Rabies in sub-Sahara Africa:

One Health-based surveillance strategy in the Kongo Central province (Democratic Republic of the Congo) and disease transmission in N'Djamena (Chad)

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Summary

Background: Rabies is a viral zoonotic disease transmitted to humans in more than 99% of cases through the bite or scratch of an infected dog (dog-mediated rabies).To date all countries on the sub-Saharan African mainland are considered endemic for dog-mediated rabies. Estimates on the worldwide burden suggest that more than a third of the annual 59'000 human rabies deaths occur in Africa, predominately in impoverished rural communities with limited access to health care.

Against this background, and in view of the global goal of eliminating human deaths from dog-mediated rabies by 2030, this PhD thesis aimed to provide new insights into the epidemiology of rabies in the DRC and rabies transmission dynamics in Chad.

Despite recurrent rabies outbreaks across the DRC, little is known about the epidemiological rabies situation in the country due to inadequate disease surveillance. In the absence of reliable incidence data, the impact of rabies on human and animal health remains obscure. Therefore, rabies is not perceived as an urgent public health problem and few resources are directed to control and prevention, contributing to endemic disease persistence.

In N'Djamena, Chad, two citywide mass dog vaccination campaigns conducted in 2012 and 2013 interrupted rabies transmission for nine months, but dog rabies cases reemerged with re-establishment of endemic rabies transmission to pre-intervention levels. It was not clear whether this was due to ongoing transmission within the city or reintroduction of rabies cases from outside the city limits.

Objectives: The objectives of this PhD thesis were to i) systematically collect data on the incidence of rabies in humans and animals and the incidence of animal bites on health facility-level, ii) estimate the owned dog population size, incidence of animal bites on community-level and assess community knowledge and practices regarding rabies, iii) contribute to validation of a rapid immunochromatographic rabies diagnostic test in the DRC, and iv) collect and analyze data on the movement and contact behavior of dogs in N'Djamena, Chad.

Methods: The PhD work applied a One Health approach to foster intersectoral collaboration and combined desk-based work with extensive field and laboratory work in a challenging research environment. A review was performed, based on published scientific literature and information obtained from rabies focal points, to provide an overview of the current rabies situation in 22 West and Central African countries and gain a better understanding of the situational context in which the research was implemented. Collection of incidence data on human and animal rabies cases and animal bites in a province of the DRC was achieved through a longitudinal observational study and strengthening of the existing rabies surveillance system using a One Health approach. In

parallel, a cross-sectional household survey was conducted to obtain estimates of the owned dog population size and gain insights into community knowledge and practices regarding rabies. In Chad, newly developed geo-located contact sensors were used to collect data on the contact and movements of dogs, which served as a basis for modeling rabies transmission dynamics.

Results: Findings from the review highlighted that most countries in West and Central Africa are still far from meeting the global goal of zero human rabies deaths by 2030 due to lack of government commitment and financial constraints. Surveillance of rabies in a province of the DRC revealed that rabies is a significant public health problem largely underestimated by official health statistics. The data generated through this study provide a sound evidence base to reflect the veterinary and public health implications of rabies and advocate for greater political commitment and policy support in rabies prevention and control. Results obtained through the cross-sectional household survey showed that the owned dog population was almost ten times larger than that assumed by local veterinary officials, with a large proportion of free-roaming unvaccinated dogs. Rabies awareness among the community was low, with knowledge gaps that result in poor practices and unnecessary rabies deaths. The rapid immunodiagnostic test validated is a promising tool for decentralized rabies surveillance in endemic settings but requires further thorough validation. In Chad, we demonstrated that the WHO-recommended dog vaccination coverage of 70% prevents major but not minor rabies outbreaks and that highly connected dogs play a critical role in transmission. Minor outbreaks may be explained by import of rabid dogs into N'Djamena from outside the city.

Conclusion: Both innovation and applied research are needed to reach the global goal of zero human rabies death by 2030, both of which were applied in this PhD thesis. We contributed vital information for rabies control in the sub-Saharan African region. Findings on the burden of rabies in humans and animals, dog ecology and community awareness in the DRC are directly relevant for veterinary and public health authorities and support planning of future rabies interventions. Applying new technologies to disease modeling helped generate new ideas on how dog vaccination could become more effective in future and be useful to set new research targets.

List of abbreviations

AfroREB:	Africa Rabies Expert Bureau
Anigen test:	Anigen Rapid Rabies Ag Test kit
CCPZ:	Centre for Control and Prevention of Zoonoses Nigeria
CDC:	United States Centers for Disease Control
CI :	Confidence Interval
CSSI :	Centre de Support en Santé International
CVL:	Central veterinary laboratory
DALY :	Disability-adjusted life years
DFA:	Direct fluorescent antibody test
DHS:	Demographic and Health Survey
DRC:	Democratic Republic of the Congo
DRIT:	Direct rapid immunohistochemical test
FAO:	Food and Agriculture Organization of the United Nations
GARC:	Global Alliance for Rabies Control
GCS:	Geo-located contact sensors
GDREP:	Global Dog Rabies Elimination Pathway
GHSA:	Global Health Security Agenda
GPS:	Global Positioning System
HDI:	Human Development Index
IBCM:	Integrated Bite Case Management
ID:	Intradermal
IDSR:	Integrated Disease Surveillance and Response
IM:	Intramuscular
INRB:	Institut National de Recherche Biomédicale Kinshasa
IP-Paris :	Institut Pasteur-Paris

List of abbreviations

IRED:	Institut de Recherche en Elevage pour le Développement
IZSVe:	Istituto Zooprofilattico Sperimentale delle Venezie
KAP:	Knowledge, Attitude and Practices
LFD:	Lateral flow device
MDV:	Mass dog vaccination
MFP:	Medical focal point
MINAGRI:	Ministry of Agriculture, Fisheries and Livestock
MPH:	Ministry of Public Health
NGO:	Non-governmental organization
NTD:	Neglected tropical disease
NZD:	Neglected zoonotic disease
ODK:	Open Data Kit
OIE:	World Organisation for Animal Health
OR:	Odds Ratio
PARACON:	Pan-African Rabies Control Network
PCR:	Polymerase chain reaction
PEP:	Post-exposure prophylaxis
PhD:	Doctor of Philosophy
PRISMA:	Preferred Reporting Items for Systematic Reviews and Meta-analyses
PWARE:	Practical Workplan towards Achieving Rabies Elimination
RABV:	Rabies Virus
REB:	Rabies Epidemiological Bulletin
RESOLAB:	Rabies Sub-network
RIDT:	Rapid immunodiagnostic test
RNA:	Ribonucleic acid
RSSI:	Received signal strength indicator

List of abbreviations

RT-PCR:	Reverse transcription polymerase chain reaction
SARE:	Stepwise Approach for Rabies Elimination
SDG:	Sustainable Development Goal
Swiss TPH:	Swiss Tropical and Public Health Institute
UHF:	Ultra-High-Frequency
UPN:	Université Pédagogique Nationale de Kinshasa
USAID:	United States Agency for International Development
VFP:	Veterinary focal point
WHO:	World Health Organization

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Patrick Martin : En tout cas ! Si Moïse l'avait vu !

Sylvain Kudiangela : Meilleur chauffeur du monde. Tokosss.

Kai Huwiler: Set the mood, it's alright!

Sanda Branca: It is a pity we have not met way earlier!

Harris Héritier : Es war so ruhig ohne dich im Büro..

Fayiz Abakar: Merci de m’avoir montré ta ville et pour les nombreuses conversations inspirantes.

Keira Spinner : z zweite macht Zug fahre vill meh Spass!

Sarah Weber: Vielen Dank, dass ich bei Dir in Kin wohnen durfte!

Wendelin Moser: E gueti inspiration damit mer s ziil nöd vor de Auge verlürt

Papa Paul et Mama Bega: Merci de m’avoir facilité la vie à Kin.

Ramona Köfler: …Ohni Wort…

Jan Hattendorf: Liest ja sowieso keiner!

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1 Thesis outline

This PhD thesis is organized into eleven chapters. The introduction (Chapter 2) provides background information supporting the rationale for the PhD research. The goals and objectives of the PhD work are summarized in Chapter 3.

Chapter 4 gives a broad overview of rabies, including rabies in wildlife, and highlights the challenges facing veterinary services for rabies control in resource-limited settings. Chapter 5 is a manuscript detailing the current rabies control landscape in 22 West and Central African countries, as submitted to *Acta Tropica*. The manuscript introduction presents recent advantages in and tools available for rabies control.

Chapter 6 and 7 present findings from the research conducted in the Democratic Republic of the Congo (DRC). They are outlined, respectively, as follows: one manuscript on rabies surveillance using a One Health approach in a DRC province, ready for submission to *PLOS Neglected Tropical Diseases;* and one publication on dog ecology, bite incidence and disease awareness among a DRC rabies-affected community, published in the journal *Vaccines*.

Chapter 8 and 9 present findings from the research conducted in Chad. Chapter 8 is a publication on the influence of dog population contact network structures on rabies transmission, published in *PLOS Neglected Tropical Diseases*. Chapter 9 is a working paper on movement and contact behavior of dogs in N'Djamena.

Chapter 10 is a book chapter on rabies as a transboundary disease in Sahelian Africa. Chapter 11 is a manuscript submitted to the *Journal of Visualized Experiments,* which presents a complete protocol for a rapid immunochromatographic rabies diagnostic test.

The last chapter, Chapter 12, gives an overview of the key findings with a discussion of the results and their implications for veterinary and public health. Finally, recommendations are provided for the human and animal health authorities in the DRC and Chad, and future research needs are identified.

2 Introduction

Infectious diseases remain the leading cause of death in sub-Saharan African countries, such as the Democratic Republic of the Congo (DRC) and Chad [1]. More than 60% of all known human pathogens and three-quarters of newly emerging infections are zoonoses spread between humans and animals [2], contributing an estimated 2.7 million human deaths and 2.5 billion cases of illness each year [3]. In recent years, outbreaks of emerging zoonotic diseases which raise concern of global spread, such as severe acute respiratory syndrome (SARS), Ebola virus disease (EVD) and highly pathogenic avian influenza, have received increased attention and repeatedly made headline news. However, it is the neglected, endemic zoonoses with low risk of pandemic spread that exert a much higher burden on human and animal health [3; 4].

Rabies, one of the oldest diseases known to humans [5], is a classic example of a neglected zoonotic disease (NZD). More than 99% of all human rabies infections are caused by rabies virus (RABV), a single-stranded, negative-sense RNA virus in the family *Rhabdoviridae*, transmitted through the bite or scratch of an infected dog [6]. After invading the peripheral nervous system at the bite site, the neurotropic RABV is transported to the brain where it causes a fatal encephalitis [7]. There is currently no cure for clinical rabies, but the disease can be prevented through timely post-exposure prophylaxis (PEP), i.e. washing the wound with water and anti-rabies vaccination [8]. PEP is indispensable to prevent rabies after potential exposures, but only control measures targeted at the animal reservoir, i.e. sustained mass vaccination of dogs, will eventually enable elimination of the disease. While dog-mediated rabies has been largely controlled in high-income countries, millions of people remain at risk of exposure in developing nations [6].

To date, every country on the sub-Saharan African mainland is considered as endemic for rabies. It is estimated that about 21’500 people die annually of rabies across the African continent [9], which is a death toll twice as high as that caused by the largest ever reported EVD outbreak in West Africa in 2014-15 [10]. One of the main drivers of endemic persistence of rabies in sub-Sahara Africa is the absence of reliable epidemiological data to demonstrate the true impact of rabies on human and animal health and advocate for greater investment in rabies control [11-13]. Rabies primarily affects impoverished, rural communities [14] with limited access to health care rendering disease surveillance extremely difficult, with many cases going unnoted [11; 12].

2.1 Rabies surveillance

Systematic surveillance is crucial to generate high-quality epidemiological data for a given disease [15]. In the World Health Organization (WHO) International Health Regulations (IHR) surveillance

is defined as “the systematic ongoing collection, collation and analysis of data for public health purposes and the timely dissemination of public health information for assessment and public health response as necessary” [16]. Effective rabies surveillance systems ideally embed a One Health approach, which fosters communication between human and animal health sectors, and are built upon decentralized, laboratory-based surveillance, together with clearly defined disease indicators and case definitions [17; 18]. When transitioning from a rabies-endemic to a post-elimination phase, the objectives of surveillance change and need to be adapted accordingly. Franka & Wallace [18] identify five stages of rabies surveillance. The first stage consists of determining rabies burden in human and animal populations (proof of burden). At the second stage, surveillance helps prevent human deaths from rabies by linking human exposures (a bite or scratch from a suspected rabid animal) and rabies diagnosis in animals to guide recommendations for human PEP. At the third stage, surveillance monitors and assesses implemented measures for prevention and control of rabies. At the fourth stage, surveillance helps to declare freedom from disease. At the fifth stage, after rabies elimination, surveillance must be maintained to ensure early detection of reintroduced rabies cases. Currently, most countries in sub-Sahara Africa are at the first stage of rabies surveillance (rabiesalliance.org/networks/paracon/ bulletin and Chapter 5).

2.1.1 Disease modeling to assist rabies control

Data generated through surveillance can be used to develop and validate epidemiological models. Such models can help to better understand the dynamics of infectious diseases and anticipate the spread of an epidemic. Simulation of different intervention strategies, e.g., mass vaccination, facilitates assessment of the impact of these interventions on human and animal health.

Most epidemiological models of rabies transmission followed ordinary differential equation (ODE) mass-action models introduced by Kermack and McKendrick in the 1920s [19]. These models divide the host population into compartments (e.g., susceptible (S), infectious (I), and recovered (R), SIR), with the underlying assumptions that each infectious individual can infect any susceptible individual with the same probability and that the population in each compartment is completely homogeneous.

Infectious disease modeling using a contact network approach has only recently gained the attention of epidemiologists [20]. Compared to SIR models, network models are appealing as they incorporate a more realistic property of heterogeneity among host contacts. Heterogeneity of contacts among hosts are important drivers of infectious disease dynamics [21], and failure to incorporate relevant properties of network structures may result in faulty predictions of disease spread [22; 23]. Network modeling is based on graph theory [24]. In its simplest form, a network (graph)

consists of a set of nodes and a set of edges (links or connections). In infectious disease modeling, the nodes usually represent the hosts of a disease and the edges correspond to contacts between the hosts. Diseases spread from an infected node to another node along the edges. An edge is “directed” if transmission of the disease only occurs in one direction (indicated through an arrow) or “weighted” if the connection is associated with a number, or value, for example the frequency or duration of a contact (Figure 1)

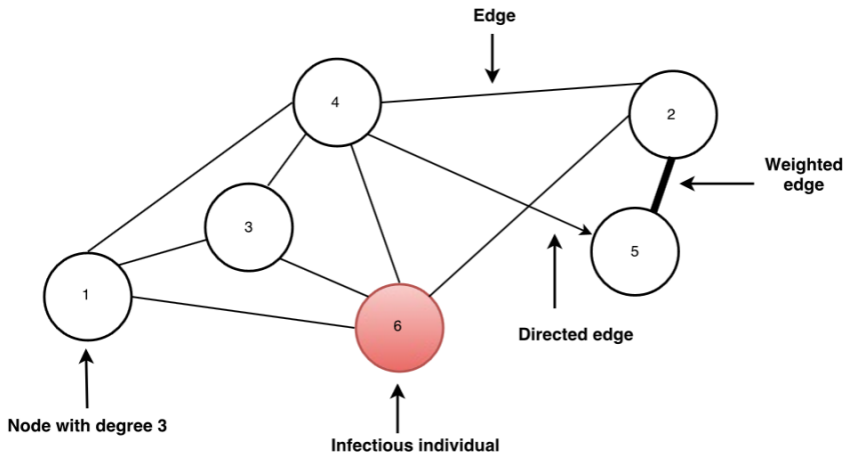

Figure 1: Illustration of a simple network with 6 nodes and 10 edges

The importance of a node within a network can be quantified by centrality metrics, such as degree, closeness and betweenness centrality [25]. The degree centrality of a node is the number of edges connected to that node. Closeness centrality looks at how close a node is to all other nodes in the network and is calculated as the inverse of the sum of the length of all shortest paths between the node and all other nodes in the network. Finally, the betweenness centrality is the proportion of all shortest paths between any two nodes in the network that pass through that node (i.e., it answers the question how often does a node lie on the shortest path between two other nodes). Additional centrality metrics include eigenvectors, katz, random-walk and harmonic centrality. Depending on the overall network structure, each of these centralities can be associated with the time to infection of a node [25-27] and can, therefore, provide useful information for targeted interventions. While centrality metrics quantify the importance of individual nodes, modularity is a measure of the network structure as a whole. Grouping a network into strongly interconnected nodes can reveal community structures that may be relevant for disease transmission [28]. Insight into the expected course of an epidemic in a given network is often gained through individual-based simulations. In comparison to SIR models, these simulations have the advantage of providing a point estimate of the expected outbreak size and of quantifying the uncertainty around that estimate.

The centrality metrics allow for characterizing networks. To make use of contact networks for transmission modeling, percolation theory contributes to an analogon from physics by estimation of the giant component of a network, reflecting the largest number of connected nodes within a network, which is interpreted as a large disease outbreak. Effective simulation of transmission dynamics using contact networks is best done using individual-based stochastic models (Chapter 8).

2.2 Tackling rabies through a One Health approach

Rabies is a prime example of a zoonotic disease that is best controlled through a One Health approach [29]. Zinsstag *et al.* [30] define One Health as "*any added value in terms of human and animal health, financial savings or sustained environmental benefit from closer cooperation of human and animal health sectors at all levels of organization.*"

Decades of experience have shown that mass dog vaccination is the most cost-effective approach for sustainable elimination of dog rabies and subsequent reduction of human disease fatalities [31-36]. Direct and indirect costs associated with PEP comprise about 35% of the overall economic impact of rabies [9]. In the absence of dog vaccination, the cumulative cost of PEP in humans continues to increase because PEP alone will never interrupt rabies transmission. A study conducted by Mindekem *et al.* [36] clearly showed a lower cumulative cost of disease intervention in the animal reservoir compared to the cumulative cost of human PEP only. The authors compared the costs of human PEP alone versus MDV combined with PEP and forecast break-even cost 6-13 years after onset of intervention if rabies is not re-introduced. Demand for PEP is likely to remain level or even increase after the start of MDV campaigns because of increased community awareness [37]. Assuming ideal communication between the animal and human health sectors (One Health communication), Mindekem *et al.* [36] showed that the costs for dog vaccination and PEP compared to PEP alone break even 6 years after the start of intervention. Therefore, effective communication between the human and animal health sectors is necessary to translate the benefits of dog vaccination into financial savings through appropriate use of PEP. Integrated Bite Case Management (IBCM) helps to differentiate true rabies exposures from non-rabid bites through follow-up investigation of the biting animal and prevents unnecessary use of costly human PEP [38].

In many developing countries, lack of infrastructure for rabies diagnosis prevents appropriate surveillance. Human diagnostic facilities are often better equipped than veterinary laboratories, and costs for rabies diagnosis can be reduced through sharing diagnostic structures, which was implemented in the context of this PhD work (Chapter 6).

2.3 Study sites

Data collection for this PhD work was conducted in the Democratic Republic of the Congo and Chad, two of the poorest countries in the world, ranking 176 and 186, respectively, out of 189 on the Human Development Index (HDI) and falling into the low human development group (HDI ≤ 152) [39]. Both countries are known for political instability, which presents a challenging research environment [40].

2.3.1 Democratic Republic of the Congo

Chapter 5 presents a detailed description of the current rabies situation in the DRC.

Research in the DRC was undertaken in the Kongo Central province in collaboration with the National Institute of Biomedical Research (INRB) and the veterinary faculty of the National Pedagogical University (UPN).

2.3.2 Research gaps and study rationale

Over the past few years, several rabies epidemics occurred in the Kongo Central province, but the scale of these outbreaks is unknown due to inadequate disease surveillance. Aside from generally weak and underfunded human and animal health systems [41], we identified major constraints to effective rabies surveillance during an exploratory visit to the Kongo Central province in July 2016:

- Weak links between the Ministry of Public Health (MPH) and the Ministry of Agriculture, Fisheries and Livestock (MINAGRI) resulting in discrepancies of reported data
- No intersectoral collaboration in the counseling of bite victims
- Low rabies awareness among medical staff and the population
- No secured supply circuit for human rabies vaccine
- Very high cost for human rabies vaccine (~50-55 USD per dose) of uncertain origin, with WHO prequalified vaccine only available in the capital city Kinshasa
- Chronic lack of office supplies and sampling equipment
- No transportation network for animal samples
- Rabies diagnostic capacity limited to Kinshasa

Recent estimates suggest that the DRC has the highest annual number of human rabies deaths (~5600) on the African continent and the third highest worldwide [9]. In the DRC's Integrated Disease Surveillance and Response (IDSR) strategy, rabies is listed among the 17 priority diseases with weekly mandatory summary notifications [42]. However, a recent study found that rabies data

captured through the IDSR should not be used for epidemiological research or public health purposes due to the low level of adequacy to reflect actual morbidity [43].

Against this background, and in view of the global goal of eliminating human deaths from dog-mediated rabies by 2030 [44], there is a need to generate reliable data on the burden and epidemiology of rabies in humans and animals in the DRC to help in designing appropriate rabies interventions and advocate for greater political commitment in rabies control.

2.3.3 Chad

Chapter 5 provides a detailed description of the current rabies situation in Chad.

Since 2000, the Swiss Tropical and Public Health Institute (Swiss TPH) has been involved in research on control of dog-mediated rabies in N'Djamena, the capital city of Chad, in close collaboration with two Chadian partner institutions (the Institut de Recherche en Elevage pour le Développement (IRED) and the Centre de Support en Santé International (CSSI)). Great progress has been made in rabies diagnosis [45; 46], surveillance [47-49] and dog vaccination [34; 36; 50-55].

2.3.4 Research gaps and study rationale

A deterministic ODE rabies transmission model developed by Zinsstag *et al.* [54] suggested that dog rabies could be eliminated in N'Djamena through a single mass dog vaccination (MDV). In 2012 and 2013, two citywide MDV campaigns were conducted in N'Djamena, reaching, in both years, the recommended coverage of >70% to interrupt RABV transmission [55; 56]. However, nine months after the end of the second MDV campaign, dog rabies cases started to reemerge with re-establishment of endemic rabies transmission to pre-intervention levels. It was not clear whether this was due to ongoing transmission within the city or reintroduction of rabies cases from outside the city limits, as has been observed, for example, in Bangui (Central African Republic) [57].

Given the resurgence of rabies cases, further investigation of rabies transmission dynamics in N'Djamena is needed to improve the strategy of upcoming vaccination campaigns. We do not have thorough knowledge of dog contact networks in African cities, nor is it known to what extent the contact structure among dogs influences the spread of rabies. Therefore, we developed geo-referenced contact sensors (GCS) that integrate both GPS and proximity loggers, together with a spin-off company of the ETH Zürich (Bonsai Systems), to study the contact and movement behavior of dogs.

Picture 1 Prototypes of housings for a GCS printed with 3D printing technology

Picture 2: Dogs in N'Djamena equipped with GCS

3 Goal and objectives

The overarching goal of this research was to provide new insights into the epidemiology and transmission of rabies in two sub-Saharan African countries, the DRC and Chad. A review was performed, based on published scientific literature and information obtained from rabies focal points, to provide an overview of the current rabies situation in 22 West and Central African countries and to gain a better understanding of the situational context in which the research was implemented (Chapter 5).

3.1 Democratic Republic of the Congo

The goal of the research conducted in the DRC was to strengthen rabies surveillance using a One Health approach to provide reliable data on the current epidemiological situation of rabies in the Kongo Central province. Towards this goal, and in view of future MDV and public awareness-raising campaigns, a household survey was conducted to obtain estimates of the dog population size and gain insights into community knowledge and practices regarding rabies.

3.1.1 Objectives

The objectives were to:

- Systematically collect data on the incidence of rabies in humans and animals and animal bite incidence on health facility-level (Chapter 6).
- Conduct a cross-sectional household survey to estimate the owned dog population size, assess community knowledge and practices regarding rabies and provide a community-level estimate of animal bite incidence (Chapter 7).
- Contribute to the validation of a rapid immunochromatographic rabies diagnostic test (Chapter 11)

3.2 Chad

The goal of the research in Chad was to use newly developed GCS to study the contact network of dogs for modeling dog rabies transmission dynamics to improve the strategy of future vaccination campaigns.

3.2.1 Objective

The objective was to:

- Collect and analyze data on the movement and contact behavior of dogs in N'Djamena (Chapter 8 and 9).

4 Rabies: a veterinary perspective – Description of rabies in dogs, livestock and wildlife

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4.1 Summary

While major progress has been made in the control of rabies in the Western Hemisphere, large parts of Europe and some parts of Asia, the disease continues to kill tens of thousands of people every year. Its highest burden is in resource-limited countries in Asia and Africa, disproportionately affecting children and poor rural communities. Today, domesticated dogs are responsible for the vast majority of human rabies cases. In late 2015, rabies experts from around the world gathered at the Rabies Global Conference in Geneva, Switzerland, and launched the ambitious initiative to end deaths from dog-mediated human rabies by 2030.The most cost-effective and sustainable approach to achieve this goal is to eliminate the disease at source through mass dog vaccination.

In this article, the role of and challenges faced by Veterinary Services in resource-limited settings in implementing the dog vaccination strategy to reduce the human rabies burden are discussed, together with the role of wildlife in disease control and why the 'One Health' approach is indispensable on the path towards a dog rabies-free future.

Keywords: Control, Dog rabies, One Health, Prevention, Rabies, Sylvatic rabies, Urban rabies**,** Veterinary medicine.

4.2 Résumé

Malgré les progrès considérables accomplis en matière de lutte contre la rage dans l'hémisphère occidental, dans une grande partie de l'Europe et en certains endroits d'Asie, la maladie continue à faire plusieurs dizaines de milliers de victimes chaque année dans le monde. Ce sont les pays à faibles ressources d'Asie et d'Afrique qui sont les plus touchés, avec une majorité écrasante de victimes parmi les enfants et dans les communautés rurales pauvres. Aujourd'hui, les chiens domestiques sont de loin la principale cause des cas de rage humaine. En décembre 2015, des experts du monde entier réunis à Genève (Suisse) à l'occasion de la Conférence mondiale sur la rage intitulée « Élimination mondiale de la rage humaine transmise par les chiens : agissons maintenant ! » ont lancé une initiative ambitieuse visant à mettre fin aux décès humains dus à la rage transmise par les chiens d'ici 2030. La méthode la plus efficace et durable pour atteindre cet objectif consiste à éliminer la maladie à sa source au moyen de la vaccination massive des chiens.

Les auteurs examinent le rôle des Services vétérinaires et les difficultés auxquelles ceux-ci sont confrontés lorsqu'ils entreprennent d'appliquer une stratégie de vaccination des chiens destinée à réduire le fardeau de la rage humaine dans un contexte de ressources limitées. Ils évoquent également l'importance de prendre en compte la faune sauvage dans le cadre du contrôle de la

rage et expliquent en quoi l'approche « Une seule santé » est incontournable pour avancer vers l'objectif d'un monde indemne de rage canine.

Mots-clés : Médecine vétérinaire, Mesures de lutte, Prévention, Rage, Rage canine, Rage sylvatique, Rage urbaine, Une seule santé.

4.3 Resumen

Aunque la lucha antirrábica ha conocido avances muy sustanciales en el hemisferio occidental, grandes partes de Europa y ciertas zonas de Asia, la enfermedad sigue matando a decenas de miles de personas al año. La carga más elevada de rabia se da en países con escasos recursos de Asia y África, donde la enfermedad afecta desproporcionadamente a los niños y a las comunidades rurales pobres. A día de hoy, los perros domésticos son responsables de la inmensa mayoría de los casos de rabia humana. A finales de 2015, especialistas del mundo entero se dieron cita en Ginebra (Suiza) para celebrar la conferencia mundial titulada «Eliminación mundial de la rabia humana transmitida por perros. ¡Actuemos ahora!» y poner en marcha la ambiciosa iniciativa de acabar con las muertes por rabia transmitida por perros como muy tarde en 2030. Para cumplir este objetivo, el método más sostenible y más eficaz en relación con el costo consiste en eliminar la enfermedad en su foco de origen, procediendo para ello a vacunaciones masivas de perros.

Los autores exponen la función de los Servicios Veterinarios y las dificultades que afrontan en situaciones de escasez de recursos a la hora de aplicar la estrategia de vacunación canina para reducir la carga de rabia humana, así como el papel de la fauna silvestre en el control de la enfermedad y la razón por la cual es indispensable aplicar los planteamientos de «Una sola salud» para avanzar hacia un futuro libre de rabia.

Palabras clave: Control, Medicina veterinaria, Prevención, Rabia, Rabia canina, Rabia selvática, Rabia urbana, Una sola salud

4.4 Introduction

The unique behavioral and cognitive abilities of dogs have long been appreciated by humans. The relationship between dogs and humans represents the oldest and most widespread form of interspecies bonding. The latest research on dog evolution suggests the onset of dog domestication to have occurred some 20,000 to 40,000 years ago during the last glacial period [58], while the location in which this took place remains subject to debate [59-62]. In human society, dogs master a variety of complex tasks ranging from assisting hearing- and vision-impaired people to being

used in law enforcement, hunting, animal therapy or animal powered transport. However, in addition to all the positive impacts of such close dog–human interactions, there also lurks the threat of animal–human disease transmission and potential adverse implications on public health. For millennia, dogs have been recognized as a source of one of the most lethal infectious diseases known to humankind: rabies [5].

Numerous wild carnivores and bats represent reservoirs for the rabies virus (RABV) (sylvatic rabies) but today almost all human rabies cases are caused by the bite of infected domesticated dogs (so-called urban rabies), and hence dog maintained RABVs are the most important target of control measures.

In some European countries, it was possible to eliminate dog-mediated human rabies at the beginning of the 20thcentury solely through enhanced legislation on responsible dog ownership (e.g. dog registration or ownership tax) and the strict implementation of veterinary sanitary measures such as stray dog population management or quarantine [63]. Europe-wide, excluding Turkey, the elimination of dog rabies was finally achieved with the development of animal rabies vaccines in the 1920s and their application in parenteral mass dog vaccination campaigns [63; 64]. However, rabies persisted in terrestrial wildlife (i.e. red foxes) and quickly spread across the European continent [63]. It became rapidly apparent that counteractive measures aimed at reducing the fox population density below the threshold that could maintain infection were inefficient in controlling the disease, triggering the need for alternative intervention strategies [63; 64]. Large parts of Western and Central Europe are deemed free of terrestrial wildlife rabies, owing to the development and sustained implementation of large-scale oral rabies vaccination programs, co-financed by the European Union (EU) [65].

The situation is similar in North America, where dog-mediated human rabies has been successfully eliminated but the control of sylvatic rabies remains especially difficult, owing to the occurrence of several primary host cycles [66; 67].

In Latin America and the Caribbean (LAC), the number of human rabies cases is at its lowest since the beginning of a regional dog rabies elimination program in 1983 coordinated by the Pan American Health Organization (PAHO) [31]. The reintroduction of RABV into the dog population through spillover from wildlife (and vice versa) poses a major challenge on the path towards dog rabies elimination in LAC, the target for which has been newly set to 2022 [31; 67-69]. With the sharp decline in human cases associated with dogs, vampire bat RABV transmitted to humans and domesticated animals has assumed increasing epidemiological importance [70]. Bats have

become the leading cause of human rabies cases reported in this region [70-72] and cause substantial economic losses in the livestock industry [73].

More than 95% of the annually estimated 25,000 to 159,000 dog-mediated human rabies deaths occur in resource-limited countries in Asia and Africa [9], disproportionately affecting children under 15 years of age and poor, marginalized, rural communities [6]. Owing to globalization, the inadequate control of dog rabies in endemic areas poses an ever-present threat to rabies-free countries. Specifically, illegal trade in infected animals between rabies-free countries and endemic areas generates the need for costly enhanced border control, quarantine or follow-up of illegal imports [74; 75]. Moreover, many popular tourist destinations are endemic for dog rabies and travellers unwittingly put themselves at risk of potential exposure to the virus through bites or scratches from infected animals [76; 77].

In late 2015, a strategic global framework to put an end to unnecessary deaths from dog-mediated human rabies by 2030 was jointly launched by the World Organisation for Animal Health (OIE) and the World Health Organization (WHO), in collaboration with the Food and Agriculture Organization of the United Nations (FAO) and the Global Alliance for Rabies Control (GARC) [78]. The ambitious initiative centers on a 'One Health' approach emphasizing that freedom from dog rabies is a feasible objective, which can be attained with currently available tools [78].

Consequently, the principal challenges, from a veterinary perspective, which must be overcome on the path towards the elimination of dog-mediated human rabies in resource-limited settings are discussed below.

4.5 Challenges for rabies control in resource-limited settings

Since RABV is transmitted through the bites of infectious animals, the rate of dog-to-dog transmission depends on: *i)* the population density of susceptible dogs, *ii)* the contact rate between dogs, *iii)* the probability of RABV transmission from the saliva of the biting dog to the brain of the victim, and *iv)* the average time before rabid dogs are killed or die. The potential for RABV transmission can therefore be reduced by mass dog vaccination campaigns (which reduce the population density of susceptible dogs) and adequate surveillance response systems. Such surveillance response systems quickly identify rabid dogs and remove them from the population, thereby shortening the average duration of the infectious period. Culling dogs may be effective in the short term in reducing the transmission potential, but the effectiveness often wears off rapidly because dog populations tend to regenerate quickly through compensatory breeding [79] and the translocation of dogs by humans in response to culling campaigns might also occur [80].

As rabies prevalence levels in dogs are usually very low, random chance events may play crucial roles in the recurrence (or not) of rabies epidemics. Transmission at these low prevalence levels may be sustained by repeated infection from wildlife species or the geographical movement of latently infected or rabid dogs [81]. Little is known thus far about the geographical translocation of RABV variants through human-mediated animal movement at smaller scales in rabies-endemic regions. A probable explanation for dispersal at short distances could be dogs accompanying their owners for hunting, which also involves the risk of contact with wildlife and potential RABV transmission. Dispersal at intermediate distances within countries was observed to be mediated by the exchange of puppies between relatives living in different geographical locations (data not shown). Phylogenetic studies on the spatial distribution of RABV in dogs in North Africa showed that the RABV strain spread was best explained by road distances, instead of actual geographical distances, pointing towards human-mediated movements of infected animals [82]. Finally, very long distances especially in Sahelian belt countries are covered by herding dogs accompanying nomadic pastoralists on their transhumance [83], which in many cases involves the crossing of borders [84]. Therefore, the control of rabies in dogs calls for national legislation on mandatory dog registration and identification, and international regulations on the movement of dogs. Such regulations are often not reinforced or do not exist in rabies-endemic countries, owing to the poor performance of Veterinary Services and a lack of appropriate legal frameworks. A fully functioning veterinary system is indispensable in long-term dog rabies control and in the uptake of dog vaccination into routine daily veterinary practice in order to maintain high vaccination coverage.

However, service is a function of supply and demand. Routine dog vaccination will only be successful if dog owners are willing to regularly seek veterinary services. Numerous *ad hoc* assumptions are made about the reasons for the low demand for routine veterinary services such as vaccination, anti-parasitic treatment or castration in resource-limited countries. Cost considerations, insufficient quality of service or the lack of value of dogs are just some of these popular assumptions. These explanations certainly do not go far enough, but there is a notable absence of in-depth studies on the value of dogs in different cultural contexts. Undoubtedly, the value and roles of dogs are extremely varied across different socio-cultural backgrounds: they are kept as work animals (guard, herding or hunting dogs), reared as companion animals or bred for consumption. Religion has been reported to have a strong influence on dog ownership and handling [55; 85; 86]. Hence, efficient rabies control through dog vaccination needs to involve the careful evaluation of socio-cultural backgrounds and values so as to reach a high level of community acceptance [87; 88]. The culling of dogs is undoubtedly considered to be an unacceptable intervention by dog owners [80]. Also, the restriction of dog ownership to populations that can afford

appropriate nutrition for their animals is controversial, owing to the need for guard dogs, especially in socio-economically weak neighborhoods with a high crime rate. Similarly, a restriction in the number of dogs permitted per owner will trigger opposition in communities where dogs are used for livestock guarding, hunting or, most importantly, as a source of food. This illustrates the complexities and challenges that any regulation or control program will encounter. Despite the fact that the proposition of responsible dog ownership is an initiative worth supporting, questions remain as to how to achieve it in the different contexts described.

Another challenge faced is the neglect of dogs by the animal health sector as their diseases are not of agricultural importance. The limited financial resources allocated to the animal health sector by governments are mainly devoted to the prevention of diseases in economically valuable livestock species such as cattle, goats or pigs [29], even though rabies can cause important economic losses in livestock-dependent communities [9]. Private veterinarians working in resource-poor or remote communities have little incentive to invest their capacities in the treatment of dogs owing to the inability, or sometimes reluctance, of dog owners to pay for their services [52; 89]. The widespread neglect of dogs and the chronic underfunding of animal health services in general, result in adverse effects on the human health sector. Half of the global human population is at risk of exposure to dog rabies. Moreover, the highest economic burden of such exposure falls upon the human health sector owing to the expensive post-exposure prophylaxis (PEP) associated with bites or scratches from suspected rabid animals [9]. Recent studies clearly show that, while the cumulative cost of PEP in humans is ever-increasing, that of mass dog vaccination combined with PEP could reach the break-even point within ten to 20 years, and that mass dog vaccination remains less costly if rabies is not reintroduced [36; 54].

The insufficient importance placed on dogs and their diseases also has significant repercussions on the health of wildlife. As control efforts progress towards the elimination of dog rabies, wildlife is sometimes perceived by the public as the ongoing offender. Rabies affects population dynamics and can pose a serious threat to biodiversity, being involved in notable declines in carnivore populations with endangered or threatened statuses. Repetitive rabies outbreaks in the world's rarest canidae, the Ethiopian wolf (*Canis simensis*), endemic to the Ethiopian Highlands, led to extensive mortalities in the wolf population [90; 91]. Similarly, the endangered wild dog (*Lycaon pictus*) has seen its population dramatically decrease during rabies outbreaks in the Serengeti ecosystem (Tanzania) and South Africa [92; 93]. In both cases, the virus was transmitted by domesticated dogs. Understanding the behavioral ecology of the wildlife species potentially involved in rabies outbreaks is of paramount importance, particularly in the wake of rapid anthropogenic and natural environmental changes, which are likely to affect their interactions with domestic reservoirs and

humans. Several studies have provided evidence for rabies exposure (i.e. protective levels of antibodies against rabies) in wildlife species that did not go on to develop the disease. These species include: lions in Zambia (*Panthera leo*) [94]; spotted hyenas in Serengeti, Tanzania (*Crocuta crocuta*) [95]; bats [96; 97]; the Arctic fox [98]; and several terrestrial wildlife mammals in Brazil, including New World monkeys (such as the capuchin [*Cebus* spp.], marmosets [*Callithrix* spp.] and black howler monkeys [*Alouatta caraya*] [99; 100].

Some wildlife species may therefore play a new role in the existing sylvatic cycles. Virus variants were sometimes shown to be species-specific [100-103]. Thus, the epidemiology of rabies can be complex and needs to be thoroughly understood before control programs are embarked upon. This close interlinkage between human health and animal health and welfare highlights the importance of a strong multisectoral collaboration for the elimination of dog rabies. An extended 'One Health' approach to control dog rabies at its source through mass dog vaccination can:

a) protect public health
b) reduce the economic impact on the public health sector owing to reduced demand for costly human vaccines
c) improve animal health and welfare
d) contribute to wildlife conservation.

The recently postulated goal of zero human rabies deaths by 2030 has caused the pursuit of dog rabies control to gather momentum and will hopefully lead to investment by national governments and international organisations in dog rabies elimination programmes based on dog vaccination. Financial savings for the public health sector can be one of the drivers for such an investment. Sustainability plays a key role in the success of elimination programmes as benefits to the human health sector from dog vaccination may take several years to accrue and donor fatigue has to be anticipated [36; 54].

Several aspects important for sustainability, such as strengthening of routine veterinary practices, sensitive socio-cultural approaches and the reinforcement of legislation, have already been discussed. Another aspect is the regional coordination of control activities to ensure that the elimination efforts of one country are not jeopardised by the reintroduction of rabies in a neighbouring country [104]. The establishment of the Pan-African Rabies Control Network (PARACON) in 2014 under the GARC secretariat is the first important step towards a unified regional approach to disease elimination and efficient cross-continental collaboration in Africa. Although joint approaches are favourable for ensuring the sustained interruption of rabies transmission, if all endemic countries strive towards mass dog vaccination at the same time, sufficient resources of personnel,

vaccines, etc. will be lacking [105]. Regional networks like PARACON will play an important role in terms of the coordination of joint geographical approaches and the regulation of resource allocation. Moreover, the lack of appropriate funding often hampers the implementation of large-scale elimination programmes in resource-limited settings. Novel sustainable investment mechanisms such as development impact bonds (DIBs) are required to mobilise 'upfront' capital. Such funding will help to achieve high-level dog vaccination coverage and ensure sustained financial support because economic benefits to the human health sector from dog vaccination may take several years to become apparent [54; 106].

In the absence of mass dog vaccination, the only measure to prevent human rabies deaths is complete coverage with PEP. The manufacture of human rabies vaccines is currently insufficient to meet demand should every bite victim in endemic countries receive PEP. This demand can be reduced through careful exposure assessment. The true proportion of rabies bites among the overall number of dog bites occurring in a given setting is often not known, but is not expected to exceed 10% [37]. To differentiate true rabies exposure from non-rabies bites, collaboration between the human health and the veterinary sectors through an integrated bite-case management (IBCM) approach is imperative [33; 38]. The routine observation of biting animals and animal rabies diagnosis has to be reinforced by public sensitisation. Enhanced rabies awareness among the community ensures that bite incidents and suspected animal and human rabies cases are reported to the relevant authority. Health personnel need to be trained in the assessment of the rabies risk of bite incidents in communication with veterinarians in order to reduce the application of unnecessary PEP. This interaction ideally also triggers further bite investigations or animal observation and hence improves animal surveillance. The absence of an IBCM can lead to a continued high demand for PEP and can thus minimise the economic benefits for the public health sector, as experienced in Chad [37].

4.6 Conclusions

Decades of experience have shown that mass dog vaccination is a highly cost-effective approach for the sustainable elimination of dog rabies and the subsequent reduction of human disease fatalities [31-33], whereas the application of PEP alone to prevent human deaths would lead to ever-increasing costs [54]. Mass dog vaccination is an essential low-cost, high-impact measure to realise the vision of zero human rabies deaths by 2030. There is an urgent need to strengthen animal health systems in resource-limited settings and to extend their services to small animals (i.e. dogs and cats) in order to attain dog vaccination coverage sufficient to interrupt virus transmission and

ensure continued disease surveillance. Public sensitisation adapted to the socio-cultural background is critical for the success of dog vaccination and for community participation in the reporting of bite incidents and detection of rabies cases. The One Health approach recognises that the health of animals and humans are interdependent and dog rabies control provides one of the best examples to showcase the benefits of such an integrated approach. Effective intersectoral communication and collaboration will optimise the administration of costly PEP and can lead to large financial savings. Wildlife is an essential part of the One Health equation when it comes to rabies surveillance and control programmes. It is, however, essential that the messages from One Health expert bodies are not ultimately detrimental to wildlife conservation efforts. Conservationists and veterinary bodies have to work together in disease surveillance and control. Conservationists often lack the resources to address and manage rabies outbreaks, whereas veterinarians lack ecological perspective on the disease.

Sustainability is a crucial component in the planning of successful dog rabies control programmes and to maintain rabies-free status once the disease is eliminated. Widespread successes in dog rabies control in South America by PAHO highlight the need for regional collaboration between countries to mitigate the risk of rabies incursion from one country to another [104]. Sustainable funding sources are a precondition to secure the success of control efforts. Development impact bonds present an innovative opportunity to overcome financial constraints.

A variety of tools are available to help countries to design, implement and steer dog rabies prevention and control programmes incorporating a One Health strategy. The Stepwise Approach towards Rabies Elimination (SARE) tool provides a standard mechanism through which countries can assess the status quo of dog-mediated human rabies and measure progress in eliminating the disease [107]. *The Blueprint for Rabies Prevention and Control* and *A Blueprint for Rabies Surveillance* are operational tool-kits providing practical guidance and summarising all relevant information in one freely available document [108; 109]. The practical application of such tools needs to be adapted to the socio-cultural background in order to guarantee the participation of the community and to achieve high community acceptance.

Difficulties may arise in answering the question of whether the human or animal health sector is responsible for leadership in the implementation of One Health interventions. Although zoonotic diseases account for 60% of all infectious diseases and 75% of emerging diseases [2], they only make up a small proportion of all the diseases addressed by the human health sector. Rabies is often not prioritised by the human health sector as other diseases may be considered more urgent or may have more direct economic importance. However, zoonotic diseases are of primary concern to the animal health sector in developing countries and thus animal health workers should

take the lead in One Health interventions in close partnership with public health and other related sectors. The incorporation of One Health thinking into the development of successful dog rabies elimination strategies is not only imperative in order to achieve the 2030 goal but the established intersectoral collaboration will also be beneficial for the control of other zoonoses and will enhance the capacity to optimally respond to newly emerging diseases in humans and animals.

5 Dog rabies control in West and Central Africa: A review.

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5.1 Abstract

Rabies is a neglected but preventable zoonotic disease that predominantly affects the most vulnerable populations living in remote rural areas of resource-limited countries. To date, every country on the African mainland is considered endemic for dog-mediated rabies with an estimated 21'500 human rabies deaths occurring each year. In 2018, the United Against Rabies collaboration launched the Global Strategic Plan to end human deaths from dog-mediated rabies by 2030. The epidemiology of rabies from most Western and Central African countries remains poorly defined, making it difficult to assess the overall rabies situation and progress towards the 2030 goal.

In this review, we attempt to provide an overview of the current rabies situation in 22 West and Central African countries based on published scientific literature and information obtained from rabies focal points. To this end, information was collected on i) diagnostic capacity, ii) established surveillance, iii) animal and human health communication (One Health), iv) dog population estimates, v) national control strategies, vi) cost estimates, vii) dog vaccination campaigns, viii) PEP availability and coverage, ix) Knowledge, Attitude and Practices (KAP) and x) molecular studies.

Although rabies is a notifiable disease in the majority of the studied countries, national surveillance systems do not adequately capture the disease. A general lack of rabies diagnostic capacity has an additional negative impact on rabies surveillance and attempts to estimate rabies burden. Recurrent shortages of human rabies vaccine are reported by all of the countries, with vaccine availability usually limited to major urban centers but no country has yet adopted the new WHO-recommended 1-week intradermal vaccination regimen. Most countries carry out subsidized mass dog vaccination campaigns on World Rabies Day. Such activities are indispensable to keep rabies in the public consciousness but are not of the scale and intensity that is required to eliminate rabies from the dog population. Countries will need to scale up the intensity of their campaigns, if they are to progress towards the 2030 goal. But more than half of the countries do not yet have reliable figures on their dog populations. Only two countries reached stage 2 on the Stepwise Approach towards Rabies Elimination ladder – indicating that their national governments have truly prioritized rabies elimination and are thus providing the necessary support and political buy-in required to achieve success.

In summary, the sub-region of West and Central Africa seems to be divided into countries that have accepted the challenge to eliminate rabies with governments committed to pushing forward rabies elimination, while other countries have achieved some progress, but elimination efforts remain stuck due to lacking government commitment and financial constraints. The possibility to meet the 2030 goal without international solidarity is low, because more than two-thirds of the

countries rank in the low human development group (HDI ≤ 152). Leading countries should act as role models, sharing their experiences and capacities so that no country is left behind. Unified and with international support it is possible to reach the common goal of zero human rabies deaths by 2030.

Keywords: Rabies, Africa, prevention, control, zoonosis, dog

5.2 Introduction

Africa is one of the continents hardest hit by rabies, accounting for more than a third of the estimated 59'000 human deaths worldwide annually [9]. Rabies, a viral disease transmitted through the bite or scratch of an infected animal, causes an inevitably fatal infection of the brain if progression is not prevented through prompt administration of post-exposure prophylaxis (PEP). Domestic dogs act as the single most important *rabies lyssavirus* (RABV) reservoir, responsible for the vast majority of human rabies cases [110]. To date, every country on the African mainland is considered endemic for dog-mediated rabies.

In 2018, the United Against Rabies collaboration (UAR), consisting of four international organizations, the World Health Organization (WHO), World Organisation for Animal Health (OIE), Food and Agriculture Organization of the United Nations (FAO) and Global Alliance for Rabies Control (GARC), launched the *Global Strategic Plan to end human deaths from dog-mediated rabies by 2030* [44], a goal set in late 2015 following the Global Rabies Conference in Geneva [78]. This target is fully aligned with the United Nations Sustainable Development Goal (SDG) No. 3: “ensure healthy lives and promote well-being for all at all ages”, specifically contributing to point 3.3: “end the epidemics of AIDS, tuberculosis, malaria and neglected tropical diseases and combat hepatitis, water-borne diseases and other communicable diseases by 2030”. In this three-phase strategic plan, UAR appeals to countries' own initiative, providing guidance and support to put generated rabies expertise and tools into action. The objectives are (1) to prevent rabies deaths through increased awareness, dog vaccination and improved access to PEP, (2) to provide guidance and generate reliable data to measure impact and inform policy decisions, and (3) to sustain commitment and resources and demonstrate the impact of activities completed under UAR [44].

5.2.1 Enhanced disease surveillance

Systematic and standardized surveillance is fundamental to demonstrate the burden of a disease and provide evidence to initiate change in health policy-making – ensuring governmental buy-in

and prioritization. For most African countries, however, reliable country-level estimates of the rabies burden are lacking because of insufficient surveillance systems and inconsistent reporting [111; 112], even though rabies is a notifiable disease in many of those countries [13]. Effective rabies surveillance systems ideally embed a One Health approach, fostering communication between public and animal health sectors and are built upon decentralized, laboratory-based surveillance, together with clearly defined disease indicators and case definitions [17; 18]. To aid countries in the design and implementation of high-quality rabies surveillance systems, the Partners for Rabies Prevention (PRP) established the “Rabies Surveillance Blueprint” which is an online resource repository that provides all of the relevant standard operating procedures (SOPs), information and guidance required by rabies-endemic countries (rabiessurveillanceblueprint.org).

5.2.2 Reinforcing laboratory-based diagnosis of rabies

Confirmatory laboratory diagnosis of animal and human rabies cases is an essential pillar of effective surveillance [17; 18; 113] because the clinical symptoms of rabies are not pathognomonic, often leading to misdiagnosis and inaccurate reporting [111; 114]. The direct fluorescent antibody (DFA) test is the most widely used post-mortem assay for rabies diagnosis in both humans and animals that is recommended by the WHO and the OIE [115; 116]. The DFA test, however, requires an expensive fluorescence microscope and CO_2 incubator, so application is often limited to urban settings or capital cities [117]. The second OIE- and WHO-recommended diagnostic assay that is widely-used test for the diagnosis of rabies in animals and humans is the direct rapid immunohistochemical test (DRIT), which can be undertaken using basic light microscopy [118]. Recently, field-ready and easy-to-use lateral flow devices (LFDs), based on immunochromatographic reactions, were developed [46; 119]. Such LFDs are promising tools to decentralize rabies surveillance to remote, underserved regions, as they do not need electricity, special storage, heavy equipment or a high level of technical expertise and yield test results within 5 to 10 minutes. While several studies [46; 120; 121] obtained satisfactory results with LFDs, others [119; 122] found a substantial number of false-negative results and batch-to-batch inconsistency making LFDs unsuitable thus far to inform decision-making on PEP. These LFDs are thus not currently recommended by the OIE or WHO and further enhancement and careful validation is recommended before promoting widespread use in routine surveillance [46; 119].

Rabies in humans is rarely diagnosed etiologically because diagnosis relies on technologies inadequate for routine use in resource-limited and remote settings [113]. This makes it difficult to estimate the burden of human rabies [123], but probability models using data on bite incidence in

humans suggest that rabies is greatly underreported [124]. For post-mortem diagnosis, brain tissue has the highest diagnostic sensitivity and the DFA and DRIT are the preferred diagnostic tests [116]. However, obtaining permission from family members in order to perform an invasive sample collection after a patient's death can be difficult [125]. A less invasive alternative that, however, requires sophisticated equipment is the detection of rabies virus nucleic acid by reverse transcription polymerase chain reaction (RT-PCR) in nuchal skin biopsy or hair follicles [113; 116]. For intra-vitam diagnosis, molecular techniques for detection of viral RNA in nuchal skin biopsies, saliva and hair follicles are the most sensitive [113; 116].

5.2.3 Improved access to biologics for animals and humans

No effective treatment for rabies exists, and death is inevitable in >99.9% of cases after onset of clinical symptoms [126]. Rabies is, however, entirely preventable after suspected exposure, and all rabies deaths result from failed prophylaxis. Risk assessment of potential rabies exposure in bite victims and rabies diagnosis in animals are inextricably linked processes, which are only achieved through close communication and collaboration between animal and human health professionals. Integrated Bite Case Management (IBCM) helps to differentiate true rabies exposures from non-rabid bites in order to guide human treatment and reduce unnecessary use of expensive PEP [37; 38]. This requires health professionals to report bite victims to the veterinary service so that biting animals can be identified and put under observation or euthanized and tested for rabies [33].

Huge progress has been made in post-exposure rabies vaccination for humans. In addition to recommending only a four dose intramuscular (IM) vaccination schedule, evidence has shown that dose-sparing intradermal (ID) vaccination regimens can be highly economical when several bite victims are treated at the same time using shared vials [8]. In 2018, the WHO recommended a shortened 1-week vaccination scheme which requires ID injection of 0.1ml vaccine in two sites on day 0, 3 and 7 [8]. This further enhances cost-effectiveness, reduces the number of clinic visits needed by patients and potentially improves patient adherence [8]. A modeling study predicted more than one million human rabies deaths in 67 rabies endemic countries between 2020 and 2035, under current PEP use conditions without intensified dog vaccination [127].

5.2.4 One Health: vaccinating dogs to save human lives

Rabies diagnosis in animals to inform the use of PEP in humans illustrates the interdependence of human and animal health and the need for a One Health approach for successful rabies surveillance and control. Zinsstag *et al.* [128] define One Health as "*any added value in terms of*

human and animal health, financial savings or environmental benefit from closer cooperation of human and animal health sectors at all levels of organization." Human rabies cases can be prevented solely through massive administration of costly PEP, but only control measures targeted at the animal reservoir will eventually allow for elimination of the disease. Sustained mass vaccination of dogs reduced the number of human rabies deaths in different parts of the world [33; 104; 129] and save costs in the long-term [130-133]. At least 70% of the dog population needs to be vaccinated to create herd immunity and interrupt rabies transmission [56]. Hence, to plan effective MDV campaigns, studies on the size of dog populations are required. Data routinely submitted to the Rabies Epidemiological Bulletin (REB) [134] by member states suggests that West and Central African countries are not implementing mass dog vaccination campaigns (MDV) of the scale and intensity required to interrupt disease transmission - most likely due to financial constrains (rabiesalliance.org/networks/paracon/bulletin).

5.2.5 International support and resources

A variety of freely available tools and mechanisms assist countries in elimination of dog-mediated rabies. The Canine Rabies Blueprint (caninerabiesblueprint.org) is a website developed by the Partners for Rabies Prevention (PRP) to provide standard operating procedures for all aspects related to dog rabies surveillance and control and the prevention of human rabies, incorporating a One Health approach [108]. In 2014, the Stepwise Approach for Rabies Elimination (SARE), a standardized self-assessment tool that allows countries to evaluate their current rabies situation and measure progress towards elimination, was integrated with the Canine Rabies Blueprint [107]. In combination, they provide detailed guidance to create national rabies control strategies [135]. The United States Centers for Disease Control (US CDC) developed the Global Dog Rabies Elimination Pathway (GDREP) to help countries estimate the resources needed to control dog-mediated human rabies based on MDV [136]. In 2012, the OIE set up a rabies vaccine bank to aid OIE Member countries to procure high-quality dog vaccine at a pre-established, affordable price, helping to implement national rabies elimination strategies [137]. Upon official request from a National Delegate to the Director General of the OIE, vaccine can be purchased and delivered to beneficiary countries within 12 weeks to 12 months or in urgent cases within 10 days. In the first six years, 20.1 million rabies vaccine doses were disseminated by the OIE, mainly in Asia and Africa.

5.2.6 Regional coordination and support of elimination efforts

a) PARACON

Rabies spreads irrespective of national borders and can only be controlled through coordinated efforts of countries. The Pan-African Rabies Control Network (PARACON) was established in

2015 under the secretariat of GARC, in partnership with the intergovernmental tripartite (WHO, OIE, FAO), CDC, NGOs (such as World Animal Protection), and industry partners (including MSD Animal Health, Boehringer Ingelheim, Sanofi Pasteur and IDT Biologika), with the support of foundations like the UBS Optimus foundation. This Pan-African network joined the major sub-Saharan African rabies groups, AfroREB (Africa Rabies Expert Bureau, Francophone Africa), SEARG (Southern and Eastern African Rabies Group), RESOLAB Rabies Sub-network and RIWA (Rabies in West Africa), and provided a rabies platform for countries that had not been included in these pre-existing rabies groups [138]. PARACON provides a new united rabies-specific network for all of sub-Saharan Africa which supports its 37 member countries by actively promoting a One Health approach towards rabies elimination. This unique transboundary platform offers African states across the continent an opportunity to showcase successes in rabies control, reflect on lessons learned, share challenges faced and discuss ways to overcome common ones. PARACON is also dedicated to introducing member countries to the developed tools and resources to support national and regional rabies control programs. PARACON meetings focus on workshops and group discussions where rabies focal persons (nominated by the governmental animal and human health sectors of their member states) work through tools and resources such as: i) the SARE [135; 138; 139]; ii) the Rabies Epidemiological Bulletin (a data repository developed to optimize disease surveillance and reporting using defined standardized disease indicators, capture vaccination data and facilitate integrated bite case management activities) [134]; GARC Education Platform (a website hosting targeted educational courses and awareness materials), Canine Rabies Blueprint [108], and GDREP [105; 140]. These tools and resources were all carefully developed to best support member countries in their efforts to control and eliminate dog rabies.

b) RIWA

The RIWA forum was inaugurated in 2012 at the University of Ibadan in Nigeria to coordinate regular meetings among West African governments and stakeholders from the West African subregion and link Anglophone and Francophone countries for rabies prevention at the human-animal interface. The inaugural conference facilitated collaboration between the Ministries of Agriculture and Health with higher education institutions in Nigeria. The forum instituted short training programs with components on rabies diagnosis, surveillance data management, spatio-temporal epidemiologic analysis, molecular characterization and phylogenetics. The Centre for Control and Prevention of Zoonoses (CCPZ) at the University of Ibadan, since co-sponsoring RIWA, has played a key role in building the network into a sustainable Society for Rabies in West Africa (SRIWA), which has successfully held five annual sub-regional RIWA conferences between 2012 and 2018 in different West African countries.

The RIWA forum addresses the control and prevention of rabies within West Africa, similar to Rabies in the Americas (rabiesintheamericas.org). For example, in response to the chronic neglect of rabies research in Liberia, the CCPZ sponsored a 3-year project to improve postgraduate programs for surveillance of human–animal diseases in West Africa. The program enrolled people from Liberia, Nigeria, and Sierra Leone, addressing the lack of reliable rabies data in these West African countries by collecting pertinent spatiotemporal and molecular data on human and animal rabies cases [141].

c) RESOLAB

The RESOLAB Rabies Sub-network was launched in December 2010, gathering twenty-three central veterinary laboratories (CVLs) geographically subdivided into West and Central Africa (WA/CA)[1]. The objectives of this sub-network focused on filling gaps in rabies diagnosis and surveillance and building a harmonized regional diagnostic capacity. Information on laboratory capability in the region was gathered from 2010 until 2016 through active networking and three technical questionnaires. Among the causes for inactivity, lack of reagents and equipment were the most frequent. Inability to guarantee equipment, such as UV microscope maintenance, was noted equally by all the members as a crucial factor hindering diagnostic activities and was partially overcome by the provision of new UV microscopes based on LED technology across the region. A further constraint equally claimed by the members was poor level of experience in rabies laboratory diagnosis and the need for practical training courses. Consequently, training activities were implemented through in country or regional training courses for about 120 recipients from 13 countries, mostly from the animal health sector. Information from 2017 was collected within the framework of a proficiency test (PT) organized by the FAO Reference Centre for rabies at the IZSVe in collaboration with FAO (Gourlaouen et al., manuscript in preparation). Of note, 10 out of 14 veterinary laboratories participating in the exercise belonged to the RESOLAB network (covering nine countries). Information collected in this activity revealed that 90% of them (9/10) were able to perform rabies detection via DFA, but only 40% (4/10) were able to perform rabies detection via RT-PCR despite possessing the equipment. Following the PT exercise, the overall RESOLAB

[1] The WA/CA Veterinary Laboratory Network for avian influenza and other transboundary diseases (RESOLAB) was created in December 2007. The WA/CA Veterinary Laboratory Network for avian influenza and other transboundary diseases (RESOLAB) was created in December 2007, within the umbrella of the FAO-ECTAD Bamako, with the objective of improving the veterinary laboratory capacity across the region through training, technical support and information sharing (http://www.fao-ectad-bamako.org/fr/?lang=en). Partners involved in supporting this initiative were USDA-APHIS, the World Organization for animal health (OIE), AU-IBAR. The language spoken within the network is mainly French (n=14), and to a lesser extent English (n=5), Portuguese (n=3) and Spanish (n=1). In 2012 the initial network was divided into two sub-regional areas: a) RESOLAB-Central Africa (RESOLAB-CA), which includes eight CVLs [the CVL of Cameroon, Central African Republic, Chad, the Democratic Republic of Congo (DRC), Equatorial Guinea, Gabon, Republic of Congo and Sao Tome et Principe]; and b) RESOLAB-West Africa (RESOLAB-WA), comprising fifteen CVLs (those from Benin, Bissau Guinea, Burkina Faso, Cape Verde, Ghana, Guinea, Côte d'Ivoire, Liberia, Mali, Niger, Nigeria, Sierra Leone, Senegal, The Gambia and Togo).
fao.org/ag/againfo/programmes/en/empres/news_201213b.html

capacity for rabies molecular diagnosis jumped to 100% (10/10 participating laboratories) (Gourlaouen et al., manuscript in preparation).

West and Central Africa is an assembly of culturally diverse countries with varying economic and political stability. Based on UN definitions, West Africa consists of 16 countries with an estimated population of 362 million people occupying about one-fifth of the area in Africa. West Africa is bordered in the north by the Sahara Desert and to the west and south by the Atlantic Ocean. The region harbors some of Africa's most stable countries with robust economic growth as well as countries that rank among the lowest on the human development index (HDI) [39]. Recent insurgencies in the Sahel and Nigeria show that the region is still fragile and prone to violent conflicts [142]. The inability of some countries' health systems to cope with the Ebola epidemic in 2014-2016 demonstrated the fragility and long-term impact of past violent conflicts on health systems and infrastructure [143]. Central Africa is a poorly defined region in the heart of Africa. In this study, we consider Central Africa to include Cameroon, Central African Republic (CAR), Chad, Republic of the Congo (Brazzaville), the Democratic Republic of the Congo (DRC), Equatorial Guinea, and Gabon.

The epidemiology of rabies from most Western and Central African countries remains poorly defined [134], making it difficult to assess the overall rabies situation and progress towards rabies elimination. This review aims to assess the current rabies situation in West and Central African countries based on published scientific literature and information provided by rabies focal points. We review the rabies situation in each country, identify research gaps and discuss the research needed to advance rabies elimination in Central and Western Africa.

5.3 Material and Methods

We searched five electronic databases (PubMed, Web of Science, Embase, the Cochrane Library and African Journal Online) for relevant literature on rabies control in West and Central Africa published between January 1, 1998 and December 31, 2018 without any language restrictions. The study was carried out according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines [144]. The search strategy for each database is included in Appendix 1: Supplementary information for chapter 5, Table 26. We included studies focused on i) diagnostic capacity, ii) established surveillance, iii) animal and human health communication (One Health), iv) dog population estimates, v) national control strategies, vi) cost estimates, vii) dog vaccination campaigns, viii) PEP availability and coverage, ix) Knowledge, Attitude and Practices (KAP) and x) molecular studies. Table 1 lists the 22 countries included in the search. The island nations of Saint Helena, Ascension and Tristan da Cunha and Cape Verde were excluded

from this study, as they are deemed free of dog rabies. EndNote reference management software version X7.7.1 was used to store results from the literature search and remove duplicate publications. Two reviewers independently screened titles and abstracts yielded by the search against the eligibility criteria. We resolved disagreement between reviewers through discussion. A list of publications identified for each country was compiled and a short questionnaire was sent to rabies focal points with a request to review the list for any missing relevant publications and fill in the questionnaire. We obtained contact details of rabies focal points from RIWA, RESOLAB Rabies Sub-network or PARACON. Full reports were obtained for all titles and abstracts that appeared to meet the eligibility criteria or where there was any uncertainty. Full-text reports were screened to determine whether they met the eligibility criteria.

Table 1: Countries in West and Central Africa included in this review

Region	Countries
West Africa	Benin, Burkina Faso, Côte d'Ivoire, Gambia, Ghana, Guinea, Guinea-Bissau, Liberia, Mali, Mauritania, Niger, Nigeria, Senegal, Sierra Leone and Togo
Central Africa	Cameroon, Central African Republic, Chad, Republic of the Congo, Democratic Republic of the Congo, Equatorial Guinea, Gabon

5.4 Results

Figure 2 shows a flow diagram summarizing the results of the review. From the database searches, we retained 1376 documents, while manual searches and documents from rabies focal points provided another 69 additional documents. Focal points from Equatorial Guinea and Guinea did not provide any information. Findings according to the inclusion criteria are summarized in Table 2 and Table 3.

Figure 2: PRISMA 2009 Flow Diagram

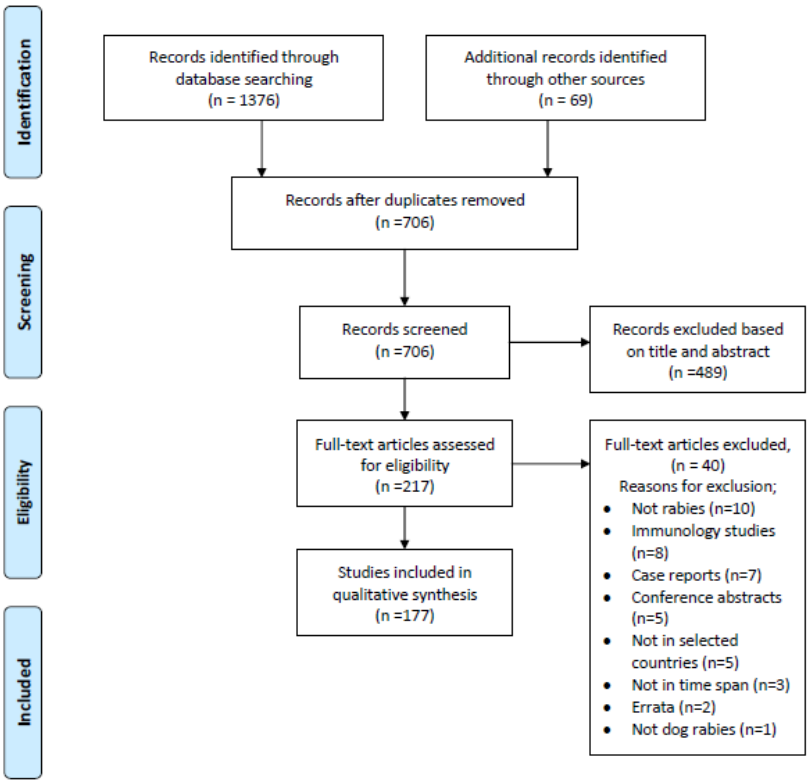

Table 2: Summary of inclusion criteria per West African country included in the review

		Benin	Burkina Faso	Côte d'Ivoire	The Gambia	Ghana	Guinea	Guinea-Bissau
Rabies notifiable	Humans	Yes	Yes	Yes	NA	Yes	NA	Yes
	Animals	Yes	Yes	Yes	NA	Yes	NA	Yes
HDI		163	183	170	174	140	175	177
Rabies legislation		Yes	Yes	Yes	NA	No	NA	Yes
National elimination strategy		Yes	No	Yes	No	Yes	NA	No
SARE (year)		1.5 (2018)	not assessed	2 (2017)	not assessed	1.5 (2018)	1.5 (2018)	0 (2016)
Diagnostic capacity	DFA	No	Yes	Yes	No	Yes	Yes	NA
	DRIT	Yes	No	No	No	No	Yes	NA
	Human	No	No	Yes	No	Yes	No	NA
	Decentralized /Centralized	Centralized	Centralized	Centralized	NA	Decentralized	Centralized	NA
Surveillance	Humans	Yes	Yes	Yes	Yes	Yes	NA	Yes
	Animals	Yes	Yes	Yes	Yes	Yes	NA	Yes
One Health	Data exchange	Yes	Yes	Yes	Yes	Yes	NA	No
	IBCM	Yes	Yes	Yes	No	Yes	NA	No
Dog population	Population estimate	Yes	No	Yes	No	Yes	NA	No
	Culling Campaigns	Yes	Yes	No	NA	No	NA	Yes
Cost estimate		No	No	Yes	No	No	NA	No
Mass dog vaccination	National	No	No	No	No	No	NA	Yes
	WRD	Yes	Yes	Yes	Yes	Yes	NA	Yes
PEP	ID	No	No	No	No	No	NA	No
	IM	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	Availability problems	Yes	Yes	Yes	Yes	Yes	NA	Yes
KAP		No	Yes	Yes	No	Yes	No	No
RABV lineage		NA	2	2	NA	1 & 2	2	NA

DFA: Direct fluorescent antibody test, **DRIT:** Direct rapid immunohistochemical test, **HDI:** Human development Index, **IBCM:** Integrated Bite Case Management, **ID:** Intradermal, **IM:** Intramuscular, **KAP:** Knowledge, attitude and practices study, **PEP:** Post-exposure prophylaxis, **RABV:** Rabies Virus, **SARE:** Stepwise Approach for Rabies Elimination**, WRD:** World rabies day

		Liberia	Mali	Mauritania	Niger	Nigeria	Senegal	Sierra Leone	Togo
Rabies notifiable	Humans	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	Animals	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
HDI		181	182	159	189	157	164	184	165
Rabies legislation		NA	Yes	No	No	NA	NA	No	No
National elimination strategy		No	Yes	No	No	Yes	No	Yes	No
SARE (year)		1.5 (2018)	2 (2016)	not assessed	1.5 (2018)	1.5 (2017)	1.5 (2018)	0 (2017)	not assessed
Diagnostic capacity	DFA	Yes	Yes	Yes	No	Yes	Yes	No	No
	DRIT	Yes	Yes	No	No	Yes	No	Yes	No
	Human	No	No	No	No	Yes	Yes	Yes	No
	Decentralized /Centralized	Centralized	Centralized	Centralized	NA	Decentralized	Centralized	Centralized	NA
Surveillance	Humans	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes
	Animals	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
One Health	Data exchange	Yes	NA	No	Yes	Yes	Yes	No	Yes
	IBCM	No	NA	Yes	No	NA	Yes	NA	Yes
Dog population	Population estimate	No	Yes	No	Yes	Yes	No	Yes	Yes
	Culling Campaigns	No	NA	Yes	No	NA	Yes	No	Yes
Cost estimate		Yes	Yes	No	No	NA	NA	No	No
Mass dog vaccination	National	No	No	No	No	No	No	No	No
	WRD	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
PEP	ID	No	No	No	No	No	No	No	No
	IM	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	Availability problems	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
KAP		No	Yes	No	No	Yes	Yes	No	No
RABV lineage		2 & 3 (China 2)	2	2	2	1 & 2	2	2	NA

DFA: Direct fluorescent antibody test, **DRIT:** Direct rapid immunohistochemical test, **HDI:** Human development Index, **IBCM:** Integrated Bite Case Management, **ID:** Intradermal, **IM:** Intramuscular, **KAP:** Knowledge, attitude and practices study, **PEP:** Post-exposure prophylaxis, **RABV:** Rabies Virus, **SARE:** Stepwise Approach for Rabies Elimination**, WRD:** World rabies day

Table 3: Summary of inclusion criteria per Central African country included in the review

		Cameroon	CAR	Chad	Republic of the Congo	DRC	Equatorial Guinea	Gabon
Rabies notifiable	Humans	Yes	Yes	Yes	Yes	Yes	NA	Yes
	Animals	Yes	Yes	Yes	Yes	Yes	NA	Yes
HDI		151	188	186	137	176	141	110
Rabies legislation		Yes	Yes	NA	No	NA	NA	NA
National elimination strategy		Yes	No	No	No	No	NA	No
SARE (year)		1.5 (2017)	0.5 (2016)	1.5 (2016)	0 (2018)	1.5 (2016)	not assessed	0 (2018)
Diagnostic capacity	DFA	Yes	Yes	Yes	Yes	Yes	NA	Yes
	DRIT	No	No	Yes	No	Yes	NA	No
	Human	Yes	Yes	No	No	No	NA	No
	Decentralized /Centralized	Centralized	Centralized	Centralized	Centralized	Centralized	NA	NA
Surveillance	Humans	Yes	Yes	Yes	Yes	Yes	NA	No
	Animals	Yes	Yes	Yes	Yes	Yes	NA	No
One Health	Data exchange	No	NA	Yes	Yes	No	NA	No
	IBCM	No	NA	Yes	No	No	NA	No
Dog population	Population estimate	Yes	Yes	Yes	No	No	NA	No
	Culling Campaigns	No	Yes	No	Yes	Yes	NA	Yes
Cost estimate		No	No	Yes	No	No	NA	No
Mass dog vaccination	National	No	No	No	No	No	NA	No
	WRD	Yes	Yes	Yes	Yes	Yes	NA	No
PEP	ID	No	No	Yes	No	No	NA	No
	IM	Yes	Yes	Yes	Yes	Yes	NA	Yes
	Availability problems	Yes	Yes	Yes	Yes	Yes	NA	Yes
KAP		Yes	No	Yes	No	No	No	No
RABV lineage		1&2	1&2	2	1	1	NA	NA

DFA: Direct fluorescent antibody test, **DRIT:** Direct rapid immunohistochemical test, **HDI:** Human development Index, **IBCM:** Integrated Bite Case Management, **ID:** Intradermal, **IM:** Intramuscular, **KAP:** Knowledge, attitude and practices study, **PEP:** Post-exposure prophylaxis, **RABV:** Rabies Virus, **SARE:** Stepwise Approach for Rabies Elimination**, WRD:** World rabies day

Countries where the rabies focal person provided all of the information as no published scientific literature was available.

Gabon

Established surveillance: Even though rabies is a notifiable disease in Gabon, there is not a specific framework covering rabies, and rabies-related activities are carried out in an uncoordinated manner. Furthermore, the little amount of epidemiological data that is available is only collected in the capital city of Libreville, where 1565 animal attacks were reported between 2013 and 2015.
Diagnostic capacity: The veterinary services in Gabon generally do not have the means to undertake grassroots-level surveillance or rabies diagnosis. As a result, the burden estimates are almost non-existent.
PEP availability and coverage: Post-exposure vaccination of bite victims is carried out in Libreville health facilities under the initiative of the Ministry of Public Health (MoPH), while the situation in rural areas of the country is unknown.
Dog population estimates: Free-roaming and ownerless dogs and cats are considered the main problem in the country, but no dog ecology studies have been implemented to date.
Dog vaccination campaigns: Vaccination is available mainly in urban centers, where some dog owners have the financial means to buy their own vaccines. The impact of these vaccination campaigns is unknown, but most likely to be negligible in terms of scale and intensity.
Animal and human health communication (One Health): Over the past years, the MoPH organized several awareness-raising activities on World Rabies Day (WRD) with participation from the Veterinary Services of the General Directorate for Livestock and sponsorship by the Sanofi Pasteur laboratory.
Molecular studies: No molecular studies had been undertaken to date.
KAP studies: No formal KAP had been published to date.
Cost estimates: No formal cost estimates had been undertaken to date.
National control strategy: In 2014, in collaboration with the Reference Center for Rabies (IZSVe), FAO organized, under the IDENTIFY project (USAID funded), a hands-on training on the FAT test for the technical staff of the CVL of Libreville. The week after, a stakeholder seminar was organized by FAO in collaboration with WHO, UNICEF and GARC [145]. A rabies technical working group was established to develop a national plan of action for rabies control in Gabon and short-, medium- and long-terms activities were prioritized and drafted at the end of the meeting.
SARE assessment undertaken: SARE assessments have only been undertaken by nominated rabies focal persons at PARACON meetings. The latest SARE score (obtained in 2018) was 0

out of 5, indicating that rabies is endemic and that the country is only compiling the basic information on the epidemiology of the disease.

Guinea-Bissau

Established surveillance: Since 2015, human rabies cases and exposures are to be notified through the National Health Information System (NHIS). However, data collection through NHIS is not very efficient given the volume of activities carried out by a minimal number of staff in the health sector and the mass of information to be collected and transmitted periodically. An epidemiological surveillance network for animal diseases with a central and regional unit exists and there are monthly data collection sheets for priority diseases including rabies.

Diagnostic capacity: The limited information available indicates that rabies surveillance is weak throughout the country. Data on the diagnostic activities submitted to the REB between 2015 and 2018 suggest that no diagnostic capacity has been established in the country [146]

PEP availability and coverage: The only anti-rabies treatment center is located in the capital city of Bissau, where the cost of rabies vaccine is incurred by the victim. People exposed in other parts of the country must travel to Bissau for adequate treatment. Data on the number of registered animal exposures submitted to the REB between 2015 and 2018 indicate that approximately 1'000 human exposures are recorded each year, but that no PEP is available through the government-subsided healthcare facilities [146].

Dog population estimates: No dog population estimates have been obtained to-date.

Dog vaccination campaigns: The vaccination of animals against rabies in Guinea-Bissau is compulsory by law. Since 2012, the Veterinary Services organize a rabies vaccination campaign in commemoration of the WRD. In 2015, approximately 16'000 dogs were vaccinated against rabies in a free-of-charge vaccination campaign. These vaccination campaigns result in an estimated vaccination coverage of 1.5% - 10% across the total dog population [146]. In the subsequent years, the vaccination was owner-charged (~ 1.7 USD per animal) and the number of vaccinated animals dropped to approximately 5'000 in 2018. Between 2011 and 2014, approximately 4'350 dogs were vaccinated against rabies by the veterinary service.

Animal and human health communication (One Health): There is not a strong synergy or exchange of information between the Ministry of Health and the National Veterinary Services.

Molecular studies: No molecular studies had been undertaken to date.

KAP studies: No formal KAP studies had been published to date.

Cost estimates: No formal cost estimates had been undertaken to date.

National control strategy: Guinea-Bissau has a national plan for the Control of Neglected Tropical Diseases, including rabies.

SARE assessment undertaken: SARE assessments have only been undertaken by nominated rabies focal persons at PARACON meetings. The latest SARE score (obtained in 2016) was 0 out of 5, indicating that rabies is endemic and that the country is only compiling the basic information on the epidemiology of the disease.

Mauritania

Established surveillance: Although rabies is one of the diseases monitored by the Mauritanian Network for Epidemiosurveillance of Animal Diseases, no specific rabies-related activities were carried out in the field except for a few dog and cat culling campaigns in response to increased incidence of animal bites.

Diagnostic capacity: The only laboratory that has the capacity for rabies diagnosis in animals using the DFA assay is based in the capital Nouakchott.

PEP availability and coverage: PEP is provided by the National Institute for Public Health Research in the capital city and by the Regional Directorate of Health Action on the intermediate (Wilayas) level with support of the WHO.

Dog population estimates: No dog population estimates have been obtained to date.

Dog vaccination campaigns: The Directorate of Veterinary Services organizes a free-of-charge dog vaccination campaign on WRD. The impact of these vaccination campaigns is unknown, but most likely to be negligible in terms of scale and intensity.

Animal and human health communication (One Health): Cooperation between the animal and human health sector is weak, but the Regional Disease Surveillance Systems Enhancement Project (REDISSE) has recently been launched in Mauritania and other Economic Community of West Africa States (ECOWAS) member countries. The project aims to strengthen the cross-sectoral capacity for infectious disease surveillance and epidemic preparedness in West Africa.

Molecular studies: No molecular studies had been undertaken to date.

KAP studies: No formal KAP studies had been published to date.

Cost estimates: No formal cost estimates had been undertaken to date.

National control strategy: A national rabies control strategy has not been developed to date.

SARE assessment undertaken: No SARE assessment undertaken to date.

Niger

Established surveillance: Rabies is included in the national network for the surveillance of animal diseases which is carried out by 250 livestock agents across the country.
Diagnostic capacity: Only the veterinary sector in Niger is capable of conducting rabies diagnosis, with all diagnoses relying on the Seller's stain test. Data on the diagnostic activities, submitted to the REB between 2015 and 2018, suggests that a limited number of samples are subjected to rabies diagnosis every year (average of 10 samples per year) and on average 81% were rabies-positive [146]. While undertaking rabies diagnosis with the use of the Seller's stain is less than ideal [147; 148], an immunofluorescence microscope was acquired in 2018 to facilitate the implementation of the DFA in the near future.
PEP availability and coverage: The availability of PEP across the country is limited and not easily assessable.
Dog population estimates: No dog population estimates have been obtained to date.
Dog vaccination campaigns: Data on dog vaccination campaigns submitted to the REB between 2015 and 2018 suggest that on average 1'500 dogs were vaccinated every year. These vaccination campaigns resulted in an estimated vaccination coverage of 0.06% - 0.14% across the total dog population between the years 2015 and 2018 [146].
Animal and human health communication (One Health): No information on inter-sectoral collaboration could be obtained.
Molecular studies: No molecular studies had been undertaken to date.
KAP studies: No formal KAP studies had been published to date.
Cost estimates: No formal cost estimates had been undertaken to date.
National control strategy: A national rabies control strategy has not been developed to date.
SARE assessment undertaken: SARE assessments have only been undertaken by nominated rabies focal persons at PARACON meetings. The latest SARE score (obtained in 2018) was 0 out of 5, indicating that rabies is endemic and that the country is only compiling the basic information on the epidemiology of the disease.

Republic of the Congo (Brazzaville)

Established surveillance: Rabies is a notifiable disease in both humans and animals in the Republic of the Congo. In 2013, a major rabies epidemic occurred in the city of Pointe-Noire with 806 bite cases registered. In 2018, 635 bite incidents and two dog rabies cases were noted in the same city. The departments of Pointe Noire and Niari report more cases of bites than the other departments with 87% and 11%, respectively.

Diagnostic capacity: The support provided by FAO through the IDENTIFY project was crucial in the detection of and response to the 2013 rabies outbreak in Pointe-Noire. Thanks to a rabies laboratory training conducted by rabies experts from the FAO Rabies Reference Centre (IZSVe) a couple of months prior to the outbreak, the first case of dog rabies was diagnosed by the Brazzaville Veterinary Diagnosis Laboratory during the Pointe-Noire rabies outbreak [149]. This represented the first ever confirmed case of rabies notified to the World Organization for Animal Health (OIE) by national authorities and the disease was declared endemic throughout the country [150; 151]. Thanks to the rabies national control plan draft during the stakeholder meeting organized by FAO in May 2013, the country was able to face the emergency situation and stop the outbreak after several months of fear and more than 806 bitten people.
PEP availability and coverage: Human rabies vaccine is only available in private pharmacies.
Dog population estimates: No dog population estimates have been obtained to date.
Dog vaccination campaigns: Data on dog vaccination campaigns submitted to the REB between 2015 and 2018 suggest that no dogs have been vaccinated by the governmental sector in the aforementioned timeframe [146].
Animal and human health communication (One Health): An inter-ministerial committee for the control of rabies under the leadership of the Ministry of the Interior with the participation of the Ministries of Health and Agriculture and Livestock has been established.
Molecular studies: No molecular studies had been undertaken to date.
KAP studies: No formal KAP studies had been published to date.
Cost estimates: No formal cost estimates had been undertaken to date.
National control strategy: A national rabies control strategy has not been developed to date.
SARE assessment undertaken: SARE assessments have only been undertaken by nominated rabies focal persons at PARACON meetings. The latest SARE score (obtained in 2018) was 0 out of 5, indicating that rabies is endemic and that the country is only compiling the basic information on the epidemiology of the disease.

Countries with published scientific literature

Benin

Established surveillance: National rabies surveillance in Benin is carried out as part of the Integrated Disease Surveillance and Response (IDSR) strategy, with the technical aspects outlined

in the national guidelines for integrated disease surveillance and response. Despite these provisions, there are shortcomings in systematic reporting of human and animal cases, with limited inter-sectoral collaboration and no existing national rabies data management system.

Diagnostic capacity: Currently, the only laboratory capable of animal rabies diagnosis is the CVL located in Parakou by means of the DRIT.

PEP availability and coverage: From 2006 to 2016, the Parakou Veterinary Clinic registered 1'856 animal bite cases and the Porto-Novo Veterinary Clinic registered 779 bite victims and 11 human rabies cases over 6 years (2010-2016). Human rabies vaccine (~14 USD) and RIG (~27 USD) is only available in major urban centers Contou, Porto-Novo and Parakou.

Dog population estimates: There is little available information on the dog population with previous findings suggesting that the dog to human ratio is 1 to 21.2 in urban areas and 1 to 7.4 in rural areas. Humane dog population management is non-existent in Benin, with sporadic dog culling campaigns implemented in response to an increase in the numbers of dog bites.

Dog vaccination campaigns: The Beninese Association for Rabies Control (ABL-Rage), founded in 2013, organizes vaccination campaigns on the occasion the WRD and information suggest that Benin has purchased rabies vaccine through the OIE rabies vaccine bank [152].

Animal and human health communication (One Health): ABL-Rage organizes rabies awareness activities on WRD, trains health professionals on PEP use and donates human rabies vaccine when funding is available.

Molecular studies: No molecular studies had been undertaken to date.

KAP studies: No formal KAP studies had been published to date.

Cost estimates: No formal cost estimates had been undertaken to date.

National control strategy: Benin adopted a national rabies elimination strategy in 2018, but has yet to secure funding for its widespread implementation.

SARE assessment undertaken: An in-country SARE assessment was done with all of the relevant governmental stakeholders in 2018. The SARE score was 1.5 out of 5 indicating that the foundational requirements for the development of a national rabies control program is in place.

Burkina Faso

Established surveillance: Rabies is not only a notifiable disease, but has also been identified as one of the top five zoonotic diseases of greatest national concern in Burkina Faso [153].

Diagnostic capacity: Laboratory diagnosis of rabies is limited to the CVL in the capital city of where FAO in collaboration with FAO reference center for rabies (IZSVe) organized a rabies laboratory training and a back to back rabies stakeholder meeting July in 2017 [154]. Between 2001 and

2013, 3816 animal rabies samples were analyzed by DFA at the National Livestock Laboratory, of which 68% were found to be rabies-positive [155]. More contemporary diagnostic data is not readily available.

PEP availability and coverage: From 2001 to 2013, a total of 54'238 bite victims visited the National Centre for Anti-Rabies Control (CNTAR) in Ouagadougou [155]. Only 31% of the bite victims had the financial means to purchase rabies vaccine which is sold for about 15 USD per dose and administered following either 4-dose intramuscular (IM) Zagreb or 5-dose IM Essen protocol [155]. Sondo *et al.* [156] observed that almost all bite victims (94.5%) at CNTAR in the year 2009 were residents of Ouagadougou and dogs were reported as the biting animal in 95% of the cases [157]. In a retrospective study conducted at the national reference hospital, Yalgado-Ouedraogo University Hospital Center, 60 human rabies cases were identified between 2004 and 2013 based on clinical confirmation, with almost half of the cases (45%) referred from other regions of the country [158].

Dog population estimates: Dogs in Ouagadougou are free-roaming and mainly kept for security purposes throughout the city and almost two-thirds of 616 households sampled in Ouagadougou owned at least one dog [155]. Humane dog population management is non-existent in Burkina Faso, with sporadic dog culling campaigns implemented in response to an increase in the numbers of dog bites [155; 159].

Dog vaccination campaigns: No information could be obtained on dog vaccination campaigns in Burkina Faso.

Animal and human health communication (One Health): An inter-ministerial One Health platform was put in place in 2017 but is not yet fully operational due to leadership crises, financial shortfalls and socio-political instability in the country [159].

Molecular studies: A phylogenetic analysis of 32 rabies-positive canine samples showed that the circulating strains in Burkina Faso in 2007 belonged to the Africa 2 lineage and were closely related to those in neighboring countries, suggesting that transboundary spread was taking place at the time [160].

KAP studies: No formal KAP studies had been published to date.

Cost estimates: No formal cost estimates had been undertaken to date.

National control strategy: A national rabies control strategy has not been developed to date.

SARE assessment undertaken: No SARE assessment done to date.

Cameroon

Established surveillance: Rabies is ranked first among the top five priority diseases of the National Program for the Prevention and Control of Emerging and Re-emerging Zoonoses (PNPLZER). Rabies has been a notifiable disease in Cameroon since 2001, with syndromic surveillance operational in the human and animal health sectors. In 2014, the Centre Pasteur of Cameroon (CPC) and Sanofi Pasteur helped implement a reinforced rabies surveillance system in the western region of Cameroon by implementing a system that allows animal bite incidents to be reported weekly by district health centers to regional health delegations that ultimately report to the Directorate of disease control of the Ministry of Public Health. To date, the system has recorded an incidence rate of 38.2 animal exposures per 100'000 population and one confirmed human rabies case out of four clinically suspected cases between 2014 and 2016 [161].

Diagnostic capacity: Two laboratories, the CPC in Yaoundé (human and animal rabies) and LANAVET in Garoua (animal rabies), both have the capacity to undertake routine laboratory diagnosis. In 2012, FAO implemented a training course for rabies and a back to back seminar. Between 1990 and 1999, a total of 38'784 suspected rabid dogs were quarantined of which 1.9% died during observation and 0.9% were tested rabies-positive [162]. A total of 93 animal samples were tested at the CPC between 2010 and 2013, of which 71% were rabies-positive [163]. More contemporary diagnostic data is not readily available.

PEP availability and coverage: Only one study focusing on PEP compliance had been undertaken to date [164]. In this study, the researchers observed that PEP completion among bite victims in an anti-rabies treatment center in Douala was approximately 30%.

Dog population estimates: Previous studies showed that the dog to human ratios were 1:8 for northwest Cameroon [165] and 1:10 for rural areas in northern Cameroon [166]. These studies also found that dogs were allowed to roam freely and were predominantly kept for safeguard and hunting [165].

Dog vaccination campaigns: Since 2009, annual mass vaccination campaigns are organized on WRD and rabies vaccine is provided at a reduced price [163]. The impact of these vaccination campaigns is unknown, but most likely to be negligible in terms of scale and intensity.

Molecular studies: One molecular epidemiological investigation undertaken to date indicated that RABVs circulating in Cameroon were part of both the Africa 1 and 2 lineages [167].

KAP studies: No formal KAP studies had been published to date.

Cost estimates: No formal cost estimates had been undertaken to date.

Animal and human health communication (One Health): Cameroon's human and animal health systems adopted the global agenda for the elimination of dog-mediated human rabies by 2030

following the one health approach, coordinated by Prevention and Fight against Emerging and Re-emerging Zoonoses (PNPLZER). The following targets have been achieved to date: i) multiplication of anti-rabies centers now open in all 10 regions of the country, ii) integration of animal bites into the weekly notification of vaccine-preventable diseases (MAPE), iii) active participation of the ministries in charge of public health, animal health, wildlife and the environment in prioritizing rabies and developing a strategic rabies elimination plan, vi) strengthening the diagnostic capacity of the reference laboratory for intra-vitam diagnosis of human rabies using non-invasive sampling techniques in suspected cases and vii) conducting projects to initiate effective rabies surveillance and control in pilot areas [161].

National control strategy: A strategic plan for the elimination of dog-mediated human rabies has been developed by national experts with the help of the Blueprint for Rabies Prevention and Control and the support of international consultants. The national rabies elimination strategy in Cameroon combines i) dog population control, ii) multi-year mass vaccination campaigns, iii) management of exposed persons and iv) awareness-raising campaigns among human and animal health professionals and the general public.

SARE assessment undertaken: An in-country SARE assessment was done with all of the relevant governmental stakeholders in 2017. The SARE score was 1.5 out of 5 indicating that the foundational requirements for the development of a national rabies control program is in place.

Central African Republic

Established surveillance: Rabies in the Central African Republic (CAR) has been a notifiable disease since 2009 after major rabies outbreaks occurred in Baboua and Bangui in 2006 and Kaga-Bandoro in 2008. Surveillance consists of observation of rabies suspected animals at the veterinary clinic of the Ministry of Agriculture. The country's political instability, poor road conditions and absence of anti-rabies treatment centers outside of Bangui prevent effective rabies surveillance in other parts of the country, and the rabies situation outside the capital thus remains largely unknown.

Diagnostic capacity: Laboratory confirmation is performed at the National Reference Laboratory at Pasteur Institute of Bangui (IPB). The IPB is the only laboratory in the country with diagnostic capability for rabies, with DFA and RT-PCR performed for both human and animal rabies samples. A description of a rabies epidemic in Bangui from 2006 and 2008 suggests that two different strains of the RABV were introduced into the capital city at two different time points [168]. A total of 101 animal rabies samples were collected during the outbreak, of which 84% tested positive

for rabies [168]. The vast majority of bite victims (90%) were from Bangui and its suburbs [169]. During the same time period, 82 animal samples were submitted to the National Reference Laboratory, of which 69 tested positive for rabies.

PEP availability and coverage: Bite victims in the CAR often present at veterinary clinics. Following an assessment of the circumstances around the exposure by an animal health technician or veterinarian, patients exposed to suspect rabid animals are referred to the IPB for treatment. Analysis of data from IPB showed that in 2012, 966 bite victims visited the Centre for Prevention and Treatment of Rabies (annual incidence of exposure of 109 per 100'000) and 632 received PEP [169]. To date, PEP is provided free-of-charge at the Centre for Prevention and Treatment of Rabies, created in 2007 at IPB.

Dog population estimates: Dog population estimates have been conducted but are not publicly available.

Dog vaccination campaigns: No official intervention strategy against rabies exists in CAR and culling campaigns are carried out in Bangui in response to increased numbers of dog bites. Dog rabies vaccine is available at the government-run veterinary clinic at a price of ~6 USD [169]. The impact of these vaccination campaigns is unknown, but most likely to be negligible in terms of scale and intensity.

Animal and human health communication (One Health): No information on inter-sectoral collaboration could be obtained.

Molecular studies: Molecular studies have shown that strains from CAR belong to both the Africa 1 and 2 clades [81; 169; 170]. A study describing the spatiotemporal dynamics of rabies in Bangui over 20 years suggests that rabies is not self-sustained but rather a result of successive incursions by rabies-infected dogs from outside the city [81]. The fact that RABV strains, which were originally only found in northern CAR, are now also present in Bangui suggests that human-mediated transport of infected dogs drives long-distance spread of rabies [81].

KAP: No formal KAP studies had been published to date.

Cost estimates: No formal cost estimates had been undertaken to date.

National control strategy: A national rabies control strategy has not been developed to date.

SARE assessment undertaken: SARE assessments have only been undertaken by nominated rabies focal persons at PARACON meetings. The latest SARE score (obtained in 2016) was 0.5 out of 5, indicating that the situational rabies data is being collected and analyzed for initiation of local-level intervention campaigns.

Chad

Established surveillance: Chad participated in a GAVI funded study on the burden of rabies between 2016 and 2018. The results of these studies are published as parallel papers in this issue of Acta Tropica (see, in this issue, article by Assandi *et al.* – Surveillance and incidence of rabies in Chad).

Diagnostic capacity: At the request of Chadian authorities, a partnership was established with the Swiss TPH to implement immunofluorescence diagnostic capacity (DFA) at the Livestock Research Institute for Development (IRED) in 2000. In 2005, the DRIT was implemented at IRED and evaluated as a reliable alternative to the DFA in Chad [45]. The first study on dog rabies in the capital N'Djamena reported an incidence of 1.4 rabid dogs per 1000 unvaccinated dogs per year, with each dog exposing on average 2.3 humans [47]. In addition, the performance of a rapid immunodiagnostic test (RIDT) was validated, with the results indicating that the RIDT under investigation had a diagnostic specificity of 93.3% and sensitivity of 95.3% compared to the DFA [46]. Efforts are made to decentralize rabies diagnosis in Chad (see, in this issue, article by Service *et al.* – Decentralization of rabies diagnostics in Chad: Duplication of the number of cases)

PEP availability and coverage: A survey of animal bite victims between 2008 and 2009 found an annual bite incidence of 12 per 100'000 and an annual human rabies incidence of 0.7 per 100'000 in N'Djamena [49]. Madjadinan *et al.* investigated risk factors for rabies exposure and PEP in Chad and Mbaipago *et al.* describe the use of a free phone hotline for treatment orientation after a dog bite in Chad (see articles in this issue).

Dog population estimates: A demographic survey of the dog population found a dog to human ratio of about 1 per 33 persons for the city of N'Djamena [53] and dog to human ratios were found to be much higher in rural northern (1 dog per 16 persons) and southern Chad (1 dog per 5 persons) [85]. Most dogs were kept as watch dogs and allowed to roam freely during the day [53].

Dog vaccination campaigns: Small scale trials of free-of-charge dog mass vaccination run in 2001 in N'Djamena reached vaccination coverage of 70% and showed that only about 10% of the dogs were ownerless and therefore not accessible for vaccination [34]. The societal cost of vaccination was calculated to be less than 3.25 USD per vaccinated dog [34]. When dog owners had to pay for the vaccination, coverage dropped to 20% [52]. A willingness to pay study further corroborated these findings, and the authors recommended that future campaigns should be free-of-charge or subsidized by the government [171]. In 2012 and 2013, two consecutive city-wide dog mass vaccination campaigns successfully interrupted rabies transmission among dogs [172; 173], but PEP demand was largely unaffected due to a lack of communication between the animal and human health sectors [37]. Rabies cases in N'Djamena resurged nine months after the last vaccination

campaign with reestablishment of endemic rabies transmission to pre-intervention levels [173]. Mathematical modeling and phylogenetic analysis suggest that rabies resurgence may be explained by importation of rabies-exposed dogs into N'Djamena from outside the city limits rather than sustained ongoing transmission [173; 174]. Data on dog vaccination campaigns submitted to the REB between 2015 and 2018 suggest that despite the resurgence of cases, dog vaccination in Chad decreased [146].

Animal and human health communication (One Health): Chad is at the heart of several studies validating the concept of One Health as added value of a closer cooperation between human and animal health, for example demonstrating that dog mass vaccination is less costly to prevent human rabies than PEP after ten years [130]. However, the Chadian authorities have so far not put in place a formal mechanism of One Health communication that would coordinate public and animal health action

Molecular studies: A phylogenetic analysis of 83 rabies-positive animal samples revealed that RABVs circulating in N'Djamena belonged to the Africa lineage 2, but they did not infer any cross-border transmission [45].

KAP studies: KAP studies conducted in rural Chad revealed that about half of the respondents would apply traditional medicine as a first aid measure in the event of a bite [85; 175]. Mbaipago et al. assessed KAP among human and veterinary workers in Chad (see article in this issue).

Cost estimates: Based on six years of animal and human rabies surveillance data, Zinsstag *et al.* [131] developed a dog-human rabies transmission model to simulate the effectiveness of different intervention strategies. Detailed cost analysis of the two campaigns run in 2012 and 2013 validated and updated the dog-human transmission model from Zinsstag *et al.* [131]. The break-even point in cost of dog mass vaccination and PEP with sole use of PEP would be reached within 10 years with maximum One Health communication [130]. Rabies surveillance is progressively being expanded to rural areas and the rest of the country. Based on the experiences during the two vaccination campaigns in N'Djamena and an estimation of the total dog population of Chad [85], the costs of a nationwide parenteral vaccination campaign were estimated at 1.9 to 4.7 million Euros [106]. A proposal for a Development Impact Bond (DIB) as an alternative financing model was proposed to cover the large sum of upfront capital necessary to reach sufficient coverage to interrupt transmission [106; 176].

National control strategy: A national rabies control strategy has not been developed to date.

SARE assessment undertaken: SARE assessments have only been undertaken by nominated rabies focal persons at PARACON meetings. The latest SARE score (obtained in 2016) was 1.5

out of 5, indicating that the foundational requirements for the development of a national rabies control program is in place.

Côte d'Ivoire

Established surveillance: In Côte d'Ivoire, dog rabies is endemic and remains largely uncontrolled. Under the framework of the GHSA, rabies was ranked among the five zoonotic diseases of greatest national concern during a One Health zoonotic disease prioritization workshop [177]. Rabies control is managed by the Ministry of Public Health and the Ministry of Animal resources. Each of the ministries has an affiliated center overseeing rabies control: the veterinary anti-rabies center which is a structure of the Directorate of Veterinary Services and the human Anti-Rabies Center in Abidjan which is part of the National Institute of Public Health (NIPH). The veterinary anti-rabies center has two main tasks: a) surveillance of biting animals by a legal provision and b) analysis of information related to human rabies cases. To achieve these tasks on a national level, it relies on 20 regional directorates. Epidemiological data on PEP and human rabies cases are collected by the NIPH and its network of 30 local units spread throughout the entire country. Data is compiled and analyzed at the Anti-Rabies Center in Abidjan. The reported annual incidence of human rabies was estimated at 0.028 cases per 100'000 inhabitants from 2001-2009, with most cases occurring in urban areas [178]. A retrospective study conducted at a referral hospital in Abidjan identified 7 human cases between 2005 and 2009 [179]. From 2006-2013, the veterinary anti-rabies center recorded an annual average of 672 biting animals and 56 dogs tested positive for rabies between 2011-2013. Tiembre *et al.* [180] reported an animal bite incidence of 5 per 10'000 population in Abidjan for the year 2008. Major challenges in the fight against rabies in the Côte d'Ivoire are: i) inadequate reporting of animal and human cases; ii) high number of cases lost to follow-up; iii) low level of public rabies awareness; iv) lack of research on the burden of rabies; v) lack of knowledge about the dog population; and vi) low level of communication between the different sectors concerned by rabies.

In 2014, the National Institute of Public Health (NIPH) initiated a program to reinforce rabies surveillance [181]. The reinforced national surveillance system captured 50 human rabies cases (annual incidence= 0.06-0.08 per 100'000) and 30'000 animal bites (41.8-48 per 100'000) during 2014 and 2016 [181]. From 2016 to 2018 Côte d'Ivoire participated in a GAVI funded study on the burden of rabies. The results of these studies are published as parallel papers in this issue of Acta Tropica (see Tetchi *et al.* Risk factors for rabies in Côte d'Ivoire).

Diagnostic capacity: Animal rabies cases are analyzed at the Pasteur Institute of Côte d'Ivoire (IPCI) and the Central Laboratory of Pathology in Bingerville (LANADA), while human rabies samples are analyzed by a nested RT-PCR at IPCI.

PEP availability and coverage: Bite victims presenting at health centers are referred to the nearest NIPH unit which are in charge of PEP administration (IM Zagreb or Essen regimen). Two studies reported high PEP drop-out rates, with 53% in 2009 and 59% between 2014-2015, mainly due to lack of financial means to complete the whole PEP regimen [182; 183]. Subjects administered the 5-dose regimen dropped out more frequently compared to subjects administered the 4-dose regimen [183]. In the course of the reinforcement of the rabies surveillance network, bite victims were followed-up by phone which improved compliance with PEP and drop-out rate decreased from 59% to 45% [184]. Studies on the impact of the Thai Red Cross protocol on compliance with PEP and the benefit of ID vaccine regimen in Côte d'Ivoire were carried out during a GAVI funded study on the burden of rabies (see, in this issue, article by Tetchi *et al.* Impact of the Thai Red Cross protocol on compliance with rabies post exposure prophylaxis (PEP) in Côte d'Ivoire)

Dog population estimates: Dog population estimates were carried out during a GAVI funded study on the burden of rabies (see, in this issue, article by Kallo *et al.* Estimation of dog population and exposure to bites in Côte d'Ivoire)

Dog vaccination campaigns: Vaccination campaigns are carried out on the occasion of WRD. Data on dog vaccination campaigns submitted to the REB between 2015 and 2018 suggest that on average 9'000 dogs were vaccinated every year. These vaccination campaigns resulted in an estimated vaccination coverage of 0.16% - 0.75% across the total dog population between the years 2015 and 2018 [146]. Pilot large-scale campaigns are planned for Bouaké and San Pédro in 2019.

Animal and human health communication (One Health): CdI is in the process of launching a National One Health Platform assembling all the ministries capable of contributing to the prevention and control of existing zoonotic diseases [177]. A joint assessment using the SARE tool was carried out in 2016 to develop a rabies control plan following OIE and WHO guidelines. The evaluation placed Côte d'Ivoire at SARE level 1.6. Following the evaluation, a study on the burden of rabies and need for PEP was conducted between 2016 and 2018 by a multidisciplinary and multisectoral team with the support of GAVI, the Vaccine Alliance, making it possible to fill some of gaps identified by the SARE assessment. At the end of this study, the following results were obtained: i) estimation of the dog population; ii) vaccination coverage of the dog population; iii) incidence of rabies; iv) estimation of the need for PEP, v) analysis of the problem of those lost to follow-up; vi) establishment of risk factors of animal bites; vii) implementation of a pilot phase for

the use of the intradermal vaccination regimen. This information has provided an updated scientific basis and provided missing data for development of a national rabies elimination strategy.

In May 2018, a workshop to evaluate and develop an integrated rabies control plan for the Côte d'Ivoire was organized with the support of technical and development partners (FAO, OIE, USAID, CDC, CSRS, Afrique One aspire and GARC). This meeting brought together all stakeholders in the fight against rabies (the Multisectoral Secretariat of the One Health platform, the public health sectors, animal health, national education, the environment, local authorities, local rabies control committees, higher education, the Ministry of the Interior, the Ministry of the Budget). A technical working group of multidisciplinary and multisectoral experts was set up to develop the National Integrated Rabies Control Program. The activity was carried out in accordance with the SARE and GDREP tool. The current program aims to eliminate dog-mediated rabies by 2030. The work of this expert group made it possible to finalize the national program which was validated by all stakeholders in September 2018. This participatory process involved several disciplines and sectors over a two-year period. It used multidisciplinary studies and a transdisciplinary approach to take into account scientific and community knowledge. It is, therefore, a program based entirely on the One Health approach.

Molecular studies: According to Talbi *et al.* [185], RABVs circulating in Côte d'Ivoire belong to the Africa 2 lineage.

KAP studies: A KAP study conducted in 2008 found that the majority (82%) of household heads in Abidjan had heard of rabies but applied inadequate care after potential rabies exposure which could predispose human rabies [186].

National control strategy: In December 2018, the National Integrated Rabies Control Program was approved by the two ministries in charge of public and animal health.

Cost estimates: As an output from the GDREP tool and its customization for use in Cote d'Ivoire in 2018, the total cost of the elimination program is estimated to be USD17 000 000 over the course of 13 years. During years 1-3, USD1 498 000 per year will be necessary to strengthen surveillance and vaccination capacity, including demonstration projects to generate data to support scale-up. In years 4-6, an estimated USD3 947 000 is required. This phase will focus in increasing national dog vaccination coverage from <18% to 70%. In years 7-13, an estimated USD12 000 000 (USD 1 700 000 per year) will be needed to eliminate dog rabies through mass dog vaccination and document rabies free status for the country.

SARE assessment undertaken: A first SARE assessment was undertaken in 2016 (see section *Animal and human health communication (One Health):* A second in-country SARE assessment was done with all of the relevant governmental stakeholders in 2017. The SARE score was 2 out

of 5 indicating that a national control strategy is being implemented. In addition, the Practical Workplan towards Achieving Rabies Elimination (PWARE) tool was used to develop a governmentally endorsed workplan based on the SARE output – enabling activities to be actioned in a timely manner.

Democratic Republic of the Congo (DRC)

Established surveillance: Initial reports of rabies in the DRC date back to 1923 [187], and the country has experienced recurrent outbreaks of dog rabies ever since. A multisectoral commission ranked rabies among the top five priority zoonotic diseases in DRC [188]. In 2000, the Ministry of Public Health (MPH) implemented the IDSR strategy with rabies included as one of the 17 diseases with mandatory weekly summary notification and immediate notification of suspected cases. Human rabies cases, identified by health workers at the health facility level (health centers, general reference hospitals) or in the community by community health workers, are reported to the provincial health division and compiled at the Directorate for Disease Control (DLM). A study revealed, however, that surveillance of zoonotic diseases is weak because health workers do not clearly understand IDSR standard case definitions and reporting tools are lacking [189]. The Ministry of Agriculture, Fisheries and Livestock (MINAGRI) has not yet established a mandatory and reliable surveillance system. The rabies situation outside the capital city remains largely unknown. It is unclear how much of a gap there is in terms of data accuracy and lack of reliable sources of information to assess the true epidemiologic situation of rabies in both animals and humans in the DRC. In this respect, the Swiss TPH, the National Institute of Biomedical Research (INRB) and the National Pedagogical University (UPN) currently implement a joint study to reinforce rabies surveillance in a province of the DRC and provide reliable data on the rabies burden.
Diagnostic capacity: Diagnostic capacity is limited to the capital city and performed by the Veterinary Laboratory of Kinshasa (LaboVet) and INRB. In 2009, after major rabies outbreaks occurred in the provinces of Kinshasa, Bandundu and Kongo Central, a partnership between the US CDC and INRB was established with introduction of the DRIT. During the same year, Muyila *et al.* [190] reported 21 clinically-confirmed rabies cases in children at the Pediatrics Department of the general reference hospital of Kinshasa in only 7 months. In a retrospective study of 5053 animal attacks between 2009 and 2013 in Kinshasa, 2.5% of attacks were found to be due to rabid animals [191]. In May 2012, FAO organized a hands-on training on rabies diagnosis at the LaboVet and a back to back seminar under IDENTIFY project in order to draft a national rabies control

plan with short-, medium- and long-term actions. In 2016, Swiss TPH equipped the rabies laboratory at INRB with reagents and laboratory material to perform the DFA.

PEP availability and coverage: The only official anti-rabies treatment center (Office for Rabies Vaccination and Control, OVCR) is located in the capital Kinshasa, under the supervision of the MINAGRI. It includes both veterinary and medical personnel responsible for the care of animal bite victims, observation of suspected rabid animals and compilation of rabies data. The current PEP regimen used in the DRC is the IM administration route whereby five doses of vaccine (administered on day 0, 3, 7, 14 and 28) are given to the exposed individual with the cost paid by the owner of the biting animal.

Dog population estimates: Previous investigations found that about two-thirds of dogs in Kinshasa were allowed to roam freely, and that the proportion of ownerless dogs was less than 2% [192]. Dog population data for the rest of the country is unknown

Dog vaccination campaigns: Studies conducted between 2014 and 2015 investigating the dog vaccination coverage in Kinshasa found mean coverages of 47% and 53% [192; 193]. Vaccination data for the rest of the country is unknown, but unlikely to be impactful in terms of rabies control and elimination. In 2017, Swiss TPH together with INRB, UPN and the national Veterinary Service organized a vaccination campaign in six cities of the province of Kongo Central on the occasion of the WRD [194]. During the campaign, 1053 animals were vaccinated against rabies [194].

Animal and human health communication (One Health): A study evaluating the One Health approach in rabies control in Kinshasa revealed significant discrepancies in rabies data reported by the MPH and MINAGRI due to lack of inter-sectoral collaboration and communication [195]. The DLM registered 114 human rabies deaths between 2013 and 2016, whereas the OVCR noted only 33 cases for the same time period [195].

Molecular studies: According to Talbi *et al.* [185], RABVs circulating in the DRC belong to the Africa 1 lineage.

KAP studies: No formal KAP studies had been published to date.

Cost estimates: No formal cost estimates had been undertaken to date.

National control strategy: No national strategy has been developed as qualified veterinary personnel and financial resources for adequate interventions are generally lacking.

SARE assessment undertaken: SARE assessments have only been undertaken by nominated rabies focal persons at PARACON meetings. The latest SARE score (obtained in 2016) was 1.5 out of 5, indicating that the foundational requirements for the development of a national rabies control program are in place.

Equatorial Guinea

Established surveillance: The rabies focal points provided no information on the rabies situation in Equatorial Guinea. A case report from 2009 applying the Milwaukee protocol to treat dog rabies in a 5-year old child is the first report of rabies in the country [196].
Diagnostic capacity: No information available to date.
PEP availability and coverage: No information available to date.
Dog population estimates: No information available to date.
Dog vaccination campaigns: No information available to date.
Animal and human health communication (One Health): No information on inter-sectoral collaboration could be obtained.
Molecular studies: No molecular studies had been undertaken to date.
KAP studies: No formal KAP studies had been published to date.
Cost estimates: No formal cost estimates had been undertaken to date.
National control strategy: No information available to date.
SARE assessment undertaken: No SARE assessment done to date.

Ghana

Established surveillance: Dog rabies has been endemic in Ghana for decades [197] and ranks second among the top six priority zoonotic diseases in the country. Animal and human rabies cases increased in all the regions after free-of-charge anti-rabies vaccination campaigns funded by the Ministry of Food and Agriculture were suspended in 1998. Dog bites and rabies cases in humans and animals are captured through a parallel surveillance system of the human and animal health sector. Between 2009 and 2012, 123 clinically-confirmed human rabies cases were reported by public health officials [198]. In addition, 22 cases were registered at the Korle-Bu Teaching Hospital in Accra between 2010 and 2014 [199], 13 cases in Techiman health facilities between 2011 and 2016 [200] and 15 cases in the Eastern Region of Ghana between 2013 and 2015 [201].
Diagnostic capacity: There are currently five laboratories across the country with capacity to perform rabies diagnosis in animals and one laboratory for rabies diagnosis in humans.
PEP availability and coverage: The annual incidence of animal bites ranged between 54-100 per 100’000 population in Techiman Municipality in 2011-2016 [200] and an annual bite incidence of 172 per 100’000 population was reported for the Eastern Region of Ghana [201]. An alarmingly

high number (76%) of public health facilities in a cross-sectional study in the Greater Accra Region between 2014 and 2015 did not have rabies vaccine in stock to treat potential bite victims [202].

Dog population estimates: Beginning in 2018, the VSD included dogs in the national livestock census.

Dog vaccination campaigns: Although dog vaccination is carried out throughout the year, it is cash based, and therefore only available to pet owners with sufficient financial means. As a result, limited vaccinations are done by VSD staff, while most is done by private Animal Health Service Provider and animal welfare organizations. All these efforts are largely uncoordinated and disjointed with minimal impact on rabies control. The climax of public awareness and free dog vaccination campaigns is during annual WRD celebrations. Data on dog vaccination campaigns submitted to the REB between 2015 and 2018 suggests that on average 45'000 dogs were vaccinated every year. These vaccination campaigns resulted in an estimated vaccination coverage of 1.5% - 2.8% across the total dog population between the years 2015 and 2018 [146].

Animal and human health communication (One Health): Joint rabies outbreak investigations are conducted and rabies data is shared officially between the major stakeholders, the Veterinary Services Directorate (VSD) and the Ghana Health Services (GHS). However, a human rabies case investigation conducted by Afakye *et al.* [203] concluded that rabies surveillance is challenged by incomplete and poor data and weak collaboration between human and animal professionals.

Molecular studies: Phylogenetic analysis of RABV from 76 dog and brain samples revealed that both Africa 1 and 2 RABV lineages are present in Ghana with probable long-distance translocation of RABV from East Africa together with transboundary spread [198].

KAP studies: A KAP survey conducted in primary healthcare facilities in Greater Accra revealed extensive gaps in rabies knowledge pertaining to and inadequate management of dog bites among frontline service providers [202].

National control strategy: Ghana accepted the global challenge to eliminate dog-mediate human rabies by 2030 and developed a national rabies elimination strategy based on the SARE tool. A joint FAO-GARC meeting was organized in May 2018. After working through the SARE assessment and determining pending activities within the country's rabies control efforts, a comprehensive workplan was generated, enabling the national stakeholders to focus their efforts on implementation. The next steps consist of implementation of the national rabies elimination strategy in pilot areas to demonstrate success before scaling-up to the rest of the country. A first rabies control project on the initiative of RIWA supported by GHS and VSD was launched in the south in

Suhum. So far, more than 2000 dogs were vaccinated and a rabies education program was initiated in 985 schools [204].

Cost estimate: According to a GDREP assessment undertaken in 2018, it was estimated that nearly $200,000 USD is currently spent on rabies vaccination programs in Ghana annually. The total cost of the elimination program will scale up over the course of 13 years. During years 1-3, an additional $443,000 USD per year will be necessary to strengthen surveillance and vaccination capacity, including demonstration projects to generate data to support scale-up. In years 4-6, additional funds needed will increase from an estimated $1.8 Million USD to $3.9 Million USD per year. This phase will focus in increasing national dog vaccination coverage from <18% to 70%. In years 7-13, and estimated $4.4 Million USD per year will be needed for to eliminate dog rabies and document rabies free status for the country.

SARE assessment undertaken: An in-country SARE assessment was done in with all of the relevant governmental stakeholders in 2018. The SARE score was 1.5 out of 5 indicating that the foundational requirements for the development of a national rabies control program is in place. In addition, the PWARE tool was used to develop a governmentally endorsed workplan based on the SARE output – enabling activities to be actioned in a timely manner.

Guinea

Established surveillance: No information was provided by the rabies focal points and very few studies address the rabies situation in Guinea. Youla *et al.* [205] carried out a retrospective study in all health and veterinary structures in Conakry. Over 11 years (2002-2012), 2916 dogs were put under observation and 0.5% were diagnosed with rabies based on clinical signs [205].

A total of 11 clinically-confirmed human rabies cases were recorded over the 11 years. In the wake of the catastrophic 2014-2016 Ebola virus outbreak in West Africa, Guinea's existing systems and structures for zoonotic disease detection and control were assessed under the GHSA and rabies was ranked among the top six priority zoonotic diseases [206].

Diagnostic capacity: In 2018, a rabies diagnostic training was hosted at the CVL through a collaboration between FAO, USAID and GARC to train 6 laboratory personnel in the use of the DRIT [207]. Moreover, in 2018, under the umbrella of FAO related activities, IZSVe trained people at the CVL for DFA. Following this training, the laboratory was able to diagnose at least three dog rabies cases that were further confirmed and typed at IZSVe as Africa 2 RABV strains and submitted to GenBank through immediate release.

PEP availability and coverage: [205]. Bite victims are managed at the National Institute of Public Health in Conakry where owner-charged rabies vaccine is provided (~20 USD) [205]. Between 2000 and 2007, 1294 bite victims were registered in Conakry, the capital of Guinea, of which 36 developed rabies. Between 2002 and 2012, 7994 human rabies exposures were noted, of which 2634 received PEP, with only half of these completing a full course of PEP [205].

Cost estimates: As an output from the GDREP and its customization for use in Guinea in 2018, the total cost of the elimination program is estimated to be USD17 500 000 over the course of 13 years. During years 1-3, USD1 498 000 per year will be necessary to strengthen surveillance and vaccination capacity, including demonstration projects to generate data to support scale-up. In years 4-6, an estimated USD3 947 000 is required. This phase will focus in increasing national dog vaccination coverage from <18% to 70%. In years 7-13, an estimated USD12 000 000 (USD 1 700 000 per year) will be needed to eliminate dog rabies through mass dog vaccination and document rabies free status for the country.

SARE assessment undertaken: An in-country SARE assessment was done with all of the relevant governmental stakeholders in 2018. The SARE score was 1.5 out of 5 indicating that the foundational requirements for the development of a national rabies control program is in place. In addition, the PWARE tool was used to develop a governmentally endorsed workplan based on the SARE output – enabling activities to be actioned in a timely manner.

The Gambia

Established surveillance: There is little information available about the rabies situation in the Gambia. A study conducted at a clinic in the Greater Banjul Area found that almost one fifth (8/49) of bites were likely to be from rabid animals [208].

Diagnostic capacity: The African Union Interafrican Bureau for Animal Resources (AU-IBAR) recently set up a rabies diagnosis unit in the Central Veterinary Laboratory in the capital of Banjul which is not yet fully operational.

PEP availability and coverage: FP report availability problems across the country.

Dog population estimates: No information available to date.

Dog vaccination campaigns: A year-round free-of-charge rabies vaccination campaign was launched on WRD 2018 with vaccine from the OIE regional vaccine bank [152].

Animal and human health communication (One Health): No information on inter-sectoral collaboration could be obtained.

Molecular studies: No molecular studies had been undertaken to date.

KAP studies: No formal KAP studies had been published to date.

Cost estimates: No formal cost estimates had been undertaken to date.
National control strategy: The Department of Livestock Services annual work plan includes an integrated approach for the control and elimination of rabies.
SARE assessment undertaken: No SARE assessment done to date.

Liberia

Established surveillance: Rabies cases in Liberia were first reported in the 1950s [209]. Prolonged civil war (1989-2003) led to near-total destruction of Liberia's health system, and little information on many public health diseases, including rabies, is available [210]. The 2014 Ebola virus outbreak is evidence of the fragility and shortfalls in Liberia's health system [143]. A rabies outbreak in 2007 that claimed ten human lives triggered humanitarian assistance from FAO to mitigate rabies epidemics in Liberia [211]. A retrospective study conducted in three counties reported 488 bite cases over five years (2008-2012) and an annual average of 81 human rabies deaths [212]. The number of annual human rabies cases for the capital Monrovia was found to be almost twice as high, at 155 cases [213] and slum settlements were identified as rabies hotspots [214]. Through improved surveillance, 1'346 animal bite cases with three related deaths and 1'645 bite cases with ten related deaths were reported, respectively, for 2017 and 2018 (Unpublished report, National Public Health Institute of Liberia, 2018).
Diagnostic capacity: Currently, rabies diagnosis is limited to the capital Monrovia and is only available for the veterinary sector carried out at the CVL. Following an FAO-led project, the CVL was recently renovated and supplied with crucial equipment and reagents necessary for rabies diagnosis. Through efforts of FAO, IZSVe, GARC, and the Swiss Tropical and Public Health Institute (Swiss TPH), the CVL is now able to perform the DFA, DRIT, rapid immunodiagnostic test (RIDT) and PCR. Technical staff is being trained with support from GAVI and the Swiss Government[2]. In 2017, the first animal rabies sample was diagnosed by DFA and subsequently confirmed and typed by means of PCR.
PEP availability and coverage: Arrangements are underway for the National Public Health Institute of Liberia to assume responsibility of procuring human rabies vaccine and providing free-of-charge PEP to bite victims.
Dog population estimates: No dog population estimate had been carried out to date.
Dog vaccination campaigns: Small scale dog vaccination campaigns are carried out on WRD celebrations mostly in the capital city of Freetown. Data on dog vaccination campaigns submitted

[2] Swiss Government Excellence Scholarships

to the REB between 2017 and 2018 suggest that 500 and 800 dogs, respectively, were vaccinated against rabies which resulted in an estimated vaccination coverage of 0.15 – 0.25% [146].

Animal and human health communication (One Health): In the aftermath of the Ebola virus outbreak in West Africa, the United States Agency for International Development (USAID) granted funding to FAO under the Global Health Security Agenda (GHSA) to enhance capacity for surveillance of zoonotic diseases including rabies [215]. Liberia established a national One Health platform bringing together many local government entities with the goal to coordinate zoonotic disease activities between sectors. As a notifiable disease in the top five priority zoonotic diseases, rabies forms one of the subgroups under the One Health Coordination platform.

Molecular studies: In 2012, CCPZ of the University of Ibadan sponsored a 3-year project to gather molecular and spatiotemporal data on animal and human rabies cases. Phylogenetic analysis of three RABV positive samples revealed co-circulation of Africa lineages 2 and 3 and China lineage 2 RABVs [141].

KAP studies: No formal KAP studies had been published to date.

Cost estimates: Following a GDREP assessment in 2018, it was estimated that approximately $1,000 USD is currently spent on rabies vaccination programs in Liberia annually. The total cost of the elimination program will scale up over the course of 13 years. During years 1-3, an additional $75,000 USD per year will be necessary to strengthen surveillance and vaccination capacity, including demonstration projects to generate data to support scale-up. In years 4-6, additional funds needed will increase from an estimated $326,000 USD to $662,000 USD per year. This phase will focus in increasing national dog vaccination coverage from <18% to 70%. In years 7-13, and estimated $746,000 USD per year will be needed to eliminate dog rabies and document rabies free status for the country. These estimates are based on an estimated cost of $2.60 per dog vaccinated, which is a value representative of published values in the region. However, due to the lack of reasonably sized dog vaccination programs in Liberia additional efforts should be made to confirm the validity of this value. Based on a sensitivity analysis varying the cost per dog vaccinated from $2 to $3 / dog vaccinated the overall additional cost for dog rabies elimination in Liberia ranged from $5.3 to $8.0 Million USD.

National control strategy: A national rabies control strategy has not been developed to date.

SARE assessment undertaken: An in-country SARE assessment was done with all of the relevant governmental stakeholders in 2018. The SARE score was 1.5 out of 5 indicating that the foundational requirements for the development of a national rabies control program is in place. In addition, the PWARE tool was used to develop a governmentally endorsed workplan based on the SARE output – enabling activities to be actioned in a timely manner.

Mali

Established surveillance: Rabies was identified in Mali as a priority disease within WHO/AFRO's framework of the Integrated Disease Surveillance and Response (SIMR) strategy in 2008, and dog vaccination is mandatory throughout the country. Under the framework of the GHSA, rabies was ranked among the five zoonotic diseases of greatest national concern during a One Health zoonotic disease prioritization workshop [216]. A retrospective study on rabies in Bamako identified 5870 animal bites and 10 clinically-confirmed human rabies cases between 2000 and 2003 [217]. During the same period, 121 animal brain samples were submitted to the CVL for diagnosis, of which 119 were found to be positive for rabies [217]. Studies in Bamako reported an annual human rabies incidence of 0.4 per 100'000 persons [218] and an annual rabies incidence in dogs of 2.3 per 1000 unvaccinated dogs [219]. A retrospective study conducted in six Malian cities (Bamako, Kayes, Koulikoro, Sikasso, Segou and Mopti) between 2007 and 2009, found 43 bites per 100'000 population per year and an annual human rabies incidence of 0.4 per 100'000 population [220]. From 2016 to 2018 Mali participated in a GAVI funded study on the burden of rabies. The results are published as parallel papers in this issue of Acta Tropica (see article by Keita *et al.* - Burden of rabies in Mali).

Diagnostic capacity: There is no human rabies diagnosis available but animal rabies diagnosis by immunofluorescence is carried out at the CVL in the capital Bamako.

PEP availability and coverage: The country's only anti-rabies treatment center is based in Bamako, although rural health centers provide consultations for rabies exposure. The current PEP protocol is IM administration of anti-rabies vaccine on Day 0, 3, 7, 14 and 28. The vaccine cost must be paid by the patient.

Dog population estimate: A study on dog ownership in Bamako estimated the dog to human ratio at 1 to 121 and the total domestic dog population of Bamako at about 14'900 [86]. A significant proportion of dogs (45%) were vaccinated by dog owners on their own initiative [86]. Traoré *et al.* estimated dog populations in urban and rural areas of Mali (see article in this issue, Dog demographic dynamics in urban and rural areas and biting risk)

Dog vaccination campaigns: In September 2013, a first pilot small-scale dog vaccination campaign took place in Bamako, with vaccination coverage of only 18% [87]. Subsequent analysis of effectiveness parameters revealed that the low coverage was mainly attributed to low participation by dog owners due to lack of information about the campaign [87; 88]. Data on dog vaccination campaigns submitted to the REB between 2015 and 2018 suggests that on average 1'400 dogs were vaccinated every year. These vaccination campaigns resulted in an estimated vaccination

coverage of 0.0% - 0.13% (no dogs were vaccinated in 2017) across the total dog population between the years 2015 and 2018 [146]

Molecular studies: Genetic characterization of 32 RABV isolates showed that all RABV isolates belonged to the Africa 2 lineage [219].

Animal and human health communication (One Health): The Malian government has created a formal One Health governance structure, bringing together representatives of the relevant ministries.

KAP studies: No formal KAP studies had been published to date.

National control strategy: In 2019, Mali adopted a national rabies elimination strategy to eliminate dog rabies by 2030.

Cost estimates: No formal cost estimates had been undertaken to date.

SARE assessment undertaken: SARE assessments have only been undertaken by nominated rabies focal persons at PARACON meetings. The latest SARE score (obtained in 2016) was 2 out of 5, indicating that a national rabies control strategy has been drafted and is being implemented.

Nigeria

Established surveillance: An immense body of scientific literature on rabies in Nigeria exists. The first cases of human rabies date back to 1912, and in 1925 the disease was reported in animals [221]. Rabies is a notifiable disease, requiring immediate notification for suspected animal and human cases and bite victims. Rabies cases are captured through the National Animal Disease Information and Surveillance (NADIS) and the IDSR strategy which is a major component of the Health Management Information System. Decision making for integrating rabies surveillance, control and stepwise elimination as a public health challenge in Nigeria is confronted by geographic imprecision in rabies exposure data at human and animal hospitals, as well as fragmentation with records kept in individual institutions, agencies and private entities of the country. It is notable that the country does not have a harmonized record of rabies outbreaks in chronological order for animal and human victims. Likewise, there is no comprehensive summary of empirically verifiable annual cases across the entire 36 states and Federal Capital Territory.

Several studies assessed the incidence of dog bite victims (DBVs) and human and animal rabies cases in northern (Bauchi [222], Kaduna [223; 224], Kano [225] and Plateau state [226-230]) and southern (Cross River [231], Edo [232; 233], Ekiti [234], Enugu [235; 236]. and Lagos state [237])

Nigeria. In northern Nigeria, a retrospective study in Bauchi state identified 44 animal and 5 human rabies cases over 15 years (1987-2001) [222]. Of 189 dog brain samples submitted to the National Veterinary Research Institute (NVRI) in Plateau state in 2006, 61% tested positive [226]. A study conducted at Vom Christian Hospital in Plateau state found that of 713 DBVs reported over a five year period (2006-2010), 42% were bitten by laboratory-confirmed rabid dogs and 95% of DBVs first sought help at a veterinary facility [227]. In the same state, 247 DBVs were registered at the ECWA Veterinary Clinic between 2009 to 2010 [228], and 554 dog brain samples were submitted to the NVRI between 2006 to 2008, of which 69% were positive [229]. Seven rabies cases in children were reported at the Pediatrics Unit of the Jos University Teaching Hospital over a 10 year period [230]. A similar number of rabies cases in children (5) and 44 cases of dog bites were recorded over 10 years (1996-2005) in the Pediatric Unit of Aminu Kano Teaching Hospital in Kano state [225]. In neighboring Kaduna state, two clinically confirmed human rabies cases and 81 DBVSs were registered over ten years (2000-2010) in a hospital [223]. A 4-year study (2008-2011) at the same hospital noted 132 DBVs [224]. In southern Nigeria, in a 15-year review of DBVs in a rural health center in Cross River state, only 11 DBVs were identified between 1990-2004, and none of the DBVs completed PEP [231]. In Lagos state, 196 DBVs were recorded between 2006 and 2011 [237]. In Benin city in Edo state, 62 DBVs (62) were registered between 1994 and 2005 [232], and 143 DBVs were recorded at the Accident and Emergency Centre of the University of Benin Teaching Hospital over 12 years (1997-2008) [233]. A study found a strong correlation between access to street food sources for dogs and the distribution of 127 DBVs and four human rabies cases identified between 2005 and 2011 in Enugu state [235]. Over a 10 year period (2004-2013), 149 DBVs and six human rabies cases were reported in the same state [236]. Six out of 84 DBVs identified at Ekiti State University Teaching Hospital between 2010-2014 developed rabies, and only 64% of DVBs completed PEP [234]. Between 2011 and 2016, 1226 dog brain samples were submitted to the NVRI from across the country, of which 686 were rabies positive. Of these positive cases, over 92% were associated with human exposure [238].

Diagnostic capacity: Each of the six geopolitical zones of Nigeria maintains a laboratory capable of routine rabies diagnosis. Activities to enhance rabies awareness in Nigeria on WRD have been carried out since the start in 2007 [239].

Dog population estimates: Dog population studies were conducted in several states of Nigeria. Relatively high dog vaccination coverage was reported from Oyo (33-57%) [240; 241], Lagos (64%), urban Kaduna (41%) [242] and urban Abuja (53%) [243], whereas low rates were found in urban Bauchi (26%) [244], rural Kaduna (9%) [242] and semi-urban Abuja (23%) [243]. Similar dog to human ratios were found in Benue, Bauchi, and Abuja state (~1 dog to 4 humans) [243-

245]. In Lagos, the dog to human ratio was 1 to 6, with most dogs confined [246], compared to other states where the majority of dogs were free roaming [241-244; 247; 248]. Dog to human ratios in Oyo and Kaduna were 1 to 11 [241] and 1 to 8 [242], respectively. The dog population of Ilorin was estimated at 1258 (dog to human ratio of 1 to 139) using a technique which combined street counts and remotely sensed imagery [249].

Dog vaccination campaigns: The NVRI produces flurry Low Egg passage dog rabies vaccine which is sold at a subsidized price [250-252]. Studies showed that maintenance of the transport cold chain from anti-rabies vaccine dispensaries to veterinary clinics is not yet fully ensured in Nigeria [253; 254]. Data on dog vaccination campaigns submitted to the REB for the years 2015, 2017 and 2018 suggests that on average 71'000 dogs were vaccinated every year. These vaccination campaigns resulted in an estimated vaccination coverage of 0.04% - 0.46% across the total dog population [146]

Animal and human health communication (One Health): Following an H5N1 outbreak in 2006 in Nigeria, an interministerial One Health platform was established, but the OH approach currently appears far from institutionalized [255; 256]. Bomoi *et al.* [257] carried out integrated human and animal vaccination delivery to reach nomadic communities.

Molecular studies: Phylogenetic analysis of RABV isolates found that RABVs circulating in Nigeria belong to Africa 2 lineage [185; 258-260], except for a strain which belonging to the Cosmopolitan clade [185]. Dog trade and slaughter for human consumption is common practice in Nigeria, and dog markets and slaughterhouses exist throughout the country [261]. Several studies identified slaughtered dogs as a source for human rabies exposure [237; 248; 262-272], and dog trade may influence human-mediated long-distance spread of rabies [261; 273].

KAP studies: In a knowledge, attitude and practice study conducted in Osun state in 2006, 62% of bite victims identified during the study never sought PEP [274]. In a similar study conducted among school children in Kaduna state, 63.7-87.5% of bite victims received PEP [275]. Overall knowledge of rabies was assessed to be satisfactory in Abuja, but gaps in knowledge regarding the appropriate treatment after a bite were identified [276].

National control strategy: In 2016, Nigeria developed the national rabies elimination guidelines which provide stakeholders with the essential guides, tools, and methods for rabies elimination in Nigeria. The guidelines suggest MDV as a rabies control measure in Nigeria.

Cost estimates: No formal cost estimates had been undertaken to date.

SARE assessment undertaken: SARE assessments have only been undertaken by nominated rabies focal persons at PARACON meetings. The latest SARE score (obtained in 2018) was 1.5

out of 5, indicating that small-scale rabies control programs are in place and the country is working towards developing a national rabies control program.

Senegal

Established surveillance: Following the first AfroREB meeting in 2008, collection of epidemiological rabies data began [11; 112]. One year later, the second AfroREB meeting was held in Dakar where 40 rabies specialists met to analyze the rabies situation in West and Central Africa. It was concluded that lack of information on the true rabies burden was the main barrier to control the disease [11]. Rabies surveillance is integrated into the surveillance of priority diseases through the IDSR strategy. Under the GHSA, Senegal ranked rabies among the top six priority zoonotic diseases [277]. A study conducted at the Infectious Disease Clinic of the National and University Hospital Centre of Fann between 1986 and 2005 identified 54 human rabies cases [278]. The reported cases were referred from across the country, with children under 15 the most affected group [278]. A retrospective study on rabies in the region of Fatick during 1998-2007 found an annual average of 15 bite victims and 0.6 human rabies deaths for an estimated population of about 675'500 [279].

PEP availability and coverage: FP report availability problems across the country.

Diagnostic capacity: Rabies diagnosis is carried out at the national rabies reference center based in the capital city at Pasteur Institute of Dakar and the National Livestock and Veterinary Research Laboratory. According to official data, 90 animal and 80 human rabies cases were registered between 1995-2017.

Dog population estimates: No dog population estimate had been carried out to date.

Dog vaccination campaigns: Dog rabies vaccine is subsidized by the government (cost ~0.85 USD) and 10'000 doses of vaccine were procured through the OIE vaccine bank in 2017. More than 10'000 dogs were vaccinated against rabies on the WRD in 2018 [280].

Animal and human health communication (One Health): Although communication between the human and animal health sectors was established under the GHSA, at the operational level actions are still carried out in an isolated manner. Rabies data from the public and veterinary health systems are rarely cross-checked which hampers a clear picture of the epidemiological situation. The main challenges are institutional leadership and resource allocation for effective communication during operationalization. Without a specific annual budget for the two concerned sectors, integrated management will only be carried out with external resources.

Molecular studies: According to Talbi *et al.* [185], RABVs circulating in Senegal belong to the Africa 2 lineage.

National control strategy: In 2018, the Senegalese government announced a national rabies control program costing 1.3 million USD [277]. In the same year, FAO in collaboration with GARC organized a rabies stakeholder meeting during which a national control plan and a rabies working group was established.

Cost estimates: As an output from the GDREP tool and its customization for use in Senegal in 2018, the total cost of the elimination program was estimated to be USD17 500 000 over the course of 13 years. During years 1-3, USD1 498 000 per year will be necessary to strengthen surveillance and vaccination capacity, including demonstration projects to generate data to support scale-up. In years 4-6, an estimated USD3 947 000 is required. This phase will focus in increasing national dog vaccination coverage from <18% to 70%. In years 7-13, an estimated USD12 000 000 (USD 1 700 000 per year) will be needed to eliminate dog rabies through mass dog vaccination and document rabies free status for the country.

SARE assessment undertaken: An in-country SARE assessment was done with all of the relevant governmental stakeholders in 2018. The SARE score was 1.5 out of 5 indicating that the foundational requirements for the development of a national rabies control program is in place. In addition, the PWARE tool was used to develop a governmentally endorsed workplan based on the SARE output – enabling activities to be actioned in a timely manner.

Sierra Leone

Established surveillance: The first case of rabies in a dog in Sierra Leone was reported from the Teko Central Veterinary Laboratory in Makeni in 1949. Several dog vaccination campaigns in response to rabies outbreaks were carried out over the following decades. Ten years of civil war and the Ebola epidemic took its toll on the country's infrastructure, and rabies diagnosis was discontinued at Teko CVL. There was a drastic increase in human rabies cases throughout the course of the civil war [281]. In 2014, rabies was included in the list of priority diseases by both the Ministry of Agriculture Forestry and Food Security (MAFFS) and the Ministry of Health and Sanitation (MOHS) following a consultative workshop to assess the country situation on rabies. In 2017, under the framework of the GHSA, rabies was ranked among the six zoonotic diseases of greatest national concern during a One Health zoonotic disease prioritization workshop [282].

Diagnostic capacity: In 2017, the CVL was rehabilitated by FAO in partnership with the MAFFS and funding from USAID as part of the Global Health Security Agenda and is expected to resume

animal rabies diagnosis in the near future. Meanwhile, Njala University One Health Serology and Molecular Diagnostic Laboratory established capacity to perform real-time PCR and ELISA for rabies diagnosis. As a means to decentralize rabies diagnosis in Sierra Leone, GARC – in collaboration with the National Livestock, Animal Welfare and Rabies Control Task Force – facilitated the implementation of the DRIT assay at the Sierra Leone Animal Welfare Society (SLAW) offices in Freetown [283].

PEP availability and coverage: FP report availability problems across the country.

Dog population estimates: According to Suluku *et al.* [284], the civil war also led to an increase in urban dog populations due to migration of internally displaced people. The dog to human ratio in Freetown was estimated at 1 to 14 [284]. Dogs were kept for security reasons and were largely unrestrained and unvaccinated [281; 284].

Dog vaccination campaigns: In 2010, the AHC conducted a nationwide dog vaccination campaign. The data generated during this event is now being used to fight rabies in the country. Data on dog vaccination campaigns submitted to the REB between 2016 and 2017 suggests that on average 1’200 dogs were vaccinated every year. These vaccination campaigns resulted in an estimated vaccination coverage of 0.07% - 1.7% across the total dog population between the years 2016 and 2017 [146].

Animal and human health communication (One Health): Collaborative efforts of the National Livestock, Animal Welfare and Rabies Control Task Force, MAFFS, the MOHS, the Freetown City Council, the Sierra Leone Animal Welfare Society, Njala University, with support from international partners including World Animal Protection, FAO, WHO, OIE, GARC, and USAID, led to the development of a national rabies elimination strategy for Sierra Leone.

Molecular studies: According to Talbi *et al.* [185], RABVs circulating in Sierra Leone belong to the Africa 2 lineage.

KAP studies: In 2008, Animal Health Clubs of the Njala University were established in response to the absence of qualified veterinary personnel (only five qualified veterinarians in the entire country). Trained AHC members visit communities to raise awareness about rabies and general animal health, husbandry and welfare [285; 286]. Transmission rabies from dogs to cattle has also been observed in parts of the country and triggered interventions [287].

Cost estimates: No formal cost estimates had been undertaken to date.

National control strategy: Sierra Leone’s national rabies control strategy aims to eliminate human rabies by 2030 through humane dog population management, MDV and appropriate PEP administration.

SARE assessment undertaken: An in-country SARE assessment was done with all of the relevant governmental stakeholders in 2017. The SARE score was 0 out of 5 indicating that the basic information on the epidemiology of the disease is being compiled and rabies recognized as an endemic disease.

Togo

Established surveillance: In Togo, surveillance of human rabies is an integral part of the IDSR strategy with monthly summary notification and immediate notification of suspected cases and bites requiring PEP. Surveillance of animal rabies is carried out through the Epidemiosurveillance Network of Animal Diseases. In 2010, the FAO supported the Togo Ministries of Health and Agriculture to improve rabies surveillance and prevention in the country [288]. A 10-year (2001-2010) retrospective study of rabies in the capital city of Lomé and the Kara region found an annual human rabies incidence of 0.4 per 100'000 population [288; 289]. The authors reported an incidence of 4 rabid dogs per 1000 in the Kara region and 2 rabid dogs per 1000 in Lomé.

Diagnostic capacity: There is currently no laboratory capable of rabies diagnosis, but the plan is to establish rabies diagnostic capacities in the framework of the REDISSE project. Of 75 biting dogs put under observation in Lomé between 2011-2012, 32 developed rabies symptoms or died during the observation period [290]. Diagnostic confirmation at a partner laboratory in Ouagadougou, Burkina Faso found 11 of 32 samples positive for rabies.

PEP availability and coverage: FP report availability problems across the country. One dose of human rabies vaccine costs about 85 USD, while dog rabies vaccine is available at one-tenth of that price.

Dog population estimates: The dog to human ratio was estimated at 1 to 4 for rural areas and 1 to 7 for urban areas, although the sampled number of households was low [289].

Dog vaccination campaigns: According to the FP, dog vaccination campaigns are conducted on the occasion of WRD, and are unlikely to be impactful in terms of rabies control and elimination

Animal and human health communication (One Health): Rabies cases are followed-up and investigated in close cooperation between the animal and human health sector [291].

Molecular studies: No molecular studies had been undertaken to date.

KAP studies: No formal KAP had been published to date.

Cost estimates: No formal cost estimates had been undertaken to date.

SARE assessment undertaken: No SARE assessment done to date.

5.5 Discussion

In this review, we summarized existing scientific literature on dog rabies in West and Central Africa in conjunction with grey literature provided by rabies focal points to shed light on the current status of dog rabies control in 22 western and central African countries.

Although rabies is a notifiable disease in humans and animals in the majority of the countries, national surveillance systems do not adequately capture the disease, as also noted by Taylor *et al.* [123]. Many countries integrate rabies surveillance into the IDRS strategy, but inadequate awareness, and unstandardized case definitions and health indicators hamper case recognition and data collection at the health facility level. Remedial actions should ensure that all of the countries adopt standard case definitions for human and animal rabies, as suggested by WHO [292], and also standardized epidemiological indicators to assess the disease burden, as indicated by PARACON [134]. Furthermore, data compiled at the central level should be submitted to the REB regularly and made publicly available to allow disease burden assessment across countries to be undertaken [134]. Paper-based surveillance systems are associated with slow data flow from peripheral to central level and render data analysis cumbersome. A mobile-phone-based surveillance system was introduced in Tanzania with frontline health and veterinary workers equipped with mobile phones to report suspected cases of human and animal rabies, dog bite victims and PEP use [293]. The system facilitated data collection and improved timeliness and completeness of reported data [293]. Cameroon and Côte d'Ivoire successfully reinforced their rabies surveillance systems recently, and the data obtained suggests that the pre-existing national systems underreported the disease [12]. Such demonstration projects are vital to showcase success, generate political support and attract necessary funding.

A lack of rabies diagnostic capacity has an additional negative impact on rabies surveillance. In the Gambia, Niger, Togo and Gabon where rabies diagnostics are not yet established, while routine diagnostic confirmation is often limited to the capital city or major urban centers in the countries where diagnosis has been established. Human rabies diagnosis is available where there are international networks present, such as the International Network of Pasteur Institutes in Côte d'Ivoire, Senegal, CAR, and Cameroon. In the absence of transportation networks to send samples to central laboratories, surveillance data likely reflects only the urban rather than national rabies situation. Identifying the rabies status of biting animals through laboratory diagnosis is crucial to guide human treatment and reduce unnecessary use of limited human rabies vaccine for PEP.

The updated WHO recommended one week intradermal vaccination scheme [8] has been tested in the GAVI supported burden of rabies project in Mali and Côte d'Ivoire (see, in this issue, articles by Tetchi *et al.* and Keita *et al.*), but none of the countries has yet adopted the new vaccination scheme. All the countries report recurrent shortages of human rabies vaccine, with vaccine availability usually limited to urban centers or capital cities, and inflated vaccine prices when the government does not subsidize vaccine. GAVI recently decided to invest in human rabies vaccine in eligible countries beginning in 2021 [294]. Modeling shows that the investment by GAVI and a shift from IM to the abridged WHO recommended ID vaccination scheme would avert approximately 1.4 million dog-mediated human rabies deaths between 2020 and 2035 [127]. Improved access to PEP is an essential pillar of the global strategic plan to eliminate dog-mediated human rabies deaths by 2030 [44]. This investment allows countries to redistribute resources directed at rabies control and catalyze MDV, which is the only mechanism that will eventually lead to dog rabies elimination.

Unfortunately, a majority of the countries still apply mass culling as an emergency response to rabies outbreaks or increased numbers of bite incidents. The culling of dogs is strongly discouraged as it is proven ineffective to reduce rabies transmission among dogs [80; 295]. In these instances, the use of humane dog population management in collaboration with rabies elimination activities should be considered and adopted.

Our study showed that subsidized dog vaccination campaigns are usually carried out on WRD. While such activities are indispensable to keep rabies in the public consciousness, and raise awareness within communities and among veterinary and health workers, they are most often not of the scale and intensity that is required to eliminate rabies from the dog population. Indeed, most of the countries reported vaccinating such small portions of the estimated dog populations that no real impact will be observed. If countries are to progress towards rabies freedom by 2030, they will need to ensure governmental buy-in towards scaling up the intensity of their MDV campaigns at a national level and would thus need to obtain improved estimates of their vaccine requirements. Knowledge of the target dog population size and ecology is an essential factor to consider in planning MDV campaigns and subsequent evaluation of intervention effectiveness. Yet, more than half of the countries do not have reliable figures on their dog populations. Uncertainty about the dog population size was stated as a limiting factor to effective rabies control in Africa [32]. While a large-scale vaccination campaign in N'Djamena, Chad reached sufficient coverage (>70%) to interrupt rabies transmission, pilot MDV campaigns in Bamako achieved coverage only between 18%-27% [88] despite a similar setting. This highlights the importance of careful

evaluation of the sociocultural context and factors that influence community participation in rabies elimination programs [296; 297]. Despite the success of MDV campaigns in N'Djamena, citywide vaccination was discontinued due to lack of financial commitment by Chadian authorities, and rabies cases surged again to the pre-intervention level [173].

Rabies control programs often lie beyond the financial capacities of western and central African governments. Most rabies control programs across Africa rely on international agencies for financial support, for example, rabies elimination demonstration projects in Tanzania and South Africa were supported by the Bill and Melinda Gates Foundation [298; 299]. MSD Animal Health, Dog Trust and Worldwide Veterinary Service assisted a citywide dog vaccination campaign in Blantyre, Malawi [300] and UBS Optimus foundation funded vaccination in N'Djamena [172]. As economic benefits of dog vaccination take several years to become apparent [130], long-term funding needs to be secured. More than two-thirds of the countries studied are in the low human development group (HDI ≤ 152), facing other pressing social, economic and health issues, making the possibility to meet the 2030 rabies elimination goals without international solidarity low. A key challenge will be to secure long-term funding for sustained rabies control in Central and West Africa. DIBs have been suggested as an innovative funding mechanism to attract investors and catalyze rabies control programs [176]. DIBs are outcome-based contracts between a private investor and aid agencies or recipient governments (outcome funders). Private investors provide the necessary initial funding, and if outcomes are achieved, the outcome funders repay the private investor the initial investment plus a rate of return [301]. According to Clarke *et al.* [301], seven DIBs were launched between 2015 and 2018 which will help evaluate the robustness of and risks associated with this novel funding scheme. A DIB structure was developed for Chad and is currently market tested with investors and donors [106]. Instead of approaching rabies in an isolated manner, integrative programs could be built up such that resources and infrastructure are shared to address other neglected zoonotic diseases, e.g., leishmaniasis and echinococcosis, at the same time [176]. As stated in the Global Strategic Plan to end human deaths from dog-mediated rabies by 2030 [44]: "*The capacity-building required for rabies elimination is an investment not only in the elimination of a fatal but preventable disease but also in building capacity in the world's most neglected regions and improving access to health services within these communities."*

The SARE score has not yet been assessed for one country in Central Africa (Equatorial Guinea) and three countries in West Africa (Burkina Faso, The Gambia, and Mauritania). These countries have thus not benefitted from the SARE tool's outputs that enable a country to identify pending

activities in their rabies control program before being able to generate a workplan for them. Gabon, Niger, Sierra Leone, and Togo are currently at SARE stage 0 and the Central African Republic is at SARE stage 0.5, meaning that these countries are in very early stages of rabies control with only limited data on the disease available. About half of the countries in Central and West Africa (Benin, Cameroon, Chad, Congo Brazzaville, DRC, Ghana, Guinea (Conakry), Liberia, Nigeria, Senegal) are between SARE stage 1 and 1.5, meaning they are currently assessing the local rabies epidemiology and working on a short-term action plan. Only two countries (Mali and Côte d'Ivoire) reached SARE stage 2– indicating that their national governments have truly prioritized rabies elimination and are thus providing the necessary support and political buy-in required to achieve success. It is possible that some reported assessments are out-dated and that some countries may have progressed to a higher level. One-third of the countries have worked out national rabies elimination strategies.

West and Central Africa seem to be divided into countries such as Cameroon, Ghana, Côte d'Ivoire and Nigeria, which have accepted the challenge to eliminate rabies with governments committed to pushing forward rabies elimination along with support from international agencies. These countries represent ideal candidates for the start-up phase of the Global Strategic Plan to end human deaths from dog-mediated rabies by 2030 [44]. On the other hand, there are countries where some progress occurred, but elimination efforts remain stuck due to lacking government commitment and financial constraints. Leading countries should act as role models, sharing their experiences and capacities so that no country is left behind. An example is Togo, where rabies diagnosis is currently not available but has been outsourced to a laboratory in Burkina Faso, thus confirming that rabies is present in the country. Such unified efforts reflect the spirit of a One Health approach and are necessary to reach the common goal of zero human rabies deaths by 2030. Ultimately, because of the transnational transmission, successful rabies control requires a high level of regional coordination as has been shown by the rabies elimination campaigns in Europe [65] and Latin America [31]. Panafrican (African Union) and regional (ECOWAS) should lead such coordination activities as they require careful science base regional planning of a West and Central African rabies mass vaccination campaign [302]. Such a regional campaign that could begin in Mauritania, situated between the geographical barriers of the Sahara desert and the Atlantic Ocean, and create a rabies free zone progressively extending towards the South and the East of the continent.

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6 A One Health-based surveillance strategy to estimate the burden of rabies in the Kongo Central province, Democratic Republic of the Congo.

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6.1 Abstract

In the Democratic Republic of the Congo (DRC), rabies has long been a public health problem with first reports dating back to 1923. Despite recurrent disease outbreaks across the country ever since, little is known about the epidemiological rabies situation in the DRC due to inadequate disease surveillance. Effective surveillance is key to reliable country-specific rabies burden estimates and a cornerstone of the global strategic plan to eliminate human deaths from dog-mediated rabies by 2030. We used a One Health approach to strengthen rabies surveillance in the Kongo Central province and gather reliable baseline epidemiological data on human and animal rabies incidence and incidence of animal bites. Between July 2017 and December 2018, we registered a total of 786 animal bite victims (overall annual bite incidence of 44 per 100'000 population) along with ten suspected human rabies cases of which five were laboratory confirmed. Based on our data and using a probability decision tree model we predict 169 human rabies deaths for the Kongo Central province each year. We analyzed 138 brain samples from rabies suspected animals of which 71% were rabies-positive. This translates into an average of 1.7 rabid animals per week or an annual incidence of 1.2 laboratory-confirmed dog rabies cases per 1000 dogs. In this study, we demonstrated that it is possible to enhance rabies surveillance in a resource-poor setting with engagement and targeted training of human and veterinary personnel and limited financial support for basic equipment and logistics. We hope the data provided through this study will contribute to political will to invest in rabies prevention and control in the DRC.

Keywords: Rabies, surveillance, Democratic Republic of the Congo, zoonosis, dog, Integrated Bite Case Management, One Health

6.2 Introduction

The majority of infectious diseases are zoonoses spread between humans and animals, causing an estimated 2.7 million human deaths and 2.5 billion cases of human illness every year [3]. Rabies is a prime example of a zoonotic disease best controlled through a One Health approach [29]. That is, the added value of a closer cooperation between the animal and human health sector for cost-effective and sustainable disease prevention and control [303].

Animal bites are the primary route of rabies transmission to humans. While rabies has been largely controlled in high-income countries, it remains endemic in domestic dog populations in developing countries causing an estimated 59'000 human deaths each year – mostly children in remote rural communities [9]. Human rabies can be prevented through prompt administration of costly post-exposure prophylaxis (PEP), but disease elimination can only be achieved through

targeted intervention, i.e. vaccination, at the animal source. It is estimated that the highest per capita rabies mortality rates are in sub-Sahara Africa [110], but reliable surveillance data to demonstrate the true impact of disease on human and animal health are scarce across the region [304; 305]. Therefore, rabies is not perceived as an urgent public health problem in many sub-Saharan African countries, thus little resources are attributed to control and prevention fueling a cycle of neglect [305].

In the Democratic Republic of the Congo (DRC), rabies has long been a public health problem with first reports dating back to 1923 [187] and recurrent epidemics across the country ever since [306]. Recent estimates suggest that the DRC has the highest annual number of human rabies deaths (~5600) on the African continent and third highest worldwide [110]. Still, due to the lack of adequate surveillance, the true number of human and animal rabies cases in the DRC is unknown and there are discrepancies in official figures reported by the Ministry of Public Health (MPH) and Ministry of Agriculture, Fisheries and Livestock (MINAGRI) highlighting the lack of intersectoral communication [195]. Two studies address rabies in humans [190] and animals [191] in the capital city of Kinshasa. Muyila *et al.* [190] report 21 clinically-confirmed rabies cases in children within 7 months in a hospital in Kinshasa in 2009 and Twabela *et al.* [191] found that 2.5% of 5053 animal bites recorded throughout the city between 2009 and 2013 were likely due to rabid animals. Outside the capital city, the rabies situation remains largely unknown, especially in rural areas where healthcare access is limited. A community-based study conducted in a province of the DRC found an annual dog bite incidence of 5.2/1000 person-years and a large proportion of unvaccinated free-roaming dogs, suggesting that exposures to rabies are likely to occur in this community [307].

Recently, a multisectoral commission ranked rabies among the top five zoonotic diseases of greatest national concern [188]. The DRC is assessed as stage 1.5 on the Stepwise Approach towards Rabies Elimination (SARE) ladder, indicating that the foundational requirements for the development of a national rabies control program are in place. In DRC's Integrated Disease Surveillance and Response (IDSR) strategy, rabies is listed among the 17 priority diseases with weekly mandatory summary notifications [42]. However, decades of civil conflicts have taken a toll on DRC's health care system and left many parts of the country with devastated infrastructure. The weak road network combined with insecurity due to armed militias render much of the country's terrain poorly accessible and disease surveillance in remote communities and timely submission of weekly disease reports are extremely difficult. A recent study found that rabies data captured through DRC's IDSR did not reflect actual morbidity and should not be used for public health purposes [43]. Moreover, inadequate training of health workers in surveillance methods,

lack of standardized report forms, unfamiliarity of health workers with standard case definitions and insufficient supervision by higher-level health authorities were among the factors contributing most to poor surveillance of zoonotic diseases in the DRC [189].

Effective surveillance is key to reliable country-specific rabies burden estimates [12] and a cornerstone of the global strategic plan proposed by the United Against Rabies (UAR) collaboration to eliminate human deaths from dog-mediated rabies by 2030 [44]. To better understand the epidemiology of rabies in the DRC and contribute to the 2030 goal, the Swiss Tropical and Public Health Institute, the National Institute of Biomedical Research (INRB) and the National Pedagogical University (UPN) of Kinshasa worked to strengthen rabies surveillance in the Kongo Central province using a One Health approach. The system was implemented to gather reliable baseline epidemiological data on human and animal rabies incidence and incidence of animal bites.

6.3 Methods

6.3.1 Study design

We report on a longitudinal observational study conducted in the Kongo Central province of the DRC over an 18 month period from July 2017 to December 2018.

6.3.2 Study site:

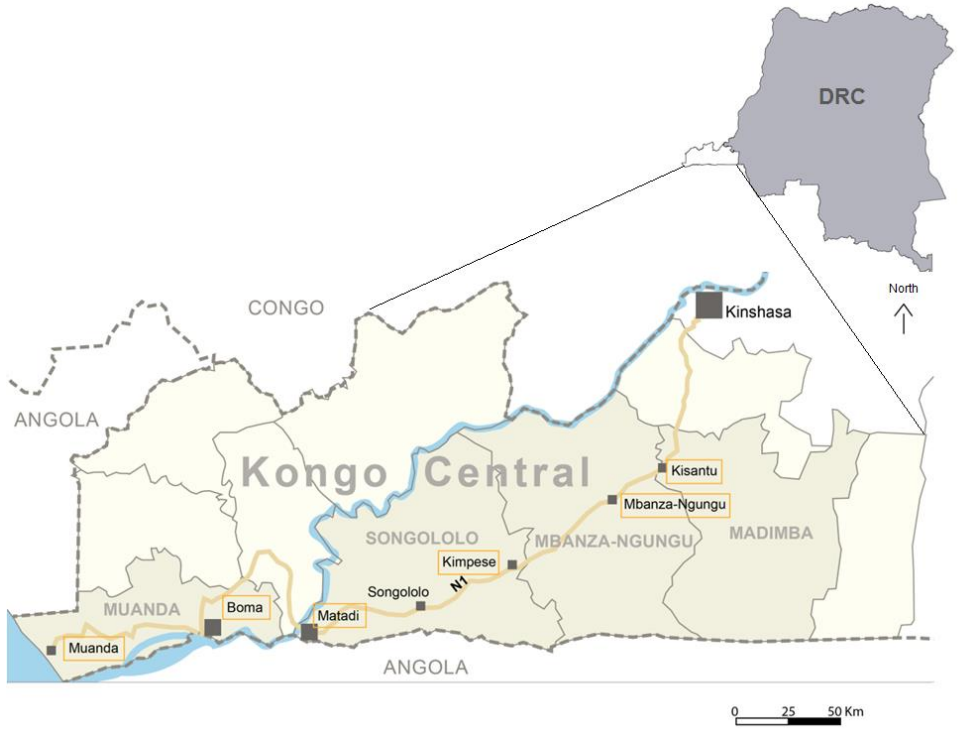
The DRC is Africa's second largest country located in the heart of the continent with a population of about 85 million. The DRC remains one of the poorest countries in the world ranking 176 out of 189 on the Human Development Index (HDI) [39], with 61% of the population living on less than 1.9 USD a day [308].

Kongo Central is the westernmost province of DRC's 26 provinces, bordering the Republic of the Congo and the Angolan enclave Cabinda in the north and Angola in the south (Figure 3). The province has an estimated population of about 5.5 million people, of whom 25% live in urban areas, and covers an area of 53'920 km^2 [309]. It is primarily tropical in climate, with a long dry season ranging from mid-May to mid-September and a short dry season from February to mid-March. Kongo Central is the only province with an ocean coastline, supporting DRC's two main ports, Matadi and Boma, which serve as major gateways for external trade. Due to its economic importance, Kongo Central is one of the most developed and accessible provinces in the country. Administratively, the province divides into 2 cities (Matadi and Boma) with 6 communes and 34 neighborhoods (urban entities) and 10 territories with 55 sectors, 376 groupings, 17 towns and 6514 villages (rural entities).

Human Health sector: DRC's health system is structured with three levels: national (or central), provincial (or intermediate) and peripheral (or operational) level. The Kongo Central Province is located at the intermediate level. At this level, the Provincial Health Division (DPS), headed by a Provincial Minister in charge of Health, supervises implementation of strategies and policies developed at the central level. The province divides into 31 health zones, with the network of health centers headed by general referral hospitals.

Animal health sector: The province registers only 18 official veterinarians, with only one veterinary clinic in Matadi, the provincial capital.

Figure 3: Map of the Kongo Central province.
Cities (Matadi and Boma) and towns (Kisantu, Mbanza-Ngungu and Muanda) where rabies units were established are outlined in orange.



6.3.3 Rabies surveillance in the DRC

Human rabies is a notifiable disease under the IDRS strategy. In theory, suspected human rabies cases and exposures are identified at the health facility level (health centers and general referral hospitals) reported to the DPS through the health zones and compiled at the Directorate for Disease Control (central level). The only official anti-rabies treatment center is in Kinshasa. The PEP costs of ~50 USD per dose are paid by the owner of the biting animal or, if the latter cannot be identified, by the bite victim. The official PEP regimen used is intramuscular injection of five doses

of vaccine, on day 0, 3, 7, 14 and 28 (Essen). Human rabies diagnoses are solely based on clinical symptoms. There is a transportation network for human samples through the health zones, but capacity is limited and shipment of samples is conducted irregularly.

The MINAGRI has not yet instituted a mandatory animal surveillance system. Laboratory diagnosis of animal rabies is limited to the capital city, performed by the rabies laboratory at INRB and the Veterinary Laboratory of Kinshasa (LaboVet). DRC's animal health sector suffers from chronic neglect. Equipment for routine rabies sample collection is lacking, and there is no official sample transportation network.

6.3.4 Surveillance reinforcement measures

We used a One Health approach involving both the human and animal health sectors in rabies surveillance. Staff of the rabies laboratory at INRB and the Faculty of Veterinary Medicine of the National Pedagogical University Kinshasa in collaboration with the Swiss Tropical and Public Health Institute supervised and coordinated surveillance activities and delivery of PEP to the study site.

In order to improve rabies surveillance in the Kongo Central province, we strategically placed rabies units at six locations along the national route 1 where i) both official veterinarians and general referral hospitals were present, ii) a large area of the province was covered, and iii) samples could easily be shipped to the rabies reference laboratory in Kinshasa (Figure 3).

A rabies unit consisted of two veterinary and two medical focal points with one supervisor each. Veterinary focal points (VFPs) and their supervisors were identified through the Provincial Inspectorate of Agriculture, Fisheries and Livestock (IPAPEL) and based at the Territorial Inspectorate of Agriculture, Fisheries and Livestock (ITAPEL) of Kisantu, Kimpese, Mbanza-Ngungu, Boma and Muanda and the government veterinary clinic in Matadi. VFPs collected animal rabies samples, put suspected rabid animals under observation and centralized data on bite victims. Medical focal points (MFPs) and their supervisor were identified through the health zones and recruited at the Pediatric and Emergency Departments of general referral hospitals of the six sites where VFPs were present (Figure 3). MFPs were responsible for administration of PEP to bite victims and data collection on rabies exposures and human rabies cases.

Integrated Bite Case Management

Only a fraction of animal bites are actual exposures to rabid animals [310]. We used Integrated Bite Case Management (IBCM) as a strategy to rapidly detect suspected rabid animals and improve rabies risk assessment of human exposures to avoid unnecessary administration of costly PEP. MFPs referred each bite victim to VFPs to trigger an investigation of biting animals. VFPs immediately tried to locate biting animals and assess their rabies status (*see Data collection*). A stock of WHO prequalified human rabies vaccine (VERORAB ®, Sanofi Pasteur) was maintained at the rabies laboratory at INRB to ensure correct storage and maintenance of the cold chain. Upon request by rabies units, vaccines were sent to the study site within 24 hours. The transport network which delivered rabies vaccine was also used to convey animal and human rabies samples from study sites to the rabies laboratory in Kinshasa. PEP was administered using the Zagreb 4-dose intramuscular regimen, consisting of 2 doses on day 0, followed by 1 dose on days 7 and 21. When several bite victims were injected simultaneously, the 2-site intradermal (ID) regimen of 0.1ml on days 0, 3, 7 and 28 was applied [311]. Because of limited availability of WHO prequalified human rabies vaccine in the DRC, PEP was delivered applying WHO recommendations applicable under advanced surveillance conditions [116], PEP was delayed if a dog was healthy at the time of the bite and available for quarantine. However, following high risk exposure, i.e. bites to children, to the head, neck or other highly innervated sites, multiple or deep wounds, ownerless animals and animals that were not available for observation, PEP was initiated immediately. Bite victims who requested PEP despite not considered exposed could purchase vaccine from the study stock at the purchase price (~18 USD per dose). Because all bite victims interacted with the VFPs to initiate investigation on biting animals, and due to excessive workloads of MFPs, VFPs were responsible for centralization of data on bite victims.

Training of veterinary and medical rabies focal points

All rabies units received on-site training through a two-day joint rabies workshop. The aims of the workshop were to establish direct communication channels between VFPs and MFPs and familiarize FPs of their respective roles and responsibilities in rabies surveillance. On the first day, rabies units heard lectures on i) rabies surveillance, prevention and control, ii) biosecurity and safety and iii) integrated bite case management. On the second day, MFPs were trained on ID administration of human rabies vaccine and saliva sample collection and VFPs were trained on brain sample collection. Each rabies unit received case report forms (CRFs), material for sample collection, preservation and shipment and a manual handout of standard operating procedure.

Awareness raising and vaccination campaign

Effective rabies surveillance depends on the veterinary and human health system but community participation is likewise important. The population recognizes and informs the veterinary services of suspected rabid animals and needs to be aware of preventive measures taken following an animal bite, such as seeking PEP. We, therefore, conducted an awareness raising and vaccination campaign on World Rabies Day 2018 to increase sample submission from suspected rabid animals and encourage people to seek treatment after a bite incident. Posters in French, Kikongo and Lingala, including drawings illustrating best practices after a bite injury, were distributed to health centers, general referral hospitals and veterinary health facilities throughout the study site. Radio broadcasts emphasized the importance of reporting suspected rabid animals to the veterinary service and PEP.

6.3.5 Data collection

Human rabies exposures and cases

We developed standardized CRFs to collect data on human rabies exposures and cases (Appendix 2: Supplementary information for chapter 6, Figure 31, Figure 32 and Box 2). Upon presentation, exposed persons had their wounds washed and disinfected. When necessary, additional wound care (e.g., tetanus vaccine and antibiotics) was administered. Data on the completion rate of PEP was not recorded in this study. Based on WHO standard case definitions for animal and human rabies, we assessed the rabies exposure status of bite victims based on the rabies status of the biting animal (Box 1).

The MFPs managed suspected clinical cases of human rabies presenting at general referral hospitals. Three saliva samples were collected at three hour intervals from each patient and stored at -20°C until they were transported to the INRB rabies laboratory accompanied by a CRF.

Box 1: Standard case definitions for human and animal rabies according to WHO [116] and human rabies exposure based on animal rabies status.

Case definition of human rabies:

- **suspected:** a case that is compatible with the clinical case definition of human rabies*;
- **probable:** a suspected case plus a reliable history of contact with a suspected, probably or confirmed rabid animal;
- **confirmed:** a suspected or probable case that is confirmed in the laboratory.

*an acute neurological syndrome (i.e. encephalitis) dominated by forms of hyperactivity (furious rabies) or paralytic syndromes (paralytic rabies) progressing towards coma and death, usually by cardiac or respiratory failure, typically within 7–10 days of the first signs if no intensive care is instituted. The syndrome may include any of the following: aerophobia, hydrophobia, paresthesia or localized pain, dysphagia, localized weakness, nausea or vomiting.

Case definition of animal rabies and human exposure:

- **suspected case/exposure:** a case that is compatible with the clinical case definition of animal rabies#/a person exposed by a suspected rabid animal
- **probable case/exposure:** a suspected case with a reliable history of contact with a suspected, probably or confirmed rabid animal and/or an animal with suspected rabies that is killed, died or disappeared within 4–5 days of illness being observed/ a person exposed by a probably rabid animal;
- **confirmed case/exposure:** a suspected or probable case that is confirmed in a laboratory/ a person exposed by a confirmed rabid animal;
- **not a case/non-exposure:** a suspected or probable case that is ruled out by laboratory tests or epidemiological investigation (i.e. appropriate quarantine period of eligible animals)/ a person exposed by a non-rabid animal.

#an animal that presents with any of the following signs: hypersalivation, paralysis, lethargy, unprovoked abnormal aggression (biting two or more people or animals and/or inanimate objects), abnormal vocalization and diurnal activity of nocturnal species.

Suspected rabid animals

MFPs informed VFPs about bite incidents by phone and asked bite victims to contact the VFPs to provide details about the biting animal. In response, VFPs initiated an investigation to identify the biting animal and conduct follow-up observations. If the animal appeared healthy at the time of capture, regardless of vaccination status, it was put under observation for 10 days. If the animal died at the time of capture, displayed symptoms consistent with rabies (Box 1), or became sick or died during the observation period, a brain sample for rabies diagnosis was collected. An animal rabies CRF was completed for every biting animal with information on the biting animal (species, ownership, vaccination and health status) and human exposure (age and sex of bite victim, type, time and place of exposure). Because VFPs did not have official transportation at their disposal, they received 25 USD per animal rabies sample collected. The amount allowed VFPs to operate in a radius of 25-30km (depending on season and road condition) from their home base and send samples to the rabies laboratory in Kinshasa. Payment was via mobile money service

(M-Pesa), with half of the amount transferred immediately upon request by VFPs and the pending amount transferred upon receipt of the sample at the laboratory.

Laboratory diagnosis of animal and human rabies samples

Prior to initiating the study, a dedicated room for processing rabies samples was established, and the laboratory was equipped with supplies required for rabies diagnosis. All animal brain samples were tested by using the direct fluorescent antibody (DFA) [312] and direct rapid immunohistochemical test (DRIT) [313]. Because diagnosis for human rabies samples was not available at INRB, human rabies samples were tested at the Pasteur Institute in Paris (IP-Paris). Viral RNA was extracted from all five human saliva samples and from a subset of 90 animal brain samples according to the Direct-zol™ RNA MiniPrep Protocol (Zymo Research). The extracted RNA was then analyzed by real-time polymerase chain reaction (RT-PCR) at IP-Paris. Laboratory results were communicated by phone to VFPs and MFPs who communicated the information to the bite victims.

Follow-up of rabies units

A rabies hotline was available at all times allowing rabies units to be in permanent contact with INRB. An agent based at the rabies laboratory at INRB, conducted a monthly phone follow-up of rabies units to obtain information on the number of exposed persons, suspected rabid animals and human rabies cases. In addition, VFPs reported the number of exposed persons and suspected rabid animals to the head of the Provincial Animal Health Office who disseminated monthly surveillance reports to the IPAPEL. Supervisory visits were conducted on average every 6 months to collect CRFs, provide rabies units with fresh material, discuss difficulties and refresh training. In urgent cases, material was shipped to the site using local transport.

6.3.6 Data analysis

Human and animal CRFs were entered into Epi Info, exported as comma-separated value files and analyzed using R software version 3.5.0 [314]. Maps were created with QGIS version 3.4.4. Incoherent or missing data were confirmed at the source whenever possible.

Bite and dog rabies incidence

Only bite (722/786) and dog rabies (694/725) cases that occurred in the six sites were included in bite and dog rabies incidence estimation. The human population of the six sites was used as the population at-risk denominator for the bite incidence estimate (see Appendix 2: Supplementary information for chapter 6,Table 27). Bite incidence was expressed as the number of bite

victims seeking health care after a bite per 100'000 population per year. We estimated dog rabies incidence based on the number of confirmed, probable and suspected dog rabies cases observed over the study period assuming that 80% of probable and suspected dog rabies cases were true rabies cases. The dog population was estimated for each site based on the dog/human ratio of 1:37.7 reported by Mbilo *et al.* [307] (see Appendix 2: Supplementary information for chapter 6,Table 27). The dog rabies incidence was expressed as the number of rabid dogs per 1000 dogs per year.

Estimate of human rabies deaths

We adapted the decision tree probability model developed by Cleaveland *et al.* [124] for human rabies mortality estimation in Tanzania to estimate the probability of death (*Pdeath*) after a bite and the number of human rabies deaths. Bite cases that occurred outside the six sites (64/786) were excluded from the estimate. *Pdeath* was calculated from the probability parameters using the formula:

$$Pdeath = Prabid * Pinfect * [(1-Pseek) + Pseek * (1-Ppep)],$$

where *Prabid* is the proportion of bites that are considered true rabies exposures due to confirmed, probable and suspected rabid animals, *Pinfect* is the probability of developing rabies after a bite from a rabid animal, *Pseek* is the proportion of bite victims that seek treatment and *Ppep* is the probability of receiving PEP. We assumed that no deaths resulted from incomplete PEP. We estimated confidence limits for Pdeath by assigning probability distributions to the input parameters and running Markov chain Monte Carlo simulations for 1000 steps. A detailed description of the parameters used for the decision tree model is provided in Appendix 2: Supplementary information for chapter 6, Figure 33 and Table 28.

The number of rabies deaths (Ndeath) per year in the six sites was calculated using the formula:

$$Ndeath=(I*Q*Pdeath/100\ 000),$$

where I is the incidence of bites per 100,000 population at-risk per year and Q the total population at-risk (n=1098575).

6.4 Results

6.4.1 Bite victims and biting animals

From July 2017 to December 2018, we registered a total of 786 animal bite victims in the study area, with a monthly average of 44 bite victims (range: 8-62). The mean number of bite victims

registered was 50.4 during the dry season and 37.7 during the rainy season. Due to missing values in some CRFs, sample size varied for each element of the CRFs analyzed. About 5% (36/784) of the bite victims reported scratch wounds in addition to bite injuries. Dogs were the most common species involved in human exposures, responsible for 96% (750/780) of all bites, followed by cats (3%) and monkeys (0.5%). Two persons (0.3%) reported bites inflicted by other humans.

Matadi reported almost half (385/786, 49%) of all bite victims, while only six bite cases were reported from Mbanza-Ngungu. About 8% (64/786) of bites were reported to have occurred in rural areas outside the six sites. More men (457/767, 60%) were attacked by animals, and 43 % (316/737) of the bitten population were less than 15 years of age. In 97 % (707/726) of cases, bite victims sustained wound category II or III injuries. Bite victims (n=775) were more often bitten on the lower extremities (leg and foot) (68%) or upper extremities (hand and arm) (23%) compared to the head, neck or face (17%) and body (9%). About 29% of bite victims sustained multiple bites by the same animal.

Eighty percent (514/718) of the bite victims presented to the clinic within the first 48 hours (range 0 to 120 days) after the bite. Upon presentation (n=775), 79% had their wounds washed with water and 82% had their wound disinfected. In addition, 65% and 62% received a tetanus toxoid and antibiotics, respectively.

A total of 725 animals were involved in the human exposures of which 59 were confirmed, 266 probable, 3 suspected and 378 non-rabid animals, while the rabies status could not be assessed for 19 animals (Table 4). The median proportion of confirmed, probable and suspected rabid animals across the six sites was 45%, with a minimum proportion of 34% in Kisantu and a maximum proportion of 75% in Mbanza-Ngungu (although the sample size was low in this site). Nearly three quarters (74%) were owned animals, but only 16% were reported to be vaccinated against rabies of which about two-thirds (61%) had a valid vaccination (vaccination <1year). More than half of the biting animals (52%) were put under observation, during the course of which 17 animals died. Of these, 6 were subsequently tested rabies-positive and 8 rabies-negative, while 3 were not submitted for laboratory analysis. Of the 59 confirmed rabid animals, 90% were immediately killed by a community member after the bite.

Table 4: Characteristics of biting animals disaggregated by rabies status.

	Confirmed	Probable	Suspected	Unknown	Non-rabid
Total (n=725)	59 (8.1%)	266 (36.7%)	3 (0.4%)	19 (2.6%)	378 (52.1%)
Species (n=719)					
Dog	54 (91.5%)	257 (97%)	3 (100%)	16 (88.9%)	364 (97.3%)
Other	5 (8.5%)	8 (3%)	0 (0%)	2 (11.1%)	10 (2.7%)
Ownership status (n=705)					
Owned	27 (45.8%)	124 (47%)	3 (100%)	11 (73.3%)	358 (98.4%)
Unowned	32 (54.2%)	140 (53%)	0 (0%)	4 (26.7%)	6 (1.6%)
Vaccination status (n=683)					
Don’t know	26 (51%)	200 (77.5%)	1 (33.3%)	5 (50%)	114 (31.6%)
No	23 (45.1%)	49 (19%)	1 (33.3%)	3 (3%)	149 (41.3%)
Yes	2 (3.9%)	9 (3.5%)	1 (33.3%)	2 (20%)	98 (27.1%)
Outcome (n=706)					
Died during observation	6 (10.2%)	0 (0%)	3 (100%)	0	8 (2.1%)
Disappeared	0 (0%)	207 (77.8%)	0 (0%)	0	0 (0%)
Killed	53 (89.8%)	59 (22.2%)	0 (0%)	0	0 (0%)
Survived observation	0 (0%)	0 (0%)	0 (0%)	0	370 (97.9%)

*__Other species:__ Cats (n=18), monkeys (n=3), humans (n=2), pigs (n=1) and goats (n=1)

Rabies exposure status of bite victims

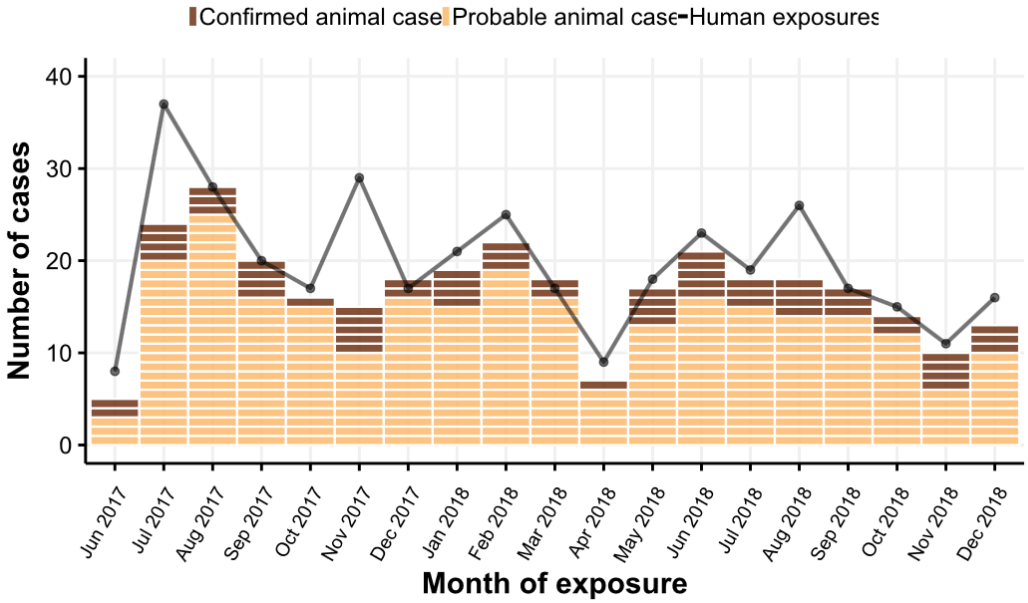
Based on the rabies status of the biting animal, we were able to assess the rabies exposure status of 767 bite victims, of which 50% were likely exposed to rabies (13% confirmed, 36.5% probable and 0.5% suspected exposures) and 50% were non-exposures (Table 5). Of all likely exposures, almost half occurred in children 15 years of age or younger and 60% occurred in males.

Table 5: Characteristics of likely exposed and non-exposed bite victims, including place and type of exposure and delay in presentation to clinic.
Bite victims for whom the exposures could not be assessed (n=19) are not included in the table.

	Likely exposed*	Not exposed
Total (n=767)	381 (49.7%)	386 (50.3%)
Sex (n=748)		
Female	147 (40.1%)	155 (40.7%)
Male	220 (59.9%)	226 (59.3%)
Age (n=719)		
<5yrs	27 (7.5%)	22 (6.2%)
5-14yrs	137 (38%)	125 (34.9%)
≥15yrs	197 (54.5%)	211 (58.9%)
Biting animal (n=762)		
Dog	362 (95.3%)	372 (97.4%)
Other	18 (4.7%)	10 (2.6%)
Zone (n=767)		
Rural	37 (9.7%)	26 (6.7%)
Urban	344 (90.3%)	360 (93.3%)
Wound category (n=712)		
Category I	11 (3.2%)	8 (2.2%)
Category II	60 (17.6%)	90 (24.3%)
Category III	271 (79.2%)	272 (73.5%)
Delay in presentation [days] (n=704)		
Median	1	1
Range	0 - 120	0 - 90

*Confirmed (n=98), probable (n=280), suspected (n=3) exposures

Figure 4: Time series of confirmed and probable animal rabies cases and human exposures captured by the surveillance system based on month of exposure.
For 12 bite victims and 9 biting animals the date of exposure was not available.



Human rabies cases

A total of ten human rabies cases were identified through the surveillance system over the 18 month study period in the general referral hospitals of Muanda (n=4), Matadi (n=3) and Kimpese (n=2). One case presented at a hospital in Kinshasa, but the bite occurred in the Kongo Central province. Two of the four cases that presented at the general referral hospital of Muanda were originally from the bordering town of Soyo, Angola (Table 6). Saliva samples were collected from five patients, of which all were confirmed rabies-positive by PCR. Of the remaining five, two were suspected and three were probable cases.

Table 6: Details of the ten human rabies cases identified through the surveillance system over the 18 months study period (July 2017-December 2018).

Case	Locality	Zone	Sex	Adult/Child	Age (years)	Biting animal	Ownership status	Date of death	Date Bite
Confirmed	Muanda*	Urban	Male	Adult	30	Dog	Owned	July 2017	Apr 17
Confirmed	-#	Urban	Male	Child	4	Dog	-		-
Confirmed	Maunda*	Urban	Male	Adult	28	Dog	-	January 2018	-
Confirmed	Muanda	Rural	Male	Child	4	Dog	-	January 2018	Sep 17
Confirmed	Muanda	Rural	Female	Adult	27	Dog	Unowned	October 2017	Aug 17
Probable	Matadi	Rural	Female	Child	8	Dog	Owned	Nov 18	-
Probable	Matadi	-	-	Child	7	Dog	Owned	February 2018	-
Probable	Matadi	Rural	-	Adult	>15	Dog	-	February 2018	January 2017
Suspected	Kimpese	Rural	-	Child	-	Dog	-	-	-
Suspected	Kimpese	-	-	Adult	-	Dog	-	-	-

* Bite victims presented at the general referral hospital in Muanda but bite occurred in Soyo, Angola
# Bite victim presented at a hospital in Kinshasa but bite occurred in the Kongo Central province
- unknown

Animal rabies samples:

Between July 2017 and December 2018, we analyzed 138 brain samples from rabies suspected animals at the rabies laboratory at INRB. The median time between sampling and receipt of sample was 3 days (IQR: 1-4). Almost three-quarters (71%) of samples were rabies-positive. This translates into an average of 1.7 rabid animals per week. The majority (93%) of samples came from domestic dogs, of which half (66/128) were owned dogs with known owners. Table 7 gives an overview of the samples collected during the study period. Boma submitted more than two-thirds (67%) of all samples. Rabies samples were predominantly collected within (54%) and around (40%) urban centers with only a minority (7%) collected in rural areas. More than half (55%) of the animals showed rabies symptoms, amongst which the most common were aggressiveness (68%) followed by dysphonia (22%). About half (48%) of the animals tested were involved in bite incidents. These 66 animals exposed a total of 113 persons. The median number of humans exposed by one animal was 1 (IQR: 1-2). The proportion of rabies-positive samples was 88% among animals involved in human exposure and 57% in non-biting animals. About one in ten animals were aggressive towards other animals.

Table 7: Details on animal rabies samples from the Kongo Central province analyzed at the rabies laboratory at INRB between July 2017 and December 2018

	Negative (n=40)	Positive (n=98)	Total (n=138)
Species			
Dog	37 (92.5%)	91 (92.9%)	128 (92.8%)
Other	3 (7.5%)	7 (7.1%)	10 (7.2%)
Site			
Boma	32 (80%)	60 (61.2%)	92 (66.7%)
Kimpese	4 (10%)	7 (7.1%)	11 (8%)
Kisantu	1 (2.5%)	1 (0%)	2 (1.4%)
Matadi	1 (2.5%)	7 (7.1%)	8 (5.8%)
Mbanza-Ngungu	0 (0%)	1 (1%)	1 (0.7%)
Muanda	2 (5%)	22 (22.4%)	24 (17.4%)
Zone			
Peri-urban	18 (45%)	37 (37.8%)	55 (39.9%)
Rural	2 (5%)	7 (7.1%)	9 (6.5%)
Urban	20 (50%)	54 (55.1%)	74 (53.6%)
Symptoms			
No	9 (22.5%)	8 (8.2%)	17 (12.3%)
Yes	12 (30%)	64 (65.3%)	76 (55.1%)
Don’t know	19 (47.5%)	26 (26.5%)	45 (32.6%)

Human exposure			
No	27 (67.5%)	36 (36.7%)	63 (45.7%)
Yes	8 (20%)	58 (59.2%)	66 (47.8%)
Don't know	5 (12.5%)	4 (4.1%)	9 (6.5%)
Animal exposure			
No	12 (30%)	14 (14.3%)	26 (18.9%)
Yes	1 (2.5%)	12 (12.2%)	13 (9.4%)
Don't know	27 (67.5%)	72 (73.5%)	99 (71.7%)

Distribution of animal rabies cases by time

The median number of samples submitted per month was 6 (range: 1-19), with a median number of 4.5 (IQR: 3-6.75) rabies-positive samples per month. The median proportion of rabies-positive samples per month was 80% (IQR: 66.7%-95.5%). The monthly average of samples submitted during the dry season was almost twice the number submitted during the rainy season (10.2 vs. 5.8). The proportion of rabies-positive samples was 72% during the dry and 70% during the wet season.Appendix 2: Supplementary information for chapter 6, Figure 34 provides a time series of rabies-positive and –negative samples analyzed at the rabies laboratory in Kinshasa between July 2017 and December 2018.

Bite and dog rabies incidence

The overall annual bite incidence was 44 per 100'000 population and ranged from a low of 3 bites/100'000 persons in Mbanza-Ngungu to 272 bites/100'000 persons in Muanda. We estimated the annual dog rabies incidence at 9.4 (95% CI: 8.8-9.9) per 1000 dogs across the six sites. The lowest incidence was found in Mbanza-Ngungu with 0.5 (95%CI: 0.1-0.67) per 1000 dogs and the highest in Muanda with 31.6 (95% CI: 25.4-38.1) per 1000 dogs. Figure 5 shows the spatial trends of bite and dog rabies incidence across the six sites in the Kongo Central province.

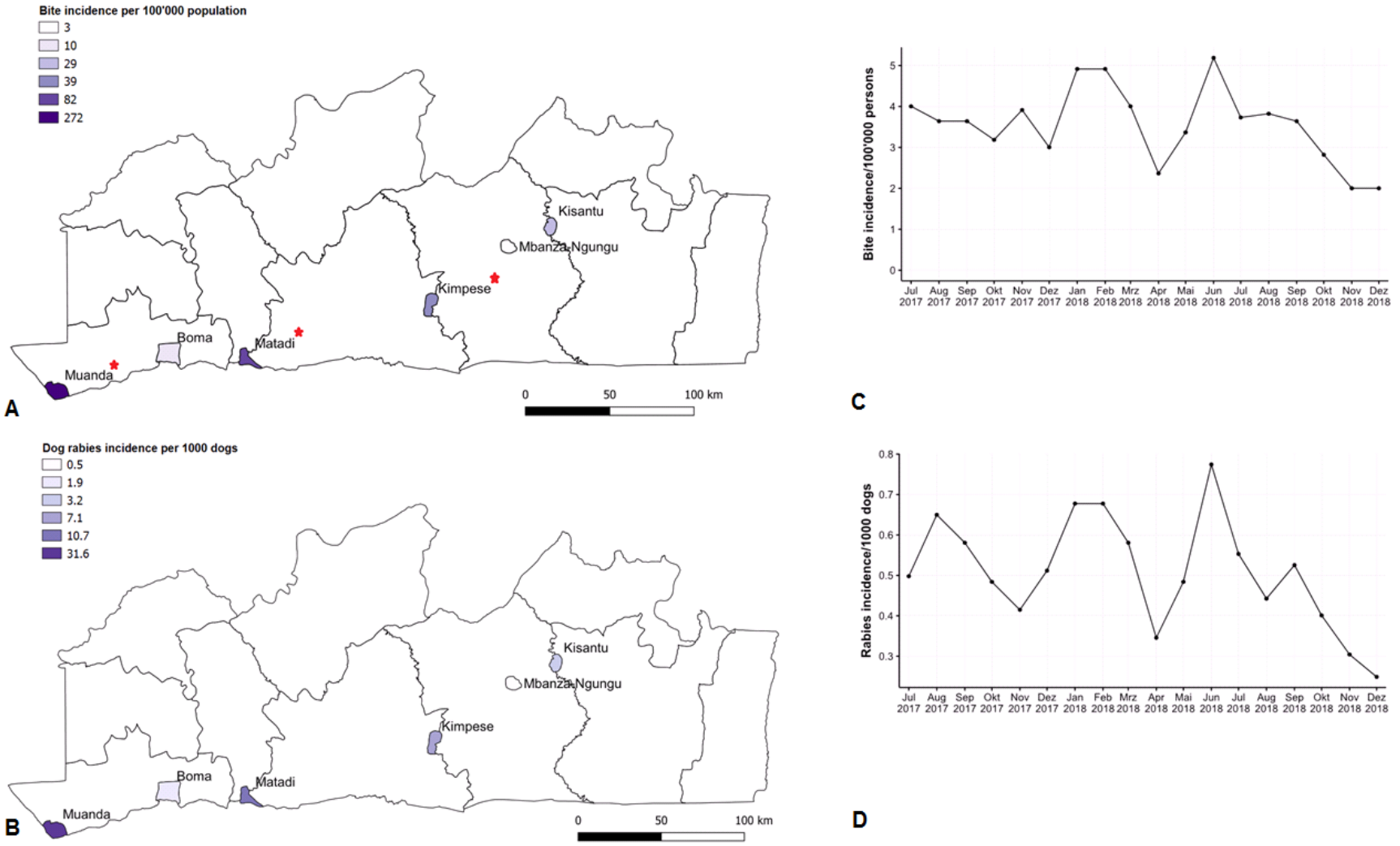


Figure 5: Spatial and temporal trends of bite and dog rabies incidence in the Kongo Central province. Maps depict (A) annual bite incidence (number of bite victims seeking health care after a bite per 100'000 persons), stars indicate locations where human rabies cases were identified between July 2017 and December 2018; (B) annual dog rabies incidence per 1000 dogs in the six sites with rabies units; (C) monthly average bite incidence per 100'000 population; and (D) monthly average dog rabies incidence per 1000 dogs

Estimate of human rabies deaths

The decision tree model estimated a probability of dying of rabies after a bite of 7% (95% CI 2% – 15%). Given the bite incidence of 44/100'000 population per year we would expect 34 human rabies deaths across the six sites (equivalent to an annual human rabies incidence of 3.1 per 100'000). Extrapolated, we predict 169 human rabies deaths for the Kongo Central province.

6.5 Discussion

We implemented a One Health-based surveillance system engaging both medical and veterinary personnel to provide reliable epidemiological data on the burden of rabies in the Kongo Central province. Prior to this study, animal rabies surveillance was not routinely conducted and human rabies surveillance was non-systematic and inconsistent with minimal collaboration between the two sectors. To our knowledge, this is the first study that assesses rabies burden in the DRC. Our results demonstrate that rabies is a significant public health problem in the DRC, which is largely underestimated by official statistics.

During the 18 month study period, 786 bite victims were reported through the surveillance system along with ten suspected human rabies cases of which five were laboratory confirmed, likely the first human rabies samples ever analyzed from the DRC. Our predictive model estimated that 169 human rabies deaths occur each year in the Kongo Central province. This is more than 80 times higher than the 2 cases officially reported in 2018 for the entire province.

Bite incidence varied considerably between the six sites, with the highest incidence found in Muanda. This may be explained by an increased level of rabies awareness among this community due to high personal engagement of the VFPs in rabies prevention in this area. Prior to the study, surveillance of bite victims included that all bite victims who presented to health centers and the general referral hospital were referred to the official veterinarian. This suggests that a higher proportion of bite victims seeking treatment after a bite was captured in this site. It is not uncommon that bite victims from neighboring Angola seek PEP in Muanda if it is not available in their country, which is also reflected in the two human rabies cases that originated in Angola but died in the general referral hospital of Muanda. However, we excluded these cases from bite incidence estimates. The lacking engagement in surveillance activities and communication between the VFPs and MFPs noticed during our follow-up visits in Mbanza-Ngungu might explain the low number of bite victims captured in this site.

Through enhanced communication between the human and animal health sectors using IBCM, we were able to assess the rabies exposure status of most (98%) bite victims based on investigation of the biting animal. About 50% of bite victims were likely exposed to rabies. Slightly higher proportions were reported from other African countries, such as Tanzania (62%) [315] and Ethiopia (73%) [316]. In line with findings from other rabies endemic settings, dogs were the source of most bites, with children and males disproportionately affected [37; 49; 310; 315-317].

The vast majority of non-exposures (96%) could be ruled out through observation of the biting animal, in contrast to only 4% ruled out based on a rabies-negative test result. Most animals (97%) that were put under observation survived the ten day period indicating that VFPs were quite competent to distinguish between animals that appeared healthy at the time of the bite and animals that displayed signs of illness. In settings with advanced rabies surveillance, PEP may be delayed for low risk exposures (i.e., when animals appear healthy at the time of the bite) to limit unnecessary PEP use [116]. IBCM is very attractive from an economic perspective [310; 318], and clinical identification of suspected rabid animals is highly sensitive [319; 320]. However, the mental stress to which bite victims are subjected while awaiting the results over a ten day observation period should not be ignored. In the DRC where rabies surveillance is limited and human and animal health professionals are not trained for IBCM, PEP should be initiated immediately after a bite. Improved access to PEP is an essential pillar of the global strategic plan to eliminate dog-mediated human deaths by 2030 [44]. GAVI recently decided to invest in human rabies vaccine, and the DRC is among the eligible countries. [294]. Hopefully, this will improve PEP availability across the DRC in the near future. A shift from IM to ID administration, as applied in this study, could further increase PEP availability and reduce costs for bite victims.

Between 2009 and 2013, 44 rabies samples from the entire DRC were tested at both the INRB and LaboVet (~9 samples per year) [191]. Ten times more rabies samples (92) were submitted to the INRB within one year of the enhanced surveillance. The proportion of rabies-positive samples (71%) is higher than that reported from other rabies endemic countries in sub-Sahara Africa (53% in Chad [37], 64% in Kenya [321], 68% in Tanzania, 67% in Namibia [322]) indicating that recognition of rabies among VFPs was high. Two-thirds of the project samples were submitted from Boma. The increased number of samples submitted from this site is explained by the fact that Boma also collected samples from animals that were not involved in human exposures (66% of samples), whereas four of the five remaining sites only submitted samples from animals involved in human exposures. On their own initiative, VFPs from Boma involved community members in the surveillance of suspected rabid animals which led to the detection of non-biting animals. Rabies-positivity was still 57% among non-biting animals, suggesting that community members are

also capable of recognizing rabid animals. The monthly average of animal samples submitted during the dry season was almost double the number of samples submitted during the rainy season (10.2 vs. 5.8), but the proportion of rabies-positive samples was similar (72% during the dry compared to 70% during the rainy season). Probably the decreased number of samples submitted during the rainy season is related to difficulties in sample collection because of poor road conditions rather than reduced rabies transmission. Similar to the bite incidence, dog rabies incidence varied across the six sites with the highest incidence also found in Muanda and the lowest in Mbanza-Ngungu. We assumed the same dog/human ratio across the six sites, and differences in incidence could be due to variations in dog population size. However, only one rabies sample was submitted from Mbanza-Ngungu and lack of engagement was noticed during follow-up visits. Considering that the same disparity was seen in bite incidence, we believe that the low dog rabies incidence seen in Mbanza-Ngungu is more likely due to poor surveillance. We found a high annual dog rabies incidence, at 9.4 rabid dogs per 1000 dogs across the study site. However, unlike other studies, we estimated incidence based on the number of confirmed plus probably and suspected rabid dogs instead of based on laboratory-confirmed dogs only. When based on confirmed rabid dogs only, the DRC incidence decreases to 1.2 per 1000 dogs, which is similar to a comparable urban setting (in terms of dog population size and human population) in Chad, at 1.4 per 1000 dogs [47].

We faced several challenges when implementing the surveillance system in the Kongo Central province. A major constraint to effective rabies surveillance is the chronic neglect of the animal health sector in the DRC. The limited financial resources allocated to the animal health sector are mainly focused on prevention of diseases in economically valuable livestock. Even if veterinary services are motivated to engage in rabies surveillance, they simply lack the equipment and means of transportation for routine collection of animal rabies samples and the human resources to conduct follow-up investigations of suspected rabid animals. To put this into perspective, there are currently only 18 official veterinarians in the Kongo Central province to cover a population of 5.5 million people and an unknown number of animals. In addition, dog owners have to pay for observation and laboratory diagnosis of suspected rabid animals. Given that over 60% of Congolese live on less than 1.9 USD a day, it is obvious that many are unable or unwilling to pay for these follow-up investigations, which is a clear disincentive for veterinarians to investigate rabies activities. It is also important to note that dog vaccination constitutes a source of income for many veterinarians. Fear of losing this valued additional revenue could cause veterinarians to not support increased rabies control activities, such as subsidized dog vaccination campaigns. The ab-

sence of an official sample transport network hampers laboratory diagnosis of animal rabies, especially from remote rural areas. Health zones were willing to assist in shipment of animal samples, but transportation was slow and conducted irregularly. For timely submission and diagnosis of animal samples to provide accurate advice for bite victims about PEP, we had to establish our own transportation network at a cost of about 25 USD per sample collected. Hence, sample submission is likely not sustainable and expected to decrease after the project end.

Community participation is a key component in effective rabies surveillance, for instance, reporting bite incidents and suspected rabid animals. Less than 50% of animals that were killed after a bite incident were brought to the veterinary service for laboratory testing. This supports findings from a previous study that there is a lack of rabies awareness among the community [307]. Likewise, rabies case recognition among health care workers is low [189] resulting in non-detection of cases. It is difficult to link a bite that occurred several months ago to the non-specific symptoms present in rabies patients, potentially leading to misdiagnosis. After training of health care workers, ten human rabies cases were identified. Subsequent laboratory confirmation in five of these cases was a rewarding motivation for the health personnel. Human rabies diagnosis is not yet possible at INRB, but efforts to install PCR are underway.

Sustainability of the surveillance system will be a challenge. While MFPs and VFPs were not remunerated for participating in the study, communication fees and sample collection consumables, coolers and packing materials for sample shipment, as well as personal protective equipment were provided by the project. Without active governmental support, scaling up surveillance to rural areas and other provinces will be difficult.

Limitations:

Most human rabies exposures (92%) and animal rabies samples (93%) came from in and around the six sites where rabies units were located. It is likely our data reflects the urban rabies situation rather than that of the entire province. However, it is well recognized that rabies mortality is higher in rural areas [14; 316]. Therefore, we believe that our figures are a conservative estimate of the human rabies burden in the Kongo Central province, and the actual number of human rabies deaths is higher. We assumed that no rabies deaths resulted from incomplete PEP, although there is no data on such outcomes currently available. In a study investigating bite incidence among a community in the Kongo Central province, Mbilo *et al.* [307] found a community-level bite incidence of 520/ 100’000 population per year. Given our health facility-level bite incidence of 44/ 100’000 population, assuming that all bite victims seeking health care were captured by the surveillance system, this indicates that less than 10% of bite victims seek treatment after a bite. However, our surveillance of bite victims was limited to general referral hospitals and the veterinary services, so

we missed bite victims who presented to health centers and pharmacies. In Ethiopia, extensive tracing of non-registered bite victims found that 71% of bite victims present to a health facility after a bite [316]. A similar proportion (84%) was found in Madagascar [323]. Enrolling more health care providers in surveillance of bite victims would help to better define the proportion of bite victims who attend a health facility after a bite and further improve estimates of human rabies deaths.

Conclusion

This study demonstrates that it is possible to enhance rabies surveillance in a resource-poor environment with engagement and targeted training of human and veterinary personnel and limited financial support for basic equipment and logistics. We hope the data provided through this study will contribute to political will to invest in rabies prevention and control. The established collaboration between the animal and health sectors builds a foundation for surveillance of other zoonotic diseases, which is an additional incentive in countries like the DRC which experience recurrent outbreaks of zoonotic diseases like Ebola, yellow fever and monkey pox.

7 Dog ecology, bite incidence and disease awareness: a cross-sectional survey among a rabies-affected community in the Democratic Republic of the Congo.

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7.1 Abstract

Despite the existence of safe and efficacious human and animal rabies vaccines, millions of people remain at risk of exposure to this deadly zoonotic disease through bites of infected dogs. Sub-Saharan African countries, such as the Democratic Republic of the Congo (DRC), bear the highest per capita death rates from rabies where dog vaccination and availability of lifesaving post-exposure prophylaxis (PEP) is scarce. Mass dog vaccination is the most cost-effective and sustainable approach to prevent human rabies deaths. We conducted a cross-sectional household survey in a rabies-affected community in Matadi, DRC, to estimate the size of the owned dog population and dog bite incidence and assess knowledge and practices regarding rabies, as preparation for future mass dog vaccination campaigns. Our study revealed that the owned dog population in Matadi was almost ten times larger than assumed by local veterinary officials, with a large proportion of free-roaming unvaccinated dogs. The annual dog bite incidence of 5.2 per 1000 person years was high, whereas community rabies knowledge was low resulting in poor practices. Given these findings, human rabies deaths are likely to occur in this community. Lack of disease awareness could negatively affect participation in future mass dog vaccination campaigns. A public sensitization campaign is needed to promote appropriate rabies prevention (washing bite wounds and PEP) and control (dog vaccination) measures in this community.

Keywords: Rabies; free-roaming dog; dog ecology; dog bite incidence; zoonosis; Democratic Republic of the Congo

7.2 Introduction

Rabies, a fatal viral disease transmitted through the bite or scratch of an infected animal, is estimated to cause about 21,500 human deaths per year in Africa [110]. Domestic dogs are the main reservoir species of the rabies virus (RABV) and by far the most common transmitter of the disease to humans [9]. Because most human rabies exposures (>99%) result from dog bites and highly efficacious dog rabies vaccines are available, the disease in humans can be prevented through vaccination of its animal source.

In 2018, the Global Strategic Plan to end human deaths from dog-mediated rabies by 2030 was launched by the United Against Rabies (UAR) collaboration of four international organizations, the World Health Organization (WHO), the World Organisation for Animal Health (OIE), the Food and Agriculture Organization of the United Nations (FAO), and the Global Alliance for Rabies Control (GARC) [44]. The global strategic plan emphasizes mass dog vaccination as key for sustainably

preventing human rabies, along with increasing disease awareness and ensuring prompt post-exposure prophylactic treatment (PEP) for bite victims.

Rabies transmission among dogs (and therefore exposure to humans) can be interrupted when a sufficient proportion of the dog population is immunized [56]. The threshold proportion p_c of the population that needs to be immunized to interrupt transmission under assumptions of homogeneity is determined by the basic reproductive number R_0 of an infectious disease as $p_c = 1\text{-}1/R_0$. R_0 of rabies transmission between dogs is estimated to be relatively low, between 1.05 and 1.72 [324; 325]. Based on empirical data and disease modeling, the UAR collaboration recommends annual vaccination campaigns that attain at least 70% of the dog population to sustain interrupted transmission [44; 56; 292]. Recent work based on social contact networks of dogs confirmed the recommended coverage of 70% [326]. Therefore, the size of the target dog population is a critical factor to consider in planning vaccination campaigns. A straightforward approach to obtain a rough estimate of the owned dog population is a household survey to determine the dog/human ratio, with subsequent extrapolation to a given area [327].

Even though the dog rabies vaccine is highly efficacious, final effectiveness in the field, in terms of vaccination coverage, depends on accessibility of the dog population for vaccination and many other effectiveness factors [87; 88; 296; 303; 328], which are influenced by the sociocultural context [172]. Accessibility of dogs for parenteral vaccination is affected by their ownership status. Increasing evidence suggests that the majority of dogs in Africa are owned but allowed to roam freely and that the proportion of ownerless dogs is low [32; 327]. Proof-of-concept studies in Africa were successful in reducing human and animal rabies cases but mainly targeted the owned dog population [172; 173; 298; 299; 329]. Lack of disease awareness and inability of dog owners to handle their dog can be a constraint to participation in vaccination campaigns [87; 172].

While dog vaccination is essential to sustainably control rabies in the reservoir population and prevent human deaths, individuals also need to be aware of preventive measures following an animal bite, such as seeking PEP and informing the veterinary service of suspected rabid animals. It is thus critical to understand the local context and assess community awareness on rabies prior to implementation of mass dog vaccination campaigns. Knowledge, attitude, and practices (KAP) studies within rabies-affected communities expose factors that influence response to vaccination campaigns and identify knowledge gaps and practices that hamper rabies prevention and control.

Dog bites have been widely used as a proxy to estimate human rabies deaths based on decision-tree probability models [110; 124; 317; 330]. Epidemiological data on dog bites are often obtained

from health facilities, but not all dog bite victims seek care in the formal health care sector. Therefore, such data fail to capture the true incidence of dog bites in the community and are likely to be biased towards more severe bites that require medical attention [304]. Community population-level estimates of actual dog bite incidence are needed to assess the burden of rabies in a given area.

In the Democratic Republic of the Congo (DRC), first reports of rabies date back to 1923 [187] and recurrent outbreaks have occurred across the country ever since [190; 306; 331]. In 2009, 21 clinically-confirmed rabies cases in children were registered in the Pediatrics Department of a single hospital in the capital city of Kinshasa in only seven months. In a retrospective study of 5053 animal attacks in Kinshasa between 2009 and 2013, 2.5% were likely due to rabid animals [191]. Despite this alarming situation, no official rabies control program is in place and very limited data on dog population and community awareness regarding rabies exist. In the DRC, dog vaccination is not governmentally subsidized. The Congolese National Order of Veterinarians (ONMVC: Ordre National des Médecins Vétérinaires du Congo) fixed the price for dog rabies vaccination at no less than 20 USD [193], although it has been shown earlier in a similar setting that cost above 2 USD cannot be borne by the community[171]. Recent studies found dog vaccination coverage between 24% and 81% in different communes of Kinshasa [192; 193]. The vaccination status of dogs appeared to depend on access to vaccine and the dog owner's economic situation [192; 193]. About two-thirds of dogs were allowed to roam freely, and the proportion of ownerless dogs was estimated to be less than 2% [192].

In this article, we report the findings from a cross-sectional household survey conducted in a rabies-affected community in the DRC to study both the human and dog population as a preparation for future dog mass vaccination campaigns in the DRC. The aims of the study were to collect baseline data on the size and characteristics of the owned urban dog population, assess community knowledge and practices regarding rabies and, provide a community-level estimate of dog bite incidence.

7.3 Methods

7.3.1 Study Site

Our study took place in Matadi (5°49′03″S 13°28′15″E, Figure 6), the capital of the province of Kongo Central, located in the western part of the DRC. Matadi was selected due to the occurrence of several rabies outbreaks over the past few years (personal communication, government veterinary clinic of Matadi) and its accessibility by road from Kinshasa. Compared to other provinces,

Kongo Central is relatively well developed as it is the only province with access to the Atlantic Ocean and harbors DRC's two main ports. In the second Demographic and Health Survey (DHS), 81% of the households in Kongo Central were categorized into the three highest quintiles of the wealth index [309]. This is still low in comparison to Kinshasa, where over 97% of households are classified in the highest quintile of the wealth index [309]. Matadi is located on the Congo River and divided into three communes comprising 17 neighborhoods. According to the 2015 annual report of the municipality, an estimated 301,644 people live in Matadi across 110km^2. The government veterinary clinic of Matadi recorded 873 dogs living in the city in 2017 (personal communication, 2018).

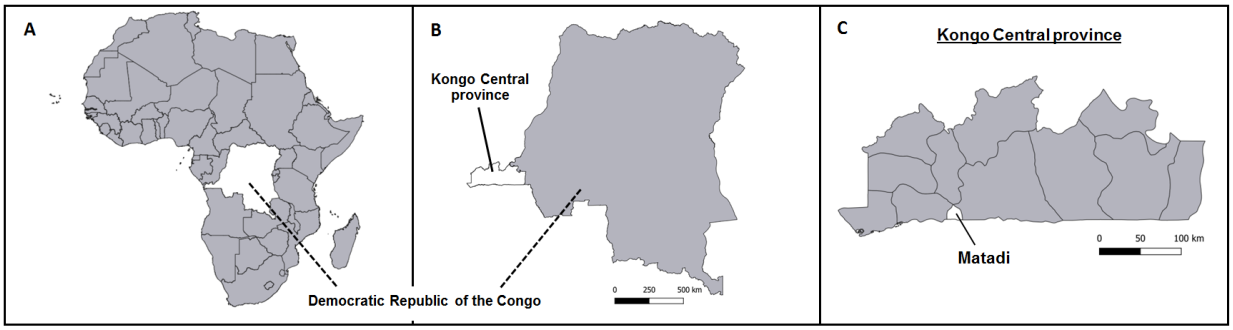

Figure 6: Maps indicating the Democratic Republic of the Congo (A) and the Kongo Central province (B) with the study site Matadi (C).

In May 2017 (dry season), we conducted a cross-sectional household survey among the residents of Matadi. A structured questionnaire in French based on the Report of WHO Consultation on Dog Ecology Studies Related to Rabies Control [332] and the Guidelines for dog population management [333] was developed and pre-tested in 40 Matadi households during an exploratory visit in July 2016. The questionnaire consisted of three sections. The first part contained questions on household sociodemographic characteristics, while the second part gathered information on the respondent's knowledge and practices regarding rabies and animal bite incidents occurring in the household in the last three years. The third part captured detailed data on individual dogs older than three months owned by the household. A household was defined as one person living alone or a group of persons, related or not, occupying a housing unit (house, apartment, a single or group of rooms), with shared living accommodation and meals under the lead of a household head.

7.3.2 Sample Size Calculation and Household Sampling Procedure

Due to the lack of a household register or clearly defined administrative boundaries of neighborhoods, we applied the sampling procedure proposed by Schelling & Hattendorf [334] using random geo-coordinates. A set of numbered random geo-coordinates that served as interview starting points for the survey teams was generated with R software version 3.3.1 [314] and exported as a kml file to Google Earth software version 7.1.2.2041. Points lying outside the city boundary were discarded.

Based on expertise gathered from research projects in Bamako (Mali), Iringa (Tanzania) and N'Djamena (Chad) [86; 335; 336], we assumed a prevalence of dog owning households of 14%. To obtain a point estimate with a precision, defined as one half-length of the confidence interval, of 4.5 percentage points, we needed to survey 50 clusters with 20 households each, assuming an intra-cluster correlation coefficient of 0.2.

Four field teams conducted the house-to-house survey. Each team consisted of an interviewer and a local guide. Three interviewers were recruited at the veterinary faculty of the National Pedagogical University in Kinshasa. One interviewer and four veterinary technicians who served as local guides were recruited at the government veterinary clinic of Matadi. Teams were trained on the household selection process and administration of the questionnaire during a pre-test in July 2016 and two days prior to the launch of the survey in May 2017. The head of the government veterinary clinic of Matadi supervised and coordinated the four field teams. After pre-testing, one question regarding household sanitary situation was removed from the questionnaire because it was considered inappropriate by respondents. Several questions were rephrased to improve understanding. Based on the experience gained during the pre-test, we deemed data collection in 21 households per team and day attainable and estimated study completion within 13 days.

The study lasted from May 15 to May 28, 2017. On the survey days, the four field teams gathered at the government veterinary clinic of Matadi at 07:30 AM. Each team was guided to a random geo-coordinate starting point using the free, open source mobile application MAPS.ME. Facing north, the nearest household on the right side of the street was chosen for a first interview, then every third household was interrogated. At cross roads, the team first turned left and then right at the next cross road and so on. After completion of seven households, the teams moved back to the starting point and repeated the same procedure facing east, south, and west until a total of 21 households were visited. If for any reason the survey could not be carried out in a household (e.g. nobody is at home, the person refuses to participate or was too young, the house was a shop),

the neighboring household continuing in the direction of movement was selected. The questionnaire was administered to the household head or another occupant over 16 years of age. Depending on the respondent's preference, interviews were conducted in French or the local languages Lingala or Kikongo. Each interviewer was equipped with a computer tablet (Trekstor SurfTab breeze) configured with Open Data Kit (ODK) software and a solar battery charger. In non-dog owning households, interviews were limited to the first two parts of the questionnaire. At the end of each survey day, completed questionnaires were downloaded from the tablets to a password protected external hard drive.

7.3.3 Data Analysis

Data collected during the study were downloaded from ODK software in comma-separated value files and analyzed using R software version 3.5.0 [314]. Maps were created with QGIS version 3.4.4.

We applied generalized estimating equations (GEE) for binary distributed outcome variables, i.e. dog ownership status (yes/no), having heard of rabies (yes/no), rabies knowledge (adequate/inadequate), and history of animal bite in the household (yes/no), with a logit link function and independent correlation structure to account for the clustered nature (random geo-coordinates) of the questionnaire data. Association with explanatory variables (age, sex, education, household position, livestock ownership, dwelling ownership status, water source, residence) were analyzed using univariable and multivariable analysis. All explanatory variables with p-values <0.2 in univariable analysis were included in the multivariable model [337]. Estimates are presented as odds ratios (OR) and adjusted OR with corresponding 95% confidence interval (CI). The significance level was set at p-value ≤ 0.05. To estimate the dog/human ratio, we used GEE for negative binomial distributed outcomes and independent correlation structure to account for the over-dispersed nature of the distribution of dog counts per household. The point estimate and CI were divided by the mean number of persons per household.

We constructed knowledge and practices scores for respondents who had heard of rabies (n = 551) based on eight and three questions, respectively (see Appendix 3: Supplementary information for chapter 7, Table 31). All questions were equally weighted with a maximum score of 4 per knowledge and 3.5 per practices question. No penalty was given for incorrect answers. A respondent could obtain overall scores of 32 for rabies knowledge and 10.5 for practices, when all questions were correctly answered. Respondents that obtained 16 points or higher were classified as knowledgeable about rabies. We assessed the relationship between the practices score

(outcome variable) and knowledge score (explanatory variable) using GEE for normal distributed outcome variables with an identity link function and independent correlation structure.

Based on proxy markers similar to those used in the DHS conducted in the DRC in 2013-2014, we assessed the socioeconomic status (SES) of households [193; 309]. We used the following five housing characteristics: availability of piped drinking water, wall material, roof type, livestock ownership, and fenced property. A score of 0 or 1 was attributed to each item: 0 in case of unavailability of piped water, straw/wooden wall, straw roof, non-ownership of livestock, and unfenced property, and 1 in case of availability of piped water, cement/brick wall, sheet roof, ownership of livestock, and fenced property. Households that scored ≤ 2 points were assigned low SES, while households that scored ≥3 were classified as middle SES. Our SES was well in line with the DHS wealth index [309].

7.3.4 Ethical Approval

We obtained research permission from *EKNZ (*Ethics Committee *for* Northwest/Central Switzerland, EKNZ BASEC Req-2017-00395*) and* ethical clearance from the Ministry of Public Health ethics committee at the Clinique Ngaliema, Kinshasa (COMETH/TKK/PRES 002/2017). Study participants were verbally informed about the purpose of the survey, and we obtained verbal consent from each participant before administering the questionnaire. Respondents were free to refuse or discontinue participation at any time. Before the beginning of the study, a meeting was held with the mayor of Matadi, who provided written permission to conduct the study.

7.4 Results

7.4.1 Household and Respondent Characteristics

In May 2017, we approached a total of 1095 households in the city of Matadi of which 1056 completed the survey (96%). The total number of occupants living in the 1056 households was 6742 with a median household size of 6 (IQR: 4–8). Details about household and respondent characteristics are given in Table 8. More women (63%) were interviewed and about half (44%) of the interviews were conducted with the wife of the head of household. Most respondents (90%) had secondary (78%) or higher education (12%) and around half (49%) were older than 40 years. Two-thirds of households were owned and slightly more than half (53%) were located in peri-urban parts of the city. Almost all households (97.5%) had no fence around their premises, meaning that dogs could enter and leave the premises without restriction. Two-thirds of households (67%) disposed of garbage so that it was easily accessible for roaming dogs.

Table 8: Characteristics of the 1056 households and respondents surveyed in Matadi, Democratic Republic of the Congo (DRC) in May 2017.

	Overall (*n* = 1056)
Sex of respondent	
Female	669 (63.4%)
Male	387 (36.6%)
Age of respondent (years)	
16-29	259 (24.5%)
30-39	281 (26.6%)
>40	516 (48.9%)
Level of education of respondent	
None	23 (2.2%)
Primary	85 (8.0%)
Secondary	818 (77.5%)
Tertiary	130 (12.3%)
Socioeconomic status	
Middle	920 (87.1%)
Low	136 (12.9%)
Position of respondent in the household	
Wife of head of household	464 (43.9%)
Head of household	317 (30.0%)
Child of head of household	211 (20.0%)
Other relative of head of household	64 (6.1%)
Occupation of respondent	
Private sector	603 (57.1%)
Public sector	316 (29.9%)
Unemployed	101 (9.6%)
Retired	36 (3.4%)
Dwelling ownership status	
Owner	629 (59.6%)
Tenant	427 (40.4%)
Source of water	

	Overall (*n* = 1056)
Improved	893 (84.6%)
Unimproved	163 (15.4%)
Waste disposal	
Open	707 (67.0%)
Closed	349 (33.0%)
Livestock ownership	
No	769 (72.8%)
Yes	287 (27.2%)
Residence	
Peri-urban	557 (52.7%)
Urban	499 (47.3%)
History of bite incident	
No	964 (91.3%)
Yes	92 (8.7%)
Dog ownership	
No	956 (90.5%)
Yes	100 (9.5%)

*Source of water was categorized as improved or unimproved according to WHO Water, sanitation and hygiene (WASH) standards [338]
Open waste disposal: public/private waste disposal site, burying waste/ **closed waste disposal**: incineration of waste

7.4.2 Dog Ownership and Dog-Keeping Practices

The percentage of dog-owning households (DOHH) was 9.5%, with 1–9 dogs per DOHH (median: 1, IQR: 1-2). We recorded a total of 178 dogs during the household survey. The dog/human ratio, when considering the 6742 persons represented in this survey, was 1:37.7 (95% CI: 32.1–44.3). An estimate of the owned dog population based on an extrapolation of the dog/human ratio predicts 8001 dogs for the city of Matadi (95% CI: 6809–9397), resulting in a dog density of 74/km^2.

More than two-thirds of dog owners (63%) allowed their dogs to roam freely at all times, while the remainder confined their dogs part time either during the day (35%) or night (2%). Dogs were primarily kept for security (92%) and livestock herding (7%). Only one household (1%) reported using dogs for hunting. The majority of dogs (93%) were fed with leftovers from human consumption and/or slaughterhouse (16%), while 3% of dogs were provided commercial dog food. Less than one in four dog owners (22%) stated that they provided some level of veterinary care to their

dogs. Dog owners reported that their dogs would spend most of their time on (86%) or in front of the dog owner's premises (8%), with only a small minority roaming the streets (6%). The most common way to acquire a dog was through purchase (52%) or as a gift (30%), followed by off-spring from another household dog (15%) or adoption from the street (3%). Among households that purchased a dog, about two-thirds (58%) were bought in another neighborhood of the city, 36% were acquired in the same neighborhood and 6% were purchased outside the city.

When asked if ownerless dogs were present in the neighborhood, the majority of respondents (82%) stated that there were no ownerless dogs, while 17% reported having seen ownerless dogs and 1% could not provide a response to this question. The median number of ownerless dogs stated by 154 out of 182 respondents who reported having seen ownerless dogs was 2 (IQR: 1–4), the remaining 28 respondents did not provide an estimate of the ownerless dog population in the neighborhood.

Among non-dog owning households (956/1056) reasons given for non-ownership were a dislike of dogs (39%), no necessity for keeping a dog (18%), not enough space (13%), or no specific reason (10%). Only 8% stated that they were afraid of the public health risks associated with dog-ownership. The remaining 12% provided various other reasons.

Factors Associated with Dog Ownership

The results of the univariable and multivariable analysis of factors associated with dog ownership are presented in Table 9. In the final multivariable model, livestock owning households had twice the odds of owning a dog as compared to households without livestock (95% CI: 1.25–3.23, p = .004). Similarly, the odds of owning a dog were greater among dwelling owners as compared to tenants (adj OR = 2.37, 95% CI: 1.36–4.15, p = 0.002). We found no association with residence or household socioeconomic status.

Table 9: Uni- and multivariable generalized estimating equation models for binomial outcome variables to determine household characteristics associated with dog ownership in Matadi, Democratic Republic of the Congo (DRC) in May 2017.

Variables		%(Npos)	OR	95% CI	*p*-value	Adj OR	95% CI	*p*-value
Socioeconomic status	Middle	10% (90/920)	reference					
	Low	7% (10/136)	0.73	0.37–1.44	.366			
Dwelling ownership status	Tenant	5% (21/427)	reference			reference		
	Owner	13% (79/629)	2.78	1.65–4.68	**<.001**	2.37	1.36–4.15	**.002**
Livestock ownership	No	7% (55/769)	reference			reference		
	Yes	16% (45/287)	2.41	1.53–3.8	**<.001**	2.01	1.25–3.23	**.004**
Residence	Peri-urban	10% (56/557)	reference					
	Urban	9% (44/499)	0.87	0.54–1.39	.55			

7.4.3 Dog Population Characteristics

Of the 178 dogs identified during the household survey, 57 (32%) were puppies (0–3 months), 31 (17%) young dogs (4–11 months), 89 (50%) adult dogs (1–7 years), and 1 old dog (> 7 years). Detailed information on 106 dogs older than 3 months was available, while data on 3 young dogs and 12 adult dogs were missing. The study dog population consisted of 53 males (50%) and 53 females (50%) with a sex ratio of exactly 1:1 (Figure 7). The 57 puppies were not sexed. None of the female dogs and only 3 out of 53 male dogs were neutered. Roughly three-quarters of dogs (71%) were local nondescript breeds, followed by mixed breeds (29%). Twenty-six out of 53 (49%) females gave birth in the 12 months preceding the study with a median litter size of 6 puppies (IQR: 4–7). Of the 142 puppies born, 37% died, and the remainder were kept in the household (42%), sold (11%) or given away (10%).

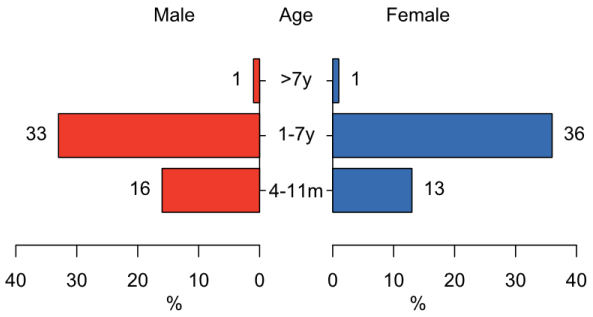

Figure 7: Age and sex distribution of surveyed dogs older than 3 months.
Note that age categories are not distributed equally.

Dog Vaccination and Accessibility for Parenteral Vaccination

According to dog owners, 25 out of 106 dogs (24%) were vaccinated against rabies at some point in their lifetime. Of these, only three-quarters of dogs (72%, 18/25) had a vaccination booklet and one-third of vaccinations (33%, 6/18) were outdated at the time of the survey. Vaccination status of the 57 puppies was not available. When asked about reasons for non-vaccination, almost one-third of dog owners (30%) refused to respond to this question. One in three dog owners lacked the money to purchase the vaccine, while 16% did not know where to find the vaccine and 14% thought that their dog was too young for vaccination. About 7% of dog owners did not know that their dog should be vaccinated against rabies. Dogs living in households assigned a high SES were more often vaccinated than dogs owned by low SES households (30% vs. 0%). Three-quarters of dog owners (74%) claimed that they would be willing to spend money on dog vaccination and the median amount they would be willing to pay for dog vaccine was 2.1 USD (IQR: 0-

3.6 USD). The majority of dog owners (92.5%) stated that they could handle their dog for parenteral vaccination.

7.4.4 Animal Bite Incidents

Almost one in ten households (9%) had at least one family member who experienced an animal bite in the previous three years. A total of 97 bite victims were reported, of which 62 % were adults and 38% children. Sex of the bite victim was not recorded. We estimated a dog bite incidence of 5.2 per 1000 person years (95% CI: 4.3–6.2). All bite victims were bitten by dogs, which were in most cases (89%) known by the bite victim, reported as a neighbor's dog (60%), a dog owned by the household (16%) or a community dog (13%). Only 7% were bitten by an unfamiliar ownerless dog. Dog ownership was positively associated with the occurrence of a bite incident in the household (OR = 2, 95% CI: 1.01–3.85, $p = 0.034$). More than one in ten bite victims (11%) died before the survey, and 64% of respondents stated that the death was related to the bite incident, i.e. the bite victims died after showing symptoms of rabies.

7.4.5 Community Rabies Knowledge

When asked if respondents knew of diseases transmitted by dogs, the most common (37%) disease mentioned was rabies, followed by tetanus (14%). Slightly more than half of respondents (52%) had previously heard of rabies, with the majority (95%) correctly describing rabies as a disease. Only study respondents who had heard of rabies were further questioned ($n = 551$). Most respondents (88%) knew that rabies was transmitted through the bite of an infected animal but were unaware of other modes of transmission. Almost all respondents (94%) mentioned dogs as the principal reservoir of rabies, followed by cats (19%). Forty percent of respondents correctly described the symptoms of rabies (mentioned one or more symptoms) in animals. Aggressiveness (95%) was the most commonly mentioned symptom, followed by behavior change (40%). Figure 8 gives an overview of animal rabies symptoms stated by the participants. About half of respondents (45%) were able to state one or more rabies symptoms in humans. A wide range of symptoms were identified by the study participants, with monologizing (23%), insanity (23%), barking like a dog (22%) and aggression (8%) most frequently mentioned. Of the 551 respondents, 33% claimed to know someone who died of rabies and 30% stated that they had encountered a rabid animal. The odds of correctly describing animal rabies symptoms were 3.43 times higher among respondents who had seen a rabid animal (95% CI: 2.14 – 5.5, $p < .001$). There was no similar association found between knowing someone who died of rabies and correctly

describing human rabies. Around three-quarters of respondents (73%) stated that rabies is a preventable disease, and half of respondents (51%) knew that human rabies is preventable through dog vaccination. However, only 3% of respondents mentioned human vaccination as a preventive measure and only 14% knew that rabies is fatal following the onset of symptoms.

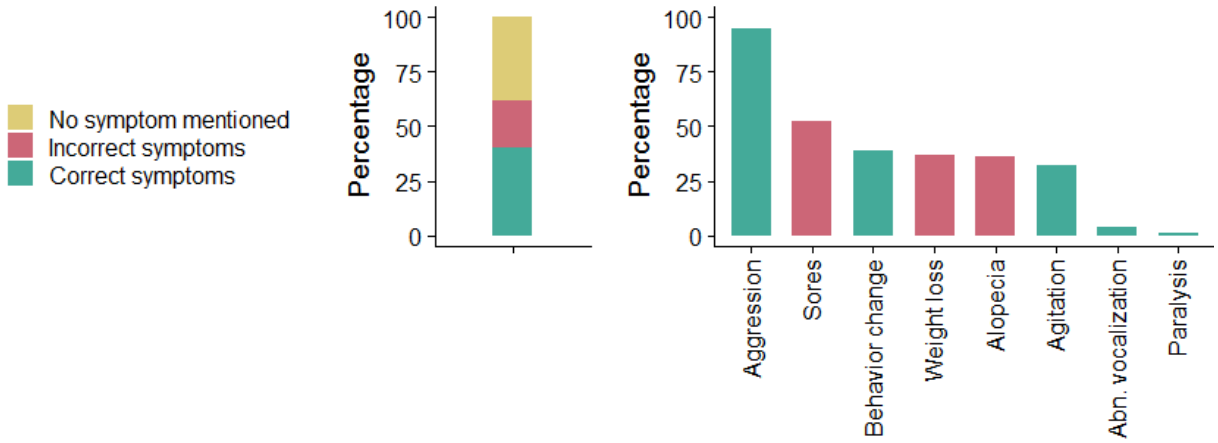

Figure 8: Animal rabies symptoms mentioned by the study participants in Matadi, Democratic Republic of the Congo (DRC) in May 2017.
The left plot displays the proportion of respondents that did not mention any rabies symptoms and the proportion of respondents that stated correct and incorrect symptoms. The right plot displays frequently mentioned correct and incorrect animal rabies symptoms.

Factors Associated with Rabies Knowledge

The mean knowledge score was 13.65 (SD: 4.85, median: 13), with a minimum of 0 and a maximum of 26 points. The most frequent knowledge scores were 12 and 13 (11.4% and 17.4%, respectively). Using the cut-off score of ≥ 16 to classify high and low level of rabies knowledge, 189 out of 551 (34%) respondents were classified as having a high knowledge about rabies. Results of the multivariable analysis indicated that rabies knowledge was higher among respondents who were i) male, ii) 40 years or older, and iii) had a high level of education, whereas urban residence was negatively associated with knowledge (Table 10).

Table 10: Uni- and multivariable generalized estimating equation models for binomial outcome variables to determine respondent and household characteristics associated with rabies knowledge in Matadi, Democratic Republic of the Congo (DRC) in May 2017.

Variable		%(Npos)	OR	95% CI	*p*-value	adjOR	95% CI	*p*-value
Sex	Female	29% (86/299)	reference			reference		
	Male	41% (103/252)	1.71	1.25–2.34	**.001**	1.44	1.02-2.03	**.038**
Age	16-29	24% (28/118)	reference			reference		
	30-39	31% (39/128)	1.41	0.78–2.53	.252	1.63	0.85–3.11	.14
	>40	40% (122/305)	2.14	1.28–3.58	**.004**	2.21	1.29–3.78	**.004**
Level of education	Low	32% (149/466)	reference			reference		
	High	47% (40/85)	1.89	1.19–3.01	**.007**	1.87	1.22–2.87	**.004**
Socioeconomic status	Middle	36% (176/493)	reference					
	Low	22% (13/58)	0.52	0.24–1.15	.107	0.49	0.22–1.1	.084
Livestock ownership	No	32% (126/392)	reference			reference		
	Yes	40% (63/159)	1.39	0.87–2.21	.17	1.14	0.71–1.83	.599
Residence	Peri-urban	41% (126/311)	reference			reference		
	Urban	26% (63/240)	0.52	0.29–0.95	**.033**	0.5	0.28–0.91	**.024**
History of bite incident	No	34% (170/494)	reference			reference		
	Yes	33% (19/57)	0.95	0.53–1.73	.874			
Dog ownership	No	35% (173/491)	reference			reference		
	Yes	27% (16/60)	0.67	0.39–1.14	.14	0.59	0.34–1.02	.06

Practices Towards Suspected Rabid Animals

When asked about measures they would take when encountering a rabid animal, two-thirds (62%) of respondents would kill the animal, while 15% would chase it away or run from it and 3% would do nothing. Twenty percent of respondents would inform the veterinary service of the suspected rabid animal. Only 2% of respondents would take the carcass of a suspected rabid to the veterinary service for laboratory testing, while the majority would bury it (82%) or throw it away (16%).

Health Seeking Behavior

After an animal bite, only 2% of respondents would wash the wound with water as a first aid measure. Respectively, 85% and 4% would report to a health facility or the veterinary service immediately after a bite. Only 2% would seek anti-rabies vaccine, while 6% would get anti-tetanus vaccination. A small fraction would contact a traditional healer (0.5%) or do nothing after a bite (0.5%). When asked about where to find human rabies vaccine, respondents stated at a health facility (41%) and/or veterinary service (39%). One-fifth of respondents indicated that they did not know where to obtain human rabies vaccine.

Relationship between Rabies Knowledge and Practices

Overall practices scores were low with a mean of 3.11 (SD: 1.05, median: 3.5), minimum of 0, and maximum of 6.5 out of 10.5 possible points. We found a significant positive correlation between the knowledge and practices score (Beta = 0.056, $p < .001$). Figure 9 shows the relationship between the knowledge and practices score by level of education.

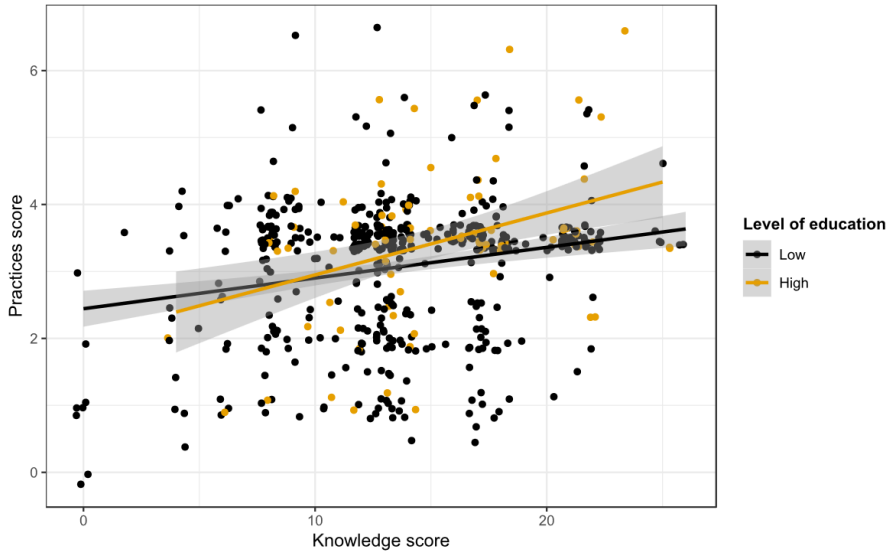

Figure 9: Relationship of knowledge and practices score by level of education. Small random noise was added to avoid over-plotting.

7.5 Discussion

Despite recurrent rabies outbreaks across the DRC, the disease remains neglected with few rabies-focused studies limited to Kinshasa [190-193]. Controlling rabies in the animal reservoir through mass dog vaccination is the most cost-effective and sustainable approach to prevent dog-mediated human rabies cases [31; 36; 44]. Knowledge of the dog population size is a prerequisite for adequately planned vaccination campaigns, and understanding community rabies awareness is critical to adapt rabies interventions to the local context and ensure high community acceptance and participation. This study revealed several important findings that help plan prospective dog rabies awareness raising and vaccination campaigns in the city of Matadi and DRC urban areas. First, the owned dog population in Matadi was almost ten times larger than assumed by veterinary officials. Secondly, a large proportion of the owned dog population was unvaccinated and free-roaming. Thirdly, the annual community-level dog bite incidence was high as compared to other sub-Saharan African settings. Lastly, rabies awareness among the community was low with knowledge gaps that result in poor practices.

7.5.1 Dog Ownership and Dog-Keeping Practices

In our study, about one in 10 households owned at least one dog. This proportion of DOHH is comparable to studies carried out in neighboring countries. For example, 7% and 14–15% of households in coastal and inland urban areas in Tanzania [336; 339] and 11% of households in a suburb of the capital city of Zambia owned dogs [340]. In other sub-Saharan African areas, the

proportion of urban DOHH was higher (17% in South Africa [341], 28% in Chad [335], 61% in Ethiopia [342], 89% in Madagascar [343], and 95% in Nigeria [246]). An exception in West Africa was Mali, where only 9% of households in the capital city of Bamako owned a dog [86]. The observed dog/human ratio of 1:37.7 is lower than the dog/human ratio of 1 to 21 (95% CI: 12.5–37.1) determined by a meta-analysis for urban Africa [14] and generally lower than dog/human ratios found in other urban sub-Saharan African areas (Chad: 1:20.7 [335], Tanzania: 1:14 [336; 339]. South Africa: 1:12.7 [341], Nigeria: 1:3.5-5.7 [243; 246], Madagascar: 1:4.5 [343]) with two exceptions, Zambia (1:45) [340] and Mali (1:121) [86]. In rural areas, dog/human ratios are generally higher but the dog density per km^2 is lower [327]. Based on the dog/human ratio, we estimated the owned dog population of Matadi at 8113 individuals. This was almost ten times higher than the official number of 873 assumed by the government veterinary clinic of Matadi. This finding has large financial implications for planning prospective dog vaccination campaigns, in terms of vaccine doses and human resources needed to reach the 70% vaccination coverage of recommended by the UAR collaboration. Although our data indicates a tenfold underestimation of the owned dog population, this figure is still a rough estimate of the actual dog population size and should be refined after vaccination campaigns using post-vaccination transects as suggested by Sambo *et al.* [344]. The study compared different methods to estimate dog populations and found that estimates based on household surveys were often imprecise due to relatively small sample sizes.

One limitation of our study is that the method used to estimate the dog population size did not take into account the unowned dog population. The majority of study participants (82%), however, reported that there were no ownerless dogs in their neighborhood, which could indicate that the proportion of ownerless dogs is low. This result is further corroborated by a study conducted in Kinshasa which found that ownerless dogs accounted for less than 2% of the entire dog population [192] and the widely accepted assumption that the proportion of unowned dogs across sub-Saharan Africa is low [327; 345].

Many of our findings concerning dog keeping practices agree with previous studies conducted in urban sub-Saharan Africa, specifically that the majority of dogs were free-roaming [86; 243; 335; 336; 340-343], fed regularly by their owners [246; 342; 343; 346; 347], and primarily kept for security and livestock herding [246; 335; 339; 340; 342; 343; 346; 347]. In contrast, in a municipality of Kinshasa, more than half of the surveyed dogs were confined. [193]. A possible explanation is the relative higher standard of living in Kinshasa compared to Matadi. Dogs in middle- and high-income residential areas in Kinshasa are typically kept within fenced properties to guard the prem-

ises [193]. Only a fraction (2.5%) of the properties in Matadi had fences that kept dogs from roaming. This difference in SES between the capital city and Matadi is also reflected in the preference of residents of Kinshasa for hybrid breeds (purchase price about 400 USD per puppy) [193], whereas the majority of dogs in Matadi were local nondescript breeds.

7.5.2 Dog Vaccination and Accessibility

Although most dogs could roam freely, they remained mainly on (86%) or in front (8%) of the dog owner's premises, indicating that most dogs would be accessible during a door-to-door vaccination campaign. Almost all dog owners (92.5%) were confident they could handle their dog for parenteral vaccination. During a vaccination campaign conducted in Matadi on World Rabies Day 2017, no problems restraining dogs and no dog bites were noted [194]. However, difficult dogs might not have been presented for vaccination. Twenty-five out of 106 (24%) dogs were reported to be vaccinated against rabies but only 11% had a valid vaccination booklet. This is far below the 70% coverage recommended by the UAR collaboration and the reported vaccination coverages from Kinshasa, which varied between 24%–81% [192; 193]. Kazadi et al. [193] explained the high vaccination coverages in Kinshasa as due to increased willingness of dog owners in middle- and high-income neighborhoods to pay for vaccination of expensive hybrid dogs. In Matadi, dog owner economic status seems to partially affect dog vaccination status. One in three dog owners stated that they lacked the money for vaccination and dogs in households with a middle SES were more often reported as vaccinated compared to dogs from low SES households (30% vs. 0%). Almost one-third of respondents refused to answer why their dog was not vaccinated, which could be explained as fearing legal consequences for non-vaccination. On average, dog owners would be willing to pay 2.1 USD for dog vaccination, which is in line with findings from Chad [51] and ten times less than the vaccination price of US$ 20 fixed by the Congolese National Order of Veterinarians. Although per dog vaccination cost seems to vary between different regions [328], the mean price willing to pay is below the actual per dog vaccination cost of $ 4.9–5.4 USD found in a similar urban setting in Chad [36]. Given that 61% of Congolese live of less than US$ 1.9 a day [308], dog vaccination is not affordable by a large part of the population. Willingness to pay surveys always have limitations because actual behavior might differ from hypothetical claims when confronted with real payment and we recommend further studies (e.g. double bounded dichotomous choice contingent valuation [348]) to validate dog owner's willingness to pay for dog rabies vaccine. Freedom of dog rabies should be declared a public good and dog rabies vaccination subsidized by the government to achieve dog rabies elimination effectively. Development Impact Bond (DIB) financing schemes should be tested for their suitability for dog rabies elimination in the DRC [106].

7.5.3 Dog Population Characteristics

Similar to other urban dog populations in sub-Saharan Africa, our dog study population was young (32% puppies, 49% < 1yr) [243; 246; 336; 340; 342; 345; 347], indicating rapid population turnover, and very few dogs were neutered [336; 341; 342]. The mean litter size per female dog was 6, higher than the average litter size of 4.7–5.7 reported from other sub-Saharan African countries [86; 335; 336; 349]. Sex ratios skewed towards male dogs are a consistent feature reported in studies on urban dog populations in sub-Sahara Africa [86; 243; 327; 336; 340; 341; 343; 345; 347; 349; 350], with the exception of Lagos State, Nigeria [246]. Possibly, dog owners have a preference for male dogs which are assumed to perform better as guard dogs [351]. Another explanation might be a male-biased birth ratio and a lower female survival rate [352]. We did not observe a predominance of male dogs in our study (sex ratio exactly 1:1), although puppies were not sexed. Hambolu *et al*. [246] explained the predominance of female dogs in Lagos State (Nigeria) as use of dogs for breeding.

7.5.4 Animal Bite Incidents

Bite victims in this study were exclusively bitten by dogs. Sex and age are two demographic characteristics frequently identified as risk factors for dog bites worldwide, with children and males being disproportionately affected [49; 124; 317; 353-360]. Our finding that 38% of bites occurred in children is in accordance with age distributions of bite victims reported in these studies. We did not record the sex of bite victims. The increased risk of dog bites in children is attributed to the fact that children enjoy playing with dogs but are unable to read their behavioral signals and emotions. Children are at higher risk of rabies because their shorter stature makes them more likely to incur dangerous bites to the neck and face and they may not report bites to caregivers [6]. Therefore, children present an important at-risk population that needs to be educated about the dangers of dog bites and appropriate care measures after a bite. Meta-analyses of educational programs on bite prevention in children found no direct evidence that such programs are effective in reducing bite rates [361; 362], but school-based interventions were successful in encouraging children to report bite injuries and increasing knowledge about appropriate PEP [363-365]. Almost all bite victims (89%) in this study were bitten by a familiar dog, meaning the majority of biting animals could be identified and subsequently put under observation or tested for rabies to inform decision-making on the need for PEP.

Dog bite incidence can be used to indirectly estimate human rabies deaths [124]. Epidemiological data on dog bites are often available from health facilities but are likely to be biased because not all bite victims seek care after a bite. To date, little data is available on community-level incidence

of dog bites. In this study, we found an annual dog bite incidence of 5.2 per 1000 population. When extrapolated to the population of the city of Matadi, we would expect 1500–1600 dog bite victims per year. The dog bite incidence found in this study is considerably higher than the annual dog bite incidence of 0.5/1000 persons and 1.2/1000 persons reported in Mali and Côte d'Ivoire, respectively [366; 367], but is similar to the annual dog bite incidence documented in Chad. Higher incidences were reported from rural areas in Asia, for example, 72.9/1000 population in Bangladesh [368], 48.4/1000 population in Cambodia [369], and 17–19.6/1000 population in India [356; 370; 371]. Previous studies have shown that dog/human ratios are typically higher in rural settings [327] potentially resulting in more frequent dog-human interactions and thus an increased risk of bite incidents.

7.5.5 Community Knowledge

Only half of our study population had heard of rabies. This proportion is considerably lower than reported in other sub-Saharan African countries, where 76%–99% of respondents had heard of rabies [85; 186; 335; 342; 372; 373]. The majority of respondents knew that rabies is primarily transmitted through infected dogs (94%) via bites (88%). Rabies seems to be typically associated with dogs, reflected in the local name of the disease "Maladie Ya Mbwa" meaning "disease of the dog", and is similar to the Arabic term داء الكَلْبِ ("dog disease") used in Sahel countries. Although respondents were familiar with rabies transmission, they were unaware of disease symptoms, prevention, and control. In order to detect suspected rabid animals within the community and take appropriate measures, such as informing the veterinary service, the public needs to be aware of the signs of rabies. Study participants who claimed to have seen a rabid animal had higher odds of correctly describing the symptoms of rabies in animals indicating that the disease really is prevalent in the study area. However, a minority (1%) mentioned paralysis as a possible symptom of rabies in animals, despite being typical for the paralytic (or dumb) form of rabies. This may result in cases of paralytic rabies going undetected. We did not find an association between knowing a person who had died of rabies and correctly describing the symptoms of human rabies. Clinical manifestation of human rabies is non-specific, which can lead to misdiagnosis of the disease [114]. It is particularly worrisome that 86% of respondents did not know that rabies is fatal after onset of symptoms. About half of respondents knew of dog vaccination and only 3% knew of PEP as a preventive measure. While dog vaccination is crucial to control rabies at its animal source, timely administration of PEP is essential to prevent rabies infection after a bite.

7.5.6 Community Practices

The general poor rabies knowledge is reflected in the community's practices towards suspected rabid animals and health seeking behavior. Although two-thirds of respondents would kill a suspected rabid animal, only one in five would report the incident to the veterinary service. This will likely affect rabies surveillance efforts because cases will go undetected. A minority of respondents would wash their wound with water and soap if bitten by a dog. Low awareness about first aid measures following a bite seems to be a common feature across sub-Saharan Africa [49; 85; 166; 342; 374; 375]. Washing a bite wound with water and soap for 15 minutes is a simple but potentially lifesaving first aid measure that can prevent an infection through mechanical removal or inactivation the RABV [311]. The knowledge gap about PEP as a preventive measure aligns with the alarmingly low proportion of respondents (2%) who would seek PEP after a bite. In contrast to other studies [85; 376], our study population would not rely on traditional medicine in case of a bite. Even though most respondents would seek medical care after an animal bite, this does not guarantee receiving adequate treatment. For example, in Ghana, 60% of primary health care providers were not aware of the importance of PEP after a bite and 76% of health care facilities did not have human rabies vaccine in stock [202]. Because a high proportion of potential bite victims would seek medical care, health care provider's knowledge about rabies and management of bite wound should be assessed. The positive correlation between the knowledge score and practices score indicates translation of knowledge into practice.

Like most observational studies, this study has potential limitations. Because of lack of alternatives, random geo-coordinates were used as starting points for the household survey. Therefore, households from less densely populated areas might be overrepresented. As mentioned, the study population represents urban communities. Dog/human ratios in rural areas are generally higher, therefore expanding data collection to rural areas is necessary to allow extrapolation to the entire DRC. In addition, we did not investigate the unowned dog population, although their number is considered to be less than 2% of the total dog population [192]. Furthermore, we asked respondents about bite incidents in the last three years, and respondent-reported data could be subject to recall bias. Finally, we did not evaluate respondents' source of rabies information. This could target common sources, for example, media, health workers, or community leaders, for future awareness raising campaigns and effective information channels. Ideally, the KAP study should be repeated after an awareness raising campaign to assess the impact on the community's knowledge and monitor changes in the practices.

7.6 Conclusion

Given the large proportion of unvaccinated, free-roaming dogs coupled with a high dog bite incidence and poor community knowledge about rabies prevention and control, human rabies deaths are likely to occur in this community. Even though findings from this study indicate that the owned dog population is accessible for parenteral vaccination, lack of disease awareness could negatively influence community participation in future dog mass vaccination campaigns. This highlights the urgent need for a public sensitization campaign to promote appropriate prevention (washing bite wounds and PEP) and control (dog vaccination) measures in this community. Our results can support veterinary and public health officials in planning dog vaccination and awareness raising campaigns in the DRC.

7.7 Acknowledgments

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8 The importance of dog population contact network structures in rabies transmission

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8.1 Abstract

Canine rabies transmission was interrupted in N'Djaména, Chad, following two mass vaccination campaigns. However, after nine months cases resurged with re-establishment of endemic rabies transmission to pre-intervention levels. Previous analyses investigated district level spatial heterogeneity of vaccination coverage, and dog density; and importation, identifying the latter as the primary factor for rabies resurgence. Here we assess the impact of individual level heterogeneity on outbreak probability, effectiveness of vaccination campaigns and likely time to resurgence after a campaign. Geo-located contact sensors recorded the location and contacts of 237 domestic dogs in N'Djaména over a period of 3.5 days. The contact network data showed that urban dogs are socially related to larger communities and constrained by the urban architecture. We developed a network generation algorithm that extrapolates this empirical contact network to networks of large dog populations and applied it to simulate rabies transmission in N'Djaména. The model predictions aligned well with the rabies incidence data. Using the model we demonstrated, that major outbreaks are prevented when at least 70% of dogs are vaccinated. The probability of a minor outbreak also decreased with increasing vaccination coverage, but reached zero only when coverage was near total. Our results suggest that endemic rabies in N'Djaména may be explained by a series of importations with subsequent minor outbreaks. We show that highly connected dogs hold a critical role in transmission and that targeted vaccination of such dogs would lead to more efficient vaccination campaigns.

8.2 Author summary

Rabies transmission between dogs and from dogs to humans can be interrupted by mass vaccination of dogs. Novel geo-referenced contact sensors tracked the contacts and locations of several hundred dogs in N'Djaména, the capital of Chad. With the data generated by the sensors we developed a contact network model for rabies transmission dynamics. The model results compared well to incidence data. The model explains the relationship between vaccination campaigns and number of cases better than previous models. Highly connected dogs play a critical role in rabies transmission and targeted vaccination of these dogs would lead to more efficient vaccination campaigns.

8.3 Introduction

The viral disease rabies, transmitted between mammals through bites, is fatal following the onset of symptoms. Although human rabies can be prevented by appropriate post-exposure prophylaxis (PEP), approximately 60,000 people die annually from rabies, mainly in Africa and Asia, [377].

The main source of exposure for human rabies is the domestic dog, so vaccinating dogs is an effective way of reducing rabies transmission among dogs and from dogs to humans [378], [379].

Rabies is endemic in N'Djaména, the capital city of Chad, with an average incidence of one laboratory-confirmed infected dog per week [37]. A deterministic model of rabies transmission predicted that mass vaccination of dogs would be sufficient to interrupt transmission for six years [378]. Vaccination campaigns in dogs were conducted in 2012 and 2013, with both campaigns exceeding 70% coverage [380]. Rabies transmission was interrupted in January 2014 after the second vaccination campaign [379], but there was a resurgence of cases nine months later. Subsequent analyses considered reasons for the quick resurgence, including spatial heterogeneity of vaccination coverage, and dog density; underreporting of cases; and importation. Simulation results from a deterministic metapopulation model suggested that importation was the most likely reason for the case resurgence [381]. Although deterministic models can predict the effect of large scale vaccination campaigns and the overall population dynamics, they do not adequately capture effects of stochasticity in low level endemic settings. This becomes important towards the end of an elimination campaign or upon re-establishment after interruption of transmission [382]. Previous models did not include fine scale heterogeneity at the individual level or the network structure of dog to dog contacts.

The importance of including host contact structure in infectious disease modeling has been highlighted in many studies [383], [384], [385]. Theoretical analysis of epidemic processes on graphs has shown that the basic reproductive ratio not only depends on the expected value but also on the standard deviation of the degree distribution of the graph [386] and that on scale-free networks diseases can spread and persist independently of the spreading rate [387]. These theoretical insights led to better understanding of disease transmission dynamics for different diseases, including pertussis [28], influenza [388], severe acute respiratory syndrome (SARS)[389], human immunodeficiency virus and acquired immune deficiency syndrome (HIV/AIDS) [390] and gonorrhea [391], and inspired novel control measures such as acquaintance immunization [392], contact tracing [393] and ring vaccination [394], [395].

Due to the substantial influence of network structure on disease transmission dynamics, many studies have collected data on host interactions. Human contact network models are generally established using contact diaries [396], [397], [398], proximity loggers [399], [400] , [401], [402], video recording [403] or mobile phones [404]. Contacts have also been studied in a wide range of animal species. The most common method for measuring animal contacts is behavioral observation, but other methods such as radio tracking, Global Positioning System (GPS) trackers, proximity loggers or powder marking are also utilized [405].

In the past decades, several rabies models with host contact structure have been published. [406] simulated fox movement pathways using home range size estimates, data from radio tracking and behavioral encounter observations to estimate contact probabilities for different seasons and fox densities. They found that the rabies front set off by an incursion of rabies into a healthy population moved more slowly than in a previous model of homogeneous fox populations. Including contact behavior in the model also resulted in a substantially higher predicted rabies control success rate. [407] used data from 30 raccoons fitted with proximity loggers to assess properties of the raccoon contact network. Unlike in earlier radio telemetry studies, they found a highly connected population and discussed possible implications of the social network on the spread of rabies. [408] used proximity logger data from 15 raccoons to build a contact network model of 90 raccoons and simulate rabies spread. They studied the effects of seasonality, differences in vaccination coverage and impact of behavioral changes in infected raccoons on disease spread. [409] used a contact network model of rabies transmission among owned free-roaming dogs in Australia to estimate the impact of a hypothetical rabies incursion from Indonesia. They differentiated transmission within households, between households and between communities. The probability of between household transmission was based on GPS data from 69 dogs, while between community transmission was estimated using questionnaire data. [410] developed a contact network model for rabies in the wild dog population in Australia. They constructed a function for dog contact probabilities, using a wide range of different values to generate contact networks and then implemented a rabies transmission model based on parameters from literature.

However, individual based models of dog rabies transmission in endemic settings are lacking, so this study equipped 300 dogs in N'Djaména with purpose developed geo-referenced contact sensors. This is the first study to collect contact data among dogs as well as the first to integrate contact data from such a large subset of an animal population into a rabies model. The individual based model of rabies transmission we developed includes distance between home locations and a degree distribution fit to a contact network structure of dogs in N'Djaména. We compared our model results to 2016 outbreak data from two quarters of N'Djamena. We examined the re-establishment probability of rabies over different vaccination coverage and compared outbreak probability over time with rabies incidence in N'Djaména from 2012 to 2016. Finally, we investigated the role of individual heterogeneity among dogs and the effect of targeted vaccination strategies.

8.4 Materials and methods

8.4.1 Contact network data collection

Contact network data was collected in three districts of N'Djaména, Chad, using 300 geo-located contact sensors (GCS) developed specifically for this study. The devices contain Global Positioning System (GPS) modules to track the location and movements of dogs and Ultra-High-Frequency (UHF) technology sensors to measure close-proximity events between dogs. The GCS devices record locations at one minute intervals. For the contact recording, the devices broadcast beacons at one minute intervals and constantly scan for beacons ensuring that no contacts with durations of at least one minute will be missed. Close proximity events were defined as records with a received signal strength indicator (RSSI) of more than -75dBm. Static tests of the devices showed that, independently of the angle between two devices, all contacts closer than 25 cm are registered when signal strength is above that value (Appendix 4: Supplementary information for chapter 8,Figure 35)

Collars fitted with the devices were placed on free roaming domestic dogs in three city districts (Table 11, Figure 10) with different dog densities (low, medium and high), that were easily accessible. The zones were chosen to include urban and peri-urban areas. Data were collected during the dry season in December 2016. In the selected districts, all dog-owning households in a predefined area of 1km^2 were identified in order to capture as many of the contacts between dogs as possible, bearing in mind that only contacts between dogs that both wear a sensor can be captured. Dog owners were asked to enroll their pets. Only one dog owner refused to participate in the study. The GCS units remained on the dogs for 3.5 days. After retrieval of the GCS units, dogs were vaccinated against rabies. We excluded study zone 3 from the network analysis due to the low proportion of devices usable for analysis.

Zone	District	Location	Dog density	Number of dogs	Deployed devices	Devices useable for analysis
1	6	urban	high	328	290	237
2	1	peri-urban	medium	94	80	66
3	8	urban	low	59	41	25

Table 11 Characteristics of the three study zones.
The reason for the discrepancy between the number of dogs and the number of deployed devices is that some collars could not be attached because dogs resisted. In study zone 1 the number of deployed devices was also limited by the fact that we had only 300 devices at our disposal. The discrepancy between deployed and usable devices is due to broken or lost GCS units, battery failure or failure in the data downloading process.

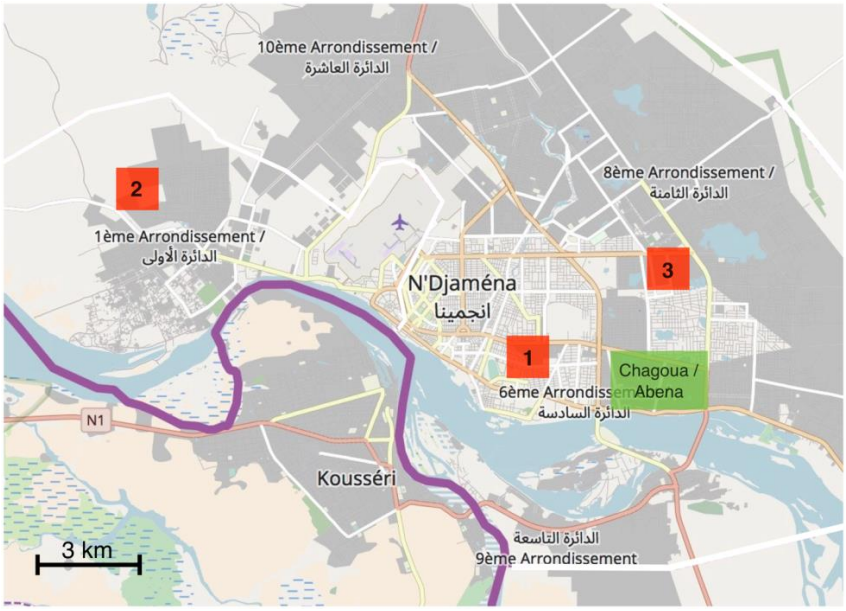

Figure 10: The location of the three study zones (red squares) and Chagoua and Abena quarters (green rectangle) in N'Djamena.
The solid purple line denotes the border to Cameroun. The maps were generated using OpenStreetMap contributors.

The data from the contact sensors were used to establish an empirical contact network, where the nodes correspond to the dogs and any two nodes are connected by an edge if at least one contact between the two dogs was registered. Appendix 4: Supplementary information for chapter 8, Figure 36 shows the number of edges in the empirical network during different subintervals of the study period.

8.4.2 Rabies cases data

Surveillance of canine rabies in N'Djaména consists of passive reporting of cases confirmed with an immunofluorescence antibody test (IFAT). In 2012, prior to the vaccination campaign, there was, on average, one case of dog rabies per week. After the vaccination campaigns in 2012 and 2013, no rabies cases were reported for nine months. In October 2014, new rabies cases were reported in district number 9, south of the Chari River. In January 2016, the first case north of the river was reported in the Chagoua quarter of district 6 (Figure 10). An additional 6 cases of dog rabies were reported in 2016 in Chagoua and the neighboring Abena quarter.

8.4.3 Dog population estimates

We simulated rabies incursion into Chagoua and Abena quarters to compare the model results to the outbreak data. Dog population estimates were derived from the 2012 mass vaccination campaign coverage assessment to determine the number of nodes in the network. A total of 2775

dogs were vaccinated during the 2012 campaign in Chagoua, Abena and the neighboring Dembe quarters [380]. A capture-mark-recapture model estimated vaccination coverage in that area at 67%. In a second stage of the campaign, additional dogs were vaccinated in Chagoua, Abena and Dembe. During the latter stage, the proportion of dogs originating from Chagoua and Abena was assessed at 86% of dogs. Assuming that this proportion was the same in the first round, we estimated the dog population in Chagoua and Abena to total 3,500 dogs. This was confirmed through a household survey conducted after the vaccination campaign, which estimated the dog/human ratio to be 1/20. The proportion of ownerless dogs was between 8% and 15% [380]. The total human population in Chagoua and Abena was 72,000 people.

8.4.4 Network construction

We developed a spatially explicit network construction algorithm to expand the empirical contact network to a synthetic network with more nodes, which allows for more realistic simulations of rabies transmission. When applied to a set of nodes of the same size as the empirical network, this algorithm generated a network with a similar degree distribution. The outbreak probability and size of a rabies transmission model on the empirical and the synthetic network were similar, meaning we captured the features of the empirical network which are relevant for disease transmission in the construction algorithm.

The steps of the algorithm to create the synthetic network are described below. We first create a graph with n nodes and zero edges. The number of nodes n corresponds to the number of nodes in the empirical network. Each node is assigned a position consisting of x and y coordinates in a square. The coordinates are sampled using Latin Hypercube sampling. Any two nodes i and j are connected with a probability p_{ij} given by $p_{ij} = \exp(-\kappa\Delta_{ij})$, where Δ_{ij} is the Euclidean distance between node i and node j and κ is a scaling parameter. Next a proportion $1 - \tau$ of the nodes are selected uniformly at random. For each node i in that subset of nodes a number m is sampled from a Poisson distribution with mean λ. The node i is then connected to exactly m other nodes out of all the nodes in the graph. The probability of selecting node j into the m nodes is given by $\tilde{p}_{ij} = \frac{k_j}{\sum_{l=1}^{n} k_l}$, where k_j is the degree of node j and $\sum_{l=1}^{n} k_l$ is the sum of the degrees of all the nodes in the graph.

The three scaling parameters, κ, τ and λ are chosen such that the Kolmogorov distance between the degree distribution of the synthetic network and the degree distribution of the empirical network is minimal. We minimize the Kolmogorov distance by using a gridsearch and confirm the results by minimizing a second metric, the χ^2 distance. The optimal values of the parameters κ, τ and λ for

the two study zones are displayed in Table 12. Larger networks are constructed by choosing the desired number of nodes in the networks and following the steps described above with the optimal values for λ, τ and κ. The properties of the empirical and the synthetic networks are displayed in Table 13. When optimizing the parameters κ, τ and λ only the degree distribution of the two networks is taken into account. Therefore, other network properties such as clustering do not necessarily align between the synthetic and the empirical network.

Table 12: Optimal values for the three scaling parameters of the network construction algorithm for the two study zones.
The optimal values minimize the distance between the degree distributions of the empirical and the simulated networks. The distance is calculated using the Kolmogorov or the Chi-Square metric.

		λ	τ	κ
Zone 1	Kolmogorov	24	0.75	25
	Chi-Square	23	0.75	25
Zone 2	Kolmogorov	7	0.7	10
	Chi-Square	6	0.7	11

Table 13: Properties of the empirical and the synthetic contact networks for the two study zones.
For the synthetic network five point summary statistics of 1000 runs of the construction algorithm are displayed.

		empirical	synthetic				
			min	p25	p50	p75	max
Zone 1	*nodes*	237			237		
	edges	1739	1630	1756	1782	1812	1914
	density	0.0622	0.0582	0.0628	0.0637	0.0648	0.0648
	av. degree	14.68	13.8	14.8	15.0	15.3	16.2
	max. degree	64	39	46	48	50	67
	clustering	0.562	0.129	0.157	0.164	0.172	0.198
Zone 2	*nodes*	66			66		
	edges	272	238	280	292	305	365
	density	0.125	0.111	0.131	0.136	0.142	0.170
	av. degree	8.24	7.2	8.5	8.8	9.2	11.1
	max. degree	19	19	20	21	29	
	clustering	0.516	0.159	0.230	0.246	0.263	0.339

8.4.5 Transmission model

We used an individual based transmission model to simulate the spread of rabies in a contact network. All nodes of the network are assigned a status; susceptible, exposed, infective or removed. Nodes infect adjacent nodes with a transmission rate β and progress from exposed to

infectious and from infectious to removed with average transition periods σ and δ. For each infected dog the individual incubation period and infectious period is sampled from a Poisson distribution, with mean σ or δ, respectively. The model ignores birth and natural mortality. The parameter values are displayed in Table 14. The incubation period, σ, is chosen from recent literature [411] and fits with the observed time between cases in the incidence data from Chagoua and Abena. The duration of the incubation period is only marginally relevant for our simulations, because it only affects the outbreak duration and not the outbreak probability or size. The infectious period, δ, is chosen based on the assumption that a rabid dog in an urban setting would be killed earlier than a natural death from rabies. Our observation that more than two thirds of all samples tested at the rabies laboratory are positive supports the hypothesis that people are likely to recognize the symptoms of rabies since they are less likely to send non-rabid dogs for testing. If people recognise rabies they are more likely to kill rabid dogs [37]. The transmission rate is chosen using (1). We calculated the mean and variance of the empirical degree distribution, choosing the transmission rate such that R_0 is smaller or equal than 1. We reasoned that rabies is endemic in N'Djaména, with a constant low number of cases and no large outbreaks observed. The transmission rate choice is further supported by the comparison of the simulation results to the outbreak data from Chagoua and Abena.

Table 14: Parameters of the rabies transmission model.
Time is measured in days

	Description	Unit	Distribution	Range/Mean
σ	incubation period	time	Poisson	90
δ	infectious period	time	Poisson	2
β	transmission rate	$time^{-1}$	Uniform	[0.015,0.02]

8.4.6 Network construction validation

We used an individual based transmission model to test whether the properties of the empirical and the reconstructed network lead to similar outbreak probability and size for different transmission rates. The results for 1000 simulation runs of this model on the empirical and the synthetic network are shown in Figure 11. We differentiate between minor outbreaks, which are outbreaks where more than one and less than one percent of the nodes gets infected, and major outbreaks, which are outbreaks where more than one percent of the nodes get infected. Incursions denote all outbreaks where more than one node gets infected and therefore include both minor and major outbreaks. The figure suggests the construction algorithm performs well since the empirical and the simulated network yield similar results in outbreak probability and size. The values of the proportion of simulation runs with outbreaks correspond to the values of the average relative outbreak size, that is the sum of all the final outbreak sizes divided by the number of nodes in the network

and the number of simulation runs. This is consistent with the theoretical result that the probability of a major outbreak and the relative size of such a major outbreak are equal [412]. This holds despite the clustering of the synthetic network being higher than in a random graph due to the spatial component of the network construction algorithm. In Figure 11 the outbreak size increases steeply for transmission rate values that are slightly larger than 0.02. This is consistent with the basic reproductive ratio R_0 given by

$$R_0 = p\left(\mu + \frac{var(D) - \mu}{\mu}\right) \quad (1)$$

where p is the transmission probability given a contact and μ and var(D) are the expected value and the variance of the degree distribution [412]. In the case of the described network R_0 takes the value of 1 if the transmission rate β is 0.02. Since major outbreaks are only possible when R_0 is greater than one, the observed increase of the average outbreak size for values of the transmission probability greater than 0.02 aligns well with the theoretical result, even though not all conditions are met in the case of the described networks.

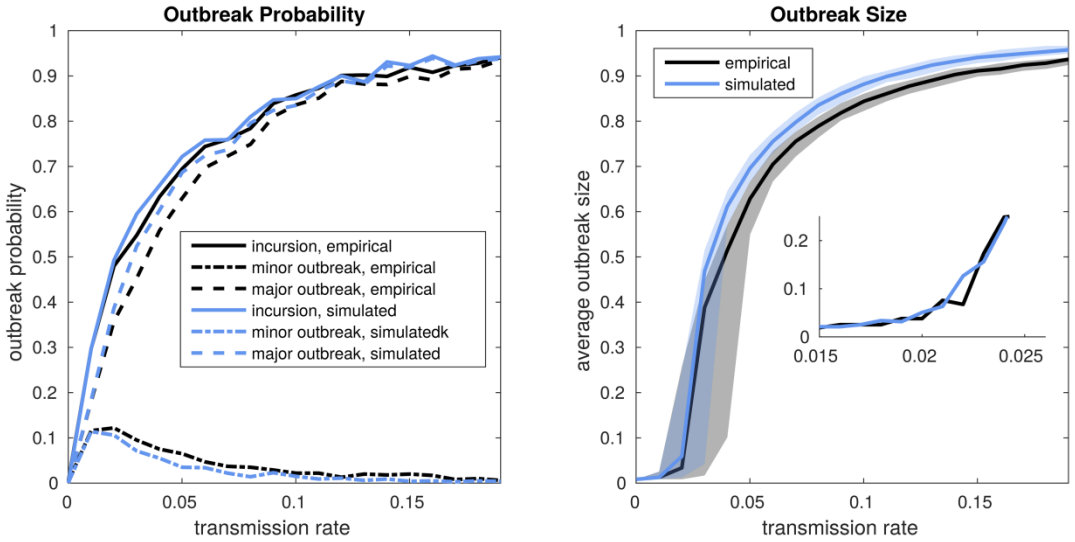

Figure 11: Outbreak probabilities and sizes of the transmission model on the empirical and the constructed network in study zone 1.
In each simulation run, one randomly chosen dog is infected from the outside. The simulation ends when there is no more transmission. The incursion probability is the proportion of simulation runs where the number of infected dogs was greater than one. The probability of a minor outbreak is the proportion of simulation runs where more than one dog and less than 1 percent of the population get infected. The major outbreak probability is the proportion of simulation runs where more than 1 percent of the population get infected. The outbreak size is the cumulative proportion of infected dogs over the whole course of the infection. In the left panel the lines correspond to the mean over 1000 simulation runs for each value of the transmission rate. In the right panel the lines correspond to the median over 1000 simulation runs for each value of the transmission rate and the shaded area corresponds to the interquartile range.

8.5 Results

8.5.1 The empirical contact networks

In study zone 1, the network consisted of 237 nodes and 1739 edges, with an average degree of 15 and maximal degree of 64. In zone 2, the network consisted of 66 nodes and 272 edges, with an average degree of 9 and a maximum degree of 20. In both zones, nearly all dogs were part of one connected component, that is a sub-graph where any two nodes are connected by a path. The network can be divided into communities using a modularity optimization algorithm [413]. This algorithm optimizes both, the number of communities and the assignment of each node to a specific community, such that the modularity, that is the density of links within communities compared to links between communities, takes the maximum possible value. When the network of study zone 1 is divided into communities using this algorithm it becomes visually obvious that communities mainly consist of dogs which live close together and do not frequently crossrange across roads with traffic (Figure 12). This suggests, that roads with high traffic intensity constitute a functional barrier which substantially reduces contact between dogs residing on either side.

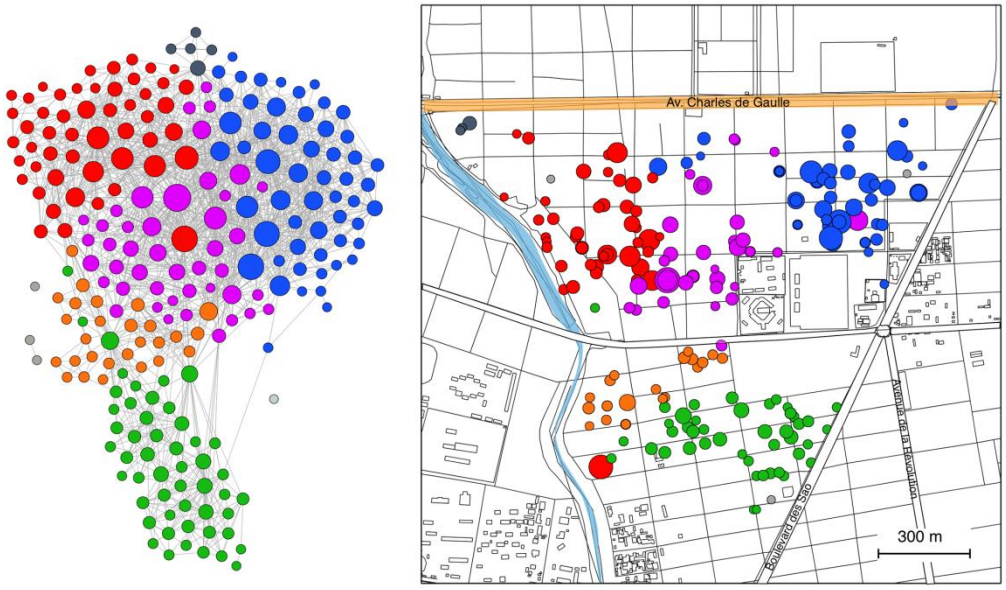

Figure 12: Contact network and home location of the 237 dogs in study zone 1.
In the left panel each node corresponds to a dog. The size of the node is proportional to the degree of the node and the color corresponds to the community the node belongs to. Contacts between dogs are shown as grey lines. In the right panel each dot on the map corresponds to a home location of a dog. The colors correspond to the community in the network. The maps were generated using OpenStreetMap contributors.

8.5.2 Comparing simulation results to outbreak data

Rabies was absent from the Chagoua and Abena quarters of N'Djaména for more than a year prior to the outbreak in 2016. The 7 cases were the first to occur north of the Chari River. Chagoua and Abena are virtually separated from other quarters to the west, north and east by main traffic roads and to the south by the Chari River. The area of these two quarters is approximately 4km^2, and the total number of dogs is estimated to be around 3,500. We simulate the course of the infection after the incursion of one rabid dog. We found that in 450 out of 1000 simulations the chain of transmission was longer than 1, in other words additional dogs get infected. Among these chains of transmission the median of the cumulative incidence of all simulation runs aligns well with the cases observed in Chagoua and Abena (Figure 13). This suggests that the transmission rate in our model is a reasonable choice and that our simulations yield realistic results. Since rabies is often underreported, the true number of cases is likely to be higher than the reported number of cases. We accounted for this in a sensitivity analysis on the reporting probability (Appendix 4: Supplementary information for chapter 8, Figure 37). We found that if more than 60% of the cases are reported, the median of the simulations does not differ more from the incidence data than with perfect reporting. The final outbreak sizes are shown in Appendix 4: Supplementary information for chapter 8, Figure 38.

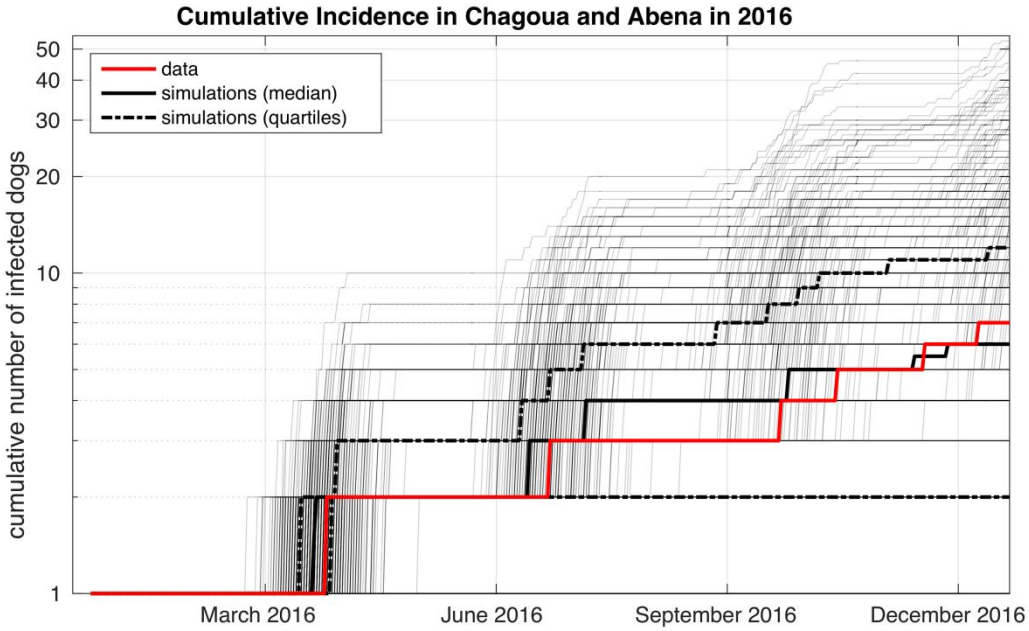

Figure 13: Incidence data and simulation results for the quarters Chagoua and Abena in 2016. The red line is the cumulative number of confirmed rabies cases. The black lines show median and quartiles the cumulative number of cases in simulation runs where the number of rabid dogs was greater than 1. Individual simulation runs are displayed as gray lines.

8.5.3 Outbreak probability, size and duration for different vaccination coverages

To assess the impact of vaccination coverage on the outbreak probability and size after the introduction of one rabid dog, we constructed a network with a large number of nodes. We considered a 4 × 4 kilometer square and a dog population with the same density as the dog population in study zone 1, which yields a network with 4930 nodes. We ran rabies incursion simulations on that network. The outbreak probabilitiy, size and duration across different vaccination coverage are shown in Figure 14. The probability of a major outbreak, defined as more than 1% of the dog population becoming infected, is substantially reduced when vaccination coverage is above 70%. The probability of minor outbreaks also decreases with vaccination coverage, but only reaches zero with nearly complete vaccination coverage. Even though a minor outbreak, by definition, could affect up to 1% of the population (50 dogs) the simulated average outbreak size is, in fact, very low. This is consistent with the theoretical result that the final number of infected nodes converges to a two point distribution. A proportion of simulation runs stays close to zero whereas the other proportion ends up near the major outbreak size (for an example see Appendix 4: Supplementary information for chapter 8, Figure 39). The minor outbreaks, therefore, only capture the short chains of transmission. These chains include, on average, 5 dogs and last approximately 20 weeks, yielding an average number of one infected dog per month which aligns well with the observed endemic situation in N'Djaména [37].

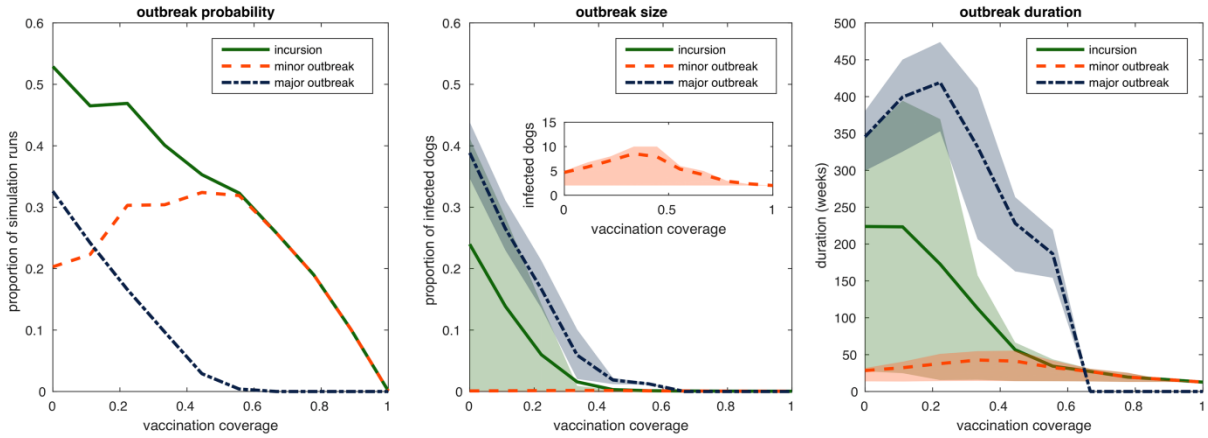

Figure 14: Fig 5. Outbreak probability, size and duration on a network of 4930 dogs for different vaccination coverages.
In each simulation run, a proportion of the dogs is randomly assigned the status vaccinated and one randomly chosen susceptible dog is infected from the outside. The simulation ends when there is no more transmission. Simulation runs where more than one dog gets infected are classified as incursion. Simulation runs where more than one dog and less than 1% of the population get infected are classified as minor outbreaks. Simulation runs where more than 1% of the population gets infected are classified as major outbreaks. Incursions include minor and major outbreaks. The outbreak probability is the proportion of simulation runs with outbreaks. The outbreak size is the cumulative proportion of infected dogs over the whole course of the infection. The outbreak duration is the number of weeks until the last infected dog dies. In the left panel the lines correspond to the mean over 1000 simulation runs for each value of the vaccination coverage. In the center and the right panel the lines correspond to the mean over 1000 simulation runs for each value of the vaccination coverage and the shaded areas correspond to the interquartile ranges. The axis of the indented figure in the center panel are the same as in the surrounding figure.

8.5.4 Time to resurgence

After the vaccination campaigns in 2012 and 2013, no rabies cases were reported north of the Chari River until October 2014. We used a deterministic model [378] to estimate vaccination coverage over time and the contact network model to calculate outbreak probability for the respective coverage. Comparing these probabilities with the incidence data (Figure 15) showed that the first case after the vaccination campaigns could not establish a chain of transmission because the probability for a major outbreak was very low at that time. Later, in February 2016, the respective probability was higher which could explain the subsequent cases.

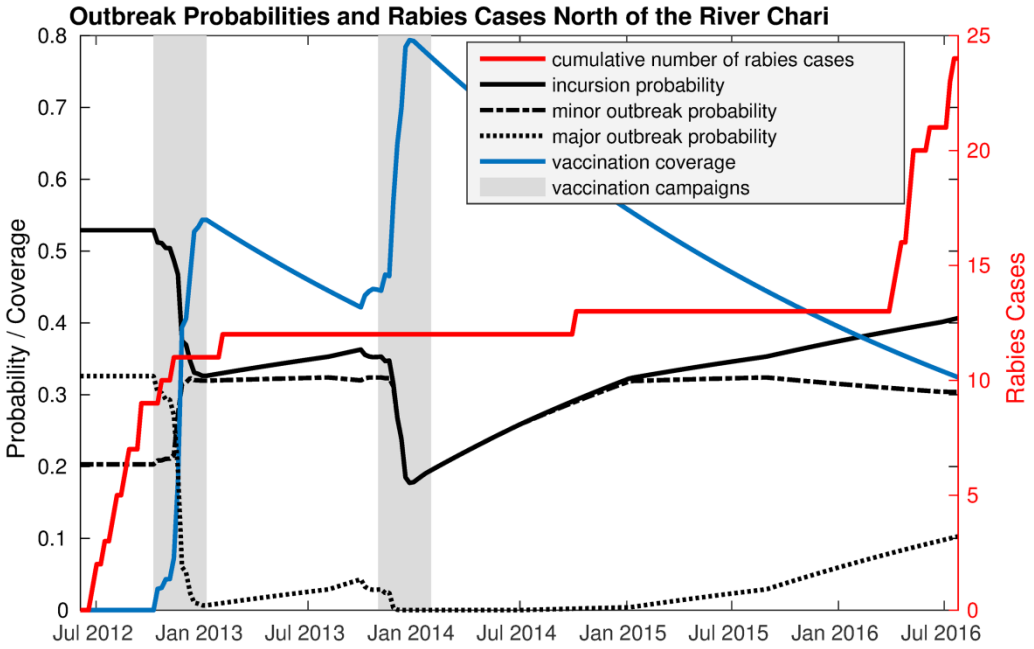

Figure 15: Outbreak probabilities and rabies cases north of the river Chari from 2012 and 2016. For each week the vaccination coverage was calculated using a deterministic transmission model. Vaccination coverages were then translated to outbreak probabilities using the contact network model.

8.5.5 Comparing vaccination strategies

We used the empirical contact network from zone 1 to compare different types of vaccination strategies. Dogs can be vaccinated at random or in a targeted way, based on the contact network structure among the dogs or based on the movements of the dogs. We considered four different ways of targeting dogs: (i) vaccination in order of the degree centrality of the nodes, (ii) vaccination in order of the betweenness centrality of the nodes, (iii) vaccinating each node with a probability that is linearly proportional to the average distance the corresponding dog spent away from the home location of the owner and (iv) vaccinating each node with a probability that is linearly proportional to the area covered by the corresponding dog, where the area was estimated by fitting a minimal convex polygon to the GPS locations of the dog. The outbreak probability and size for each type of vaccination and different coverages are shown in Figure 16. Consistent with previous findings [414], [415] we observed that targeted vaccination reduces the outbreak probability and size more than random vaccination. Targeting nodes by degree yields a lower outbreak probability and size than targeting nodes by betweenness. The betweenness centrality of a node i is the proportion of shortest paths between any pair of nodes in the network that pass through node i. Nodes with high betweenness centrality are therefore part of many short paths between nodes, which is why removing them affects the global network structure and reduces the size of the largest component, while targeting nodes by degree operates on a local level and reduces the total number of edges more rapidly. In our case, chains on average are short, so the local structure is more

important than the global structure. Vaccination based on movement also reduces the outbreak probability and sizes.

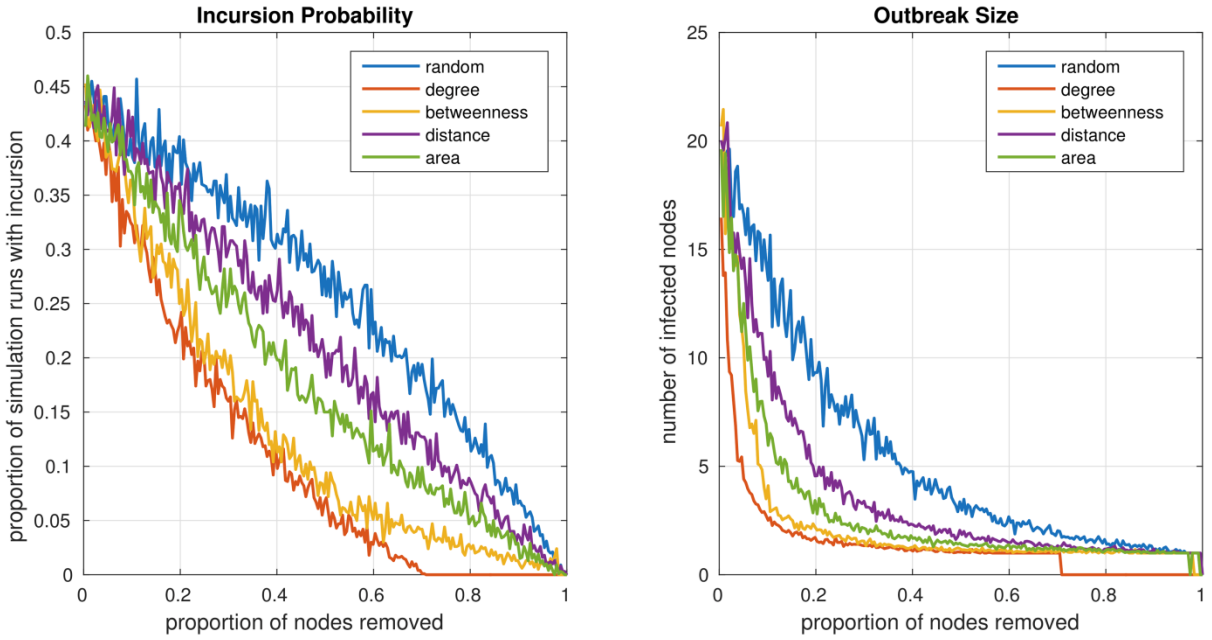

Figure 16: Outbreak probability and size for random and targeted vaccination and different coverages.
For each coverage a fraction of nodes is considered as immunized and therefore removed from the network. These nodes are chosen either randomly (blue lines), in descending order of degree (red lines) or descending order of betweenness (yellow line). To simulate the effect of oral vaccination, the probability of a node being immunized was chosen to be linearly proportional to the average distance from the home location (purple line) or the area of the minimal convex polygon fitted into the gps logs (green line). The centrality values of the nodes are recalculated after each node removal. For each strategy and coverage 1000 simulation runs are conducted.

8.5.6 Sensitivity analysis

We conducted a Partial Rank Correlation Coefficient (PRCC) sensitivity analysis [416] to assess the impact of the network construction and transmission model parameters on the model output, with ranges as displayed in Appendix 4: Supplementary information for chapter 8, Table 32. The results are shown in Figure 17. The most sensitive parameter is τ, a scaling parameter of the network construction algorithm. For low values of τ, a large proportion of nodes are sampled to connect both to spatially close nodes and any other node in the network. These nodes have a higher degree and betweenness centrality than the other nodes in the network, resulting in an overall larger outbreak size and duration. The remaining two network construction parameters, κ and λ, do not have a large effect on the model output. Among the parameters of the transmission model the infectious period, δ, is most sensitive. Since the model ignores birth and natural mortality, the incubation period σ is only relevant for the outbreak duration and not for the outbreak

size. A sensitivity analysis of the outbreak probability, size and duration for different vaccination coverages is shown in Appendix 4: Supplementary information for chapter 8, Figure 40 and Figure 41.

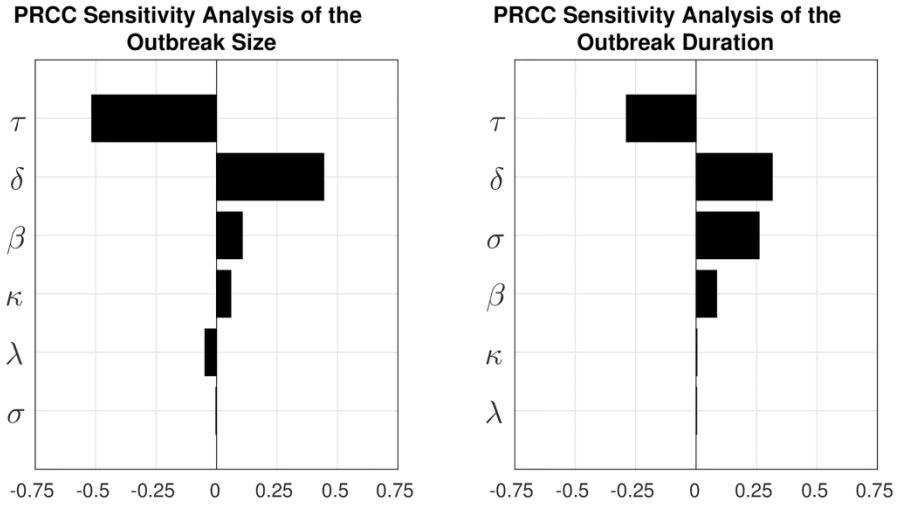

Figure 17: Partial Rank Correlation Coefficient sensitivity analysis of the outbreak size and outbreak duration.
The parameter κ is a network construction parameter involved in the scaling of the spatial connection. The parameter τ is a network construction parameter that alters the proportion of far roaming dogs. The parameter λ is a network construction parameter that alters the mean number of peers of far roaming dogs. The parameter δ is the infectious period. The parameter σ is the incubation period. The parameter β is the transmission rate.

8.6 Discussion

This study used empirical contact data to develop a contact network model of dog rabies transmission. We validated the simulation results with 2016 outbreak data from N'Djaména. We used the model to compare the probability of rabies establishment after incursion across different vaccination coverage. We showed that vaccination coverage above 70% prevents major outbreaks, which is consistent with previous findings [379].

In contrast to deterministic models, our individual-based model allowed us to investigate the whole possibility space of outbreak scenarios. Differentiating between minor and major outbreaks revealed that even though the probability of major outbreaks is very low for high vaccination coverage, minor outbreaks can still occur even at nearly complete vaccination coverage. These minor rabies outbreaks consist of approximately 5 dogs, which aligns well with current observations from N'Djaména [37]. The endemicity of rabies in N'Djaména could be explained as a series of rabies introductions with subsequent minor rabies outbreaks, as has been observed in Bangui [57].

We showed that targeting dogs by degree centrality, betweenness centrality or based on their movement substantially increases the impact of vaccination. Targeted vaccination based on betweenness centrality does not perform better than targeted vaccination based on degree centrality.

The observation that vaccination by degree performs as well as vaccination according to other network centralities is consistent with previous findings in humans [415]. The degree or betweenness centrality can only be assessed using expensive methods like the tagging with geo-located contact sensors conducted in this study. Such methods cannot be used in routine surveillance. We have shown that vaccination based on movement also reduces the outbreak probabilities and sizes. This might indicate that oral vaccination would be an effective intervention because dogs which cover a lot of territory would be more likely to encounter oral vaccine baits. Oral vaccination has been shown to effectively prevent rabies in dogs [417] and is currently recommended by the WHO as a complementary measure to increase coverage in mass vaccination campaigns [418]. Oral vaccination must be carefully planned with regard to biosafety, for example by assuring that vaccinators retrieve unconsumed baits [419]. It has been successfully implemented to eliminate fox rabies in central Europe [420]. Further consideration of oral vaccination of dogs is warranted based on these results.

We observed a dog population where only a few dogs were not part of the largest component, similar to [407], who used proximity loggers to reveal a highly connected population in raccons.

In contrast to the raccoon rabies model of [408], which concluded that with vaccination coverage of 65% the probability of a large outbreak remains around 60–80%, we noted a substantial drop in the probability of a major outbreak. This might be due to the fact that, while raccoons remain infectious until death from rabies, we assumed that dogs remain rabid for only two days on average because we hypothesized that in an urban setting a rabid dog would be killed by the community. Therefore, major rabies outbreaks could be prevented by rabies awareness and locally reactive interventions.

Unlike [409] who found that even at a vaccination coverage of 70% approximately half the dog population dies from rabies, we found outbreak sizes of less than 1% of the population for high vaccination coverage. This might be due to the fact that Dürr *et al.* considered reactive vaccination after incursion rather than preventive vaccination.

There are several limitations to our study. Our simulations are based on the assumption that rabid dogs stay infective for two days on average, which does not consider the fact, that rabid dogs can be infectious for several days before they show symptoms. Previous models of rabies in wildlife indicated an effect of seasonality on outbreak sizes and durations. Collecting contact data at different times of the year is currently planned, and subsequent analyses will explore the impact of seasonality on contact rates. Dog contacts were only measured for a period of 3.5 days, the extent of battery life. While this observation window is longer than the average infectious period, we

cannot be certain that the structure of the network would remain the same when measured for a longer time. Also, contacts with untagged owned dogs and unowned dogs (approx. 8% to 15% of the dog population) were not recorded. Furthermore, we did not include the change of behavior of a rabid animal. However, [408] found that assuming a combination of paralytic and furious rabies in the population leads to little quantitative change in the outbreak size.

We found that major rabies outbreaks are unlikely when vaccination coverage is above 70%. Our results suggest that the endemicity of rabies in N'Djaména might be explained as a series of importations with subsequent minor outbreaks. Further investigation of determinants of dog roaming and contact behavior could inform potential targeted vaccination strategies.

8.7 Acknowledgments

Calculations were performed at sciCORE (scicore.unibas.ch) scientific computing core facility at University of Basel. The maps were generated using OpenStreetMap contributors. Katya Galactionova and Lisa Crump are greatly acknowledged for language editing.

9 Exploring the contact and roaming behavior of free-roaming dogs in an African city.

9.1 Introduction

In N'Djamena, the capital of Chad, dogs are the principal reservoir of rabies. Although this is also the case throughout Africa, little is known about the contact and roaming behavior of owned free-roaming dog populations on the continent, and how these might affect the spread of rabies. Individual variation in contact patterns has considerable implications for the epidemiology of an infectious disease [21; 421], and the presence of highly connected individuals may substantially influence the course of a disease outbreak [422; 423]. The aim of this study was to investigate the contact and movement of dogs and identify intrinsic and extrinsic factors associated with highly connected dogs.

9.2 Material and Method

We collected ata on the contact and movement behavior of owned free-roaming dogs during the dry season (December 2016) and rainy season (August 2017) in N'Djamena, Chad, using geo-located contacts sensors (GCS). The GCS and sampling methodology are described in detail elsewhere [326]. In short, GCS contain Ultra High-Frequency (UHF) technology sensors to measure close-proximity events between dogs and Global Positioning System (GPS) modules to track the location and movement of dogs. Using four field teams, we did a census of all owned dogs within pre-defined 1km^2 areas in three city districts with high (study zone 1), medium (study zone 2) and low (study zone 3) dog density. The goal of the study was explained to the dog owners and, upon approval, collars with GCS units were fitted to their dogs. Only one dog owner refused to participate in the study. For each participating dog, a structured questionnaire was completed to gather information on the religion of the dog owner and sex, age (categorized as young dog <1 year, adult dog: 1-7 years and old dog > 7 years), neutering status, confinement and diet of the dog. The GCS remained on the dogs for 3.5 days. All dogs were immunized against rabies after retrieval of the GCS. We recruited data collectors from the Livestock Research Institute for Development (IRED). They were trained in administration of the questionnaire and deployment of the GCS collars in a pre-test which included 30 dogs in July 2016.

Contact and GPS data downloaded from the GCS units to an iOS device using the Dog Logger application were finally stored on an online password-protected server. For data analysis, data were downloaded from the server in comma-separated value files and analyzed using R software 3.5.0 [314].

9.3 Data analysis

We quantified the space use of each dog over the study period (i.e., a dog's home range) by applying dynamic Brownian Bridge movement models (dBBmm) using the adehabitatHR package in R. Compared to traditional Kernel Density estimators, these models have the advantage that they incorporate the temporal information contained in movement data rather than only the position of the GPS fixes [424]. Home range (HR) size was derived from the 95% isopleth of the utilization distribution (UD) which quantifies the probability of encountering a tracked dog in a given area during the study period. HR size was estimated for a subset of 32 dogs (n=19 dry season and n=13 rainy season) from study zone 3. We used the net squared displacement (NSD) to investigate the activity patterns of dogs during the study period. The NSD calculates the squared distance between the starting location of a dog's movement path (in our case, likely the dog's home location) and each subsequent location of a dog's trajectory.

Table 15: Characteristics of the three study zones and devices available for analysis in dry and rainy season

Study zone	Location	Majority religion	Number of dogs counted/km^2	Number of devices available for analysis dry season	Number of devices available for analysis rainy season
1	Urban	Christian	328	228	158
2	Peri-urban	Christian	94	61	64
3	Urban	Muslim	59	19	13

9.4 Results

In the dry season, we counted a total of 481 dogs across the three study zones, of which 411 dogs from 242 households were successfully collared with a GCS unit. Due to lost or damaged GCS and battery failure, only 308 GCS were available for final data analysis corresponding to 64% of the originally identified dog population (Table 15). The median recording time of the GCS units was 2.82 days (IQR: 2.4-3.5). Table 16 provides an overview of the study population characteristics. The majority of dogs were intact males (73%) and adult dogs (81%) aged between 1 and 7 years. About three-quarters of dogs (74%) were allowed to roam freely at any time of the day. According to dog owners, all dogs were fed at home. Half of the dogs lived in a multi-dog household, i.e., households owning more than one dog.

In the rainy season, we collared a total of 458 dogs from 201 households. A total of 235 GCS units were available for final data analysis. The composition of the study population was similar to the dry season study population (Appendix 5: Supplementary information for chapter 9, Table 33).

Table 16: Characteristics of the study dog population in the dry season (December 2016).

	Overall (n=308)
Religion of the owner (n=306)	
Christian	274 (89.5%)
Muslim	32 (10.5%)
Sex (n=307)	
Female	49 (16.0%)
Male	223 (72.6%)
Neutered male	35 (11.4%)
Age (n=307)	
Young dog (< 1y)	38 (12.4%)
Adult dog (1-7 y)	250 (81.4%)
Old dog (> 7y)	19 (6.2%)
Confinement (n=306)	
None	225 (73.5%)
Daytime	45 (14.7%)
Nighttime	36 (11.8%)
Multi-dog household (n=308)	
Yes	155 (50.3%)
No	153 (49.7%)

9.4.1 Contact behavior

The median daily number of contact partners (“peers”) per dog was 3.4 and ranged from 0 to 42.4 across the three study zones (IQR: 1.8-5.8). The median daily time spent with each peer was 42.8 minutes (IQR: 9.1-104.8 minutes). The median daily number of peers in study zone 1 was four times higher as compared to study zone 3 (4 vs 1) and about two times higher as compared to study zone 2 (4 vs 2.4). When excluding contacts to dogs living in the same household, the daily number of peers dropped slightly to 3.2 (IQR: 1.7 to 4.6). We observed no noteworthy difference in the mean daily number of peers according to sex, age confinement, and religion of the owner (Appendix 5: Supplementary information for chapter 9, Figure 42 and Figure 43). The few dogs with a very high number of daily peers (> 20) were all male (Figure 18).

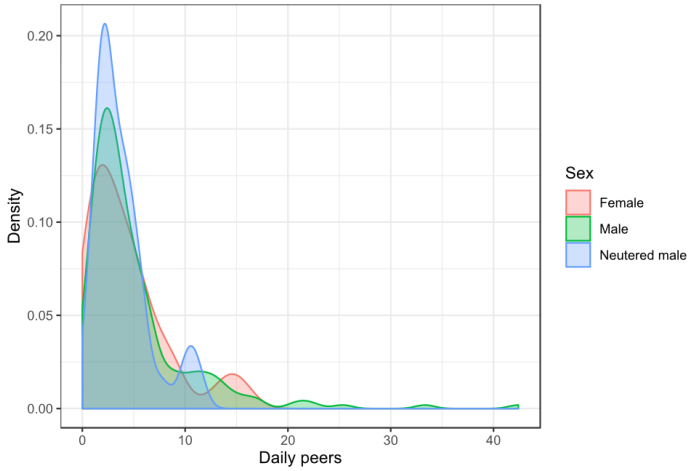

Figure 18: Density plot of number of daily peers grouped by sex

In the rainy season, the median daily number of peers per dog dropped to 0.8 with a range from 0 to 19.5 across the three study zones. The median daily time spent with each peer was shorter, at 16 minutes (IQR: 0.01 -151.6 minutes). The median daily number of peers was 1.4 (IQR: 0.5-3) in study zone 1 and 0.2 (IQR: 0-0.7) in study zone 2. No contacts were recorded in study zone 3.

NSD revealed a daily movement pattern, with dogs being most active throughout the night. Figure 19 shows the movement pattern of the 228 tracked dogs in study zone 1 over the 3.5 day study period.

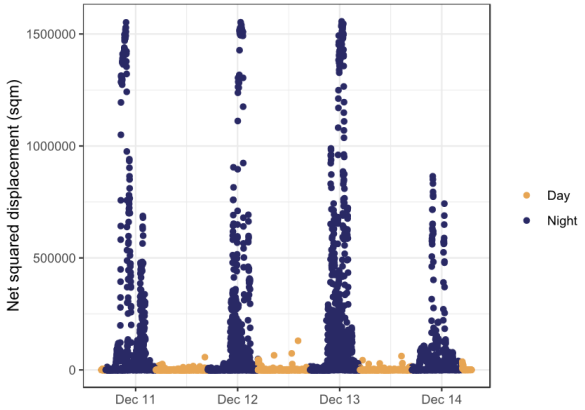

Figure 19: Net squared displacement of 228 dogs collared in study zone 1 during the dry season (December 2016).

The median home range size was similar in the two seasons, with a median size of 1.42 hectares (ha) (IQR: 0.68-2.92 ha) in the dry and 1.24 ha (IQR: 0.64- 4.92 ha) in the rainy season.

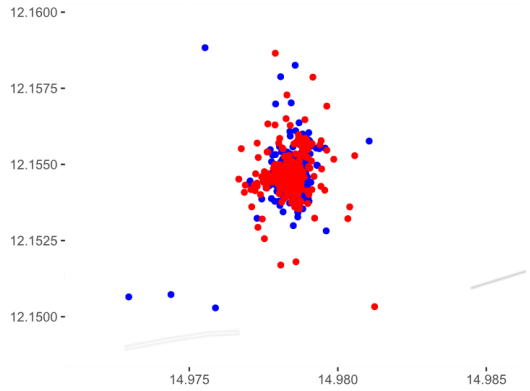

Figure 20: GPS fixes of the same dog tracked during both the dry season (blue dots) and rainy season (red dots)

Through visual inspection, we identified two roaming patterns of dogs: stay-at-home dogs with circular HRs around their home location and far roaming dogs with variably shaped HRs. Figure 21 depicts the HR of a far roaming and a stay-at-home dog. The daily number of peers did not differ between far roaming and stay-at-home dogs, and both male and female dogs exhibited both patterns.

Figure 21: GPS location fixes (black dots) and the corresponding HR (blue lines, 95% isopleth of the UD) of a far roaming dog (left) and stay-at-home dog (right).
The red dot indicates the dog's home location.

9.5 Discussion

We investigated the contact and movement behavior of owned free-roaming dogs in N'Djamena, Chad during two different seasons. We found that the number of contact partners per dog was higher in study zones with higher dog densities and during the dry season, while HR sizes were similar between the two seasons. Incidence data on dog rabies cases in N'Djamena do not suggest seasonality of disease spread (personal communication Monique Lechenne), however, Frey *et al.* [49] reported more dog bites in N'Djamena during the dry season as compared to the rainy

season. HR sizes are similar to those found in other studies [425-427]. Dürr *et al.* [425] reported that male dogs had larger HR sizes as compared to female and neutered male dogs. We did not investigate predictors for HR size in this study. Hudson *et al.* [428] identified similar roaming patterns (stay-at-home, roamer and explorer dogs) among a free-roaming domestic dog population in Australia. No intrinsic (sex, age, neutering status) or extrinsic (religion of the owner or diet) factor was clearly associated with highly connected dogs. Dogs were more active during the night as compared to the day. This might be explained by the fact that dogs are primarily kept for security reasons, guarding the house while dog owners are away. In addition, temperatures in N'Djamena throughout the day are extremely high which likely affects the nocturnal activity seen in this dog population.

Limitations

The dogs were tracked for 3.5 days which is rather short to estimate HR sizes. Moreover, fewer GCS were available for analysis in the rainy season which might influenced the number of contacts observed.

10 Rabies with a special attention to the 'One Health' aspect.

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10.1 Abstract

The inevitably fatal outcome of rabies makes it one of the most well-known and feared zoonoses. But rabies is still a neglected zoonotic disease (NZD), despite being one of the oldest diseases known. Dog mediated rabies is the main cause of human rabies and is globally responsible for approximately 59 000 human deaths per year, nearly all occurring in low- and middle-income countries (LMICs). Most African countries and connected regions are high risk areas for contracting rabies. Highly effective vaccines and post exposure prophylaxis (PEP) for humans allow for the disease to be 100% preventable. But PEP is often unavailable or too expensive for affected persons in LMIC. Recently several new and low tech diagnostic solutions have been developed, which offer opportunities to establish rabies diagnosis in remote areas and decentralize rabies laboratories.

Rabies has gained more global attention in recent years with development of new tools, formation of regional networks and implementation of a realistic global drive to eliminate canine-mediated human rabies by 2030. Canine rabies elimination is biologically feasible due to efficacious, safe and cheap vaccines and the low basic reproductive ratio (R_0) of the disease. It is well known that mass dog vaccination is a cost-effective, sustainable measure to eliminate the disease at its source. Domestic dogs are tied to human populations, so the role of humans in rabies spread needs to be further investigated, for example through anthropogenic landscape features like roads or vaccine corridors, human movements and sociocultural factors. Combining powerful approaches, such as landscape epidemiology and genetics, can facilitate strategic control programs and, for example, enable appropriate placement of vaccine barriers and surveillance points. It is known that major landscape features, such as oceans, mountains and deserts can act as natural barriers to disease spread. However, very little is known about barrier effects at smaller scales. Long-distance transport of infected dogs by humans poses a risk of rabies introduction in novel places. For successful rabies control, it is also important to guarantee access to pre- and post-exposure prophylaxis, to build capacity in disease diagnosis and to conduct educational campaigns. Well-functioning, continuous rabies surveillance systems are crucial to provide reliable data to increase political commitment, which is eminently important for successful, sustainable disease control. For elimination of rabies in Africa and the connected regions, a multidisciplinary, transdisciplinary and regionally well-coordinated approach, with sustained vaccination programs to maintain sufficient vaccination coverage, across political boundaries is required. To foster reflection on strategic rabies elimination on the African continent, one scenario of a possible spatio-temporal dynamic of dog rabies elimination in West Africa is proposed. Dog rabies elimination might, for example, start in northwestern Mauritania. The approach should be highly coordinated

between the involved countries, to avoid cross-border transmission and return of disease in rabies-free zones. It is difficult to mobilize large sums initially, but development impact bonds (DIB) offer an alternative funding mechanism. The total cost is estimated at 800 million to one billion Euros for rabies elimination in sub-Sahara West Africa, including Chad and Cameroon.

Keywords: Rabies, dogs, rabies control, vaccination campaigns, zoonoses, PARACON, 'One Health'

10.2 History

The inevitably fatal outcome of rabies, a disease caused by a neurotropic virus, makes it one of the most well-known and feared zoonoses. A zoonotic disease is defined as a disease which can be transmitted between humans and vertebrate animals. The majority of rabies cases around the world are caused by the canine-associated classical rabies virus (Family *Rhabdoviridae*, Genus *Lyssavirus*), but rabies-related viruses of the same genus, mostly circulating in bat species, can also cause rabies (Lagos bat virus, Mokola virus, Duvenhage virus, European bat lyssavirus-1 & 2, Australian bat lyssavirus and others). In general, all mammalian species can be infected by the rabies virus [429]. In Western and Central Africa, rabies virus mainly belongs to the lineage 'Africa 2' [185].

10.3 Geographical distribution, economic/public-health impact and epidemiology

Canine rabies is the main cause of human rabies, being globally responsible for approximately 59 000 human deaths per year, the majority which are in Asia (60%) and Africa (36%) [9]. Almost all human cases occur in low- and middle-income countries (LMICs), with children being the most affected group. In resource poor countries, the virus is mainly transmitted through dog bites, but transmission is also possible through infected wild animals [429; 430]. It is believed that rural areas are more affected by rabies deaths than urban areas [14]. Most African countries and connected regions are high risk areas for contracting rabies [431].

Highly effective vaccines and post exposure prophylaxis (PEP) for humans allow for the disease to be 100% preventable. PEP consists of local wound care, vaccination and immunoglobulin application. Every year, over 15 million people globally are exposed to rabies and should receive PEP, at considerable monetary cost. But PEP is often unavailable or too expensive for affected persons in LMIC. In some contexts, inappropriate recommendation of PEP use by health personnel was observed [9; 36; 430].

Different factors contribute to the burden of rabies, including: mortality and productivity lost due to premature death, morbidity from adverse events following vaccination (nerve tissue vaccines), psychological effects and direct and indirect costs of PEP. In 2015, the global rabies burden was estimated to be 3.7 million disability-adjusted life years (DALYs), with over 95% lost in Africa and Asia [9]. Since political will and interest often depends on the economic burden of a disease, the annual economic losses due to premature death, costs of PEP and costs to the veterinary sector were estimated, reaching 8.6 billion USD globally [9]. In Africa alone, the costs of rabies were estimated to be 1.3 billion USD annually [432].

Today, rabies is classified as a neglected zoonotic disease (NZD), despite being one of the oldest diseases known to humankind. In many affected countries, the control of the disease is still hampered by a low level of political commitment, most likely due to lack of reliable data [430; 433]. The fact that many bite victims in LMICs do not visit medical facilities and lack of laboratory confirmation of cases leads to underreporting of the disease, contributing to the cycle of neglect [111; 430]. Poor disease surveillance prevents decision-makers and stakeholders from making informed decisions regarding allocation of funding and resources towards better control and intervention strategies. However, because of lack of funding, it is difficult to improve surveillance [434]. In Tanzania, a 10 to 100 fold higher incidence of human rabies cases was estimated when incidence was extrapolated from animal bite occurrence by using a probability decision tree modeling method to determine the likelihood of clinical rabies in humans after the bite of a rabid dog, compared to the officially reported human incidence estimated through passive surveillance data [124]. Ninety-nine per cent of human rabies cases are likely to be unreported in Tanzania [435]. Knobel *et al.* [14] estimated a rate of underreporting of 160 times for Africa in general. Misdiagnosis also adds to underreporting, as seen in Malawi where children with rabies were falsely diagnosed with cerebral malaria [114].

10.4 Symptoms and Lesions

After saliva containing virus comes in contact with peripheral nerve endings through a bite, skin lesion or mucous membrane, the virus affects the central nervous system where it causes fatal encephalitis with dramatic symptoms. Incubation periods last from a few days to several months depending on factors such as the site of virus inoculation and viral load. Symptoms in humans and animals result from brain dysfunction, leading either to a furious (mad) or a dumb (paralytic) form of rabies [429; 436].

10.5 Diagnostic

The ‘gold standard’ for detection of rabies virus antigen in the brain is the direct fluorescent antibody (DFA) test. However, proper application in LMICs remains limited due to inappropriate laboratory facilities, uncooled sample transportation and lack of quality management systems. Because animal rabies diagnosis is typically only conducted at Central Veterinary Laboratories (CVLs) in LMICs, existing surveillance data mainly reflects the rabies situation of urban areas. Several new and low tech diagnostic solutions have been developed in recent years. A promising one for rabies diagnosis in LMICs is the direct rapid immunohistochemical test (dRIT), which has diagnostic efficacy equal to that of the DFA. Benefits include ease of differentiation between a positive and a negative result so the test is simple to interpret by inexperienced readers. Unlike the DFA test, the dRIT does not rely on fluorescence, another advantage because fluorescence is difficult to interpret in degraded or archived samples. Since fluorescent microscopes are not needed, in-house calibration is possible and maintenance of equipment is reduced. Furthermore, storage of brain samples in glycerol seems to influence the DFA more than the dRIT, improving diagnosis in archival samples and samples maintained at room temperature. The dRIT requires a smaller initial capital investment and is cheaper to perform [45; 138; 437-439]. Another recent test is the rapid immunodiagnostic test (RIDT), a pre-manufactured diagnostic kit based on the lateral flow principle. This test is extremely easy to conduct, does not require a microscope for interpretation and can be stored at ambient temperature. However, currently RIDTs are not considered as reliable as the FAT or the DRIT [119]. Lechenne *et al.* [46] showed, on the other hand, higher reliability compared to the FAT when applied in a resource poor laboratory. Nevertheless, both the DRIT and the RIDT offer opportunity to establish rabies diagnosis in remote areas and therefore decentralize rabies laboratories [46].

10.6 Application to prevention and control/adopted surveillance and control strategies

Rabies has gained more global attention in recent years, with development of new tools, formation of regional networks and a realistic global drive to eliminate canine-mediated human rabies by 2030, in line with the United Nations Sustainable Development Goals (SDGs) [78; 440]. The Blueprint for Rabies Prevention and Control [108] was developed to provide guidelines and strategies on rabies control (http://www.rabiesblueprint.org/). In 2014, the Pan-African Rabies Control Network (PARACON) was established under the secretariat of the Global Alliance for Rabies Control (GARC). PARACON represents a unified coordinated approach to eliminate canine rabies in sub-Saharan Africa on a regional, national and continental level, with support from global human and

animal health organizations such as the World Health Organization (WHO), the World Organisation for Animal Health (OIE), the Food and Agriculture Organization of the United Nations (FAO) and World Animal Protection (WAP). North African countries are incorporated into the Middle East and Eastern Europe Rabies Expert Bureau (MEEREB), but collaborating closely with PARACON [138]. The ‘One Health’ concept must be strengthened to result in an added-value of closer cooperation between human and animal health. The added-value can be defined as health benefits, financial savings or environmental services [138; 441]. To date, interaction and collaboration between the veterinary sector and the public health departments is often non-existent in LMICs even though with zoonotic diseases both sectors are dealing with the same epidemiological complexities. In general, the ministry of health is responsible for prevention of the disease in humans, the ministry of agriculture is in charge of rabies control in animals and the ministry of local government and the ministries of commerce, industry or science and technologies are involved in rabies vaccine production and imports, dog population management and dog immunization [442]. A ‘One Health’ perspective for rabies brings a clear added value by strengthening intersectoral cooperation, for instance, through better disease surveillance and communication. The approach should always be adapted to the local setting and its human-animal relationship which is governed by the cultural and religious context [30; 443]. The PARACON network encourages collaboration amongst African countries through regular meetings and workshops held in member countries throughout Africa. Different tools are provided through the PARACON network, like capacity building in rabies diagnosis, focusing on the implementation of the dRIT and provision of material, expertise and assistance in rabies control programs. Additionally, an online African rabies epidemiological bulletin was launched in 2016, which captures high quality, pan-African data on human and animal rabies cases in a timely manner, and assesses number of vaccine doses administered, vaccination coverage and dog population estimates. The gathered data from the bulletin will be further used for advocacy purposes in order to draw support from governmental authorities and stakeholders for the implementation of National Control Strategies within countries allowing them to take ownership for control and elimination of canine-mediated human rabies [138]. The Stepwise Approach towards Rabies Elimination (SARE), developed by GARC and the FAO in 2012, helps member countries evaluate their progress towards rabies control and elimination. The SARE tool provides countries with measurable stages to progress from Stage 0 to Stage 5 towards becoming canine-rabies free. A country typically begins at Stage 0, with little or no epidemiological understanding of, or control efforts for, rabies in place. The country progresses to the next stage once certain critical and non-critical activities have been achieved, eventually reaching Stage 5 – being canine rabies free [107; 135; 138].

Canine rabies elimination is biologically feasible due to efficacious, safe and cheap vaccines and the low basic reproductive ratio (R_0) of the disease [34; 80; 324; 444]. It is well known that mass dog vaccination is a cost-effective, sustainable measure to eliminate the disease at its source and prevent humans from exposure [9; 36; 54]. In many African communities sufficient dogs are accessible for vaccination in order to achieve herd immunity [442]. PEP alone will never be able to interrupt human exposure. Mindekem *et al.* [36] demonstrated in an African city that canine vaccination in combination with PEP becomes more cost-effective after 15 years in comparison to use of PEP alone. With ideal One Health communication, the cost for dog vaccination and PEP compared to PEP alone breaks even within the timeframe of 10 years. In Western Europe and North America, rabies has been successfully eliminated from domestic dog populations through mass vaccination and legislation [64; 66; 445]. Disease control through dog vaccination is even feasible in situations where large wildlife populations are prevalent [324]. For efficient rabies control, the World Health Organization (WHO) recommends vaccinating at least 70% of the dog population, which is rarely reached in African countries [56]. In Bamako, the capital of Mali, a vaccination coverage of only 24% was estimated in the domestic dog population, which is insufficient coverage to interrupt virus transmission [446]. In N'Djamena, the capital of Chad, rabies cases in dogs decreased by more than 90% within one year after a free mass vaccination campaign which achieved coverage at the recommended 70% level [55]. In both the Malian and the Chadian context, an important constraint to vaccination was cost of the vaccination of the dog [51; 446]. In Chad, it was found that vaccination coverage dramatically dropped when dog owners had to pay for the vaccination [52]. In Latin America and the Caribbean, canine rabies was successfully reduced due to mass dog vaccination. The Pan American Health Organization (PAHO) played an important role in this process, through its constant support and coordination of dog rabies elimination programs. It is important that not only urban centers are vaccinated, as was the case for Bolivia which prioritized urban areas for vaccination due to financial limitations and the fact that higher levels of rabies vaccination in urban areas are more easily achieved compared to rural areas [31; 447]. Despite some notable efforts and achievements, we are still far from global elimination of canine rabies. Rabies vaccination campaigns risk failure when they are not adequately presented and accepted within different socio-cultural contexts. The vaccine 'final' effectiveness in a specific setting is determined by several additional parameters, such as availability, accessibility, affordability, adequacy and acceptability [297; 303; 448]. Prior to attempting rabies control in a target area, it is important to know what the achievable vaccination coverage and the level of community participation is likely to be. Through a deeper qualitative assessment, as in the intervention effectiveness model, an explanatory framework for a specific area can be elaborated. This

was recently done by Muthiani *et al.* [87] and Mosimann *et al.* [88] in Bamako, Mali. Lack of information and inability to handle the dogs were the most frequently stated reasons for not bringing a dog to the vaccination posts. Knowledge generated in specific local contexts, which accounts for cultural practices and social and political realities, is fundamental to design successful effective, sustainable dog rabies control programs [443].

Another important factor in canine rabies elimination is better understanding of affected dog populations. It is crucial to know more about dog ecology, size of the dog population in a specific area, dog density, sex ratio, population turnover/growth and roles of dogs in human societies [332; 449]. Mass vaccination campaigns should be repeated at short intervals because of the high turnover rate in dog populations in sub-Saharan African countries which leads to a rapid decrease of population-level immunity [446]. In Chad, information on the size of dog populations was used for planning dog rabies elimination on the country level [106]. It is important that dog population reduction through culling and poisoning is not only socially unacceptable but has also been found to be counter-productive in dog rabies elimination campaigns. Such practices should be discontinued [80; 450]. As domestic dogs are tied to human populations, the role of humans in rabies spread needs to be further investigated. This includes for example anthropogenic landscape features like roads or vaccine corridors, human movements and sociocultural factors. Free-roaming dogs in rabies endemic countries need to be considered during implementation of control measures. Genetic work can identify main routes of viral dissemination. Combining landscape epidemiology and genetics approaches would be a powerful method to facilitate strategic control programs and identify the appropriate placement of vaccine barriers and surveillance points. It is known that major landscape features, such as oceans, mountains or deserts can act as natural barriers to disease spread. However, very little is known about barrier effects on smaller scales. Long-distance transport of infected dogs by humans poses a risk of rabies reintroduction or introduction to novel places [81; 451]. Hampson *et al.* [445] and Talbi *et al.* [82] demonstrated long-distance translocation of infected dogs by humans in North and sub-Saharan Africa. In rabies-free areas a focus should, therefore, be put on the risk of rabies re-emergence through dog movement and possible spill over from wild to domesticated animals [447]. In rabies free areas, reintroduction control needs to be applied as well as controlled dog movement. Transboundary spread of the rabies virus need to be considered during the disease elimination process, so control programs should include neighboring countries [444]. In North Africa, there was restricted movement across geopolitical boundaries [82], but the model of Hampson *et al.* [445] demonstrated large-scale synchronous cycles of domestic dog rabies, with a period of 3-6 years, in southern and eastern Africa.

In their examination, climate did not influence the observed synchronous pattern of rabies outbreaks. Epidemiological analyses from Ghana also demonstrated frequent cross-border incursions [198].

For successful rabies control, it is vital to guarantee access to pre- and post-exposure prophylaxis, to build capacity in disease diagnosis and to conduct educational campaigns. Responsible dog ownership through community education and legislative measures should be promoted [452]. Well-functioning continuous rabies surveillance systems are crucial to provide reliable data to increase political commitment, which is necessary for successful and sustainable disease control. Countries with limited financial resources could significantly benefit from international support, including from other countries. One example could be vaccine donations, as the Dominican Republic has provided to Haiti. In addition, NGOs and the public and private sectors need to be included in rabies control programs [447]. Remote and hard to access areas should not be ignored, as these areas could harbor remaining foci of infection. A sustained focus on communities where rabies incidence has declined is critical, so that decreased investment does not prevent continued control measures which are necessary to achieve complete elimination in those communities [444].

10.7 Conclusion

It is evident that rabies elimination in Africa and elsewhere in the dog rabies endemic world requires a multidisciplinary, transdisciplinary and regionally well co-ordinated approach with effective, sustained vaccination programs which span political boundaries. Freedom from rabies should be recognized as a public good. Canine mass vaccinations should be free for dog owners in order to reach sufficiently high coverage. Given the importance of such regional approaches, international organizations like the African Union (AU-IBAR) and the Economic Union of the West African States (ECOWAS) should be involved from the outset, working in close collaboration with PARACON. To illustrate strategic rabies elimination in Africa, a scenario of one possible spatio-temporal dynamic of elimination in West Africa is shown in Figure 22. This proposal begins with dog rabies elimination in North-western Mauritania, continuing simultaneously in a south and east-ward direction. Natural barriers like the Atlantic Ocean and the Sahara desert are taken into account. Alternatively, elimination efforts could start in Liberia with a simultaneous north, south and east-ward progression. The approach should be highly coordinated between the involved countries to avoid cross-border transmission and return of disease in rabies-free zones. It is difficult to mobilize large sums initially, but development impact bonds (DIB) offer an alternative funding mechanism.

Total cost for rabies elimination in sub-Sahara West Africa, including Chad and Cameroon, is estimated at 800 million to one billion Euros.

Figure 22: Scenario of a possible spatio-temporal dynamic of dog rabies elimination in West Africa, in the context of a Pan-African campaign (Map data from Google, arrows included by S. Mauti).

To identify the optimal operational unit for disease intervention campaigns and optimize existing resources, the role humans play in pathogen spread, natural barriers, virus genetics and host ecology should be assessed for each country. Higher-resolution data from the continent and connected regions would enable better understanding of spatio-temporal virus dynamics. Such information would allow effective buffer zones for dog rabies to be designed and implemented [445; 451]. Where control measures deteriorate, rabies epidemics rapidly re-emerge through dispersal from endemic areas. Integrated and effective surveillance systems allow for early disease detection and quick response [453]. Surveillance is a key element in rabies control, and weak surveillance systems jeopardize disease elimination efforts. Proactive mass dog vaccination followed by two years of monitoring and vaccination is recommended for countries with poor surveillance systems. This approach is more effective at controlling rabies in isolated areas compared to vaccinating in response to case detection. Surveillance levels that detect at least 10% of all cases are recommended for the control and elimination of rabies [454].

11 Field postmortem rapid immunochromatographic rabies diagnostic test for resource-limited settings with further molecular applications

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Results from the DRC are presented in Appendix 6: Supplementary information for chapter 11

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11.1 Abstract:

Functional rabies surveillance systems are crucial to provide reliable data and increase the political commitment necessary for disease control. To date, animals suspected as rabies-positive must be submitted to a postmortem confirmation using classical or molecular laboratory methods. However, most endemic areas are in low- and middle-income countries where animal rabies diagnosis is restricted to central veterinary laboratories. Poor availability of surveillance infrastructure leads to serious disease underreporting from remote areas. Several diagnostic protocols requiring low technical expertise have been recently developed, providing opportunity to establish rabies diagnosis in decentralized laboratories. We present here a complete protocol for field postmortem diagnosis of animal rabies using a rapid immunochromatographic diagnostic test (RIDT), from brain biopsy sampling to the final interpretation. We complete the protocol by describing a further use of the device for molecular analysis and viral genotyping. RIDT easily detects rabies virus and other lyssaviruses in brain samples. The principle of such tests is simple: brain material is applied on a test strip where gold conjugated antibodies bind specifically to rabies antigens. The antigen-antibody complexes bind further to fixed antibodies on the test line, resulting in a clearly visible purple line. The virus is inactivated in the test strip, but viral RNA can be subsequently extracted. This allows the test strip, rather than the infectious brain sample, to be safely and easily sent to an equipped laboratory for confirmation and molecular typing. Based on a modification of the manufacturer's protocol, we found increased test sensitivity, reaching 98% compared to the gold standard reference method, the direct immunofluorescence antibody test. The advantages of the test are numerous: rapid, easy-to-use, low cost and no requirement for laboratory infrastructure, such as microscopy or cold-chain compliance. RIDTs represent a useful alternative for areas where reference diagnostic methods are not available.

Keywords: Rabies, diagnosis, field, postmortem, brain RIDT, lateral flow device, low- and middle-income countries, rapid, remote areas, RT-qPCR, genotyping

11.2 Author summary:

We present a complete protocol for postmortem diagnosis of animal rabies under field conditions using a rapid immunochromatographic diagnostic test (RIDT), from brain biopsy sampling to final interpretation. We also describe further applications using the device for molecular analysis and viral genotyping.

11.3 Introduction:

Canine rabies is the main cause of human rabies, globally responsible for approximately 59 000 human deaths per year, nearly all occurring in low- and middle-income countries (LMICs) in Asia and Africa [9]. The main etiological agent is a neurotropic canine-associated classical rabies virus (RABV, family *Rhabdoviridae*, genus *Lyssavirus,* species *Rabies lyssavirus*). However, other rabies-related lyssaviruses, mostly circulating in bat species, also cause disease [455; 456]. In affected regions, disease surveillance and control are often hampered by low level political commitment likely due to lack of reliable data [113; 116; 457]. One reason for disease underreporting is absence of laboratory diagnosis, due in part to limited access to equipped laboratories and trained staff as well as the difficulties of shipment of the specimens. Laboratory diagnosis is necessary to confirm rabies cases and additionally allows for genetic characterization of the involved strains, providing insight on virus transmission at the regional level [113; 116; 458].

The current gold standards for postmortem rabies diagnosis, approved by both the World Health Organization (WHO) and the World Organisation for Animal Health (OIE), are the direct fluorescent antibody test (DFAT), the direct rapid immunohistochemistry test (DRIT) and molecular methods (e.g. reverse transcription polymerase chain reaction (RT-PCR))[116; 439]. However, proper application in LMICs remains limited due to inadequate laboratory facilities with inconsistent power supply, uncooled sample transportation and lack of a quality management system. Because animal rabies diagnosis is typically only conducted at central veterinary laboratories in LMICs, existing surveillance data mainly reflects the rabies situation in urban areas.

Recently developed low technology diagnostic alternatives offer opportunities to establish rabies diagnosis in remote areas and decentralized rabies laboratories [116; 439; 459]. The DRIT is a lateral flow test based on immunochromatography using gold conjugated detector antibodies and is a very promising rabies diagnostic tool [460-462]. The principle is simple: after dilution, brain material is mixed in the provided buffer, and a few drops are applied on the test strip where gold conjugated monoclonal antibodies bind specifically to rabies antigens, mainly the nucleoproteins (Figure 23). The antigen-antibody complexes then undergo lateral flow migration, binding at the test line (T-line) to fixed antibodies against rabies antigens, resulting in a clearly visible purple line. The remaining gold conjugated antibodies not bound to rabies antigens continue migrating and fix to the membrane through additional targeting antibodies, resulting in a clearly visible purple control line (C-line).

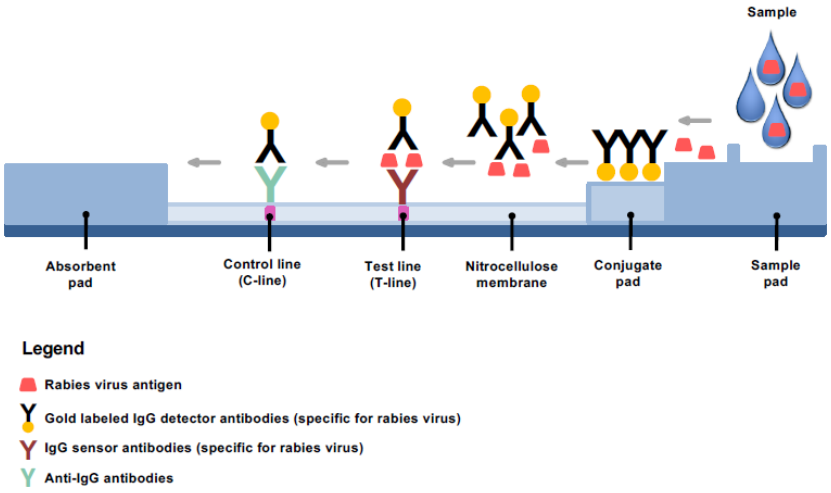


Figure 23: Schematic representation of the structure of an RIDT for rabies diagnosis.

The one-step, low cost method is rapid, extremely easy and does not require expensive equipment or special storage conditions. With modification of the manufacturer protocol to eliminate the dilution step, nearly all equipment and reagents required to perform the test are included in the kit [46]. The result is read after 5-10 minutes without a microscope. This is a major advantage over the DFAT test, which requires a fluorescence microscope and immunofluorescence conjugate, along with refrigerated transportation and sample storage. Even the DRIT test, which can be performed using a light microscope, requires a continuous cold chain to store the anti-rabies antibodies, which are also not yet commercially available. In comparison to the DRIT, the RIDT requires no toxic chemicals, a particular advantage in countries where waste disposal is poorly regulated. The rapid test is less time-consuming with much easier interpretation compared to the gold standard tests DFAT and DRIT. This allows for on-site testing by personnel with limited technical expertise.

Based on these test properties, prompt diagnosis of suspected animals in remote areas becomes feasible, facilitating implementation of post exposure prophylaxis (PEP) for exposed people as soon as possible. In addition, distance transport of rabies samples is not necessary, resulting in better sample quality at the time of testing. However, the results obtained with the RIDT tests should be confirmed using a reference diagnostic test such as DFAT or DRIT.

RIDT techniques for detection of RABV and other lyssaviruses have been evaluated. One of the first studies was conducted by Korean researchers in 2007 [460]. Compared to the DFAT method,

in 51 animal samples and 4 RABV isolates, the RIDT showed a sensitivity and specificity of 91.7% and 100%, respectively. These results were later confirmed with 110 animal brain samples from Korea, with sensitivity and specificity, compared to DFAT, of 95% and 98.9%, respectively [463]. More recently, other studies assessed the performance of this RIDT using virus isolates and/or infected brain samples from various animals with different geographical origins. A panel of 21 samples, including African RABV and other African lyssaviruses (Duvenhage virus (DUVV), Lagos bat virus (LBV) and Mokola virus (MOKV)), were successfully detected, with sensitivity of 100% compared to the DFAT [121]. Similar high sensitivity (96.5%) and specificity (100%) values were obtained from a panel of 115 brain samples from Ethiopia [464]. Another study evaluated European RABV isolates, two other European lyssaviruses (European bat lyssavirus type 1 (EBLV-1) and type 2 (EBLV-2)), and the Australian bat lyssavirus (ABLV) [465]. Based on analysis of 172 animal brain samples, the RIDT kit had 88.3% sensitivity and 100% specificity compared to DFAT, and the three rabies-related lyssaviruses were successfully detected. In this study, some of the false negative results came from brain samples stored in glycerol buffer, suggesting that improper glycerol removal influenced capillary flow or antibody binding. A recent analysis of 43 clinical samples from Australian bats confirmed previous test results, with complete concordance to DFAT [466]. Two studies were conducted in India using the RIDT on a limited number of clinical samples (11 and 34 samples). Compared to DFAT, sensitivity was between 85.7% and 91.7% and specificity was 100% [122; 467]. Another evaluation of this kit using 80 animal brain samples from Africa, Europe and the Middle East obtained complete concordance with DFAT for specificity (100%) but a higher sensitivity (96.9%) compared to the previous studies [120]. In a recent interlaboratory comparison of this RIDT performed in 22 different laboratories using a panel of 10 samples overall concordance was 99.5% [468].

Only one recent multicentric study showed unsatisfactory overall RIDT performance [119]. Samples from three different datasets were tested and provided variable sensitivity and specificity values compared to DFAT. For example, sensitivity and specificity obtained with the first panel (n= 51) and the second panel (n=31) of samples from experimental infected animals, all tested in laboratory A, gave a sensitivity of 16% and 43%, respectively, whereas the specificity was 100% for both. Conversely, the results of the third panel (n=30) of field clinical samples analyzed by laboratory B provided a complete concordance with the results of DFAT, which was further nearly completely confirmed by laboratory A (85% sensitivity and 100% specificity). Batch-to-batch variation was suggested as a possible explanation for the fluctuating relatively low sensitivity with RIDT [119].

At the same time, another study performed a similar validation process of the above described RIDT, with a modification of the manufacturer recommended protocol [46]. The pre-dilution step (1:10) in PBS was omitted during preparation of the brain material. Based on this simpler modified protocol, the authors obtained sensitivity and specificity of 95.3% and 93.3%, respectively, compared to DFAT by testing, under laboratory conditions, a dataset of 73 animal brain samples, naturally or experimentally infected with various RABV strains. The study presented the first evaluation of this RIDT in a field setting (Chad, Africa). In 48 clinical brain samples, sensitivity and specificity were 94.4% and 100%, respectively. The discrepancies between DFAT and RIDT were due to false positive results with DFAT, determined after confirmation with RT-PCR. When these results were deleted, there was complete concordance, and it demonstrated that the RIDT was more reliable that DFAT under these field conditions [46]. No batch-to-batch variation was observed using the modified protocol. When the modified protocol was applied to a small number of the DFAT/ RIDT divergent samples (n=8) in the study of Eggerbauer et al. [119], all were found concordant (100% sensitivity).

Another major advantage of the RIDT is secondary use for detecting viral RNA fixed on the strip using molecular techniques (such as RT-PCR) and subsequent genotyping [46; 119]. Following an extraction step, Lechenne *et al.* [46] demonstrated viral RNA fixed on the Anigen device membrane using RT-PCR with 86.3% sensitivity in a panel of 51 samples (including 18 samples tested and shipped from Chad at ambient temperature). Subsequent genotyping was possible in 93% of the 14 samples tested. Sanger sequencing of PCR amplicons of at least 500 nucleotides in length were used. In addition to RABV isolates, the test detected four other lyssavirus species, DUVV, EBLV-1, EBLV-2 and Bokeloh bat lyssavirus (BBLV), during a fully concordant international inter laboratory test [46]. The sensitivity of viral RNA detection was even higher (100%) in the study of Eggerbauer et al., based on laboratory samples examination [119]. The latter study also demonstrated that the buffer used in the RIDT kit inactivated virus. Thereby, the devices can be shipped easily, at ambient temperature without specific biosafety precautions to reference laboratories, for molecular confirmation and genotyping.

Based on the previous evaluations, RIDT tools offer numerous advantages for use in field settings, especially when the reference diagnostic techniques are not available. However, this test also has some limitations, in particular, low sensitivity of antigen detection [46; 119]. The test is applicable for samples containing high quantities of viral antigens, such as brain samples. However, it is not appropriate for other samples such as saliva or other body fluids. Another drawback is cost of the device (around 5-10 Euros in Europe), which is less expensive compared to the cost of performing DFAT, RT-PCR or DRIT, but which still remains high for LMICs. However, future development and

validation of similar RIDTs from other companies could lead to a price decrease. One study reported batch-to-batch variations. Although not reported by others, strict quality controls should nevertheless be performed when testing a new batch, as for any reagent used in a quality management environment. The use of the modified protocol was not altered when using different batches [46]. All except one study demonstrated that sensitivity of RDIT was high compared to DFAT (around 90%-95%). Because rabies is always fatal, it is still strongly recommended to confirm any negative results with RDIT using a reference diagnostic test such as DFAT, DRIT or RT-PCR [46].

In this manuscript, we present a complete protocol for field postmortem diagnosis of animal rabies based on an example of a commercialized RIDT, from brain sample collection to application of a modified protocol compared to the manufacturer recommendations (which were previously validated [46]) and subsequent molecular analysis. This protocol was applied and validated many times under field conditions in West- and Central Africa, where the RIDT was used routinely for rabies diagnosis alongside the DFA test. We additionally demonstrate a second application for the device, in laboratory settings, for extraction and detection using RT-PCR of viral RNA fixed on the device.

11.4 Protocol:

11.4.1 Sample collection via the foramen magnum (occipital route) [469]

Note: This technique can be implemented under laboratory conditions or in field settings. Samples should be processed as soon as possible after death of the suspected animal or kept at cool temperature to avoid decomposition.

CAUTION: All samples should be considered as potentially infectious. Safety regulations and procedures should be strictly followed, even in field settings [116]. In particular, wear appropriate personal protective equipment including mask, glasses, gloves and a lab coat. Use appropriate disinfectant for material and sample decontaminations (e.g. sodium hypochlorite with recommended manufacturer dilutions, 70% alcohol - ethanol or isopropanol, 1% soap solution). All personnel handling samples should be vaccinated against rabies.

1. Remove the animal head with a knife before the first cervical vertebra (atlas vertebra) to access the foramen magnum.

Note: To minimize infective aerosol, avoid using a manual saw or similar tool.

2. Collect brainstem (medulla oblongata) sample using a disposable plastic pipette (Figure 24A), a drinking straw (Figure 24B), a clamp (Figure 24C) or a dropper (supplied with the RIDT) (Figure 24D).

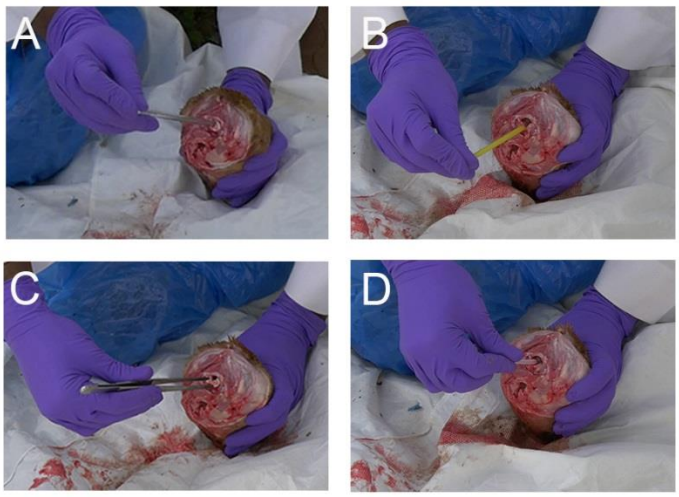


Figure 24: Examples of rapid simple techniques for collection of brain samples (brainstem with medulla oblongata) in animals (dog shown here) via the occipital foramen in field settings (Mali). **A.** Collection with a disposable plastic pipette. **B.** Collection with a plastic drinking straw. **C.** Collection with a clamp. **D.** Collection with the disposal dropper provided in the RIDT kit.

Optionally, or if medulla oblongata is not available, other parts of the brain (brainstem, cerebellum, hippocampus, thalamus and cortex) can be collected by the same occipital route by pushing and rotating the plastic pipette or straw towards the eye socket (Figure 25).

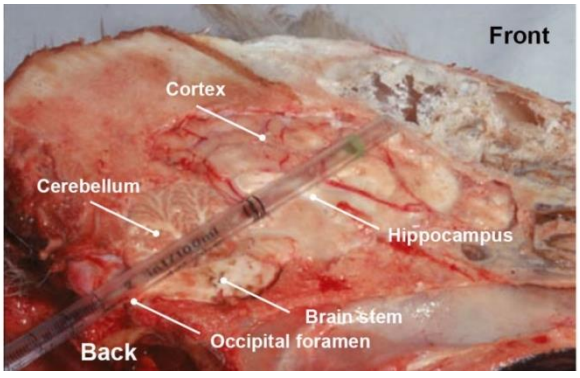


Figure 25: Longitudinal anatomical section of dog head, showing the different parts of the brain (brainstem, cerebellum, hippocampus, thalamus and cortex) collected when pushing, in a rotational movement, a disposable plastic pipette through the occipital foramen route.

If using a straw or pipette, gently squeeze it to deposit the brain sample (0.5-2 g) in a tube for subsequent analysis and/or biobanking.

Note: Sample storage in glycerol is not recommended, as it seems to affect capillary flow or the antibody binding step of the RIDT [465].

11.4.2 Execution of the modified RIDT protocol [46]

Note: This modification omits a dilution step (1:10) into PBS, as specified in manufacturer protocol (all versions), and can be implemented under laboratory or field settings.

1. Use the swab to collect the equivalent of half a peanut or pea (0.1-0.5 g) of brain material and place it in the buffer sample tube.

Note: For the modified protocol, all reagents/consumables are included in the kit (no PBS or additional tube is needed) (Figure 26). Document the batch number of the kit and check validity of the expiration date.

Figure 26: Description of the contents of RIDT kit, including the device, a disposable plastic dropper, a disposable swab, and the assay diluent.
The tube where the sample will be collected and stored is not provided.

2. Carefully crush the brain material directly in the tube with the swab for about 30 s until a homogeneous suspension is obtained.

Note: The buffer reaction inactivates the infectivity of the virus [119].

3. Using the dropper, deposit four drops (approximately 100 µL) of the suspension in the sample inlet on the test device.
4. Wait for complete sample migration (1-5 min) before reading the test device. The migration should start rapidly after deposit of the sample (1-5 min).

5. In case of delay (due to high viscosity suspension), gently scratch the bottom of the deposit site of the device with the dropper (1-5 times), and add 1-2 more drops. Migration should start immediately thereafter.
6. Read the test result in the detection window after 5-10 min, and no more than 20 min, after the end of the migration.
7. Interpret the result based on presence or absence of the control line (C-line) and test line (T-line) (purple lines) in the detection window, according to Figure 27. Consider the sample positive when two lines are visible (Figure 27A), negative if only the C-line is present (Figure 27B) and invalid if only the T-line is present or if no lines are visible (Figure 27C).

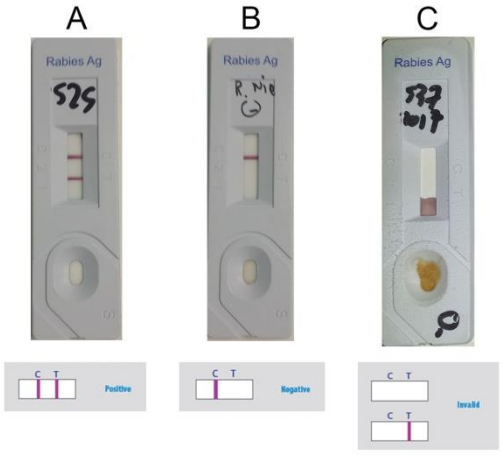


Figure 27: Representative results for interpretation of the Anigen RIDT.
A. Positive results (visible presence of two lines, C-line and T-line). B. Negative results (visible presence of C-line only). C. Invalid results (absence of visible C-line).

Note: Invalid results should be repeated at least once. Other techniques should be performed if results remain invalid. Negative results obtained with RIDT need to be subsequently confirmed using a gold standard reference method, like DFAT, DRIT and molecular methods (polymerase chain reaction or PCR). Even though the sensitivity of this test is high (see Representative results section), it is not 100%.

8. Store devices at room temperature, or refrigerate/freeze when possible, for subsequent molecular analysis (see Genotyping after RNA extraction from the RIDT device 11.4.4). Freeze the remaining sample suspension at -20°C/-80°C in the buffer tube to repeat the test if necessary or for subsequent molecular analysis.

11.4.3 RNA extraction and detection by RT-qPCR from the RIDT device

Note: This step can only be implemented under laboratory conditions with adapted environment and suitable equipment for molecular diagnosis. It can be done soon after the RIDT test or retrospectively on archived RIDT devices, stored at room temperature (15-30 °C), refrigerated or frozen.

RNA extraction

Note: To monitor the extraction step, it is recommended to use an internal control which can be an endogenous mRNA (such as ß-actin) or an exogenous control (such as eGFP synthetic RNA) directly spiked into the sample during the first steps of the extraction [125; 470].

Carefully open the device and remove the filter paper.

Cut the deposit area of the sample and place it into a tube containing 1 mL of Tri-Reagent LS. Incubate at RT for 1 hour with gentle regular manual agitation.

1. Perform the extraction in accordance with manufacturer recommendations, as previously described [125]. At this step, the exogenous internal control can be added.
2. During the process, add 2 µL of glycogen for facilitating precipitation of RNA, according to the manufacturer recommendations.
3. The final volume for RNA resuspension in nuclease-free water can be adjusted, with a volume of 50 µL generally used.

Note 1: At the end of the centrifugation step for aqueous and organic phase separation (after addition of 200 µL of chloroform into the Tri-Reagent LS), the piece of membrane from the device will be at the bottom of the tube and not interfere with collection of the upper aqueous phase.

Note 2: Alternatively, other easy and rapid protocols can be used, for instance, using phenol-based reagents and silica membranes (for example [471]).

Detection by RT-qPCR [470]

Note 1: Detection of potential viral RNA present in extracted samples can be done using different molecular techniques, such as reverse-transcription PCR, conventional (end-point) or real time PCR (qPCR). Several methods are available, such as conventional RT-PCR [125; 472] or RT-qPCR [470; 473] targeting the viral polymerase gene.

Note 2: One example will be presented below based on a dual combined pan-lyssavirus RT-qPCR targeting a conserved region among the viral polymerase. This RT-qPCR technique associates two different RT-qPCR: one based on the TaqMan probe technology (pan-RABV RT-qPCR) and

the other using the SyBR Green detection (pan-lyssa RT-qPCR). In addition, the detection of an exogenous internal control (eGFP RNA) directly spiked during the extraction process is done by a specific TaqMan probe-based RT-qPCR (eGFP RT-qPCR).

Note 3: Careful on site validation of the molecular techniques selected for detection of viral RNA is important, in particular, to verify that primers, and probes for real-time RT-PCR, are adapted for detection of the strains circulating in the region of interest [116].

1. Dilute RNA sample to 1:10 in nuclease free water. Each RNA sample is tested in duplicate, using a 96-well reaction plate or other formats. Positive and negative controls should be used for each assay and tested in duplicate.
2. Prepare the master mix reaction solution for the three different RT-qPCR assays according to Table 17, and with the primers/probes indicated in Table 18.
3. Add 5 µL of diluted RNA samples and 15 µL of master mix to each of the three different assays. The pan-RABV RT-qPCR assay and the eGFP RT-qPCR assay can cycling in the same plate.
4. Run the different assays following the thermal cycling conditions indicated in Table 19. If only one PCR thermal cycler is available, start with the pan-RABV RT-qPCR and keep the plate for the pan-lyssa RT-qPCR at 4°C until the end of the pan-RABV RT-qPCR.
5. Analyze the results obtained with the three assays according to **Table 20**.

11.4.4 Genotyping after RNA extraction from the RIDT device

Reverse transcription RT [125; 472]

1. Prepare a master mix with 6 µL of RNA, 2 µL of pd(N)6 random primers (200 µg/µL) and 2 µL of nuclease-free water for a final volume of 10 µL.
2. Incubate at 65°C for 10 min in a heat-block and then store on ice.
3. Prepare a master mix with 6 µL of 5X First-Strand Buffer, 2 µL of 0.1 M dithiothreitol (DTT), 1 µL (200 U) of Superscript II reverse transcriptase, 2 µL (80 U) of RNasin, 2 µL of dNTP mix (10 µM) and complete with nuclease-free water to obtain a final volume of 20 µL for each sample.
4. Add the master mix (20 µL) to the sample (10 µL) (final volume of 30 µL) and incubate at 42°C for 90 min in a heat-block.
5. Proceed to the next step with PCR amplification or store the cDNA at -20°C.

Conventional PCR [125; 185; 472]

Note: Different techniques of conventional PCR are available for genotyping. Two are presented, both hemi-nested PCR, targeting a part of the nucleoprotein or a part of the viral protein of the lyssavirus. The protocol is the same for each of these assays, except for the primers and cycling conditions. Positive (positive RNA) and negative (negative cDNA and/or nuclease-free water) controls should be included in each series and each round of PCR.

1. Prepare for each sample in a 0.2 mL microtube a master mix reaction solution for the first PCR step. This mix contains 5 µL of 10x NH4 Reaction Buffer, 2.5 µL of $MgCl_2$ Solution (50 mM), 1 µL of dNTP Mix (10 µM), 1 µL of each primer (10 µM), 0.2 µL (1 U) of Biotaq DNA polymerase and 37.3 µL of nuclease-free water (final volume of 48 µL). The primers are indicated in Table 21.
2. Add 2 µL of cDNA in every tube and cycle on a separate conventional PCR thermal cycler for each assay, according to Table 22.
3. Prepare a second master mix reaction solution identical to the previous one with using the appropriate primers (Table 21) for the hemi-nested PCR reaction.
4. Add 2 µL of the first round PCR product and cycle on a conventional PCR thermal cycler using the cycling parameters indicated in Table 22
5. Visualize the different PCR products (first and second round PCR) after loading them on a 1% agarose gel (100 mL of Tris-acetate EDTA buffer 1x – TAE 1x) with ethidium bromide (final concentration around 0.01%) and run the gel during 30 min at 120 V. A positive PCR result is observed in the form of a bright band of the expected size (Table 21).

Sanger sequencing

Perform a Sanger sequencing of the amplicons obtained with the pan-lyssavirus hemi-nested PCR and complete the genotyping analysis.

11.5 Representative results:

As with any diagnostic method, sample collection is of paramount importance for reliability of the results, especially when performed in field settings. The collection process needs to be as simple as possible to guarantee collection of high quality samples. The collection of a brain biopsy (mostly brainstem with medulla oblongata) via the foramen magnum route for postmortem diagnosis of animal rabies fulfills this requirement, as indicated in Figure 24A-D [469].

After collection, the brain sample is submitted to the modified protocol of the RIDT, summarized in Figure 28. As indicated in the Protocol section, the major adaptation from the manufacturer

provided protocol is omission of the dilution step in PBS, which simplifies the procedure and necessary consumables/reagents, thus all included in the kit (Figure 26).

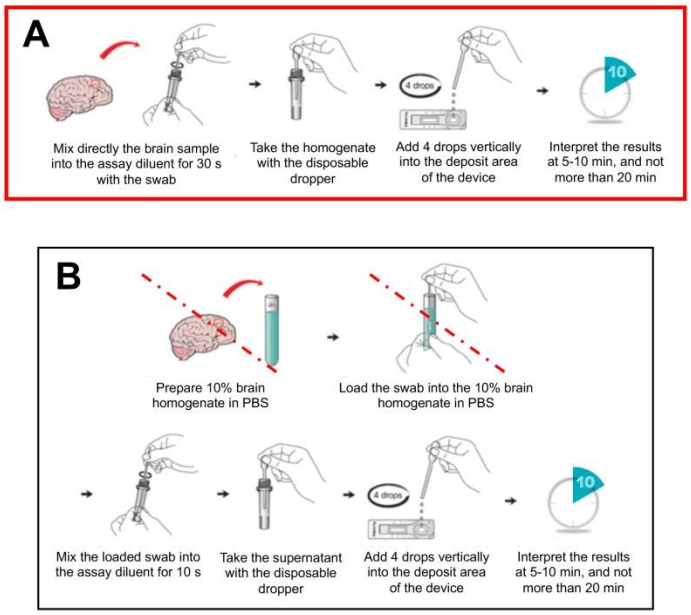


Figure 28: Schematic representation of RIDT protocol, adapted from manufacturer instructions. A. Modified version of the protocol, with deletion of the dilution step recommended by the manufacturer. B. Initial protocol recommended by manufacturer, with a pre 1:10 dilution step in PBS of the brain samples. The steps deleted in the modified version of the protocol (presented in **Figure 28**A) are indicated with a red line

This modified protocol was implemented and evaluated in five different laboratories, including one WHO collaborative center on rabies (Lab 1, France), one FAO reference center for rabies (Lab 5, Italy) and three reference laboratories located in enzootic African countries, Chad (Lab 2), Ivory Coast (Lab 3) and Mali (Lab 4). In Chad, an evaluation of the RIDT was done in both laboratory and field settings.

Compared to the reference technique DFAT, sensitivity and specificity of the RDIT were high for all laboratories, with 96% to 100% and 93.7% to 100%, respectively (Table 23). The lowest sensitivity and specificity of the RDIT was obtained for Lab 1 (France) during the laboratory validation step. Based on the cumulative number of tested samples (n=162) (Appendix 6: Supplementary information for chapter 11**,** Table 35), the overall sensitivity and specificity compared to DFAT were 98.2% and 95.8%, respectively (Table 23). Representative results of RIDT tests performed in Lab 1 (France) and Lab 2 (Chad) are presented in Figure 29A and Figure 29B, respectively (from [46]).

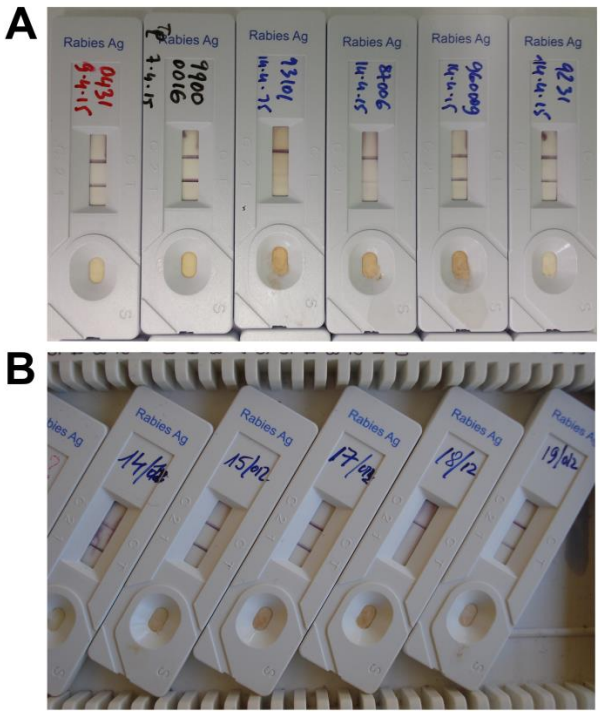


Figure 29 : Examples of RIDT results (from [46]).
A. Results obtained by Lab 1 (France) with six positive results including the two middle devices (samples 93101 and 87006) demonstrating only a weak test line in the results window. B. Results obtained by Lab 2 (Chad) with one negative result (sample 18/12) and four positive results.

The RIDT test is suitable to detect lyssavirus in brain biopsies from infected animals, where the level of lyssavirus antigens is important. However, the test limit of detection remains high when testing titrated virus suspension (Table 24) (Figure 8).

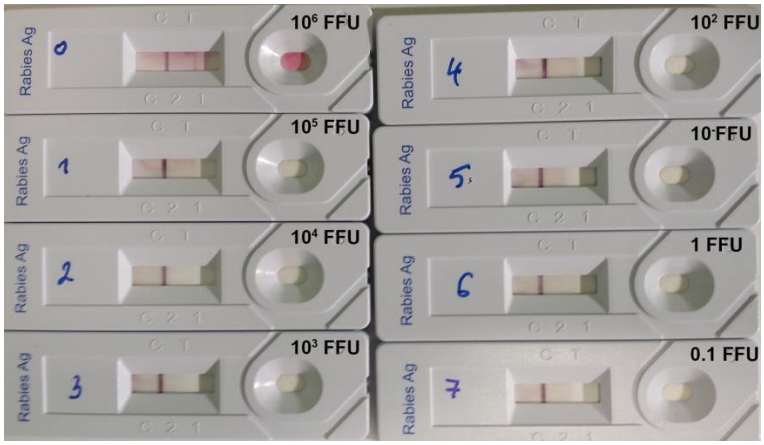


Figure 30 Example of determination of the limit of detection of RIDT (from [46]).
A serial 10:1 dilution of a titrated rabies virus of the strain 9704ARG was used. The quantity of virus deposited on each device is indicated in FFU (fluorescent focus-forming units).

Table 25 (from [46]) shows an example of results obtained after RNA detection by the dual combined pan-lyssavirus RT-qPCR targeting the viral polymerase of lyssavirus. A panel of 51 positive RIDT tests performed in laboratory conditions (Lab 1, n=32) or in Chad (Lab 2, n=19) and then shipped at ambient temperature to Lab 1, was tested. Positive detection was obtained for 18 (94.7%), 26 (81.2%) and 44 (86.3%) samples from Lab 1, Lab 2 and the two combined, respectively. In addition, genotyping was performed for 14 of these samples (10 from Lab 1 and 4 from Lab 2) using the hemi-nested PCR targeting the partial nucleoprotein gene and was successful for 13 of them (93%) (from [46]).

11.6 Discussion:

The RIDT is a simple, rapid and low-cost method for postmortem rabies diagnosis and a promising field alternative to laboratory testing. The application of such a test, especially for decentralized areas of low- and middle-income countries, would improve understanding of rabies virus prevalence and transmission on a local and potentially national scale. When combined with the rapid brain sample collection method (without full necropsy), a great advantage is that the test can be entirely performed in the field setting, away from laboratory facilities. Brain samples collected via the foramen magnum can be used for testing, thus it is not required to completely open the animal skull. The test is simple to perform and interpret and is particularly suitable for field surveillance activities [46]. Other advantages of the RIDT over the DFA or DRIT are no need for positive and negative controls and kit storage at room temperature. In addition, the modified protocol, where

the dilution step (1:10) into PBS is omitted, does not require extra reagents to perform the test and further simplifies the procedure under field conditions.

A key point is the quality of the brain samples. Samples should be collected and tested as soon as possible after death of the suspected animal, or kept at cool temperature before testing, to avoid degradation. Although no data are yet available regarding the loss of sensitivity of RIDT over time for brain samples, we hypothesize that it is similar compared to the DFAT test [474]. However, time between the death of the animal and performing the test can be reduced, as the test can be done quickly and directly in the field. So there is in general a lower risk of decomposed samples.

Another critical step within the protocol is the sample suspension migration. The migration should start directly after deposit of the sample (1-5 min). High viscosity of the suspension could therefore negatively influence the migration. Gently scratching the bottom of the device deposit site with the dropper and adding 1-2 more drops often solves this problem, and the migration begins immediately after.

Most of the RIDT tests performed in African laboratories (Chad, Ivory Coast and Mali) were performed at ambient temperature which can exceed 30°C, whereas the range of temperature for storage and use recommended by the manufacturer is 15°C - 30°C. Although we did not identify any impact of high temperature on RIDT test performance, it is necessary to evaluate it more carefully. Similarly, the impact of high temperature during storage and transportation of the device after use for viral RNA detection and genotyping needs additional evaluation. The sensitivity of the viral RNA detection by RT-qPCR from the RIDT strip can be affected by the quality of the brain sample initially used in the test, but also by the condition of storage of the RIDT tests after use. For example, the sensitivity of the RNA detection was higher when used RIDT tests were stored under controlled laboratory conditions (94.7%) compared to under field conditions (e.g. Chad) (81.2%) [46]. These conditions might also affect the integrity (especially the length) of RNA fixed on the strip, possibly explaining the moderate sensitivity for genotyping based on longer PCR amplicons (e.g. > 500 nucleotides) [46]. The sensitivity of RT-qPCR performed on the test strip was lower than that obtained using FTA Whatman cards (80.6%) [46]. Similar to other molecular techniques, the viral load can also impact the success of genotyping based on RDIT strips, with potential negative results for samples with low viral load [46].

The test is not currently recommended by WHO and OIE for routine diagnosis and disease surveillance, and a result cannot be used on its own to guide PEP decision making. Further test validation is still needed. However, accurate quick rabies diagnosis is a crucial element of well-

functioning continuous rabies surveillance systems and is instrumental to increase political commitment, which is eminently important for successful sustainable rabies control. RIDT tests offer new rabies diagnostic opportunities in this context and are a useful tool to expand animal rabies surveillance in the field in low or middle income enzootic areas.

11.7 Acknowledgments:

This work was supported through the Global Alliance for Vaccines and Immunisation (GAVI), the Wolfermann Nägeli Foundation, the Swiss African Research Cooperation (SARECO), the SWF Stiftung für wissenschaftliche Forschung, the Freiwillige Akademische Gesellschaft (FAG) Basel, the Bilateral Science and Technology Cooperation Programme of Switzerland with Asia and the Novartis Foundation for biomedical research.

We thank especially the dog owners, the veterinary personnel and the laboratory staff for their great commitment. We also want to acknowledge Lisa Crump for the language editing.

11.8 Tables

Table 17: Description of the master mix reaction solution for the three different RT-qPCR assays (pan-RABV RT-qPCR, pan-lyssa RT-qPCR and eGFP RT-qPCR).

Master mix reaction solution for pan-RABV RT-qPCR assay	
Reagent	**µL/Reaction**
2X Reaction Mix (a buffer containing 0.4 mM of each dNTP and 6 mM MgSO4)	**10**
Nuclease free water	**1.5**
Taq3long (Forward) [10 µM]	**1**
Taq17revlong (Reverse) [10 µM]	**1**
RABV4 [10 µM]	**0.3**
RABV5 [10 µM]	**0.3**
$MgSO_4$ [50-mM] (provided in the kit)	**0.25**
ROX Reference Dye (25 µM) (provided in the kit)	**0.05**
RNasin (40U/µL) (Promega)	**0.2**
SuperScript® III RT/Platinum® Taq Mix	**0.4**
Total per reaction	**15**

Master mix reaction solution for eGFP RT-qPCR assay	
Reagent	**µL/Reaction**
2X Reaction Mix (a buffer containing 0.4 mM of each dNTP and 6 mM MgSO4)	**10**
Nuclease free water	**2.8**
EGFP1F (Forward) [10 µM]	**0.5**
EGFP2R (Reverse) [10 µM]	**0.5**
eGFP probe [10 µM]	**0.3**
$MgSO_4$ [50-mM] (provided in the kit)	**0.25**
ROX Reference Dye (25 µM) (provided in the kit)	**0.05**
RNasin (40U/µL) (Promega)	**0.2**
SuperScript® III RT/Platinum® Taq Mix	**0.4**
Total per reaction	**15**

Master mix reaction solution for pan-lyssa RT-qPCR assay	
Reagent	**µL/Reaction**
2X SYBR® Green Reaction Mix	**10**
Nuclease free water	**2.1**
Taq5long (Forward) [10 µM]	**1**
Taq16revlong (Reverse) [10 µM]	**1**
$MgSO_4$ [50-mM] (provided in the kit)	**0.25**
ROX Reference Dye (25 µM)	**0.05**
RNasin (40U/µL) (Promega)	**0.2**
SuperScript® III RT/Platinum® Taq Mix	**0.4**
Total per reaction	**15**

Table 18: Description of the primers/probes for the three different RT-qPCR assays (pan-RABV RT-qPCR, pan-lyssa RT-qPCR and eGFP RT-qPCR). [a] According to the Pasteur virus (PV) RABV genome sequence (GenBank accession number M13215). [b] According to the cloning vector pEGFP-1 sequence (GenBank accession number U55761).

RT-qPCR assay	Name	Type	Length	Sequence (5'-3')	Sense	Position
pan-RABV RT-qPCR assay	Taq3long	Primer	22	ATG AGA AGT GGA AYA AYC ATC A	S	7273-7294[a]
	Taq17revlong	Primer	25	GAT CTG TCT GAA TAA TAG AYC CAR G	AS	7390-7414[a]
	RABV4	Probe (FAM/TAMRA)	29	AAC ACY TGA TCB AGK ACA GAR AAY ACA TC	AS	7314-7342[a]
	RABV5	Probe (FAM/TAMRA)	32	AGR GTG TTT TCY AGR ACW CAY GAG TTT TTY CA	S	7353-7384[a]
Pan-lyssa RT-qPCR assay	Taq5long	Primer	23	TAT GAG AAA TGG AAC AAY CAY CA	S	7272-7294[a]
	Taq16revlong	Primer	25	GAT TTT TGA AAG AAC TCA TGK GTY C	AS	7366-7390[a]
eGFP RT-qPCR assay	EGFP1F	Primer	20	GAC CAC TAC CAG CAG AAC AC	S	637-656[b]
	EGFP2R	Primer	19	GAA CTC CAG CAG GAC CAT G	AS	768-750[b]
	EGFP	Probe (FAM/TAMRA)	22	AGC ACC CAG TCC GCC CTG AGC A	S	703-724[b]

Table 19: Description of the thermal cycling conditions for the three different RT-qPCR assays (pan-RABV RT-qPCR, pan-lyssa RT-qPCR and eGFP RT-qPCR).

Thermal cycling conditions for pan-RABV RT-qPCR and eGFP RT-qPCR assays				
Step	**Cycle**	**Temp**	**Time**	**Data Collection**
Reverse Transcription	1	45ºC	15 min	
RT inactivation/initial denaturation	1	95ºC	3 min	
Amplification	40	95ºC	15 sec	
		61ºC	1 min	End point

Thermal cycling conditions for pan-lyssa RT-qPCR assay				
Step	**Cycle**	**Temp**	**Time**	**Data Collection**
Reverse Transcription	1	45ºC	15 min	
RT inactivation/initial denaturation	1	95ºC	3 min	
Amplification	40	95ºC	15 sec	
		55ºC	1 min	End point
Dissociation curve	1	95°C	15 sec	Increase 0.1° C/s, 55–95°C
		55ºC	1 min	
		95°C	15 sec	
		55ºC	15 sec	

Table 20: Overall interpretation of the dual combined pan-lyssavirus RT-qPCR assay.

Overall interpretation after dual combined pan-lyssavirus RT-qPCR assay			
Assay	**Analysis**	**Results**	**Interpretation**
eGFP RT-qPCR	Cq in the interval of acceptance	Extraction validated	Analysis of other assays can be done
	Cq out of the interval of acceptance	Extraction not validated	Retest the sample (repeat the run or/and the extraction), request another sample if necessary
pan-RABV RT-qPCR	Cq <38	Positive	Positive detection of viral RNA
	Cq ≥38	Negative	Analysis the pan-lyssa RT-qPCR assay
pan-lyssa RT-qPCR	Melting curve considered as positive	Positive	Positive detection of viral RNA
	Melting curve considered as negative	Negative	Absence of detection of viral RNA

Table 21: Description of the primers used for the conventional hemi-nested PCR.

Hemi-nested conventional PCR assay	PCR round	Name	Length	Sequence (5'-3')	Sense	Position[a]	Amplicon size (bp)
Hemi-nested PCR targeting the polymerase gene	1st round	PVO5m	20	ATG ACA GAC AAY YTG AAC AA	S	7170-7189	320
		PVO9	19	TGA CCA TTC CAR CAR GTN G	AS	7471-7489	
	2nd round	PVO5m	20	ATGA CAG ACA AYY TGA ACA A	S	7170-7189	250
		PVO8	22	GGT CTG ATC TRT CWG ARY AAT A	AS	7398-7419	
Hemi-nested PCR targeting the nucleoprotein gene	1st round	N127	20	ATG TAA CAC CTC TAC AAT GG	S	55-74	1532
		N8m	19	CAG TCT CYT CNG CCA TCT C	AS	1568-1586	
	2nd round	N127	20	ATG TAA CAC CTC TAC AAT GG	S	55-74	845
		N829	19	GCC CTG GTT CGA ACA TTC T	AS	881-899	

Table 22: Description of the thermal cycling conditions for the conventional hemi-nested PCR.

	Thermal cycling conditions for hemi-nested PCR targeting the polymerase gene			
	Step	**Cycle**	**Temp**	**Time**
First and second rounds	Initial denaturation	1	94ºC	3 min
	Denaturation	35	94ºC	30 sec
	Hybridation		56ºC	45 sec
	Elongation		72ºC	40 sec
	Final elongation	1	72ºC	3 min

	Thermal cycling conditions for hemi-nested PCR targeting the nucleoprotein gene			
	Step	**Cycle**	**Temp**	**Time**
First round	Initial denaturation	1	94ºC	3 min
	Denaturation	35	94ºC	30 sec
	Hybridation		56ºC	30 sec
	Elongation		72ºC	45 sec
	Final elongation	1	72ºC	3 min
Second round	Initial denaturation	1	94ºC	3 min
	Denaturation	35	94ºC	30 sec
	Hybridation		58ºC	30 sec
	Elongation		72ºC	30 sec
	Final elongation	1	72ºC	3 min

Table 23: Determination of the intrinsic parameters (sensitivity, specificity) of the RIDT test compared to the reference DFAT method, based on the analysis of a total of 162 samples and with the participation of 5 different laboratories.

Lab	Country	Period of evaluation	Nb of samples	DFAT results		RIDT results		Sensitivity	Specificity
				Pos	Neg	Pos	Neg		
Lab 1	**France**	2015	82	50	32	50	32	96%	93.7%
Lab 2	**Chad**	2012-2015	44	33	11	33	11	100%	100%
Lab 3	**Ivory Coast**	2017	10	8	2	8	2	100%	100%
Lab 4	**Mali**	2017	18	15	3	15	3	100%	100%
Lab 6	**Italy**	2016	8	8	0	8	0	100%	-
All		**2015-2017**	**162**	**114**	**48**	**114**	**48**	**98.2%**	**95.8%**

Table 24: Limit of detection of the RIDT using 8 different titrated rabies virus suspensions (from [46]).

[a] CVS: Challenge virus strain, SAD: Street Alabama Dufferin, PV: Pasteur virus. [b] Number of fluorescent focus-forming units (FFU) per mL. [c] Number of fluorescent focus-forming units (FFU) deposited on the strip.

Virus strain[a]	Original host	Location	Initial concentration (FFU/mL)[b]	Limit of detection (FFU/mL)[c]
9147FRA	Red fox	France	3.1×10^7	10^6
CVS	Lab isolate	-	1.6×10^7	10^6
8743THA	Human	Thailand	8.1×10^7	$> 8.1 \times 10^6$
9508CZK (SAD)	Lab isolate	-	5.4×10^8	10^7
PV	Lab isolate	-	4.3×10^7	10^6
9001FRA	Dog	French Guiana	2.4×10^6	$> 2.4 \times 10^5$
9704ARG	Bat	Argentina	9.5×10^7	10^5
04030PHI	Human	Philippines	2.5×10^7	10^5

Table 25: Detection of viral RNA with RT-qPCR on Anigen test strip used in laboratory conditions (Lab 1), in field conditions and shipped at ambient temperature (Lab 2) or combined (from [46]).

		RIDT performed in Lab 1			RIDT performed in Lab 2			Combined		
		Positive	Negative	Total	Positive	Negative	Total	Positive	Negative	Total
Viral RNA detection	Positive	18	1	19	26	0	32	44	7	51
	Negative	0	0	0	0	0	3	0	3	3
	Total	18	1	19	26	0	35	44	10	54

12 Discussion

12.1 Overall significance

This PhD thesis provides new insights into the epidemiology and transmission of rabies in two sub-Saharan African countries through applied and innovative research. We established new research partnerships between the Swiss TPH and INRB and the INRB and IP-Paris. The PhD work applied a One Health approach to foster intersectoral collaboration and combined desk-based work (review and statistical analyses) with extensive field (cross-sectional household surveys, longitudinal surveillance study and application of novel GCS) and laboratory work (on-site training for DFA, DRIT and Anigen) in a challenging research environment. The findings are directly relevant for national and regional disease control efforts and are discussed and translated into recommendations in detail in the following section.

12.2 Objectives and research output

12.2.1 Review on the current rabies situation in West and Central Africa

Findings from the review highlighted the need for a regional approach to rabies control, as the disease spreads irrespective of national borders. Progress on elimination efforts within a single country is constantly threatened by potential reintroduction of rabies from neighboring countries. Most countries in West and Central Africa are still far from meeting the global goal of zero human rabies deaths by 2030 due to lacking government commitment and financial constraints. Nonetheless, elimination efforts received a huge boost in 2018 when GAVI, the Vaccine Alliance, agreed to invest in human rabies vaccines for PEP, beginning in 2021 [294]. A shift from IM to the new abridged WHO recommended ID vaccination scheme, as applied in this research (Chapter 6), should further increase PEP availability and reduce costs for bite victims. This investment allows countries to redistribute resources directed at rabies control and catalyze MDV. Making dog vaccination mandatory by law might increase routine vaccination of dogs and sustain vaccination coverage at a sufficient level. Eligible governments can procure dog vaccine through the OIE Rabies Vaccine Bank at a reduced cost. The tools for rabies elimination are available and proof of concept studies show that MDV campaigns in sub-Sahara Africa are feasible and reduce human rabies mortality [298; 299]. Economic benefits of MDV may take several years to accrue [130], so a key challenge will be to secure long-term funding for sustained rabies control. DIBs are suggested as an innovative funding mechanism [106; 176; 301]. However, until the robustness and risks of such funding schemes are evaluated and more institutional donors engage, the benefits of rabies control for the simultaneous control of other zoonotic diseases, such as echinococcosis, leishmaniasis or snakebites, should be highlighted. This is an incentive for governments to allocate

more resources to the animal health sector. A better understanding of the societal role and value of dogs in different cultural settings in West and Central Africa is needed for targeted promotion of responsible dog ownership to positively influence private vaccination uptake [475].

Future research:

Future applied research is needed on how access to available tools and their implementation in the community can be improved (better program-delivery) and the incremental profitability of integrating rabies control with control of other zoonotic diseases.

Recommendations:

- Legislation change to require mandatory dog vaccination
- Shift from IM to ID administration of human rabies vaccine and utilize the new abridged WHO-recommended vaccination scheme
- Highlight benefits of rabies control for simultaneous control of other zoonotic diseases and use as advocacy tool to strengthen the animal health system

12.2.2 Systematic data collection on the incidence of rabies in humans and animals and the incidence animal bites on health facility-level in a province of the DRC

This is the first study to assess rabies burden in the DRC. We used a One Health approach to strengthen rabies surveillance in the Kongo Central province and gather reliable epidemiological data on human and animal rabies incidence and incidence of animal bites. The data generated through this study provide a sound evidence base to reflect the veterinary and public health implications of rabies and advocate for greater political commitment and policy support in rabies prevention and control. The first steps for advocacy were taken: on the World Rabies Day 2018, we were invited by the FAO to present our results to a panel of 30 experts from the MINAGRI, MPH, the Ministry of Environment and Sustainable Development and partners of the GHSA (www.fao.org/africa/news/detail-news/fr/c/1164818/). The meeting resulted in adoption of a procedure for the integrated management of bite cases in the DRC, with the IBCM applied in our study serving as the model. In the next step, results will be further disseminated to important stakeholders at the International Congress on Parasitic and Infectious Diseases (CIPIP), which takes place from 14-16 November 2019 in Kinshasa.

Laboratory diagnosis is an essential pillar for effective rabies surveillance [17; 454]. Diagnostic capacity at the rabies laboratory at INRB was improved through provision of reagents and equipment, on-site refresher training on the DFA and DRIT and introduction of the Anigen test (see also 12.3). In addition, we trained VFPs on the sampling of brain material from suspected rabid animals

and use of the Anigen test. A North-South research partnership was established with the Institute Pasteur in Paris, for analysis of human rabies samples collected during the study and phylogenetic analysis on animal rabies samples. Efforts to install PCR for rabies diagnosis at INRB are currently underway.

The ethics committee of the MPH agreed to shift from IM to ID administration of human rabies vaccine following the new abridged WHO-recommended vaccination scheme. The DRC, a GAVI-eligible country, should adopt the new ID scheme nationwide, which would increase PEP availability and reduce costs for bite victims.

Although we faced several challenges implementing rabies surveillance in the Kongo Central province (as outlined in Chapter 6), we successfully put One Health into practice. Through investigations of the biting animal and enhanced communication between the human and animal health sectors, we were able to assess the rabies exposure status of most bite victims captured by the surveillance system. This shows that intersectoral collaboration in the DRC is possible through targeted training of veterinary and medical personnel. This is an important finding, because only with effective intersectoral communication will the benefits of dog vaccination campaigns be translated to financial savings for the human health sector [130]. It should be noted that willingness to collaborate between VFPs and MFPs varied throughout the study area, highlighting that achieving good One Health practice also depends on interpersonal relationships and individual dedication (Chapter 6).

The collaboration established between the animal and human health sectors offers the ideal starting point to address other zoonotic diseases. For example, the same infrastructures and resources are required for surveillance of another NTD – snakebites. As is the case with rabies, snakebites mostly affect poor, rural communities, with children and males most at risk. We did not register any snakebites during our studies, but this is because rabies surveillance was primarily conducted in urban sites (see Limitations).

Limitations

The DRC is a vast country with a poor road network. For perspective, the DRC is two-thirds the size of Western Europe with less paved roads than Luxembourg, which is 1000 times smaller than the DRC. Reaching populations in remote areas, especially during the rainy season, is extremely difficult and associated with high costs. Collecting rabies samples in the most remote parts of the Kongo Central province could easily take up to one week. Due to the limited budget of this project, rabies surveillance was mainly conducted in and around urban sites where VFPs and MFPs were located, so the data collected likely reflects only the urban rabies situation.

Future research

This research focused primarily on measurements of mortality to estimate rabies burden. Future studies should also address the economic impacts of a One Health approach on PEP use and a shift from IM to ID administration of human rabies vaccine. This would greatly support rabies advocacy in the DRC.

Recommendations:

- Expand rabies surveillance in the Kongo Central to rural areas to allow for more accurate extrapolation of rabies data to the national level
- Include snakebites in surveillance
- Upload rabies data to the Rabies Epidemiological Bulletin on a regular basis
- Shift from IM to ID administration of human rabies vaccine
- Prepare a short term rabies action plan with clearly defined tasks, realistic timeline and budget for each activity using the SARE assessment and the PWARE tool (refer to Chapter 5 for a description and the aims of these tools)
- Institute legal mandate for dog vaccination

12.2.3 Conduct a cross-sectional household survey to estimate the owned dog population size, community-level dog bite incidence and assess community knowledge and practices regarding rabies in the DRC

Results obtained through the cross-sectional household survey are directly relevant to veterinary and public health authorities for planning of future MDV and public sensitization campaigns. Veterinary officials grossly underestimated the owned dog population, and a large proportion of dogs were unvaccinated. Only half of the study participants had heard of rabies and of the remainder only 50% knew about dog vaccination and 3% knew about PEP for prevention. Lack of awareness among the community affects all aspects of rabies control: surveillance (i.e., reporting suspected rabid animals and bite injuries), participation in dog vaccination and human treatment (i.e., seeking PEP after a bite). The low level of community awareness had an impact on the surveillance activities conducted under 12.2.2. Less than 50% of animals killed following a bite incident were submitted to the veterinary service for laboratory testing. Likewise, bite incidence found at the health-facility level was ten times smaller than the community-level bite incidence, indicating that only a small proportion of the population seeks treatment after a bite. However, our surveillance of bite victims was limited to general referral hospitals and the veterinary services, so we certainly missed bite victims who presented to health centers and pharmacies. Future studies could include tracing non-registered bite victims [316] and extending surveillance of bite injuries to include basic health

care providers (health centers and pharmacies) to better define the proportion of bite victims seeking care at a health facility after a bite and further improve estimates of human rabies deaths (Chapter 6).

Given our findings, public sensitization is warranted to encourage bite victims to seek treatment after a bite, report rabid suspected animals and highlight the need for dog vaccination. The GARC Education Platform hosts targeted educational courses and awareness materials. By working together with anthropologists and ethnologists, available material could be adapted to the socio-cultural context of the DRC.

Limitations:

The household survey was conducted in an urban setting. Dog/human ratios in rural areas are generally higher [327]; therefore, expanding data collection to rural areas is necessary to allow extrapolation to the entire DRC. In addition, we did not investigate the unowned dog population, although their number is likely less than 2% of the total dog population [192]. A capture-mark-recapture study would better estimate the unowned dog population [55; 344].

Recommendations:

- Reduce the private cost for dog vaccination to support routine vaccination of dogs
- Institute awareness-raising campaign targeting schools and/or churches with continued dissemination of information through radio, posters and leaflets
- Hold a participatory stakeholder meeting involving communities and local authorities to disseminate the findings from this study and plan a pilot small-scale vaccination campaign
- Conduct a pilot small-scale vaccination campaign and assess intervention effectiveness using quantitative and qualitative epidemiological methods to optimize future large-scale vaccination campaigns [88]

12.3 Contribute to the validation of a rapid immunochromatographic rabies diagnostic test

The validation of a RIDT (Anigen Rapid Rabies Ag Test kit), directly applied in the field, allowed VFPs to be more engaged in rabies surveillance. All field test results were confirmed by both the DRIT and DFA. In contrast to studies that performed validation of the Anigen test in other sub-Saharan settings (Chapter 11), the test yielded a high number of false negatives (i.e., the test indicated that the animal was not rabid when it actually was rabid) resulting in a low sensitivity of 83.8%. Specificity (i.e., a low number of false positives and a high number of true negatives) was high (99.1%), with a small 95% confidence interval (98.1%-99.6%). We hope phylogenetic analy-

sis, ongoing at IP-Paris, will provide more insight into the genetic variability and phylogenetic relatedness of the viral strains collected in the DRC to allow for a deeper interpretation of obtained results. We anticipate publishing these findings in a future separate publication, so results from the DRC were not included in the Chapter 11 publication.

Rabies is a fatal disease. If a new diagnostic test is intended to rule out the disease with high certainty, it needs to have high diagnostic sensitivity (a negative result in a test with high sensitivity is reliable because the test gives a low number of false negatives and a high number of true positives). Basing PEP recommendations on a test with a low sensitivity has serious implications: wrongly assuming that the animal is not rabid and, therefore, not recommending PEP can result in unnecessary human rabies deaths. Therefore, our results indicate that the Anigen test is not suitable to confirm the absence of rabies. However, like in other validation studies [122; 460; 463; 465; 467; 468] and Chapter 11, Anigen test specificity was very high which could be beneficial in rabies-endemic settings where it is expected that a high proportion of biting animals tested for rabies will actually be positive (71% in this study, see Chapter 6). In a test with a high specificity, a positive result is reliable in confirming the presence of a disease due to the low number of false positives and the high number of true negatives. With regards to rabies, this means if a positive test result is obtained in the field with the Anigen, the test result is reliable and the bite victim should start PEP immediately and no secondary (more expensive) confirmatory test is needed. Only a fraction of patients will be subject to unnecessary costs for PEP (i.e., pay for PEP when it is not needed, because the test was false positive). This is also favorable in view of the GAVI decision to invest in human rabies vaccine, which should reduce PEP costs for bite victims. Therefore, in rabies-endemic countries, such as the DRC, where shipment of samples to central laboratories is difficult and associated with high costs, the Anigen test is a promising tool for decentralized rabies surveillance. During progress towards rabies elimination, however, the number of rabies-negative animals will increase, so a field test with a high sensitivity would be required. Based on our results, only positive test results are reliable, while negative results always need to be further confirmed using a test with high diagnostic sensitivity (e.g., DFA, DRIT or PCR).

Future research:

Future studies should address the economic aspects of RIDTs in rabies-endemic settings focusing on the following questions: how many samples need to be double tested because of negative results and what are the associated costs (shipment costs and costs for the secondary confirmatory test)? How many patients are subject to unnecessary costs due to a false positive result?

Recommendation:

- Further validation of the Anigen test (and alternative RIDTs), according to international standards including circulating RABVs variants and other lyssaviruses

12.4 Data collection and analysis on the movement and contacts of dogs in N'Djamena, Chad

We applied innovative research to investigate the contact network and roaming behavior of free-roaming dogs in N'Djamena through tracking the location and contacts of dogs using newly developed GCS. Using the collected empirical data, we extrapolated the contact network to adequate size to simulate rabies outbreaks and investigate the effectiveness of different vaccination coverages. Using this approach, we demonstrated that the WHO-recommended vaccination coverage of 70% prevents major but not minor rabies outbreaks and that highly connected dogs play a critical role in transmission (Chapter 8). Minor outbreaks may be explained by import of rabid dogs into N'Djamena from outside the city. Phylodynamic analysis of RABV strains from N'Djamena further corroborated these findings [325]. Targeted vaccination of highly connected dogs could substantially increase the effectiveness of vaccination campaigns. We postulated that an intrinsic (e.g., sex or neutering status) or extrinsic (e.g., religion of the owner or diet) factor was associated with highly connected dogs, through which these dogs could easily be identified and targeted for vaccination. Preliminary analysis suggests that, unfortunately, there is no such association (Chapter 9). However, the daily number of contact partners was more than three times higher during the dry season as compared to the rainy season. A next step in the analysis is to develop a contact network based on the data collected during the rainy season to investigate if the probability of rabies transmission is lower as compared to the dry season.

Future research:

Our results suggest that humans may play an important role in the transmission of rabies. Future studies should investigate how human movement influences the spread of rabies. For example, N'Djamena provides an ideal setting to investigate the frequency of importing dogs into the city. There are only four main roads entering the city, and all vehicles pass through a toll checkpoint before entering the city. Together with local authorities, these toll checkpoints could help in surveillance of import of dogs into the city.

Recommendations:

- Continuation of annual MDV along with vaccination of dogs entering the city via toll checkpoints subsidized through the Chadian government

12.5 Conclusion

Both innovation and applied research are needed to reach the global goal of zero human rabies death by 2030, both of which were applied in this PhD thesis. We contributed vital information for rabies control in the sub-Saharan African region. Findings on the burden of rabies in humans and animals, dog ecology and community awareness in the DRC are directly relevant for veterinary and public health authorities and support planning of future rabies interventions. Applying new technologies to disease modeling helped generate new ideas on how dog vaccination could become more effective in future and be useful to set new research targets.

13 References

[1] World Health Organization (2018). – Global Health Estimates 2016: Deaths by Cause, Age, Sex, by Country and by Region, 2000-2016. .

[2] Taylor L.H., Latham S.M. & Woolhouse M.E. (2001). – Risk factors for human disease emergence. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences,* **356** (1411), 983-989. doi: 10.1098/rstb.2001.0888.

[3] Grace D., Mutua F., Ochungo P., Kruska R., Jones K., Brierley L., Lapar L., Said M., Herrero M., Phuc D.P., Thao N.B., Akuku I. & Ogutu F. (2012). – Mapping of poverty and likely zoonoses hotspots. Zoonoses Project 4. *In* Report to Department for International Development, UK.

[4] Maudlin I., Eisler M.C. & Welburn S.C. (2009). – Neglected and endemic zoonoses. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences,* **364** (1530), 2777-2787. doi: 10.1098/rstb.2009.0067.

[5] Tarantola A. (2017). – Four Thousand Years of Concepts Relating to Rabies in Animals and Humans, Its Prevention and Its Cure. *Tropical Medicine and Infectious Disease,* **2** (2), 5.

[6] World Health Organization (2019). – Rabies Fact Sheet. WHO. Available at: https://www.who.int/news-room/fact-sheets/detail/rabies (accessed on 16 July 2019).

[7] Fooks A.R., Cliquet F., Finke S., Freuling C., Hemachudha T., Mani R.S., Müller T., Nadin-Davis S., Picard-Meyer E., Wilde H. & Banyard A.C. (2017). – Rabies. *Nature Reviews Disease Primers,* **3**, 17091. doi: 10.1038/nrdp.2017.91

https://www.nature.com/articles/nrdp201791#supplementary-information.

[8] World Health Organization (2018). – Rabies vaccines: WHO position paper – April 2018. *Weekly epidemiological record: Releve epidemiologique hebdomadaire,* **93** (16), 201-220.

[9] Hampson K., Coudeville L., Lembo T., Sambo M., Kieffer A., Attlan M., Barrat J., Blanton J.D., Briggs D.J., Cleaveland S., Costa P., Freuling C.M., Hiby E., Knopf L., Leanes F., Meslin F.X., Metlin A., Miranda M.E., Muller T., Nel L.H., Recuenco S., Rupprecht C.E., Schumacher C., Taylor L., Vigilato M.A., Zinsstag J. & Dushoff J. (2015). – Estimating the global burden of endemic canine rabies. *PLoS Negl Trop Dis,* **9** (4), e0003709. doi: 10.1371/journal.pntd.0003709.

[10] World Health Organization (2016). – Situation Report Ebola Virus Disease. Available at: apps.who.int/iris/bitstream/10665/208883/1/ebolasitrep_10Jun2016_eng.pdf?ua=1.

[11] Dodet B., Adjogoua E.V., Aguemon A.R., Baba B.A., Adda S.B., Boumandouki P., Bourhy H., Brahimi M., Briggs D., Diallo M.K., Diarra L., Diop B., Diop S.A.G., Fesriry B.,

Gosseye S., Kharmachi H., Le Roux K., Yandoko E.N., Nel L., Ngome J.M., Nzengue E., Ramahefalalao I.E.F., Ratsitorahina M., Rich H., Simpore L., Soufi A., Tejiokem M.C., Thiombiano R., Tiembre I., Traore A.K., Wateba M.I., Yahaye H. & Zaouia I. (2010). – The fight against rabies in Africa: From recognition to action. *Bull. Soc. Pathol. Exot.,* **103** (1), 51-59. doi: 10.1007/s13149-009-0034-3.

[12] Broban A., Tejiokem M.C., Tiembre I., Druelles S. & L'Azou M. (2018). – Bolstering human rabies surveillance in Africa is crucial to eliminating canine-mediated rabies. *PLoS Negl Trop Dis,* **12** (9), e0006367. doi: 10.1371/journal.pntd.0006367.

[13] Taylor L.H. & Knopf L. (2015). – Surveillance of Human Rabies by National Authorities--A Global Survey. *Zoonoses and public health,* **62** (7), 543-552. doi: 10.1111/zph.12183.

[14] Knobel D.L., Cleaveland S., Coleman P.G., Fevre E.M., Meltzer M.I., Miranda M.E., Shaw A., Zinsstag J. & Meslin F.X. (2005). – Re-evaluating the burden of rabies in Africa and Asia. *Bulletin of the World Health Organization,* **83** (5), 360-368. doi: /S0042-96862005000500012.

[15] M'ikanatha N.M., Lynfield R., Julian K.G., Van Beneden C.A. & de Valk H. (2013). – Infectious disease surveillance: a cornerstone for prevention and control. *In* Infectious disease surveillance (N.M. M'ikanatha, R. Lynfield, C.A. Van Beneden & H. de Valk, eds), John Wiley & Sons, Ltd

[16] World Health Organization (2008). – International Health Regulations (2005). 10.

[17] Banyard A.C., Horton D.L., Freuling C., Müller T. & Fooks A.R. (2013). – Control and prevention of canine rabies: The need for building laboratory-based surveillance capacity. *Antiviral Research,* **98** (3), 357-364. doi: http://dx.doi.org/10.1016/j.antiviral.2013.04.004.

[18] Franka R. & Wallace R.M. (2018). – Rabies Diagnosis and Surveillance in Animals in the Era of Rabies Elimination. *OIE Scientific and Technical Review,* **37** (2), 359-370.

[19] Kermack W.O. & McKendrick A.G. (1927). – A Contribution to the Mathematical Theory of Epidemics. *Proceedings of the Royal Society A: Mathematical, Physical and Engineering Sciences,* **115** (772), 700-721. doi: 10.1098/rspa.1927.0118.

[20] Klovdahl A.S. (1985). – Social networks and the spread of infectious diseases: the AIDS example. *Social science & medicine (1982),* **21** (11), 1203-1216.

[21] Keeling M.J. & Eames K.T.D. (2005). – Networks and epidemic models. *Journal of the Royal Society Interface,* **2** (4), 295-307. doi: 10.1098/rsif.2005.0051.

[22] Pellis L., Ball F., Bansal S., Eames K., House T., Isham V. & Trapman P. (2015). – Eight challenges for network epidemic models. *Epidemics,* **10**, 58-62. doi: 10.1016/j.epidem.2014.07.003.

[23] Nishiura H., Chowell G., Safan M. & Castillo-Chavez C. (2010). – Pros and cons of estimating the reproduction number from early epidemic growth rate of influenza A (H1N1) 2009. *Theor Biol Med Model,* **7**, 1. doi: 10.1186/1742-4682-7-1.

[24] Bondy A. & Murty U.S.R. (2008). – Graph Theory. 1 edn., Springer London.

[25] Bell D. & Atkinson J. (1999). – Centrality measures for disease transmission networks. *Soc Networks,* **21** doi: 10.1016/s0378-8733(98)00010-0.

[26] Donker T., Smieszek T., Henderson K.L., Johnson A.P., Walker A.S. & Robotham J.V. (2017). – Measuring distance through dense weighted networks: The case of hospital-associated pathogens. *PLOS Computational Biology,* **13** (8), e1005622. doi: 10.1371/journal.pcbi.1005622.

[27] Christley R., Pinchbeck G., Bowers R., Clancy D., French N., Bennett R. & Turner J. (2005). – Infection in social networks: using network analysis to identify high-risk individuals. *Am J Epidemiol,* **162** doi: 10.1093/aje/kwi308.

[28] Rohani P., Zhong X. & King A.A. (2010). – Contact network structure explains the changing epidemiology of pertussis. *Science,* **330** (6006), 982-985.

[29] Cleaveland S., Lankester F., Townsend S., Lembo T. & Hampson K. (2014). – Rabies control and elimination: a test case for One Health. *The Veterinary record,* **175** (8), 188-193. doi: 10.1136/vr.g4996.

[30] Zinsstag J., Waltner-Toews D. & Tanner M. (2015). – Theoretical Issues of One Health. *In* One Health: the theory and practice of integrated health approaches (J. Zinsstag, E. Schelling, D. Waltner-Toews, M. Whittaker & M. Tanner, eds), CABI, Wallingford.

[31] Velasco-Villa A., Escobar L.E., Sanchez A., Shi M., Streicker D.G., Gallardo-Romero N.F., Vargas-Pino F., Gutierrez-Cedillo V., Damon I. & Emerson G. (2017). – Successful strategies implemented towards the elimination of canine rabies in the Western Hemisphere. *Antiviral Res,* **143**, 1-12. doi: 10.1016/j.antiviral.2017.03.023.

[32] Lembo T., Hampson K., Kaare M.T., Ernest E., Knobel D., Kazwala R.R., Haydon D.T. & Cleaveland S. (2010). – The Feasibility of Canine Rabies Elimination in Africa: Dispelling Doubts with Data. *PLOS Neglected Tropical Diseases,* **4** (2), e626. doi: 10.1371/journal.pntd.0000626.

[33] Lapiz S.M., Miranda M.E., Garcia R.G., Daguro L.I., Paman M.D., Madrinan F.P., Rances P.A. & Briggs D.J. (2012). – Implementation of an intersectoral program to eliminate human and canine rabies: the Bohol Rabies Prevention and Elimination Project. *PLoS Negl Trop Dis,* **6** (12), e1891. doi: 10.1371/journal.pntd.0001891.

[34] Kayali U., Mindekem R., Hutton G., Ndoutamia A.G. & Zinsstag J. (2006). – Cost-description of a pilot parenteral vaccination campaign against rabies in dogs in N'Djamena, Chad. *Tropical medicine & international health : TM & IH,* **11** (7), 1058-1065. doi: 10.1111/j.1365-3156.2006.01663.x.

[35] Zinsstag J., Durr S., Penny M.A., Mindekem R., Roth F., Menendez Gonzalez S., Naissengar S. & Hattendorf J. (2009). – Transmission dynamics and economics of rabies control in dogs and humans in an African city. *Proceedings of the National Academy of Sciences of the United States of America,* **106** (35), 14996-15001. doi: 10.1073/pnas.0904740106.

[36] Mindekem R., Lechenne M.S., Naissengar K.S., Oussiguere A., Kebkiba B., Moto D.D., Alfaroukh I.O., Ouedraogo L.T., Salifou S. & Zinsstag J. (2017). – Cost Description and Comparative Cost Efficiency of Post-Exposure Prophylaxis and Canine Mass Vaccination against Rabies in N'Djamena, Chad. *Frontiers in veterinary science,* **4**, 38.

[37] Lechenne M., Mindekem R., Madjadinan S., Oussiguere A., Moto D.D., Naissengar K. & Zinsstag J. (2017). – The Importance of a Participatory and Integrated One Health Approach for Rabies Control: The Case of N'Djamena, Chad. *Trop Med Infect Dis,* **2** (3) doi: 10.3390/tropicalmed2030043.

[38] Undurraga E.A., Meltzer M.I., Tran C.H., Atkins C.Y., Etheart M.D., Millien M.F., Adrien P. & Wallace R.M. (2017). – Cost-Effectiveness Evaluation of a Novel Integrated Bite Case Management Program for the Control of Human Rabies, Haiti 2014–2015. *The American journal of tropical medicine and hygiene,* **96** (6), 1307-1317. doi: 10.4269/ajtmh.16-0785.

[39] United Nations Development Programme (2018). – Latest Human Development Index (HDI) Ranking. Available at: http://hdr.undp.org/en/2018-update (accessed on 2 August 2019).

[40] Bonfoh B., Raso G., Koné I., Dao D., Girardin O., Cissé G., Zinsstag J., Utzinger J. & Tanner M. (2011). – Research in a war zone. *Nature,* **474**, 569. doi: 10.1038/474569a.

[41] Pavignani E., Michael M., Murru M., E. Beesley M. & Hill P. (2014). – Making sense of apparent chaos: Health-care provision in six country case studies. *International Review of the Red Cross,* **95**, 41-60. doi: 10.1017/S1816383113000726.

[42] Ministère de la Santé Publique. RDC (2011). – Guide Technique pour la surveillance intégrée de la maladie et riposte : SIMR.

[43] Mandja B.M., Bompangue D., Handschumacher P., Gonzalez J.P., Salem G., Muyembe J.J. & Mauny F. (2019). – The score of integrated disease surveillance and response adequacy (SIA): a pragmatic score for comparing weekly reported diseases based on a systematic review. *BMC public health,* **19** (1), 624. doi: 10.1186/s12889-019-6954-3.

[44] World Health Organization, Food and Agriculture Organization of the United Nations, World Organisation for Animal Health & Global Alliance for Rabies Control (2018). – Zero by 30: the global strategic plan to end human deaths from dog-mediated rabies by 2030.

[45] Dürr S., Naissengar S., Mindekem R., Diguimbye C., Niezgoda M., Kuzmin I., Rupprecht C.E. & Zinsstag J. (2008). – Rabies diagnosis for developing countries. *PLoS Negl Trop Dis,* **2** (3), e206. doi: 10.1371/journal.pntd.0000206.

[46] Lechenne M., Naissengar K., Lepelletier A., Alfaroukh I.O., Bourhy H., Zinsstag J. & Dacheux L. (2016). – Validation of a Rapid Rabies Diagnostic Tool for Field Surveillance in Developing Countries. *PLoS Negl Trop Dis,* **10** (10), e0005010. doi: 10.1371/journal.pntd.0005010.

[47] Kayali U., Mindekem R., Yemadji N., Oussiguere A., Naissengar S., Ndoutamia A.G. & Zinsstag J. (2003). – Incidence of canine rabies in N'Djamena, Chad. *Prev Vet Med,* **61** (3), 227-233.

[48] Lechenne M., Mindekem R., Madjadinan S., Oussiguéré A., Moto D.D., Naissengar K. & Zinsstag J. (2017). – The Importance of a Participatory and Integrated One Health Approach for Rabies Control: The Case of N'Djaména, Chad. *Tropical Medicine and Infectious Disease,* **2** (3), 43.

[49] Frey J., Mindekem R., Kessely H., Doumagoum Moto D., Naissengar S., Zinsstag J. & Schelling E. (2013). – Survey of animal bite injuries and their management for an estimate of human rabies deaths in N'Djamena, Chad. *Trop Med Int Health,* **18** (12), 1555-1562. doi: 10.1111/tmi.12202.

[50] Kayali U., Mindekem R., Yemadji N., Vounatsou P., Kaninga Y., Ndoutamia A.G. & Zinsstag J. (2003). – Coverage of pilot parenteral vaccination campaign against canine rabies in N'Djamena, Chad. *Bulletin of the World Health Organization,* **81** (10), 739-744.

[51] Dürr S., Meltzer M.I., Mindekem R. & Zinsstag J. (2008). – Owner valuation of rabies vaccination of dogs, Chad. *Emerging infectious diseases,* **14** (10), 1650-1652. doi: 10.3201/eid1410.071490.

[52] Dürr S., Mindekem R., Kaninga Y., Doumagoum Moto D., Meltzer M.I., Vounatsou P. & Zinsstag J. (2009). – Effectiveness of dog rabies vaccination programmes: comparison of owner-charged and free vaccination campaigns. *Epidemiology and infection,* **137** (11), 1558-1567. doi: 10.1017/s0950268809002386.

[53] Mindekem R., Kayali U., Yemadji N., Ndoutamia A.G. & Zinsstag J. (2005). – Impact of canine demography on rabies transmission in N'djamena, Chad. *Medecine tropicale : revue du Corps de sante colonial,* **65** (1), 53-58.

[54] Zinsstag J., Durr S., Penny M.A., Mindekem R., Roth F., Gonzalez S.M., Naissengar S. & Hattendorf J. (2009). – Transmission dynamics and economics of rabies control in dogs

and humans in an African city. *Proceedings of the National Academy of Sciences of the United States of America,* **106** (35), 14996-15001. doi: 10.1073/pnas.0904740106.

[55] Lechenne M., Oussiguere A., Naissengar K., Mindekem R., Mosimann L., Rives G., Hattendorf J., Moto D.D., Alfaroukh I.O. & Zinsstag J. (2016). – Operational performance and analysis of two rabies vaccination campaigns in N'Djamena, Chad. *Vaccine,* **34** (4), 571-577. doi: 10.1016/j.vaccine.2015.11.033.

[56] Coleman P.G. & Dye C. (1996). – Immunization coverage required to prevent outbreaks of dog rabies. *Vaccine,* **14**, 185-186. doi: 10.1016/0264-410X(95)00197-9.

[57] Bourhy H., Nakouné E., Hall M., Nouvellet P., Lepelletier A., Talbi C., Watier L., Holmes E.C., Cauchemez S. & Lemey P. (2016). – Revealing the micro-scale signature of endemic zoonotic disease transmission in an African urban setting. *PLoS pathogens,* **12** (4), e1005525.

[58] Botigue L.R., Song S., Scheu A., Gopalan S., Pendleton A.L., Oetjens M., Taravella A.M., Seregely T., Zeeb-Lanz A., Arbogast R.M., Bobo D., Daly K., Unterlander M., Burger J., Kidd J.M. & Veeramah K.R. (2017). – Ancient European dog genomes reveal continuity since the Early Neolithic. *Nature communications,* **8**, 16082. doi: 10.1038/ncomms16082.

[59] Wang G.-D., Zhai W., Yang H.-C., Wang L., Zhong L., Liu Y.-H., Fan R.-X., Yin T.-T., Zhu C.-L., Poyarkov A.D., Irwin D.M., Hytonen M.K., Lohi H., Wu C.-I., Savolainen P. & Zhang Y.-P. (2016). – Out of southern East Asia: the natural history of domestic dogs across the world. *Cell Res,* **26** (1), 21-33. doi: 10.1038/cr.2015.147.

[60] Shannon L.M., Boyko R.H., Castelhano M., Corey E., Hayward J.J., McLean C., White M.E., Abi Said M., Anita B.A., Bondjengo N.I., Calero J., Galov A., Hedimbi M., Imam B., Khalap R., Lally D., Masta A., Oliveira K.C., Perez L., Randall J., Tam N.M., Trujillo-Cornejo F.J., Valeriano C., Sutter N.B., Todhunter R.J., Bustamante C.D. & Boyko A.R. (2015). – Genetic structure in village dogs reveals a Central Asian domestication origin. *Proceedings of the National Academy of Sciences of the United States of America,* **112** (44), 13639-13644. doi: 10.1073/pnas.1516215112.

[61] Thalmann O., Shapiro B., Cui P., Schuenemann V.J., Sawyer S.K., Greenfield D.L., Germonpre M.B., Sablin M.V., Lopez-Giraldez F., Domingo-Roura X., Napierala H., Uerpmann H.P., Loponte D.M., Acosta A.A., Giemsch L., Schmitz R.W., Worthington B., Buikstra J.E., Druzhkova A., Graphodatsky A.S., Ovodov N.D., Wahlberg N., Freedman A.H., Schweizer R.M., Koepfli K.P., Leonard J.A., Meyer M., Krause J., Paabo S., Green R.E. & Wayne R.K. (2013). – Complete mitochondrial genomes of ancient canids suggest a European origin of domestic dogs. *Science,* **342** (6160), 871-874. doi: 10.1126/science.1243650.

[62] Anderson T.M., Von Holdt B.M., Candille S.I., Musiani M., Greco C., Stahler D.R., Smith D.W., Padhukasahasram B., Randi E., Leonard J.A., Bustamante C.D., Ostrander E.A., Tang H., Wayne R.K. & Barsh G.S. (2009). – Molecular and evolutionary history of

melanism in North American gray wolves. *Science,* **323** (5919), 1339-1343. doi: 10.1126/science.1165448.

[63] Pastoret PP, Kappeler A & Aubert M (2004). – Euopean Rabies Control and its History. *In* Historical Perspective of Rabies in Europe and the Mediterranean (F.A.R. King A.A., Aubert M., Wandeler A.I.,, ed), OIE, Paris. 337-345.

[64] Müller T., Demetriou P., Moynagh J., Cliquet F., Fooks A.R., Conraths F.J., Mettenleiter T.C. & Freuling C.M. (2012). – Rabies elimination in Europe - a success story. *In* Rabies control: towards sustainable prevention at the source. Compendium of the OIE Global Conference on Rabies Control, World Organisation for Animal Health (OIE), Incheon, Seoul (Republic of Korea). 31-43.

[65] Müller T., Freuling C.M., Wysocki P., Roumiantzeff M., Freney J., Mettenleiter T.C. & Vos A. (2015). – Terrestrial rabies control in the European Union: Historical achievements and challenges ahead. *The Veterinary Journal,* **203** (1), 10-17. doi: https://doi.org/10.1016/j.tvjl.2014.10.026.

[66] Slate D., Algeo T.P., Nelson K.M., Chipman R.B., Donovan D., Blanton J.D., Niezgoda M. & Rupprecht C.E. (2009). – Oral Rabies Vaccination in North America: Opportunities, Complexities, and Challenges. *PLOS Neglected Tropical Diseases,* **3** (12), e549. doi: 10.1371/journal.pntd.0000549.

[67] Velasco-Villa A., Mauldin M.R., Shi M., Escobar L.E., Gallardo-Romero N.F., Damon I., Olson V.A., Streicker D.G. & Emerson G. (2017). – The history of rabies in the Western Hemisphere. *Antiviral Res,* doi: 10.1016/j.antiviral.2017.03.013.

[68] Cordeiro R.d.A., Duarte N.F.H., Rolim B.N., Soares Junior F.A., Franco I.C., Ferrer L.L., Almeida C.P., Duarte B.H., de Araujo D.B., Rocha M.F., Brilhante R.S., Favoretto S.R. & Sidrim J.J. (2016). – The Importance of Wild Canids in the Epidemiology of Rabies in Northeast Brazil: A Retrospective Study. *Zoonoses and public health,* **63** (6), 486-493.

[69] Escobar L.E., Restif O., Yung V., Favi M., Pons D.J. & Medina-Vogel G. (2014). – Spatial and temporal trends of bat-borne rabies in Chile. *Epidemiology and infection,* **143** (7), 1486-1494. doi: 10.1017/S095026881400226X.

[70] Vigilato M.A.N., Cosivi O., Knöbl T., Clavijo A. & Silva H.M.T. (2013). – Rabies Update for Latin America and the Caribbean. *Emerging infectious diseases,* **19** (4), 678-679. doi: 10.3201/eid1904.121482.

[71] Schneider M.C., Belotto A., Ade M.P., Hendrickx S., Leanes L.F., Rodrigues M.J., Medina G. & Correa E. (2007). – Current status of human rabies transmitted by dogs in Latin America. *Cadernos de saude publica,* **23** (9), 2049-2063.

[72] Schneider M.C., Romijn P.C., Uieda W., Tamayo H., Silva D.F.d., Belotto A., Silva J.B.d. & Leanes L.F. (2009). – Rabies transmitted by vampire bats to humans: an emerging

zoonotic disease in Latin America? *Revista Panamericana de Salud Pública,* **25**, 260-269.

[73] Acha P.N. (1967). – Epidemiology of paralytic bovine rabies and bat rabies. *Bulletin - Office international des epizooties,* **67** (3), 343-382.

[74] Johnson N., Freuling C., Horton D., Muller T. & Fooks A.R. (2011). – Imported rabies, European Union and Switzerland, 2001-2010. *Emerging infectious diseases,* **17** (4), 753-754. doi: 10.3201/eid1706.101154.

[75] Fèvre E.M., Bronsvoort B.M.d.C., Hamilton K.A. & Cleaveland S. (2006). – Animal movements and the spread of infectious diseases. *Trends in Microbiology,* **14** (3), 125-131. doi: https://doi.org/10.1016/j.tim.2006.01.004.

[76] Ross R.S., Wolters B., Viazov S.O. & Roggendorf M. (2006). – Awareness of Rabies Risks and Knowledge About Preventive Measures Among Experienced German Travel Health Advisors. *Journal of Travel Medicine,* **13** (5), 261-267. doi: 10.1111/j.1708-8305.2006.00058.x.

[77] Sibunruang S., Tepsumethanon S., Raksakhet N. & Tantawichien T. (2013). – Rabies Immunization of Travelers in a Canine Rabies Endemic Area. *Journal of Travel Medicine,* **20** (3), 159-164. doi: 10.1111/jtm.12023.

[78] World Health Organization (2016). – Global elimination of dog-mediated human rabies. *In* Report of the Rabies Global Conference, World Health Organization and World Organisation for Animal Health, Geneva, Switzerland.

[79] Bogel K. & Meslin F.X. (1990). – Economics of human and canine rabies elimination: guidelines for programme orientation. *Bulletin of the World Health Organization,* **68** (3), 281-291.

[80] Morters M.K., Restif O., Hampson K., Cleaveland S., Wood J.L. & Conlan A.J. (2013). – Evidence-based control of canine rabies: a critical review of population density reduction. *J Anim Ecol,* **82** (1), 6-14. doi: 10.1111/j.1365-2656.2012.02033.x.

[81] Bourhy H., Nakoune E., Hall M., Nouvellet P., Lepelletier A., Talbi C., Watier L., Holmes E.C., Cauchemez S., Lemey P., Donnelly C.A. & Rambaut A. (2016). – Revealing the Micro-scale Signature of Endemic Zoonotic Disease Transmission in an African Urban Setting. *PLoS Pathog,* **12** (4), e1005525. doi: 10.1371/journal.ppat.1005525.

[82] Talbi C., Lemey P., Suchard M.A., Abdelatif E., Elharrak M., Jalal N., Faouzi A., Echevarría J.E., Vazquez Morón S., Rambaut A., Campiz N., Tatem A.J., Holmes E.C. & Bourhy H. (2010). – Phylodynamics and Human-Mediated Dispersal of a Zoonotic Virus. *PLOS Pathogens,* **6** (10), e1001166. doi: 10.1371/journal.ppat.1001166.

[83] Brunker K., Marston D.A., Horton D.L., Cleaveland S., Fooks A.R., Kazwala R., Ngeleja C., Lembo T., Sambo M., Mtema Z.J., Sikana L., Wilkie G., Biek R. & Hampson K. (2015). – Elucidating the phylodynamics of endemic rabies virus in eastern Africa using whole-genome sequencing. *Virus Evol,* **1** (1), vev011. doi: 10.1093/ve/vev011.

[84] Dean A.S., Bonfoh B., Kulo A.E., Boukaya G.A., Amidou M., Hattendorf J., Pilo P. & Schelling E. (2013). – Epidemiology of Brucellosis and Q Fever in Linked Human and Animal Populations in Northern Togo. *PloS one,* **8** (8), e71501. doi: 10.1371/journal.pone.0071501.

[85] Mbilo C., Lechenne M., Hattendorf J., Madjadinan S., Anyiam F. & Zinsstag J. (2017). – Rabies awareness and dog ownership among rural northern and southern Chadian communities-Analysis of a community-based, cross-sectional household survey. *Acta tropica,* **175**, 100-111. doi: 10.1016/j.actatropica.2016.06.003.

[86] Mauti S., Traore A., Sery A., Bryssinckx W., Hattendorf J. & Zinsstag J. (2017). – First study on domestic dog ecology, demographic structure and dynamics in Bamako, Mali. *Prev Vet Med,* **146**, 44-51. doi: 10.1016/j.prevetmed.2017.07.009.

[87] Muthiani Y., Traore A., Mauti S., Zinsstag J. & Hattendorf J. (2015). – Low coverage of central point vaccination against dog rabies in Bamako, Mali. *Prev Vet Med,* **120** (2), 203-209. doi: 10.1016/j.prevetmed.2015.04.007.

[88] Mosimann L., Traore A., Mauti S., Lechenne M., Obrist B., Veron R., Hattendorf J. & Zinsstag J. (2017). – A mixed methods approach to assess animal vaccination programmes: The case of rabies control in Bamako, Mali. *Acta Trop,* **165**, 203-215. doi: 10.1016/j.actatropica.2016.10.007.

[89] Schelling E., Mahamat Bechir M., Zinsstag J. & Tanner M. (2015). – Integrated One Health Services. *In* One Health: Theory and Practice of Integrated Health Approaches (J. Zinsstag, E. Schelling, D. Waltner-Toews, M. Whittaker & M. Tanner, eds), CABI Books.

[90] Johnson N., Mansfield K.L., Marston D.A., Wilson C., Goddard T., Selden D., Hemson G., Edea L., van Kesteren F., Shiferaw F., Stewart A.E., Sillero-Zubiri C. & Fooks A.R. (2010). – A new outbreak of rabies in rare Ethiopian wolves (Canis simensis). *Archives of virology,* **155** (7), 1175-1177. doi: 10.1007/s00705-010-0689-x.

[91] Randall D.A., Williams S.D., Kuzmin I.V., Rupprecht C.E., Tallents L.A., Tefera Z., Argaw K., Shiferaw F., Knobel D.L., Sillero-Zubiri C. & Laurenson M.K. (2004). – Rabies in Endangered Ethiopian Wolves. *Emerging infectious diseases,* **10** (12), 2214-2217. doi: 10.3201/eid1012.040080.

[92] Gascoyne S.C., Laurenson M.K., Lelo S. & Borner M. (1993). – Rabies in African wild dogs (Lycaon pictus) in the Serengeti region, Tanzania. *Journal of wildlife diseases,* **29** (3), 396-402. doi: 10.7589/0090-3558-29.3.396.

[93] Flacke G., Becker P., Cooper D., Szykman Gunther M., Robertson I., Holyoake C., Donaldson R. & Warren K. (2013). – An Infectious Disease and Mortality Survey in a Population of Free-Ranging African Wild Dogs and Sympatric Domestic Dogs. *International Journal of Biodiversity,* **2013**, 9. doi: 10.1155/2013/497623.

[94] Berentsen A.R., Dunbar M.R., Becker M.S., M'Soka J., Droge E., Sakuya N.M., Matandiko W., McRobb R. & Hanlon C.A. (2013). – Rabies, canine distemper, and canine parvovirus exposure in large carnivore communities from two Zambian ecosystems. *Vector Borne Zoonotic Dis,* **13** (9), 643-649. doi: 10.1089/vbz.2012.1233.

[95] East M.L., Hofer H., Cox J.H., Wulle U., Wiik H. & Pitra C. (2001). – Regular exposure to rabies virus and lack of symptomatic disease in Serengeti spotted hyenas. *Proceedings of the National Academy of Sciences of the United States of America,* **98** (26), 15026-15031. doi: 10.1073/pnas.261411898.

[96] Davis A., Gordy P., Rudd R., Jarvis J.A. & Bowen R.A. (2012). – Naturally acquired rabies virus infections in wild-caught bats. *Vector Borne Zoonotic Dis,* **12** (1), 55-60. doi: 10.1089/vbz.2011.0674.

[97] Zieger U., Cheetham S., Santana S.E., Leiser-Miller L., Matthew-Belmar V., Goharriz H. & Fooks A.R. (2017). – Natural exposure of bats in Grenada to rabies virus. *Infection Ecology & Epidemiology,* **7** (1), 1332935. doi: 10.1080/20008686.2017.1332935.

[98] Ballard W.B., Follmann E.H., Ritter D.G., Robards M.D. & Cronin M.A. (2001). – Rabies and canine distemper in an arctic fox population in Alaska. *Journal of wildlife diseases,* **37** (1), 133-137. doi: 10.7589/0090-3558-37.1.133.

[99] Jorge R.S., Pereira M.S., Morato R.G., Scheffer K.C., Carnieli P., Jr., Ferreira F., Furtado M.M., Kashivakura C.K., Silveira L., Jacomo A.T., Lima E.S., de Paula R.C. & May-Junior J.A. (2010). – Detection of rabies virus antibodies in Brazilian free-ranging wild carnivores. *Journal of wildlife diseases,* **46** (4), 1310-1315. doi: 10.7589/0090-3558-46.4.1310.

[100] Marcelo Azevedo de Paula Antunes J., de Castro Demoner L., Morosini de Andrade Cruvinel T., Paula Kataoka A., Fatima Alves Martorelli L., Puglia Machado G. & Megid J. (2017). – Rabies Virus Exposure of Brazilian Free-ranging Wildlife from Municipalities without Clinical Cases in Humans or in Terrestrial Wildlife. *Journal of wildlife diseases,* **53** (3), 662-666. doi: 10.7589/2016-09-204.

[101] Nel L., Jacobs J., Jaftha J. & Meredith C. (1997). – Natural spillover of a distinctly Canidae-associated biotype of rabies virus into an expanded wildlife host range in southern Africa. *Virus genes,* **15** (1), 79-82.

[102] Sterner R.T., Meltzer M.I., Shwiff S.A. & Slate D. (2009). – Tactics and Economics of Wildlife Oral Rabies Vaccination, Canada and the United States. *Emerging Infectious Diseases journal - CDC,* **15** (8), 1176-1184. doi: https://dx.doi.org/10.3201/eid1508.081061.

[103] Mork T., Bohlin J., Fuglei E., Asbakk K. & Tryland M. (2011). – Rabies in the arctic fox population, Svalbard, Norway. *Journal of wildlife diseases,* **47** (4), 945-957. doi: 10.7589/0090-3558-47.4.945.

[104] Del Rio Vilas V.J., Freire de Carvalho M.J., Vigilato M.A.N., Rocha F., Vokaty A., Pompei J.A., Molina Flores B., Fenelon N. & Cosivi O. (2017). – Tribulations of the Last Mile: Sides from a Regional Program. *Frontiers in veterinary science,* **4**, 4. doi: 10.3389/fvets.2017.00004.

[105] Wallace R.M., Undurraga E.A., Blanton J.D., Cleaton J. & Franka R. (2017). – Elimination of Dog-Mediated Human Rabies Deaths by 2030: Needs Assessment and Alternatives for Progress Based on Dog Vaccination. *Frontiers in veterinary science,* **4**, 9. doi: 10.3389/fvets.2017.00009.

[106] Anyiam F., Lechenne M., Mindekem R., Oussigere A., Naissengar S., Alfaroukh I.O., Mbilo C., Moto D.D., Coleman P.G., Probst-Hensch N. & Zinsstag J. (2017). – Cost-estimate and proposal for a development impact bond for canine rabies elimination by mass vaccination in Chad. *Acta tropica,* **175**, 112-120. doi: 10.1016/j.actatropica.2016.11.005.

[107] Food and Agriculture Organization of the United Nations & Global Alliance for Rabies Control (2012). – Developing a stepwise approach for rabies prevention and control. *In* FAO/GARC Workshop, Rome, Italy.

[108] Lembo T. on behalf of the Partners for Rabies Prevention (2012). – The Blueprint for Rabies Prevention and Control: A Novel Operational Toolkit for Rabies Elimination. *PLoS Negl Trop Dis,* **6** (2), e1388. doi: 10.1371/journal.pntd.0001388.

[109] Müller T. (2015). – A blueprint for rabies surveillance. Available at: https://rabiesalliance.org/news/blueprint-rabies-surveillance (accessed on 30 October 2017).

[110] Hampson K., Coudeville L., Lembo T., Sambo M., Kieffer A., Attlan M., Barrat J., Blanton J.D., Briggs D.J., Cleaveland S., Costa P., Freuling C.M., Hiby E., Knopf L., Leanes F., Meslin F.X., Metlin A., Miranda M.E., Muller T., Nel L.H., Recuenco S., Rupprecht C.E., Schumacher C., Taylor L., Vigilato M.A., Zinsstag J., Dushoff J. & Global Alliance for Rabies Control Partners for Rabies P. (2015). – Estimating the global burden of endemic canine rabies. *PLoS neglected tropical diseases,* **9** (4), e0003709. doi: 10.1371/journal.pntd.0003709.

[111] Nel L.H. (2013). – Discrepancies in data reporting for rabies, Africa. *Emerging infectious diseases,* **19** (4), 529-533. doi: 10.3201/eid1904.120185.

[112] Dodet B., Adjogoua E.V., Aguemon A.R., Amadou O.H., Atipo A.L., Baba B.A., Ada S.B., Boumandouki P., Bourhy H., Diallo M.K., Diarra L., Diop B.M., Diop S.A., Fesriry B., Gosseye S., Hassar M., Kingé T., Kombila Nzamba T.E., Yandoko E.N., Nzengué E.,

Ramahefalalao E.F., Ratsitorahina M., Simpore L., Soufi A., Tejiokem M., Thiombano R., Tiembré I., Traoré A.K. & Wateba M.I. (2008). – Fighting rabies in Africa: the Africa Rabies Expert Bureau (AfroREB). *Vaccine,* **26**, 6295-6298. doi: 10.1016/j.vaccine.2008.04.087.

[113] Dacheux L., Wacharapluesadee S., Hemachudha T., Meslin F.X., Buchy P., Reynes J.M. & Bourhy H. (2010). – More accurate insight into the incidence of human rabies in developing countries through validated laboratory techniques. *PLoS Neglected Tropical Diseases,* **4** (11) doi: 10.1371/journal.pntd.0000765.

[114] Mallewa M., Fooks A.R., Banda D., Chikungwa P., Mankhambo L., Molyneux E., Molyneux M.E. & Solomon T. (2007). – Rabies encephalitis in malaria-endemic area, Malawi, Africa. *Emerging infectious diseases,* **13** (1), 136-139. doi: 10.3201/eid1301.060810.

[115] World Organisation for Animal Health (2018). – Rabies (infection with rabies virus and other lyssaviruses). *In* Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, OIE, Paris, France. 35 pp.

[116] World Health Organization (2018). – WHO Expert Consultation on Rabies. Third Report. *WHO Technical Report Series, No. 1012*.

[117] Dodet B., Tejiokem M.C., Aguemon A.R. & Bourhy H. (2015). – Human rabies deaths in Africa: breaking the cycle of indifference. *Int Health,* **7** (1), 4-6. doi: 10.1093/inthealth/ihu071.

[118] Rupprecht C.E., Xiang Z., Servat A., Franka R., Kirby J. & Ertl H.C.J. (2018). – Additional Progress in the Development and Application of a Direct, Rapid Immunohistochemical Test for Rabies Diagnosis. *Veterinary sciences,* **5** (2), 59. doi: 10.3390/vetsci5020059.

[119] Eggerbauer E., de Benedictis P., Hoffmann B., Mettenleiter T.C., Schlottau K., Ngoepe E.C., Sabeta C.T., Freuling C.M. & Müller T. (2016). – Evaluation of Six Commercially Available Rapid Immunochromatographic Tests for the Diagnosis of Rabies in Brain Material. *PLoS Negl Trop Dis,* **10** (6), e0004776. doi: 10.1371/journal.pntd.0004776.

[120] Voehl K.M. & Saturday G.A. (2014). – Evaluation of a rapid immunodiagnostic rabies field surveillance test on samples collected from military operations in Africa, Europe, and the Middle East. *U.S. Army Medical Department journal*, 27-32.

[121] Markotter W., York D., Sabeta C.T., Shumba W., Zulu G., Le Roux K. & Nel L.H. (2009). – Evaluation of a rapid immunodiagnostic test kit for detection of African lyssaviruses from brain material. *The Onderstepoort journal of veterinary research,* **76** (2), 257-262.

[122] Ahmad A. & Singh C.K. (2016). – Comparison of rapid immunodiagnosis assay kit with molecular and immunopathological approaches for diagnosis of rabies in cattle. *Veterinary world,* **9** (1), 107-112. doi: 10.14202/vetworld.2016.107-112.

[123] Taylor L.H., Hampson K., Fahrion A., Abela-Ridder B. & Nel L.H. (2017). – Difficulties in estimating the human burden of canine rabies. *Acta tropica,* **165**, 133-140. doi: https://doi.org/10.1016/j.actatropica.2015.12.007.

[124] Cleaveland S., Fèvre E.M., Kaare M. & Coleman P.G. (2002). – Estimating human rabies mortality in the United Republic of Tanzania from dog bite injuries. *Bulletin of the World Health Organization,* **80**, 304-310.

[125] Dacheux L., Reynes J.M., Buchy P., Sivuth O., Diop B.M., Rousset D., Rathat C., Jolly N., Dufourcq J.B., Nareth C., Diop S., Iehle C., Rajerison R., Sadorge C. & Bourhy H. (2008). – A reliable diagnosis of human rabies based on analysis of skin biopsy specimens. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America,* **47** (11), 1410-1417. doi: 10.1086/592969.

[126] Jackson A.C. (2016). – Human Rabies: a 2016 Update. *Current Infectious Disease Reports,* **18** (11), 38. doi: 10.1007/s11908-016-0540-y.

[127] Hampson K., Ventura F., Steenson R., Mancy R., Trotter C., Cooper L., Abela-Ridder B., Knopf L., Ringenier M., Tenzin T., Ly S., Tarantola A., Moyengar R., Oussiguéré A., Bonfoh B., Narayana D.H.A., Sudarshan M.K., Muturi M., Mwatondo A., Wambura G., Andriamandimby S.F., Baril L., Edosoa G.T., Traoré A., Jayme S., Kotzé J., Gunesekera A., Chitnis N., Hattendorf J., Laager M., Lechenne M., Zinsstag J., Changalucha J., Mtema Z., Lugelo A., Lushasi K., Yurachai O., Metcalf C.J.E., Rajeev M., Blanton J., Costa G.B., Sreenivasan N., Wallace R., Briggs D., Taylor L., Thumbi S.M. & Huong N.T.T. (2019). – The potential effect of improved provision of rabies post-exposure prophylaxis in Gavi-eligible countries: a modelling study. *The Lancet Infectious Diseases,* **19** (1), 102-111. doi: 10.1016/S1473-3099(18)30512-7.

[128] Zinsstag J., Meisser A., Schelling E., Bonfoh B. & Tanner M. (2012). – From 'two medicines' to 'One Health' and beyond. *The Onderstepoort journal of veterinary research,* **79** (2), 492. doi: 10.4102/ojvr.v79i2.492.

[129] Lushasi K.S., Cleaveland S., Changalucha J.J., Haydon D., Kazwala R., Lembo T., Masoud M., Maziku M., McHau G., Mtema Z., Omar K., Maganga S., Rysava K. & Hampson K. (2017). – Progress towards rabies elimination from Pemba Island, Southern Tanzania. *Online Journal of Public Health Informatics,* **9** (1), e118. doi: 10.5210/ojphi.v9i1.7702.

[130] Mindekem R., Lechenne M.S., Naissengar K.S., Oussiguéré A., Kebkiba B., Moto D.D., Alfaroukh I.O., Ouedraogo L.T., Salifou S. & Zinsstag J. (2017). – Cost Description and Comparative Cost Efficiency of Post-Exposure Prophylaxis and Canine Mass Vaccination against Rabies in N'Djamena, Chad. *Frontiers in veterinary science,* **4**, 38. doi: 10.3389/fvets.2017.00038.

[131] Zinsstag J., Dürr S., Penny M.A., Mindekem R., Roth F., Menendez Gonzalez S., Naissengar S. & Hattendorf J. (2009). – Transmission dynamics and economics of rabies control in dogs and humans in an African city. *Proceedings of the National Academy of*

Sciences of the United States of America, **106** (35), 14996-15001. doi: 10.1073/pnas.0904740106.

[132] Fitzpatrick M.C., Hampson K., Cleaveland S., Mzimbiri I., Lankester F., Lembo T., Meyers L.A., Paltiel A.D. & Galvani A.P. (2014). – Cost-effectiveness of canine vaccination to prevent human rabies in rural Tanzania. *Annals of internal medicine,* **160** (2), 91-100. doi: 10.7326/m13-0542.

[133] Tenzin, Wangdi K. & Ward M.P. (2012). – Human and animal rabies prevention and control cost in Bhutan, 2001-2008: the cost-benefit of dog rabies elimination. *Vaccine,* **31** (1), 260-270. doi: 10.1016/j.vaccine.2012.05.023.

[134] Scott T.P., Coetzer A., Fahrion A.S. & Nel L.H. (2017). – Addressing the Disconnect between the Estimated, Reported, and True Rabies Data: The Development of a Regional African Rabies Bulletin. *Frontiers in veterinary science,* **4**, 18. doi: 10.3389/fvets.2017.00018.

[135] Coetzer A., Kidane A.H., Bekele M., Hundera A.D., Pieracci E.G., Shiferaw M.L., Wallace R. & Nel L.H. (2016). – The SARE tool for rabies control: Current experience in Ethiopia. *Antiviral Research,* **135**, 74-80. doi: https://doi.org/10.1016/j.antiviral.2016.09.011.

[136] Undurraga E.A., Blanton J.D., Thumbi S.M., Mwatondo A., Muturi M. & Wallace R.M. (2017). – Tool for Eliminating Dog-Mediated Human Rabies through Mass Dog Vaccination Campaigns. *Emerging infectious diseases,* **23** (12), 2114-2116. doi: 10.3201/eid2312.171148.

[137] Mace J., Renaudin S., Dieuzy-Labaye I. & Dehove A. (2018). – Vaccine banks for controlling dog-mediated rabies. *OIE Scientific and Technical Review,* **37** (2), 511-518.

[138] Scott T.P., Coetzer A., de Balogh K., Wright N. & Nel L.H. (2015). – The Pan-African Rabies Control Network (PARACON): A unified approach to eliminating canine rabies in Africa. *Antiviral Research,* **124** (Supplement C), 93-100. doi: https://doi.org/10.1016/j.antiviral.2015.10.002.

[139] Coetzer A., Scott T.P., Amparo A.C., Jayme S. & Nel L.H. (2018). – Formation of the Asian Rabies Control Network (ARACON): A common approach towards a global good. *Antiviral Research,* **157**, 134-139. doi: https://doi.org/10.1016/j.antiviral.2018.07.018.

[140] Pieracci E.G., Scott T.P., Coetzer A., Athman M., Mutembei A., Kidane A.H., Bekele M., Ayalew G., Ntegeyibizaza S., Assenga J., Markalio G., Munyua P., Nel L.H. & Blanton J. (2017). – The Formation of the Eastern Africa Rabies Network: A Sub-Regional Approach to Rabies Elimination. *Trop Med Infect Dis,* **2** (3), 29.

[141] Olarinmoye A.O., Kamara V., Jomah N.D., Olugasa B.O., Ishola O.O., Kamara A. & Luka P.D. (2019). – Molecular detection of rabies virus strain with N-gene that clustered with China lineage 2 co-circulating with Africa lineages in Monrovia, Liberia: first reported case in Africa. *Epidemiology and infection,* **147**, e85. doi: 10.1017/S0950268818003333.

[142] Annan N. (2014). – Violent Conflicts and Civil Strife in West Africa: Causes, Challenges and Prospects. *Stability: International Journal of Security and Development,* **3** (1) doi: http://doi.org/10.5334/sta.da.

[143] Nyenswah T., Engineer C.Y. & Peters D.H. (2016). – Leadership in Times of Crisis: The Example of Ebola Virus Disease in Liberia. *Health Systems & Reform,* **2** (3), 194-207. doi: 10.1080/23288604.2016.1222793.

[144] Moher D., Liberati A., Tetzlaff J. & Altman D.G. (2009). – Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med,* **6** (7), e1000097. doi: 10.1371/journal.pmed.1000097.

[145] Food And Agriculture Organization of the United Nations (2014). – Atelier national pour la prévention et le contrôle de la rage au Gabon: « Comment Améliorer la Collaboration entre les Acteurs ». Available at: https://afro.who.int/fr/news/atelier-national-pour-la-prevention-et-le-controle-de-la-rage-au-gabon-comment-ameliorer-la (accessed on 28 June 2019).

[146] Global Alliance for Rabies Control (2019). – A rabies epidemiological database for sub-Saharan Africa Available at: https://rabiesalliance.org/networks/paracon/bulletin (accessed on 28 June 2019).

[147] Tekki I.S., Ponfa Z.N., Nwosuh C.I., Kumbish P.R., Jonah C.L., Okewole P.A., Shamaki D. & Ahmed S.M. (2016). – Comparative assessment of seller's staining test (SST) and direct fluorescent antibody test for rapid and accurate laboratory diagnosis of rabies. *African health sciences,* **16** (1), 123-127. doi: 10.4314/ahs.v16i1.16.

[148] Tepsumethanon V., Lumlertdacha B. & Wilde H. (2004). – Microscopic Diagnosis of Rabies (Sellers' Stain Compared With the Fluorescent Antibody Test). *Infectious Diseases Journal of Pakistan,* **13** (2), 39-44.

[149] Food and Agriculture Organization of the United Nations (2014). – FAO's contribution to improve rabies control in West and Central Africa. *In* EMPRES-Animal Health 360, Rome.

[150] Istituto Zooprofilattico Sperimentale delle Venezie (2015). – Rabies in Africa: the RESOLAB network. Available at: https://www.izsvenezie.com/rabies-in-africa-the-resolab-network/ (accessed on 04 April 209).

[151] World Organisation for Animal Health (2013). – Rabies, Congo (Rep. of the). Available at: https://www.oie.int/wahis_2/public/wahid.php/Reviewreport/Review?page_refer=MapFullEventReport&reportid=14438.

[152] World Organisation for Animal Health (2018). – Pilot projects to support the elimination of dog mediated human rabies. Available at: http://www.rr-africa.oie.int/projects/SVS/en_index.html (accessed on 28 June 2019).

[153] Centers for Disease Control and Prevention (2017). – One Health Zoonotic Disease Prioritization for Multisectoral Engagement in Burkina Faso. Available at: https://www.cdc.gov/onehealth/pdfs/burkinafaso-french-508.pdf.

[154] Istituto Zooprofilattico Sperimentale delle Venezie (2018). – Rabies, the IZSVe supports the veterinary services of Liberia and Burkina Faso. Available at: https://www.izsvenezie.com/rabies-izsve-supports-veterinary-services-liberia-burkina-faso/ (accessed on 10 March 2019).

[155] Savadogo M. (2015). – Amélioration de la connaissance de l'épidémiologie de la rage au Burkina Faso: Situation épidémiologique et connaissance-attitudes-perceptions sur la rage dans la commune de Ouagadougou, Université Cheikh Anta Diop de Dakar, Dakar.

[156] Sondo K.A., Diallo I., Savadogo M., Kaboré B.E., Ouédraogo G.A., Zoungrana J., Diendéré E.A., Da L., Bazié K., Kyelem N., Basshono J., Bidima P., Ouédraogo/Traoré R., Ouédraogo M., Ouédraogo S.M. & Drabo J.Y. (2018). – Enquête transversale sur les cas de rage reçus de 2003 à 2014 au service des Maladies infectieuses du Centre Hospitalier Universitaire Yalgado Ouédraogo (Burkina Faso). *Science et Technique, Sciences de la Santé,* **41** (1).

[157] Sondo K.A., Zoungrana J., Bazié K., Kyelem C.G., Diallo I., Korsaga N., Basshono J., Simporé I., Thiombiano R. & Traoré A. (2014). – Prise en charge post exposition des victimes d'agression par un animal à Ouagadougou. *Science et Technique, Sciences de la Santé,* **37** (1-2).

[158] Sondo K.A., Yonaba/Okengo C., Diop S.A., Kaboré B.E., Diallo I., Kyelem N., Basshono J., Thombiano R. & Kam L. (2015). – Rabies in Children: Report of 24 Cases at the Yalgado Ouedraogo University Hospital Center of Ouagadougou in Burkina Faso. *Journal of Tropical Diseases,* **3** (3) doi: 10.4172/2329-891X.1000168.

[159] Zangre H. (2018). – Enquête sur l'approche une seule santé et son application dans les stratégies de lutte contre la rage à Ouagadougou (Burkina Faso), Université Cheikh Anta Diop de Dakar, Dakar.

[160] De Benedictis P., Sow A., Fusaro A., Veggiato C., Talbi C., Kabore A., Dundon W.G., Bourhy H. & Capua I. (2010). – Phylogenetic analysis of rabies viruses from Burkina Faso, 2007. *Zoonoses and public health,* **57** (7-8), e42-46. doi: 10.1111/j.1863-2378.2009.01291.x.

[161] Sofeu C.L., Broban A., Njifou Njimah A., Blaise Momo J., Sadeuh-Mba S.A., Druelles S., L'Azou M. & Tejiokem M.C. (2018). – Improving systematic rabies surveillance in Cameroon: A pilot initiative and results for 2014-2016. *PLoS Neglected Tropical Diseases,* **12** (9) doi: 10.1371/journal.pntd.0006597.

[162] Awah Ndukum J., J T. & J. C T. (2002). – Canine and Human Rabies in Cameroon. *Tropical Veterinarian,* **20**, 162-168. doi: 10.4314/tv.v20i3.4497.

[163] Sadeuh-Mba S.A., Besong L., Demanou M., Loul S., Nchare A. & Njouom R. (2014). – Laboratory data of dog rabies in southern Cameroon from 2010 to 2013. *BMC Res Notes,* **7**, 905. doi: 10.1186/1756-0500-7-905.

[164] Bita A., Etapelong Sume G., Essomba N., Nguemne E., Anani C., Mbida D., Eboa E., Feuhgouo2 Gertrude Noufack Zambou A. & Owona J. (2014). – Uptake of Anti rabies vaccine by users of the Anti rabies centre Douala, Cameroon: a descriptive cross sectional study.

[165] Awah Ndukum J. (2003). – Ecological aspects of dogs in relation to rabies control and public health significance in North-West Cameroon. *Journal of the Cameroon Academy of Sciences,* **3** (1).

[166] Barbosa Costa G., Gilbert A., Monroe B., Blanton J., Ngam Ngam S., Recuenco S. & Wallace R. (2018). – The influence of poverty and rabies knowledge on healthcare seeking behaviors and dog ownership, Cameroon. *PloS one,* **13** (6), e0197330. doi: 10.1371/journal.pone.0197330.

[167] Sadeuh-Mba S.A., Momo J.B., Besong L., Loul S. & Njouom R. (2017). – Molecular characterization and phylogenetic relatedness of dog-derived Rabies Viruses circulating in Cameroon between 2010 and 2016. *PLoS Neglected Tropical Diseases,* **11** (10), e0006041. doi: 10.1371/journal.pntd.0006041.

[168] Nakoune E., Digol M., Konamna X., Selekon B. & Le Faou A. (2012). – New introduction and spread of rabies among dog population in Bangui. *Acta tropica,* **123** (2), 107-110. doi: 10.1016/j.actatropica.2012.04.005.

[169] Tricou V., Bouscaillou J., Kamba Mebourou E., Koyanongo F.D., Nakoune E. & Kazanji M. (2016). – Surveillance of Canine Rabies in the Central African Republic: Impact on Human Health and Molecular Epidemiology. *PLoS Negl Trop Dis,* **10** (2), e0004433.

[170] Tricou V., Berthet N., Nakoune E. & Kazanji M. (2014). – Complete genome sequence of a rabies virus isolated from a human in central african republic. *Genome announcements,* **2** (3) doi: 10.1128/genomeA.00598-14.

[171] Durr S., Meltzer M.I., Mindekem R. & Zinsstag J. (2008). – Owner valuation of rabies vaccination of dogs, Chad. *Emerg Infect Dis,* **14** (10), 1650-1652. doi: 10.3201/eid1410.071490.

[172] Lechenne M., Oussiguere A., Naissengar K., Mindekem R., Mosimann L., Rives G., Hattendorf J., Moto D.D., Alfaroukh I.O., Zinsstag J. & technical c. (2016). – Operational performance and analysis of two rabies vaccination campaigns in N'Djamena, Chad. *Vaccine,* **34** (4), 571-577. doi: 10.1016/j.vaccine.2015.11.033.

[173] Zinsstag J., Lechenne M., Laager M., Mindekem R., Naïssengar S., Oussiguéré A., Bidjeh K., Rives G., Tessier J., Madjaninan S., Ouagal M., Moto D.D., Alfaroukh I.O., Muthiani Y., Traoré A., Hattendorf J., Lepelletier A., Kergoat L., Bourhy H., Dacheux L., Stadler T. & Chitnis N. (2017). – Vaccination of dogs in an African city interrupts rabies transmission and reduces human exposure. *Science translational medicine,* **9** (421) doi: 10.1126/scitranslmed.aaf6984.

[174] Laager M., Mbilo C., Madaye E.A., Naminou A., Lechenne M., Tschopp A., Smieszek T., Zinsstag J., Chitnis N. & Serv Kemdongarti N. (2018). – The importance of dog population contact network structures in rabies transmission. *Plos Neglected Tropical Diseases,* **12** (8), 18. doi: 10.1371/journal.pntd.0006680.

[175] Mindekem R., Lechenne M., Alfaroukh I.O., Moto D.D., Zinsstag J., Ouedraogo L.T. & Salifou S. (2017). – Evaluation of Knowledge-Attitudes-Practices of the populations in the health districts of Benoye, Laoukassy, Moundou and South N'Djamena towards canine rabies in Chad. *Pan Afr. Med. J.,* **27**, 8. doi: 10.11604/pamj.2017.27.24.11464.

[176] Welburn S.C., Coleman P.G. & Zinsstag J. (2017). – Rabies Control: Could Innovative Financing Break the Deadlock? *Frontiers in veterinary science,* **4**, 32. doi: 10.3389/fvets.2017.00032.

[177] Centers for Disease Control and Prevention (2017). – One Health Zoonotic Disease Prioritization for Multi-Sectoral Engagement in Côte d'Ivoire. Available at: https://www.cdc.gov/onehealth/pdfs/cote-dlvoire-english-508.pdf.

[178] Tiembre I., Dagnan S., Douba A., Adjogoua E.V., Bourhy H., Dacheux L., Kouassi L., Dosso M. & Odehouri-Koudou P. (2010). – Epidemiologic monitoring of human rabies in an endemic canine rabies area in the Ivory Coast. *Medecine et maladies infectieuses,* **40** (7), 398-403. doi: 10.1016/j.medmal.2010.01.008.

[179] Ouattara S.I., Cissé H., Kouakou G., Kolia-Diafouka P., Doumbia A., Yokoué A.D., Eholié S.P. & Bissagnené E. (2012). – Human rabies in Abidjan (Côte d'Ivoire): Recent cases. *Medecine et sante tropicales,* **22** (2), 157-161.

[180] Tiembre I., Vroh Benie Bi J., N'Cho Dagnan S., Kouadio Ekra D., Zebe S. & Tagliante-Saracino J. (2011). – The epidemiological profile of subjects exposed to rabies in Abidjan, Ivory Coast. *Sante publique (Vandoeuvre-les-Nancy, France),* **23** (4), 279-286.

[181] Tiembre I., Broban A., Benie J., Tetchi M., Druelles S. & L'Azou M. (2018). – Human rabies in Cote d'Ivoire 2014-2016: Results following reinforcements to rabies surveillance. *PLoS neglected tropical diseases,* **12** (9), e0006649. doi: 10.1371/journal.pntd.0006649.

[182] Tiembré I., Aka-Kone D.M., Konan Y.E., Vroh J.B., Kouadio D.E., N'Cho S D., Aka J., Tagliante-Saracino J. & Odehouri-Koudou P. (2009). – Adherence to rabies vaccine

treatment for people exposed to rabies in Abidjan (Côte d' Ivoire). *Sante publique (Vandoeuvre-les-Nancy, France),* **21** (6), 595-603.

[183] Zamina B.Y.G., Tiembre I., Attoh-Toure H., N'Guessan K.E., Tetchi S.M. & Benie B.V.J. (2018). – Factors associated with the abandonment of postexposure prophylaxis at the antirabies center in Abidjan, Cote d'Ivoire. *Medecine et sante tropicales,* **28** (2), 212-218. doi: 10.1684/mst.2018.0796.

[184] Zamina B.Y.G., Tiembre I., N'Guessan K., Attoh-Touré H., Tétchi S.M. & Benié B.V.J. (2018). – Amélioration de l'observance de la prophylaxie post-exposition par l'usage de la téléphonie mobile, Côte d'Ivoire. *Sante publique (Vandoeuvre-les-Nancy, France),* **30** (4), 545-554. doi: 10.3917/spub.185.0545.

[185] Talbi C., Holmes E.C., de Benedictis P., Faye O., Nakoune E., Gamatie D., Diarra A., Elmamy B.O., Sow A., Adjogoua E.V., Sangare O., Dundon W.G., Capua I., Sall A.A. & Bourhy H. (2009). – Evolutionary history and dynamics of dog rabies virus in western and central Africa. *The Journal of general virology,* **90** (Pt 4), 783-791. doi: 10.1099/vir.0.007765-0.

[186] Tiembre I., Vroh Benie Bi J., Kouassi P., Attoh-Toure H., Ekra K.D., Diane A., Dagnan N.S. & Tagliante-Saracino J. (2014). – Knowledge, attitudes and practices of household heads regarding rabies in the Abobo district (Abidjan, Cote d'Ivoire) in 2008. *Sante publique (Vandoeuvre-les-Nancy, France),* **26** (4), 547-553.

[187] Courtois G., Ninane G. & Thys A. (1964). – [ON CASES OF RABIES DIAGNOSED IN THE LABORATORY AT STANLEYVILLE FROM 1939 TO 1958]. *Annales des Societes belges de medecine tropicale, de parasitologie, et de mycologie,* **44**, 405-414.

[188] Salyer S.J., Silver R., Simone K. & Barton Behravesh C. (2017). – Prioritizing Zoonoses for Global Health Capacity Building—Themes from One Health Zoonotic Disease Workshops in 7 Countries, 2014–2016. *Emerging Infectious Disease journal,* **23** (13) doi: 10.3201/eid2313.170418.

[189] Stolka K.B., Ngoyi B.F., Grimes K.E.L., Hemingway-Foday J.J., Lubula L., Nzanzu Magazani A., Bikuku J., Mossoko M., Manya Kitoto L., Mpangi Bashilebo S., Lufwa Maya D., Kebela Ilunga B., Rhea S. & MacDonald P.D.M. (2018). – Assessing the Surveillance System for Priority Zoonotic Diseases in the Democratic Republic of the Congo, 2017. *Health security,* **16** (S1), S44-s53. doi: 10.1089/hs.2018.0060.

[190] Muyila D.I., Aloni M.N., Lose-Ekanga M.J., Nzita J.M., Kalala-Mbikay A., Bongo H.L., Esako M.N., Malonga-Biapi J.P., Mputu-Dibwe B., Aloni M.L. & Ekila M.B. (2014). – Human rabies: a descriptive observation of 21 children in Kinshasa, the Democratic Republic of Congo. *Pathogens and global health,* **108** (7), 317-322. doi: 10.1179/2047773214y.0000000161.

[191] Twabela A.T., Mweene A.S., Masumu J.M., Muma J.B., Lombe B.P. & Hankanga C. (2016). – Overview of Animal Rabies in Kinshasa Province in the Democratic Republic of Congo. *PloS one,* **11** (4), e0150403. doi: 10.1371/journal.pone.0150403.

[192] Kazadi E.K., Marcotty T., Muylkens B., Antoine-Moussiaux N., van Gucht S., Mulumba L. & Kirschvink N. (2019). – Factors of rabies maintenance in dog population in Kinshasa, Democratic Republic of Congo (DRC). *International Journal of Infectious Diseases,* **79**, 56. doi: 10.1016/j.ijid.2018.11.147.

[193] Kazadi E.K., Tshilenge G.M., Mbao V., Njoumemi Z. & Masumu J. (2017). – Determinants of dog owner-charged rabies vaccination in Kinshasa, Democratic Republic of Congo. *PloS one,* **12** (10), e0186677. doi: 10.1371/journal.pone.0186677.

[194] Bureau Provincial de la Production et Sante Animales (PSA) (2017). – Rapport de la journee mondiale contre la rage. *In*, Inspection Provinciale de l'Agriculture, Peche et Elevage.

[195] Généus L.V. (2017). – Evaluation de l'approche One Health dans la gestion de la rage à Kinshasa, République Démocratique du Congo, Université de Namur.

[196] Rubin J., David D., Willoughby Jr R.E., Rupprecht C.E., Garcia C., Guarda D.C., Zohar Z. & Stamler A. (2009). – Applying the Milwaukee Protocol to treat canine rabies in Equatorial Guinea. *Scandinavian journal of infectious diseases,* **41** (5), 372-375. doi: 10.1080/00365540902798333.

[197] Belcher D.W., Wurapa F.K. & Atuora D.O. (1976). – Endemic rabies in Ghana. Epidemiology and control measures. *The American journal of tropical medicine and hygiene,* **25** (5), 724-729.

[198] Hayman D.T.S., Johnson N., Horton D.L., Hedge J., Wakeley P.R., Banyard A.C., Zhang S.F., Alhassan A. & Fooks A.R. (2011). – Evolutionary History of Rabies in Ghana. *Plos Neglected Tropical Diseases,* **5** (4), 10. doi: 10.1371/journal.pntd.0001001.

[199] Elieza S. (2016). – Trends in dog bites and Human Rabies in Greater Accra Region, University of Ghana, Accra.

[200] Punguyire D.T., Osei-Tutu A., Aleser E.V. & Letsa T. (2017). – Level and pattern of human rabies and dog bites in Techiman Municipality in the Middle Belt of Ghana: a six year retrospective records review. *The Pan African medical journal,* **28**, 281. doi: 10.11604/pamj.2017.28.281.14218.

[201] Adomako B.Y., Baiden F., Sackey S., Ameme D.K., Wurapa F., Nyarko K.M., Kenu E. & Afari E. (2018). – Dog bites and rabies in the Eastern Region of Ghana in 2013-2015: A call for a one-health approach. *Journal of tropical medicine,* **2018** doi: 10.1155/2018/6139013.

[202] Kenu E., Ganu V., Noora C.L., Adanu R. & Lartey M. (2018). – Management of dog bites by frontline service providers in primary healthcare facilities in the Greater Accra Region of Ghana, 2014-2015. *Infect Dis Poverty,* **7** (1), 18. doi: 10.1186/s40249-018-0398-3.

[203] Afakye K., Kenu E., Nyarko K.M., Johnson S.A., Wongnaah F. & Bonsu G.K. (2016). – Household exposure and animal-bite surveillance following human rabies detection in Southern Ghana. *The Pan African medical journal,* **25** (Suppl 1), 12. doi: 10.11604/pamj.supp.2016.25.1.6200.

[204] World Animal Protection (2018). – Ghana launches rabies prevention project. Available at: https://www.worldanimalprotection.us/news/ghana-launches-rabies-prevention-project (accessed on 08 March 2019).

[205] Youla A.S., Traore F.A., Sako F.B., Feda R.M. & Emeric M.A. (2014). – Canine and human rabies in Conakry: epidemiology and preventive aspects. *Bull Soc Pathol Exot,* **107** (1), 18-21. doi: 10.1007/s13149-013-0321-x.

[206] Standley C.J., Carlin E.P., Sorrell E.M., Barry A.M., Bile E., Diakite A.S., Keita M.S., Koivogui L., Mane S., Martel L.D. & Katz R. (2019). – Assessing health systems in Guinea for prevention and control of priority zoonotic diseases: A One Health approach. *One Health,* **7**, 100093-100093. doi: 10.1016/j.onehlt.2019.100093.

[207] Global Alliance for Rabies Control (2018). – Diagnosing the problem and planning for the future: Bolstering rabies control in Guinea. Available at: https://rabiesalliance.org/news/diagnosing-problem-and-planning-future-bolstering-rabies-control-guinea (accessed on 05 April 2019).

[208] Thomas E., Jallow B.J., Carayol I., Ideh R.C. & Anderson S.T. (2014). – Rabies post-exposure prophylaxis at a clinic in Fajara, The Gambia. *Trans R Soc Trop Med Hyg,* **108** (8), 513-515. doi: 10.1093/trstmh/tru069.

[209] Poindexter H.A. (1953). – An analytical study of 45,000 consecutive admissions to a clinic in Monrovia, Liberia, West Africa. *Journal of the National Medical Association,* **45** (5), 345-349.

[210] Kruk M., C Rockers P., H Williams E., Varpilah S., Macauley R., Saydee G. & Galea S. (2010). – Research Availability of essential health services in post-conflict Liberia.

[211] Food And Agriculture Organization of the United Nations (2008). – Liberia Critical Humanitarian Gaps Appeal 2008. Available at: http://www.fao.org/emergencies/appeals/detail/en/c/168111/ (accessed on 09 March 2019).

[212] Jomah N.D., Ososanya T.O., Mulbah C.K. & Olugasa B.O. (2013). – A descriptive and categorical analysis of age, gender and seasonal pattern of dog bite cases and rabies-like-illness among humans in Liberia, 2008-2012. *Epizootiology and Animal Health in West Africa,* **9** (2), 113-124.

[213] Olarinmoye A.O., Ojo J.F., Fasunla A.J., Ishola O.O., Dakinah F.G., Mulbah C.K., Al-Hezaimi K. & Olugasa B.O. (2017). – Time series analysis and mortality model of dog bite victims presented for treatment at a referral clinic for rabies exposure in Monrovia, Liberia, 2010–2013. *Spatial and spatio-temporal epidemiology,* **22**, 1-13. doi: 10.1016/j.sste.2017.04.003.

[214] Olarinmoye A., G. Dakinah F., Ishola O. & Olugasa B. (2017). – Mapping of dog bite cluster alarms and hotspots of rabies in Monrovia, Liberia, 2010-2013.

[215] Food and Agriculture Organization of the United Nations (2017). – Liberia, Evaluation for action - Assessing animal disease surveillance capacities. Available at: www.fao.org/3/CA1770EN/ca1770en.pdf.

[216] Food and Agriculture Organization of the United Nations & United States Agency International Development (2019). – Protecting people and animals from disease threats. Available at: http://www.fao.org/3/i8747en/I8747EN.pdf (accessed on 18 July 2019).

[217] Dao S., Abdillahi A.M., Bougoudogo F., Toure K. & Simbe C. (2006). – Epidemiological aspects of human and animal rabies in the urban area of Bamako, Mali. *Bulletin de la Societe de pathologie exotique (1990),* **99** (3), 183-186.

[218] Kone O. (2013). – Aspect épidémiologique de la rage humaine dans le district de Bamako de 2007 à 2012, Université des Sciences, des Techniques et des Technologies de Bamako, Bamako, Mali.

[219] Traoré A., Picard-Meyer E., Mauti S., Biarnais M., Balmer O., Samaké K., Kamissoko B., Tembely S., Sery A., Traoré A.K., Coulibaly A.P., Robardet E., Zinsstag J. & Cliquet F. (2016). – Molecular characterization of canine rabies virus, Mali, 2006–2013. *Emerging infectious diseases,* **22** (5), 866-870. doi: 10.3201/eid2205.150470.

[220] Kone O. (2010). – L'Epidémiologie de la Rage dans les Localités Urbaines du Mali, Université de Bamako, Bamako, Mali.

[221] Boulger L.R. & Hardy J. (1960). – Rabies in Nigeria. *West African Medical Journal,* **9** (6), 223-234.

[222] Bello M., M Lukshi B. & Usman B. (2008). – A Fifteen-Year Retrospective Study Of The Prevalence Of Rabies In Bauchi State, Nigeria.

[223] Abubakar S.A. & Bakari A.G. (2012). – Incidence of dog bite injuries and clinical rabies in a tertiary health care institution: A 10-year retrospective study. *Annals of African medicine,* **11** (2), 108-111. doi: 10.4103/1596-3519.93534.

[224] Joshua I.A., Bello M., Adeiza A. & Danbirni S. (2012). – Analysis of 4-year dog-bite cases treated at ahmadu bello university health centre, Zaria, Nigeria. *TAF Preventive Medicine Bulletin,* **11** (6), 661-666. doi: 10.5455/pmb.1322410404.

[225] Adeleke S.I. (2010). – Impact of dog bite in Kano city a retrospective study. *Nigerian journal of clinical practice,* **13** (1), 67-69.

[226] Ehizibolo D.O., Ogunsan E.A., Muhammad M.J., Nwosuh C.I., Olaleye S., Chuckwu O.O.C., Sugun M.Y., Sati N.M., Waziri N.E., Egwu O.K., Kamani J., Meseko C.A., Idachaba S.E. & Dogo G.I. (2008). – Diagnosis of canine rabies by the direct fluorescent antibody technique in Plateau State, Nigeria. *Nigerian Veterinary Journal,* **29** (2).

[227] Aworh M.K., Nwosuh C.I., Ajumobi O.O., Okewole P.A., Okolocha E.C., Akanbi B.O. & Nguku P. (2011). – A Retrospective Study of Rabies Cases Reported at Vom Christian Hospital, Plateau State, Nigeria, 2006 – 2010. *Nigerian Veterinary Journal,* **32** (4).

[228] Bata S.I., Dzikwi A. & Ayika D.G. (2011). – Retrospective study of dog bite cases reported to ECWA Veterinary Clinic, Bukuru, Plateau state, Nigeria. *Science World Journal,* **6** (4).

[229] Alabi O., Nguku P., Chukwukere S., Gaddo A., Nsubuga P. & Umoh J. (2014). – Profile of dog bite victims in Jos Plateau State, Nigeria: a review of dog bite records (2006-2008). *The Pan African medical journal,* **18 Suppl 1**, 12. doi: 10.11694/pamj.supp.2014.18.1.4341.

[230] Okpe E.S., Bode-Thomas F., Ocheke I.E., Oguche S., Toma B. & Okolo S. (2011). – Childhood Rabies: A 10 Year Review of Management and Outcome in a Tertiary Centre.

[231] Asuquo M., Ndifon W., Ugare G. & Mwankon J. (2009). – Prevalence Of Dog Bites In A Rural Community: A 15 Year Review Of Cases In Okoyong, Cross River State, Nigeria. *Global Journal of Community Medicine,* **2** (1-2), 65-69. doi: 10.4314/gjcm.v2i1-2.47932.

[232] Osaghae D.O. (2011). – Animal and human bites in children. *West African journal of medicine,* **30** (6), 421-424.

[233] Aghahowa S.E. & Ogbevoen R.N. (2010). – Incidence of dog bite and anti-rabies vaccine utilization in the, University of Benin Teaching Hospital, Benin City, Nigeria: A 12-year assessment. *Vaccine,* **28** (30), 4847-4850. doi: 10.1016/j.vaccine.2010.04.004.

[234] Ogundare E.O., Olatunya O.S., Oluwayemi I.O., Inubile A.J., Taiwo A.B., Agaja O.T., Airemionkhale A. & Fabunmi A. (2017). – Pattern and outcome of dog bite injuries among children in Ado-Ekiti, Southwest Nigeria. *The Pan African medical journal,* **27**, 81. doi: 10.11604/pamj.2017.27.81.7360.

[235] Olugasa B.O., Okeke O.S. & Ishola O.O. (2014). – Geographic access to street food sources for dogs and its association with spatial patterns of animal bite injuries in Enugu, Nigeria, 2005-2011. *African journal of medicine and medical sciences,* **43 Suppl**, 79-86.

[236] Eke C.B., Omotowo I.B., Ukoha O.M. & Ibe B.C. (2015). – Human rabies: still a neglected preventable disease in Nigeria. *Nigerian journal of clinical practice,* **18** (2), 268-272. doi: 10.4103/1119-3077.151064.

[237] Hambolu S.E., Dzikwi A.A., Kwaga J.K., Kazeem H.M., Umoh J.U. & Hambolu D.A. (2013). – Rabies and dog bites cases in lagos state Nigeria: a prevalence and retrospective studies (2006-2011). *Global journal of health science,* **6** (1), 107-114. doi: 10.5539/gjhs.v6n1p107.

[238] Odita C.I., Tekki I.S., Moses G.D., Egwu O.K., Idachaba S.E., Barde I.J., Ifende I.V., Makanju O.A., Ahmed J.S., Dashe Y.G., Nwosuh C.I. & Okewole P.A. (2017). – Dog owners' attitude, a risk factor for human rabies in Nigeria Rabies in Nigeria. *Am. J. Trop. Med. Hyg.,* **97** (5), 573-574.

[239] Dzikwi A.A., Garkida A.D. & Umoh J.U. (2011). – World Rabies Day: Efforts Towards Rabies Awareness in Zaria, Nigeria. *Nigerian Veterinary Journal,* **32** (2).

[240] Awoyomi O.J., Adeyemi F.S. & Awoyomi F.S. (2007). – Socioeconomic factors associated with non-vaccination of dogs against rabies in Ibadan: Nigeria. *Nigerian Veterinary Journal,* **28** (3), 59-63.

[241] Faleke O.O. (2003). – Studies on dog population and its implication for rabies control. *Nigerian Journal of Animal Production,* **30** (2).

[242] Luga I.I., Enemuneme O.V. & Apaa T.T. (2018). – Dog population and ecology in Ahmadu Bello University (ABU) main campus and Bomo village, Kaduna state, Nigeria. *Sokoto Journal of Veterinary Sciences,* **16** (1).

[243] Mshelbwala P.P., Akinwolemiwa D.K., Maikai B.V., Otolorin R.G., Maurice N.A. & Weese J.S. (2018). – Dog ecology and its implications for rabies control in Gwagwalada, Federal Capital Territory, Abuja, Nigeria. *Zoonoses and public health,* **65** (1), 168-176. doi: 10.1111/zph.12385.

[244] Atuman Y.J., Ogunkoya A.B., Adawa D.A.Y., Nok A.J. & Biallah M.B. (2014). – Dog ecology, dog bites and rabies vaccination rates in Bauchi State, Nigeria. *International Journal of Veterinary Science and Medicine,* **2** (1), 41-45. doi: 10.1016/j.ijvsm.2014.04.001.

[245] Omudu E.O., Otache E.O. & Adelusi S.M. (2010). – Studies on Dog Population in Makurdi, Nigeria (I): Demography and Survey of Pet Owners' Beliefs and Attitudes. *Journal of Research in Forestry, Wildlife and Environment,* **2** (1).

[246] Hambolu S.E., Dzikwi A.A., Kwaga J.K., Kazeem H.M., Umoh J.U. & Hambolu D.A. (2014). – Dog ecology and population studies in Lagos State, Nigeria. *Global journal of health science,* **6** (2), 209-220. doi: 10.5539/gjhs.v6n2p209.

[247] Aiyedun J.O. & Olugasa B.O. (2012). – Identification and analysis of dog use, management practices and implications for rabies control in Ilorin, Nigeria. *Sokoto Journal of Veterinary Sciences,* **10** (2) doi: 10.4314/sokjvs.v10i2.1.

[248] Ameh V.O., Dzikwi A.A. & Umoh J.U. (2014). – Assessment of knowledge, attitude and practice of dog owners to canine rabies in wukari metropolis, taraba state Nigeria. *Global journal of health science,* **6**, 35085. doi: 10.5539/gjhs.v6n5p226.

[249] Aiyedun J.O., BO. (2012). – Use of aerial photograph to enhance dog population census in Ilorin, Nigeria. *Sokoto Journal of Veterinary Sciences,* **10** (1), 22-27.

[250] Onoja A.B., Meseko C.A. & Tekki S.I. (2014). – Rabies unending malady--Nigeria in perspective. *African journal of medicine and medical sciences,* **43 Suppl**, 105-109.

[251] Osinubi M.O., Ogunkoya A.B. & Umoh J.U. (2005). – Evaluation of antibody response by dogs vaccinated with low egg passage, flurry strain anti-rabies vaccine using single and multiple sites. *Sokoto Journal of Veterinary Sciences,* **6**.

[252] Igado O.O., Omobowale T.O. & Nottidge H.O. (2010). – The Effect of Honey and Vitamin C on the Response of Dogs to Anti-Rabies Vaccination. *Sahel Journal of Veterinary Sciences,* **9** (2).

[253] Oladokun A.T., Meseko C.A., Lazarus D.D., Sati L.D., Ekong P.S. & Nwosuh C.M. (2010). – Field Handling and Anti-Rabies Vaccine Efficacy. *Nigerian Veterinary Journal,* **31** (4), 275-278. doi: 10.4314/nvj.v31i4.68984.

[254] Adeiga A. & Harry T. (2005). – Cold chain facility status and the potency of animal rabies vaccine used in Nigeria. *Nigerian Journal of Health and Biomedical Sciences,* **4** (2).

[255] Okello A.L., Bardosh K., Smith J. & Welburn S.C. (2014). – One Health: past successes and future challenges in three African contexts. *PLoS Negl Trop Dis,* **8** (5), e2884. doi: 10.1371/journal.pntd.0002884.

[256] Idachaba S.E. & Bolajoko M.B. (2018). – The need for one health approach towards rabies control in Plateau State, Nigeria. *Sokoto Journal of Veterinary Sciences,* **16** (2).

[257] Bomoi I.M., Waziri N.E., Nguku P. & Tsofo A. (2016). – Integrated human and animal vaccination delivery to Nomadic Fulani communities in Northern Nigeria 2015. *International Journal of Infectious Diseases,* **45**, 22. doi: 10.1016/j.ijid.2016.02.082.

[258] Zhou M., Zhou Z., Kia G.S., Gnanadurai C.W., Leyson C.M., Umoh J.U., Kwaga J.P., Kazeem H.M. & Fu Z.F. (2013). – Complete genome sequence of a street rabies virus

isolated from a dog in Nigeria. *Genome announcements,* **1** (1) doi: 10.1128/genomeA.00214-12.

[259] Ogo M.F., Nel L. & Sabeta C.T. (2011). – Phylogenetic Evidence of the Public and Veterinary Health Threat of Dog Rabies in Nigeria. *Nigerian Veterinary Journal,* **32** (1) doi: 10.4314/nvj.v32i1.68996.

[260] Kia G.S.N., Huang Y., Zhou M., Zhou Z., Gnanadurai C.W., Leysona C.M., Umoh J.U., Kazeem H.M., Ehizibolo D.O., Kwaga J.K.P., Nwosu C.I. & Fu Z.F. (2018). – Molecular characterization of a rabies virus isolated from trade dogs in Plateau State, Nigeria. *Sokoto Journal of Veterinary Sciences,* **16** (2) doi: 10.4314/sokjvs.v16i2.8.

[261] Ehimiyein A., Audu S.W. & Ikhide E. (2014). – The Role of Dog Trading and Slaughter for Meat in Rabies Epidemiology with Special Reference to Nigeria-A Review. *Journal of Experimental Biology and Agricultural Science,* **2** (2).

[262] Ajayi B.B., Rabo J.S. & Baba S.S. (2006). – Rabies in apparently healthy dogs: histological and immunohistochemical studies. *The Nigerian postgraduate medical journal,* **13** (2), 128-134.

[263] Aliyu T.B., De N., Yenda E.N. & Lynn M. (2010). – Prevalence of Rabies Virus Antigens in Apparently Healthy Dogs in Yola, Nigeria. *Researcher*.

[264] Ehimiyein A., Niezgoda M., Orciari L., Kuzmin I., Osinubi M., Ehimiyein I.O., Adawa D., Abdullahi S., Ogunkoya A. & Rupprecht C.E. (2010). – Rabies cases in dog markets in Kaduna state, northern Nigeria. *International Journal of Infectious Diseases,* **14**, e476. doi: 10.1016/j.ijid.2010.02.675.

[265] Garba A., Oboegbulem, Elsa, Magaji A., Umoh, Yahya, Danbirni, Ali H. & Masdooq (2008). – A Comparative Rabies Laboratory Diagnosis: Peculiar features of samples from apparently healthy dogs in Nigeria.

[266] Garba A., Oboegbulem S.I., Junaidu A.U., Magaji A.A., Umoh J.U., Ahmed A., Danbirni S., Chiko K.L., Habu A.K. & Masdooq A.A. (2010). – Rabies' virus antigen in the brain of apparently healthy slaughtered dogs in Sokoto and Katsina States, Nigeria. *Nigerian Journal of Parasitology,* **31** (2), 123-125. doi: 10.4314/njpar.v31i2.69483.

[267] Ekanem E.E., Eyong K.I., Philip-Ephraim E.E., Eyong M.E., Adams E.B. & Asindi A.A. (2013). – Stray dog trade fuelled by dog meat consumption as a risk factor for rabies infection in Calabar, southern Nigeria. *African health sciences,* **13** (4), 1170-1173. doi: 10.4314/ahs.v13i4.44.

[268] Mshelbwala P.P., Ogunkoya A.B. & Maikai B.V. (2013). – Detection of rabies antigen in the saliva and brains of apparently healthy dogs slaughtered for human consumption and its public health implications in abia state, Nigeria. *ISRN veterinary science,* **2013**, 468043. doi: 10.1155/2013/468043.

[269] Isek T.I., Umoh J.U. & Dzikwi A.A. (2013). – Detection of Rabies Antigen in the Brain Tissues of Apparently Healthy Dogs Slaughtered in Ogoja - Cross River State, Nigeria. *Nigerian Veterinary Journal,* **34** (2).

[270] Odeh L.E. & Umoh J.U. (2014). – Assessment of risk of possible exposure to rabies among processors and consumers of dog meat in Zaria and Kafanchan, Kaduna state, Nigeria. *Global journal of health science,* **6** (1), 142-153. doi: 10.5539/gjhs.v6n1p142.

[271] Ehizibolo D.O., Nwosuh C.I., Ehizibolo E.E. & Kia G.S.N. (2009). – Comparison of the Fluorescent Antibody Test and Direct Microscopic Examination for Rabies Diagnosis at the National Veterinary Research Institute, Vom, Nigeria. *African Journal of Biomedical Research,* **12** (1).

[272] Audu S.W., Adawa D., Mshelbwala P. & Ya S. (2017). – Field and Laboratory Detection of Rabies Antigens in Saliva and Brains of Dogs in Nigeria: An Approach Using Rapid Immunochromatographic Test.

[273] Ogunkoya A., Audu S.W., Mshelbwala P., Atuman Y.J., Ehimiyein A.M., Osinubi M., Garba A., Abidoye E.O. & Onoja I. (2013). – Problems of Rabies in Nigeria: A Review. *Epizootiology and Animal Health in West Africa,* **9**, 86-105.

[274] Opaleye O.O., Adesiji Y.O., Olowe O.A. & Fagbami A.H. (2006). – Rabies and antirabies immunization in South Western Nigeria: knowledge, attitude and practice. *Tropical doctor,* **36** (2), 116-117. doi: 10.1258/004947506776593305.

[275] Dzikwi A.A., Ibrahim A.S. & Umoh J.U. (2012). – Knowledge, attitude and practice about rabies among children receiving formal and informal education in Samaru, Zaria, Nigeria. *Global journal of health science,* **4** (5), 132-139. doi: 10.5539/gjhs.v4n5p132.

[276] Edukugho A.A., Umoh J.U., Diem M., Ajani O., Uba B., Okeke L., Adedire E., Adefisoye A., Edukugho C. & Nguku P. (2018). – Knowledge, attitudes and practices towards rabies prevention among residents of abuja municipal area council, federal Capital Territory, Nigeria. *Pan Afr. Med. J.,* **31** doi: 10.11604/pamj.2018.31.21.15120.

[277] Food and Agriculture Organization of the United Nations (2018). – Le Sénégal élabore un plan d'action intégré de contrôle de la rage basée sur l'approche «Une seule santé». Available at: http://www.fao.org/senegal/actualites/detail-events/en/c/1136594/ (accessed on 03 April 2019).

[278] Diop S.A., Manga N.M., Dia N.M., Ndour C.T., Seydi M., Soumare M., Diop B.M. & Sow P.S. (2007). – The point on human rabies in Senegal from 1986 to 2005. *Medecine et maladies infectieuses,* **37** (12), 787-791. doi: 10.1016/j.medmal.2007.01.010.

[279] Ndao D.M. (2009). – Contribution à une meilleure gestion des cas de morsure et de rage dans la région de Fatick, UNIVERSITE CHEIKH ANTA DIOP DE DAKAR.

[280] Food and Agriculture Organization of the United Nations (2018). – Plus de 10 000 chiens vaccinés contre la rage grâce à un groupe whatsapp créé par les vétérinaires nationaux Available at: http://www.fao.org/senegal/actualites/detail-events/en/c/1169821/ (accessed on 03 April 2019).

[281] Hatch C., Sneddon J. & Jalloh G. (2004). – A descriptive study of urban rabies during the civil war in Sierra Leone: 1995-2001. *Trop Anim Health Prod,* **36** (4), 321-334.

[282] Prepardeness & Response (2017). – Sierra Leone Identifies Priority Diseases. Available at: http://preparednessandresponse.org/news/sierra-leone-identifies-priority-diseases/.

[283] Awoko (2018). – Sierra Leone News: Rabies can now be confirmed – Animal Welfare Society. Available at: https://awoko.org/2018/02/14/sierra-leone-news-rabies-can-now-be-confirmed-animal-welfare-society/ (accessed on 18 July 2019).

[284] Suluku R., Abu-Bakarr I., Johnny J. & Jonsyn-Ellis F. (2012). – Post-war Demographic and Ecological Survey of Dog Populations and Their Human Relationships in Sierra Leone. (A Case Study of Urban Freetown).

[285] Suluku R. (2010). – "Dogs of War": Animal Health Clubs are Protecting Livelihoods, food security and lives. FAO Agriculture and Consumer Protection Department. Available at: http://www.fao.org/ag/againfo/home/en/news_archive/AGA_in_action/2010_Animal_Health_Clubs_3.html (accessed on 21 February 2019).

[286] Suluku R. (2012). – "Dogs of War": Animal Health Clubs champion rabies prevention to protect livelihoods and lives in Sierra Leone. FAO Agriculture and Consumer Protection Department. Available at: http://www.fao.org/ag/againfo/home/en/news_archive/AGA_in_action/2010_Animal_Health_Clubs_2.html (accessed on 21 February 2019).

[287] Suluku R., Nyandeboh J.P.J., Kallon M.N., Barrie A., Kabba B., Koroma B.M. & Emikpe B.O. (2017). – First reported case of dog associated cattle rabies in Koinadugu district, Northern Sierra Leone. *African Journal of Biomedical Research,* **20** (3), 325-327.

[288] Zingeser J., Pato P., Bebay C. & Debalogh K. (2011). – A One Health Approach to Rabies Control in Togo. *EcoHealth,* **7**, S19-S19.

[289] Pato P.S. (2011). – Etude épidémiologique de la rage humaine et canine au Togo: Régions sanitaires de Lomé commune et Kara

[290] Pato P.S. (2012). – Epidémiologie de la rage canine et diagnostic par la réaction d'immunofluorescence directe dans la Commune de Lomé, Togo, 2011, Programme Régional de Formation en Epidémiologie d'Intervention et Laboratoire.

[291] Naba M. P.P., Adom S. (2017). – Investigation of human rabies cases and estimation of the risk of rabies virus spread among dog population in commune of Niamtougou, (Northern Togo) in 2017 *In* Second PENAPH Technical Conference, Khon Kaen, Thailand.

[292] World Health Organization (2018). – WHO expert consultation on rabies: third report. *WHO Technical Report Series, No. 1012*.

[293] Mtema Z., Changalucha J., Cleaveland S., Elias M., Ferguson H.M., Halliday J.E.B., Haydon D.T., Jaswant G., Kazwala R., Killeen G.F., Lembo T., Lushasi K., Malishee A.D., Mancy R., Maziku M., Mbunda E.M., McHau G.J.M., Murray-Smith R., Rysava K., Said K., Sambo M., Shayo E., Sikana L., Townsend S.E., Urassa H. & Hampson K. (2016). – Mobile Phones As Surveillance Tools: Implementing and Evaluating a Large-Scale Intersectoral Surveillance System for Rabies in Tanzania. *PLOS Medicine,* **13** (4), e1002002. doi: 10.1371/journal.pmed.1002002.

[294] GAVI (2019). – Gavi Board starts framing Alliance's approach to 2021-2025 period. Available at: https://www.gavi.org/library/news/press-releases/2018/gavi-board-starts-framing-alliance-s-approach-to-2021-2025-period/ (accessed on 10 April 2019).

[295] Taylor L.H., Wallace R.M., Balaram D., Lindenmayer J.M., Eckery D.C., Mutonono-Watkiss B., Parravani E. & Nel L.H. (2017). – The Role of Dog Population Management in Rabies Elimination-A Review of Current Approaches and Future Opportunities. *Frontiers in veterinary science,* **4**, 109. doi: 10.3389/fvets.2017.00109.

[296] Obrist B., Iteba N., Lengeler C., Makemba A., Mshana C., Nathan R., Alba S., Dillip A., Hetzel M.W., Mayumana I., Schulze A. & Mshinda H. (2007). – Access to Health Care in Contexts of Livelihood Insecurity: A Framework for Analysis and Action. *PLOS Medicine,* **4** (10), e308. doi: 10.1371/journal.pmed.0040308.

[297] Zinsstag J., Bonfoh B., Cissé G., Nguyen Viet H., Silué B., N'Guessan T.S., Weibel D., Schertenleib R., Obrist B. & Tanner M. (2011a). – Towards Equity Effectiveness in Health Interventions. *In* NCCR North-South, Research for Sustainable Development: Foundations, Experiences, and Perspectives (U.W.a.H. Hurni, ed), Swiss National Centre of Competence in Research North-South, Switzerland.

[298] Mpolya E.A., Lembo T., Lushasi K., Mancy R., Mbunda E.M., Makungu S., Maziku M., Sikana L., Jaswant G., Townsend S., Meslin F.X., Abela-Ridder B., Ngeleja C., Changalucha J., Mtema Z., Sambo M., McHau G., Rysava K., Nanai A., Kazwala R., Cleaveland S. & Hampson K. (2017). – Toward Elimination of Dog-Mediated Human Rabies: Experiences from Implementing a Large-scale Demonstration Project in Southern Tanzania. *Frontiers in veterinary science,* **4**, 21. doi: 10.3389/fvets.2017.00021.

[299] LeRoux K., Stewart D., Perrett K.D., Nel L.H., Kessels J.A. & Abela-Ridder B. (2018). – Rabies control in KwaZulu-Natal, South Africa. *Bulletin of the World Health Organization,* **96** (5), 360-365. doi: 10.2471/BLT.17.194886.

[300] Gibson A.D., Handel I.G., Shervell K., Roux T., Mayer D., Muyila S., Maruwo G.B., Nkhulungo E.M.S., Foster R.A., Chikungwa P., Chimera B., Bronsvoort B.M.d., Mellanby R.J. & Gamble L. (2016). – The Vaccination of 35,000 Dogs in 20 Working Days Using Combined Static Point and Door-to-Door Methods in Blantyre, Malawi. *PLOS Neglected Tropical Diseases,* **10** (7), e0004824. doi: 10.1371/journal.pntd.0004824.

[301] Clarke L., Chalkidou K. & Cassandra Nemzoff C. (2019). – Development Impact Bonds Targeting Health Outcomes. *Journal*, Available at: https://www.cgdev.org/publication/development-impact-bonds-targeting-health-outcomes.

[302] Zinsstag J. (2013). – Towards a science of rabies elimination. *Infectious diseases of poverty,* **2** (1), 22. doi: 10.1186/2049-9957-2-22.

[303] Zinsstag J., Schelling E., Waltner-Toews D. & Tanner M. (2011). – From "one medicine" to "one health" and systemic approaches to health and well-being. *Preventive veterinary medicine,* **101**, 148-156. doi: 10.1016/j.prevetmed.2010.07.003.

[304] Taylor L.H., Hampson K., Fahrion A., Abela-Ridder B. & Nel L.H. (2015). – Difficulties in estimating the human burden of canine rabies. *Acta tropica,* doi: 10.1016/j.actatropica.2015.12.007.

[305] Dodet B. (2009). – The fight against rabies in Africa: From recognition to action. *Vaccine,* **27** (37), 5027-5032. doi: 10.1016/j.vaccine.2009.06.030.

[306] Food and Agriculture Organization of the United Nations (2012). – FAO taking steps to address rabies situation in DRC. Available at: http://www.fao.org/ag/againfo/home/en/news_archive/AGA_in_action/2012_FAO_address_rabies_situation_in_DRC.html (accessed on 22 July 2019).

[307] Mbilo C., Kabongo J.B., Pyana P.P., Nlonda L., Nzita R.W., Luntadila B., Badibanga B., Hattendorf J. & Zinsstag J. (2019). – Dog ecology, bite incidence and disease awareness : a cross-sectional survey among a rabies-affected community in Matadi, the Democratic Republic of the Congo. *Manuscript submitted for publication*.

[308] The World Bank (2018). – The World Bank in DRC. Available at: https://www.worldbank.org/en/country/drc/overview (accessed on 16 July 2019).

[309] Ministère du Plan et Suivi de la Mise en oeuvre de la Révolution de la Modernité (MPSMRM), Ministère de la Santé Publique (MSP) & ICF International (2014). – Enquête Démographique et de Santé en République Démocratique du Congo 2013-2014. *In*, Rockville, Maryland, USA: MPSMRM, MSP et ICF International.

[310] Wallace R.M., Reses H., Franka R., Dilius P., Fenelon N., Orciari L., Etheart M., Destine A., Crowdis K., Blanton J.D., Francisco C., Ludder F., Del Rio Vilas V., Haim J. & Millien M. (2015). – Establishment of a High Canine Rabies Burden in Haiti through the Implementation of a Novel Surveillance Program [corrected]. *PLoS Negl Trop Dis,* **9** (11), e0004245.

[311] World Health Organization (2010). – Rabies vaccines: WHO position paper-Recommendations. *Vaccine,* **28** (44), 7140-7142. doi: 10.1016/j.vaccine.2010.08.082.

[312] Dean D.J., Abelseth M.K. & Atanasiu P. (1996). – The fluorescent antibody test. *In* Laboratory techniques in rabies., Eds: F.X. Meslin, M.M. Kaplan & H. Koprowski, World Health Organization pp 88-89.

[313] Patrick E.M., Bjorklund B.M., Kirby J.D., Nelson K.M., Chipman R.B. & Rupprecht C.E. (2019). – Enhanced Rabies Surveillance Using a Direct Rapid Immunohistochemical Test. *JoVE,* (146), e59416. doi: doi:10.3791/59416.

[314] R Development Core Team (2010). – R: A language and environment for statistical computing. *In*, R Foundation for Statistical Computing, Vienna, Austria.

[315] Hampson K., Dobson A., Kaare M., Dushoff J., Magoto M., Sindoya E. & Cleaveland S. (2008). – Rabies Exposures, Post-Exposure Prophylaxis and Deaths in a Region of Endemic Canine Rabies. *PLOS Neglected Tropical Diseases,* **2** (11), e339. doi: 10.1371/journal.pntd.0000339.

[316] Beyene T.J., Mourits M.C.M., Kidane A.H. & Hogeveen H. (2018). – Estimating the burden of rabies in Ethiopia by tracing dog bite victims. *PloS one,* **13** (2), e0192313. doi: 10.1371/journal.pone.0192313.

[317] Tenzin, Dhand N.K., Gyeltshen T., Firestone S., Zangmo C., Dema C., Gyeltshen R. & Ward M.P. (2011). – Dog Bites in Humans and Estimating Human Rabies Mortality in Rabies Endemic Areas of Bhutan. *PLoS Neglected Tropical Diseases,* **5** (11), e1391. doi: 10.1371/journal.pntd.0001391.

[318] Rysava K., Miranda M.E., Zapatos R., Lapiz S., Rances P., Miranda L.M., Roces M.C., Friar J., Townsend S.E. & Hampson K. (2018). – On the path to rabies elimination: The need for risk assessments to improve administration of post-exposure prophylaxis. *Vaccine,* doi: 10.1016/j.vaccine.2018.11.066.

[319] Tepsumethanon V., Wilde H. & Meslin F.X. (2005). – Six criteria for rabies diagnosis in living dogs. *Journal of the Medical Association of Thailand = Chotmaihet thangphaet,* **88** (3), 419-422.

[320] Medley A.M., Millien M.F., Blanton J.D., Ma X., Augustin P., Crowdis K. & Wallace R.M. (2017). – Retrospective Cohort Study to Assess the Risk of Rabies in Biting Dogs, 2013⁻2015, Republic of Haiti. *Tropical medicine and infectious disease,* **2** (2), 14. doi: 10.3390/tropicalmed2020014.

[321] Bitek A., Osoro E., Munyua P., Nanyingi M., Muthiani Y., Kiambi S., Muturi M., Mwatondo A., Muriithi R., Cleaveland S., Hampson K., Njenga M., Kitala P. & Thumbi S. (2019). – A hundred years of rabies in Kenya and the strategy for eliminating dog-mediated rabies by

2030 [version 2; peer review: 4 approved]. *AAS Open Research,* **1** (23) doi: 10.12688/aasopenres.12872.2.

[322] Hikufe E.H., Freuling C.M., Athingo R., Shilongo A., Ndevaetela E.-E., Helao M., Shiindi M., Hassel R., Bishi A., Khaiseb S., Kabajani J., van der Westhuizen J., Torres G., Britton A., Letshwenyo M., Schwabenbauer K., Mettenleiter T.C., Denzin N., Amler S., Conraths F.J., Müller T. & Maseke A. (2019). – Ecology and epidemiology of rabies in humans, domestic animals and wildlife in Namibia, 2011-2017. *PLOS Neglected Tropical Diseases,* **13** (4), e0007355. doi: 10.1371/journal.pntd.0007355.

[323] Rajeev M., Edosoa G., Hanitriniaina C., Andriamandimby S.F., Guis H., Ramiandrasoa R., Ratovoson R., Randrianasolo L., Andriamananjara M., Heraud J.-M., Baril L., Metcalf C.J.E. & Hampson K. (2018). – Healthcare utilization, provisioning of post-exposure prophylaxis, and estimation of human rabies burden in Madagascar. *Vaccine,* doi: https://doi.org/10.1016/j.vaccine.2018.11.011.

[324] Hampson K., Dushoff J., Cleaveland S., Haydon D.T., Kaare M., Packer C. & Dobson A. (2009). – Transmission dynamics and prospects for the elimination of canine rabies. *PLoS biology,* **7** (3), e53. doi: 10.1371/journal.pbio.1000053.

[325] Zinsstag J., Lechenne M., Laager M., Mindekem R., Naissengar S., Oussiguere A., Bidjeh K., Rives G., Tessier J., Madjaninan S., Ouagal M., Moto D.D., Alfaroukh I.O., Muthiani Y., Traore A., Hattendorf J., Lepelletier A., Kergoat L., Bourhy H., Dacheux L., Stadler T. & Chitnis N. (2017). – Vaccination of dogs in an African city interrupts rabies transmission and reduces human exposure. *Science translational medicine,* **9** (421) doi: 10.1126/scitranslmed.aaf6984.

[326] Laager M., Mbilo C., Madaye E.A., Naminou A., Lechenne M., Tschopp A., Naissengar S.K., Smieszek T., Zinsstag J. & Chitnis N. (2018). – The importance of dog population contact network structures in rabies transmission. *PLoS neglected tropical diseases,* **12** (8), e0006680. doi: 10.1371/journal.pntd.0006680.

[327] Davlin S.L. & Vonville H.M. (2012). – Canine rabies vaccination and domestic dog population characteristics in the developing world: a systematic review. *Vaccine,* **30** (24), 3492-3502. doi: 10.1016/j.vaccine.2012.03.069.

[328] Kaare M., Lembo T., Hampson K., Ernest E., Estes A., Mentzel C. & Cleaveland S. (2009). – Rabies control in rural Africa: Evaluating strategies for effective domestic dog vaccination. *Vaccine,* **27** (1), 152-160. doi: 10.1016/j.vaccine.2008.09.054.

[329] Cleaveland S., Kaare M., Tiringa P., Mlengeya T. & Barrat J. (2003). – A dog rabies vaccination campaign in rural Africa: impact on the incidence of dog rabies and human dog-bite injuries. *Vaccine,* **21** (17-18), 1965-1973.

[330] Ly S., Buchy P., Heng N.Y., Ong S., Chhor N., Bourhy H. & Vong S. (2009). – Rabies situation in Cambodia. *PLoS neglected tropical diseases,* **3** (9), e511.

[331] Global Alliance for Rabies Control (2012). – Rabies in the Democratic Republic of Congo. Available at: https://rabiesalliance.org/resource/rabies-democratic-republic-congo.

[332] World Health Organization. Veterinary Public Health Unit (1988). – Report of WHO Consultation on Dog Ecology Studies Related to Rabies Control. *In* WHO Consultation on Dog Ecology Studies Related to Rabies Control, World Health Organization, Geneva.

[333] World Health Organization. Veterinary Public Health Unit (1990). – Guide pour la Gestion des Populations Canines.

[334] Schelling E. & Hattendorf J. (2015). – One Healh Study Designs. *In* One Health: the theory and practice of integrated health approaches (J. Zinsstag, E. Schelling, D. Waltner-Toews, M. Whittaker & M. Tanner, eds), CAB International, UK.

[335] Mindekem R., Kayali U., Yemadji N., Ndoutamia A.G. & Zinsstag J. (2005). – [Impact of canine demography on rabies transmission in N'djamena, Chad]. *Medecine tropicale : revue du Corps de sante colonial,* **65** (1), 53-58.

[336] Gsell A.S., Knobel D.L., Kazwala R.R., Vounatsou P. & Zinsstag J. (2012). – Domestic dog demographic structure and dynamics relevant to rabies control planning in urban areas in Africa: the case of Iringa, Tanzania. *BMC veterinary research,* **8**, 236. doi: 10.1186/1746-6148-8-236.

[337] Mickey R.M. & Greenland S. (1989). – The impact of confounder selection criteria on effect estimation. *Am J Epidemiol,* **129** (1), 125-137. doi: 10.1093/oxfordjournals.aje.a115101.

[338] World Health Organization & UNICEF (2013). – Progress on sanitation and drinking-water. Available at: https://www.who.int/iris/bitstream/10665/81245/1/9789241505390_eng.pdf?ua=1 (accessed on 04 July 2019).

[339] Knobel D.L., Laurenson M.K., Kazwala R.R., Boden L.A. & Cleaveland S. (2008). – A cross-sectional study of factors associated with dog ownership in Tanzania. *BMC veterinary research,* **4**, 5. doi: 10.1186/1746-6148-4-5.

[340] De Balogh K.K., Wandeler A.I. & Meslin F.X. (1993). – A dog ecology study in an urban and a semi-rural area of Zambia. *The Onderstepoort journal of veterinary research,* **60** (4), 437-443.

[341] Conan A., Akerele O., Simpson G., Reininghaus B., van Rooyen J. & Knobel D. (2015). – Population Dynamics of Owned, Free-Roaming Dogs: Implications for Rabies Control. *PLoS Negl Trop Dis,* **9** (11), e0004177. doi: 10.1371/journal.pntd.0004177.

[342] Tschopp R., Bekele S. & Aseffa A. (2016). – Dog Demography, Animal Bite Management and Rabies Knowledge-Attitude and Practices in the Awash Basin, Eastern Ethiopia. *PLoS Negl Trop Dis,* **10** (2), e0004471. doi: 10.1371/journal.pntd.0004471.

[343] Ratsitorahina M., Rasambainarivo J.H., Raharimanana S., Rakotonandrasana H., Andriamiarisoa M.P., Rakalomanana F.A. & Richard V. (2009). – Dog ecology and demography in Antananarivo, 2007. *BMC veterinary research,* **5**, 21. doi: 10.1186/1746-6148-5-21.

[344] Sambo M., Hampson K., Changalucha J., Cleaveland S., Lembo T., Lushasi K., Mbunda E., Mtema Z., Sikana L. & Johnson P.C.D. (2018). – Estimating the Size of Dog Populations in Tanzania to Inform Rabies Control. *Veterinary sciences,* **5** (3) doi: 10.3390/vetsci5030077.

[345] Jibat T., Hogeveen H. & Mourits M.C. (2015). – Review on dog rabies vaccination coverage in Africa: a question of dog accessibility or cost recovery? *PLoS Negl Trop Dis,* **9** (2), e0003447. doi: 10.1371/journal.pntd.0003447.

[346] Mauti S., Traoré A., Sery A., Bryssinckx W., Hattendorf J. & Zinsstag J. (2017). – First study on domestic dog ecology, demographic structure and dynamics in Bamako, Mali. *Preventive Veterinary Medicine,* **146** (Supplement C), 44-51. doi: https://doi.org/10.1016/j.prevetmed.2017.07.009.

[347] Otolorin G.R., Umoh J.U. & Dzikwi A.A. (2014). – Demographic and ecological survey of dog population in aba, abia state, Nigeria. *ISRN veterinary science,* **2014**, 806849. doi: 10.1155/2014/806849.

[348] Hanemann M., Loomis J. & Kanninen B. (1991). – Statistical Efficiency of Double-Bounded Dichotomous Choice Contingent Valuation. *American Journal of Agricultural Economics,* **73** (4), 1255-1263. doi: 10.2307/1242453.

[349] Morters M.K., McKinley T.J., Restif O., Conlan A.J., Cleaveland S., Hampson K., Whay H.R., Damriyasa I.M. & Wood J.L. (2014). – The demography of free-roaming dog populations and applications to disease and population control. *The Journal of applied ecology,* **51** (4), 1096-1106. doi: 10.1111/1365-2664.12279.

[350] Brooks R. (1990). – Survey of the dog population of Zimbabwe and its level of rabies vaccination. *Vet Rec,* **127** (24), 592-596.

[351] Kitala P., McDermott J., Kyule M., Gathuma J., Perry B. & Wandeler A. (2001). – Dog ecology and demography information to support the planning of rabies control in Machakos District, Kenya. *Acta tropica,* **78** (3), 217-230.

[352] Czupryna A.M., Brown J.S., Bigambo M.A., Whelan C.J., Mehta S.D., Santymire R.M., Lankester F.J. & Faust L.J. (2016). – Ecology and Demography of Free-Roaming Domestic Dogs in Rural Villages near Serengeti National Park in Tanzania. *PloS one,* **11** (11), e0167092.

[353] Fèvre E.M., Kaboyo R.W., Persson V., Edelsten M., Coleman P.G. & Cleaveland S. (2005). – The epidemiology of animal bite injuries in Uganda and projections of the burden of rabies. *Tropical medicine & international health : TM & IH,* **10** (8), 790-798. doi: 10.1111/j.1365-3156.2005.01447.x.

[354] Salomão C., Nacima A., Cuamba L., Gujral L., Amiel O., Baltazar C., Cliff J. & Gudo E.S. (2017). – Epidemiology, clinical features and risk factors for human rabies and animal bites during an outbreak of rabies in Maputo and Matola cities, Mozambique, 2014: Implications for public health interventions for rabies control. *PLoS neglected tropical diseases,* **11** (7), e0005787-e0005787. doi: 10.1371/journal.pntd.0005787.

[355] Mustiana A., Toribio J.A., Abdurrahman M., Suadnya I.W., Hernandez-Jover M., Putra A.A. & Ward M.P. (2015). – Owned and unowned dog population estimation, dog management and dog bites to inform rabies prevention and response on Lombok Island, Indonesia. *PloS one,* **10** (5), e0124092. doi: 10.1371/journal.pone.0124092.

[356] Sudarshan M.K., Mahendra B.J., Madhusudana S.N., Ashwath Narayana D.H., Rahman A., Rao N.S.N., Meslin F.X., Lobo D., Ravikumar K. & Gangaboraiah (2006). – An epidemiological study of animal bites in India: Results of a who sponsored national multi-centric rabies survey. *Journal of Communicable Diseases,* **38** (1), 32-39.

[357] Overall K.L. & Love M. (2001). – Dog bites to humans--demography, epidemiology, injury, and risk. *J Am Vet Med Assoc,* **218** (12), 1923-1934.

[358] Rosado B., Garcia-Belenguer S., Leon M. & Palacio J. (2009). – A comprehensive study of dog bites in Spain, 1995-2004. *Vet J,* **179** (3), 383-391. doi: 10.1016/j.tvjl.2008.02.002.

[359] Westgarth C., Brooke M. & Christley R.M. (2018). – How many people have been bitten by dogs? A cross-sectional survey of prevalence, incidence and factors associated with dog bites in a UK community. *Journal of epidemiology and community health,* **72** (4), 331-336. doi: 10.1136/jech-2017-209330.

[360] Esmaeilzadeh F., Rajabi A., Vahedi S., Shamsadiny M., Ghelichi Ghojogh M. & Hatam N. (2017). – Epidemiology of Animal Bites and Factors Associated With Delays in Initiating Post-exposure Prophylaxis for Rabies Prevention Among Animal Bite Cases: A Population-based Study. *J Prev Med Public Health,* **50** (3), 210-216.

[361] Shen J., Rouse J., Godbole M., Wells H.L., Boppana S. & Schwebel D.C. (2017). – Systematic Review: Interventions to Educate Children About Dog Safety and Prevent Pediatric Dog-Bite Injuries: A Meta-Analytic Review. *Journal of pediatric psychology,* **42** (7), 779-791. doi: 10.1093/jpepsy/jsv164.

[362] Duperrex O., Blackhall K., Burri M. & Jeannot E. (2009). – Education of children and adolescents for the prevention of dog bite injuries. *The Cochrane database of systematic reviews,* (2), Cd004726. doi: 10.1002/14651858.CD004726.pub2.

[363] Auplish A., Clarke A.S., Van Zanten T., Abel K., Tham C., Bhutia T.N., Wilks C.R., Stevenson M.A. & Firestone S.M. (2017). – Estimating the intra-cluster correlation coefficient for evaluating an educational intervention program to improve rabies awareness and dog bite prevention among children in Sikkim, India: A pilot study. *Acta tropica,* **169**, 62-68. doi: https://doi.org/10.1016/j.actatropica.2016.12.032.

[364] Burdon Bailey J.L., Gamble L., Gibson A.D., Bronsvoort B.M.d., Handel I.G., Mellanby R.J. & Mazeri S. (2018). – A rabies lesson improves rabies knowledge amongst primary school children in Zomba, Malawi. *PLoS neglected tropical diseases,* **12** (3), e0006293-e0006293. doi: 10.1371/journal.pntd.0006293.

[365] Deray R., Rivera C., Gripon S., Ulanday C., Roces M.C., Amparo A.C., Attlan M., Demont C., Kieffer A. & Miranda M.E. (2018). – Protecting children from rabies with education and pre-exposure prophylaxis: A school-based campaign in El Nido, Palawan, Philippines. *PloS one,* **13** (1), e0189596. doi: 10.1371/journal.pone.0189596.

[366] Keita Z. G., F., Léchenne, M., Thiero, O., Hattendorf, J., Zinsstag, J., Traoré, A., Traoré, A.K. (2019). – Burden of rabies in Mali. *Manuscript submitted for publication*.

[367] Kallo V. (2019). – Estimation of dog population and exposure to bites in Côte d'Ivoire. *Manuscript submitted for publication*.

[368] Rumana R., Sayeed A.A., Basher A., Islam Z., Rahman M.R. & Faiz M.A. (2013). – Perceptions and treatment seeking behavior for dog bites in rural Bangladesh. *The Southeast Asian journal of tropical medicine and public health,* **44** (2), 244-248.

[369] Ponsich A., Goutard F., Sorn S. & Tarantola A. (2016). – A prospective study on the incidence of dog bites and management in a rural Cambodian, rabies-endemic setting. *Acta tropica,* **160**, 62-67. doi: 10.1016/j.actatropica.2016.04.015.

[370] Sharma S., Agarwal A., Khan A.M. & Ingle G.K. (2016). – Prevalence of Dog Bites in Rural and Urban Slums of Delhi: A Community-based Study. *Annals of medical and health sciences research,* **6** (2), 115-119. doi: 10.4103/2141-9248.181836.

[371] Murugan V., Amol D. & Kalaiselvan G. (2016). – A Community based cross sectional study of dog bites in children in a rural district of Tamil Nadu. *International Journal of Medical Science and Public Health*.

[372] Digafe R.T., Kifelew L.G. & Mechesso A.F. (2015). – Knowledge, attitudes and practices towards rabies: questionnaire survey in rural household heads of Gondar Zuria District, Ethiopia. *BMC Res Notes,* **8**, 400. doi: 10.1186/s13104-015-1357-8.

[373] Hergert M., Le Roux K. & Nel L.H. (2016). – Risk factors associated with nonvaccination rabies status of dogs in KwaZulu-Natal, South Africa. *Vet. Med.-Res. Rep,* **7**, 75-83. doi: 10.2147/vmrr.s103859.

[374] Sambo M., Lembo T., Cleaveland S., Ferguson H.M., Sikana L., Simon C., Urassa H. & Hampson K. (2014). – Knowledge, attitudes and practices (KAP) about rabies prevention and control: a community survey in Tanzania. *PLoS Negl Trop Dis,* **8** (12), e3310. doi: 10.1371/journal.pntd.0003310.

[375] Hergert M. & Nel L.H. (2013). – Dog Bite Histories and Response to Incidents in Canine Rabies-Enzootic KwaZulu-Natal, South Africa. *PLOS Neglected Tropical Diseases,* **7** (4), e2059. doi: 10.1371/journal.pntd.0002059.

[376] Jemberu W.T., Molla W., Almaw G. & Alemu S. (2013). – Incidence of rabies in humans and domestic animals and people's awareness in North Gondar Zone, Ethiopia. *PLoS neglected tropical diseases,* **7**, e2216. doi: 10.1371/journal.pntd.0002216.

[377] Hampson K., Coudeville L., Lembo T., Sambo M., Kieffer A., Attlan M., Barrat J., Blanton J.D., Briggs D.J., Cleaveland S., Costa P., Freuling C.M., Hiby E., Knopf L., Leanes F., Meslin F.X., Metlin A., Miranda M.E., Müller T., Nel L.H., Recuenco S., Rupprecht C.E., Schumacher C., Taylor L., Vigilato M.A., Zinsstag J., Dushoff J. & Prevention G.A.f.R.C.P.f.R. (2015). – Estimating the global burden of endemic canine rabies. *PLoS Negl Trop Dis,* **9** (4), e0003709. doi: 10.1371/journal.pntd.0003709.

[378] Zinsstag J., Dürr S., Penny M.A., Mindekem R., Roth F., Menendez Gonzalez S., Naissengar S. & Hattendorf J. (2009). – Transmission dynamics and economics of rabies control in dogs and humans in an African city. *Proc Natl Acad Sci U S A,* **106** (35), 14996-15001. doi: 10.1073/pnas.0904740106.

[379] Zinsstag J., Lechenne M., Laager M., Mindekem R., Naïssengar S., Oussiguéré A., Bidjeh K., Rives G., Tessier J., Madjaninan S., Ouagal M., Moto D.D., Alfaroukh I.O., Muthiani Y., Traoré A., Hattendorf J., Lepelletier A., Kergoat L., Bourhy H., Dacheux L., Stadler T. & Chitnis N. (2017). – Vaccination of dogs in an African city interrupts rabies transmission and reduces human exposure. *Sci Transl Med,* **9** (421) doi: 10.1126/scitranslmed.aaf6984.

[380] Léchenne M., Oussiguere A., Naissengar K., Mindekem R., Mosimann L., Rives G., Hattendorf J., Moto D.D., Alfaroukh I.O., Zinsstag J. & committee t. (2016). – Operational performance and analysis of two rabies vaccination campaigns in N'Djamena, Chad. *Vaccine,* **34** (4), 571-577. doi: 10.1016/j.vaccine.2015.11.033.

[381] Laager M., Léchenne M., Naissengar K., Mindekem R., Oussiguere A., Zinsstag J. & Chitnis N. (2019). – A metapopulation model of dog rabies transmission in N'Djamena, Chad. *Journal of theoretical biology,* **462**, 408-417. doi: 10.1016/j.jtbi.2018.11.027.

[382] Smieszek T., Fiebig L. & Scholz R.W. (2009). – Models of epidemics: when contact repetition and clustering should be included. *Theor Biol Med Model,* **6**, 11. doi: 10.1186/1742-4682-6-11.

[383] Newman M.E.J. (2006). – The Structure and Function of Complex Networks. *http://dx.doi.org/10.1137/S003614450342480,* doi: 10.1137/S003614450342480.

[384] Pastor-Satorras R., Castellano C., Van Mieghem P. & Alessandro V. (2019). – Rev. Mod. Phys. 87, 925 (2015) - Epidemic processes in complex networks. doi: doi:10.1103/RevModPhys.87.925.

[385] Silk M.J., Croft D.P., Delahay R.J., Hodgson D.J., Weber N., Boots M. & McDonald R.A. (2017). – The application of statistical network models in disease research. *Methods in Ecology and Evolution,* **8** (9), 1026-1041. doi: 10.1111/2041-210x.12770.

[386] Andersson H. (1999). – Epidemic models and social networks. *Mathematical Scientist,* **24** (2), 128-147.

[387] Pastor-Satorras R. & Vespignani A. (2001). – Epidemic spreading in scale-free networks. *Physical review letters,* **86** (14), 3200.

[388] Barclay V.C., Smieszek T., He J., Cao G., Rainey J.J., Gao H., Uzicanin A. & Salathé M. (2014). – Positive network assortativity of influenza vaccination at a high school: implications for outbreak risk and herd immunity. *PloS one,* **9** (2), e87042.

[389] Meyers L.A., Pourbohloul B., Newman M.E., Skowronski D.M. & Brunham R.C. (2005). – Network theory and SARS: predicting outbreak diversity. *Journal of theoretical biology,* **232** (1), 71-81.

[390] Morris M. & Kretzschmar M. (1995). – Concurrent partnerships and transmission dynamics in networks. *Social Networks,* **17** (3-4), 299-318.

[391] Yorke J.A., Hethcote H.W. & Nold A. (1978). – Dynamics and control of the transmission of gonorrhea. *Sex Transm Dis,* **5** (2), 51-56.

[392] Cohen R., Havlin S. & Ben-Avraham D. (2003). – Efficient immunization strategies for computer networks and populations. *Physical review letters,* **91** (24), 247901.

[393] Kretzschmar M., van Duynhoven Y.T. & Severijnen A.J. (1996). – Modeling prevention strategies for gonorrhea and chlamydia using stochastic network simulations. *Am. J. Epidemiol.,* **144** (3), 306-317.

[394] Greenhalgh D. (1986). – Optimal control of an epidemic by ring vaccination. *Stochastic Models,* **2** (3), 339-363.

[395] Müller J., Schönfisch B. & Kirkilionis M. (2000). – Ring vaccination. *Journal of mathematical biology,* **41** (2), 143-171.

[396] Wallinga J., Teunis P. & Kretzschmar M. (2006). – Using data on social contacts to estimate age-specific transmission parameters for respiratory-spread infectious agents. *Am. J. Epidemiol.,* **164** (10), 936-944.

[397] Mossong J., Hens N., Jit M., Beutels P., Auranen K., Mikolajczyk R., Massari M., Salmaso S., Tomba G.S. & Wallinga J. (2008). – Social contacts and mixing patterns relevant to the spread of infectious diseases. *PLoS medicine,* **5** (3), e74.

[398] Smieszek T. (2009). – A mechanistic model of infection: why duration and intensity of contacts should be included in models of disease spread. *Theoretical Biology and Medical Modelling,* **6** (1), 25.

[399] Stehlé J., Voirin N., Barrat A., Cattuto C., Isella L., Pinton J.-F., Quaggiotto M., Van den Broeck W., Régis C. & Lina B. (2011). – High-resolution measurements of face-to-face contact patterns in a primary school. *PloS one,* **6** (8), e23176.

[400] Cattuto C., Van den Broeck W., Barrat A., Colizza V., Pinton J.-F. & Vespignani A. (2010). – Dynamics of Person-to-Person Interactions from Distributed RFID Sensor Networks. *PloS one,* **5** (7), e11596. doi: 10.1371/journal.pone.0011596.

[401] Salathé M., Kazandjieva M., Lee J.W., Levis P., Feldman M.W. & Jones J.H. (2010). – A high-resolution human contact network for infectious disease transmission. *Proceedings of the National Academy of Sciences,* **107** (51), 22020-22025.

[402] Machens A., Gesualdo F., Rizzo C., Tozzi A.E., Barrat A. & Cattuto C. (2013). – An infectious disease model on empirical networks of human contact: bridging the gap between dynamic network data and contact matrices. *BMC infectious diseases,* **13** (1), 185.

[403] Rainey J.J., Cheriyadat A., Radke R.J., Crumly J.S. & Koch D.B. (2014). – Estimating contact rates at a mass gathering by using video analysis: a proof-of-concept project. *BMC public health,* **14** (1), 1101.

[404] Read J., Edmunds W., Riley S., Lessler J. & Cummings D. (2012). – Close encounters of the infectious kind: methods to measure social mixing behaviour. *Epidemiology & infection,* **140** (12), 2117-2130.

[405] White L.A., Forester J.D. & Craft M.E. (2017). – Using contact networks to explore mechanisms of parasite transmission in wildlife. *Biological Reviews,* **92** (1), 389-409.

[406] White P.C., Harris S. & Smith G.C. (1995). – Fox contact behaviour and rabies spread: a model for the estimation of contact probabilities between urban foxes at different population densities and its implications for rabies control in Britain. *Journal of Applied Ecology*, 693-706.

[407] Hirsch B.T., Prange S., Hauver S.A. & Gehrt S.D. (2013). – Raccoon social networks and the potential for disease transmission. *PLoS One,* **8** (10), e75830.

[408] Reynolds J.J., Hirsch B.T., Gehrt S.D. & Craft M.E. (2015). – Raccoon contact networks predict seasonal susceptibility to rabies outbreaks and limitations of vaccination. *Journal of Animal Ecology,* **84** (6), 1720-1731.

[409] Dürr S. & Ward M.P. (2015). – Development of a novel rabies simulation model for application in a non-endemic environment. *PLoS neglected tropical diseases,* **9** (6), e0003876.

[410] Johnstone-Robertson S.P., Fleming P.J., Ward M.P. & Davis S.A. (2017). – Predicted spatial spread of canine rabies in Australia. *PLoS neglected tropical diseases,* **11** (1), e0005312.

[411] Singh R., Singh K.P., Cherian S., Saminathan M., Kapoor S., Manjunatha Reddy G., Panda S. & Dhama K. (2017). – Rabies–epidemiology, pathogenesis, public health concerns and advances in diagnosis and control: a comprehensive review. *Veterinary Quarterly,* **37** (1), 212-251.

[412] Britton T., Janson S. & Martin-Löf A. (2007). – Graphs with specified degree distributions, simple epidemics, and local vaccination strategies. *Advances in Applied Probability,* **39** (4), 922-948.

[413] Blondel V.D., Guillaume J.-L., Lambiotte R. & Lefebvre E. (2008). – Fast unfolding of communities in large networks. *Journal of statistical mechanics: theory and experiment,* **2008** (10), P10008.

[414] Albert R., Jeong H. & Barabási A.-L. (2000). – Error and attack tolerance of complex networks. *Nature,* **406** (6794), 378.

[415] Smieszek T. & Salathé M. (2013). – A low-cost method to assess the epidemiological importance of individuals in controlling infectious disease outbreaks. *BMC medicine,* **11** (1), 35.

[416] Wu J., Dhingra R., Gambhir M. & Remais J.V. (2013). – Sensitivity analysis of infectious disease models: methods, advances and their application. *Journal of The Royal Society Interface,* **10** (86), 20121018.

[417] Estrada R., Vos A. & De Leon R. (2001). – Acceptability of local made baits for oral vaccination of dogs against rabies in the Philippines. *BMC infectious diseases,* **1** (1), 19.

[418] WHO (2018). – WHO Expert Consultation on Rabies: Third Report. *In*, World Health Organization.

[419] Smith T.G., Millien M., Vos A., Fracciterne F.A., Crowdis K., Chirodea C., Medley A., Chipman R., Qin Y. & Blanton J. (2017). – Evaluation of immune responses in dogs to oral rabies vaccine under field conditions. *Vaccine*.

[420] Freuling C.M., Hampson K., Selhorst T., Schröder R., Meslin F.X., Mettenleiter T.C. & Müller T. (2013). – The elimination of fox rabies from Europe: determinants of success and lessons for the future. *Philosophical Transactions of the Royal Society B: Biological Sciences,* **368** (1623), 20120142.

[421] Newman M.E. (2002). – Spread of epidemic disease on networks. *Physical review. E, Statistical, nonlinear, and soft matter physics,* **66** (1 Pt 2), 016128. doi: 10.1103/PhysRevE.66.016128.

[422] Lloyd-Smith J.O., Schreiber S.J., Kopp P.E. & Getz W.M. (2005). – Superspreading and the effect of individual variation on disease emergence. *Nature,* **438** (7066), 355-359. doi: 10.1038/nature04153.

[423] May R.M. & Lloyd A.L. (2001). – Infection dynamics on scale-free networks. *Physical Review E,* **64** (6), 066112.

[424] Kranstauber B., Kays R., Lapoint S.D., Wikelski M. & Safi K. (2012). – A dynamic Brownian bridge movement model to estimate utilization distributions for heterogeneous animal movement. *J Anim Ecol,* **81** (4), 738-746. doi: 10.1111/j.1365-2656.2012.01955.x.

[425] Dürr S., Dhand N.K., Bombara C., Molloy S. & Ward M.P. (2017). – What influences the home range size of free-roaming domestic dogs? *Epidemiology and infection,* **145** (7), 1339-1350. doi: 10.1017/s095026881700022x.

[426] Van Kesteren F., Mastin A., Mytynova B., Ziadinov I., Boufana B., Torgerson P.R., Rogan M.T. & Craig P.S. (2013). – Dog ownership, dog behaviour and transmission of Echinococcus spp. in the Alay Valley, southern Kyrgyzstan. *Parasitology,* **140** (13), 1674-1684. doi: 10.1017/s0031182013001182.

[427] Meek P. (1999). – The movement, roaming behavior and home range of free-roaming domestic dogs, Canis lupus familiaris, in coastal New South Wales.

[428] Hudson E.G., Brookes V.J., Durr S. & Ward M.P. (2017). – Domestic dog roaming patterns in remote northern Australian indigenous communities and implications for disease modelling. *Prev Vet Med,* **146**, 52-60. doi: 10.1016/j.prevetmed.2017.07.010.

[429] Banyard A.C. & Fooks A.R. (2011). – Oxford Textbook of Zoonoses: Biology, Clinical Practice, and Public Health Control. *In* Rabies and rabies-related lyssaviruses, Oxford University Press.

[430] World Health Organization (2006). – The control of neglected zoonotic diseases : a route to poverty alleviation, report of a joint WHO/DFID-AHP meeting, 20 and 21 September 2005, with the participation of FAO and OIE.

[431] World Health Organization (2014). – Rabies: Epidemiology and burden of disease. Available at: https://www.who.int/rabies/epidemiology/en/.

[432] Global Alliance for Rabies Control (2016). – Africa rabies costs. Available at: https://rabiesalliance.org/media/photo-gallery/africa-rabies-costs.

[433] World Health Organization (2015). – Investing to overcome the global impact of neglected tropical diseases. Third report on neglected tropical diseases. Available at: apps.who.int/iris/bitstream/10665/152781/1/9789241564861_eng.pdf.

[434] Nel L.H. (2013). – Factors Impacting the Control of Rabies. *Microbiology spectrum,* **1** (2) doi: 10.1128/microbiolspec.OH-0006-2012.

[435] World Health Organization (2010). – The control of neglected zoonotic diseases: community based interventions for NZDs prevention and control, report of the third conference organized with ICONZ, DFID-RiU, SOS , EU,TDR and FAO with the participation of ILRI and OIE; 23–24 November 2010.

[436] Rupprecht C.E., Hanlon C.A. & Hemachudha T. (2002). – Rabies re-examined. *Lancet Infect Dis,* **2** (6), 327-343.

[437] Coetzer A., Sabeta C.T., Markotter W., Rupprecht C.E. & Nel L.H. (2014). – Comparison of biotinylated monoclonal and polyclonal antibodies in an evaluation of a direct rapid immunohistochemical test for the routine diagnosis of rabies in southern Africa. *PLoS Negl Trop Dis,* **8** (9), e3189. doi: 10.1371/journal.pntd.0003189.

[438] Weyer J. & Blumberg L. (2007). – Rabies: Challenge of Diagnosis in Resource Poor Countries. *Infect Dis J Pak,* **16**.

[439] World Organisation for Animal Health (OIE) (2012). – Rabies. *In* Manual of Diagnostic Tests and Vaccines for Terrestrial Animals.

[440] United Nations (2016). – About the Sustainable Development Goals. Available at: https://www.un.org/sustainabledevelopment/sustainable-development-goals/.

[441] Zinsstag J., Schelling E., Wyss K. & Mahamat M.B. (2005). – Potential of cooperation between human and animal health to strengthen health systems. *The Lancet,* **366** (9503), 2142-2145. doi: 10.1016/S0140-6736(05)67731-8.

[442] World Health Organization (2005). – Expert Consultation on Rabies. First report. *Technical Report Series*.

[443] Léchenne M., Miranda M.E. & Zinsstag J. (2015). – Integrated Rabies Control. *In* One Health: the theory and practice of integrated health approaches (J. Zinsstag, E. Schelling, D. Waltner-Toews, M. Whittaker & M. Tanner, eds), CABI, Wallingford.

[444] Klepac P., Metcalf C.J.E., McLean A.R. & Hampson K. (2013). – Towards the endgame and beyond: complexities and challenges for the elimination of infectious diseases.

Philosophical transactions of the Royal Society of London. Series B, Biological sciences, **368** (1623), 20120137-20120137. doi: 10.1098/rstb.2012.0137.

[445] Hampson K., Dushoff J., Bingham J., Brückner G., Ali Y.H. & Dobson A. (2007). – Synchronous cycles of domestic dog rabies in sub-Saharan Africa and the impact of control efforts. *Proceedings of the National Academy of Sciences,* **104** (18), 7717-7722. doi: 10.1073/pnas.0609122104.

[446] Mauti S., Traore A., Hattendorf J., Schelling E., Wasniewski M., Schereffer J.L., Zinsstag J. & Cliquet F. (2015). – Factors associated with dog rabies immunisation status in Bamako, Mali. *Trop. Med. Int. Health,* **20**, 393-393.

[447] Vigilato M.A., Clavijo A., Knobl T., Silva H.M., Cosivi O., Schneider M.C., Leanes L.F., Belotto A.J. & Espinal M.A. (2013). – Progress towards eliminating canine rabies: policies and perspectives from Latin America and the Caribbean. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences,* **368** (1623), 20120143. doi: 10.1098/rstb.2012.0143.

[448] Bardosh K., Sambo M., Sikana L., Hampson K. & Welburn S.C. (2014). – Eliminating Rabies in Tanzania? Local Understandings and Responses to Mass Dog Vaccination in Kilombero and Ulanga Districts. *PLOS Neglected Tropical Diseases,* **8** (6), e2935. doi: 10.1371/journal.pntd.0002935.

[449] World Health Organization (1987). – Guidelines for dog rabies control. *In*, World Health Organization, Geneva.

[450] World Health Organization (2013). – WHO Expert Consultation on Rabies. Second Report. *Technical Report Series*.

[451] Brunker K., Hampson K., Horton D.L. & Biek R. (2012). – Integrating the landscape epidemiology and genetics of RNA viruses: rabies in domestic dogs as a model. *Parasitology,* **139** (14), 1899-1913. doi: 10.1017/s003118201200090x.

[452] Lembo T., Attlan M., Bourhy H., Cleaveland S., Costa P., de Balogh K., Dodet B., Fooks A.R., Hiby E., Leanes F., Meslin F., #xE7, ois-Xavier, Miranda M.E., #xFC, ller T., Nel L.H., Rupprecht C.E., Tordo N., #xEB, Tumpey A., Wandeler A. & Briggs D.J. (2011). – Renewed Global Partnerships and Redesigned Roadmaps for Rabies Prevention and Control. *Veterinary Medicine International,* **2011**, 18. doi: 10.4061/2011/923149.

[453] Russell C.A., Smith D.L., Childs J.E. & Real L.A. (2005). – Predictive spatial dynamics and strategic planning for raccoon rabies emergence in Ohio. *PLoS biology,* **3** (3), e88. doi: 10.1371/journal.pbio.0030088.

[454] Townsend S.E., Lembo T., Cleaveland S., Meslin F.X., Miranda M.E., Putra A.A., Haydon D.T. & Hampson K. (2013). – Surveillance guidelines for disease elimination: a case study of canine rabies. *Comparative immunology, microbiology and infectious diseases,* **36** (3), 249-261. doi: 10.1016/j.cimid.2012.10.008.

[455] Rupprecht C., Kuzmin I. & Meslin F. (2017). – Lyssaviruses and rabies: current conundrums, concerns, contradictions and controversies. *F1000Research,* **6**, 184. doi: 10.12688/f1000research.10416.1.

[456] Walker P.J., Blasdell K.R., Calisher C.H., Dietzgen R.G., Kondo H., Kurath G., Longdon B., Stone D.M., Tesh R.B., Tordo N., Vasilakis N., Whitfield A.E. & Ictv Report C. (2018). – ICTV Virus Taxonomy Profile: Rhabdoviridae. *The Journal of general virology,* **99** (4), 447-448. doi: 10.1099/jgv.0.001020.

[457] Welburn S.C., Beange I., Ducrotoy M.J. & Okello A.L. (2015). – The neglected zoonoses—the case for integrated control and advocacy. *Clinical Microbiology and Infection,* **21** (5), 433-443. doi: https://doi.org/10.1016/j.cmi.2015.04.011.

[458] Dacheux L. & Bourhy H. (2018). – Diagnostic tests for human rabies. *Revue scientifique et technique (International Office of Epizootics),* **37** (2), 581-593. doi: 10.20506/rst.37.2.2826.

[459] Kasempimolporn S., Saengseesom W., Lumlertdacha B. & Sitprija V. (2000). – Detection of rabies virus antigen in dog saliva using a latex agglutination test. *Journal of clinical microbiology,* **38** (8), 3098-3099.

[460] Kang B., Oh J., Lee C., Park B.K., Park Y., Hong K., Lee K., Cho B. & Song D. (2007). – Evaluation of a rapid immunodiagnostic test kit for rabies virus. *J Virol Methods,* **145** (1), 30-36. doi: 10.1016/j.jviromet.2007.05.005.

[461] Nishizono A., Khawplod P., Ahmed K., Goto K., Shiota S., Mifune K., Yasui T., Takayama K., Kobayashi Y., Mannen K., Tepsumethanon V., Mitmoonpitak C., Inoue S. & Morimoto K. (2008). – A simple and rapid immunochromatographic test kit for rabies diagnosis. *Microbiology and immunology,* **52** (4), 243-249. doi: 10.1111/j.1348-0421.2008.00031.x.

[462] Ahmed K., Wimalaratne O., Dahal N., Khawplod P., Nanayakkara S., Rinzin K., Perera D., Karunanayake D., Matsumoto T. & Nishizono A. (2012). – Evaluation of a monoclonal antibody-based rapid immunochromatographic test for direct detection of rabies virus in the brain of humans and animals. *The American journal of tropical medicine and hygiene,* **86** (4), 736-740. doi: 10.4269/ajtmh.2012.11-0332.

[463] Yang D.-K., Shin E.-K., Oh Y.-I., Lee K.-W., Lee C.-S., Kim S.-Y., Lee J.-A. & Song J.-Y. (2012). – Comparison of four diagnostic methods for detecting rabies viruses circulating in Korea. *J Vet Sci,* **13** (1), 43-48. doi: 10.4142/jvs.2012.13.1.43.

[464] Ali U., Minhas R.A., Awan M.S., Ahmed K.B., Qamar Q.Z. & Dar N.I. (2016). – Human-Grey Wolf (Canis lupus Linnaeus, 1758) Conflict in Shounther Valley, District Neelum, Azad Jammu and Kashmir, Pakistan. *Pak. J. Zool.,* **48** (3), 861-868.

[465] Servat A., Picard-Meyer E., Robardet E., Muzniece Z., Must K. & Cliquet F. (2012). – Evaluation of a Rapid Immunochromatographic Diagnostic Test for the detection of rabies from brain material of European mammals. *Biologicals : journal of the International Association of Biological Standardization,* **40** (1), 61-66. doi: 10.1016/j.biologicals.2011.12.011.

[466] Certoma A., Lunt R.A., Vosloo W., Smith I., Colling A., Williams D.T., Tran T. & Blacksell S.D. (2018). – Assessment of a Rabies Virus Rapid Diagnostic Test for the Detection of Australian Bat Lyssavirus. *Tropical medicine and infectious disease,* **3** (4), 109. doi: 10.3390/tropicalmed3040109.

[467] Sharma P., Singh C.K. & Narang D. (2015). – Comparison of immunochromatographic diagnostic test with Hheminested Reverse transcriptase polymerase chain reaction for detection of rabies virus from brain samples of various species. *Veterinary world,* **8** (2), 135-138. doi: 10.14202/vetworld.2015.135-138.

[468] Servat A., Robardet E. & Cliquet F. (2019). – An inter-laboratory comparison to evaluate the technical performance of rabies diagnosis lateral flow assays. *J Virol Methods,* **272**, 113702. doi: 10.1016/j.jviromet.2019.113702.

[469] Barrat J. (1996). – Simple technique for the collection and shipment of brain specimens for rabies diagnosis. *In* Laboratory techniques in rabies (F.X. Meslin, M.M. Kaplan & H. Koprowski, eds), World Health Organization. 425–432

[470] Dacheux L., Larrous F., Lavenir R., Lepelletier A., Faouzi A., Troupin C., Nourlil J., Buchy P. & Bourhy H. (2016). – Dual Combined Real-Time Reverse Transcription Polymerase Chain Reaction Assay for the Diagnosis of Lyssavirus Infection. *PLOS Neglected Tropical Diseases,* **10** (7), e0004812. doi: 10.1371/journal.pntd.0004812.

[471] World Health Organization (2019). – Application of next generation sequencing to rabies virus and other lyssaviruses. *In* Laboratory techniques in rabies (C. Rupprecht, A.R. Fooks & B. Abela-Ridder, eds). 49-61.

[472] World Health Organization (2019). – Conventional pan-lyssavirus reverse transcriptase polymerase chain reaction. *In* Laboratory techniques in rabies (C. Rupprecht, A.R. Fooks & B. Abela-Ridder, eds). 1-16.

[473] World Health Organization (2019). – Rabies real-time reverse transcriptase polymerase chain reaction. *In* Laboratory techniques in rabies (C. Rupprecht, A.R. Fooks & B. Abela-Ridder, eds). 17-34.

[474] McElhinney L.M., Marston D.A., Brookes S.M. & Fooks A.R. (2014). – Effects of carcase decomposition on rabies virus infectivity and detection. *Journal of Virological Methods,* **207**, 110-113. doi: https://doi.org/10.1016/j.jviromet.2014.06.024.

[475] Mpolya E.A. (2019). – Encouraging responsible dog ownership in Africa. *Vet. Rec.,* **184** (9), 278-280. doi: 10.1136/vr.l862.

[476] Petersen B.W. & Rupprecht C.E. (2011). – Human Rabies Epidemiology and Diagnosis. *In* Non-Flavivirus Encephalitis (S. Tkachev, ed), IntechOpen.

Appendix 1: Supplementary information for chapter 5

Table 26: Search syntax for data base search.
Last search conducted 28 November 2019

1. PubMed

	Search Syntax	Hits
1	(("rabies"[MeSH Terms] OR "rabies"[All Fields]) OR "rage"[All Fields]) AND ("Cameroon"[All Fields] OR "Cameroun"[All Fields] OR "Central African Republic"[All Fields] OR "Republique centrafricaine"[All Fields] OR "Chad"[All Fields] OR "Congo "[All Fields] OR "Equatorial Guinea"[All Fields] OR "Guinee equatoriale"[All Fields] OR "Guinea Ecuatorial"[All Fields] OR "Gabon"[All Fields] OR (Sao[All Fields] AND Tome[All Fields] AND Principe[All Fields]) OR "Benin"[All Fields] OR "Burkina Faso"[All Fields] OR "Cape Verde"[All Fields] OR "Cabo verde"[All Fields] OR "Ivory Coast"[All Fields] OR "Cote d'Ivoire"[All Fields] OR "Gambia"[All Fields] OR "Ghana"[All Fields] OR "Guinea"[All Fields] OR (La[All Fields] AND Guinee[All Fields]) OR "Guinea-Bissau"[All Fields] OR "Guine-Bissau"[All Fields] OR "Liberia"[All Fields] OR "Mali"[All Fields] OR "Mauritania"[All Fields] OR "Mauritanie"[All Fields] OR "Niger"[All Fields] OR "Nigeria"[All Fields] OR "Saint Helena"[All Fields] OR "St Helena"[All Fields] OR "Senegal"[All Fields] OR "Sierra Leone"[All Fields] OR "Togo"[All Fields]) AND ("1998/01/01"[PDAT] : "2018/12/31"[PDAT])	276
2	"Rabies"[Mesh] AND "Africa, Central"[Mesh] AND ("1998/01/01"[PDAT] : "2018/12/31"[PDAT])	30
3	"Africa, Western"[Mesh] AND "Rabies"[Mesh] AND ("1998/01/01"[PDAT] : "2018/12/31"[PDAT])	58
	Sub-Total	**364**

2. Web of Science

	Search Syntax	Hits

1	(("rabies" OR "rage") AND ("Cameroon" OR "Cameroun" OR "Central African Republic" OR "Republique centrafricaine" OR "Chad" OR "Congo" OR "Equatorial Guinea" OR "Guinee equatoriale" OR "Guinea Ecuatorial" OR "Gabon" OR "Sao Tome & Principe" OR "Benin" OR "Burkina Faso" OR "Cape Verde" OR "Cabo verde" OR "Ivory Coast" OR "Cote dIvoire" OR "Gambia" OR "Ghana" OR "Guinea" OR "La Guinee" OR "Guinea-Bissau" OR "Guine-Bissau" OR "Liberia" OR "Mali" OR "Mauritania" OR "Mauritanie" OR "Niger" OR "Nigeria" OR "Saint Helena" OR "St Helena" OR "Senegal" OR "Sierra Leone" OR "Togo"))	341
2	("rabies" AND "Central Africa")	11
3	("rabies" AND "West Africa")	38
	Sub-Total	**390**

3. Embase

	Search Syntax	Hits
1	('rabies'/exp OR 'rabies' OR 'rage'/exp OR 'rage') AND ('cameroon'/exp OR 'cameroon' OR 'cameroun'/exp OR 'cameroun' OR 'central african republic'/exp OR 'central african republic' OR 'republique centrafricaine' OR 'chad'/exp OR 'chad' OR 'congo'/exp OR 'congo' OR 'equatorial guinea'/exp OR 'equatorial guinea' OR 'guinee equatoriale' OR 'guinea ecuatorial' OR 'gabon'/exp OR 'gabon' OR 'sao tome & principe' OR 'benin'/exp OR 'benin' OR 'burkina faso'/exp OR 'burkina faso' OR 'cape verde'/exp OR 'cape verde' OR 'cabo verde'/exp OR 'cabo verde' OR 'ivory coast'/exp OR 'ivory coast' OR 'cote divoire' OR 'gambia'/exp OR 'gambia' OR 'ghana'/exp OR 'ghana' OR 'guinea'/exp OR 'guinea' OR 'la guinee' OR 'guinea-bissau'/exp OR 'guinea-bissau' OR 'guine-bissau' OR 'liberia'/exp OR 'liberia' OR 'mali'/exp OR 'mali' OR 'mauritania'/exp OR 'mauritania' OR 'mauritanie' OR 'niger'/exp OR 'niger' OR 'nigeria'/exp OR 'nigeria' OR 'saint helena'/exp OR 'saint helena' OR 'st helena'/exp OR 'st helena' OR 'senegal'/exp OR 'senegal' OR 'sierra leone'/exp OR 'sierra leone' OR 'togo'/exp OR 'togo') AND [1998-2018]/py	426
2	'rabies' AND 'central africa' AND [1998-2018]/py	15
3	'rabies' AND 'west africa' AND [1998-2018]/py	30
	Sub-Total	**471**

4. Cochrane Library

	Search Syntax	Hits
1	'rabies and Africa'	23
	Sub-Total	**23**

5. African Journal Online

	Search Syntax	Hits
1	'rabies'	107
2	''rage'	21
	Sub-Total	**128**

	Total	**1376**

Appendix 2: Supplementary information for chapter 6

Figure 31: Case report form for human rabies exposures

Formulaire Morsures d'Animaux

Merci de remplir ce formulaire pour toute personne mordue ou griffée par un animal !!

Formulaire rempli par : _____ Date : ____/___/_____

Type de la structure de santé :

☐Zone de santé ☐Hôpital ☐Centre de santé ☐Clinique Vétérinaire ☐Autre, précisez_____

Ville_____ Commune_____ Quartier_____

Territoire :_____ Secteur_____ Groupement :_____ Village_____

Identité de la victime :

(ne remplir qu'avec l'accord explicite de la victime ou de son tuteur)

Nom, prénom_____Adresse_____

Ville/Village_____ Quartier_____ Téléphone_____

Signature de la victime ou de son tuteur : _____

Données obligatoires de la victime :

Sexe : ☐Féminin ☐ Masculin Age : ____ (si possible)

Tranches d'âge (ans): ☐ 0-4 ☐ 5-9 ☐ 10-14 ☐ 15-19

☐ 20-29 ☐ 30-39 ☐ 40-49 ☐ >50

Informations relatives au cas :

Date de la morsure : ____/___/_____

Lieu de l'incident : Territoire _____ Ville/Village _____

Quartier _____

Informations sur l'animal mordeur :

Espèce animale : _____ ☐ Animal domestique ☐ Sans propriétaire ☐ Animal sauvage

Est-ce que l'animal présentait des symptômes de la rage ? ☐ Oui ☐ Non ☐ Ne sait pas

A qui appartenait l'animal mordeur ? _____ Adresse_____

L'animal mordeur, est-il vacciné contre la rage ? ☐Oui ☐ Non ☐ Ne sait pas

Carnet de vaccination ? ☐Oui ☐ Non Date de la dernière vaccination :___/____/_____

Où se trouve l'animal mordeur ?

☐ Abattu ☐ Décédé ☐ Mis sous surveillance ☐Ne sait pas

Au moment de l'incident l'animal mordeur était : ☐Enfermé/Attaché ☐Libre de mouvement

L'animal mordeur, a-t-il mordu/griffé d'autres personnes ?

☐Oui ☐ Non ☐Ne sait pas Indiquez le nombre : ___

L'animal mordeur, a-t-il mordu/griffé d'autres animaux ?

☐Oui ☐ Non ☐Ne sait pas Indiquez le nombre : ___

^

➔ **Veuillez continuer avec la page suivante, s'il vous plaît !**

Page **1** de **2**

Contact: INRB, Av. de la Démocratie, Kin/Gombe, B.P. 1197, www.inrb-rdc.org
Prof. Pati Pyana : 081815106213/0852882534 email : p.pyana@inrb.cd

Détails sur la blessure :

☐Une seule morsure ☐Plusieurs morsures Indiquez le nombre de morsures : _____

☐Une seule griffure ☐Plusieurs griffures Indiquez le nombre de griffures : _____

Catégorie de(s) la blessure(s) selon l'OMS:

☐Catégorie I : Contact avec l'animal (la personne l'a touché ou nourri) ou léchage de la peau Intacte → Mesures : Observation de l'animal, pas de traitement

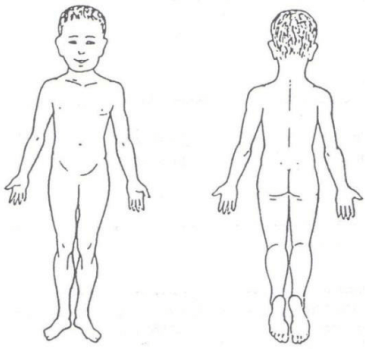
☐Catégorie II : Mordillement de la peau nue, griffures ou égratignures superficielles sans Saignement → Mesures : Vaccination immédiate et traitement de la plaie

☐ Catégorie III : Morsures ou griffures uniques ou multiples ayant traversé le derme, léchage de la peau lésée ; contamination des muqueuses par la salive après léchage, exposition à des chauves-souris → Mesures : Vaccination immédiate, administration d'immunoglobuline antirabique et traitement de la plaie

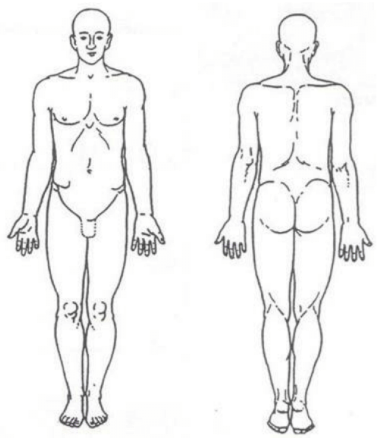
Localisation de la blessure :

(Indiquez le(s) lieu(s) de la blessure avec un X)

Enfant :



Adulte :



Informations sur la prophylaxie postexpositionelle :

Combien de temps après la morsure/griffure la victime s'est-elle présentée à votre structure de santé?

☐ Le même jour |__|__| jours après (Indiquez le nombre de jours)

Quel traitement a été administré au sein de votre structure de santé ?

☐ Nettoyage de la plaie avec de l'eau ☐Désinfection de la plaie ☐AINS

☐ Vaccination antirabique Si vacciné, indiquez le nombre de doses : |__|__|

Si ne pas vacciné, notez la raison_____

☐ Administration d'immunoglobuline antirabique ☐ Sérum anti-tétanique ☐Antibiothérapie

☐ Autre, précisez_____

Observation :_____

Merci pour votre collaboration !

Contact: INRB, Av. de la Démocratie, Kin/Gombe, B.P. 1197, www.inrb-rdc.org
Prof. Pati Pyana : 081815106213/0852882534 email : p.pyana@inrb.cd

Figure 32: Case report form for human rabies cases

Formulaire d'Envoi d'Echantillons Rabiques (Hôpital Général de Réference)

Merci de remplir COMPLETEMENT ce formulaire si possible !!

Expéditeur : ☐ Matadi 2 ☐ Kimpese 4 ☐ Kisantu 6

Date de l'expédition : ____/____/_____ Numéro de l'échantillon : |__|__|__|

Identité de la personne exposée:

(ne remplir qu'avec l'accord explicite de la victime ou de son représentant légal)

Nom, prénom_____ Adresse_____

Ville/Village_____Téléphone_____

Signature de la victime ou de son représentant légal : _____

Données anonymes de la victime :

Sexe : ☐Féminin ☐ Masculin Age : ☐ Enfant ☐ Adulte

Tranches d'âge (ans): ☐ 0-4 ☐ 5-9 ☐ 10-14 ☐ 15-19

☐ 20-29 ☐ 30-39 ☐ 40-49 ☐ >50

Espèce de l'animal mordeur : _____

☐ Animal domestique ☐ Sans propriétaire ☐ Animal sauvage

Date de la morsure : ____/___/_____

Lieu de l'incident : District_____Territoire_____

Ville/Village _____ Quartier_____

Zone : ☐ Urbaine ☐ Peri-urbaine ☐ Rurale

Est-ce que la personne exposée présente-t-elle des symptômes de rage ? : ☐ Oui ☐Non

Si oui, décrivez les symptômes : _____

Depuis quand présente-t-elle ces symptômes ? |__|__| jours

La personne exposée, a-t-elle reçu une prophylaxie postexpositionelle ? ☐ Oui ☐Non

Si oui, indiquez le nom de la structure de santé et la date du traitement :

_____ Date : ____/___/_____

➔ *Veuillez s'il vous plait remplir le formulaire « Morsure d'animaux » si applicable*

Section réservée au laboratoire de la rage à l'INRB :
Date de réception____/____/_____ Initiales____ Identification de l'échantillon : |__|__|__|__| Type d'échantillon : ☐ Salive ☐ Sérum ☐ Conservé au froid ☐Préservé en solution saline tamponée au glycérol ☐Papier filtre (FTA) Condition de l'échantillon :☐Bonne ☐Mauvaise

Epreuve	Résultat	Date de l'appel	Initiales
Anticorps immunofluorescent (IFAT)	☐Positif ☐Négatif		
Test immunohistochimique direct rapid (dRIT)	☐Positif ☐ Négatif		
Anigen Rapid Rabies Ag Test Kit	☐Positif ☐Négatif		
Autre, précisez_____	☐Positif ☐Négatif		
Commentaires du laboratoire :			

Légende :

1 : Service vétérinaire de Matadi
2 : Hôpital général de référence de Matadi
3 : Service vétérinaire de Kimpese
4 : Hôpital général de référence de Kimpese
5 : Service vétérinaire de Kisantu
6 : Hôpital général de référence de Kisantu

Résultat du test de diagnostic rapide (Anigen Kit) sur place :

☐Positif ☐Négatif ☐Douteux Executé le : ____/___/_____

Section réservée au laboratoire de la rage à l'INRB :
Date de réception____/____/_____ Nom Lab.Tech_____ **Identification de l'échantillon :** |__|__|-|__|__|-|__||__||__| *Expéditeur(XX)-Espèce(YY)-Numéro de l'échantillon(ZZZ)* **Type d'échantillon :** ☐ Cerveau ☐ Autre, précisez :_____ **Conservation :** ☐ Conservé au froid ☐Préservé en solution saline tamponée au glycérol ☐Papier filtre (FTA) **Conformité de l'échantillon :** ☐Bonne ☐Mauvaise

Test effectué	**Résultat**	**Executé le**	**Init. Lab.Tech.**
Anticorps immunofluorescent (IFAT)	☐Positif ☐Négatif ☐Douteux		
Test immunohistochimique direct rapid (dRIT)	☐Positif ☐Négatif ☐Douteux		
Anigen Rapid Rabies Ag Test Kit	☐Positif ☐Négatif ☐Douteux		
Autre, précisez_____	☐Positif ☐Négatif ☐Douteux		
Commentaires du laboratoire :			

Légende :

1 : Service vétérinaire de Matadi **2** : Hôpital Général de Référence de Kikanda **Chien** : Cn **Porc** : Pc
3 : Service vétérinaire de Kimpese **4** : Hôpital Général de Référence de Kimpese **Chat** : Ct **Chèvre** : Ce
5 : Service vétérinaire de Kisantu **6** : Hôpital Général de Référence de Kisantu **Primate** : Pe **Autre** : Au
7 : Service vétérinaire de Boma **8** : Hôpital Général de Référence de Boma **Humain** : Hu
9 : Service vétérinaire de Muanda **10** :Hôpital Général de Référence de Muanda

Contact: **Numéro Rage** : 0842508020
INRB : Av. de la Démocratie, Kin/Gombe, B.P. 1197, www.inrb-rdc.org
Prof. Pati Pyana : 081815106213/0852882534 email : ppyana@yahoo.fr, inrb_rdc@yahoo.fr.

Box 2: English traduction of parameters included in the case report forms for human rabies exposures and cases

1. Case report form for human rabies exposures

- Age
- Sex
- Date of bite
- Geographical location of bite incident
- Biting animal (species)
- Status of biting animal (owned, unowned, wild)
- Symptoms in biting animal
- Vaccination status of biting animal
- Outcome of the biting animal (killed, died, under observation)
- Other people/animals offended by the same animal
- Bite site
- Bite severity (category I-III)
- Number of bites
- Delay in presentation

2. Case report form for human rabies cases

- Age
- Sex
- Biting animal (species)
- Status of biting animal (owned, unowned, wild)
- Date of bite
- Geographical location of bite incident
- Clinical symptoms
- Duration of symptoms
- If patient received PEP

Table 27: Human population and estimated owned and unowned dog population of the six sites in the Kongo Central province.

Site	Human population[1]	Owned dog population[2]	Unowned dog population[3]	Total dog population
Matadi	301644	8001	160	8161
Boma	459361	12185	244	12428
Mbanza-Ngungu	146749	3893	78	3970
Kimpese	62821	1666	33	1700
Kisantu	78000	2069	41	2110
Muanda	50000	1326	27	1353
Total	**1098575**	**29140**	**583**	**29723**

1 Based on population extrapolation utilised by the health zones and information provided by the rabies units
2 Based on the dog/human ratio of 1:37.7 estimated by Mbilo *et al.* [307]
3 Based on findings from Kazadi *et al.* [192]

Figure 33: Decision tree probability model to estimate human rabies deaths from bite injuries.

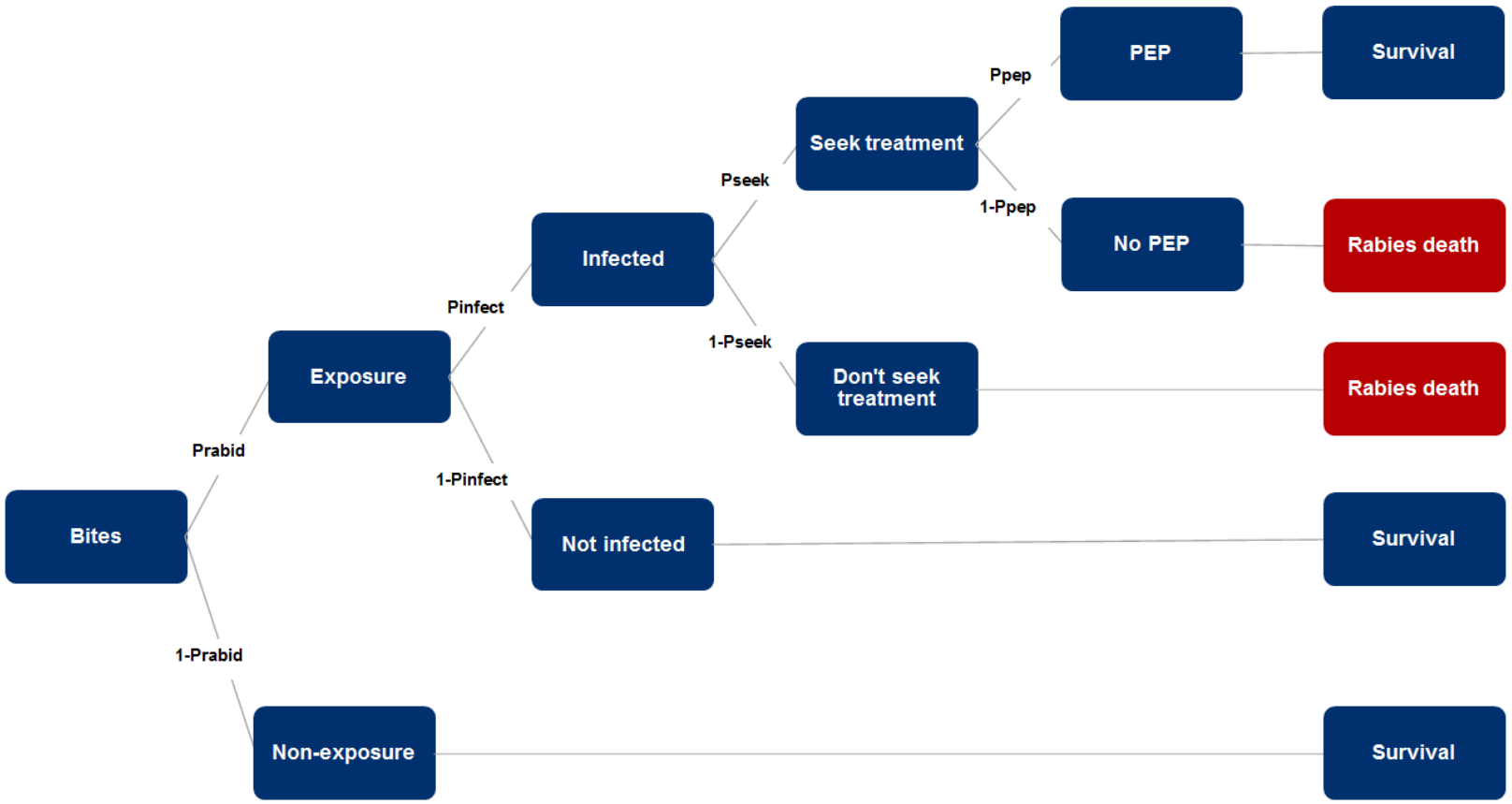


Table 28: Parameters to derive the probability of death (Pdeath) after a bite and the total number of rabies deaths.

All parameters are assumed to follow a Pert distribution.

Parameter		Min	Mode	Max	Information source
Prabid	Proportion of bites due to rabid animals	0.45	0.495	0.54	Own data mode = 381/767, min and max = 99% confidence limits
Pinfect	Development of rabies following a bite injury from a rabid dog	0.02	0.07	0.5	Estimated from[1]
Pseek	Probability of seeking treatment	0.1	0.4	0.7	Min: Estimated from community-level bite incidence of 5.2/ 1000 person-years [307] Max: Proportion of bite victims seeking help identified by Beyene et al. in contact tracing study in Ethiopia [316].
Ppep	Probabilty of receiving PEP after seeking treatment	0.5	0.56	0.6	Hampson *et al.* [9]

[1]**Pinfect**=(Pbite_head*Pinfect_head) + (Pbite_arm*Pinfect_arm) + (Pbite_trunk* Pinf_trunk) + (Pbite_trunk*Pinf_trunk)

Pbite_head	Bite injury to the head	Binomial: p=0.15 n=344
Pbite_arm	Bite injury to the arms	Binomial: p=0.22 n=344
Pbite_trunk	Bite injury to the trunk	Binomial: p=0.11 n=344
Pbite_leg	Bite injury to the legs	Binomial: p=0.52 n=344
Pinf_head	Development of rabies following a bite injury to the head	Pert: minimum = 0.3, mode = 0.45, maximum = 0.6
Pinf_arm	Development of rabies following a bite injury to the arms	Pert: minimum = 0.15, mode = 0.275, maximum = 0.4
Pinf_trunk	Development of rabies following a bite injury to the trunk	Pert: minimum = 0, mode = 0.05, maximum = 0.1
Pin_leg	Development of rabies following a bite injury to the legs	Pert: minimum = 0, mode = 0.05, maximum = 0.1

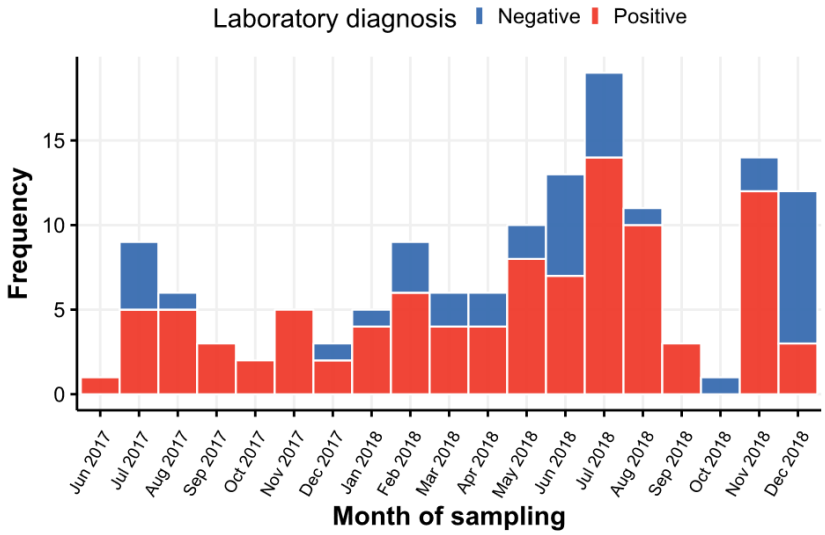
Table 29: Characteristics of bite victims disaggregated by their exposures status, including type and place of exposure and delay in presentation to clinic.

	Confirmed (N=98)	Probable (N=280)	Suspected (N=3)	Unknown (N=19)	Non-exposure (N=386)	Total (N=786)
Sex						
Female	34 (39.1%)	113 (40.8%)	0 (0.0%)	8 (42.1%)	155 (40.7%)	310 (40.4%)
Male	53 (60.9%)	164 (59.2%)	3 (100.0%)	11 (57.9%)	226 (59.3%)	457 (59.6%)
N-Miss	11	3	0	0	5	19
Age						
<5yrs	6 (6.1%)	21 (8.1%)	0 (0.0%)	1 (5.6%)	22 (6.1%)	50 (6.8%)
5-14yrs	43 (43.9%)	93 (35.8%)	1 (33.3%)	4 (22.2%)	125 (34.9%)	266 (36.1%)
>15yrs	49 (50.0%)	146 (56.2%)	2 (66.7%)	13 (72.2%)	211 (58.9%)	421 (57.1%)
N-Miss	0	20	0	1	28	49
Biting animal						
Dog	90 (91.8%)	269 (96.4%)	3 (100.0%)	16 (88.9%)	372 (97.4%)	750 (96.2%)
Other	8 (8.2%)	10 (3.6%)	0 (0.0%)	2 (11.1%)	10 (2.6%)	30 (3.8%)
N-Miss	0	1	0	1	4	6
Site						
Boma	21 (21.4%)	27 (9.6%)	0 (0.0%)	1 (5.3%)	24 (6.2%)	73 (9.3%)
Kimpese	10 (10.2%)	9 (3.2%)	0 (0.0%)	1 (5.3%)	32 (8.3%)	52 (6.6%)
Kisantu	6 (6.1%)	10 (3.6%)	0 (0.0%)	0 (0.0%)	23 (6.0%)	39 (5.0%)
Matadi	16 (16.3%)	164 (58.6%)	3 (100.0%)	4 (21.1%)	198 (51.3%)	385 (49.0%)
Mbanza-Ngungu	4 (4.1%)	1 (0.4%)	0 (0.0%)	0 (0.0%)	1 (0.3%)	6 (0.8%)
Muanda	41 (41.8%)	69 (24.6%)	0 (0.0%)	13 (68.4%)	108 (28.0%)	231 (29.4%)
Zone						
Rural	8 (8.2%)	29 (10.4%)	0 (0.0%)	1 (5.3%)	26 (6.7%)	64 (8.1%)
Urban	90 (91.8%)	251 (89.6%)	3 (100.0%)	18 (94.7%)	360 (93.3%)	722 (91.9%)
Wound category						
Category I	3 (3.8%)	8 (3.1%)	0 (0.0%)	0 (0.0%)	8 (2.2%)	19 (2.6%)
Category II	12 (15.4%)	46 (17.6%)	2 (100.0%)	1 (7.1%)	90 (24.3%)	151 (20.8%)
Category III	63 (80.8%)	208 (79.4%)	0 (0.0%)	13 (92.9%)	272 (73.5%)	556 (76.6%)
N-Miss	20	18	1	5	16	60
Delay in presentation (days)						
Median	0.0	1.0	0.5	1.0	1.0	1.0
Range	0.0 - 21.0	0.0 - 120.0	0.0 - 1.0	0.0 - 14.0	0.0 - 90.0	0.0 - 120.0
N-Miss	16	19	1	5	27	68

Table 30: Annual dog rabies incidence across the six sites in the Kongo Central province.

Site	Dog rabies incidence per 1000 dogs (95% CI) based on confirmed, probable and suspected cases	Dog rabies incidence per 1000 dogs based on confirmed cases
Boma	1.9 (1.5-2.4)	1.1
Matadi	10.7(9.2-12.2)	0.6
Kimpese	7.1 (4.5-9.8)	3.1
Kisantu	3.2 (1.6-5.1)	0.3
Muanda	31.6 (25.4-38.1)	8.7
Mbanza-Ngungu	0.5 (0.1-0.67)	0.17
Overall	9.4 (8.8-9.9)	1.2

Figure 34: Time series of rabies-positive and –negative samples analyzed at the rabies laboratory in Kinshasa during the study period shown by month of sampling.



Appendix 3: Supplementary information for chapter 7

Table 31: Questions included to assess respondents' knowledge regarding rabies transmission, its symptoms in animals and humans, prevention and control, and practices towards suspected rabid animals and carcasses.
No negative scores were given for wrong answers.

Question	Answer	Score correct
1. What is rabies?	Rabies described as a disease	4
	Wrong answer/Unknown	0
2. How is rabies transmitted? (multiple answers possible)	Bite	2
	Scratch	2
	Wrong answer/ Unknown	0
3. Which animals can be infected with rabies?	Dog	2
	Three or more animals mentioned	2
	One or two animals mentioned	1
	Wrong answer /Unknown	0
4. Rabies symptoms in animals[1]	Three or more symptoms mentioned	4
	One or two symptoms mentioned	2
	Wrong answer/ Unknown	0
5. Rabies symptoms in humans[1]	Three or more symptoms mentioned	4
	One or two symptoms mentioned	2
	Wrong answer/ Unknown	0
6. Is rabies preventable?	Yes	4
	No	0
	Unknown	0
7. Methods of prevention and control (multiple answers possible)	Vaccination of humans	1
	Vaccination of animals	1
	Dog population management	1
	Dog bite prevention	1
	Wrong answer / Unknown	0
8. Is rabies curable?	Yes	0
	No	4
	Unknown	0
Overall score knowledge		32
Action taken after exposure (multiple answers possible)	Wound cleaning with water and soap	1
	Post-exposure prophylaxis	1
	Seek medical attention	1
	Anti-tetanus treatment	0.5
	Wrong answer/ Unknown	0
Practice towards suspected rabid animal (multiple answers possible)	Kill the animal	1.5
	Inform veterinary service	2
	Do nothing	0
	Unknown	0
Practice towards carcass of an animal	Inform veterinary service	2
	Take carcass to veterinary service	3.5

	Burry or burn the carcass	1
	Do nothing	0
	Unknown	0

[1] Based on standard case definitions for rabies in the WHO Expert Consultation on Rabies. Third Report [116; 319; 476].

Appendix 4: Supplementary information for chapter 8

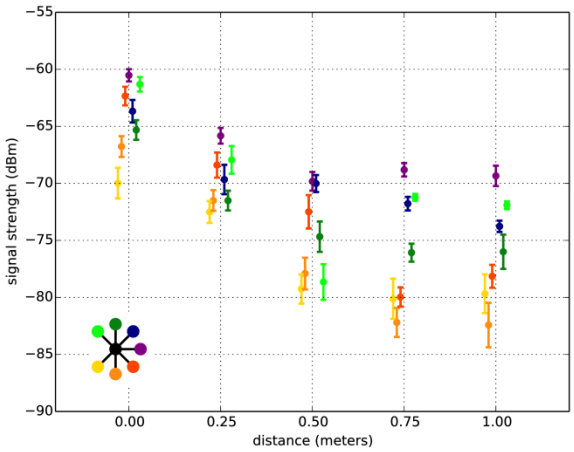


Figure 35: Signal strength of contacts between devices in a static test in N'Djaména.
The devices were set up on the ground in a circular arrangement around a central device and contact were recorded for different distances over a period of 1 hour per distance. The colors correspond to different angles from the central device (black dot).

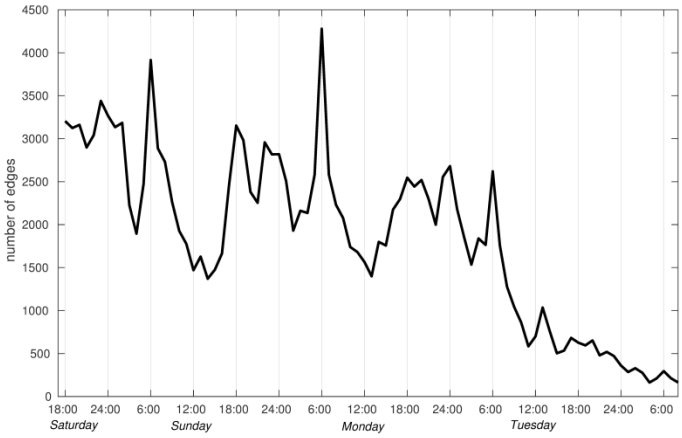


Figure 36: Contacts over time in study zone 1.
For each 1 hour interval during the study period, ranging from Saturday 17:00 to Tuesday 7:00, the number of edges in the network is shown. The network for each 1 hour interval was constructed based on all contacts recorded during that interval and an edge was established if at least one contact between the two dogs was registered.

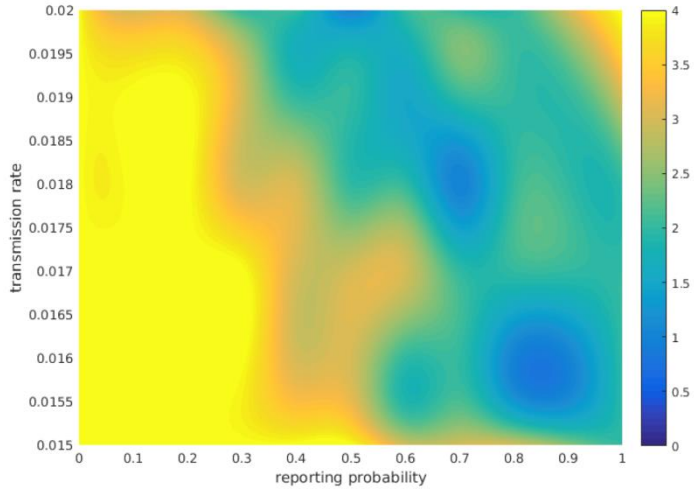


Figure 37: Sensitivity analysis of the simulation results for Chagoua and Abena on the probability of detecting rabid dogs.
For each value of the transmission rate (ranging from 0.015 to 0.02 with steps of 0.01) 1000 simulation runs were conducted. Each case in the simulated incidence was randomly assigned as either reported or not reported for different values of the reporting probability (ranging from 0 to 1 with steps of 0.1). The color of each pixel corresponds to the maximum absolute difference between the median of the simulated reported cumulative incidence and the outbreak data.

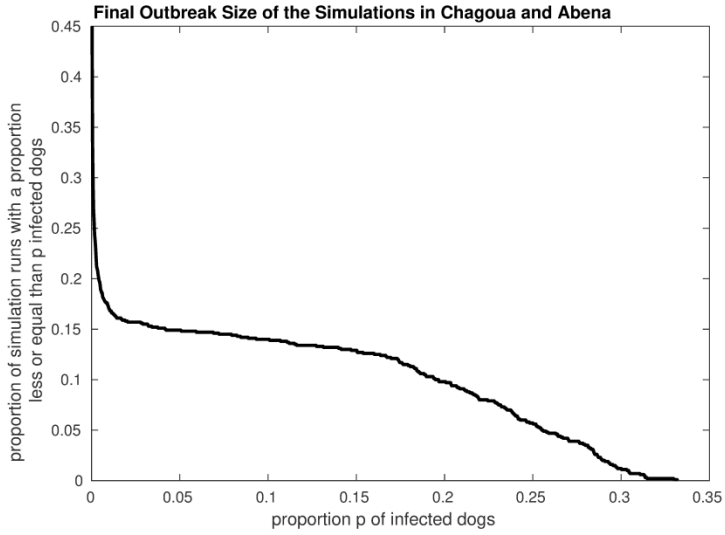


Figure 38: Final outbreak size of the simulations in the quarters Chagoua and Abena

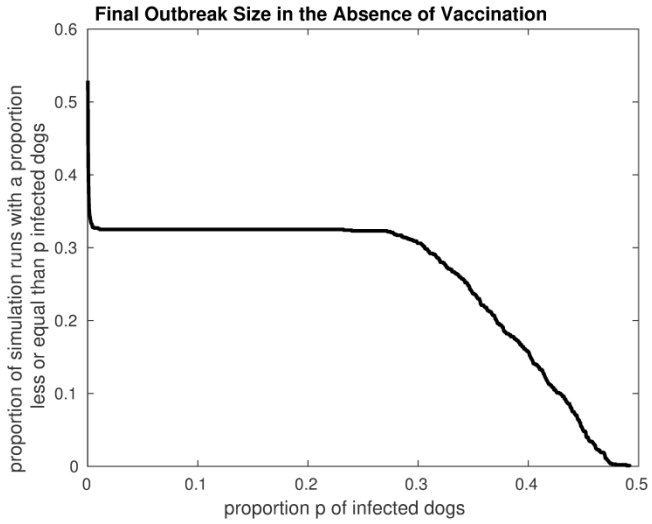


Figure 39: Final outbreak size of the simulations on a network of 4930 dogs, showing the two point distribution expected from theory.

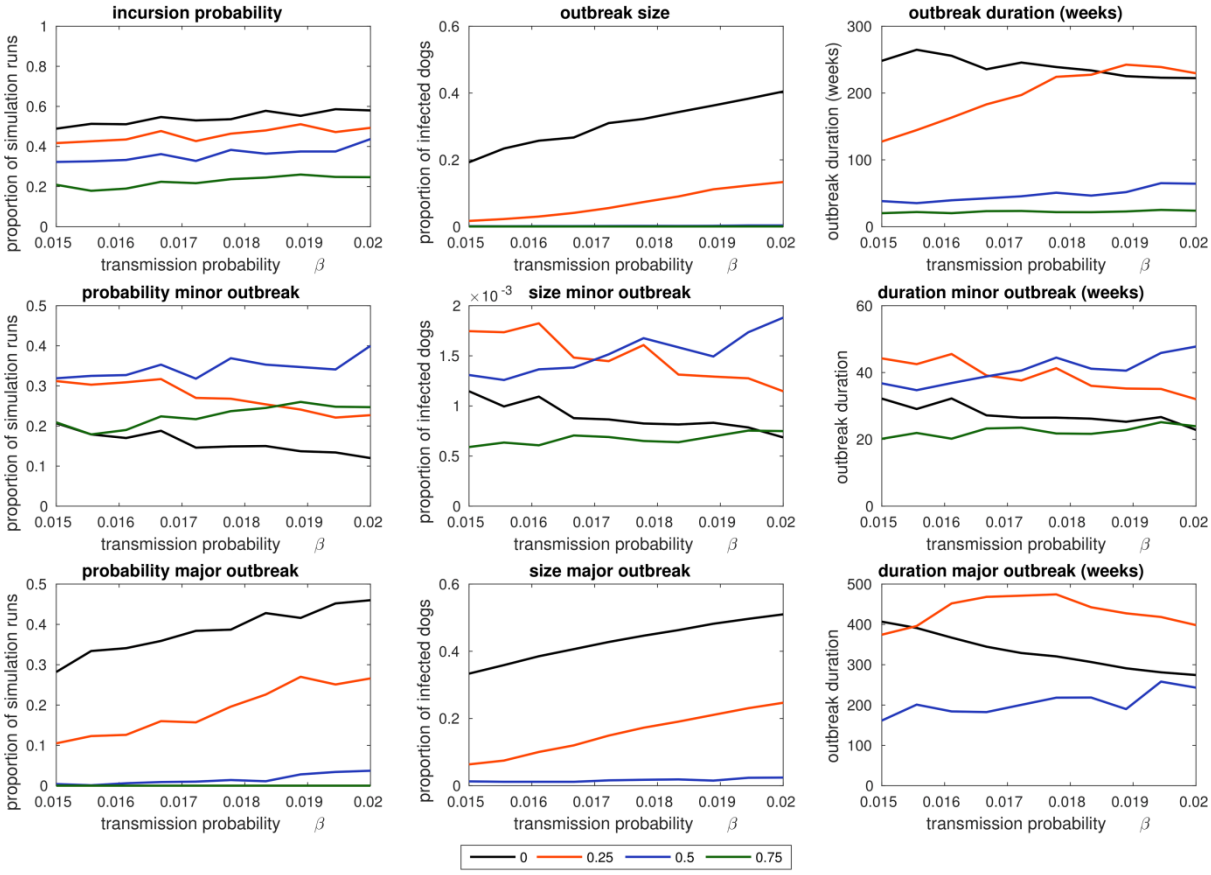


Figure 40: Sensitivity analysis of the outbreak probability, duration and size.
The colors correspond to different vaccination coverages. For each vaccination coverage and parameter value the mean of 1000 simulation runs is shown. Simulation runs where more than one dog gets infected are classified as incursion. Simulation runs where more than one dog and less than 1% of the population get infected are classified as minor outbreaks. Simulation runs where more than 1% of the population gets infected are classified as major outbreaks. Incursions include minor and major outbreaks.

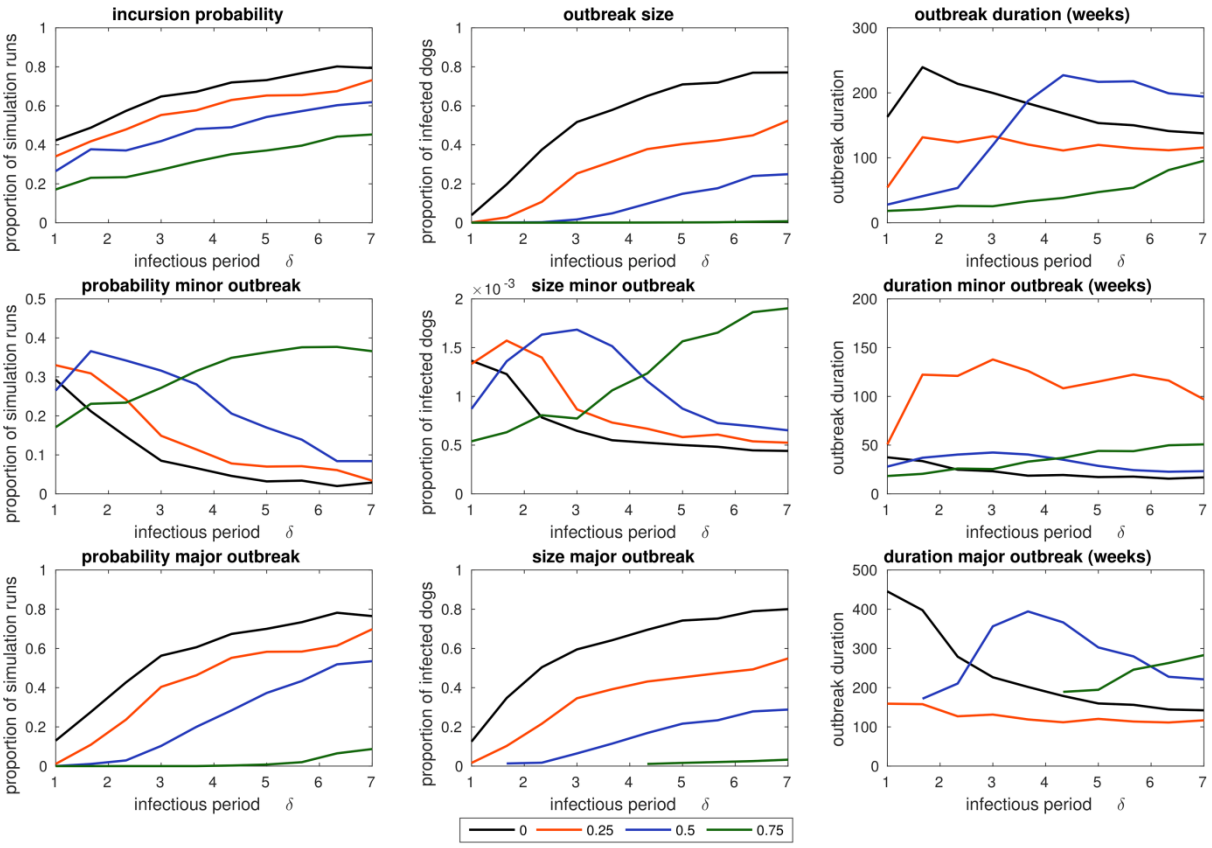


Figure 41: Sensitivity analysis of the outbreak probability, duration and size.
The colors correspond to different vaccination coverages. For each vaccination coverage and parameter value the mean of 1000 simulation runs is shown. Simulation runs where more than one dog gets infected are classified as incursion. Simulation runs where more than one dog and less than 1% of the population get infected are classified as minor outbreaks. Simulation runs where more than 1% of the population gets infected are classified as major outbreaks. Incursions include minor and major outbreaks.

Table 32: Parameter ranges for the PRCC sensitivity analysis.

Parameter	Usage	Description	Range	Type
κ	network construction	scaling of spatial connection	[20,30]	discrete
⊠	network construction	proportion of far roaming dogs	[0.5, 1]	continuous
λ	network construction	mean number of peers of far roaming dogs	[20,30]	discrete
δ	transmission model	infectious period	[1,7]	discrete
σ	transmission model	incubation period	[7, 730]	discrete
β	transmission model	transmission rate	[0.015, 0.02]	continuous

Appendix 5: Supplementary information for chapter 9

Table 33: Composition of the study dog population during the rainy season (August 2017).

Rainy season	
	Overall (n=235)
Religion of the owner	
Christianity	211 (89.8%)
Islam	24 (10.2%)
Sex (n=231)	
Female	37 (16.0%)
Male	179 (77.5%)
Neutered male	15 (6.5%)
Age (n=231)	
Young dog (< 1y)	187 (81.0%)
Adult dog (1-7 y)	40 (17.3%)
Old dog (> 7y)	4 (1.7%)
Confinment (n=231)	
None	203 (87.9%)
Daytime	17 (7.3%)
Nighttime	11 (4.8%)
Multi-dog household	
No	118 (50.2%)
Yes	117 (49.8%)

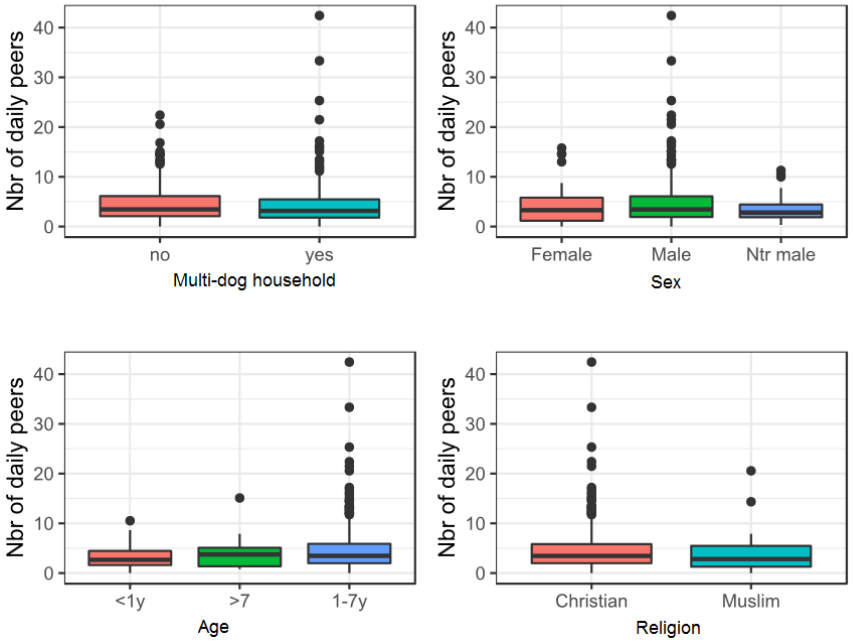


Figure 42: Daily number of contacts by multi-dog household, sex, age and religion, study 1 (December 2016).

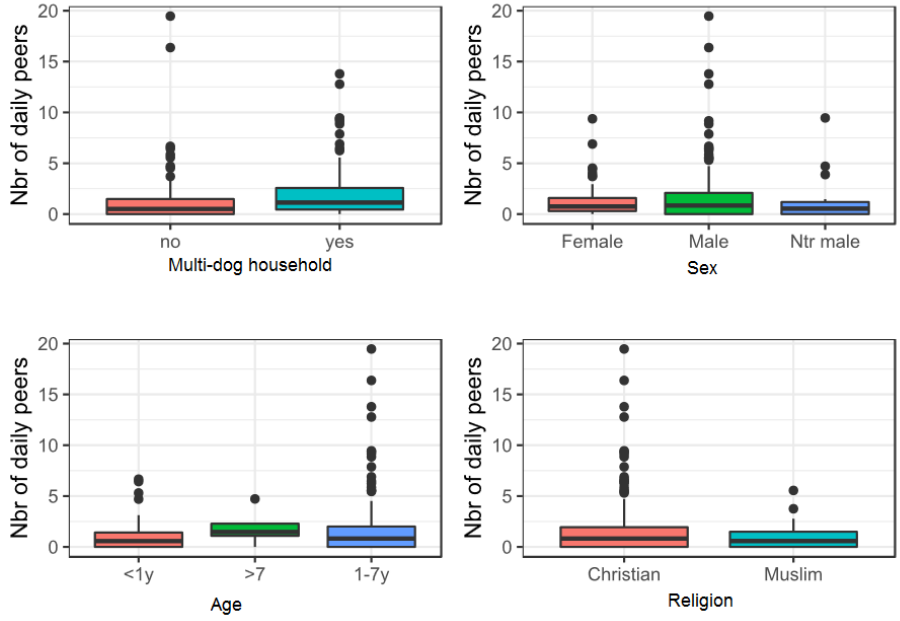


Figure 43: Daily number of contacts by multi-dog household, sex, age and religion, study 2 (December 2017).

Appendix 6: Supplementary information for chapter 11

Results from the Democratic Republic of the Congo

We validated the Anigen Rapid Rabies Ag Test kit test in comparison with the WHO and OIE-reference test, the DFA. Viral RNA was extracted from a subset of 90 animal brain samples according to the Direct-zol™ RNA MiniPrep Protocol (Zymo Research). Inconsistent results between the Anigen test and the DFA were confirmed through the detection of viral RNA by real-time polymerase chain reaction (RT-PCR) at IP-Paris. Phylogenetic analysis is currently performed at IP-Paris. Results from the DRC will be published in a separate manuscript together with the phylogenetic analysis.

Sensitivity and specificity was estimated using a Bayesian model for 2 conditional dependent tests in a single population. Because the model is over-parameterized we used informative priors for the standard test assuming a median test sensitivity and specificity of 99% (beta distribution with alpha = 600 and beta = 6).

Compared to the DFA, the Anigen showed a sensitivity and specificity of 83.8% (95% CI: 68.9% – 92.3%) and 99.1% (95% CI: 98.1%-99.6%), respectively.

Table 34: List of animal brain samples collected in the DRC during the PhD work.

SampleID	Species	DFA	DRIT	Anigen_Labo	Anigen_Field	PCR
1-Dog-1	Dog	Positive	Positive	Positive	Positive	
1-Dog-2	Dog	Positive	Positive	Positive	Positive	
1-Dog-3	Dog	Positive	Positive	Positive	Douteux	
1-Dog-4	Dog	Positive	Positive	Positive	Positive	
1-Dog-5	Dog	Negative	Negative	Negative	Negative	
1-Dog-6	Dog	Positive	Positive	Positive	Positive	
3-Dog-1	Dog	Positive	Positive	Positive	Not performed	
3-Dog-2	Dog	Negative	Negative	Negative	Negative	
3-Dog-3	Dog	Not performed	Positive	Negative	Negative	Negative
3-Dog-4	Dog	Positive	Positive	Positive	Positive	
3-Dog-5	Dog	Not performed	Negative	Negative	Negative	

3-Dog-6	Dog	Positive	Douteux	Positive	Positive	
5-Dog-1	Dog	Positive	Positive	Positive	Douteux	
7-Dog-1	Dog	Negative	Negative	Negative	Negative	
7-Dog-2	Dog	Positive	Positive	Positive	Positive	
7-Dog-3	Dog	Negative	Positive	Negative	Negative	
7-Dog-4	Dog	Negative	Positive	Negative	Negative	Negative
7-Dog-5	Dog	Positive	Positive	Positive	Positive	
7-Dog-6	Dog	Positive	Positive	Positive	Positive	
7-Dog-7	Dog	Positive	Positive	Positive	Positive	
7-Dog-8	Dog	Positive	Positive	Positive	Douteux	
7-Dog-11	Dog	Negative	Positive	Negative	Not performed	Positive
7-Dog-12	Dog	Positive	Negative	Negative	Not performed	Positive
7-Dog-13	Dog	Negative	Negative	Negative	Not performed	
7-Dog-14	Dog	Positive	Positive	Positive	Not performed	
7-Dog-15	Dog	Positive	Negative	Negative	Not performed	Positive
7-Goat-16	Goat	Positive	Negative	Negative	Not performed	Positive
5-Dog-2	Dog	Negative	Negative	Negative	Negative	
3-Dog-7	Dog	Not performed	Douteux	Positive	Not performed	Negative
7-Dog-9	Dog	Negative	Negative	Negative	Negative	
7-Dog-21	Dog	Negative	Positive	Negative	Negative	Positive
7-Dog-20	Dog	Negative	Negative	Negative	Negative	
7-Mn-19	Mn	Negative	Negative	Negative	Douteux	
7-Dog-18	Dog	Positive	Positive	Positive	Negative	
7-Dog-17	Dog	Positive	Positive	Positive	Douteux	
9-Dog-4	Dog	Positive	Positive	Positive	Positive	
9-Dog-5	Dog	Positive	Positive	Positive	Positive	
9-Dog-1	Dog	Positive	Positive	Positive	Positive	
9-Dog-2	Dog	Positive	Positive	Positive	Positive	
9-Dog-3	Dog	Negative	Negative	Negative	Negative	
9-Dog-6	Dog	Positive	Positive	Positive	Positive	
9-Dog-7	Dog	Positive	Positive	Positive	Positive	

9-Dog-8	Dog	Positive	Positive	Positive	Positive	
9-Dog-10	Dog	Positive	Positive	Positive	Negative	
9-Dog-12	Dog	Positive	Positive	Positive	Positive	
9-Cat-9	Cat	Positive	Positive	Positive	Positive	
9-Dog-11	Dog	Positive	Positive	Positive	Positive	
9-Dog-13	Dog	Positive	Positive	Positive	Positive	
9-Dog-14	Dog	Positive	Positive	Positive	Positive	
9-Goat-15	Goat	Positive	Positive	Positive	Positive	
9-Cat-16	Cat	Positive	Positive	Positive	Positive	
9-Dog-17	Dog	Negative	Negative	Negative	Negative	
9-Cat-18	Cat	Positive	Positive	Positive	Positive	
9-Dog-19	Dog	Positive	Positive	Positive	Positive	
9-Jackal-1	Jackal	Negative	Negative	Negative	Not performed	
9-Jackal-2	Jackal	Negative	Negative	Negative	Not performed	
9-Jackal-3	Jackal	Negative	Negative	Negative	Not performed	
9-Jackal-4	Jackal	Negative	Negative	Negative	Not performed	
9-Jackal-5	Jackal	Negative	Negative	Negative	Not performed	
9-Jackal-6	Jackal	Negative	Negative	Negative	Not performed	
9-Jackal-10	Jackal	Negative	Negative	Negative	Not performed	
9-Jackal-11	Jackal	Negative	Negative	Negative	Not performed	
9-Jackal-12	Jackal	Negative	Negative	Negative	Not performed	
7-Goat-10	Goat	Positive	Positive	Positive	Not performed	
7-Dog-25	Dog	Positive	Positive	Positive	Positive	
7-Dog-26	Dog	Positive	Positive	Positive	Positive	
7-Pc-22	Pc	Negative	Negative	Negative	Douteux	
7-Dog-23	Dog	Positive	Positive	Positive	Positive	
11-Dog-1	Dog	Positive	Positive	Positive	Douteux	
7-Dog-24	Dog	Positive	Positive	Positive	Positive	
7-Dog-27	Dog	Positive	Positive	Positive	Positive	
7-Dog-28	Dog	Positive	Positive	Positive	Positive	
9-Cat-20	Cat	Positive	Positive	Positive	Positive	

7-Dog-29	Dog	Negative	Negative	Negative	Douteux	
7-Dog-30	Dog	Negative	Negative	Negative	Negative	
7-Dog-31	Dog	Negative	Negative	Negative	Negative	
7-Dog-32	Dog	Negative	Negative	Negative	Negative	
7-Dog-33	Dog	Negative	Negative	Negative	Negative	
7-Dog-34	Dog	Negative	Negative	Negative	Negative	
7-Dog-35	Dog	Positive	Positive	Positive	Positive	
7-Dog-36	Dog	Positive	Positive	Positive	Positive	
7-Dog-37	Dog	Positive	Positive	Positive	Positive	
7-Goat-38	Goat	Negative	Negative	Negative	Negative	
7-Dog-39	Dog	Positive	Positive	Positive	Positive	
7-Dog-40	Dog	Positive	Not performed	Negative	Negative	Positive
7-Dog-41	Dog	Positive	Not performed	Negative	Negative	Positive
7-Dog-42	Dog	Positive	Not performed	Negative	Negative	Positive
7-Dog-43	Dog	Positive	Not performed	Negative	Negative	Positive
7-Dog-44	Dog	Negative	Not performed	Positive	Negative	
7-Dog-45	Dog	Positive	Not performed	Negative	Negative	Positive
7-Dog-46	Dog	Positive	Not performed	Negative	Negative	Positive
7-Dog-47	Dog	Positive	Not performed	Positive	Positive	
7-Dog-48	Dog	Positive	Not performed	Positive	Positive	
7-Dog-49	Dog	Positive	Not performed	Positive	Positive	
7-Dog-50	Dog	Negative	Not performed	Negative	Negative	
7-Dog-51	Dog	Negative	Not performed	Negative	Negative	
7-Dog-52	Dog	Positive	Not performed	Positive	Douteux	
7-Dog-54	Dog	Positive	Not performed	Positive	Positive	
7-Dog-55	Dog	Positive	Not performed	Positive	Douteux	
7-Dog-56	Dog	Positive	Not performed	Positive	Positive	
7-Dog-57	Dog	Negative	Not performed	Negative	Negative	
7-Dog-58	Dog	Negative	Not performed	Negative	Negative	
7-Dog-59	Dog	Positive	Not performed	Positive	Positive	
7-Dog-60	Dog	Positive	Not performed	Positive	Positive	

7-Dog-61	Dog	Positive	Not performed	Positive	Positive	
7-Dog-62	Dog	Positive	Not performed	Positive	Positive	
7-Dog-63	Dog	Negative	Not performed	Negative	Not performed	
7-Dog-64	Dog	Positive	Not performed	Positive	Positive	
7-Dog-65	Dog	Positive	Not performed	Positive	Positive	
7-Dog-66	Dog	Positive	Not performed	Positive	Positive	
7-Dog-67	Dog	Positive	Not performed	Positive	Positive	
7-Dog-68	Dog	Positive	Not performed	Positive	Positive	
1-Dog-7	Dog	Positive	Not performed	Positive	Positive	
3-Dog-9	Dog	Positive	Not performed	Positive	Positive	
3-Dog-10	Dog	Positive	Not performed	Positive	Douteux	
9-Dog-23	Dog	Positive	Not performed	Positive	Positive	
7-Dog-53	Dog	Positive	Not performed	Positive	Douteux	
9-Jackal-9	Jackal	Negative	Negative	Negative	Not performed	
9-Dog-21	Dog	Positive	Positive	Positive	Positive	
9-Dog-22	Dog	Positive	Positive	Positive	Positive	
3-Dog-8	Dog	Positive	Positive	Positive	Positive	
9-Jackal-7	Jackal	Negative	Negative	Negative	Not performed	
9-Jackal-8	Jackal	Negative	Negative	Negative	Not performed	
9-Jackal-13	Jackal	Negative	Negative	Negative	Not performed	
9-Jackal-14	Jackal	Negative	Negative	Negative	Not performed	
9-Jackal-15	Jackal	Negative	Negative	Negative	Not performed	
9-Jackal-16	Jackal	Negative	Negative	Negative	Not performed	
7-Dog-69	Dog	Positive	Not performed	Positive	Positive	
7-Dog-70	Dog	Positive	Not performed	Positive	Douteux	
7-Dog-71	Dog	Positive	Not performed	Positive	Douteux	
7-Dog-73	Dog	Positive	Not performed	Positive	Positive	
9-Dog-24	Dog	Not performed	Not performed	Not performed	Negative	
7-Dog-74	Dog	Positive	Not performed	Positive	Positive	
7-Dog-73	Dog	Positive	Not performed	Positive	Douteux	
7-Dog-75	Dog	Positive	Not performed	Positive	Positive	

7-Dog-76	Dog	Positive	Not performed	Positive	Positive	
7-Dog-77	Dog	Positive	Not performed	Positive	Positive	
7-Dog-78	Dog	Positive	Not performed	Positive	Positive	
7-Dog-79	Dog	Positive	Not performed	Positive	Positive	
7-Dog-80	Dog	Positive	Not performed	Positive	Negative	
7-Dog-81	Dog	Negative	Not performed	Negative	Negative	
7-Dog-82	Dog	Negative	Not performed	Negative	Not performed	
7-Dog-83	Dog	Positive	Not performed	Positive	Not performed	
01-Dog-8	Dog	Positive	Not performed	Positive	Positive	
KIN LEMBA	Dog	Positive	Positive	Positive	Not performed	
KIN DELVAUX	Dog	Positive	Positive	Positive	Not performed	

Table 35: Description of the 162 samples tested with the RIDT test for determination of its intrinsic parameters presented in Table 6

Lab 1	France	2015	150007	Cat	Negative	2015	Brain	Primary	Negative	Negative	1801111	Results from Léchenne et al., 2016
Lab 1	France	2015	150036	Dog	Negative	2015	Brain	Primary	Negative	Negative	1801111	Results from Léchenne et al., 2016
Lab 1	France	2015	150038	Cat	Negative	2015	Brain	Primary	Negative	Negative	1801111	Results from Léchenne et al., 2016
Lab 1	France	2015	150041	Cat	Negative	2015	Brain	Primary	Negative	Negative	1801111	Results from Léchenne et al., 2016
Lab 1	France	2015	150042	Ferret	Negative	2015	Brain	Primary	Negative	Negative	1801111	Results from Léchenne et al., 2016
Lab 1	France	2015	150043	Dog	Negative	2015	Brain	Primary	Negative	Negative	1801111	Results from Léchenne et al., 2016
Lab 1	France	2015	150044	Dog	Negative	2015	Brain	Primary	Negative	Negative	1801111	Results from Léchenne et al., 2016
Lab 1	France	2015	150049	Dog	Negative	2015	Brain	Primary	Negative	Negative	1801111	Results from Léchenne et al., 2016
Lab 1	France	2015	150050	Dog	Negative	2015	Brain	Primary	Negative	Negative	1801111	Results from Léchenne et al., 2016
Lab 1	France	2015	150051	Dog	Negative	2015	Brain	Primary	Negative	Negative	1801111	Results from Léchenne et al., 2016
Lab 1	France	2015	150052	Cat	Negative	2015	Brain	Primary	Negative	Negative	1801111	Results from Léchenne et al., 2016
Lab 1	France	2015	150053	Dog	Negative	2015	Brain	Primary	Negative	Negative	1801111	Results from Léchenne et al., 2016
Lab 1	France	2015	150054	Cat	Negative	2015	Brain	Primary	Negative	Negative	1801111	Results from Léchenne et al., 2016
Lab 1	France	2015	150055	Red fox	Negative	2015	Brain	Primary	Negative	Negative	1801111	Results from Léchenne et al., 2016
Lab 1	France	2015	150056	Cat	Negative	2015	Brain	Primary	Negative	Negative	1801111	Results from Léchenne et al., 2016
Lab 1	France	2015	150057	Dog	Negative	2015	Brain	Primary	Negative	Positive	1801111	Results from Léchenne et al., 2016
Lab 1	France	2015	150058	Dog	Negative	2015	Brain	Primary	Negative	Negative	1801111	Results from Léchenne et al., 2016
Lab 1	France	2015	150059	Dog	Negative	2015	Brain	Primary	Negative	Negative	1801111	Results from Léchenne et al., 2016
Lab 1	France	2015	150060	Cat	Negative	2015	Brain	Primary	Negative	Negative	1801111	Results from Léchenne et al., 2016
Lab 1	France	2015	150061	Cat	Negative	2015	Brain	Primary	Negative	Negative	1801111	Results from Léchenne et al., 2016
Lab 1	France	2015	150062	Cat	Negative	2015	Brain	Primary	Negative	Negative	1801111	Results from Léchenne et al., 2016
Lab 1	France	2015	150119	Ferret	Negative	2015	Brain	Primary	Negative	Negative	1801111	Results from Léchenne et al., 2016
Lab 1	France	2015	150125	Cat	Negative	2015	Brain	Primary	Negative	Positive	1801111	Results from Léchenne et al., 2016

Lab 1	France	2015	150127	Cat	Negative	2015	Brain	Primary	Negative	Negative	1801111	Results from Léchenne et al., 2016
Lab 1	France	2015	150129	Cat	Negative	2015	Brain	Primary	Negative	Negative	1801111	Results from Léchenne et al., 2016
Lab 1	France	2015	150132	Dog	Negative	2015	Brain	Primary	Negative	Negative	1801111	Results from Léchenne et al., 2016
Lab 1	France	2015	150133	Dog	Negative	2015	Brain	Primary	Negative	Negative	1801111	Results from Léchenne et al., 2016
Lab 1	France	2015	150134	Red fox	Negative	2015	Brain	Primary	Negative	Negative	1801111	Results from Léchenne et al., 2016
Lab 1	France	2015	150148	Horse	Negative	2015	Brain	Primary	Negative	Negative	1801111	Results from Léchenne et al., 2016
Lab 1	France	2015	150230	Red fox	Negative	2015	Brain	Primary	Negative	Negative	1801111	Results from Léchenne et al., 2016
Lab 1	Nigeria	2015	8670NIG	Human	RABV	NA	Brain	Experimental (mouse)	Positive	Positive	1801111	Results from Léchenne et al., 2016
Lab 1	Greenland	2015	8683GRO	Fox	RABV	1980	Brain	Primary	Positive	Positive	1801111	Results from Léchenne et al., 2016
Lab 1	Greenland	2015	8684GRO	Fox	RABV	1981	Brain	Primary	Positive	Positive	1801111	Results from Léchenne et al., 2016
Lab 1	Egypt	2015	8692EGY	Human	RABV	1979	Brain	Experimental (mouse)	Positive	Positive	1801111	Results from Léchenne et al., 2016
Lab 1	Benin	2015	8697BEN	Cat	RABV	1986	Brain	Primary	Positive	Positive	1801111	Results from Léchenne et al., 2016
Lab 1	Saudi Ara-bia	2015	8706ARS	Fox	RABV	1987	Brain	Primary	Positive	Positive	1801111	Results from Léchenne et al., 2016
Lab 1	Cameroon	2015	8801CAM	Dog	RABV	1987	Brain	Primary	Positive	Positive	1801111	Results from Léchenne et al., 2016
Lab 1	Ethiopia	2015	8807ETH	Hyena	RABV	1988	Brain	Primary	Positive	Positive	1801111	Results from Léchenne et al., 2016
Lab 1	Ethiopia	2015	8808ETH	Dog	RABV	1987	Brain	Primary	Positive	Positive	1801111	Results from Léchenne et al., 2016
Lab 1	Ivory Coast	2015	9003CI	Dog	RABV	1989	Brain	Primary	Positive	Positive	1801111	Results from Léchenne et al., 2016
Lab 1	Niger	2015	9010NIG	Dog	RABV	1990	Brain	Primary	Positive	Positive	1801111	Results from Léchenne et al., 2016
Lab 1	Chad	2015	9021TCH	Dog	RABV	1990	Brain	Primary	Positive	Positive	1801111	Results from Léchenne et al., 2016
Lab 1	Guinea	2015	9024GUI	Dog	RABV	1990	Brain	Primary	Positive	Positive	1801111	Results from Léchenne et al., 2016
Lab 1	USA	2015	9104USA	Skunk	RABV	1991	Brain	Primary	Positive	Positive	1801111	Results from Léchenne et al., 2016
Lab 1	Mexico	2015	9115MEX	Dog	RABV	1991	Brain	Primary	Positive	Positive	1801111	Results from Léchenne et al., 2016
Lab 1	Mauritania	2015	9136MAU	Goat	RABV	1991	Brain	Primary	Positive	Positive	1801111	Results from Léchenne et al., 2016
Lab 1	Russia	2015	9141RUS	Polar fox	RABV	1988-90	Brain	NA	Positive	Positive	1801111	Results from Léchenne et al., 2016

Lab 1	Germany	2015	9217ALL	Red fox	RABV	1991	Brain	Primary	Positive	Negative	1801111	Results from Léchenne et al., 2016
Lab 1	Chad	2015	9218TCH	Dog	RABV	1992	Brain	Primary	Positive	Positive	1801111	Results from Léchenne et al., 2016
Lab 1	Central African Republic	2015	9228CAR	Dog	RABV	1992	Brain	Primary	Positive	Positive	1801111	Results from Léchenne et al., 2016
Lab 1	Namibia	2015	9231NAM	Jackal	RABV	1992	Brain	Primary	Positive	Positive	1801111	Results from Léchenne et al., 2016
Lab 1	Somalia	2015	9302SOM	Dog	RABV	1993	Brain	Primary	Positive	Positive	1801111	Results from Léchenne et al., 2016
Lab 1	SeNegativeal	2015	9305SEN	Dog	RABV	1992	Brain	Primary	Positive	Positive	1801111	Results from Léchenne et al., 2016
Lab 1	Mauritania	2015	9312MAU	Dog	RABV	1993	Brain	Primary	Positive	Positive	1801111	Results from Léchenne et al., 2016
Lab 1	Iran	2015	9319IRA	Jackal	RABV	NA	Brain	Primary	Positive	Positive	1801111	Results from Léchenne et al., 2016
Lab 1	Hungary	2015	9391HON	Fox	RABV	1993	Brain	Primary	Positive	Positive	1801111	Results from Léchenne et al., 2016
Lab 1	Turkey	2015	93101TUR	Fox	RABV	1993	Brain	Primary	Positive	Negative	1801111	Results from Léchenne et al., 2016
Lab 1	Estonia	2015	93105EST	Fox	RABV	1993	Brain	Primary	Positive	Positive	1801111	Results from Léchenne et al., 2016
Lab 1	Zimbabwe	2015	93119ZIM	Dog	RABV	NA	Brain	Primary	Positive	Positive	1801111	Results from Léchenne et al., 2016
Lab 1	Rwanda	2015	94289RWA	Dog	RABV	1994	Brain	Primary	Positive	Positive	1801111	Results from Léchenne et al., 2016
Lab 1	Brazil	2015	9522BRE	Dog	RABV	1995	Brain	Primary	Positive	Positive	1801111	Results from Léchenne et al., 2016
Lab 1	Burkina Faso	2015	9547HAV	Dog	RABV	1995	Brain	Primary	Positive	Positive	1801111	Results from Léchenne et al., 2016
Lab 1	Chad	2015	9609TCH	Dog	RABV	1996	Brain	Primary	Positive	Positive	1801111	Results from Léchenne et al., 2016
Lab 1	Tanzania	2015	9613TAN	Dog	RABV	1996	Brain	Primary	Positive	Positive	1801111	Results from Léchenne et al., 2016
Lab 1	Poland	2015	96178POL	Fox	RABV	1994	Brain	Primary	Positive	Positive	1801111	Results from Léchenne et al., 2016
Lab 1	India	2015	9702IND	Human	RABV	1997	Brain	Primary	Positive	Positive	1801111	Results from Léchenne et al., 2016
Lab 1	French Guiana	2015	9705FRA	Bovine	RABV	1997	Brain	Primary	Positive	Positive	1801111	Results from Léchenne et al., 2016
Lab 1	Myanmar	2015	9915BIR	Dog	RABV	1999	Brain	Primary	Positive	Positive	1801111	Results from Léchenne et al., 2016
Lab 1	Cambodia	2015	9916CAM	Dog	RABV	1999	Brain	Primary	Positive	Positive	1801111	Results from Léchenne et al., 2016
Lab 1	China	2015	02041CHI	Dog	RABV	1987	Brain	Primary	Positive	Positive	1801111	Results from Léchenne et al., 2016

Lab 1	Afghanistan	2015	02052AFG	Dog	RABV	2002	Brain	Primary	Positive	Positive	1801111	Results from Léchenne et al., 2016
Lab 1	France	2015	04031FRA	Dog	RABV	2004	Brain	Primary	Positive	Positive	1801111	Results from Léchenne et al., 2016
Lab 1	Madagas-car	2015	04033MAD	Human	RABV	2004	Brain	NA	Positive	Positive	1801111	Results from Léchenne et al., 2016
Lab 1	Lab strain	2015	CVS 27 14-10	-	RABV	-	Brain	Experimental (mouse)	Positive	Positive	1801111	Part of the panel of samples from the proficiency test 2015 by Anses, results from Lé-chenne et al., 2016
Lab 1	France	2015	GS7	Red fox	RABV	1986	Brain	Experimental (mouse)	Positive	Positive	1801111	Part of the panel of samples from the proficiency test 2015 by Anses, results from Lé-chenne et al., 2016
Lab 1	Greece	2015	DR627	Red fox	RABV	2012	Brain	Experimental (mouse)	Positive	Positive	1801111	Part of the panel of samples from the proficiency test 2015 by Anses, results from Lé-chenne et al., 2016
Lab 1	France	2015	127900	Bat (My-otis nat-tereri)	BBLV	2012	Brain	Experimental (mouse)	Positive	Positive	1801111	Part of the panel of samples from the proficiency test 2015 by Anses, results from Lé-chenne et al., 2016
Lab 1	South Af-rica	2015	DUVV 05-11	Human	DUVV	1971	Brain	Experimental (mouse)	Positive	Positive	1801111	Part of the panel of samples from the proficiency test 2015 by Anses, results from Lé-chenne et al., 2016
Lab 1	France	2015	122938	Bat (Epte-sicus seroti-nus)	EBLV-1	2002	Brain	Experimental (mouse)	Positive	Positive	1801111	Part of the panel of samples from the proficiency test 2015 by Anses, results from Lé-chenne et al., 2016
Lab 1	United Kingdom	2015	EBL2 RV1787	Bat (My-otis dauben-tonii)	EBLV-2	2004	Brain	Experimental (mouse)	Positive	Positive	1801111	Part of the panel of samples from the proficiency test 2015 by Anses, results from Lé-chenne et al., 2016
Lab 1	France	2015	Negative 17-13	Red fox	Negative	2012	Brain	Primary	Negative	Negative	1801111	Part of the panel of samples from the proficiency test 2015 by Anses, results from Lé-chenne et al., 2016
Lab 1	France	2015	Negative 17-13	Red fox	Negative	2012	Brain	Primary	Negative	Negative	1801111	Part of the panel of samples from the proficiency test 2015 by Anses, results from Lé-chenne et al., 2016
Lab 2	Chad	2012	342	Dog	RABV	2012	Brain	Primary	Negative	Negative	1801076	Results from Léchenne et al., 2016, lyssavirus species not confirmed by molecular geno-typing
Lab 2	Chad	2012	344	Dog	RABV	2012	Brain	Primary	Positive	Positive	1801076	Results from Léchenne et al., 2016, lyssavirus species not

												confirmed by molecular geno-typing
Lab 2	Chad	2012	345	Dog	RABV	2012	Brain	Primary	Positive	Positive	1801076	Results from Léchenne et al., 2016, lyssavirus species not confirmed by molecular geno-typing
Lab 2	Chad	2012	346	Dog	RABV	2012	Brain	Primary	Positive	Positive	1801076	Results from Léchenne et al., 2016, lyssavirus species not confirmed by molecular geno-typing
Lab 2	Chad	2012	347	Dog	RABV	2012	Brain	Primary	Positive	Positive	1801076	Results from Léchenne et al., 2016, lyssavirus species not confirmed by molecular geno-typing
Lab 2	Chad	2012	348	Dog	RABV	2012	Brain	Primary	Positive	Positive	1801076	Results from Léchenne et al., 2016, lyssavirus species not confirmed by molecular geno-typing
Lab 2	Chad	2012	349	Dog	RABV	2012	Brain	Primary	Positive	Positive	1801076	Results from Léchenne et al., 2016, lyssavirus species not confirmed by molecular geno-typing
Lab 2	Chad	2012	350	Dog	RABV	2012	Brain	Primary	Positive	Positive	1801076	Results from Léchenne et al., 2016, lyssavirus species not confirmed by molecular geno-typing
Lab 2	Chad	2012	354	Dog	RABV	2012	Brain	Primary	Positive	Positive	1801076	Results from Léchenne et al., 2016, lyssavirus species not confirmed by molecular geno-typing
Lab 2	Chad	2012	355	Dog	RABV	2012	Brain	Primary	Positive	Positive	1801076	Results from Léchenne et al., 2016, lyssavirus species not confirmed by molecular geno-typing
Lab 2	Chad	2012	356	Dog	RABV	2012	Brain	Primary	Positive	Positive	1801076	Results from Léchenne et al., 2016, lyssavirus species not confirmed by molecular geno-typing
Lab 2	Chad	2012	357	Dog	RABV	2012	Brain	Primary	Positive	Positive	1801076	Results from Léchenne et al., 2016, lyssavirus species not confirmed by molecular geno-typing
Lab 2	Chad	2012	358	Dog	RABV	2012	Brain	Primary	Positive	Positive	1801076	Results from Léchenne et al., 2016, lyssavirus species not confirmed by molecular geno-typing
Lab 2	Chad	2012	359	Dog	RABV	2012	Brain	Primary	Positive	Positive	1801076	Results from Léchenne et al., 2016, lyssavirus species not

												confirmed by molecular geno-typing
Lab 2	Chad	2012	361	Dog	RABV	2012	Brain	Primary	Positive	Positive	1801076	Results from Léchenne et al., 2016, lyssavirus species not confirmed by molecular geno-typing
Lab 2	Chad	2012	363	Dog	RABV	2012	Brain	Primary	Positive	Positive	1801076	Results from Léchenne et al., 2016, lyssavirus species not confirmed by molecular geno-typing
Lab 2	Chad	2012	364	Dog	RABV	2012	Brain	Primary	Positive	Positive	1801076	Results from Léchenne et al., 2016, lyssavirus species not confirmed by molecular geno-typing
Lab 2	Chad	2012	365	Dog	RABV	2012	Brain	Primary	Negative	Negative	1801076	Results from Léchenne et al., 2016, lyssavirus species not confirmed by molecular geno-typing
Lab 2	Chad	2012	366	Dog	RABV	2012	Brain	Primary	Positive	Positive	1801076	Results from Léchenne et al., 2016, lyssavirus species not confirmed by molecular geno-typing
Lab 2	Chad	2013	367	Dog	RABV	2013	Brain	Primary	Positive	Positive	1801076	Results from Léchenne et al., 2016, lyssavirus species not confirmed by molecular geno-typing
Lab 2	Chad	2013	368	Dog	RABV	2013	Brain	Primary	Positive	Positive	1801076	Results from Léchenne et al., 2016, lyssavirus species not confirmed by molecular geno-typing
Lab 2	Chad	2013	369	Dog	RABV	2013	Brain	Primary	Positive	Positive	1801076	Results from Léchenne et al., 2016, lyssavirus species not confirmed by molecular geno-typing
Lab 2	Chad	2013	371	Dog	RABV	2013	Brain	Primary	Positive	Positive	1801076	Results from Léchenne et al., 2016, lyssavirus species not confirmed by molecular geno-typing
Lab 2	Chad	2013	372	Dog	RABV	2013	Brain	Primary	Positive	Positive	1801076	Results from Léchenne et al., 2016, lyssavirus species not confirmed by molecular geno-typing
Lab 2	Chad	2013	373	Dog	RABV	2013	Brain	Primary	Positive	Positive	1801076	Results from Léchenne et al., 2016, lyssavirus species not confirmed by molecular geno-typing
Lab 2	Chad	2013	379	Dog	RABV	2013	Brain	Primary	Positive	Positive	1801076	Results from Léchenne et al., 2016, lyssavirus species not

												confirmed by molecular geno-typing
Lab 2	Chad	2013	380	Dog	RABV	2013	Brain	Primary	Negative	Negative	1801076	Results from Léchenne et al., 2016, lyssavirus species not confirmed by molecular geno-typing
Lab 2	Chad	2014	381	Dog	RABV	2014	Brain	Primary	Positive	Positive	1801076	Results from Léchenne et al., 2016, lyssavirus species not confirmed by molecular geno-typing
Lab 2	Chad	2014	383	Dog	RABV	2014	Brain	Primary	Negative	Negative	1801076	Results from Léchenne et al., 2016, lyssavirus species not confirmed by molecular geno-typing
Lab 2	Chad	2014	384	Dog	RABV	2014	Brain	Primary	Negative	Negative	1801076	Results from Léchenne et al., 2016, lyssavirus species not confirmed by molecular geno-typing
Lab 2	Chad	2014	386	Dog	RABV	2014	Brain	Primary	Negative	Negative	1801076	Results from Léchenne et al., 2016, lyssavirus species not confirmed by molecular geno-typing
Lab 2	Chad	2014	387	Ovine	RABV	2014	Brain	Primary	Negative	Negative	1801076	Results from Léchenne et al., 2016, lyssavirus species not confirmed by molecular geno-typing
Lab 2	Chad	2014	390	Dog	RABV	2014	Brain	Primary	Negative	Negative	1801076	Results from Léchenne et al., 2016, lyssavirus species not confirmed by molecular geno-typing
Lab 2	Chad	2014	392	Dog	RABV	2014	Brain	Primary	Negative	Negative	1801076	Results from Léchenne et al., 2016, lyssavirus species not confirmed by molecular geno-typing
Lab 2	Chad	2014	393	Dog	RABV	2014	Brain	Primary	Negative	Negative	1801076	Results from Léchenne et al., 2016, lyssavirus species not confirmed by molecular geno-typing
Lab 2	Chad	2014	394	Dog	RABV	2014	Brain	Primary	Positive	Positive	1801076	Results from Léchenne et al., 2016, lyssavirus species not confirmed by molecular geno-typing
Lab 2	Chad	2014	395	Dog	RABV	2014	Brain	Primary	Positive	Positive	1801076	Results from Léchenne et al., 2016, lyssavirus species not confirmed by molecular geno-typing
Lab 2	Chad	2014	401	Dog	RABV	2014	Brain	Primary	Positive	Positive	1801076	Results from Léchenne et al., 2016, lyssavirus species not

												confirmed by molecular genotyping
Lab 2	Chad	2014	402	Singe	RABV	2014	Brain	Primary	Negative	Negative	1801076	Results from Léchenne et al., 2016, lyssavirus species not confirmed by molecular genotyping
Lab 2	Chad	2014	403	Dog	RABV	2014	Brain	Primary	Positive	Positive	1801076	Results from Léchenne et al., 2016, lyssavirus species not confirmed by molecular genotyping
Lab 2	Chad	2015	405	Dog	RABV	2015	Brain	Primary	Positive	Positive	1801076	Results from Léchenne et al., 2016, lyssavirus species not confirmed by molecular genotyping
Lab 2	Chad	2015	406	Dog	RABV	2015	Brain	Primary	Positive	Positive	1801076	Results from Léchenne et al., 2016, lyssavirus species not confirmed by molecular genotyping
Lab 2	Chad	2015	407	Dog	RABV	2015	Brain	Primary	Positive	Positive	1801076	Results from Léchenne et al., 2016, lyssavirus species not confirmed by molecular genotyping
Lab 2	Chad	2015	408	Dog	RABV	2015	Brain	Primary	Positive	Positive	1801076	Results from Léchenne et al., 2016, lyssavirus species not confirmed by molecular genotyping
Lab 3	Ivory Coast	2017	Viro 012	Dog	RABV	2017	Brain	Primary	Positive	Positive	NA	Lyssavirus species not confirmed by molecular genotyping
Lab 3	Ivory Coast	2017	Viro 013	Cat	RABV	2017	Brain	Primary	Positive	Positive	NA	Lyssavirus species not confirmed by molecular genotyping
Lab 3	Ivory Coast	2017	Viro 014	Dog	RABV	2017	Brain	Primary	Positive	Positive	NA	Lyssavirus species not confirmed by molecular genotyping
Lab 3	Ivory Coast	2017	Viro 68	Dog	RABV	2017	Brain	Primary	Positive	Positive	NA	Lyssavirus species not confirmed by molecular genotyping
Lab 3	Ivory Coast	2017	Viro 71	Dog	RABV	2017	Brain	Primary	Negative	Negative	NA	Lyssavirus species not confirmed by molecular genotyping
Lab 3	Ivory Coast	2017	Viro 89	Dog	RABV	2017	Brain	Primary	Positive	Positive	NA	Lyssavirus species not confirmed by molecular genotyping
Lab 3	Ivory Coast	2017	Viro 97	Dog	RABV	2017	Brain	Primary	Positive	Positive	NA	Lyssavirus species not confirmed by molecular genotyping

Lab 3	Ivory Coast	2017	Viro 006	Dog	RABV	2017	Brain	Primary	Positive	Positive	NA	Lyssavirus species not confirmed by molecular genotyping
Lab 3	Ivory Coast	2017	Viro 020	Dog	RABV	2017	Brain	Primary	Positive	Positive	NA	Lyssavirus species not confirmed by molecular genotyping
Lab 3	Ivory Coast	2017	Viro 035	Cat	RABV	2017	Brain	Primary	Negative	Negative	NA	Lyssavirus species not confirmed by molecular genotyping
Lab 4	Mali	2017	042/01/17	Dog	RABV	2017	Brain	Primary	Positive	Positive	NA	Lyssavirus species not confirmed by molecular genotyping
Lab 4	Mali	2017	106/01/17	Dog	RABV	2017	Brain	Primary	Positive	Positive	NA	Lyssavirus species not confirmed by molecular genotyping
Lab 4	Mali	2017	143/02/17	Dog	RABV	2017	Brain	Primary	Positive	Positive	NA	Lyssavirus species not confirmed by molecular genotyping
Lab 4	Mali	2017	174/02/17	Dog	RABV	2017	Brain	Primary	Positive	Positive	NA	Lyssavirus species not confirmed by molecular genotyping
Lab 4	Mali	2017	216/03/17	Dog	RABV	2017	Brain	Primary	Negative	Negative	NA	Lyssavirus species not confirmed by molecular genotyping
Lab 4	Mali	2017	285/03/17	Dog	RABV	2017	Brain	Primary	Negative	Negative	NA	Lyssavirus species not confirmed by molecular genotyping
Lab 4	Mali	2017	389/05/17	Dog	RABV	2017	Brain	Primary	Positive	Positive	NA	Lyssavirus species not confirmed by molecular genotyping
Lab 4	Mali	2017	410/05/17	Dog	RABV	2017	Brain	Primary	Positive	Positive	NA	Lyssavirus species not confirmed by molecular genotyping
Lab 4	Mali	2017	530/08/17	Dog	RABV	2017	Brain	Primary	Positive	Positive	NA	Lyssavirus species not confirmed by molecular genotyping
Lab 4	Mali	2017	537/08/17	Dog	RABV	2017	Brain	Primary	Positive	Positive	NA	Lyssavirus species not confirmed by molecular genotyping
Lab 4	Mali	2017	541/08/17	Jackal	RABV	2017	Brain	Primary	Positive	Positive	NA	Lyssavirus species not confirmed by molecular genotyping
Lab 4	Mali	2017	581/08/17	Dog	RABV	2017	Brain	Primary	Negative	Negative	NA	Lyssavirus species not confirmed by molecular genotyping
Lab 4	Mali	2017	610/09/17	Dog	RABV	2017	Brain	Primary	Positive	Positive	NA	Lyssavirus species not confirmed by molecular genotyping

Lab 4	Mali	2017	640/09/17	Dog	RABV	2017	Brain	Primary	Positive	Positive	NA	Lyssavirus species not confirmed by molecular genotyping
Lab 4	Mali	2017	646/09/17	Dog	RABV	2017	Brain	Primary	Positive	Positive	NA	Lyssavirus species not confirmed by molecular genotyping
Lab 4	Mali	2017	663/10/17	Dog	RABV	2017	Brain	Primary	Positive	Positive	NA	Lyssavirus species not confirmed by molecular genotyping
Lab 4	Mali	2017	682/10/17	Dog	RABV	2017	Brain	Primary	Positive	Positive	NA	Lyssavirus species not confirmed by molecular genotyping
Lab 4	Mali	2017	692/11/17	Dog	RABV	2017	Brain	Primary	Positive	Positive	NA	Lyssavirus species not confirmed by molecular genotyping
Lab 5	Italy (ex-Nepal)	2016	117/1996	Human (ex-dog)	RABV	-	Brain	-	Positive	Positive	1801077/1801081	Results from Eggerbauer et al., 2016
Lab 5	Italy (ex-India)	2016	3570/2011	Italy (ex-India)	RABV	-	Brain	-	Positive	Positive	1801077/1801081	Results from Eggerbauer et al., 2016
Lab 5	Botswana	2016	2871/2009	Bovine	RABV	-	Brain	-	Positive	Positive	1801077/1801081	Results from Eggerbauer et al., 2016
Lab 5	Botswana	2016	6665/2009	Honey badger	RABV	-	Brain	-	Positive	Positive	1801077/1801081	Results from Eggerbauer et al., 2016
Lab 5	Brazil	2016	351/2010	Bovine	RABV	-	Brain	-	Positive	Positive	1801077/1801081	Results from Eggerbauer et al., 2016
Lab 5	Brazil	2016	5B1/2011	Kinkajou (Potos flavus)	RABV	-	Brain	-	Positive	Positive	1801077/1801081	Results from Eggerbauer et al., 2016
Lab 5	Brazil	2016	343/2011	Equine	RABV	-	Brain	-	Positive	Positive	1801077/1801081	Results from Eggerbauer et al., 2016
Lab 5	Italy	2016	6944/2009	Red fox	RABV	-	Brain	-	Positive	Positive	1801077/1801081	Results from Eggerbauer et al., 2016

Appendix 7: Pictures from the field, DRC

Picture 3: Exploratory study in July 2016, DRC. Getting a first impression on rabies awareness and case management at the General Referral Hospital of Muanda.

Picture 4: Joint rabies workshops, DRC. Training and setting a foundation for future collaboration between veterinary and medical focal points.

Picture 5:Animal rabies sampling training in the field with VFPs from Muanda

Picture 6: Enhancement of the rabies laboratory at INRB, DRC

Picture 7: World rabies day 2017, awareness campaign in the DRC

Appendix 8: Pictures from the field, Chad

Picture 8: There are about 30 000 dogs in N'Djamena, Chad, most of them owned, but free-roaming

Picture 9: Dog collared with a GCS unit in N'Djamena, Chad

Picture 10: Explaining the goal of the study to dog owners

Picture 11: Preparing for the field survey and becoming acquainted with GPS

Appendix 9: Curriculum Vitae

Name: med.vet. Céline Mbilo, Phd

Date of birth: 29.05.1988

Nationality: Swiss

Academic Background

03/2016 – 09/2019	**University of Basel, PhD in Epidemiology** Supervisor: Prof. Dr. Jakob Zinsstag, Swiss TPH
09/2013 – 01/2016	**University of Zurich, Master of Veterinary Medicine** Specialization in Veterinary Public Health
09/2009 – 09/2012	**University of Zurich, Bachelor of Veterinary Medicine**

Publications

1. Mauti, S., Léchenne, M., Naïssengar, S., Traoré, A., Kallo, V., Kouakou, C., Couacy-Hymann, E., Gourlaouen, M., **Mbilo, C**., Pyana, P. P., Madaye, E., Dicko, I., Cozette, P., De Benedictis, P., Bourhy, H., Zinsstag, J., Dacheux, L. **Field Postmortem Rabies Rapid Immunochromatographic Diagnostic Test for Resource-Limited Settings with Further Molecular Applications**. e60008, doi:10.3791/60008 (2020).
2. **Mbilo C.** et al (2020) - **Dog rabies control in West and Central Africa: A review. Acta Tropica**
3. **Mbilo, C.,** Kabongo, J.-B., Pyana, P.P., Nlonda, L., Nzita, R.W., Luntadila, B., Badibanga, B., Hattendorf, J., Zinsstag, J. (2019). – **Dog ecology, bite incidence, and disease awareness: a cross-sectional survey among a rabies-affected community in the Democratic Republic of the Congo**.Vaccines, 7 (3), doi: 10.3390/vaccines7030098.
4. **Mbilo C**., Lechenne M., Mauti S., Chitnis N., Tschopp R., & Zinsstag J. (2018). – **Rabies in dogs, livestock and wildlife: a veterinary perspective.** Rev. Sci. Tech. Off. Int. Epiz., 37 (2), doi: 10.20506/rst.37.2.2806.
5. Laager M., **Mbilo C.**, Madaye E.A., Naminou A., Lechenne M., Tschopp A., Naissengar S.K., Smieszek T., Zinsstag J. & Chitnis N. (2018). – **The importance of dog population contact network structures in rabies transmission**. PLoS Negl Trop Dis, 12 (8), doi: 10.1371/journal.pntd.0006680.
6. **Mbilo C.**, Lechenne M., Hattendorf J., Madjadinan S., Anyiam F. & Zinsstag J. (2016). – **Rabies awareness and dog ownership among rural northern and southern Chadian communities - Analysis of a community-based, cross-sectional household survey.** Acta tropica, doi: 10.1016/j.actatropica.2016.06.003