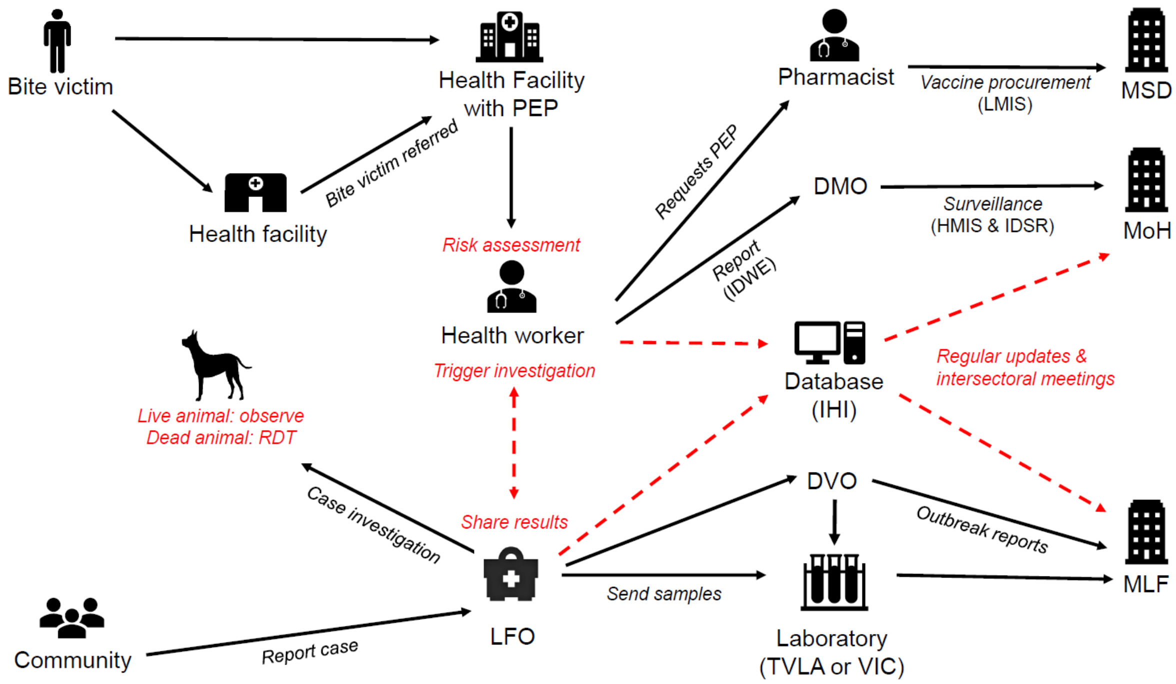

Figure 3: **Integrated Bite Case Management framework in Tanzania**

Red text and arrows indicate interventions introduced as part of IBCM. The existing health systems and reporting structures under the Ministry of Health (MoH) and Ministry of Livestock and Fisheries (MLF) are shown in black and include: The Medical Stores Department (MSD), District Medical Offices (DMO), District Veterinary Offices (DVO), the Tanzania Veterinary Laboratories Agency (TVLA), Livestock Field Officers (LFO), the Integrated Disease Weekly Ending (IDWE) surveillance and reporting system, the Logistic Management Information System (LMIS), the Integrated Disease Surveillance and Response system (IDSR) and the Health Management Information System (HMIS). Ifakara Health Institute (IHI) hosts the database server for the IBCM. The RDTs are Rapid Diagnostic Tests.

3.3.1 The Integrated Bite Case Management Application

A mobile phone-based surveillance system for rabies previously developed and set up in southern Tanzania (Mtema *et al.*, 2016) was adopted as the basis for an IBCM application (app) for android phones with a web-based interface (dashboard). The app included risk assessment forms for completion by health workers (Appendix 1) and epidemiological investigation forms for LFOs that also cover sample collection (Appendix 2). The forms use mainly multichoice selections to minimize free-text data entry. The dashboard was developed to monitor submitted records and is accessible via the app or a password-protected website.

The app was developed using Waterfall development methodology starting with requirement solicitation followed by design, testing, deployment, and maintenance (Ian, 2003). The app is hosted on Google Playstore and has been updated to fix bugs and add new features as required. Data can be accessed via the dashboard by government stakeholders including regional and district veterinary and health officers, who provide feedback to their respective health workers and LFOs on the data being collected.

In February and March 2019, additional functionality was added to the app so that 'high-risk' bites were identified following risk assessments by health workers. In response to a high-risk bite being identified, an automated alert would be sent to designated LFOs to trigger an investigation. A 'high-risk' investigation was triggered if one of the following criteria were met: (a) a person was bitten by an animal that displayed at least one sign suggestive of rabies (e.g. excessive salivation, paralysis [Appendix 1]), (b) a person was bitten by an animal that subsequently disappeared or died or was of unknown origin, (c) a person was bitten by a wild animal and, (d) a person presented to a health facility with symptoms of rabies. If one of these conditions were met, an automated alert would be triggered when the health worker submitted the risk assessment form. The generated message for the LFO contained the patient ID, their name and location details, including their village and phone number to facilitate the investigation. Automated alerts to LFOs were only generated on a bite patient's first visit to a health facility and not for their subsequent visits. Bite patients were given a vaccination card on receipt of their 1st PEP dose and were required to present the card on subsequent visits. The card contained the patient's name, age, village and district, date bitten, and PEP dates following the newly recommended 1-week ID regimen (Tarantola *et al.*, 2019). If a bite victim sought care but PEP was unavailable, a patient ID was still generated and an alert sent to trigger the investigation. The victim was advised to travel to another health facility for PEP and was given a vaccination card containing their ID and other details indicating that they required PEP. This enabled the next facility to provide PEP to the victim without triggering another investigation.

3.3.2 Training of Government Personnel (Health and Veterinary Workers)

At least one government health facility offers PEP in each district, with a few districts having more than one government facility providing PEP. In each district in the study area, two health workers from each government facility that offers PEP and one LFO was chosen and trained to be focal rabies personnel. The trained health workers were from the immunization departments of each district hospital. To ensure all bite victims who presented to the hospital for treatment were captured, all health and medical attendants working at the Outpatient Department (OPD) were also informed by the respective hospital authorities to refer bite victims to the immunization departments. A joint on-job training was held with LFOs brought to health facilities in their districts and together with health workers they were trained in IBCM during the first 6 months of IBCM introduction in July 2018. Specifically, health workers were trained to undertake risk assessments of bite patients, while the LFOs were trained on how to conduct epidemiological investigations. To maintain implementation of IBCM monthly phone credit was provided to all focal persons (1GB per month) and reimbursement or advance payment to LFOs for fuel to undertake investigations (typically 10 000 to 20 000 Tsh per investigation).

The protocol for LFOs involved first conducting a phone consultation with either the animal bite victim or a relative of the victim whose phone number was recorded during the health worker's risk assessment. In scenarios where the biting animal's information could not be obtained through phone consultation, LFOs were advised to visit the household of the animal owner. If multiple people were attacked by the same animal the LFO was required to record their names, patient ID (if the person had sought care), village and PEP status on the investigation form. If the biting animal was vaccinated against rabies and did not appear sick, no further investigation was undertaken, however the owner was instructed to observe the animal for 10 days following the bite incident and immediately inform the LFO if any health or behavioural changes were observed. If the investigation revealed the animal was suspected to be rabid, the LFO was advised to check within the community to determine whether any other persons or animals had been exposed. The LFOs were trained to collect samples from animals that had been killed or died, and were provided with BioNote rapid diagnostic test (RDT) to test for rabies where possible. Following investigations, LFO were advised to inform the health worker of the investigation result. Consent was not sought from patients for undertaking risk assessments or for animal investigations, as both activities are considered part of government duties. However, patients were informed that their data was being recorded electronically to inform an investigation of the biting animal.

To quantify baseline incidence of bite patient presentations, prior to the introduction of IBCM, paper records from health facilities in the study from the 1st of January 2018 were collected. To determine the impact of introducing IBCM, analysis of records from the IBCM database up until October 2021, providing 3 years of data following the introduction of IBCM was performed. A chi-squared test was used to investigate differences in risk classifications pre- and post-implementation of IBCM.

3.4 Investigations of Reservoir Dynamics of Rabies Infection in Southeast Tanzania (Lindi and Mtwara Regions)

3.4.1 Contact Tracing

A mobile phone-based surveillance system was used across the area to record animal-bite victims presenting to health facilities requiring PEP, on animal cases suspected of being rabid or bitten by other suspect rabid animals reported by LFOs, and mass dog vaccination campaign information coordinated through each district livestock office (Mtema *et al.*, 2016). Records from this system for the study areas of Lindi and Mtwara were extracted and validated against paper-based records from health facilities and livestock offices. Animal-bite victims and owners of biting animals from the mobile systems were exhaustively traced and interviewed to obtain details of each bite incident, using previously established contact tracing methodologies (Hampson *et al.*, 2008). The aim of contact tracing was to assess whether the biting animal was rabid using clinical and epidemiological criteria. For all possible and suspect rabies exposures identified, the source of the biting animal and all known persons and animals bitten were investigated as well as households the animal was reported to have visited. Information collected during interviews included: the date and coordinates of the person bitten; if possible, the origin of the biting animal; the species; the dog owner if known; and whether the animal was known to have bitten other people or animals. Details regarding the animal's behaviour and the bite circumstances were used to assess whether the animal was considered likely to have been rabid. If additional biting animals or bite victims were identified during investigations, they were also traced and interviewed. The resulting data on probable rabies cases and exposures were used to examine trends in incidence and infer transmission within and between species. The information collected during the interviews were on cases or exposures that were reported from 2011 to 2019.

3.4.2 Mass Dog Vaccination Campaigns

Five rounds of mass dog vaccination campaigns were conducted in each study district of Lindi and Mtwara regions (13 districts) between 2011 and 2017 (Mpolya *et al.*, 2017). Temporary vaccination stations were set up within villages at points chosen to be accessible to most villagers, often within a ward or village office, school, or other central village location. Each vaccination point was operated by two LFOs and either a health worker or a local primary school teacher. On arrival at the vaccination station, dogs were registered, and their age, sex, and prior vaccination history recorded. Following vaccination dogs were marked with a temporary collar to distinguish them from unvaccinated dogs and owners were provided with a vaccination certificate. Campaigns ran from 9.00 am to 3.00 pm on a single day at each vaccination station. From 2017 onwards no additional mass dog vaccinations campaigns have been conducted on Tanzania mainland, except for some localized vaccinations in Lindi Municipal Council and Kilwa Districts in 2019, 2020, and 2021 in response to outbreaks (these data have not been included in the analysis).

3.4.3 Post-Vaccination Transects

Following the completion of vaccination campaigns, transects were conducted to record the numbers of vaccinated dogs (marked by temporary collars) and unvaccinated dogs in two randomly selected sub-villages in each of a subset of villages. Transects changed direction at the village boundaries to avoid counting dogs from other villages. Transects were completed by LFOs who walked or cycled along transect routes on the evening of the campaign day as detailed in Sambo *et al.* (2017). These data together with vaccination registers were used for estimating dog population sizes and vaccination coverage.

3.4.4 Analysis

(i) Parameter Estimation

Serial interval is defined as the interval between the onset of clinical signs in a primary case to the onset of clinical signs in a secondary case infected by the primary case. The distance kernel represents the distance between the locations of the primary and secondary cases. The probability distribution of the serial interval and distance kernel for transmission of RABV in domestic dogs were estimated using data on probable rabies cases from a long-term contact tracing study in Serengeti District, northern Tanzania. These data included the date and location of the bite incident for the primary rabid animals and the secondary cases that they infected, with information available for serial interval and distance kernel estimation in 1139 and 958 cases respectively. Only 25 cases from southeast Tanzania included this information so these data were excluded from parameter estimation. For both parameters, maximum likelihood-based approaches were used for estimation and the best-fitting distribution was selected using Akaike’s Information Criteria (AIC).

Estimation of the serial interval distribution was carried out using a maximum likelihood-based approach, specifically through fitting gamma, Weibull and lognormal distributions to the times between the onset of clinical signs in a primary rabies case and the onset of clinical signs in known secondary cases in the Serengeti data. Model comparison and identification of the best fitting distribution was carried out using AIC. The distance kernel was estimated by fitting to the distances between the locations of known primary cases and secondary probable cases that they contacted. Of the 958 pairs of locations of primary cases and their secondary contacts, 301 had a distance of zero recorded. This was due to both primary cases and secondary contacts being from the same household. For these cases interval censoring was applied over a distance of 0-50 metres. Gamma, Weibull and lognormal distributions, as well as two-component mixtures of gamma, Weibull and lognormal distributions were fitted to these distance data to accommodate potential bimodality. Distribution fitting was carried out using maximum likelihood methods and the best fitting distributions selected using AIC.

An epidemic tree of rabies spread was constructed from the timing and location of identified rabies cases. Previously estimated parameters describing the generation time (between one animal becoming infected and causing an infection in a new animal) and the dispersal kernel were used by the algorithm (Hampson *et al.,* 2009). The tree-building algorithm was run 1000 times to incorporate uncertainty in the timing of cases and generate probability estimates of the most likely progenitors for each rabies case and their bootstrap support. The effective reproduction number (Re), which describes the average transmission probability when control measures are being implemented, was calculated as well as the number of cases attributed to each rabies case. Re was examined through time and credible intervals calculated. I employed a GLMM with a negative binomial error structure to examine how Re (for individual rabies cases) varied with vaccination coverage.

(ii) Transmission Trees

The estimates of the serial interval G, and distance kernel K, described above were used within a previously developed algorithm (Hampson *et al.,* 2009) to generate putative epidemic trees. A 'progenitor' is defined as a case that was inferred to be the source of infection for another case. For each probable case *i*, a progenitor *j* was chosen at random with probability p_{ij} from all cases within southeast Tanzania with a date of onset of clinical signs prior to the date of onset of the case (n), where:

$$p_{ij} = \frac{G(t_{ij})K(d_{ij})}{\sum_{k=1}^{n} G(t_{ik})K(d_{ik})} \qquad (1)$$

t_{ij} is the days between the onset of clinical signs in case *i* and its potential progenitor *j;* and d_{ij} is the distance between the locations of cases *i* and *j*. For probable cases in wildlife or for dogs where the owner was not known, the convolution of two distance kernels were used to better incorporate the greater uncertainty in reported locations for these cases.

Due to uncertainty around the dates and locations of some cases, 50 000 bootstrapped datasets of plausible progenitors were generated. In each iteration, for cases with uncertainty around the date of onset of clinical signs and/or their location, dates and/or locations were selected randomly from a uniform distribution within the period or radius of uncertainty, respectively. The most likely progenitor for each case was the case selected most frequently as the progenitor within the 50 000 bootstrapped datasets.

Cases from all species were included in the analysis. As data to estimate the serial interval and distance kernel for wildlife were lacking, we assumed these distributions for wildlife were the same as those for domestic dogs.

(iii) Assessing within- and between-Species Transmission

The algorithm assigning progenitors does not account for unobserved cases. Attempts to adjust for unobserved cases were made by analysing a subset of inferred transmissions considered most likely to represent direct transmission. Only inferred transmissions below the 99^{th} percentile value of the serial interval and the (convolution of two) distance kernel distributions were analysed to assess within- and between-species transmission, corresponding to cut-off values of 156 days and 9803 metres, respectively. Transmissions with serial intervals and/or distances above these cut-off values were considered less likely to represent direct transmission.

Within this subset of inferred transmissions, relative frequencies of within- and between-species transmission were estimated. Weighted random sampling was used to select a single progenitor for each case from the set of bootstrapped progenitors for that case (selected with replacement from all cases) and the species recorded and used to construct a contingency table of inferred transmissions. Fisher's exact test statistic was calculated to test whether the inferred levels of inter-species transmission would be expected under random mixing. This procedure was repeated 1000 times and median levels of inferred transmission and p-values calculated.

To assess the robustness of the transmission tree results, sensitivity analyses for different scenarios were conducted. These included using an alternative upper limit for the interval censoring of the distance data, using the 95^{th} percentile values of the distributions as the cut-off values, using only the single most likely progenitor in construction of the transmission trees rather than considering all possible progenitors, alternative approaches to addressing the uncertainty in the dates reported, and subsampling dog rabies cases to assess how case detection affects inference of within- and between-species transmission. The additional scenarios explored during sensitivity analysis are outlined below:

(a) Using exact dates of clinical signs onset, without incorporating uncertainty.

(b) Incorporating uncertainty in recorded dates (0, +/- 7, +/- 14 or +/- 28 days) but allowing progenitors to have a date of onset up to 56 days after the primary case onset. An upper limit of 56 days was chosen to allow for the maximum uncertainty of 28 days recorded in both the primary and secondary case.

(c) Evaluating only the single most likely progenitor for each case rather than all possible progenitors.

(d) Using parameters for the distance kernel but with 100 metres as the upper limit for interval censoring instead of 50 metres.

(e) Using the 95th percentile of the distributions for serial interval and spatial kernel.

(f) Dog rabies cases were posited to be better observed than wildlife cases given their proximity to humans. To explore the impact that observation bias might have on inferred species-to-species transmission, analyses were undertaken on subsampled data. Trees were constructed using 60%, 75% or 90% of dog cases including all wildlife and domestic cat cases. For each scenario, sampling with replacement was used to generate a population for transmission tree construction and repeated 10 000 times with mean levels of species-to-species transmission calculated as described in the main text.

(iv) Chains of Transmission and Cluster Size

Using the most likely progenitor identified for each case (highest bootstrap support), chains of transmission were constructed and examined for evidence of sustained transmission amongst domestic animals and/or wildlife. Clusters of cases linked by directly inferred transmissions were identified and the sizes of clusters consisting of a single species or mixture of species evaluated. The mean cluster size (including clusters of one) per six-month period from the first case recorded was calculated and a weighted linear spline regression performed to test for a temporal trend. A six-month period was selected to allow full use of the data whilst allowing a long enough time window for clusters to be observed. Sensitivity analyses were performed using periods of three-months and one-year, with the 2019 data excluded from the one-year analysis as data for the full year were not available.

(v) Regression Analysis of Monthly Incidence by Species

Negative binomial regression models were fitted to the monthly probable rabies cases observed amongst all species; amongst domestic animals only and amongst wildlife only. Linear splines were used within the regression analyses where visual inspection of the data suggested a change in trend. The correlation between the monthly time series of cases in domestic dogs and in jackals was also examined, evaluating lags of 0 to 11 months for both time series.

(vi) Logistic Regression of Cases in Relation to Population Composition

Whether the proportion of wildlife cases within a district was related to their relative availability within the susceptible population was examined, focusing on only dogs and jackals (95% of all cases) and including data from other districts where cases had been traced using the same methods, specifically from Serengeti district (cases between January 2002 - June 2019), Ngorongoro district (January 2002 - March 2019), and Pemba Island (January 2010 - January 2019). The four districts of Pemba Island were considered a single population given the small numbers of dogs and limited geographical area.

The susceptible population (jackals and dogs) was estimated as follows. Jackals were assigned to grid cells at a density of 0.3 per km^2 for all districts following a literature review (Durant *et al.*, 2011; Maddox, 2003; Yarnell *et al.*, 2013), except to cells with human population density below 2.5 per km^2 or over 500 per km^2 (using gridded population data from: http://worldpop.org.uk). Areas with lower human densities were excluded assuming negligible case detection from these largely uninhabited settings; the upper density limit was applied assuming unsuitable habitat. Alternative lower limits of 1.25 and 5 humans per km^2 were explored as were alternative jackal densities of 0.15 and 0.50 jackals per km^2. Dog numbers were estimated from post-vaccination transect data (Sambo *et al.*, 2018). To account for dog vaccination on the availability of susceptible animals, three scenarios were applied to the estimated dog populations: (a) Zero vaccination coverage; (b) Median coverage recorded during the period and; (c) Maximum recorded coverage. The combined susceptible (jackal and dog) population for each district was estimated. Using logistic regression, the proportion of probable cases (those in both dogs and jackals) that occurred in jackals was regressed against the proportion of the susceptible population consisting of jackals to assess evidence for a relationship.

(vii) Assessment of Vaccination Coverage

Data from the post-vaccination transects were used, along with reported numbers of dogs vaccinated during campaigns, to estimate the dog population in each village at the time of each vaccination campaign, using the approach described by Sambo *et al.* (2018). Where transect data were not available for a given village and campaign, population estimates were obtained indirectly, based on transects conducted in the same village but during other campaigns or (if no transects were available for the village) on the overall human/dog ratio for the district, estimated from the projected human population size and a district dog population estimate from all available transects for the district in that vaccination round. Vaccination coverage in each village and campaign was then estimated by dividing the recorded numbers of dogs vaccinated by the associated dog population estimates. District-level coverage estimates (Table 1) were similarly obtained after summing numbers of dogs vaccinated and overall dog population estimates from villages in a district. Coverage achieved by each round of mass dog vaccination campaigns is shown for each district. Values shown are an average of the level achieved across the entire district and do not show the heterogeneity in coverage.

Table 1: Dog vaccination coverage by district for mainland Tanzania

District	Vaccination coverage (%) by campaign				
	1st	2nd	3rd	4th	5th
Kilwa	23.5	36.0	39.1	38.2	47
Lindi Rural	23	31.4	21.9	33.9	33.2
Lindi Urban	60.1	52.5	48.9	58	50.1
Liwale	13.1	18.6	16.9	25.2	19.7
Masasi	29.1	28.2	34.2	34.5	28.2
Masasi Township Authority	3.3	15.4	0.8	26.5	15.8
Mtwara Rural	14.5	25.7	25.8	33.5	26.8
Mtwara Urban	34.9	24	33.7	32.7	37.6
Nachingwea	34.1	47.2	54.5	54.9	46.8
Nanyumbu	21	24.8	44.3	42.4	42
Newala	24.2	26.7	39.2	22.1	19.8
Ruangwa	27	27.5	29.8	45.4	54.8
Tandahimba	22.8	23.9	31.4	35.2	32.7
Mean (standard deviation)	25.4 (13.5)	29.4 (10.5)	32.3 (14.1)	37.1 (10.7)	35 (12.6)
Median (range)	23.5 (3.2-60.1)	26.7 (15.4-52.5)	33.7 (0.8-54.4)	34.5 (22.1-58)	33.2 (15.8-54.8)

3.5 A Case Study of Rabies Elimination from Pemba Island

3.5.1 Epidemiology and Laboratory Investigations

Records of bite patients presenting to health facilities were used to initiate contact tracing (Hampson *et al.,* 2008). Bite victims and, if known, the owners of biting animals were exhaustively traced, recording the details of biting incidents, including the date and coordinates of each incident, and the biting animal. Other people or animals bitten that were identified were then further traced. The status of animals was assessed from their reported behaviour and outcome (whether they died, disappeared or survived), and classified according to WHO case definitions (World Health Organization, 2018). Briefly, an animal showing any clinical signs of rabies was considered a suspect case; if a suspect case had a reliable history of contact with a suspect rabid animal and/or was killed, died or disappeared within 10 days of observation of illness, the animal was considered a probable case. Animals that remained alive for more than 10 days after biting a person, were considered healthy. Brain tissue samples were collected from animal carcasses whenever possible. Prior to 2014, these samples were tested using the fluorescent antibody test (FAT) (Mayes & Rupprecht, 2015). Samples from 2016 onwards were tested using the direct rapid immunohistochemical test (DRIT) (Niezgoda & Rupprecht, 2006) following training by the Global Alliance for Rabies Control.

Two batches of sequencing were performed to obtain near-whole genome sequences (WGS) from the collected brain samples of rabid dogs, with the approach changing as protocols and capacity for in-country sequencing developed (Brunker *et al.*, 2020). Archived 2012 Pemba samples (4) and samples (6) collected during early outbreak surveillance (September/October 2016) that had been confirmed FAT positive at PVLD were shipped to the Animal and Plant Health Agency (APHA), UK. Total RNA was extracted using Trizol (Invitrogen) and a real-time PCR assay (Marston *et al.*, 2019) was performed to confirm the presence of rabies virus and indicate viral load. Metagenomic sequencing libraries were prepared and sequenced on an Illumina MiSeq (Brunker *et al.*, 2015). Subsequent sequencing of 8 additional samples (September 2016 to May 2017) was conducted in-country in August 2018 at Tanzania Veterinary Laboratory Agency (TVLA) in Tanzania following an end-to-end protocol using a multiplex PCR approach (Quick *et al.*, 2016) to sequence RABV genomes on a MinION platform (Oxford Nanopore Technology, Oxford, UK), which have previously been published (Brunker *et al.,* 2020). Fourteen previously unpublished WGS (via metagenomic approach) from mainland Tanzania (2009 to 2017) are also published here and included in subsequent analyses.

3.5.2 Control and Prevention Measures

Data on rabies control and prevention measures implemented on Pemba were compiled. Dog vaccinations carried out between 2010 and 2021 comprised the first small-scale campaign (705 dogs vaccinated) in 2010. This was followed by four consecutive annual island-wide campaigns that were undertaken as part of the elimination demonstration project (Mpolya *et al.,* 2017) organised as follows. Four vaccination teams were formed to conduct dog vaccination across the island. Each team consisted of two LFOs and Community Animal Health Workers (CAHW). One week before each campaign, a meeting was held between District Veterinary Officers, LFOs, and CAHW to review protocols and distribute vaccination equipment. The CAHWs for each village (shehia) then moved door-to-door inviting dog owners to bring their dogs to their nearest vaccination point and distributed posters. One day before the campaign, the CAHWs walked repeatedly through each shehia announcing the forthcoming vaccination over a loudspeaker. Vaccination points were organised such that each three shehias had on average one vaccination station located at central convenient locations. Campaigns ran from 9.00 am to 3.00 pm on a single day. In 2016, a central point vaccination strategy of dogs was employed in shehias where dog rabies cases had been reported as a response to control the outbreak of rabies. However, these efforts were not effective in preventing further spread of rabies across the island. An evaluation of the vaccination campaign was conducted immediately by the district veterinary officers and remedial campaigns followed. A door-to-door vaccination strategy was adopted in some shehias where dog owners could not bring their dogs to allocated central points. This strategy increased the number of shehias where vaccination campaigns were conducted to cover the whole island from 2017 onwards.

From 2010, the Gates Foundation procured PEP for free provisioning at the four district hospitals on Pemba and delivered training in administering both intradermal and intramuscular PEP. After the closure of this project in 2015, bite patients paid >30 000 TSh ($12.9) per PEP vaccine vial. However, in 2017 the government of Zanzibar decided to subsidise PEP again, making the PEP vaccine available to bite patients for free at the island's main hospital (Chake Chake) and in hospitals in Zanzibar and the Tanzanian mainland (both 1-day's ferry travel away). Data on the numbers and timing of all vaccination campaigns were collated, as well as costs of dog vaccination and provisioning of PEP, derived from local prices and government salaries.

3.5.3 Analysis

(i) Rabies Exposures and Cases

The number of human exposures per rabid dog was calculated as the ratio of human exposures to dog rabies cases. A comparison was made between routine surveillance that involves official data collection of human bites and suspected animal rabies cases by healthy and veterinary workers and suspected rabies exposures identified from contact tracing using linear regression.

(ii) Dog Population and Vaccination Coverage Analyses

Monthly dog populations in each *shehia* were estimated and vaccination coverage and the level of vaccine-induced immunity projected monthly from the dog population estimates and vaccination campaign records. To estimate time-varying vaccination coverage at the shehia level, it was necessary to first estimate dog population sizes. This was achieved using two datasets: (a) government dog population surveys for the years 2012 and 2017-2019, and (b) post-vaccination transects from the 2013-2014 vaccination campaigns, with associated numbers of dogs vaccinated in the preceding campaigns. Where at least one collared (i.e. vaccinated) dog and >10 total dogs were observed on a transect, the dog population of a shehia at the time of the transect was estimated as:

$$D = \frac{V_d(1+PAR)}{\left(\frac{C_d}{(C_d+U_d)}\right)} \quad (2)$$

where D is the dog population size, Vd is the number of dogs vaccinated in the campaign preceding the transect, Cd is collared dogs, Ud is unmarked dogs, and PAR is the ratio of pups (<3 months) to adult dogs (Sambo *et al.,* 2018). The PAR was estimated to be 0.256 from a census of the Serengeti District dog population in Northern Tanzania between 2008-2016 (Sambo *et al.*, 2017). By multiplying by (1+PAR), assuming that both vaccination campaigns fail to reach pups, and that pups are not counted during transects (Sambo *et al.,* 2018).

At least one Government or transect-based dog population estimate was available for each shehia, with some having estimates at up to six time points. For each shehia, the dog population in every month throughout the study period for which there were no already an estimate was then projected. For months that lay between two known population estimates, a population projection was obtained via the exponential population growth rate calculated between those two estimates. For months where there was only a preceding or subsequent dog population estimate available, the population based on a human: dog ratio calculated from this preceding/subsequent estimate and the human population projected from the 2012 national census (Tanzania National Bureau of Statistics, 2013) was estimated. In some cases, the projected dog population obtained for a month using this approach was lower than the number of dogs vaccinated during a campaign in that month. Where this occurred, the population estimates were adjusted as necessary to prevent coverage estimates exceeding 100%.

The coverage achieved by each vaccination campaign in each shehia was obtained by dividing the number of dogs vaccinated by the estimated dog population for the month when the campaign occurred. The monthly number of dogs with vaccine-induced immunity was estimated as follows:

$$\lambda = e^{-\left(\frac{1}{v}+d\right)\left(\frac{1}{12}\right)} \min\left(1, \frac{D_m}{D_{m-1}}\right) \tag{3}$$

$$P_m = \begin{cases} \max(0, V_{m-1}\lambda - N_m), & if\ January \\ \max(0, P_{m-1}\lambda - N_m), & if\ any\ other\ month \end{cases} \tag{4}$$

$$V_m = \min\left(D_m, V_{m-1}\lambda + \max(0, N_m - P_{m-1}\lambda)\right) \tag{5}$$

Whereby, Vm is the number of immune dogs at month m, Nm is the number of newly vaccinated dogs at m, Dm is the dog population at m estimated using the methods described above, and Pm is the number of immune dogs that were vaccinated during campaigns in previous years, not in the current year. Immunity wanes according to both v, the mean duration of vaccine-induced immunity (assumed to be 3 years), and d=0.595, the annual dog death rate (Czupryna *et al.*, 2016). This approach conservatively assumes both that dogs that are immune from previous campaigns are preferentially vaccinated in subsequent campaigns and that, if the dog population declines between months, then this is a consequence of an above average death rate, rather than a below average birth rate. It also assumes that any top-up campaigns in a shehia in the current year focus on vaccinating susceptible dogs, avoiding re-vaccination of already vaccinated animals.

(iii) Assessing the Impact of Mass Dog Vaccinations on Rabies Cases

Vaccination coverages were grouped into one of three categories: low coverage of between 0 and 20%; medium coverage between 20 and 70% and high coverage of >70% as recommended by WHO (World Health Organization, 2018). The relationship between monthly vaccination coverage and number of rabies cases in each village was explored using a generalised linear mixed model (GLMM) with a negative binomial error structure. A zero-inflation model was employed to account for the zero values.

(iv) Phylogenetic Analyses

Resulting RABV consensus sequence data were analysed using an existing sequence data resource for rabies virus maintained by University of Glasgow collaborators. The RABV-GLUE is a newly developed data-centric bioinformatics software system, which organizes RABV genomic data along evolutionary lines (Singer *et al.*, 2018), so that trees can be constructed using publicly available sequences consolidated with new sequences from Tanzania.

The platform can be accessed as a web version for basic analysis (http://rabv.glue.cvr.ac.uk/) or installed offline for more advanced work depending on the user's expertise. Within GLUE modular tools are available to produce high-quality alignments from viral genomes, maximum likelihood phylogenetic trees, and RABV clade assignment based on the genetic clusters defined in (Singer *et al.*, 2018). This enabled the diversity and distribution of RABV lineages in the study area to be quantified and identify the most closely related viruses for each new sample obtained. The spatiotemporal relationships of the identified viruses were assessed to determine sources of incursions.

Pemba sequences were submitted to RABV-GLUE (Campbell *et al.*, 2022) to determine which global RABV subclade they belonged to. Then, to contextualise the sequences from Pemba more broadly, they were compared to all publicly available RABV sequence data from the same subclade (Cosmopolitan-Africa 1b). The genome region and number of sequences varied widely in these data; therefore, subsets were used to extract the most relevant data for comparison.

(v) Transmission Tree Analyses

Using the case data, transmission trees were constructed building on previously described methods (Mancy *et al.*, 2022). Traced progenitors were assigned, otherwise links between cases were inferred probabilistically from dispersal kernel and serial interval distributions incorporating uncertainties in timings (Mancy *et al.*, 2022). Distributions previously parameterized from contact tracing in northwest Tanzania (Lognormal serial interval, meanlog 2.85, sdlog 0.966, n=1107 rabid dog case histories; Weibull distance kernel, shape 0.698, scale 1263.954, n=6626 rabid dog biting incidents, with 3275 right-censored due to the unknown start location of the biting dog) were used (Mancy *et al.*, 2022).

The tree-building algorithm was refined to generate trees consistent with the phylogeny. This required creating a pairwise patristic distance matrix from the maximum likelihood phylogeny using the ape package (Campbell *et al.*, 2022) in R, from which genetic clusters were assigned using the adegenet package (Campbell *et al.*, 2022; Faye *et al.*, 2015), with a cutoff value of 0.002. A directed graph of the transmission tree and sequentially sampled edges connecting mismatched genetic clusters to rebuild these paths to generate trees consistent with phylogenetic assignments was built. First, sampled by frequency, i.e. how often edges occur in paths with mismatches, then by the scaled probability of the spatiotemporal distance to the assigned progenitor, generally selecting lower probability links to resample. For edges that were broken, sequentially a progenitor was resampled from those that generated trees consistent with the phylogenetic assignments.

To further resolve transmission chains additional pruning steps were applied to filter out case pairs where the time interval or distance exceeded the 99th percentile of the serial interval and distance kernel distributions (without pruning or integration of phylogenetic information, the tree reconstruction results in a single large chain). The tree reconstruction methods are wrapped into an R package, available at github.com/mrajeev08/treerabid and archived on Zenodo (DOI: 10.5281/zenodo.5269062). Pruned trees (split into transmission chains) were compared to transmission trees reconstructed to be consistent with the phylogeny. For each pruning algorithm, the Maximum Clade Credibility (MCC) trees (the tree within the bootstrap that had the highest product of progenitor probabilities) and the majority transmission trees (the tree within the bootstrap that had the highest number of consensus progenitors) were compared across the consensus trees (i.e. the most frequently assigned progenitors for each case).

The effective reproduction number Re, which describes transmission in the presence of control measures, was estimated from the number of secondary cases per case in the transmission trees. Re over time was examined by fitting a LOESS regression with time as our predictor and Re as my response. Individual Re estimates were also examined in relation to vaccination coverage at the time of symptoms in the shehia where each case occurred and compared the distributions of Re from different tree summaries.

Case detection achieved from contact tracing was estimated using recently developed analytical methods (Faye *et al.*, 2015; Mancy *et al.*, 2022). Specifically, the times between statistically or directly-linked cases from the transmission tree reconstructions and the serial interval distribution for rabies were used (Mancy *et al.*, 2022) to fit the simulated distribution of numbers of unobserved intermediates, assuming all infected individuals have the same probability of being detected. To account for the long-tailed distribution of serial intervals, simulated values were sorted for initial intervals to most closely match observed values (i.e. so long incubators are accounted for and not always taken to be cases with multiple generations separating them from their progenitors). This approach with sorting generally performs better than the unsorted approach (Mancy *et al.*, 2022) but tends to underestimate detection probabilities by about 10%, in particular for values between 0.3 - 0.75. The fit across a range of detection probabilities was examined for the endemic period (2010-2014), the subsequent outbreak (2016-2018) and overall, applying the method to 100 bootstrapped trees generated by the pruning strategies (with and without genetic information), and to the majority tree and the MCC tree, taking the mean of 10 estimates as the detection probability for each tree.

3.5.4 Cost-Effectiveness Analyses

Contact tracing data were used to inform a probabilistic decision tree model to estimate the impacts and cost-effectiveness of interventions on Pemba. A baseline scenario without dog vaccination and with patients charged for PEP was compared (as was initially the case on Pemba), with scenarios of free PEP provisioning but without dog vaccination, and with both free PEP and sustained island-wide dog vaccination carried out annually, i.e. a One Health approach, over a ten-year time horizon. From compiled cost data (Table 2) the per campaign cost of island-wide dog vaccination and the per patient cost of PEP for use in the model were estimated. The probability of rabies-exposed bite victims starting and completing PEP (defined as at least 3 doses) from 2010-2015 (when most bite victims paid for PEP) and 2016-2020 (when most bite victims received free PEP), and the frequency of healthy dog bite victims presenting for PEP were estimated. After adjusting for case detection, the time series of rabid dogs on Pemba were estimated, to generate rabies incidence under scenarios with and without dog vaccination. For scenarios with dog vaccination, the first campaign was assumed to take place in year one, translating to reduced incidence from year two onwards, as per the contact tracing data, sampled from 2010-2015 and from 2016-2020 with zero incidence thereafter. Using negative binomial parameters fitted to the offspring distribution of bite victims per rabid dog, adjusted for case detection, the corresponding time series of rabies exposures were simulated. The simulated incidence of healthy bite patients were tuned to match the data under these scenarios. Parameter estimates for probabilities of starting and completing PEP and for rabies progression in the absence of PEP16 were used to estimate deaths and deaths averted. The perspective of the health provider were taken into account and report cost-effectiveness per death averted, with costs discounted at 3%. All monetary values presented are in 2023 US dollars. All analyses were undertaken using the R statistical computing language (Wickham & Bryan, 2023).

Exchange rate: 1 USD: 2296 Tsh (bank of Tanzania, 05/05/2022 https://www.bot.go.tz/). MoLDF = Ministry of Livestock Development and Fisheries, Tanzania; LTRA = Land transport regulatory authority; DoLD = Department of Livestock Development, Pemba. MSD

= Medical Stores Department. LFO = Livestock Field Officer. Costs of vaccine collection from the airport are not included. Each injection requires 5 minutes of health worker time and up to 8 injections per PEP course.

Table 2: Costs of rabies control and prevention activities

Intervention	Cost variables	Unit Cost (USD)	Number	Source
Mass dog vaccination	Dog vaccine	0.65	Per dog	MoLDF
	Consumables (syringes, needles)	0.05	Per dog	MSD price catalogue 2022/23
	Stationary (registers, certificates)	4.36	Per district	Local prices
	Advertising for campaigns	7.45	Per vaccination day	DoLD
	Transport (fuel) for team*	7.45	Per central point	DoLD
	Assistant allowance	2.18	Per vaccination day	DoLD
	LFO allowance	13.07	Per vaccination day	DoLD
Post-exposure vaccination	Consultation & wound care	10.9	Per patient	National health Insurance scheme
	Post-exposure vaccine	10.98	Per vaccine vial	MSD price catalogue 2022/23
	Health worker time	2.11**	Per patient	Tanzania Public service management and good governance

3.5.5 Ethical Clearance

The study was approved by the Zanzibar Medical Research and Ethics Committee (ZAMREC/0001/JULY/018), the Medical Research Coordinating Committee of the National Institute for Medical Research of Tanzania (NIMR/HQ/R.8a/vol.IX/2788), the Ministry of Regional Administration and Local Government (AB.81/288/01), and Ifakara Health Institute Institutional Review Board (IHI/IRB/No:22-2018).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Results

4.1.1 Integrated Bite Case Management

(i) Bite Patient Risk Assessments

Bite patients and assessments of high-risk bites increased with IBCM. Prior to the introduction of IBCM, an average of 57.6 (range: 15-91) new bite patients presented per month in these regions, with only 26.3% indicating a risk of rabies by the health worker who completed the record (Fig. 4). Following the introduction of IBCM, an average of 113.9 (range: 15-202) bite incidents were reported per month, with 62.8% assessed by health workers to be by suspect rabid animals. Overall, bite patient presentations corresponded to an incidence of 22.1 bites per 100 000 persons per annum over the study period from January 2018 until April 2022 (ranging from 0.3 to 226.6 among districts). However, there was a risk of 14.2 rabies exposures per 100 000 persons per year (ranging from 0 to 209.7 among districts) under IBCM (from June 2018 until April 2022), assuming that the health workers' risk assessments provide a more accurate indicator of rabies compared to routine records of bite patients (compared to 4.2 per 100 000 persons per year pre-IBCM from January 2018 until June 2018) prior to the introduction of IBCM (Table 3). Significant differences in the proportions of high-risk patients pre-and-post-IBCM are indicated by * <0.05 and ** <0.001, as detected by a chi-squared test.

Table 3: Patient presentations in study regions before and after the introduction of IBCM

Region	Pre-IBCM		Post-IBCM	
	Patient presentation per 100 000 persons per annum	% high-risk	Patient presentation per 100 000 persons per annum	% high-risk
Lindi	15.3	32.7	16.7	77.1**
Mara	5.2	26.3	29.9	41.2
Morogoro	29.3	28.0	28.6	76.8**
Mtwara	7.4	7.7	4.8	68.8**

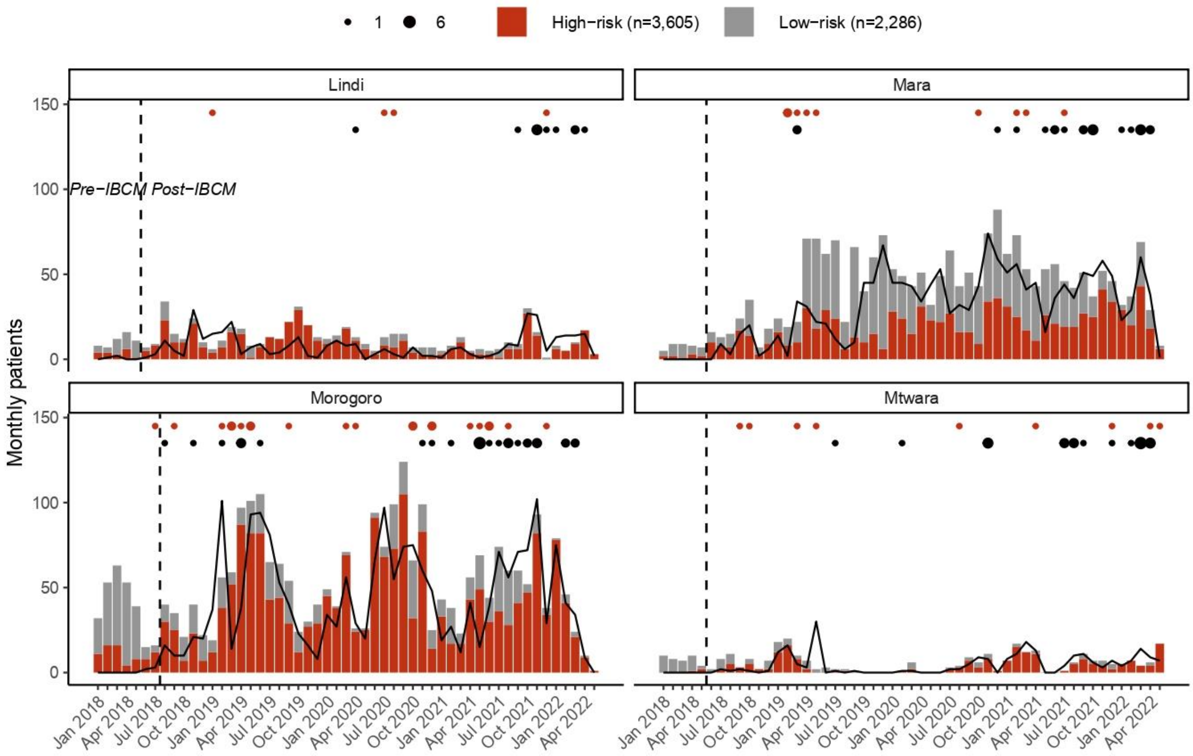

Figure 4: **Regional reporting of bites assessed as high risk (red) vs low risk (grey) and investigations of biting animals (lines) in the study regions**

The dashed line as shown in Fig. 4 indicates the period when IBCM was not implemented in each region (Pre-IBCM from Jan-May 2018); red dots indicate the number of human deaths (n=46) attributable to rabies; black dots indicate the number of positive animal rabies cases (n= 96) confirmed through rapid diagnostic tests scaled by numbers (1 and 6).

Of the bite victims that presented to health facilities following the introduction of IBCM (between July 2018 and April 2022; n=5446), most were due to bites from domestic dogs (96.2%) with only a few being bitten by wild animals (Lindi, n=10; Morogoro, n=1, Mtwara, n=24 and Mara, n=10). Most bite patients were recorded with scratches or minor wounds (83.8%, n=4566), while 15% (n=816) had more severe wounds and 0.4% (n=23) required hospitalisation due to broken bones or infection. One child (aged 2) and an adult person (aged 46) died as a result of bite injuries. Throughout the study regions, PEP was unavailable for 240 bite patients (4.4%) upon presentation to a health facility, during the period of IBCM implementation. Only 189 of these bite patients were referred to other facilities for PEP with 139 assessed as being suspected rabies exposures. Forty human deaths due to rabies were reported within the IBCM study districts between July 2018 and April 2022 and these were confirmed epidemiologically based on dog diagnosis and human clinical presentation (Fig. 4) from: Kilwa (1), Lindi District Council (1), Nachingwea (1) and Ruangwa (1) in Lindi region; Bunda (5), Butiama (1), Rorya (1), Tarime (1) and Serengeti (1) in Mara region; Kilombero (3), Kilosa (2), Morogoro Municipal (6), Morogoro District Council (1), and Ulanga (8) in

Morogoro region; and Mtwara Rural (3), Nanyumbu (1) and Newala (3) in Mtwara region. These deaths were also confirmed through the investigations done by LFOs after the health worker's alert.

(ii) Animal Investigations

Prior to the introduction of IBCM, investigations were not carried out as standard by LFOs but were only carried out on an *ad hoc* basis. However, since IBCM began in the study area, 3251 investigations have been conducted by LFOs. The 2760 investigations were conducted following an alert of a potentially high-risk bite while 491 investigations were carried out following community reports of sick, dead or biting animals (Fig. 3). The number of investigations undertaken following the introduction of IBCM differed between regions, with LFOs investigating an average of 26.2 cases/month in Mara, 8.1/month in Lindi, 36/month in Morogoro and 5.8/month in Mtwara (Fig. 4). An outbreak of rabies that began in February 2019 resulted in a surge of investigations in Morogoro region (Fig. 3). Out of all the investigations, 1172 were carried out in person, and 2079 were completed via a phone consultation. From the 1172 in-person investigations, samples 4.5% (97/215) were collected between August 2018 and March 2022 from animals that were found dead (Fig. 5) and were tested with a rapid diagnostic test.

From the investigations, 57.2% (1860/3251) of biting animals showed at least one clinical sign consistent with rabies and/or were positive following a rapid diagnostic test (n=59/89; six specimens tested negative and eighteen tests were inconclusive), while 12.3% (401/3251) were determined to be healthy and 8.8% (286/3251) to be sick from other causes that were not rabies. The remaining 21.7% (704/3251) were classified as unknown status due to insufficient evidence. The 15.4% (502/3251) of the animals investigated were alive at the time of investigation, allowing for observation of clinical signs; the remaining 84.4% (2743/3251) had either disappeared 69% (2242/3251) or were already dead 15.4% (501/3251) at the time of investigation, with a large proportion killed by community members (n=301) or their owners (n=31). Almost all domestic dogs are owned in rural Tanzania, but also almost all domestic dogs roam freely. Therefore, investigations were difficult to resolve if the owner of the biting dog was not known and the dog disappeared following the bite, but such circumstances were assumed to be high-risk and potentially indicative of rabies.

(iii) Veterinary and Health Data Combined (One Health)

The high-risk bites and animals assessed as suspect for rabies were generally widespread across the study regions (Fig. 6). Both health workers and LFOs reported similar criteria about biting animals that they assessed to be suspect rabid. Health workers considered rabid animals showing unprovoked aggression (including attempting to bite and grip people, animals, or objects without feeding (51.3%), excessive salivation (33.9%), restlessness (2.1%) and/or abnormal vocalization (11.3%). In 18.4% (1017/5519) of bite patients, the health worker did not report any clinical signs for the biting animal, yet still classified 276 of them as suspect rabies, apparently because the animal was unknown or the attack unprovoked. On investigation of high-risk bites, LFOs also reported animals displaying unprovoked aggression (61.6%), abnormal vocalisation (16.6%), restlessness (2.8%) and/or excessive salivation (41.7%). The LFOs did not report any clinical signs in 17.2% (567/3291) of investigations but considered 61 of these animals to be suspect rabid on the basis of other unreported information. The outcomes of rapid diagnostic tests are highlighted in Fig. 5.

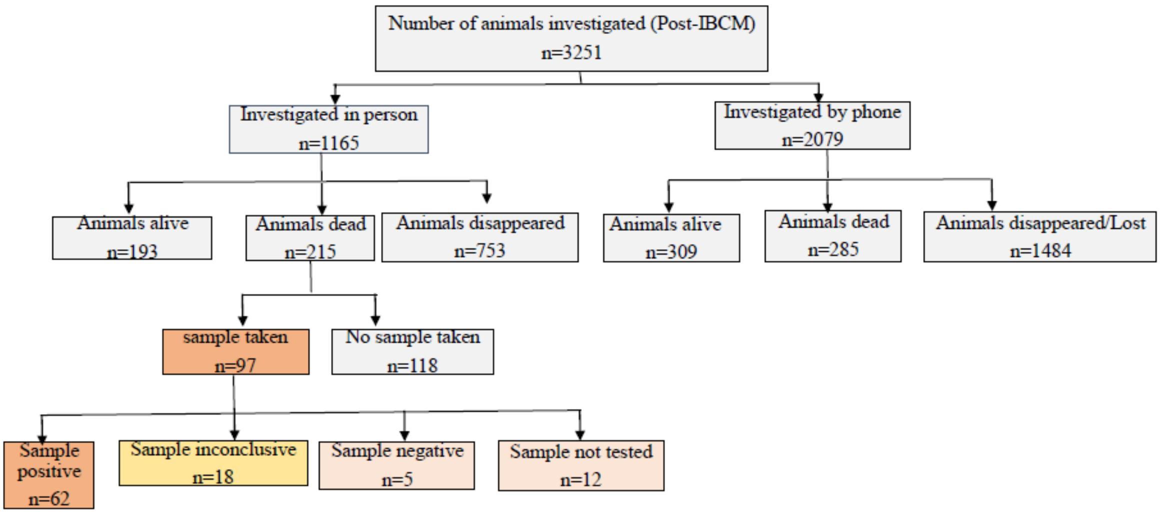

Figure 5: Number of investigations carried out by LFOs between August 2018 and May 2022

(iv) Assessment of Health Worker Knowledge

The proficiency testing indicated that all health workers could identify at least 3 clinical signs in animals consistent with rabies. Excessive salivation (94%), restlessness (86%), unprovoked aggression (86%) and abnormal vocalisation (74%) were the most commonly identified signs, but a few respondents also identified paralysis (34%), and diurnal activity amongst nocturnal wildlife (24%) as well as a lack of fear among wildlife (16%) as clinical signs. However, only 66% of respondents considered a bite from an unknown animal as suspicious for rabies.

During proficiency testing most health workers stated that the wound severity would affect their recommendation for PEP and whether they would inform LFOs to investigate. Health workers reported that they were most likely to classify an exposure as high-risk and recommend PEP when treating severe wounds (wounds requiring hospitalisation 90%, large wounds 96%, minor wounds 86%, and scratches 62%) and were also more likely to request LFOs to investigate severe bites (fatal wounds 78%, wounds requiring hospitalisation 90%, large wounds 88%, minor wounds 70%, and scratches 56%). Only 78% of health workers indicated they would inform an LFO if they received a patient presenting with clinical signs of rabies. Following the refresher training, 74% of health workers were able to correctly recommend PEP to a patient bitten by an unknown suspected dog who had been delayed in seeking treatment. High-risk bites per ward reported through health facilities and probable cases confirmed through LFO investigations are presented in Fig. 6. Protected areas are overlaid in grey.

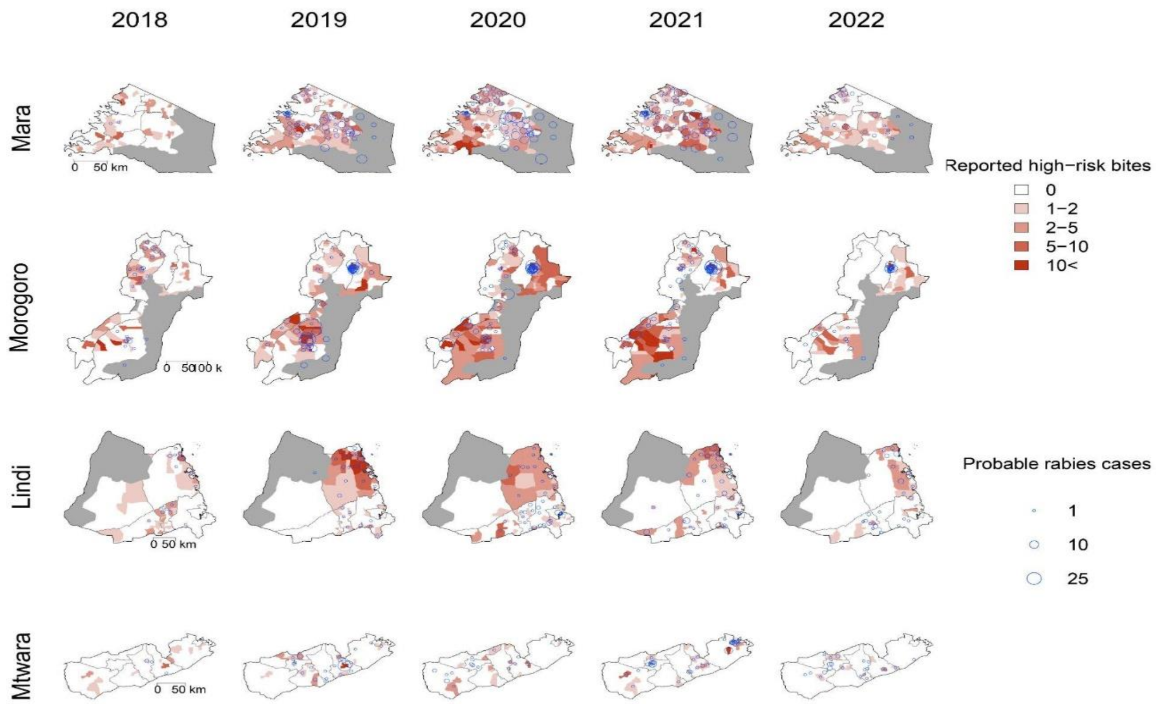

Figure 6: High-risk bites per ward reported through health facilities (red polygons) and probable cases confirmed through LFO investigations (blue circles) scaled by size from 2018 to May 2022

4.1.2 Exploring the Reservoir Dynamics of Rabies Infection in Lindi and Mtwara Regions, Southeast Tanzania

(i) Human Exposures, Deaths and Rabies Cases

Over the nine-year study period (2011-2019), 688 human exposures to probable rabid animals were recorded within the Lindi and Mtwara regions from the contact tracing. Of these exposures, 47 (6.8%) resulted in death due to probable rabies (none were laboratory-confirmed). The number of probable animal rabies cases recorded over the same period was 549, comprising 313 cases (57.0%) in domestic animals and 236 (43.0%) in wildlife (Table 3). Only two of the animal rabies cases were laboratory confirmed. Domestic dogs accounted for the majority of human exposures to probable rabid animals (389/688, 56.5%) but jackals were responsible for a large proportion of the remaining exposures (262/688, 38.1%) (Table 4). The highest incidence of exposures was found in Kilwa district (mean of 6.7/100 000 people/ year), with a cluster of dog bites in 2018/19 (Fig. 7). Mtwara Rural had the highest incidence of wildlife exposures (mean of 4.0/ 100 000/ persons/ year). In addition, four people died from bite injuries from probable rabid hyenas (3) and a probable rabid jackal (1).

Table 4: Probable animal rabies cases, human exposures, and human rabies deaths by infecting species detected from January 2011 to July 2019 in Lindi and Mtwara regions

Group	Species	Probable animal rabies cases (%)	Human rabies exposures by species (%)	Human rabies deaths by infecting species (%)
Domestic animals	Dog	303 (55.2)	389 (56.5)	32 (68.1)
	Cat	10 (1.8)	12 (1.7)	0 (0)
Wildlife	Jackal	221 (40.3)	262 (38.1)	12 (25.5)
	Hyena	8 (1.5)	16 (2.3)	3 (6.4)
	Honey badger	5 (0.9)	6 (0.9)	0 (0)
	Leopard	2 (0.4)	3 (0.4)	0 (0)

The incidence of human exposures by species and district are as well reported in Table 5. Mean incidence of exposures to suspected rabid animals per 100 000 people for each district are presented over the course of the study period.

Table 5: Incidence of human rabies exposures

District	Exposures from all species per 100 000 people	Exposure from domestic animals only per 100 000 people	Exposures from wildlife only per 100 000 people
Kilwa	6.6	6.4	0.2
Lindi Rural	2.2	1.7	0.5
Lindi Urban	1.3	1.3	0
Liwale	3.9	2.7	1.1
Masasi	2.2	1.5	0.7
MasasiTownship Authority	3.4	3.1	0.3
Mtwara Rural	5.4	1.4	4.0
Mtwara Urban	0.9	0.7	0.2
Nachingwea	5.0	2.0	2.9
Nanyumbu	3.6	0.9	2.7
Newala	3.0	1.7	1.3
Ruangwa	4.3	2.4	1.9
Tandahimba	3.7	1.6	2.0

Over time, probable rabies exposures from all species, and probable animal rabies cases in both domestic animals and wildlife decreased across all districts (Fig. 8). Most human rabies deaths and exposures occurred in 2011 (18 deaths, 218 exposures), whilst fewest exposures (15) were recorded in 2017 and fewest deaths in 2016 and 2019 (one each year). Probable animal rabies cases declined from 2011 to 2017, but then began to rise in 2018. In the first two years of the study, dogs accounted for over 1.5 times more human rabies exposures than wildlife. However, from 2013 onwards the number of human exposures from domestic dogs and wildlife became more even, with wildlife accounting for more exposures than dogs in 2013 and 2014 (Fig. 8). Throughout the study period there were districts with wildlife cases detected in the absence of domestic dog cases and vice versa. Probable rabies cases were identified in mainly inhabited areas (Fig. 7).

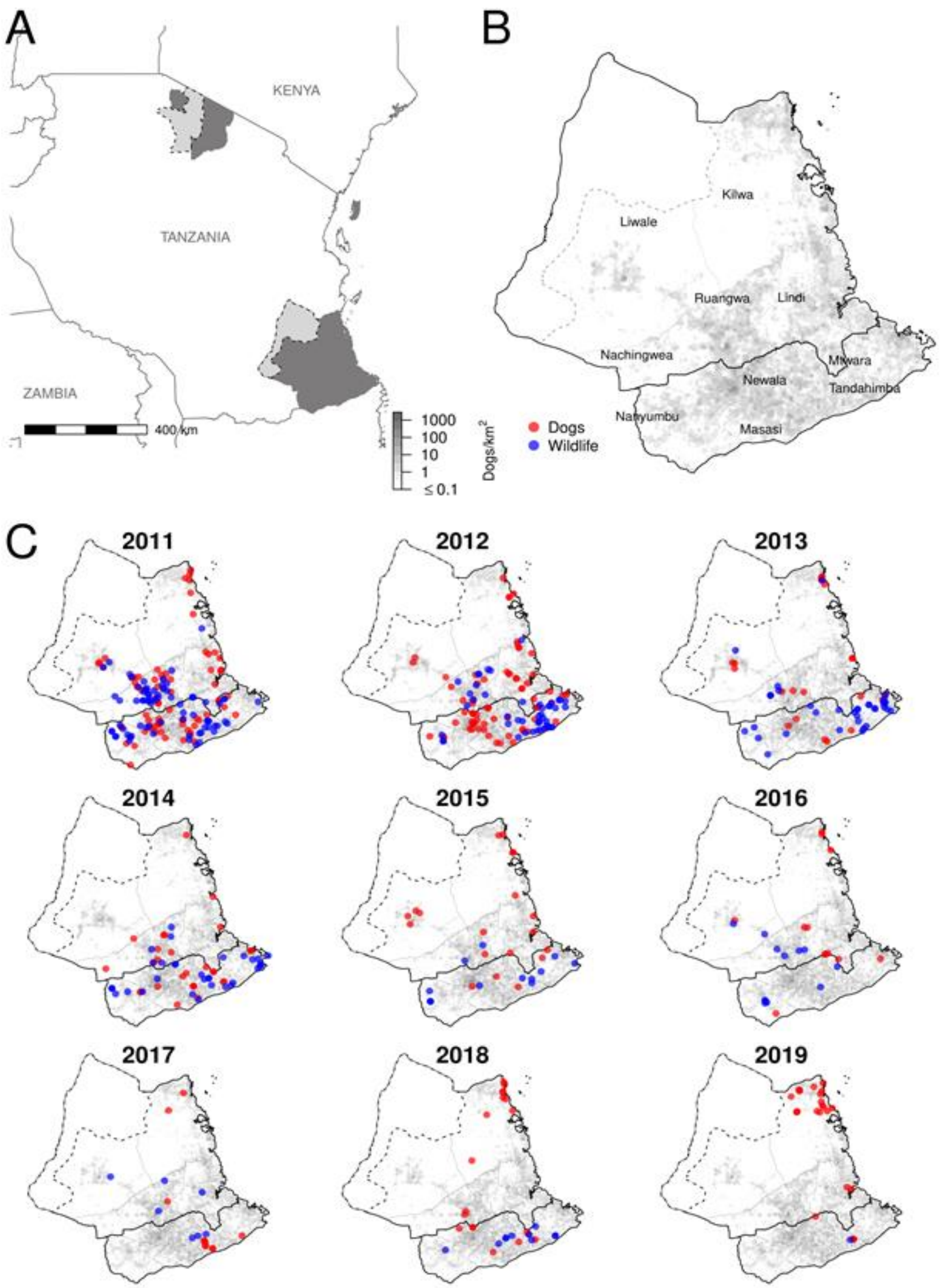

Figure 7: Study districts and locations of probable rabies cases

The study area (dark grey) and protected areas where no human settlements are allowed (light grey): Selous Game Reserve in southeast Tanzania and Serengeti National Park in northern Tanzania (A). Districts in Lindi and Mtwara regions (labelled) with estimated dog density on a 4km^2 raster (grey shading) (B). Urban districts within Masasi, Lindi and Mtwara are not labelled to improve readability. Probable rabies cases in dogs (blue) and wildlife (red) each year in Lindi and Mtwara regions (C).

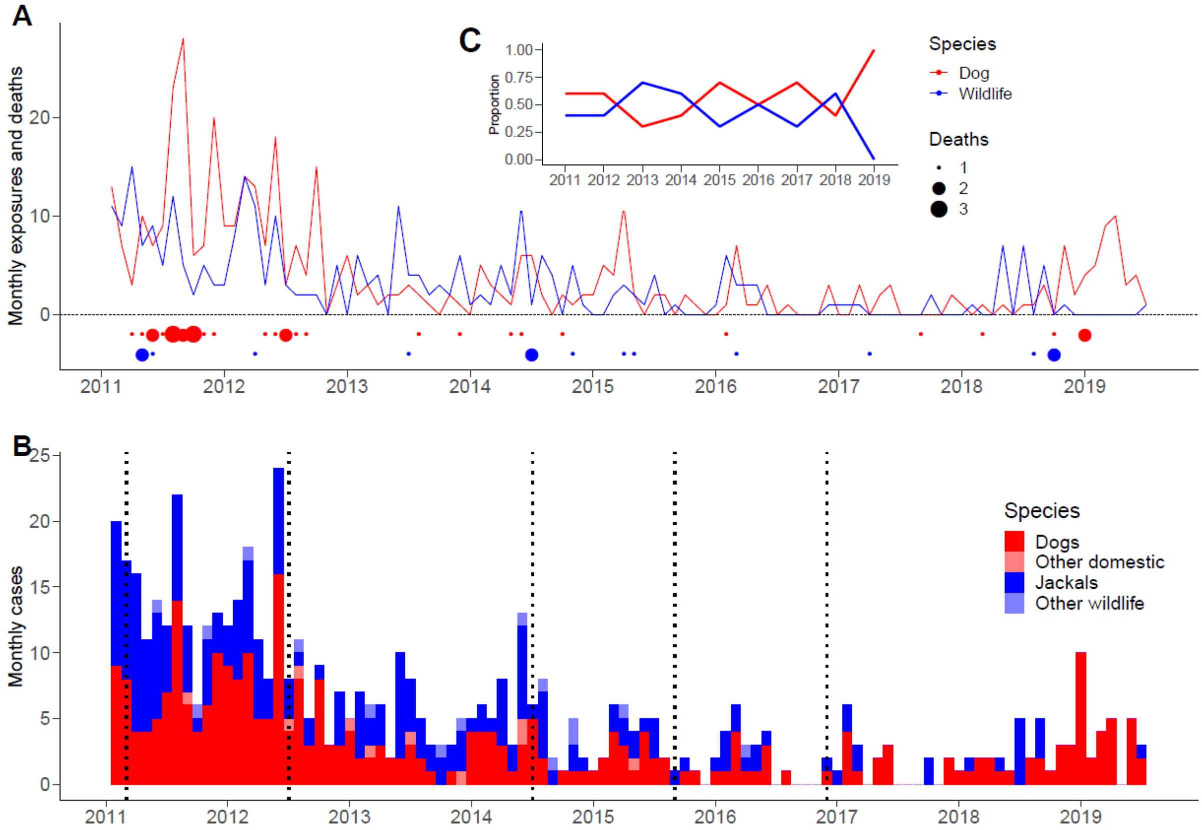

Figure 8: **Probable animal rabies cases, human rabies exposures, and deaths by species from January 2011 to July 2019**

Exposures (lines) and deaths (dots scaled by the number) from domestic dogs (red) and wildlife, mainly jackals (blue) (A). Cases in domestic dogs (red), jackals (blue), domestic cats (pink), and other wildlife (pale blue) (B). Dashed lines indicate vaccination campaigns from 2011 to 2016. The proportion of human exposures by species (dogs in red, wildlife in blue) (C).

(ii) Parameter Estimates

The best-fitting distribution to the serial intervals recorded from Serengeti District, northern Tanzania was a lognormal with mu and sigma parameters of 2.80 and 0.97, respectively, corresponding to a mean interval of 26.3 days with a standard deviation of 25.4 days (Fig. 9A). Using a 50 m upper limit for the interval censoring of recorded zero values, the best-fitting distribution for the distance kernel was a gamma distribution with shape and scale parameters of 0.34 and 2560, respectively, giving a mean distance of 873 metres with a standard deviation of 1495 metres (Fig. 9B). Likelihood ratio tests indicated no significant differences in how well the parameters derived from the Serengeti data fitted to the southeast Tanzania data compared to those derived from the southeast Tanzania data alone (p = 0.171 for the serial interval distributions and p = 0.080 and p = 0.128 for the distance kernel with an upper limit of 50m and 100m for the interval censoring, respectively (Appendix 3 [Fig. S1 – S3]).

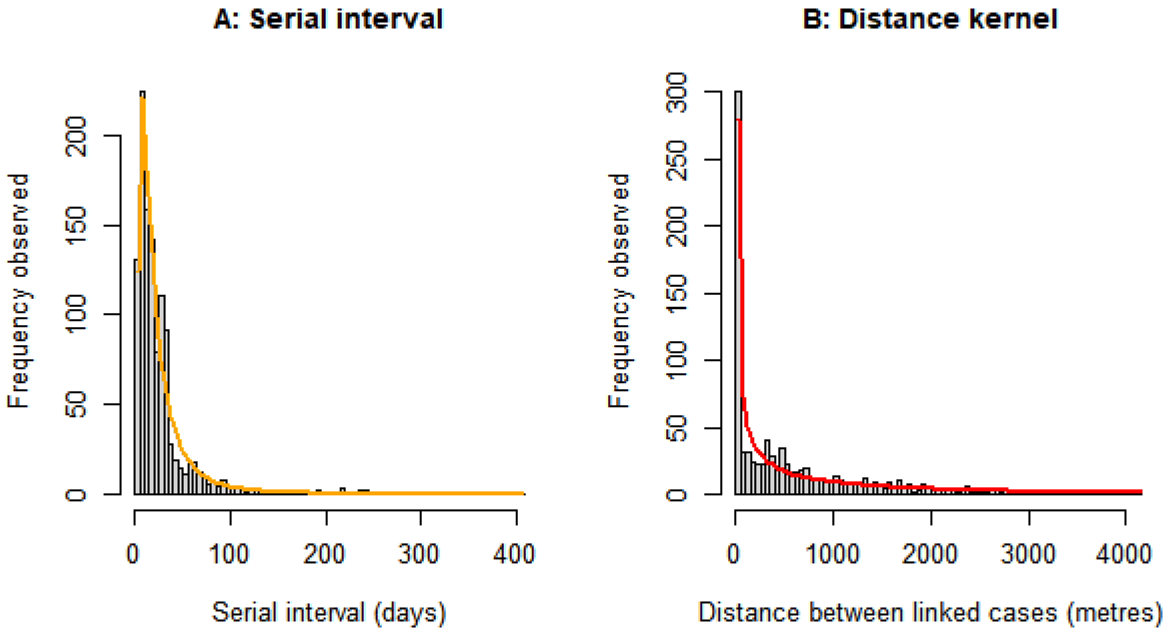

Figure 9: Best fitting distributions for rabies serial intervals and distance kernels

Data on serial intervals and distance kernels from contact tracing of rabid domestic dogs in Serengeti District, northern Tanzania were used for parameter estimation. Data are illustrated by the histograms with the best fitting distributions represented by the overlying lines. Observed serial intervals between dog rabies cases with the best-fitting lognormal distribution (A). Observed distances between domestic dog rabies cases with the best-fitting gamma distribution shown (B). An upper limit of 50 m was used for the interval censoring of recorded zero values. The x-axis has been truncated at 4000 m to allow easier visualisation of the data. The maximum observed distance was 20 713 m.

(iii) Transmission Trees

Of the 549 inferred transmissions, 304 had values within the 99th percentile of the distributions for the serial interval and the convolved distance kernel and were included in subsequent analysis.

(iv) Within- and between-Species Transmission

Dog-to-dog transmission events were inferred to occur most frequently and represented 123/304 transmission events (40.5%, 95% confidence interval (CI) 35.2% - 45.7%). Wildlife-to-wildlife transmission was the next most frequent accounting for 99/304 transmission events

(32.6%, 95% CI 27.6% - 37.8%). Dog-to-wildlife and wildlife-to-dog transmission events were inferred to occur with similar frequency at 10.5% (95% CI 7.2% - 14.1%) and 13.5% (95% CI 10.2% - 17.1%) of transmission events respectively (Table 6).

Fisher's exact test values were highly significant, with p-values of less than 0.001 for all of the 1000 contingency tables of inferred transmission, suggesting that the observed patterns did not occur by chance mixing of species. Similar results were observed for all the scenarios examined as part of sensitivity analyses. Using lower cut-offs (95th percentile) for the serial interval and distance kernel to assign likely direct transmission events resulted in a slight increase in the percentage of dog-to-dog transmissions and a slight decrease in the percentage of dog-to-wildlife and wildlife-to-dog transmissions.

Very little effect was seen on the percentage of wildlife-to-wildlife transmission (Appendix 3 [Table S1]). Subsampling dog cases appeared to reduce the percentage of transmission inferred to occur from dog-to-dog (and correspondingly increased wildlife transmission as a percentage of all transmission), but did not affect interspecific transmission (Appendix 3 [Fig. S4]).

Results from analysis of inferred transmissions with values within the 99th percentile of the serial interval and convolution of two distance kernel distributions (156 days and 9803 m, respectively) as presented in Table 6.

Table 6: Number and percentage of inferred direct transmissions between species

Transmission	Transmissions (% of total)	
	Median	**Bootstrap 95% Confidence Interval**
Dog-Dog	122 (40.1)	119 – 126 (39.1 – 41.4)
Dog-Wildlife	33 (10.9)	29 – 37 (9.5 – 12.2)
Wildlife-Dog	41 (13.5)	38 – 45 (12.5 – 14.8)
Wildlife-Wildlife	99 (32.6)	95 – 103 (31.3 – 33.9)
Cat-Dog	2 (0.7)	2 -3 (0.7 – 1.0)
Dog-Cat	1 (0.3)	1 – 2 (0.3 – 0.7)
Cat-Wildlife	1 (0.3)	1 – 1 (0.3 – 0.3)
Wildlife-Cat	4 (1.3)	3 – 4 (1.0 – 1.3)
Cat-Cat	0 (0.0)	0 (0.0 – 0.0)

(v) Chains of Transmission and Cluster Size

Chains of transmission were constructed from the most likely inferred progenitors and indicated clusters of dog-to-dog and wildlife-to-wildlife transmission (Fig. 10). Clusters composed solely of dog-to-dog transmission were observed more frequently than those of solely wildlife-to-wildlife transmission. The largest clusters involved a mixture of species: the largest cluster of 13 comprised two dogs and 11 jackals, whilst clusters of 12 comprised 11 dogs and one jackal in one chain and two dogs and 10 jackals in the other. The 163 cases could not be linked to other cases (>99th percentile of the serial interval or convolved distance kernel distribution): The 95 cases in dogs (58.3%), 65 in wildlife (40.5%) and 3 in cats. Chains of transmission occurred more frequently and were longer during the first half of the study (Fig. 10C). Whilst almost all districts had wildlife cases, some appeared to have very little sustained wildlife transmission (Kilwa, Liwale, Lindi, Masasi) whereas others had much greater wildlife involvement (Mtwara, Tandahimba). A weighted linear spline regression with a single knot at the July-December 2017 six-month period demonstrated a statistically significant decrease in mean cluster size over the first six-and-a-half years of the study ($p = 0.001$, decrease in mean cluster size of 0.12 per six-month period, 95% CI: 0.06 - 0.17), followed by a statistically significant increase ($p = 0.028$, increase in mean cluster size of 0.52 per six-month period, 95% CI: 0.10 - 0.93, Fig. 11). Sensitivity analyses using three-month and one-year periods were consistent, with statistically significant decreases in cluster size over the first six-and-a-half and seven years of the study respectively. After the initial period, cluster size increases significantly using three-month periods but this increase is not significant using one-year periods likely due to the omission of the incomplete 2019 data.

Of the 32 cases where the progenitor was known, the correct biting animal was not always assigned with the highest bootstrap probability. In 16/32 cases the biting animal was correctly identified with less than 5% bootstrap probability. All of these cases involved dogs that were part of clusters within households. The algorithm identified a different dog but always one within the same household and cluster meaning the assigned species-to-species transmission was correct.

(vi) Regression Analysis of Monthly Incidence

Negative binomial regression models with a linear spline at August 2017 supported a statistically significant downward trend in monthly probable rabies cases between January 2011 and August 2017 ($p < 0.001$, 3.1% reduction per month in all species (95% CI: 2.6% - 3.6%) and in domestic animals only ($p < 0.001$, 3.1% reduction per month (95% CI: 2.4% -

3.7%) when fitted to cases from all species or from domestic animals only. The change in slopes from August 2017 was statistically significant in both models ($p < 0.001$, 5.5% increase per month in all species (95% CI: 2.9% - 8.1%); 8.2% increase in domestic animals only (95% CI: 5.1% - 11.3%)). For probable cases in wildlife, the slope did not change significantly ($p = 0.63$), therefore a single trend was maintained (3.0% reduction per month in wildlife (95% CI: 2.4% - 3.6%)). Plots of the fitted models are shown in the supplementary information (Appendix 3 [Fig. S5]).

When assessing correlations between monthly domestic dog and jackal cases with lags ranging from 0 to 11 months, all scenarios had significant positive correlation coefficients. The largest coefficients occurred with no lag applied between monthly cases and when jackal cases were leading with a four-month lag applied to dog cases (coefficient 0.525, $p < 0.001$ for both lags). Full results are presented in Appendix (Table S2).

(vii) Logistic Regression of Cases in Relation to Population Composition

Logistic regression suggested a statistically significant positive relationship ($p < 0.001$, Fig. 12) between jackals as a proportion of the susceptible population and the proportion of probable cases that were in jackals (when jackals were distributed across areas with >2.5 and <500 humans / km^2). Results obtained using minimum and maximum district-level vaccination coverages, different cut-offs for human densities used for estimating jackal populations and different jackal density estimates were all statistically significant (Appendix 3 [Fig. S6] and Appendix 4).

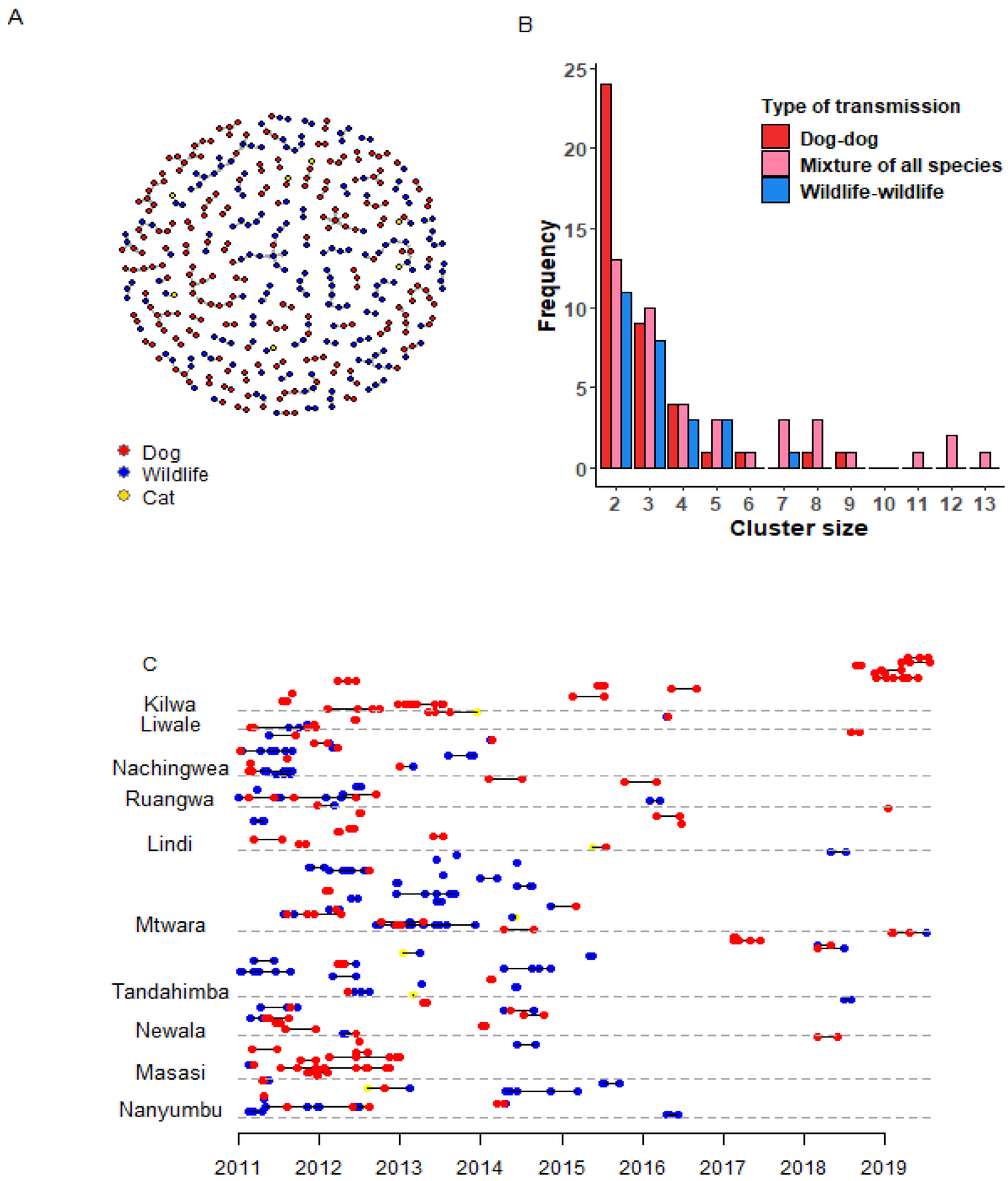

Figure 10: Inferred transmission chains and corresponding clusters according to species involved

Inferred transmission events within the 99th percentile of the serial interval and convolution of two distance kernel distributions (156 days and 9803 metres), using the single most likely progenitor for each case. Inferred transmission chains showing domestic dogs (red); wildlife (blue) and cats (yellow) (A); Frequency and composition of clusters by size (B) and inferred transmission chains by date of cases and district (C) (coloured as for (A)).

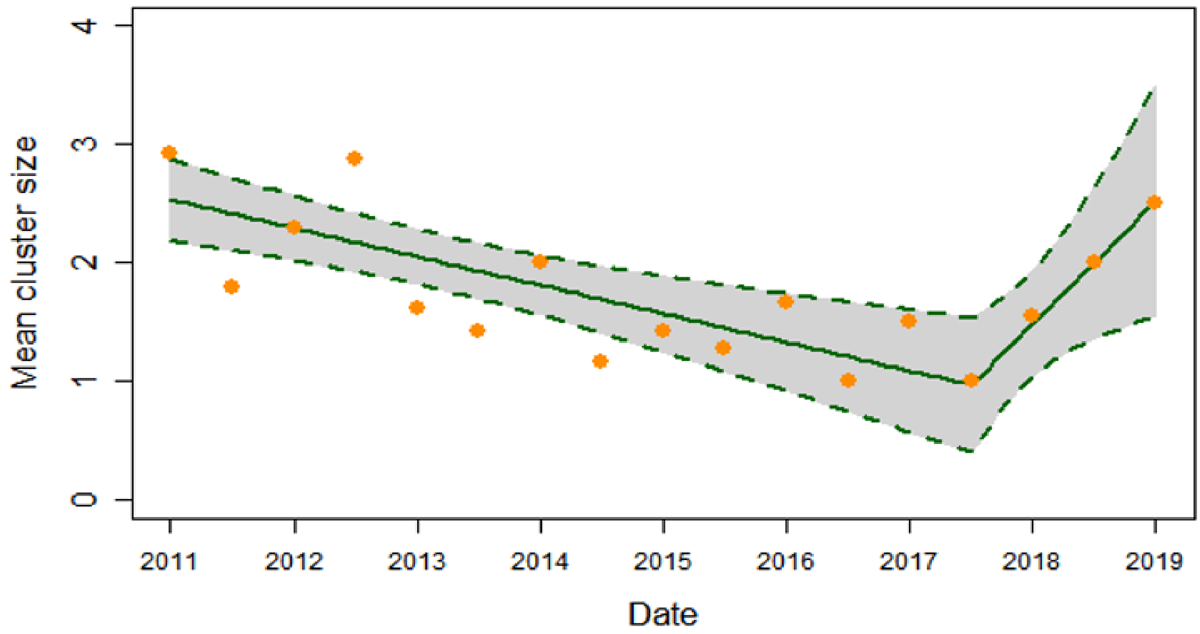

Figure 11: **Trend in mean cluster size per six-month period from January 2011 until July 2019**

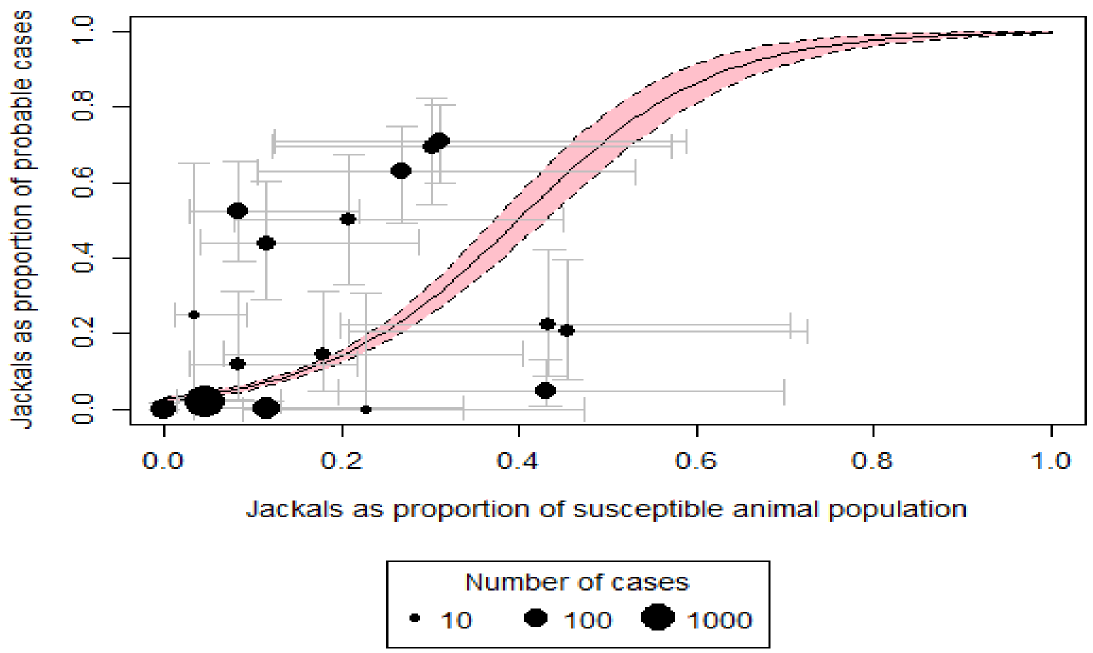

Figure 12: **Relationship between the proportion of jackals in the susceptible animal population and the proportion of probable rabies cases in jackals**

4.1.3 How Can Genomic Surveillance Inform Rabies Elimination Programmes? A case Study of Rabies Elimination from Pemba Island

(i) Incidence Data

When rabies control programmes began to implemented in 2010, rabies was endemic on Pemba Island. 32 probable human rabies exposures, 33 probable rabid dogs and three human rabies deaths diagnosed from clinical signs and history of exposure (6.6 exposures and 0.63

deaths/ 100 000 persons and 10.5 cases/ 1000 dogs) that were all reported to have occurred in 2010 were traced. Initial dog vaccination campaigns were patchy and achieved only low and heterogeneous coverage (13% in 2011, ranging from 7-20% across districts), but by 2014 campaigns were island-wide and achieved higher coverage (mean 50%, range: 46-60%, Fig. 13). Correspondingly, human rabies exposures and dog rabies cases declined each year to just 2 each in 2014 and no exposures, deaths or animal cases were detected from May 2014 until July 2016. Phylogenetic analyses indicated considerable viral diversity during this period. Five distinct chains of transmission between 2010 and 2014 (Fig. 15) were resolved and detected 50% of circulating dog rabies cases through contact tracing. Figure 17 shows how the effective reproduction number, R_e, declined from ~1.5 in 2010 to <1 in 2014 and more broadly with increasing vaccination coverage in the dog population.

Starting from August 2016, bite patients began to surge. By the end of the year, 35 human rabies exposures and 27 probable dog rabies cases had been traced. In response, the Ministry of Livestock and Fisheries Development at the time, initiated dog vaccination in *shehias* with recorded dog cases. However, because the outbreak spread very rapidly, island-wide dog vaccination was reinstated. Viruses sequenced from the outbreak belonged to two lineages (Fig. 16). The time-stamped phylogeny and discrete phylogeographic analysis pointed to two independent introductions in 2016 that took hold and spread widely (Fig. 16), i.e. not continued transmission of the previous lineages. In 2017, I traced 3 further probable human rabies deaths, 126 rabies exposures and 62 rabid dogs (26.6 exposures/100 000 people and 19.6 cases/1000 dogs). High dog vaccination coverage was achieved consecutively over four annual campaigns (median 61%, range: 46-78% in 2019, Fig. 14), and incidence rapidly declined after the 2017 peak. In 2018, 19 human rabies exposures and 8 dog rabies cases were detected, and no any other exposures or rabid dogs since (as of March 2022) have been identified or reported. The R_e was high (>1.5) at the outbreak start but declined to <1 with all transmission interrupted by October 2018 (Fig. 17).

(ii) Post-Exposure Prophylaxis Seeking Behaviour among Bite Victims

A large proportion of bite patients presenting to the island's four hospitals from 2010-2014 (n=117) were bitten by probable rabid dogs (45-72% depending upon the status of unclassified biting dogs), and only few patients who were bitten by apparently healthy dogs sought care (6.6-12.8 per year, or 1.4-2.7/100 0000/year). Based on the probability of rabies progression following exposure (17% in the absence of PEP) (Changalucha *et al.*, 2018) and the occurrence of three human rabies deaths, 10-31 rabid bite victims did not receive complete or timely PEP during this period were estimated. Correspondingly, 10-21 such rabid bite victims were traced, who did not receive or received only inadequate PEP (late and/or incomplete). The total number of rabies exposures detected (63-94) were within expectations from triangulating case detection and rabid dog behaviour (Hampson *et al.*, 2016) (70 exposures, range 37-93), and consistent with a 0.66-0.89 probability of receiving PEP, if exposed (Table 7).

Table 7: Individual deaths, ages, and reason for not getting Post Exposure Prophylaxis (PEP) for the victims who died in 2010 and 2017

Year	Victim's age	Body part injury	Type of wound	Reasons for not seeking PEP
2010	13	Both hands and on the left palm	Extremely severe wounds with broken bones	After the first hospital visit, the mother of the victim was not advised by health workers to take the victim back for the subsequent PEP dosages; and the family members were not aware if more than 1 dosages of PEP were required until when the victim developed rabies
2010	65	Lower left leg and upper thigh	The wounds were very deep with several tooth penetrations	The victim sought care at a health facility (dispensary) that did not provide PEP where he ended up receiving only first aid without being referred by the health workers to the main hospital that provided PEP
2010	75	Head (nose) and on the right arm	The nose was severely affected (broken) and bite wound on the arm was large with deep tooth penetration	The victim ignored seeking the second and subsequent doses of PEP after the wounds healing
2017	9	On the neck	Large wound	There were PEP shortages in the main hospitals
2017	11	Face/head and shoulders	Severe wounds that led to hospitalisation	PEP shortages at the hospital where the victim was admitted but also, health workers did not advise immediate PEP to be sought from elsewhere
2017	70	Shoulders, legs and chest	Large wounds with deep tooth penetrations	The victim thought only a single dose of PEP was enough to provide enough protection against developing rabies and ignored seeking the subsequent doses

During the 2016-2018 outbreak higher case detection was estimated, approaching 70% of dog rabies cases (97 of an estimated 138 rabid dogs identified during the outbreak). Probable exposures per rabid dog were also higher than in 2010-2014 (1.3 vs 0.34-0.51, both adjusted for case detection) driven in part by the variable dog biting behaviour; two rabid dogs in 2017 each bit more than 10 people. Similarly 10-31 rabid bite victims failing to obtain PEP based on the 3 human deaths were projected and traced 39 rabid bite victims who did not obtain adequate PEP (late and/or incomplete). During the outbreak, exposures with probability 0.72-0.78 were estimated to receive appropriate PEP.

Reasons reported for lack of, or inadequate, PEP varied. No shortages were reported during the period of free provision (2010-2014), but in 2016 when the outbreak began, patients had to buy PEP (~30 000 TSh/vial equivalent to $12.9) as free provision ended after vaccines purchased by the Gates Foundation project were used up. One child bitten in early 2017 by a confirmed rabid dog did not receive PEP due to a shortage. After the child showed symptoms of rabies (at which point PEP is ineffectual), the health authorities sought PEP from Zanzibar, where there was also none available and so the family took the child to the mainland in further search of help. Following the child's death, Zanzibar's Ministry of Health imported PEP and reinstated free-of-charge PEP provisioning (one patient reported the cost as a barrier at the start of the outbreak). This policy change as well as sensitization around the outbreak likely contributed to increased health seeking, and understanding of the critical need for early administration of PEP, potentially even amongst those who might not otherwise have sought` PEP despite rabid animal contact. In fact, 17.5% of exposures reported not being aware of the importance of PEP early on (2010-2014) compared to <4% during the outbreak. During the outbreak, only 3% of PEP was not advised by health workers, compared to 20% before the outbreak. Patients presenting for healthy dog bites also increased during the outbreak to ~37/year (7.8/100 0000).

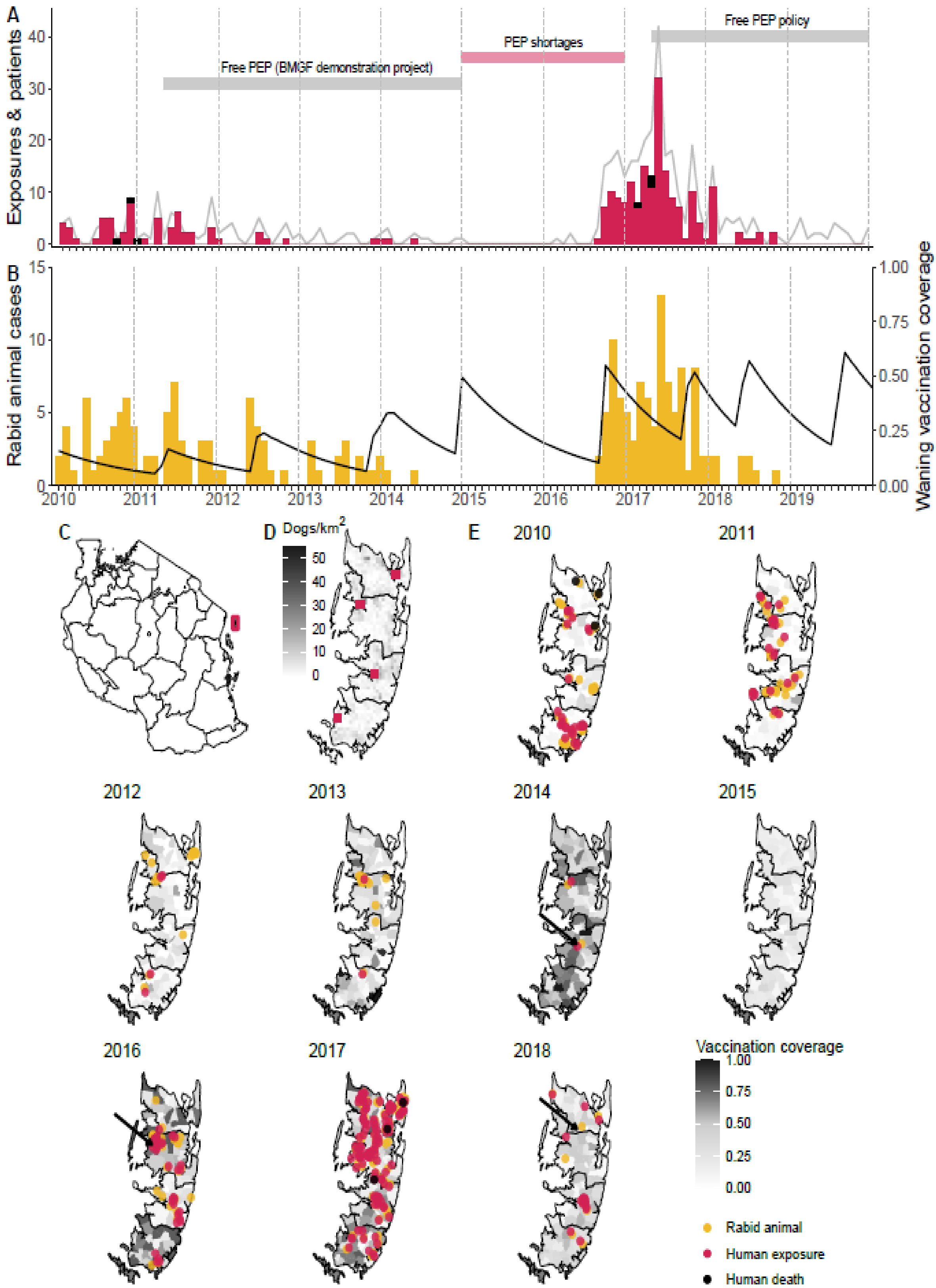

Figure 13: Timeline of rabies on Pemba island in relation to control and prevention measures; A)

Monthly time series of traced human rabies exposures (red) and deaths (black), and patients presenting to clinics from bites by both healthy and rabid dogs (grey line). Periods when PEP was provided free of charge are indicated by the grey horizontal bars, as well as periods of shortages (red horizontal bar, **B)** Dog rabies cases (orange) in relation to average dog vaccination coverage across the island (black line, **C)** Location of Pemba (red) off the coast of mainland Tanzania, **D)** Density of Pemba's dog population and location of the four government hospitals that provide PEP (red squares), one in each district, **E)** Dog rabies cases (orange circles) and human rabies exposures (red circles) and deaths (black circles) each year. Shading indicates dog vaccination coverage in December of each year, projected from the timing of *shehia*-level campaigns, dog turnover and a mean vaccine-induced immunity duration of three years. The arrows point to the last detected animal case in 2014, first detection in the 2016 outbreak and the final case found in 2018.

(iii) Case Detection by Species

Domestic dogs constituted 93.5% (n=188) of all probable cases, while 5% (n=10) and 1.5% (n=3) of the cases were in cows and goats, respectively. Of the confirmed positive rabies cases 18.7% (n=35) were from domestic dogs and 23.1% (n=3) were from livestock i.e. cows and goats. Overall, an average of 1.3 human rabies exposures per rabid dog was observed, but the ratio varied considerably over time from 0.13 to 2.38.

(iv) Comparison of Contact Tracing Versus Routine Passive Surveillance Programmes

Using contact tracing, 22.2% more bite victims (n=54) for whom there were no records at the health facilities were identified. Similarly, 76.6% (n=154) of the animal rabies cases were identified through contact tracing (based on the clinical signs) with the remaining 23.4% (n=47) identified through routine passive surveillance (laboratory confirmation).

(v) Impacts of Mass Dog Vaccination on Rabies Cases

An observed correlation between monthly rabies cases per village and vaccination coverage was initially not significant (p =0.619) but became significant when the effects of time and village were included as random effects (p = 0.001). Accounting for the large number of months with no cases across all villages using a zero-inflation model, a significant relationship (p = 0.0142) between vaccination coverage and cases was evident. Whether low, medium or high levels of vaccination coverage affected the incidence of rabies was explored and significant variations were detected (Fig. 15, P=0.0001).

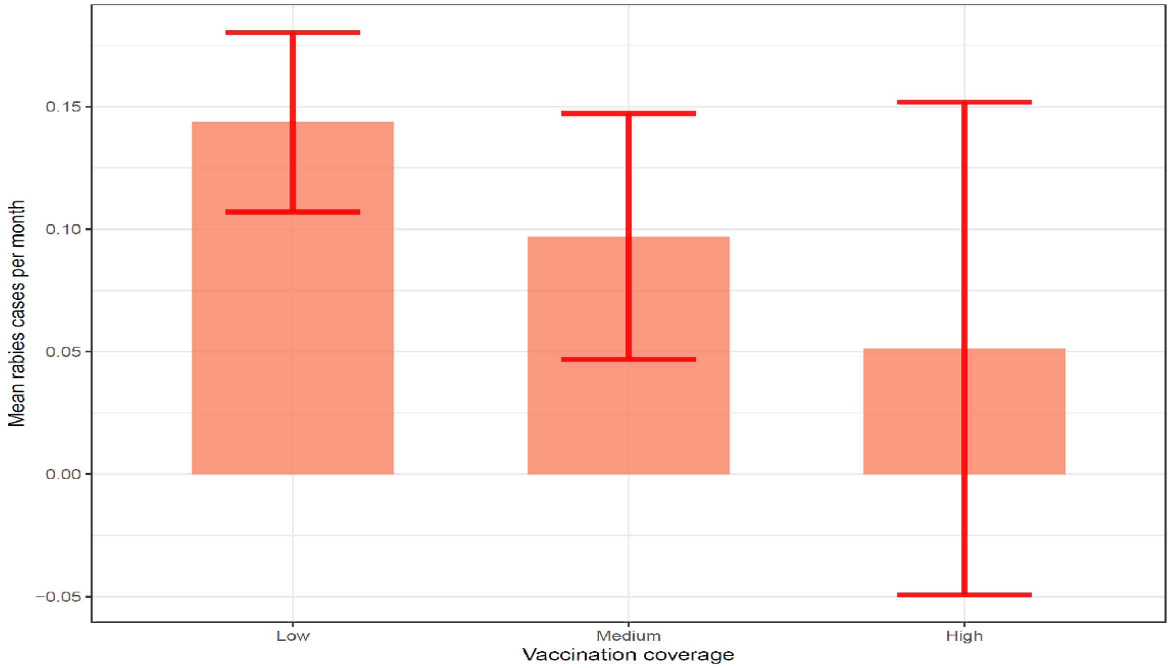

Figure 14: Rabies cases occurring every month per village and vaccination coverage in the dog population

Vaccination coverage was categorized as either low if the attained coverage fell between 0 and 20%, medium between 20% and 60%, while 60% was the high coverage. As vaccination coverage increases, the number of rabies cases occurring every month decreases. Ninety five percent confidence intervals are shown for rabies cases per month per village.

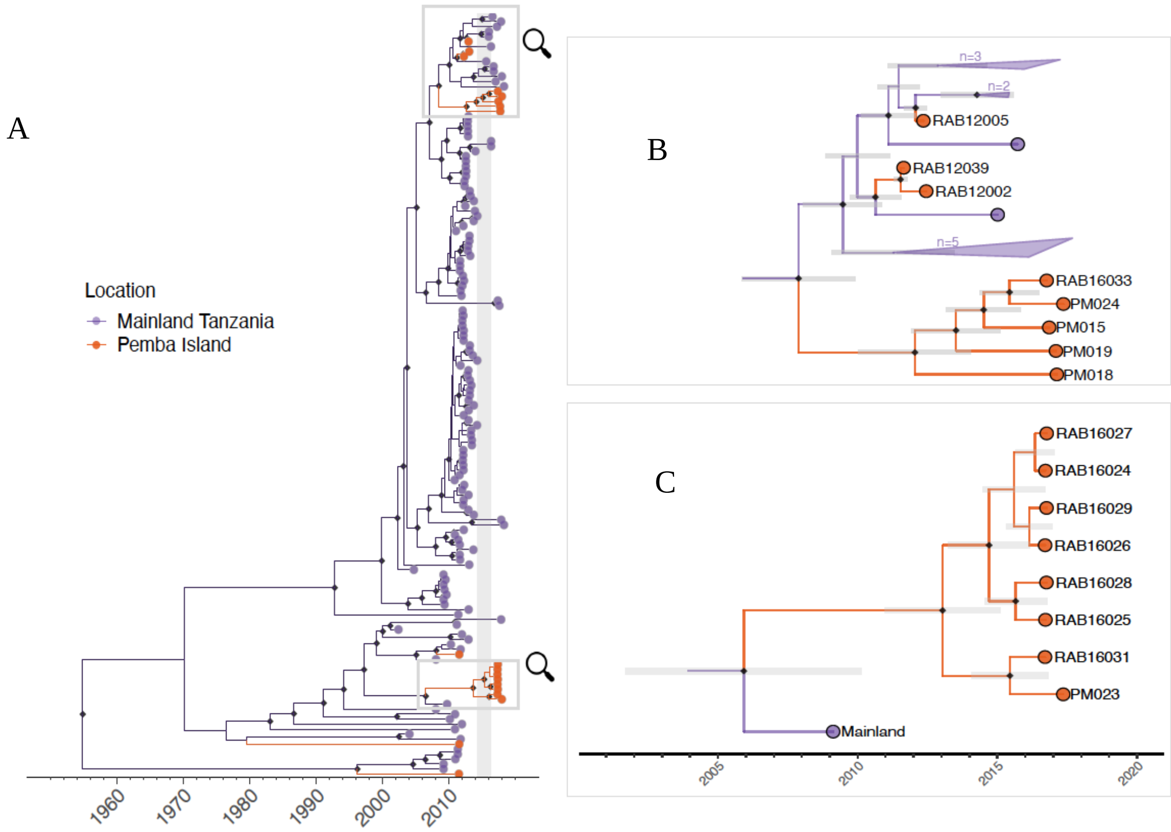

Figure 15: Phylogenetic analysis to identify independent virus introductions to Pemba

Temporal tree of 153 whole-genome sequences from Tanzania, including 22 from Pemba island (A). Grey vertical bar highlights the window of emergence for the most recent common ancestors of the two introductions that led to the 2016 outbreak (2014.33-2016.29). The expanded subtrees [(B) and (C)] show the Pemba cases one node back from the MRCA of the 2016 introductions, with branches coloured according to the inferred ancestral location and posterior support for key nodes indicated. Mainland clusters of more than one identical sequence are collapsed. Grey bars represent HPD of node heights, i.e. estimated age of ancestral nodes. Names of sequences are shown so they can be related to metadata tables (Supplementary materials). Uncertainty in ancestral node estimates, especially in the second lineage, is due to under sampling, yet still shows the distinction of the 2016 outbreak from previous Pemba samples. Viruses from the 2016 outbreak appear in the same larger cluster as those sampled in 2012, so are somewhat genetically related but their common ancestor is prior to 2010 (B).

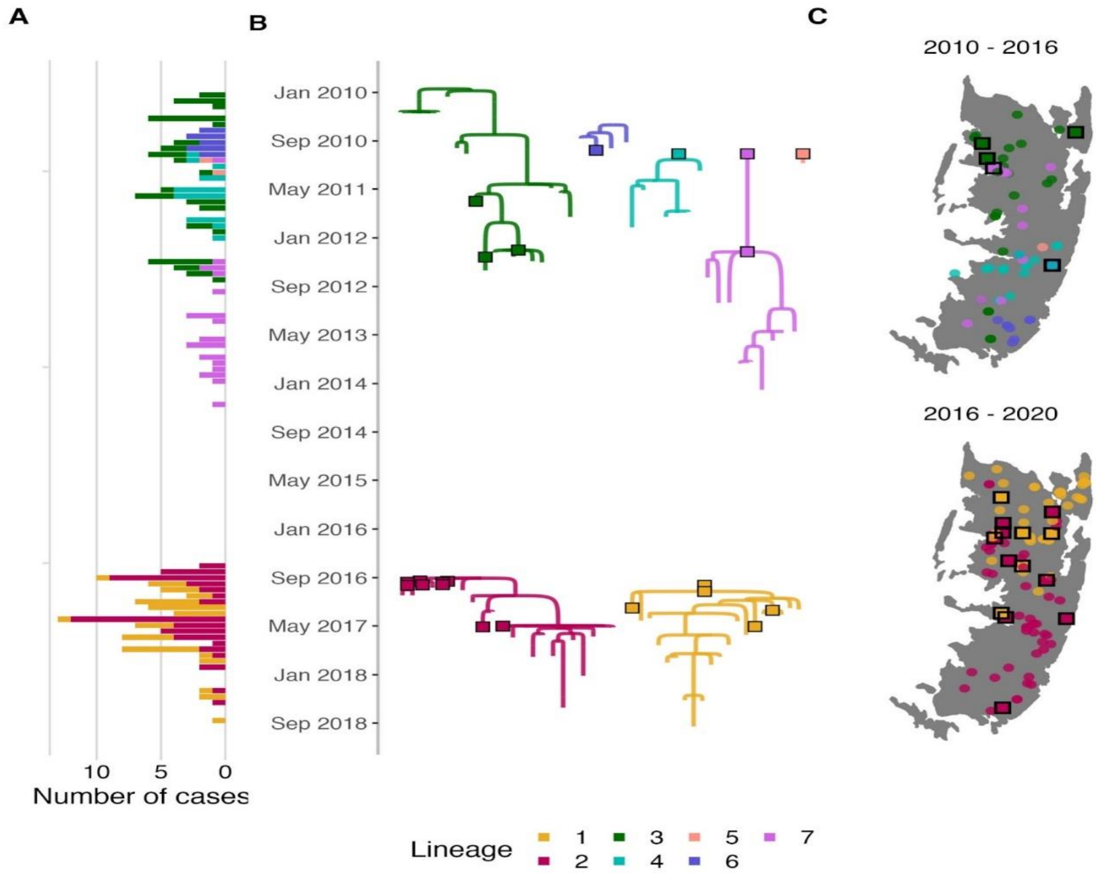

Figure 16: Rabies virus transmission chains inferred from epidemiological and phylogenetic data

Time series of cases coloured by their transmission chain (A). Consensus transmission tree (the highest probability transmission links that generate a tree consistent with the phylogeny) with chains pruned by lineage assignments such that all unsampled cases are assigned to a sample lineage (B). Spatial distribution of these cases over the two periods. In (B), sequenced viruses from sampled cases are indicated by squares with a black outline, while only the tips are shown for unsampled cases(C). In (C), unsampled cases are shown by a filled circle. In all panels, the data are coloured by the lineage they belong to.

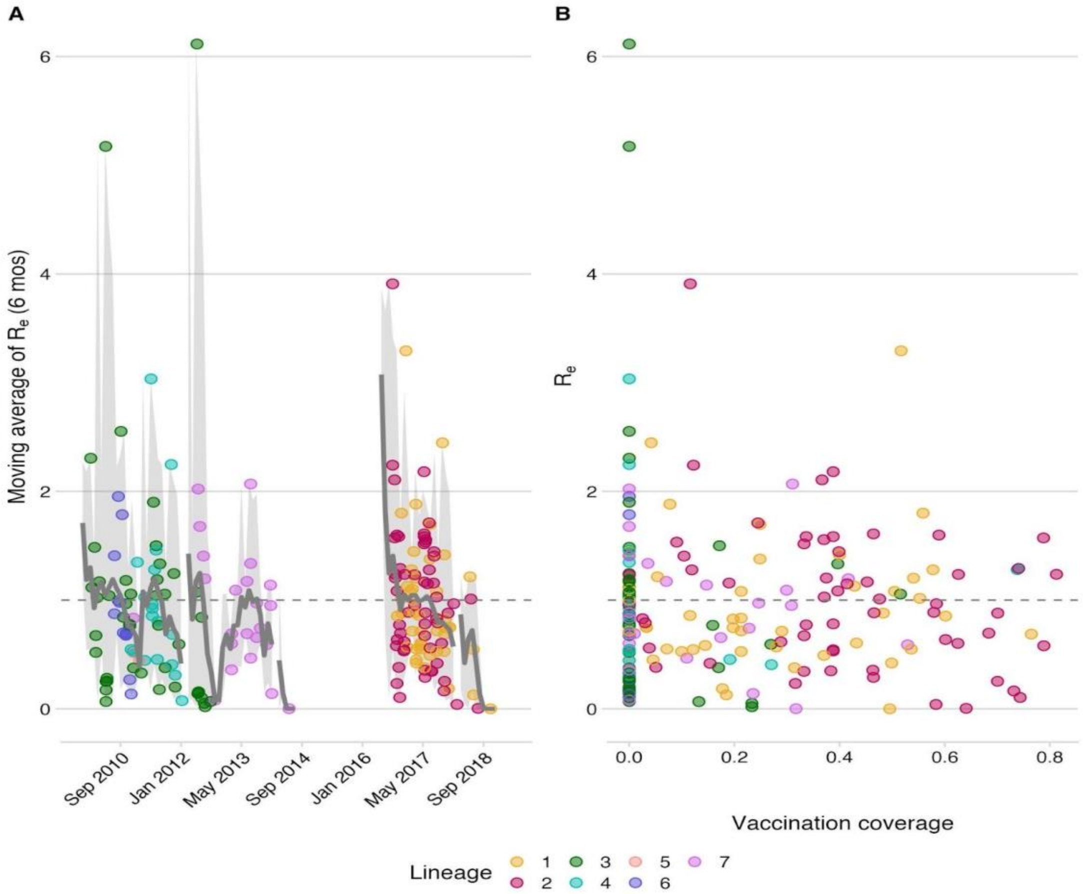

Figure 17: The effective reproductive number on Pemba Island over time and in relation to dog vaccination coverage

The mean R, for each case, across the bootstrapped set of transmission trees generated by pruning to be consistent to the phylogeny, and coloured by viral lineage, over time (A) and versus vaccination coverage (B). The points are the mean values for each case coloured by their consensus lineage assignment. In (A), the grey line is the 6-month moving average of Re (averaging over all cases in the two months prior, the current month, and the 3 subsequent months), with the envelope showing the 95% quantiles. In (B), the mean Re is plotted against vaccination coverage at the time of symptoms in the Ward where the case occurred. The grey dashed line in both panels indicates a Re of 1.

(vi) Costs Effectiveness of a One Health Approach to rabies elimination on Pemba Island

In the decision tree, estimates of the probabilities of rabies-exposed bite victims starting and completing PEP for the period 2010-2014 when most patients paid for PEP, and 2016-2020 when most patients received free PEP were used. Probabilities for starting and completing PEP were 0.667 and 0.397, respectively, for 2010-2014 and, 0.783 and 0.84 for 2016-2020, respectively (Fig. 18).

The cost of a complete intramuscular post-exposure vaccination course (4-dose Essen regimen) was approximately $56 versus $25 for an intradermal course (updated Thai Red Cross). Over the 11 years of the study around $17 800 was spent on PEP for 542 bite patients who received a combination of intramuscular and intradermal regimens and had varying levels of compliance. PEP was estimated to prevent around 42 rabies deaths (95% confidence intervals: 32-55) costing around $424 per death averted. From 2019 onwards, in the aftermath of the 2016-2018 outbreak when all transmission had been interrupted, approximately $876 was spent annually on PEP for patients presenting with bites from healthy dogs (Fig. 13), i.e. precautionary expenditure post-elimination. Islandwide dog vaccination cost approximately $12 122 per campaign ($13 145 for the campaign that reached most dogs), with a cost of $6.5 per dog vaccinated (range: $4.2-10.8 depending on the campaign). Dog vaccination campaigns interrupted transmission in the dog population within four years of implementation, first in 2014 and again in 2018. However, the lapse in dog vaccination from 2014 allowed the two introductions in 2016 to spread widely.

A probabilistic decision tree model was parameterized and projected rabies incidence, exposures and deaths under counterfactual scenarios (Fig. 18). Without dog vaccination and with PEP charged to patients (i.e. the status quo prior to the rabies elimination demonstration project) estimated that around 27 deaths (95% prediction intervals (95% PIs): 16-39) would occur on Pemba over a 10-year time horizon. On average 48 deaths (95% PIs: 31-67) would be prevented by PEP, at a cost of $300 per death averted (95% PIs: $263-374, with costs discounted at 3%) incremental to a counterfactual without PEP, i.e. in the absence of interventions, 75 human rabies deaths would be expected to occur over ten years on Pemba. Providing PEP for free to patients (as during the rabies elimination demonstration project and by Pemba's government from 2017 onwards) was projected to prevent an additional 10 deaths at a cost of $256 per death averted (95% PIs: $217-333), but still result in 17 rabies deaths (95% PIs: 9-26) over the ten years, with intradermal regimens always more cost-effective than intramuscular regimens. Introducing and sustaining mass dog vaccination, whilst charging for PEP, was projected to prevent 20 deaths relative to the status quo (68 deaths averted overall, 95% PIs: 45-92) costing $1684 per death averted (95% PIs: $1264-2515). Dog vaccination together with free PEP was projected to result in fewest deaths (4 overall, 95% PIs: 1-9), with no deaths after year four (Fig. 7), and preventing 71 deaths overall (95% PIs: 46-97) at a cost of $1657 per death averted (95% PIs: $1228-2526). Since dog vaccination interrupts transmission, routine dog vaccination would mitigate ongoing risks from introductions and keep Pemba rabies-free, and thus prevent over 300 rabies exposures over the ten years (95% PIs: 263-401) sparing around 30 families each year from rabid dog bites and the anxiety of needing to urgently obtain life-saving PEP.

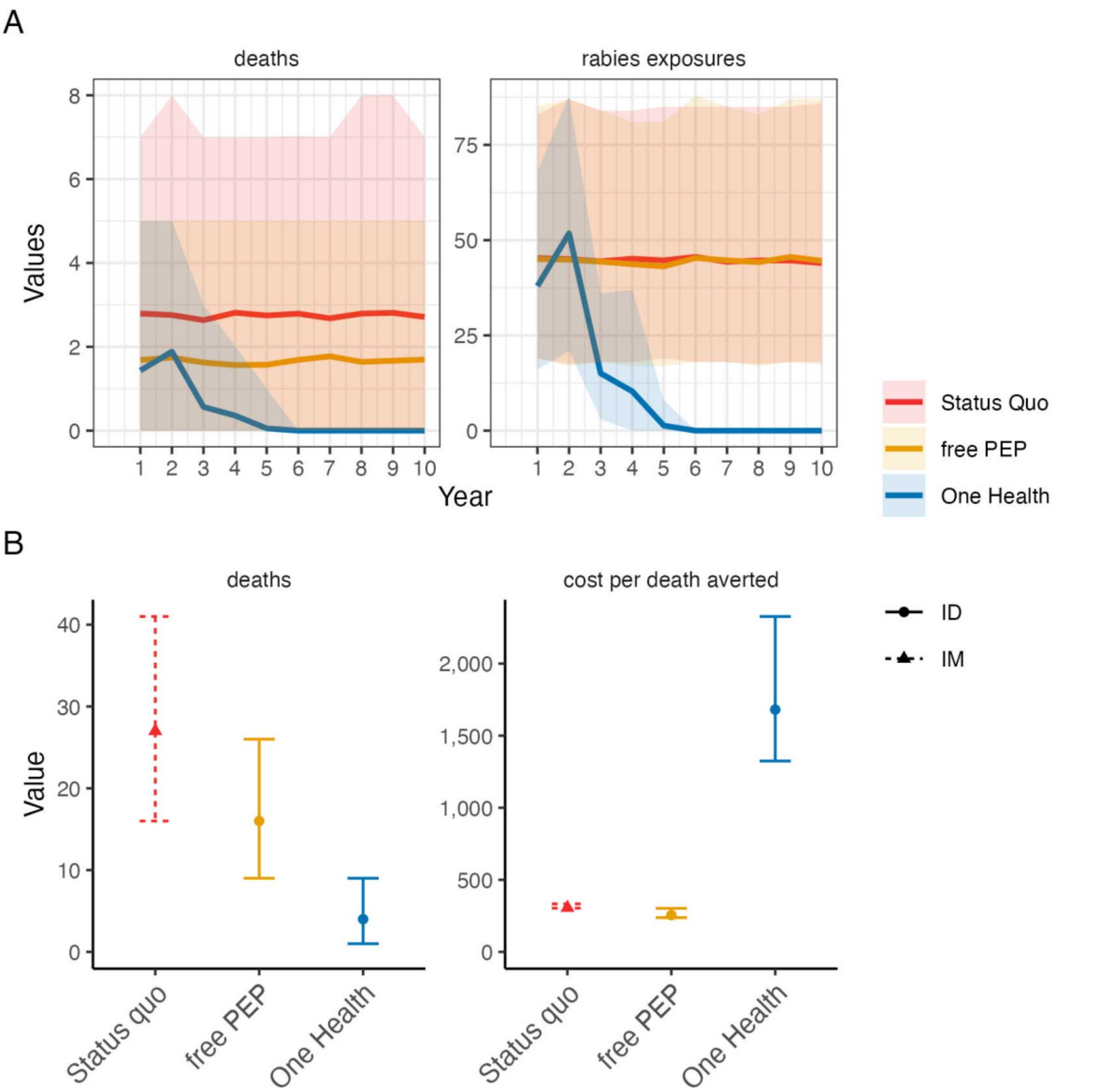

Figure 18: Comparison of cost-effectiveness of rabies control and prevention scenarios

(A) Projected human rabies deaths (left) and rabies exposures (right) over ten-year time horizon under: (a) status quo without dog vaccination and with PEP charged to patient; (b) free intradermal (ID) post-exposure vaccines, and (c) a One Health approach with free PEP and routine dog vaccination. Solid lines indicate mean values and shaded envelopes show 95% prediction intervals (PIs). (B) Resulting deaths and cost per death averted with 95% PIs. Costs were modelled from estimates of annual island-wide dog vaccination campaigns and of intramuscular (IM) PEP regimens (4-dose Essen, used under status quo) and ID PEP (updated Thai Red Cross, introduced with rabies demonstration project).

4.2 Discussion

4.2.1 Integrated Bite Case Management

Implementing IBCM demonstrated important public health impacts of rabies in Tanzania and the need to improve PEP access to prevent human rabies deaths as well as mass dog vaccination to control the disease at source. Reports of bites by suspected rabid dogs more than doubled under IBCM, and a large proportion of biting animals were identified as probable rabies cases upon investigation. Over half of patients presenting to clinics were assessed to have been bitten by suspect rabid dogs and therefore urgently required PEP. But, shortages of PEP occurred and human rabies deaths were reported from every region. Although it was possible to implement IBCM across this large geographic area, some activities were challenging, including recognition of indicative signs of rabies by health workers and investigations leading to sample collection by LFOs. Extended training could go some way to addressing these difficulties, but limited resources are a constraint. Nonetheless, IBCM shows considerable promise for improving case detection and communication between sectors, and further implementation research is warranted.

The IBCM showed promise as a tool to support rabies surveillance. Specifically, IBCM increased case detection, and generated data from health facilities that is much more useful for assessing the impact of PEP than numbers of bite patients alone, which may often not reflect rabies incidence directly (Rajeev *et al.,* 2019; Rysava *et al.,* 2019; Wambura *et al.,* 2019). The use of the mobile phone application was generally successful and both health workers and LFOs were enthusiastic about how IBCM improved intersectoral collaboration and understanding of the rabies problem, with LFOs particularly positive about using RDTs to confirm rabies. This was most evident during the response to a rabies outbreak in the first half of 2019 in Morogoro region, where several deaths occurred and dog cases were confirmed. The incidence of bite patients, suspect rabies exposures and deaths identified through IBCM in Morogoro was similar to numbers reported from the investigation of a previous outbreak in the region in 2007 (Sambo *et al.,* 2013), whereas incidence in the other districts was relatively low, likely because of previous dog vaccination campaigns.

Limitations of this study restricted the conclusions that were drawn. For example, IBCM was introduced to the government designated hospital in each district that offers PEP, but private referral hospitals, such as St. Francis in Kilombero District or Maneromango in Nachingwea district, were not included in the study, though they also offer PEP. Bite victims who directly attended these facilities (sometimes due to PEP stockouts elsewhere) were therefore not captured by IBCM. In addition, bite victims who never attended any of the health facilities and developed rabies and died at home were not captured by either IBCM or routine surveillance, leading to underestimation of the disease burden. Integration of private facilities may be needed in future if Tanzania is to bring rabies under control and IBCM is used to verify rabies freedom. Training was given to government workers and follow up provided by the research team, with one assistant remotely supporting all 4 regions. Without such technical support it may be difficult for the government to scale up IBCM to other parts of the country. More generally, district councils may vary in terms of follow up, levels of staff training and availability of funds that affects the quality of operations. These points likely apply to other LMIC settings and should be considered if efforts are made to improve PEP access and introduce IBCM (Cauchemez & Bourhy, 2019). Nonetheless, once trained, both health workers and LFOs were able to fully implement IBCM, and IBCM activities were mostly adopted and integrated within routine duties. Further work will be required to fully understand sustainability of IBCM.

Trained practitioners are indispensable to an effective health system and this applies directly to IBCM. Two or three health workers were trained to implement IBCM in each facility. This increased the workload of these health workers and they felt deserving extra payment from the project. Some health workers were also re-assigned to other facilities or departments, which required the recruitment of replacements who were trained remotely via phone. The level of knowledge and familiarity with smartphones also differed between users (both health workers and LFOs), and for a few using an app was challenging. In some facilities in Southern Tanzania only very few bite victims presented, and as a result, health workers in these areas (7 out of 63) needed reminding to conduct risk assessments and were encouraged to use the IBCM guide provided during their training to recall procedures. Generally, health workers receive only limited professional training in rabies in Tanzania. The proficiency training provided aimed to boost their ability to recognize signs of rabies, because of the difficulty that health workers showed in fully understanding rabies risks and indicative clinical signs in animals. Providing regular incentives such as training outside their workplace or in monetary terms could potentially help improve their performance but is a challenge for sustainability. Government supported training to reinforce IBCM, particularly over time and with staff turnover, could benefit sustainability. But it is likely that an ongoing support person would be required for troubleshooting, ideally a designated government employee.

From the animal health side, obtaining samples for diagnosis was difficult. Timely investigations are critical for confirming cases as well as for detecting other exposures, in both animals and people. Delays compromise sample collection opportunities and heighten risks for those who have not sought care. However, many cases that require investigation are far from district headquarters and the focal LFO responsible for sample collection. It is difficult for LFOs with limited resources and inadequate transport to reach these cases. Nevertheless, experience from this study suggests that sample collection could improve, with emphasis on timely submission of risk assessments by health workers, and additional training of LFOs based in more remote areas. Unfortunately, at the start of the study LFOs were not fully equipped with RDTs and so not all samples were collected and tested, but feedback from LFOs suggested that the ability to test samples was also a strong incentive for collection. One Health is widely promoted (Tiensin & Chuxnum, 2015) and is highly recommended for rabies control and prevention (Lechenne *et al.*, 2017). The IBCM represents a formal means of practicing intersectoral collaboration. Further joint discussions about surveillance findings amongst practitioners, including engagement with the regional and council health management teams, could help reinforce IBCM and ultimately promote better implementation of One Health and rabies control and prevention activities. Surveillance investments typically focus on laboratory diagnostics and infrastructure, but resourcing health workers to conduct risk assessments and LFOs to carry out investigations would be critical first to improve rabies case detection.

Vaccination of all persons exposed to a suspected rabid animal is an effective approach to protect people from rabies (World Health Organization, 2018). However, rabies vaccines in Tanzania are in short supply, so unnecessary use can also limit availability for those most in need (Etheart *et al.*, 2017; Medley *et al.*, 2017). While risk assessments indicate some potential for more judicious use of PEP in patients bitten by clearly healthy animals, the number and proportion of those presenting due to healthy animal bites is small compared to some settings, particularly in Asia and the Americas (Rysava *et al.*, 2019). Risk assessments to determine PEP decisions need to be both sensitive and specific. The PEP should always be recommended if there is any doubt concerning the risk of rabies, and therefore risk assessments with low sensitivity could lead to human rabies cases if PEP is either not initiated or delayed in a genuine rabies exposure, whilst risk assessments with low specificity could lead to people receiving PEP unnecessarily, incurring expenses and potentially limiting supply for those in need. A challenge for judicious PEP administration is that bite victims may demand PEP for bites from healthy animals, particularly in areas with recent rabies cases, and will cover any costs required or demand PEP and associated costs be covered by dog owners. These findings suggest that there is quite limited scope for more prudent PEP use in Tanzania, and that increasing PEP access should be the first priority. Nonetheless, the use of IBCM in such highly endemic settings could sensitize practitioners to the risks of rabies, and given limited diagnostic capacity and PEP availability, may be useful to guide PEP recommendations and prevent unnecessary overuse, particularly with a view to progressing towards elimination (Medley *et al.,* 2017; Undurraga *et al.,* 2017).

4.2.2 Exploring reservoir dynamics of rabies in Lindi and Mtwara regions, Southeast Tanzania

To examine whether wildlife could present an obstacle to rabies elimination under the hypothesis that if domestic dogs are the sole maintenance host, then control strategies directed at dogs alone should interrupt transmission. The observation throughout the eight-and-a-half-year study was most human rabies exposures and probable animal rabies cases being detected in domestic dogs. However, wildlife were a key source of human rabies exposures and comprised a large proportion of probable animal rabies cases. Wildlife-to-wildlife transmission accounted for approximately one-third of inferred transmissions and cross-species transmission among dogs and jackals were inferred to occur frequently. Both probable animal rabies cases and human rabies exposures decreased during the period of dog vaccinations, as did the size of inferred transmission clusters among all species. The initial decreased transmission observed across all species was attributed to the implementation of widespread dog vaccination and suggests that the increased cases in domestic dogs in 2018/19 resulted from waning herd immunity, coincident with the cessation of widespread dog vaccination. While domestic dogs are the main reservoir host for the maintenance of rabies in southeast Tanzania, data suggest that wildlife can sustain transmission chains and pose a substantive public health risk. In contrast to work from northern Tanzania showing that domestic dogs are the only species in which rabies appears capable of persisting (Tiziana *et al.,* 2008), here the findings show much greater involvement of jackals, but still conclude that targeting dogs through mass vaccination should eliminate rabies in this area.

One challenge faced during this study was limited information on jackal populations. Jackals numbers were extrapolated using density estimates from studies elsewhere in Africa, but this approach does not incorporate geographical population differences. More accurate jackal numbers would underpin a more confident assessment of the relationship between the susceptible population and cases. This, in turn, could provide further insight into what species drive transmission and whether assortative mixing underpins transmission pathways or if transmission depends more on the availability of susceptible animals regardless of species. Additional data will be needed to conclude whether jackals can maintain RABV independently over the longer term and this will be the future research question to be addressed.

A further limitation is related to case detection and confirmation. A low proportion of probable rabies cases were confirmed through laboratory diagnosis. Of the 549 clinically diagnosed animals, samples were collected in only two cases, both of which tested positive. The low rate of sample submission was primarily due to delays in reporting across this large area such that on follow up the animal had been lost or the carcass decomposed. Low rates of sample submission also meant that genomic data were not available. The assigned progenitors in 303 of the 549 probable cases were considered likely to represent direct transmission. This suggests that despite the intensive effort, over one-third of circulating cases were not observed (i.e., progenitors not found for the remaining 246 cases). Despite unobserved transmission, results were nonetheless robust under sensitivity analyses.

Genetic sequencing should prove useful in resolving transmission chains by determining whether RABV lineages include cases in both dogs and wildlife and for identifying introductions of RABV via human-mediated dog movement. Although several RABV lineages have been detected across Tanzania, there is currently no evidence of species-specific lineage associations (Brunker *et al.,* 2015; Tiziana *et al.,* 2008). Translocations of dogs has been shown to be important in the spread of RABV (Denduangboripant *et al.*, 2005) and genomic approaches have revealed substantial human-mediated RABV movement in Tanzania (Brunker *et al.,* 2015) which may explain how some apparently unconnected clusters and cases arose.

Whilst spill overs from RABV maintenance hosts into other species are common, most do not result in ongoing transmission (Mollentze *et al.,* 2020). However, host-shifts (establishment of novel cycles of transmission in new host species) occasionally occur and have important implications for control. Although the mechanisms that drive host-shifts are poorly understood, if RABV continues to circulate within domestic dogs in southeast Tanzania, spill over to wildlife is likely and opportunities for a host-shift remain. Establishment of sustained transmission within wildlife would have a serious impact on the effectiveness of control strategies currently focused on dog vaccination, which lends further urgency to eliminating rabies in dogs now.

4.2.3 Tracking the Dynamics of Rabies Elimination from Pemba Island

Using detailed genomic and epidemiological data, findings show that endemic rabies was first eliminated from Pemba in 2014 by effective implementation of four consecutive dog vaccination campaigns. Two independent introductions to the island in 2016, at a time when dog vaccination coverage was low, seeded a large outbreak. Further dog vaccination campaigns eliminated rabies again in 2018 and the island remains rabies-free. However, communities on Pemba experienced a severe burden, with many people exposed and preventable deaths occurring. When the BMGF rabies elimination demonstration project was initially rolled out in 2010, the context was of limited awareness amongst local communities, and both health and veterinary workers. At the start of the programme, only low dog vaccination coverage was achieved (Fig. 13) and prevention efforts only started to reach their goal after initial challenges and barriers had been overcome, including a lack of experience in dog vaccination and poor health-seeking, conflated by expensive, inaccessible and poorly provisioned PEP. By 2014, although vaccination coverage had not reached recommended levels (70%) (World Health Organization, 2018), the transmission was interrupted. Initial attempts to respond locally to re-emergence were ineffective, but when Pemba's government committed to delivering dog vaccinations island-wide, rabies was rapidly eliminated once again.

The detailed data from the contact-tracing studies highlight how routine surveillance activities in Pemba, as throughout much of Africa, can result in massive underreporting of rabies, with low case detection leading to underestimation of the disease burden, lack of prioritisation, and difficulty ascertaining impacts of control, including whether the disease has been eliminated, or is circulating undetected. In contrast, the high case detection provided through contact tracing generated confidence (Townsend *et al.*, 2013) that rabies was eliminated in both 2014 and 2018, and was further confirmed from viral genomes.

Accumulating evidence illustrates how metapopulation dynamics maintain the circulation of dog-mediated rabies, manifested in endemically co-circulating lineages (Bourhy *et al.*, 2016; Mancy *et al.*, 2022). Despite Pemba being relatively isolated, as an island with a small dog population, genomic data revealed endemic viral diversity, likely arising from historical introductions, as well as the two human-mediated introductions that spread in 2016. These introductions illustrate the fragility of elimination (Brunker *et al.*, 2020). Genomic approaches are increasingly affordable, and capacity should be in place given experience from SARS-CoV-2. Enhancing rabies surveillance with genomics, should reveal these metapopulation dynamics and could inform elimination, by identifying introductions and resolving their role in further spread.

While dog rabies remains uncontrolled in nearby populations, reintroduction risks remain high (Bourhy *et al.*, 2016; Tenzin *et al.*, 2010; Jakob Zinsstag *et al.*, 2017). Re-emergence is most likely if dog vaccination coverage is low and such incursions have major public health and economic consequences (Castillo-Neyra *et al.*, 2017; Tohma *et al.*, 2016; Jakob *et al.*, 2017).

It may be possible to reduce introductions through improved border control, but scaling up coordinated larger-scale dog vaccination would accelerate elimination, accruing and sustaining long-term benefits. Examples from Latin America show dramatic contractions in the range of dog-mediated rabies, with the last foci on the continent remaining in the poorest communities where dog vaccinations were not prioritised (Kristyna *et al.,* 2020). These dynamics also emphasise the need to maintain surveillance and vaccination coverage where the risk of introductions from connected populations remains.

Dog-mediated rabies is the quintessential zoonotic disease that requires coordinated public health and veterinary interventions, i.e. One Health. Inequitable vaccine access is epitomised by continued dog-mediated rabies in poor communities of poor countries. The cost of preventing rabies in Pemba's population (~0.5 million) is negligible, in terms of both human and dog vaccines and their delivery. By tracing transmission within the dog population and to humans, lives saved by PEP were directly quantified, and show how PEP alone does not prevent the trauma experienced by rabid bite victims, both in relation to the rabies risk and the horrific injuries that can be inflicted by rabid dogs. Consistent with other studies, evidence showed that PEP is extremely cost-effective in preventing rabies deaths and particularly so because in Pemba, PEP administration is targeted effectively to people with likely rabies exposures rather than being given more indiscriminately for any animal bite injury. However, on its own, PEP is insufficient to protect the entire at-risk population, nor would it address the suffering caused from injuries inflicted. Only dog vaccination interrupts transmission in dogs and thus prevents rabid dog attacks and rabies exposures and this therefore represents a more equitable approach to prevention. The minimal investments needed to support access to life-saving emergency vaccines for humans and to maintain rabies freedom in source dog populations, have not yet been made by either national governments or internationally, effectively ignoring those affected. Yet, lessons from Pemba should build confidence in the feasibility of eliminating rabies elsewhere on the African continent, given sustained investment and commitment. Coordinated dog vaccination over sufficiently large scales will have the greatest and most long-lasting impacts in equitably tackling this preventable disease.

The analyses highlight the cost-effectiveness of PEP as an emergency medicine critical for rabies prevention. A very low cost per death averted for free PEP provisioning on Pemba was estimated, even when considered incrementally to the status quo where PEP is charged to patients. The estimate from Pemba is amongst the highest cost-effectiveness estimates of PEP from across Gavi-eligible countries (Hampson *et al.*, 2019) (translating to a cost of $13 per DALY averted) and results from the high proportion of bite patients presenting with rabies exposures rather than bites from healthy dogs. In settings with more patients seeking care for healthy dog bites, PEP cost-effectiveness declines, although this can be slightly offset by increased vial sharing opportunities under intradermal dose-sparing regimens. Even though PEP is an essential emergency medicine, PEP does not address the suffering caused from injuries inflicted by rabid animals and is insufficient to protect the entire at-risk population. This study shows how, in practice, lack of awareness, expense and supply issues still prevent access to these emergency vaccines for marginalised populations.

In contrast to PEP sustained mass dog vaccination reduces the risk of exposure and by interrupting transmission in the reservoir is able to achieve the equitable goal of elimination. Mass dog vaccination inevitably comes at a higher cost per death averted particularly given the relatively high cost per dog vaccinated in this setting. Nonetheless compared to other health interventions, this One Health approach remains extremely cost-effective (Castillo-Neyra *et al.,* 2017). In denser, more connected populations than Pemba rabies elimination is likely to take longer and be more fragile, while conversely the cost per dog vaccinated is likely to reduce in areas with larger dog populations and with opportunities for optimising the delivery of dog vaccination. The findings on cost-effectiveness estimates of this study lie within expectations for countries in sub-Saharan Africa (Hampson *et al.*, 2019), but these considerations limit their transferability. To improve health economic models, the relationship between dog vaccination coverage and risk reduction needs to be better quantified, and research is needed on health seeking behaviours following bites by both healthy and rabid dogs that impact cost-effectiveness. Moreover, realised cost-effectiveness depends on the stochastic nature of outbreaks and the degree to which interventions are delivered as intended, including how dog vaccination coverage is maintained, since vaccination campaigns can often lapse, as seen from Pemba. The COVID-19 pandemic highlights how such disruption can severely set back rabies programmes (French multidisciplinary investigation team, 2008; Townsend *et al.*, 2013).

To conclude, investment is needed for a One Health approach, to support access to life-saving emergency vaccines for bite victims and to achieve and maintain rabies freedom in source dog populations is very cost-effective and can bring rapid success. Lessons from Pemba should build confidence in the feasibility of eliminating rabies elsewhere on the African continent but highlight the importance of sustaining commitment. Coordinated dog vaccination over sufficiently large scales will have the greatest and most long-lasting impacts in equitably tackling this preventable disease.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

In Tanzania, animal disease surveillance falls under the Ministry of Livestock and Fisheries, while the Ministry of Health deals with bite victims. An intersectoral programme, such as the One Health Coordination Unit, under the Prime Minister’s office, encourages both sectors to work together practically but coordinating rabies prevention and control between the two sectors has always been a challenge. The IBCM has helped to integrate these sectors and generates more accurate surveillance data that can guide policy decisions and public health measures. The IBCM improved intersectoral communication helped to identify rabies-exposed bite victims requiring PEP, facilitated follow-up of cases, and encouraged LFOs to rapidly test cases during investigations. Surveillance is crucial to guiding effective patient management decisions, disease control interventions, and for verifying disease elimination. A well-established surveillance system will be essential to evaluate the impact of mass dog vaccination programmes and to ensure rapid responses to outbreaks. The IBCM appears to be a practical and promising approach to improve case detection and was extremely useful during the outbreak of rabies in Morogoro region. Whether IBCM can confirm the interruption of disease transmission will depend on implementation. Until now, many practices for rabies control and prevention are still weak in LMICs with endemic rabies and will need strengthening to achieve the zero by 30 goal.

In addition, this thesis provides insights into the epidemiology of rabies in multi-host communities and highlights the potential importance of wildlife as sources of rabies exposure. The data highlights the frequent transmission of rabies from sparsely distributed domestic dog populations, to and from sympatric wildlife, specifically jackals. Yet even in areas with relatively high proportions of wildlife cases, domestic dog vaccination still reduced the risk to humans. Maintaining dog vaccination campaigns in LMICs is challenging and the results show that if vaccination campaigns are not maintained, the resurgence of rabies can rapidly occur. Herd immunity wanes quickly with high demographic turnover in the dog population, and infection circulating in nearby populations can seed introductions. Continued dog vaccination is needed to eliminate rabies from this region and should shed more light on the involvement of wildlife in rabies maintenance.

Even in the areas with relatively high proportions of wildlife rabies cases and evidence of cross-species transmission, the findings indicate that domestic dog vaccination appears to be effective in reducing exposure risks in humans and decreasing rabies incidence among all species. The importance of sustained annual vaccinations is highlighted by the observed increase in probable dog cases following the cessation of widespread vaccination campaigns in Southern Tanzania in 2017. This increase in domestic dog rabies and likely subsequent increase in wildlife rabies represents a significant public health threat. These findings have implications for Tanzania's National Rabies Control strategy and suggest that focusing on domestic dog vaccination will have major public health benefits, and if sustained and coordinated may eliminate RABV. Ongoing effective surveillance will be essential to monitor the impacts of dog vaccination, which needs scaling up to reach the 'Zero by Thirty' target. Engaging the wildlife sector and building genomic surveillance capacity would further resolve transmission dynamics within domestic dogs and wildlife and inform progression toward elimination.

Rabies kills thousands of people every year in East Africa and is a major concern for communities who need to pay for emergency vaccines following dog bites to prevent the fatal onset of the disease. This research shows how dog vaccinations on Pemba controlled rabies, but dogs brought from the mainland re-introduced infection and caused re-emergence. Nonetheless, through strengthened surveillance and island-wide dog vaccination, the local government successfully eliminated rabies in 2018. This case study illustrates how feasible rabies elimination is, but also how important scaling up of dog vaccination is to bring wider benefits across large, connected populations.

5.2 Recommendations

(i) Regular mass dog vaccinations can eliminate rabies at source: This study recommends that mass dog vaccination campaigns have to be conducted annually in collaboration with all authorities and sectors to ensure rabies is eliminated at the source, just like in Pemba.

(ii) Increasing PEP availability and accessibility for bite patients: All government-based facilities need to be able to provide PEP to exposed individuals. The PEP should always be recommended if there is any doubt concerning the risk of rabies. The findings suggest that increasing PEP access should be a priority in Tanzania. The use of IBCM in highly endemic settings could better sensitize practitioners to the risks of rabies, and given the limited diagnostic capacity and PEP availability, these risk assessments could be used to prioritize PEP use where necessary.

(iii) Incorporate molecular epidemiological studies into disease surveillance control programmes: Molecular epidemiology can detect sporadic introductions from genetic information and can reveal how often such spill over events occur. Furthermore, genomic surveillance would resolve transmission dynamics within domestic dogs and wildlife and inform progression towards elimination. Therefore, such studies should be incorporated into routine rabies control strategies in the future and should be continued as a part of the rabies control programmes.

(iv) Regular training of practitioners i.e. livestock field workers and health workers on how to control rabies and prevent human rabies deaths: Health workers should be trained to conduct risk assessments and LFOs to undertake investigations to improve rabies case detection. Strengthening the risk assessment capacity through regular training among health practitioners will enable proper recommendations for PEP among animal bite victims. It will also allow immediate investigation and rapid response to control the disease at the source.

(v) Strengthening rabies surveillance also improves the collaboration between health and veterinary sectors (One Health): There is a need for improving and strengthening the capacity for One Health. Integrated Bite Case Management (IBCM) is a way to do this and at the same time improve rabies surveillance, enhancing the capacity to detect and respond to rabies outbreaks.

(vi) Improved border control can reduce introductions but scaled up dog vaccination is even more effective for controlling rabies. In this way, mainland Tanzania can realise the wider benefits in achieving the global ‘zero by 30’ goal to eliminate dog-mediated human rabies deaths.

(vii) Engage with stakeholders regularly to improve and sustain rabies control programmes: Joint discussions about surveillance findings amongst stakeholders and practitioners, including engagement with the regional and council health management teams is highly encouraged. This could promote better implementation of One Health and rabies control and prevention activities.

(viii) Continue outreach to communities impacted by rabies to reduce the burden and secure and maintain buy-in for control efforts. Wide-scale awareness-raising is important so that people know what to do if they are bitten by a dog or encounter any suspected animal, and know the importance of dog vaccination and rabies control efforts.

(ix) Train and equip livestock field officers and veterinarians to vaccinate, assess coverage and adapt. Vaccinators should be trained to routinely assess coverage by marking dogs and doing post-vaccination coverage surveys. Where they find gaps, they should conduct supplement door to door vaccination to achieve sufficient coverage.

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APPENDICES

Appendix 1: Data Collection Tools (IBCM Protocols)

HUMAN INVESTIGATION FORM

1. Name of Health facility: __________***(entered automatically)***
2. Visit date: ____________ ***(entered automatically)***

Names of the victim:

3.1 Sir Name (Jina la ukoo):__ 3.2 given Names (2): ___

3.3 Common name known; ___3.4 Age: ______ 3.5 Sex: __

3.6 Can you provide phone number ... Yes/No?

3.7 If yes, Phone No; ___________ ***(restrict to 10 digits)***

3.8 Phone status (Own/neighbour/no phone) *select*

4. Human ID: ***(created automatically by Zac/Frank)***
5. **Location** *(Select from the list)*

Region: __________District:______ Village: ________ Type write Village if not indicated on the list ______

Bite history

6.1 Bite status visit (If 1st dose complete the rest of questions, if 2nd,3rd or 4th ***select that corresponds and skip to qn. 14***; or positive clinical signs): Tick that corresponds

6.2 Date bitten: ___________ 6.3. Date reported to hospital: ____________

6.4 Biting animal: *Select from the list below*

Dogs, cats, livestock (cow, goat/sheep, pig), wildlife (specify), human

7. **Risk assessment (Tick)**

7.1 Type of the animal: domestic/ wildlife (Tick)

7.1.1 animal signs (Tick):

- Unprovoked aggression (incl. attempting to bite and grip people, animals, or objects, without feeding
- Excessive salivation
- Unexplained dullness/lethargy
- Hyper sexuality
- Paralysis
- Abnormal vocalization
- Restlessness
- Running without reason
- Tameness/loss of fear of humans (wildlife)
- Active during day (wildlife)

None of the above

7.1.2 Feeding puppies (Yes /No), Eating (Yes /No), Normal behaviour i.e. aggressive dog (Yes /No)

7.1.3 is the dog/animal known in the community? (Yes, proceed to 7.1.4 /No)

7.1.4 If yes, name of the dog owner: ____________, Village: _____________

7.2 The **victim:** Noise (speaking/shouting) (Yes/No); running (Yes/No); Aggressive (Yes/No); scared of dogs (Yes/No); throw anything at the dog (Yes/No); playing (Yes/No); approaching the dog (Yes/No); NO provocation (Yes/No)

7.3 Environment**:** Chained (Yes/No); Fenced (Yes/No); with no owner (Yes/No); with owner (Yes/No); Lots of people (Yes/No), Lots of dogs (Yes/No); on its property (Yes/No),

Dog came out of nowhere: Ticks

8. Is the animal still alive? Yes/No (select)
9. Veterinary department consulted for follow up; (Yes/No): Tick
10. Rabies Assessment decision: ☐ Healthy ☐ suspicious for of rabies ☐ Sick, not rabies ☐ Unknown (Tick)
11. **Bite site:** Tick that applies

☐ Head/neck ☐ Trunk ☐ Arms/Hands ☐ Legs/ Feet

12. **Bite details:** Tick the level that applies

☐ Scratch: ☐ Minor wounds ☐ large wounds ☐ Severe (broken bones)

☐ Severe (hospitalization) ☐ fatal bite (bitten to death).

13. Treatment of victim (*check all that apply*)

☐ Nothing ☐ Tetanus ☐ Wound washing

☐ Antibiotics ☐ Immunoglobulin

14. PEP availability and recommendations

1. PEP advised & available (If yes, how used? (ID/IM) *select the correct*

b. PEP advised & referred (If yes, where? *Free text*)

c. PEP not advised

15. Comment (if any):

Appendix 2: Animal Investigation Form

Date of investigation: _ _/_ _ /_ _ _ _ (automatically entered)

Name of investigation officer: (automatically entered)

Animal ID: (Is investigation linked to bite incident, if Y use ID from bite patient, otherwise create ID automatically): This needs to be automatically created

1. Location of event: (*select from the list provided*)

Region__________ District_______ Village _________ other village ________ *(if not in the list hand type the village)*

2. Type of Investigation (Tick): Phone Consultation / In-Person Investigation

3. Reported from (mark all that apply)

☐ Health centre/worker

☐ Veterinarian

☐ Community members' ____________________

☐ other (*please specify*) ________________

4. Reason for report *(Tick)*:

Human Exposure (bite/scratch) ☐ Suspect rabid animal ☐ Hit by car ☐ animal found dead

☐ animal is sick ☐ person is sick ☐ other (*specify*)

5. Type of animal: Select

Dogs, cats, livestock (cow, goat/sheep, pig), wildlife (specify), human

6. Was this animal: ☐ Unowned ☐ Owned ☐ Unknown (TICK)

6.1 if owned, names of animal owner______

7. How many people were bitten/scratched by the animal? ___________

8. Dropdown to record name of each person bitten, sought PEP Y/N, advised Y/N

8.1 Name of the victim

8.2 Victim ID (enter the patient ID sent automatically) / or Insert text "ID not assigned "if patient has not sought treatment

8.3 Village

8.4 PEP status: 1) PEP initiated (1st dose received); 2) PEP completed; 3) PEP not sought – advised to seek – URGENT; 4) seeking PEP (1st dose NOT received) – URGENT; 5) PEP not advised.

9. What other animals were bitten by this animal? How many? ☐ None ☐ Dog #___ ☐Cat #___ ☐ Cattle # ___ ☐ Other (please specify)#________

10. a. Was the animal found? ☐ Yes, ☐ No

b. what was the animal outcome: ☐ alive, ☐ dead, ☐ disappeared **(Tick)**

c. If dead, cause of death: ☐ killed by owner, ☐ by community, ☐ car, ☐ natural causes, ☐ unknown ☐ Killed by animals **(Tick)**

11. What is the animal's age? (Tick)

☐ Puppy ☐ Puppy (< 3months) ☐ juvenile (<1y) ☐ Adult ☐Unknown

12. What is the animal's sex? ☐ Male ☐ Female ☐ Unknown

13. Has the animal been vaccinated for rabies?

☐ Yes, what year: __________ ☐ Not vaccinated ☐ Unknown ☐ Not applicable

14. **Risk assessment**

14.1 The Dog/Animal

14.1.1 animal signs (Tick):

- Unprovoked aggression (incl. attempting to bite and grip people, animals, or objects, without feeding
- Excessive salivation
- Unexplained dullness/lethargy
- Hyper sexuality
- Paralysis
- Abnormal vocalization
- Restlessness
- Running without reason
- Tameness/loss of fear of humans (wildlife)
- Active during day (wildlife)

None of the above

14.2 Feeding puppies (Yes /No), Eating (Yes /No), Normal behavior i.e. aggressive dog (Yes /No)

14.3 **The victim:** Noise (speaking/shouting) (Yes/No); running (Yes/No); Aggressive (Yes/No); scared of dogs (Yes/No); throw anything at the dog (Yes/No); playing (Yes/No); approaching the dog (Yes/No); NO provocation (Yes/No)

14.4 **Environment:** Chained (Yes/No); Fenced (Yes/No); with no owner (Yes/No); with owner (Yes/No); Lots of people (Yes/No), Lots of dogs (Yes/No); on its property (Yes/No), Dog came out of nowhere: Tick

15. Rabies Assessment decision: ☐ Healthy ☐ suspicious for of rabies ☐ Sick, not rabies ☐ Unknown (Tick)

16. Was a sample collected? ☐ Yes, date:____________ ☐ No , see 16.1,

16.1 If Yes, Location where the sample is stored? ☐ DVO's office, ☐ house location of LFO, ☐ Vet investigation centre ☐ Other (Specify)

16.2 No: Animal disappeared ☐ Decomposed ☐ Body thrown ☐ Burned ☐ Consumed ☐ hyenas ate ☐ Not applicable

17. Lateral flow test done? Yes/ No: Select
18. Test Results: ☐ Positive ☐ Negative ☐ Inconclusive ☐ Unsatisfactory for testing
19. Specimen sent to lab, date: ______________ select
20. Comments if any: _______________

Appendix 3: Supplementary Figures and Tables

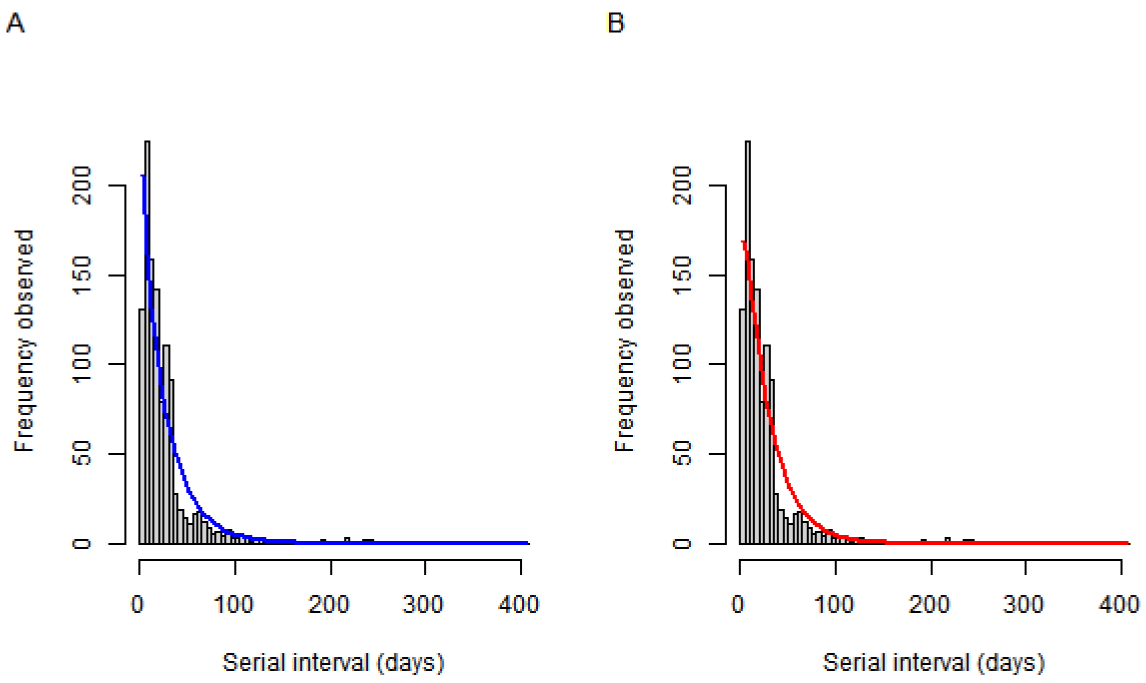

Figure S1: Alternative distributions for the serial interval

Data from dogs in Serengeti District, Northern Tanzania are illustrated by the histograms with the fitted distributions overlying. (A) Blue line illustrates the best-fitting Weibull distribution. (B) Red line illustrates the best-fitting gamma distribution

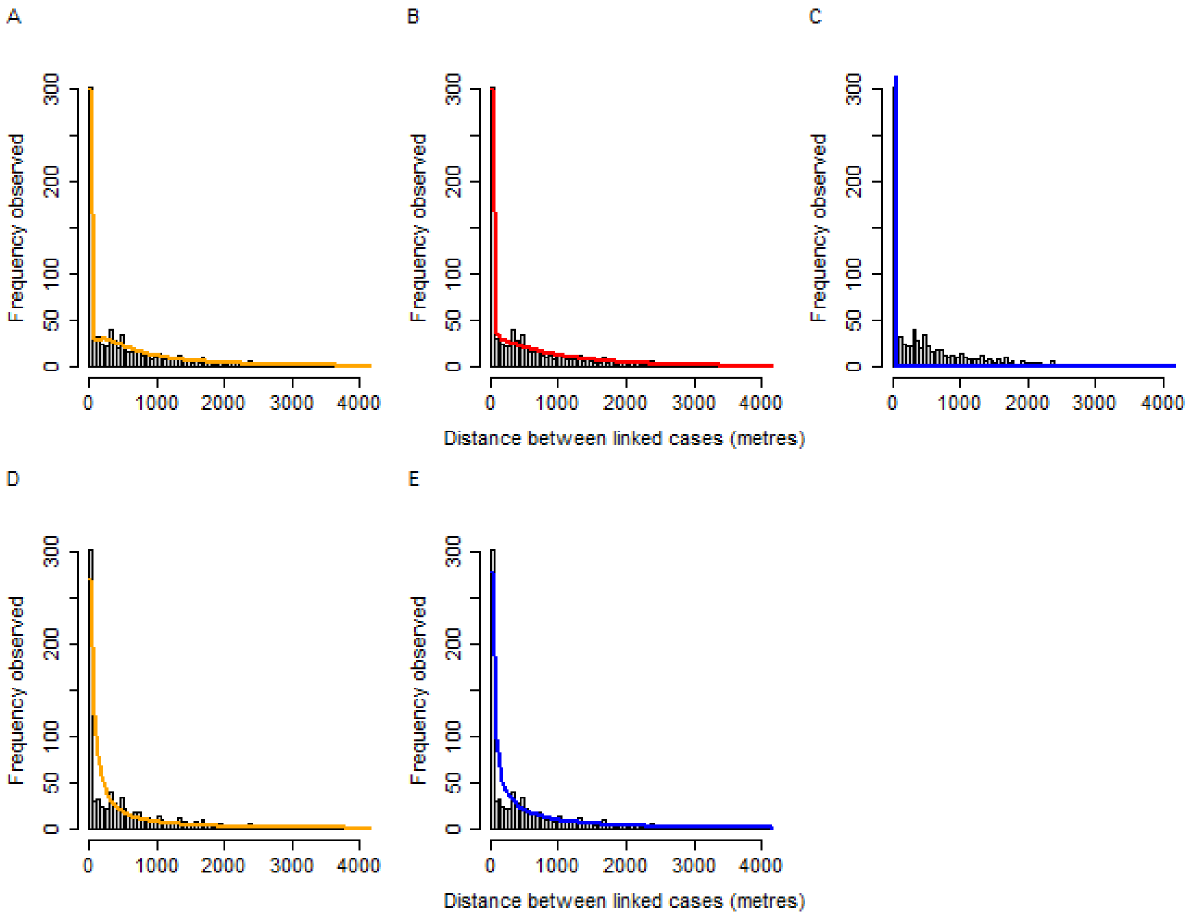

Figure S2: Distance kernel with fitted distributions using interval censored data at 50m.

The distance between biting animals from Serengeti District, Northern Tanzania are plotted with fitted distributions overlying using an upper limit of 50m for interval censored data. The x axes have been truncated at 4000 metres to allow easier visualisation of the data. The maximum observed distance was 20713m. (A) mixture distribution composed of two lognormal distributions (B) mixture distribution composed of two gamma distributions (C) mixture distributions composed of two Weibull distributions (D) single lognormal distribution (E) single Weibull distribution

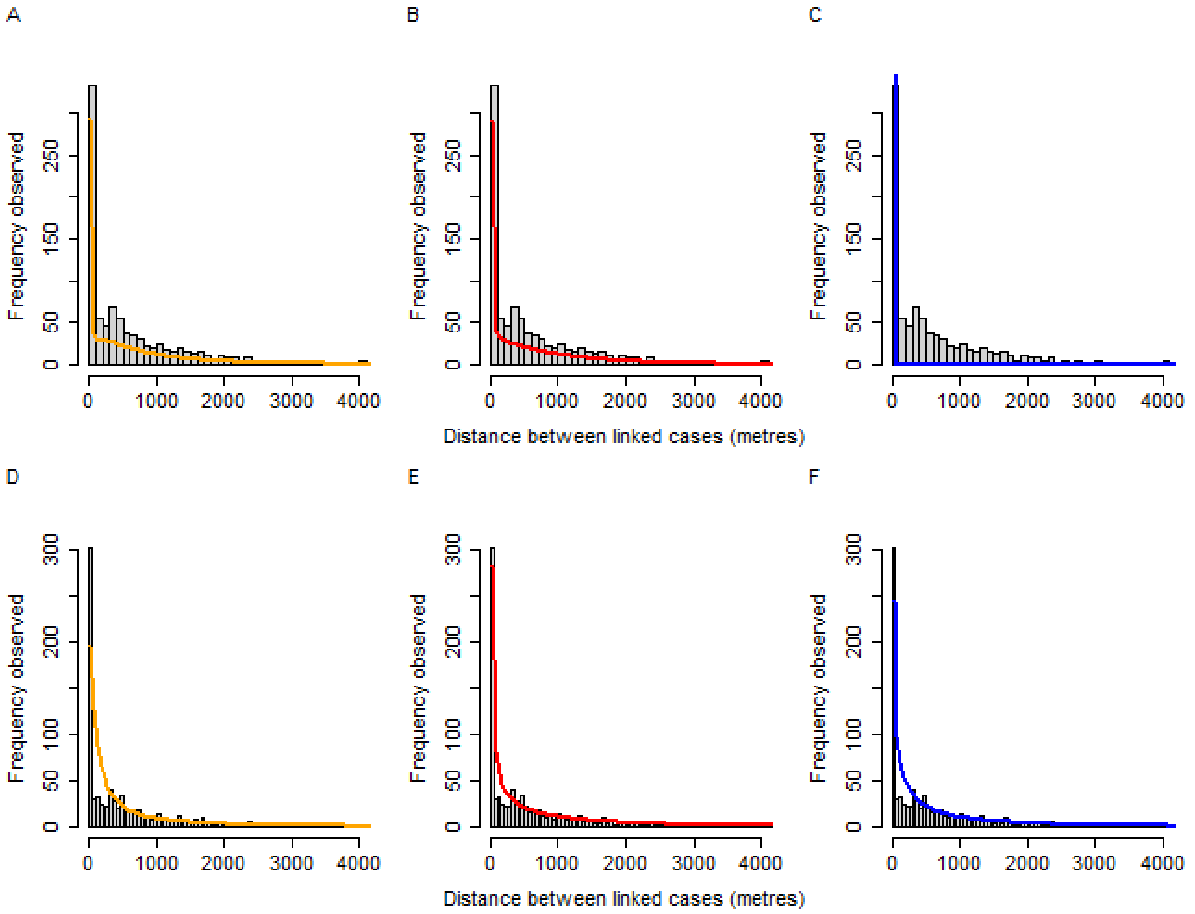

Figure S3: Distance kernel with fitted distributions using interval censored data at 100 m

The distance between biting animals from Serengeti District, Northern Tanzania are plotted with fitted distributions overlying using an upper limit of 100m for interval censored data. The x axes have been truncated at 4000 metres to allow easier visualisation of the data. The maximum observed distance was 20713m. (A) mixture distribution composed of two lognormal distributions (B) mixture distribution composed of two gamma distributions (C) mixture distributions composed of two Weibull distributions (D) single lognormal distribution (E) single gamma distribution (F) single Weibull distribution

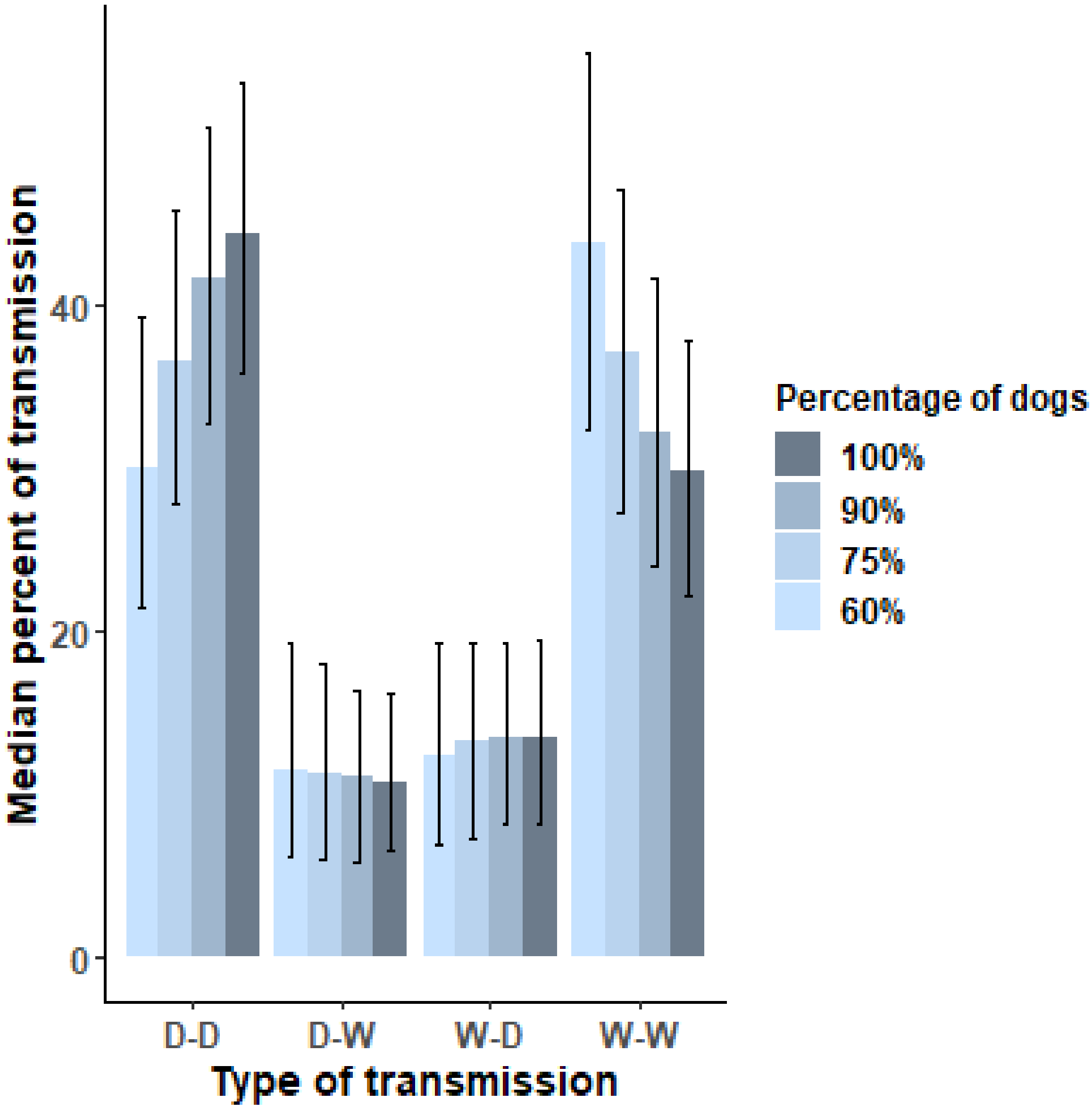

Figure S4: Subsampled transmission tree analysis

Results are displayed for the four most common types of transmission. Median percentage of all inferred transmission coloured by the percentage of dogs used within the construction of transmission trees. D-D: Dog-to-dog transmission; D-W: Dog-to-wildlife transmission; W-D: Wildlife-to-dog transmission; W-W: Wildlife-to-wildlife transmission. The results from the transmission trees produced by the subsampling analyses are shown in Fig. 4. These results suggest that as the number of dog cases observed decreases, the percentage of inferred transmissions that are dog-to-dog transmissions decreases whilst the percentage that are wildlife-to wildlife increases. However, the 95% confidence intervals are wide and overlap for all scenarios explored

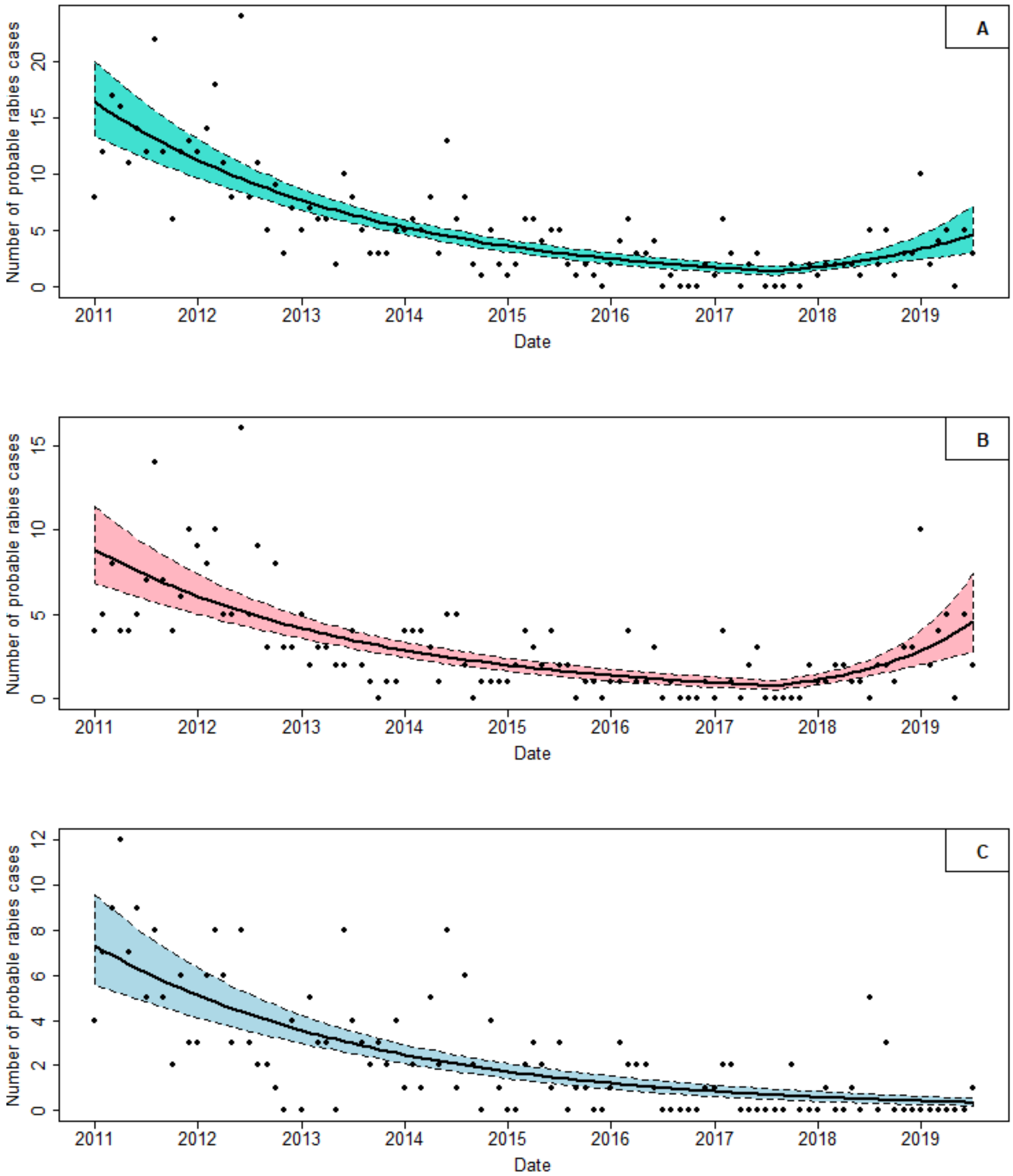

Figure S5: Regression analysis of probable animal rabies cases per month

Dots represent the number of probable animal rabies cases for each month. Fitted regression lines from negative binomial regression models are shown in black with the shaded area representing the 95% confidence interval. A) Probable rabies cases in all animal species; B) in domestic animals only and C) in wildlife species only. A statistically significant downward trend in monthly probable rabies cases was found in all three models from January 2011 ($p < 0.001$, 3.2% (95% CI: 2.7% - 3.7%) reduction per month in all species; $p<0.001$, 3.2% (95% CI: 2.5% - 3.8% reduction per month in domestic animals only; 3.1% (95% CI: 2.4% - 3.7%) reduction per month in wildlife only). A linear spline was fitted with a knot placed at August 2017 and the change in slopes was found to be significant in the models fitted to cases from all species and to cases in domestic animals only ($p < 0.001$, 6.3% (95% CI: 3.6% - 9.0%) increase per month in all species; 9.1% (95% CI: 5.8% - 12.4%) increase per month in domestic animals only). For probable cases in wildlife, the slope did not change significantly ($p = 0.543$) and therefore a single trend was maintained

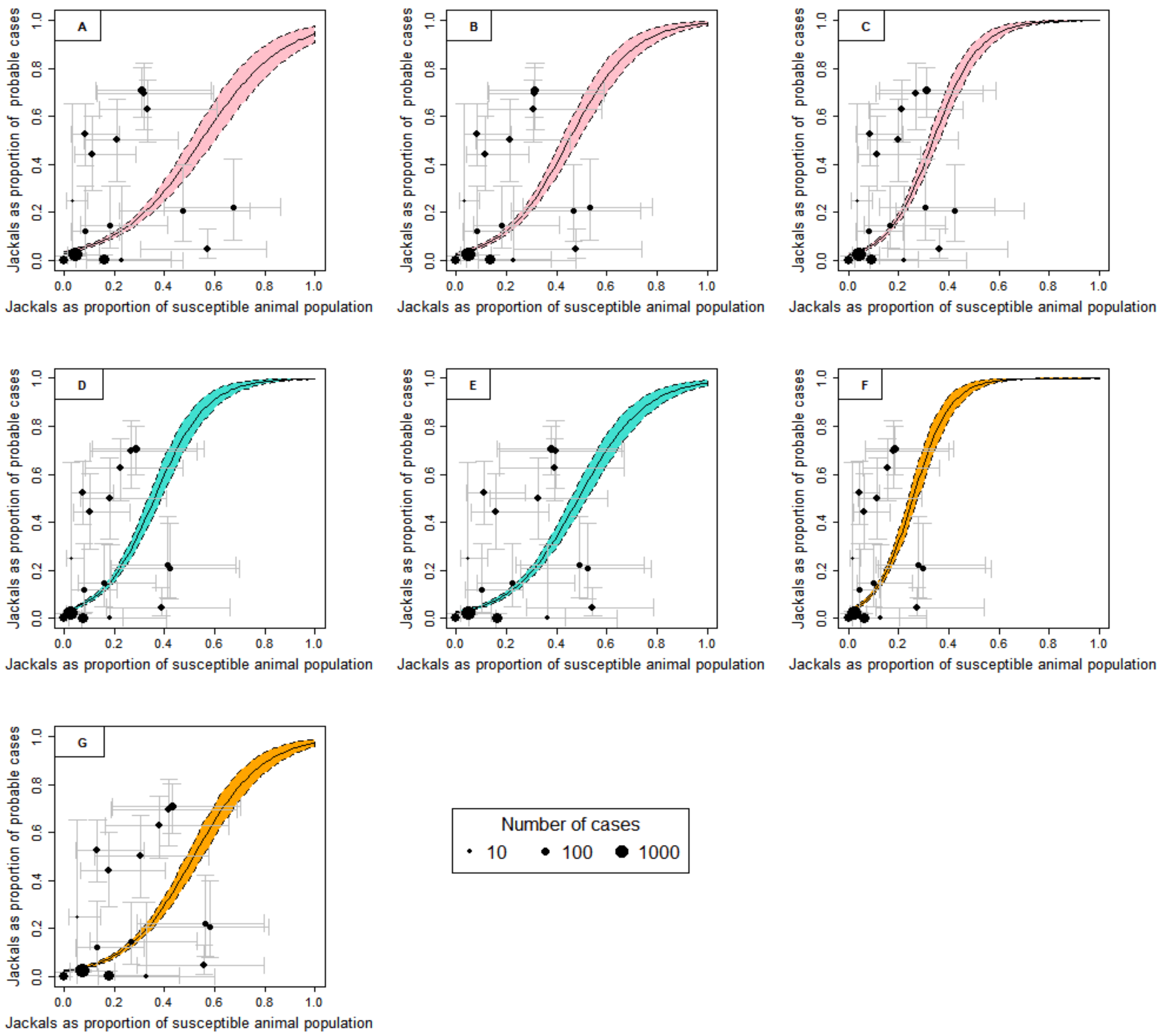

Figure S6: Jackals as a proportion of the susceptible population versus cases

The relationship between the proportion of jackals in the susceptible animal population and the proportion of the probable rabies cases observed in jackals was fitted. The susceptible population consists of jackals and unvaccinated dogs assuming A, B, C, F and G) the median level of vaccination coverage achieved in a district; D) zero vaccination coverage and E) the maximum level of dog vaccination coverage. Jackals are applied at a density of 0.3 jackals per km² to areas with between A) 0 and 500 people per km², B) 1.25 and 500 people per km², C) 5 and 500 people per km² and D and E) 2.5 and 500 people per km² and to areas with between 2.5 and 500 people per km² at densities of F) 0.15 jackals per km² and G) 0.50 jackals per km². Probable rabies cases refers to those in jackals and domestic dogs only. Dots represent the 16 districts included in this analysis, scaled by the log10 number of probable cases in that district. Grey bars around the points represent 95% confidence intervals (CIs). The CIs around the proportion of probable cases that occur in jackals are the exact binomial 95% CIs. The CIs around jackals as a proportion of the susceptible animal population was calculated keeping jackal estimates and levels of vaccination coverage constant but incorporating the lower and upper limits of the 95% CIs of the dog number estimates. The fitted logistic regression line is shown in black with the associated 95% CI shown in pink (A, B and C), turquoise (D and E) and orange (F and G).

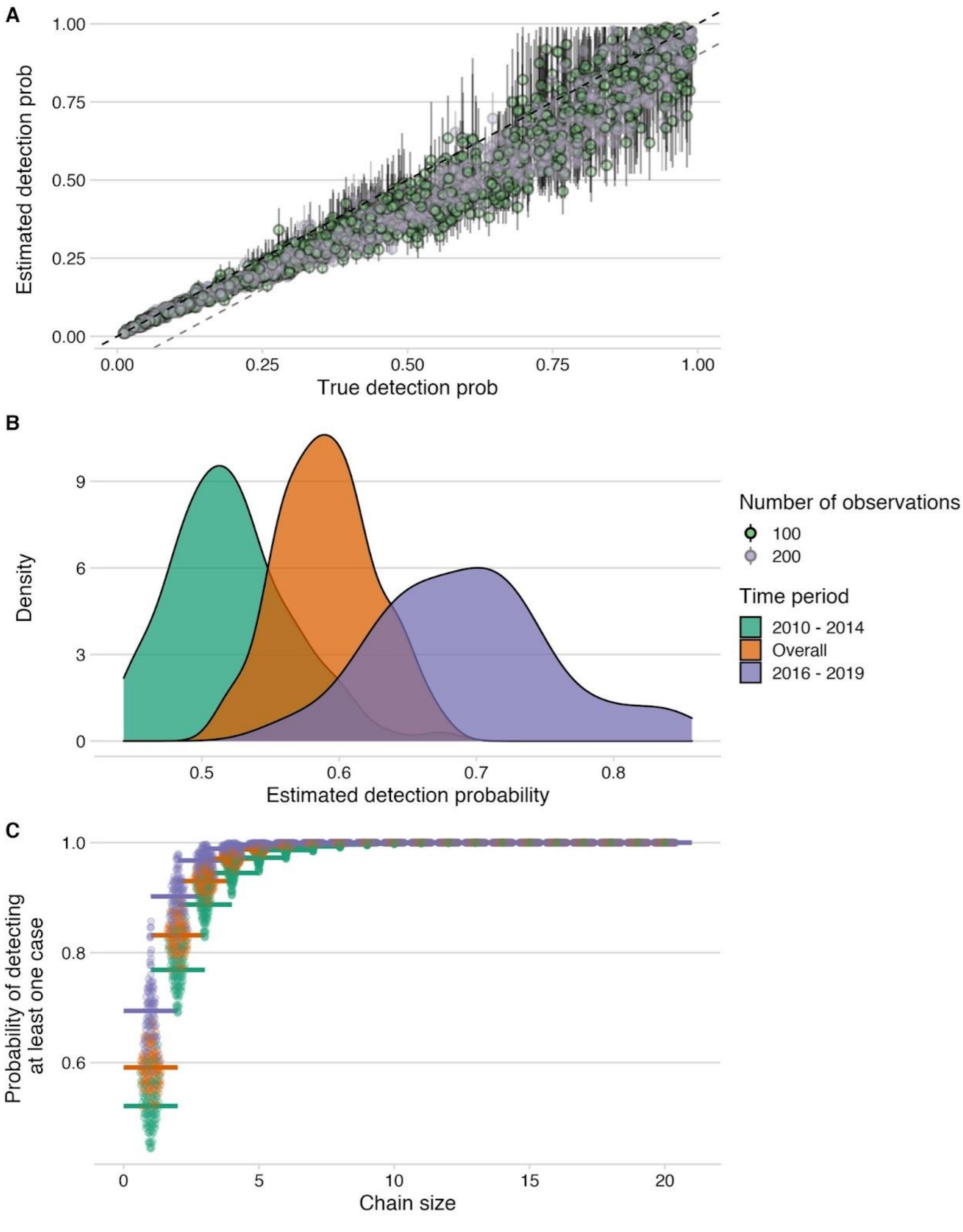

Figure S7: Estimation of detection probabilities

Estimated detection probabilities from simulated times between linked cases given a known detection probability (x-axis) (A). Colors indicate the sample size used in the simulations with N = 100 or N = 200 cases. The points show the mean and the lines the range of 10 estimates per simulation. The black dashed line shows the 1:1 line and the grey dashed line the 1.1:1 line. Detection probabilities estimated from times between linked cases using the tree algorithm pruning by phylogenetic data. For the estimation, the times between linked cases for a subsample of bootstrapped trees (N = 100), as well as the MCC and the majority tree were used (B). The colors indicate the time period for which estimates were generated, 2010-2014 (the pre-elimination period, N ~ 100 cases), 2016 - 2019 (the reemergence period, N ~ 100 cases) and overall combining cases (N ~ 200). Probability of detecting at least one case given estimated detection probabilities and chain sizes (x-axis) with colors corresponding to the period for which estimates were generated (C).

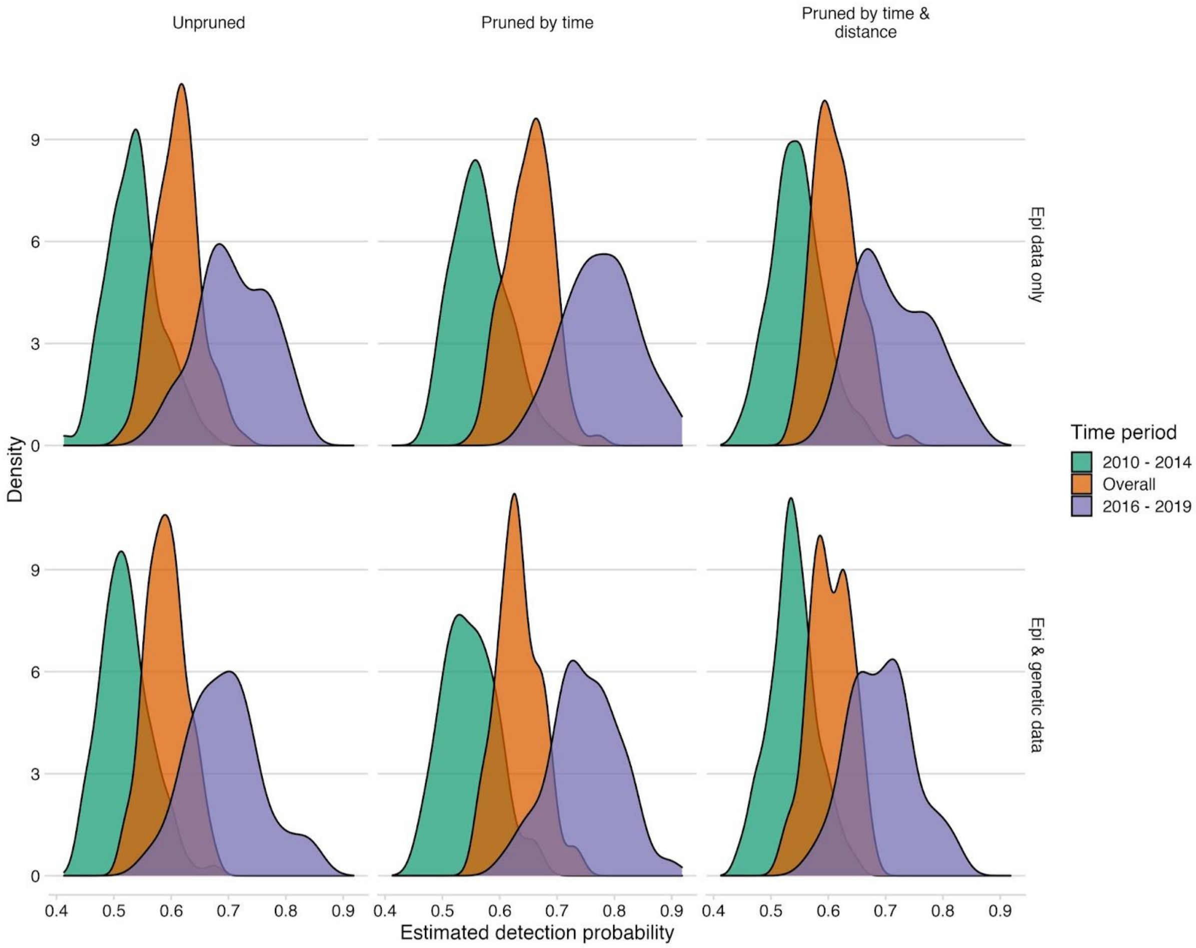

Figure S8: Comparison of detection estimates across pruning algorithms.

For the estimation, the times between linked cases for a subsample of bootstrapped trees (N = 100), as well as the MCC and the majority tree were used. The colors indicate the time period for which estimates were generated, 2010-2014 (the pre-elimination period, N ~ 100 cases), 2016 - 2019 (the reemergence period, N ~ 100 cases) and overall combining cases (N = 202)

Table S1: Inferred transmissions between species

Transmission between species	Median number of transmission events (% of overall transmission)	Bootstrap 95% confidence interval around median (% of transmissions)
Dog - Dog	77 (45.6)	65 - 90 (38.5 - 53.3)
Dog - Wildlife	13 (7.7)	7 - 20 (4.1 - 11.8)
Wildlife - Dog	21 (12.2)	12 - 29 (7.1 - 17.2)
Wildlife - Wildlife	55 (32.5)	43 - 67 (25.4 - 39.6)
Cat - Dog	1 (0.6)	0 - 4 (0.0 - 2.4)
Dog - Cat	0 (0.0)	0 - 1 (0.0 - 0.6)
Cat - Wildlife	0 (0.0)	0 - 0 (0.0 - 0.0)
Wildlife - Cat	2 (1.2)	0 - 5 (0.0 - 3.0)
Cat - Cat	0 (0.0)	0 - 0 (0.0 - 0.0)

The scenario with the lowest cut-off values for serial interval and transmission distance is shown. Cut-off values were generated using the 95th percentile of the serial interval and convolution of two distance kernel distributions and using 100m as the upper limit for interval censoring for transmission distances recorded as zero within the northern Tanzania reference data. These correspond to an upper limit of 80.8 days for serial interval and 5803 metres for transmission distance. Fisher's exact test values were highly significant, with p-values of less than 0.001 for all of the 1000 contingency tables of inferred transmission

Table S2: Lagged correlation analysis of monthly cases

Lag period (months)	**Correlation coefficient with domestic dog cases per month leading and jackal cases lagged (p-value)**	**Correlation coefficient with jackal cases per month leading and domestic dog cases lagged (p-value)**
0	**0.525 (<0.001)**	**0.525 (<0.001)**
1	0.434 (<0.001)	0.402 (<0.001)
2	0.349 (<0.001)	0.410 (<0.001)
3	0.361 (<0.001)	0.458 (<0.001)
4	0.406 (<0.001)	**0.525 (<0.001)**
5	0.256 (0.011)	0.392 (<0.001)
6	0.424 (<0.001)	0.467 (<0.001)
7	0.298 (0.003)	0.476 (<0.001)
8	0.398 (<0.001)	0.433 (<0.001)
9	0.295 (0.004)	0.509 (<0.001)
10	0.432 (<0.001)	0.512 (<0.001)
11	0.262 (0.012)	0.519 (<0.001)

Appendix 4: Logistic Regression of Cases in Relation to Population Composition

Results for monthly cases with lags from 0-11 months are shown. Scenarios with the highest value for the correlation coefficient are highlighted in bold. Sensitivity analyses were undertaken by exploring different scenarios affecting the proportion of the susceptible population composed of domestic dogs or jackals. The scenarios evaluated were:

(i) Jackal population estimated by assigning them to grid cells with human population density between 0 and 500 per km² at a density of 0.3 jackals per km². Domestic dog vaccination was applied at the median rate of coverage per district to the median estimated domestic dog population.

(ii) Jackal population estimated by assigning them to grid cells with human population density between 1.25 and 500 per km² at a density of 0.3 jackals per km². Domestic dog vaccination was applied at the median rate of coverage per district to the median estimated domestic dog population.

(iii) Jackal population estimated by assigning them to grid cells with human population density between 5 and 500 per km² at a density of 0.3 jackals per km². Domestic dog vaccination was applied at the median rate of coverage per district to the median estimated domestic dog population.

(iv) Jackal population estimates kept constant from the baseline analysis (assigned to grid cells at a density of 0.3 jackals per km² with human population density between 2.5 and 500 per km² at a density of 0.3 jackals per km²). Domestic dog vaccination was assumed to be zero for the median estimated domestic dog population.

(v) Jackal population estimates kept constant from the baseline analysis (assigned to grid cells with human population density between 2.5 and 500 per km² at a density of 0.3 jackals per km²). Domestic dog vaccination was applied at the maximum rate achieved in each district.

(vi) Jackal population estimated by assigning them to grid cells with human population density between 2.5 and 500 per km² at a density of 0.15 jackals per km². Domestic dog vaccination was applied at the median rate of coverage per district to the median estimated domestic dog population.

(vii) Jackal population estimated by assigning them to grid cells with human population density between 2.5 and 500 per km² at a density of 0.5 jackals per km². Domestic dog vaccination was applied at the median rate of coverage per district to the median estimated domestic dog population

All scenarios showed a highly statistically significant ($p<0.001$) positive association between the proportion of jackals in the susceptible population and the proportion of the total cases occurring in jackals (Fig. S6).

RESEARCH OUTPUTS

(i) Publications

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(ii) Poster Presentation