Rabies control in N'Djamena, Chad

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« Ce n’est pas une vie que de ne pas bouger !»

Alexandre Yersin

<What was life, if you don’t commit to something?>

<Das ist doch kein Leben, wenn man nichts unternimmt>

To the anonymous children with the puppy on my desktop picture

- my daily motivation

(Plate 1)

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ABC	Animal Birth Control Approach
AREB	Asian Rabies Expert Bureau
ASEAN	Association for Southeast Asian Nations
BBLV	Bokeloh bat lyssavirus
BGTO	Bali Government Tourism Office
BSR cells	Baby Hamster Kidney cells
CBPP	Contagious Bovine Pleuro-Pneumonia
CDV	Canine Distemper Virus
CSSI	Centre de Support en Santé International
DIB	Development Impact Bond
DRIT	Direct Rapid Immunohistochemical Test
DUVV	Duvenhage Virus
EBLV	European bat Lyssavirus
FAT	Fluorescent Antibody Test
FFU	Florescent Focus-forming Units
FMD	Foot and Mouth disease
GAVI	Global Alliance for Vaccine Investment
HDCV	Human Diploid Cell Vaccine
ICONZ	Network on integrated control of zoonoses in Africa
IBCM	Integrated Bite Case Managment
IEC	Information Education Communication
INSEED	Institut National de la Statistique, des Etudes Economiques et Demographiques, Chad
IRED	Insitut de Recherche en Elevage pour le Développement
LCV	Laboratoire Central Vétérinaire
MARD	Ministry of Agriculture and Rural Development, Vietnam
MOH	Ministry of Health, Vietnam
NGO	Non-Governmental Organization
NRC-R	National Reference Centre for Rabies
OIE	World Organization for Animal Health
OR	Odds Ratio
PaRaCON	Pan African Rabies Control Network
PARC	Pan-African Rinderpest Campaign
PBS	phosphate-buffered saline
PCR	Polymerase Chain Reaction
PEP	Post Exposure Prophylaxis
PreP	Pre-Exposure Prophylaxis
PRP	Partners for Rabies Prevention
R_e	Effective reproductive ratio
R_0	Basic reproductive ratio
RABV	Classical Rabies Virus
RIDT	Rapid Immune Diagnostic Test

RIG	Rabies Immunoglobulin
RNA	Ribonucleic Acid
RNT	Radiodiffusion Nationale Tchadienne
RPO	Responsible Pet Ownership
RR	Risk Ratio
RT-qPCR	Real time reverse transcription polymerase chain reaction
SAARC	South Asian Association for Regional Cooperation
SARE	Stepwise Approach towards Rabies Elimination
SD	Standard deviation
SEARG	Southern and Eastern African Rabies Group
SDC	Swiss Agency for Development and Cooperation
Swiss TPH	Swiss Tropical and Public Health Institute
TE	Translational Epidemiology
TR	Transdisciplinary research
UNWTO	United Nations World Tourism Organization
USA	United States of America
USD	US Dollar
VP	Vaccination Post
VSF	Vétérinaires Sans Frontières
WHO	World Health Organization
WRD	World Rabies Day
YLL	Years of Life Lost

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II. SUMMARY

Rabies is a viral disease that induces invariably fatal encephalitis. Transmission of the virus to humans occurs in the vast majority of cases through bite exposure to a rabies attained animal, mainly the domestic dog. Although there exists no treatment against rabies the disease can be prevented by pre- (PreP) and post exposure prophylaxis (PEP) with highly potent vaccines. However, PreP is not routinely applied in endemic countries and access to vaccination after exposure is often hindered through low availability and high costs. Most affected by the disease are resource poor countries, which do not have the capacities, the infrastructure nor the means needed to adequately fight against rabies. At the same time the canine population in these countries are mostly unsupervised or even stray, their population turnover is high and they find their food in the streets. All these factors contribute to the endemic persistence of rabies in the canine population and facilitate the transmission to humans. In consequence, estimates on the worldwide burden of rabies reveal that the disease claims one human victim approximately every ten minutes causing a yearly loss of 1.74 million disability-adjusted life years (DALY). Despite these impacts rabies remains neglected by many national and international decision makers and is overshadowed by other big public health priorities. The main obstacle for the promotion of rabies control is the absence of incidence data. At the origin of huge surveillance gaps are not only the absence of diagnostic structures or the lack of public awareness but also an insufficient communication between veterinary and public health institutions.

The tools to eliminate rabies in dogs are well established and at hand. Several efficacious vaccines, oral and parenteral are on the market. Mass vaccination campaigns together with measures to control the canine population (castration, registration) have yielded great success. Models show that vaccination of dogs is the most cost-effective method to fight against canine rabies, and it is the only intervention that can lead to the elimination of the disease at its source. The obstacles for the control of rabies could therefore be overcome by a "one health" approach based on the concept of one medicine.

The rabies control program in Chad is dedicated to show that cooperation between all stakeholders (veterinarians, medical doctors, the public, the government, the media…) is advantageous. Since the year 2000 the Swiss Tropical and Public Health Institute (Swiss TPH) works in close research partnership with the Institut de Recherche en Élevage pour le Développement (IRED) and the Centre de Support en Santé Internationale (CSSI). Huge progress has since been made in terms of reinforcement of diagnostic capacities and the proof of feasibility of dog vaccination. Previous studies have introduced the OIE standard rabies diagnostic test, the Fluorescent Antibody Test (FAT), at the laboratory of IRED. Thereafter the epidemiology of rabies in N'Djamena has been evaluated. The description of the dog demography and two pilot vaccination campaigns have shown that a high coverage can be achieved through free immunisation with a fixed post approach. The cost estimation

done with the results of the pilot studies helped to prepare the citywide campaign and to estimate the cost-effectiveness of such an intervention with a dog to human transmission model.
The current rabies control project in N'Djamena has led to the present doctoral thesis, which spans over many aspects of rabies control, from the contribution of valuable data at the intervention level and the enhancement of laboratory capacities, to the contribution of insights into accessibility of the public and the communication between health practitioners. In 2012 and 2013 two mass vaccination campaigns have been conducted that covered all 10 districts of town and reached consecutively over 70% of the total canine population. The planning, performance and analysis of the campaign is presented in chapter 1. The intervention lead to a drastic drop of dog rabies incidence, but did not translate into lower demand for PEP in the human medical sector due to insufficient communication between veterinary and human health workers as described in Chapter 2.
Data on animal rabies incidence, bite exposure incidence and PEP demand in humans obtained during the 3 years research program allow for the validation of the previous dog to human transmission model (Chapter 4) and an updated cost-effectiveness analysis. Preliminary phylogeographic data obtained from samples collected in and around N'Djamena support the hypothesis that reintroduction is occurring quite rapidly after the halt of the vaccination but also suggests that spread is hindered through landscape barriers (working paper). The laboratory aspect presents the validation of the performance and reliability of a rapid, simple rabies diagnostic test that has the potential to considerably enhance surveillance in resource poor settings (Chapter 3).

The fight against rabies in N'Djamena constitutes a pilot phase, which will be extended to the country level in the coming years. Therefore, in addition to the core objectives concentrating on rabies control in N'Djamena, subsequent objectives are included in the thesis, which address economic aspects of countrywide surveillance and vaccination (Chapter 6), Knowledge Attitude and Practices (KAP) of the Chadian population, as well as estimates of the Chadian dog population (Chapter 5). Finally social determinants of accessibility and effectiveness of rabies vaccination campaigns are discussed in Chapter 7, by comparing the results from N'Djamena with a pilot program for rabies control in Bamako, Mali.

III. RÉSUMÉ

La rage est une maladie virale qui provoque l'encéphalite mortelle. Dans la majorité des cas, le virus est transmis à travers une morsure d'un animal enragé, principalement des chiens domestiques. Alors qu'il n'existe pas de traitement contre la rage, la maladie peut être prévenue à l'aide de prophylaxie pré- (PreP) et post-exposition (PEP) avec des vaccins très puissants. Cependant, PreP n'est pas appliquée systématiquement dans les pays endémiques et l'accès à la vaccination après exposition est souvent entravé par une faible disponibilité et des coûts élevés. Les pays à faible revenu qui n'ont ni les capacités et infrastructures, ni les moyens nécessaires pour lutter de manière adéquate contre la rage sont les plus affectés. De plus, dans ces pays la population canine est souvent non surveillée ou même errante, le renouvellement de la population est élevé et les chiens cherchent la nourriture dans les rues. Tous ces facteurs contribuent à la persistance endémique de la rage dans la population canine et facilite la transmission aux humains. En conséquence, les estimations du fléau mondial de la rage révèlent que la maladie cause environ une victime humaine toutes les dix minutes, ce qui revient à une perte annuelle de 1.74 espérance de vie corriger de l'incapacité (EVCI). Malgré ces impacts, la rage continue à être négligée par de nombreux décideurs nationaux et internationaux, et reste à l'ombre d'autres grandes priorités de santé publiques. Les obstacles majeurs pour la promotion du contrôle de la maladie est l'absence de données d'incidence. A l'origine de ces énormes lacunes de surveillance, ce ne sont pas seulement l'absence de structures de diagnostiques ou le manque de conscience publique, mais également une communication insuffisante entre les institutions vétérinaires et de santé publique.

Les outils pour éliminer la rage canine existent et sont bien établis. Plusieurs vaccins efficaces (oraux ou parentéraux) sont disponibles sur le marché. La combinaison de campagnes de vaccination massives avec des mesures de contrôle de la population canine telles que la castration ou le recensement ont abouti à des réussites importantes. Des modèles montrent que la vaccination des chiens est la méthode avec le meilleur rapport coût-efficacité pour lutter contre la rage, et la seule qui permet d'éliminer la maladie à sa source. C'est pourquoi les obstacles qui empêchent le contrôle de cette maladie pourraient être surmontés grâce à une approche « *one health* » (santé unie) basé sur le concept de « *one medicine* » (médicine unie).

Notre programme de lutte contre la rage au Tchad démontre qu'une coopération entre tous les acteurs (vétérinaires, médecins, le gouvernement, les médias, etc.) présente beaucoup d'avantages. Depuis 2000, l'Institut Tropical et de Santé Publique Suisse (Swiss TPH) collabore étroitement avec l'Institut de Recherche en Elevage pour le Développement (IRED) et le Centre de Support en Santé International (CSSI) dans un partenariat de recherche. Des progrès importants ont été atteints en termes de renforcement des capacités de diagnostic et la preuve de la faisabilité de vaccination des chiens. Des études préalables ont introduit le test de diagnostic de rage selon les standards de l'OIE, le test d'Immunofluorescence (FAT), au laboratoire de l'IRED. De plus, l'épidémiologie de la rage au niveau de la ville de N'Djaména a été évaluée. La description de la démographie canine et deux campagnes pilotes de vaccination ont montré qu'une couverture vaccinale élevée peut être atteinte grâce à la vaccination gratuite à l'aide d'une approche de points fixes de vaccination. Les estimations de coûts effectuées sur la base des résultats des études pilotes ont permis de préparer la campagne à l'échelle de la ville, ainsi que d'estimer le rapport coût-efficacité d'une telle intervention à l'aide d'un modèle de transmission chien-homme.

Ce projet de lutte contre la rage à N'Djaména a débouché sur la présente thèse de doctorat qui porte sur différents aspects de la lutte contre la rage, tels que la contribution à des données valables au niveau de l'intervention, le renforcement des capacités au laboratoire, la contribution de connaissances sur l'accessibilité de la population, et la communication entre les professionnels de la santé. En 2012 et 2013, deux campagnes de vaccination massive ont été menées dans les dix arrondissements de la capitale et ont successivement atteint une couverture vaccinale supérieure à 70% dans l'ensemble de la population canine. Le chapitre 1 présente la planification, la performance et l'analyse des deux campagnes. L'intervention a provoqué une chute drastique de l'incidence rabique, mais suite à un manque de communication entre les agents vétérinaires et de santé humaine, la demande de PEP dans le secteur de la médecine humaine n'a pas diminué. Ces faits sont expliqués dans le chapitre 2.

Les données acquises durant les trois ans sur l'incidence de la rage canine, sur l'incidence d'exposition des hommes aux morsures d'animaux et sur la demande de PEP permettent de valider le précédent modèle de transmission entre chien et homme, ainsi qu'une analyse actualisée de coûts-efficacité (chapitre 4). Des données phylogéographiques obtenues d'échantillons collectés à N'Djaména et aux alentours soutiennent l'hypothèse qu'après la vaccination, le virus est rapidement réintroduit depuis l'extérieur, mais suggère que la propagation est empêchée par des barrières physiques du paysage (document de travail). Au niveau du laboratoire, nous présentons la validation de la performance et la fiabilité d'un test de diagnostic de la rage rapide et simple, qui montre un grand potentiel pour augmenter la surveillance de la maladie dans des pays à faible revenu (chapitre 3).

La lutte contre la rage à N'Djaména est dans une phase pilote que nous aimerions étendre au niveau du pays dans les années à venir. C'est pourquoi, en plus des principaux objectifs qui se concentrent sur la lutte contre la rage à N'Djaména, d'autres objectifs, qui visent les aspects économiques de la surveillance et la vaccination au niveau national, sont présentés (chapitre 5). De plus, des études sur les connaissances, attitudes et pratiques (CAP) de la population tchadienne, ainsi que des estimations sur la population canine au Tchad (chapitre 6) sont inclues dans la thèse. Enfin, le chapitre 7 présente les déterminants sociaux d'accessibilité et d'efficacité des campagnes de vaccination antirabiques, à l'aide d'une comparaison des résultats de N'Djaména avec un programme pilote de lutte contre la rage à Bamako, Mali.

IV. GENERAL INTRODUCTION

IV.1 RESEARCH SUBJECT

IV.1.1 RABIES: INVARIABLY FATAL BUT ENTIRELY PREVENTABLE

Rabies is probably the oldest and most widely known but certainly the most feared zoonotic infection over centuries. Zoonoses are infectious diseases that can be transmitted from animals to humans either directly or through vector species. Rabies is caused by negative stranded RNA viruses of the genus Lyssavirus which belongs to the Rhabdoviridae family (Schnell et al., 2010). Many different genotypes have been identified in this genus most of which circulates in chiropteran species[1] (Delmas et al., 2008). The classical rabies virus (RABV), genotype 1, has its main reservoir in canidae around the world among which the domestic dog is the predominant species for transmission to humans (Cleaveland et al., 2006; Fooks et al., 2014). In Greek mythology Lyssa was the goddess of raging madness and frenzy[2] which is in accordance with the distinct symptoms of the disease and indicates that rabies has been known since the antiquity. The first well described accounts of rabies date back to the 4th century B. C, when Aristotle wrote that rabies *"….is fatal to the dog itself, and to any animal it bites"* (King et al., 2004). This quote depicts in essence the epidemiology of classical rabies to which all mammal species are susceptible and which has the highest mortality rate of all infectious diseases. During the short symptomatic period before death, rabies virus (RABV) is shed in the saliva of rabies infected individuals. Because the virus is vulnerable to UV light it cannot survive in the environment but is transmitted directly through contact of saliva with mucosa or broken skin (Schnell et al., 2010). Rabies virus is highly specialized to evade the host immune system. This is partly explained by its unique neurotropism but also by the ability to prevent cell apoptosis during incubation (Schnell et al., 2010). Depending on the distance of the viral entry site to the brain, this incubation period can last from a few days to several months (Jackson, 2013a). Once viral particles enter peripheral neurons they are transported via the axoplasm through the spinal cord to the brain where they cause encephalitis (Dietzschold et al., 2005). Exposure to rabies does not invariably lead to disease; however, once the virus has reached the brain the infection is inevitably fatal as there is still no cure (Jackson, 2014). The commonly known symptoms of the disease are agitation and hydrophobia in humans and extreme aggression in animals. Hydrophobia results from an inspiratory muscle spasm associated with terror, provoked by the sight of water, which results in the inability to swallow (Warrell and Warrell, 2015). Humans and animals can also develop the paralytic form of rabies that is associated with

[1] http://www.who-rabies-bulletin.org/about_rabies/classification.aspx
[2] http://www.maicar.com/GML/Madness.html

quadriparesis. Often this syndrome is not attributed to rabies especially when the exposure history is not evident due to the long incubation time.

In contrast to the lack of effective treatment, both pre and post exposure vaccination are highly effective in preventing disease (Warrell and Warrell, 2015). Pre-exposure Prophylaxis (PreP) that consists of 3 to 4 doses (day 0, 7, 21-28 and a 12 month booster) of cell culture vaccine (CCV) is recommended both for high risk groups like veterinarians and also for people travelling to endemic areas in developing countries (WHO, 2013). After accurate initial immunisation, only two booster doses (days 0, 3) are required in case of exposure (Warrell and Warrell, 2015). In resource poor countries PreP is not adopted for the general public despite daily close contact of people with dogs. Besides immediate thorough wound washing with soap and water to reduce viral load at the inoculation site, bite victims who are not previously immunised should receive Post-Exposure Prophylaxis (PEP) as early as possible to avert a fatal outcome of the exposure, which occurs in about 1 out of 5 untreated cases depending on the site of viral inoculation (Cleaveland et al., 2002). Several active CCV immunisation schemes are approved by the World Health Organization (WHO) for PEP (WHO, 2013). Intradermal protocols are advantageous over intramuscular because they require fewer visits to health centres and use lower vaccine volumes (Hampson et al., 2011). In addition to active immunisation, WHO recommends the inclusion of passive immunisation via rabies immunoglobulin (RIG) in the PEP protocol for cases of category III exposure (transdermal injuries or contact of saliva with mucosa) (WHO, 2013). Fully completed PEP is 100% effective in preventing human rabies (Quiambao et al., 2005). However RIG is virtually unavailable in Africa and far more expensive than CCV. WHO estimates that less than 1% of PEP administered in developing countries includes vaccine and serum[3]. One dose of 5ml Equine derived Immunoglobulin (ERIG) exceeds the prize of 40 USD in Ivory Coast and Mali and the safer, human derived immunoglobulin product is five times more costly (Prof. Bassirou Bonfoh and Dr. Abdallah Traore, personnel communication). In addition ERIG and human RIG are not manufactured in quantities that would meet the demand of Africa and Asia if it was routinely included in the PEP schedule[3]. Cost of PEP treatment even without RIG is one of the biggest limitations to access for people living in poverty in addition to lack of awareness and reduced availability due to ineffective health systems (Dodet et al., 2015). The in-depth and extensive problem of rabies in the developing world is best illustrated with two examples personally encountered during field studies.

In Chad, PEP (excluding RIG) is subsidised but the remaining total cost of 40’000 FCFA (5 doses Essen scheme) for a full treatment is still more than half of a monthly minimum wage (70’000 FCFA). For women and children PEP is available for free at the women and children’s hospital in N’Djamena. In February 2015 a vaccine shortage occurred at this hospital and the vaccine had to be procured from

[3] http://www.who.int/rabies/vaccines/other_rabies_biolog_product/en/

pharmacies. A refugee family was unable to cover these costs for her 8 year old boy who was bitten by a rabid dog, so she sought help at the rabies laboratory. The exposure history was highly suspicious of rabies because the dog had bitten four people before he was killed. However the dog carcass was not brought to the laboratory for diagnosis. Laboratory personnel initiated a monetary collection for the boy and thus saved him from a looming fate.

In Beboto, Chad, a woman died of rabies in February 2014 after a short four day period of suffering from the inability to swallow (hydrophobia) and restlessness followed by coma. She was bitten on the toe in December 2013 by a puppy which disappeared one day later. Vaccine for PEP is available at the local hospital only a short distance from the town, but out of ignorance the victim only applied tamarind juice, a local traditional wound treatment. When symptoms appeared, help was sought at the hospital, but it was too late to initiate preventive treatment. Not wanting to give up hope, the family took the victim to different traditional healers before she succumbed.

In both cases vaccine was available, but in the first case accessibility to PEP was almost prevented due to high costs and an inefficient health service and in the latter case traditional beliefs led to non-adherence to the only effective prevention measure. Both instances occurred in an urban setting where health facilities were relatively close. In rural areas, distance is yet another factor negatively influencing accessibility to life saving PEP. In addition, remoteness enhances the described barriers of misconception and poverty.

Improved access can contribute to lower disease burden through reduced economic impact of Years of Life Lost (YLL), but investment solely on the human side is short-sighted and neglects the root cause of the disease. As over 95% of rabies in humans is caused through dog bites, the most sustainable and cost-effective disease control can only be achieved by mass immunisation of domestic dog populations (Cleaveland et al., 2014a; Hampson et al., 2015). Such a One Health approach for disease control has been proposed as early as 1882 by Louis Pasteur, the renowned pioneer celebrated for the discovery of rabies vaccine (Rosset, 1985). Despite often raised concerns about the accessibility of dog populations in developing countries, the feasibility of interventions targeting dogs has recently been proven by numerous studies in many different contexts (Davlin and Vonville, 2012). A milestone to prove the long term effectiveness of dog vaccination is the impending elimination of dog mediated rabies from Latin America (Vigilato et al., 2013a).

Continued endemicity with a consequently high burden of rabies is observed in Asia where policy is shifting only slowly from the focus on human PEP to a control policy targeting the animal reservoir (VII.2 of this thesis). In Africa, which records the highest per capita death rate reported among all continents (Hampson et al., 2015), measures on both the human and animal level are deficient (Anderson and Shwiff, 2015).

IV.1.2 The destructive dynamics of neglect

In the above mentioned example from Beboto, the victim died at home and like many others is therefore not listed in a hospital death record. Even if the woman had died at a health facility, the event would not have been reported at the national level because rabies is not notifiable in Chad, which is not exceptional. In more than half of the highest rabies risk countries, rabies is not notifiable or the surveillance system is ineffective (Taylor et al., 2015). This makes it virtually impossible to gain accurate worldwide burden data. The most recent extrapolative study estimated that about 59'000 people die of rabies annually (Hampson et al., 2015). However, the reported 95% Confidence Interval reaching from 25'000 to 159'000 cases is very wide, mirroring the weak data situation. The data situation is particularly critical in Africa where the real number of rabies cases are believed to be over 100 times higher than reported, especially in rural areas (Dodet, 2009). Reasons for the ineffectiveness of surveillance as stated by countries participating in the study of Taylor et al. (2015) were (1) underreporting in remote areas; (2) lack of follow up of suspected cases for confirmation; (3) inadequate financial investments into the surveillance system; (4) lack of enforcement and implementation of legislation on rabies prevention and dog management; (5) human deaths occurring at home; (6) inadequate training of medical staff in surveillance and case definitions, leading to (7) poor recognition of rabies by health workers; (8) competing priorities of politicians; (9) no coordination between veterinary and medical authorities for the control and prevention of zoonotic diseases; (10) lack of best treatment knowledge in victims.

This list is exhaustively long and each single factor enhances neglect on one or more connected levels. Figure IV.1 shows the elements of the wheelwork needed to control rabies and the main blockages preventing the wheels from turning. Even when blockages are removed, positive input has to be given on all interrelated levels for a dynamic spinning of the mechanism to gain the highest impact on rabies virus control and ultimately achieve elimination.

One of the major shortcomings leading to underreporting is lack of access to diagnostic methods that can attribute a suspected case with certainty to rabies as a cause of death. In Chad, virus detection in brain samples by the international standard method the Fluorescent Antibody Test (FAT) is only possible at the central rabies laboratory in N'Djamena. However the sampling of brain material from human victims is ethically not accepted by the bereaved in the majority of cases. Virus can also be detected by Polymerase Chain Reaction (PCR) in saliva through a biopsy of the skin at the nape of the neck, but this method is not yet established in N'Djamena. Sampling of animals is not ethically critical, but there are other barriers like negligence or the impossibility to get samples from an animal that is unavailable. Usually when a bite exposure victim presents at a health facility, the biting dog has vanished or has been killed and buried. In cases where victims already show symptoms of rabies, the identification of the animal can be impossible because of the large time lapse between exposure and onset of the disease. For both human and animal samples, transport to a distant laboratory is another huge challenge. Transportation of potentially infective material needs special precautions.

In addition, in the tropical climate decay is accelerated and sample decomposition limits the validity of the FAT and PCR test (McElhinney et al., 2014), meaning the cold chain should be sustained during the whole transport time. A rapid and easy, yet still highly reliable test for animal and/or human use is needed to equip veterinary and human health workers in remote locations. In addition, it would provide a quick response to bite victims, which would lead to a higher perceived benefit of diagnostic testing for the greater public. In chapter 3 of this thesis, we present the validation of a diagnostic tool that would, when approved as a standard diagnostic test by OIE (World Organization for Animal Health) and WHO, greatly enhance rabies field diagnostics.

In the absence of diagnostic tests, detection and diagnosis of rabies in humans relies on clinical diagnosis and/or verbal autopsy by physicians. However, low disease prioritisation and the general weakness of health systems in rabies endemic countries lead to misdiagnosis, especially for the paralytic form of rabies, again negatively influencing data reporting (Mallewa et al., 2007; Mallewa et al., 2013). In addition misdiagnosis has negative effects on individual, household and national levels in regard to medical, economic and social aspects of disease (Amexo et al., 2004).

Also contributing to the poor data situation is a prevailing confusion about the responsibilities for rabies control on the human and animal side, which was reflected in the inconsistent data reporting on the OIE and WHO reporting platforms over several years (Nel, 2013). Veterinary officials reported their data to OIE whereas human health officials sent their data to WHO. Discrepancy in data reporting to these two organisations therefore point directly towards the lack of communication between the two sectors, which could be enhanced through a One Health approach to disease surveillance discussed in the book chapter on integrated rabies control (Léchenne et al., 2015; Chapter VII.1 this thesis).

As long as surveillance is not enhanced, data reporting on national and international levels will remain weak and policy priority will be on other more prominent diseases. Prominence however is not only gained by the collection of case figures alone. Ebola[4] and previously avian influenza[5] and Severe Acute Respiratory Syndrome[6] have had a high media response, but to date all together do not reach half the estimated yearly death toll due to rabies. According to Nel (2013) the lack of newsworthiness of rabies compared to other emerging diseases results from the fact that humans have lived with the disease for centuries. In addition rabies is not easily transmitted between humans and generally not easily spread over large distances by reservoir species. Therefore, the disease is not perceived as an immediate threat to the globalised world, despite being omnipresent in resource poor countries.

[4] http://apps.who.int/ebola/ebola-situation-reports
[5] http://www.who.int/influenza/human_animal_interface/EN_GIP_20150623cumulativeNumberH5N1cases.pdf?ua=1
[6] http://www.who.int/csr/sars/country/table2004_04_21/en/

Nevertheless the impact on global tourism as well as the risk of introduction to rabies free countries by human mediated dog movement is not insignificant, as discussed in the book chapter on Rabies in South East Asia (VII. 2, this thesis).

The absence of prioritisation on the international and national policy levels stands in contrast to the fear rabies evokes in people residing in endemic areas. Several studies have shown that awareness of the danger of the disease is generally high and rabies would possibly gain higher priority if disease prioritisation mirrored the public risk perception. A problem however is the high misconception regarding treatment of rabies and ignorance of the importance of seeking adequate help immediately after exposure. Public awareness raising and education is therefore another lever to break the cycle of neglect.

Finally, rabies control suffers from a double neglect. Not only is the disease itself not a priority, but also the main reservoir species, the domestic dog, is not economically important. Veterinary medicine in developing countries focuses on livestock, which is important for the livelihood of the majority of people. The loss of livestock due to rabies is, although also believed to be underreported, not significant compared to other infectious diseases with high economic impact (bovine tuberculosis, trypanosomiasis, foot and mouth disease, brucellosis). The fight against the virus itself would be easy enough given the tools at hand, but the fight against the neglect of both the disease and its reservoir on the policy level make control a challenging enterprise.

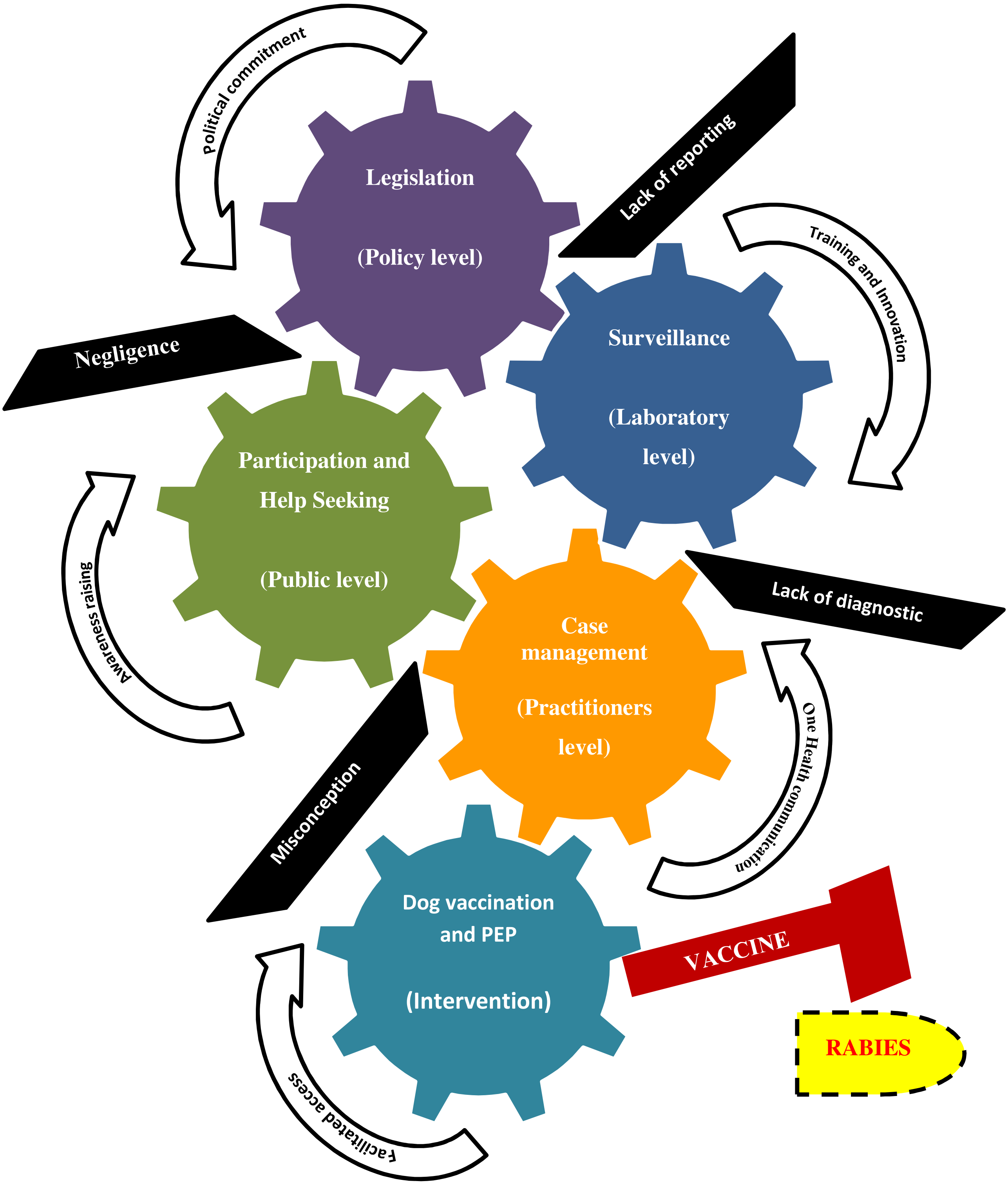

Figure IV.1: Wheelwork depicting the simplified mechanism to rabies control, the main blockages and needed inputs.

IV.1.3 PROSPECTS AND TOOLS FOR RABIES ELIMINATION

As rabies affects disproportionally poor and marginalised communities, the mechanisms of disease neglect showcase the worldwide health inequity. Provision of quality health service for all should of course remain the highest aspiration of public health. Nevertheless such a goal is not only extremely visionary to accomplish but also nearly impossible to be retained. By contrast, eradication of any disease, defined as the complete and permanent reduction of worldwide cases, provides lasting global health equity because of the overall elimination of the risk pertaining to the respective infection. The constellation of conditions that make eradication of infectious diseases feasible, as described in Dowdle et al. (2011), includes: (1) Biological feasibility; (2) Adequate public health infrastructure; (3) Sufficient funding; and (4) Sustained political and societal will. The successful eradication of small pox and rinderpest has proven that such undertakings are possible within a human as well as an animal background (Klepac et al., 2013), but never before has the eradication of a zoonotic disease been attempted. Dog mediated rabies might well be the first zoonosis to be tackled for control on the global level. However, RABV is also circulating in wildlife species, and the disease can further be caused by all other lyssaviruses. Therefore, the term elimination, defined as the reduction of cases in a defined geographical area to zero, is more fitting than eradication.

The biological feasibility for canine rabies elimination is given through availability of safe vaccines and effective intervention measures[7]. As described in the previous section, adequate public health infrastructure has yet to be established through reinforcement of the surveillance and management capacities where disease reporting and accessibility to vaccines is inefficient. Sufficient funding as well as sustained political will can only be gained through continued advocacy facilitated by an improved data reporting situation (Lembo et al., 2011). Such an advocacy has to combine top down approaches from academic, industry and political stakeholders as well as be based on and nurtured by strong bottom up initiatives of the stakeholder communities. Furthermore, close communication with the public to build trust, overcome ignorance and reduce misconceptions is prerequisite to secure societal will. The benefits of global dog rabies elimination are evident and are in line with the multiple attractions of disease eradication as referred to in Dowdle et al. (2011): (1) Reduction of human rabies cases by over 90% as ultimate health goal; (2) infinite positive benefit-cost relationship; (3) building of infrastructure, cooperation and experience for zoonosis control; (4) Advancement of a culture of prevention of zoonotic diseases; (5) Improvement of acceptance of other disease interventions in pet animals; (6) Provision of social justice.

During and after elimination, it will be crucial to secure the sustainability of improvements in health infrastructure, prevention and public acceptance. This will serve not only for the control of other

[7] http://caninerabiesblueprint.org/

zoonotic diseases but also for the prevention of re-establishment of the disease transmission in dogs through wildlife reservoirs.

Momentum is gaining on the international level for the control of dog rabies and several countries and regions have established action plans with defined target dates for rabies elimination. The Global Alliance for Rabies Control (GARC) (Nel et al., 2017) founded the international World Rabies Day, which has been celebrated annually since 20007 on 28th September (Balaram et al., 2016). Additionally GARC is enhancing public awareness and promoting rabies control through their internet based educational platform[8] and social media presence. GARC also provides a stakeholder platform bringing together WHO, OIE, the Food and Agriculture Organization of the United Nations (FAO), academia, donors, industry and animal welfare organisations under the umbrella of the Partners for Rabies Prevention (PRP) (Taylor et al., 2013, Nel et al., 2017). Through this partnership several initiatives and tools have been created: blueprints for dog control in dogs and foxes and a blueprint for rabies surveillance are available online[9] (Lembo et al., 2012); together with FAO the Stepwise Approach towards Rabies Elimination (SARE) (Coetzer et al., 2016) has been developed to assist the development of national rabies control action plans and to measure progress[10,11]; in 2015 the two African rabies surveillance networks (AfroREB and SEARG) were brought together in one Pan African Rabies Control Network (PaRaCON)[12](Scott et al., 2015).

Despite all these efforts, breakthrough has not yet been achieved. The International Task Force for Disease Eradication[13] (ITFDE) driven by the two major private funding bodies in the field of disease control, the Carter Center and the Bill and Melinda Gates Foundation, lists currently eight eradicable diseases: Guinea worm (dracunculiasis), poliomyelitis, mumps, rubella, lymphatic filariasis, cysticercosis, measles and yaws. Rabies meanwhile remains among the WHO currently listed 17 neglected tropical diseases[14] for which a combined resolution has been adopted[15] urging member states to take action.

[8] https://education.rabiesalliance.org
[9] http://www.rabiesblueprint.com/
[10] http://caninerabiesblueprint.org/6-4-Overview-of-the-stages?lang=en
[11] http://www.fao.org/docrep/019/i3467e/i3467e.pdf
[12] https://paracon.rabiesalliance.org/
[13] http://www.cartercenter.org/health/itfde/index.html
[14] http://www.who.int/neglected_diseases/diseases/en/
[15] http://www.who.int/neglected_diseases/mediacentre/WHA_66.12_Eng.pdf

IV.1.4 The potential for One Health ambassadorship

Long before the causative viral agent of rabies had been identified the disease was recognised as a condition induced through the contact with a mad animal, in particular the dog. It might even be the one disease to date that first comes to mind in the majority of people around the globe, when asked about disease transmitted by animals. Diseases emerging from animal reservoirs, in particular, cause anxiety in lay people, because there is increased perception of a risk being induced from beyond the rationally comprehensible borders of humankind. The madness witnessed in raging dogs and induced in rabies victims amplifies such a notion to the extent of fear. However, despite presenting in a terrible manner, rabies is far from being invincible, and control is rationally easy to grasp once disciplinary boundary thinking is overcome (Léchenne et al., 2015; chapter VII.1 this thesis).

All zoonotic infections demand a holistic, integrated approach to disease control and cannot be handled by one discipline alone. The absence of a zoonotic disease in the above mentioned eradication successes and the prominence of human centred pathogens in the list of diseases targeted for eradication highlight the scepticism towards investment into the seemingly complex undertaking of fighting disease in humans and animals at the same time. Most often the biggest challenge does not lie in the epidemiologic complexity of zoonotic disease nor the tools for their control, but in the absence of a culture of interdisciplinary communication. Yet, 75% of all the emerging infectious diseases are zoonotic (Grace et al., 2012), which shows that species barriers exist more prominently in human thinking than in the nature of infectious diseases. The recent detection of Guinea worm in dogs in Chad during the end phase of worldwide elimination is one example of that fact (Eberhard et al., 2014). If this mind-set barrier is not overcome science will be unable to confront over half of all infectious diseases afflicting humans (Grace et al., 2012).

One Health provides a vision and path for integrated thinking. It is defined by Zinsstag et al. (2015a) as: "*Any added value in terms of health of humans and animals, financial savings or environmental services achievable by the cooperation of human and veterinary medicine when compared to the two medicines working separately*".

Rabies is an exemplary disease to advocate the One Health concept. Close communication and collaboration between veterinarians and physicians regarding rabies will have direct positive outcomes for the well-being of animals and humans alike through dog immunisation and timely adherence of bite victims to PEP, both of which lead to disease risk reduction (Léchenne et al., 2015; chapter VII.1 this thesis). The added value extends also to financial savings through reduced need for costly PEP and reduction of the YLL burden. Lastly, the environment benefits from rabies control and the parallel dog population control through reduced risk of rabies and other dog-transmitted infectious diseases for wildlife and, in consequence, positive effects on the conservation of biodiversity (MacDonald, 1993; Prager et al., 2012).

In its definition One Health is an interdisciplinary concept which also strongly reaches out to transdisciplinarity involving multiple stakeholders (communities, public and private institutions) and considering all forms of useful knowledge, both academic and non-academic. Following the definition of One Health, Zinsstag points out that *"…..the strongest leverage of One Health is observed when it is applied to practical societal problem solving"* (Zinsstag et al., 2015a). Solving the societal problem induced by rabies and the subsequent gain of health equity makes rabies control an ambassador for One Health thinking among practitioners, in policy and in society at large.

The transmission cycle of canine rabies does not involve a chronic or latent stage, asymptomatic shedders or vector species. The virus is unable to persist in the environment, and immunisation provides 100% protection. For all these reasons, rabies should rise to the top on the list of zoonotic infections to be eliminated. During the first PaRaCON meeting in South Africa in July 2015, Sarah Cleaveland stated: "*If we can't manage elimination of rabies, we will not manage elimination of any other zoonotic disease"* (author's personnel communication).

Understood within a background of One Health, the science of rabies elimination is not complex but becomes tangible.

IV.2 Research approach

IV.2.1 From desk to field: Translational Research

Translational research is defined by Ogilvie et al. (2009) as: *translation of evidence into population health improvements*. Epidemiology, the science of the systematic and unbiased approach to collection, analysis, and interpretation of data is not only at the source of the production of evidence but also provides the tool to accompany scientific discoveries into applicability in practice. Therefore, epidemiology is an important building block of translational research but may also contribute to basic science. Kourhy (2010) describes five stages of translational epidemiology (TE) which form a continuum and centre on knowledge synthesis (Figure IV.2). On the way from scientific discovery, through all stages to outcomes in population health, TE passes and encounters many different disciplines and is therefore in its nature interdisciplinary. Exemplifying the TE steps for rabies control in dogs, the discovery of a vaccines stays at T0; the testing and approval of the vaccine (T1) has led to recommendation of its use in every day veterinary practice (T2). In countries and areas where canine rabies is controlled the proper paths have been discovered for successful implementations of the recommendation (T3).

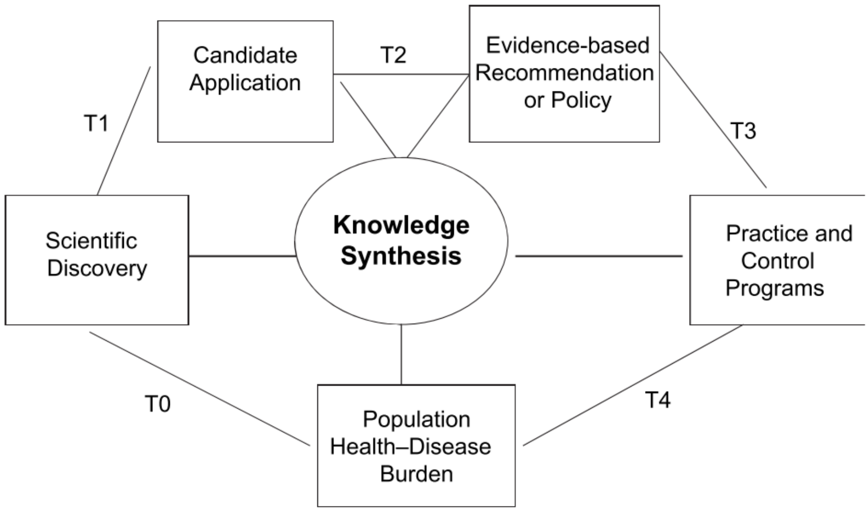

Figure IV.2: Epidemiology and the phases of translational research taken from Khoury et al. 2010. T0, scientific discovery research; T1, translational research from discovery to candidate application; T2, translational research from candidate application to evidence-based recommendation or policy; T3, translational research from recommendation to practice and control programs; T4, translational research from practice to population health impact.

Subsequently, the usefulness of dog immunisation at high population coverage has proven to be efficient to interrupt rabies transmission (T4). However, there remain research efforts to be undertaken addressing the aspects of accessibility and effectiveness of vaccination interventions. For example in Bamako it is not fully understood how a high vaccination coverage can be achieved (Muthiani et al., 2015). Hopefully, steps T0, T1 and T2 do not have to be repeated any time soon. Unlike other RNA viruses, the genome of RABV is very constrained (Holmes et al., 2002). In consequence, escape mutations are not anticipated, and it is expected that the efficacy of the vaccine will remain stable. Stage T3 and T4, however, are still subject to research and also provide the frame of the epidemiological study conducted through the vaccination campaign in N'Djamena.

Implementation of dog vaccination into routine daily veterinary practice is not efficient in countries where dog owners do not regularly seek veterinary services because of negligence and cost considerations. Although their feasibility and effectiveness have been proven, large scale dog mass vaccination campaigns still have to be defended against prevailing misconceptions about the accessibility of dogs on the one hand and the potential of a dog target intervention for the control of rabies on the other hand (Cleaveland et al., 2014b). The most frequently asked questions at meetings with authorities during the project were: (1) What about the many feral dogs in town which are inaccessible, and (2) what about the cats that can also transmit rabies? The evidence based knowledge that the majority of dogs in Africa are owned and that the cat does not play a reservoir role in rabies has to be translated into common public knowledge through constant widespread evidence communication and diffusion. In addition, results from our project and campaigns conducted in Mali show that translation of the provision of the vaccine into high immunisation coverage in dog populations has to closely study not only the geographical and financial determinants but also the cultural and societal aspects of accessibility (Muthiani et al., 2015; Chapter 7 this thesis). Research into these different aspects of health seeking (dog owner compliance to vaccination), service provision (best intervention strategy) and knowledge management (dissemination of best practice recommendations) can bridge the gap, identified in Ogilvie et al. (2009), between health technology assessment (efficacy of the vaccine) and the ultimate delivery of healthcare (immunisation of dogs).

The example can also be elaborated starting with the discovery of the human vaccine and RIG for PEP and ending with the prevention of human deaths. Some gaps along this path, that cannot be addressed by conventional scientific methods but rather call for transdisciplinary approaches, will be identified in T3 and T4.

IV.2.2 The common goal: Transdisciplinary research

As already briefly indicated above One Health, as well as TE, strives to reach beyond simply communication between academic disciplines into the field of transdisciplinarity. According to the definition of Pohl and Hirsch Hadorn (2008), transdisciplinary research (TR) "…*develops descriptive, normative and practice-oriented knowledge in order to help solve, mitigate or prevent life-world problems*". The need for such a new approach to research was identified because of an increasing mismatch between the fragmentation and sophistication of academic knowledge as opposed to knowledge requests of greater society (Hirsch Hadorn et al., 2008). Penetration into the most detailed macro and micro mechanisms of elementary processes in natural science do not necessarily entail subsequent application of this knowledge for the benefit of solving societal problems. To be able to impact real changes in everyday life empirically obtained expertise in science (systems knowledge) has to be merged with target knowledge that addresses questions about needs and interests of stakeholders affected by the intended change. Finally, transformation knowledge contributes answers to the questions of how technical, social, legal and cultural mechanisms of action are best exploited to bring forth desired change (Hirsch Hadorn et al., 2008). To unite these three knowledge demands of TR it is necessary not only to overcome barriers between different disciplines of natural sciences but also to interrelate closely with humanities.

Applied to rabies control, systems knowledge encompasses the science of epidemiology on the population and molecular level. Target knowledge evaluates the beneficial effects of rabies control on economy and public health, whereas transformation knowledge analyses the many aspects determining accessibility to intervention (Chapter 7, this thesis).

Because TR steps out of the known conformities of science to span over a wide range of contexts, new criteria and metrics are called for in order to evaluate transdisciplinary studies. Hoffmann et al. (in

Hirsch Hadorn et al., 2008) suggest a list of cross cutting issues that are of relevance for transdisciplinary projects: Participation; Values and uncertainties; Learning from Case studies; Management; Education; Integration of concepts and integration of systems analysis. In complement, Klein (2008) describes seven principles for evaluation of inter- and transdisciplinarity: (1) Variability of goals; (2) Variability of criteria and indicators; (3) Leveraging of integration; (4) Interactions of social and cognitive factors in collaboration (5) Management, leadership, and coaching; (6) Iteration in a comprehensive and transparent system; (7) Effectiveness and impact.

Since the project in which the present thesis is embedded purports to be of a transdisciplinary nature, the results of the research will be critically reviewed relative to these cross cutting issues and principles in the discussion section (VIII.2).

Management and communication of the project closely followed the 11 principles for transboundary research partnerships set forth by the Swiss Commission for Research Partnerships with Developing Countries[16]: The agenda was set together in a tripartite contract between the Swiss Tropical and Public Health Institute (Swiss TPH), Institut de Recherche en Elevage pour le Développement (IRED) and the Centre de Support en Santé International (CSSI); regular interactions with stakeholders were provided through meetings with authorities on World Rabies Day and through meetings with district leaders throughout the campaign; clear responsibilities were assigned in the contract and between campaign committee members; vaccination certificates as well as official results of diagnostic tests were delivered to beneficiaries and form an aspect of accounting; mutual learning was an important pillar of the project as evidenced by the joint training of Swiss and Chadian doctoral and Master's students; as a result laboratory and research capacities were enhanced; data and networks were shared closely through joint publications and participation at international meetings; consequently results were widely disseminated and profits and merits were pooled between all partners; and finally application of results and securing of outcomes is on-going.

[16] http://www.naturalsciences.ch/service/publications/9505-a-guide-for-transboundary-research-partnerships-2nd-edition---2014-

IV.2.3 THE PATH TO SUSTAINABILITY: COMMUNITY PARTICIPATION

The term community participation (CP) has its roots in the Alma Ata declaration made at the International Conference on Primary Health Care in 1978[17]. Point IV of this declaration states: *The people have the right and duty to participate individually and collectively in the planning and implementation of their health care.* Ten years later, the importance of CP as a way to promote empowerment and ownership was stressed in the Ottawa Charter[18] which defined a new way of health promotion as: *the process of enabling people to increase control over, and to improve, their health.* CP has since become a very popular term but critics argue that it serves too often as a fig leaf for projects and programs that claim to involve communities but do not step out of the conventional top-down approaches to knowledge transfer. Consequently, two different approaches to CP are identified: the target oriented, viewing CP as a means to achieve the end (health improvements); and the empowerment oriented, viewing CP as the end itself (Morgan, 2001; Pérez et al., 2009). Between these two extremes, multiple nuances occur which have been depicted in ladders of CP involvement. As an example, Table IV.1 shows the ladder of Pretty derived from Jacobs et al. (2010). Empowerment can only be achieved through the stages 4 to 6 on this ladder. Ultimately, sustainability of promoted changes can only be achieved through such empowerment and ownership taking of the community (Rifkin, 2014). Long term uptake and the experiences made during the CP process are also proposed to lead to higher resilience of communities not only in regard to the program subject but also to other aspects of health, e.g. infectious disease control (Rifkin, 2014; Tambo et al., 2014).

7. Self-mobilization	Community members set their own agenda and organize for action. Professionals have a role in the background, are facilitative and supportive but only if asked.
6. Interactive participation	Professionals and community members work as equal partners in defining the problems or needs and the strategies for change. There is a sharing of knowledge and valuing of 'local' or 'lay' knowledge. Professionals facilitate and support the process.
5. Functional participation	Community members are involved in decision-making and the development and execution of programmes or activities. Professionals are in control and take responsibility for the process.
4. Participation by consultation	Community members are asked to give their opinions on the program plans. The professionals decide what to do.
3. Participation by information	Community members are informed in an early stage about the program plans and are given the opportunity to ask questions.
2. Passive participation	Professionals are in control of the program; community members are informed about the program.
1. No participation	Community members are not informed about the program, only about the activities for which they have been recruited.

Table IV.1: the Ladder of Pretty derived from Jacobs 2010

[17] http://www.euro.who.int/__data/assets/pdf_file/0009/113877/E93944.pdf
[18] http://www.who.int/healthpromotion/conferences/previous/ottawa/en/

Problematic aspects of CP are identified in the general complexity of a community that is not only defined geographically but also through social identity of people which can take multiple forms (Stephens, 2007). Further, the proof of the direct link between CP and positive health outcomes is hard to furnish because the most generally used randomised clinical trials (RCT) method with CP as intervention do not capture well the causal chains of transformation (Rifkin, 2014). Rather than focussing on static outcome measures, Rifkin (2014) proposes an evaluation framework that gives justice to the dynamic nature of CP understood as a process and not as an intervention. The CP process can then be analysed around five assessment factors (needs assessment, leadership, organisation, resource mobilisation, and management) using a spidergram as depicted in Molyneux et al. (2012). This assessment is best made at different time points, both during the project and beyond. Because lack of power is a given state in poor communities, initial input to empowerment in the form of promotion and encouragement comes most often from outside (Morgan, 2001). As a project progresses, the weight of CP can move up the ladder or expand the spidergram. This is illustrated by Perez et al. (2009) who evaluated a vector control project aiming at CP at different time points through the process oriented framework. This helps to critically review the progress of CP and allows for adaptation of the implementation strategies.

In the discussion section of this thesis (VIII.2), the present project is evaluated using the ladder of Pretty and the spidergram. Strict application of CP is not favourable for all aspects of rabies control. Expertise knowledge about the best method for interruption of transmission being immunisation of dogs should not be compromised by publically popular beliefs about the solution being culling of dogs. Likewise, compromise cannot be made towards the misconception of the treatability of rabies through traditional remedies. The potential for integration of CP lies in the field of surveillance, accessibility of dogs to vaccination and dog population management. Also, identifying priorities of a community before the start of an intervention as done by Cediel et al. (2013) helps to prevent misunderstandings and assist in strategic planning. If, for example, in our national household survey, the most often stated transmissible disease from dogs was bad skin condition and not rabies, it would be good to include an antiparasitic in a future rabies control program to enhance acceptability. Another example is the low performance of our vaccination campaign in Muslim dominated districts, owing to the fact that the dog is not a priority. On different occasions, vaccination teams were confronted with animosity and criticised for dealing with dogs, when nearby children are sick. The link between intervention in animals and better health outcome in humans (in the case of rabies, especially for children), must be emphasised particularly within such a background to promote dog vaccination interventions.

IV.3 Research Context

IV.3.1 N'Djamena: Split between dearth and Boom

It was during my study time at university when I worked on a voluntary basis for Vétérinaires Sans Frontières (VSF) that I first heard of a town called N'Djamena. VSF colleagues who travelled to Chad described it as the ugly, dusty and ever too hot capital of the Sahel zone development agency business, where the only attraction was the hotel pool. Like the majority of people, I was indifferent towards this spot on earth, and it was not until I started working for the project that I became bonded to the place. I can now confirm the town is not a gem, but beyond the walls of 5 star hotels and villas one discovers the aching authenticity of ordinary daily life struggle in urban Africa which holds an inexplicable attraction. N'Djamena signifies "the place where we rested". It is the youngest capital city on the African continent, only founded in 1900 by the French who initially named the city Fort Lamy. It is situated on the Chari River which shapes, together with the Logone River, the border to Cameroon in the south. Two bridges, one for cars and one only suitable for pedestrians and motorcycles, connect the north part of the town with the only district south of the Chari (Walia, disctrict 9). From there a single bridge over the Logone River connects Chad with Cameroon. Due to high population growth and migration into cities, N'Djamena is growing at a fast pace. Reported to be only 500'000 in 1993, the population doubled in only twenty years to one million by 2013. The total population of Chad is estimated at 13 million[19], which means that roughly 1 out of 10 Chadians live in the capital city. Situated in the junction between the arid Sahel zone in the north which is inhabited predominantly by nomadic people and the Savannah zone to the south which is populated by sedentary farmers, N'Djamena reflects the ethnic diversity of the whole country. About 1/3 of the capital's inhabitants have a Christian background and 2/3 follow Islamic culture. These different contexts influenced the results of the mass vaccination campaigns we conducted (Léchenne et al., 2016a; Chapter, 1 this thesis).

Undeniably, N'Djamena is a tough working environment that is not only challenging because of the harsh climatic conditions but most importantly demands a steep learning curve in inter-cultural communication. Another challenge is the socio-economical background of the country. Chad ranks 184 of 187 countries on the United Nations development index[20]. Data of the World Bank from 2012[21] show that the broader part of Chad's inhabitants, almost 60% of people, live on less than 2 USD a day. The urban poverty ratio at national poverty lines is 21%[6]. Such underprivileged conditions are partially explained by the history of the country. After Chad gained independence in 1960, the country suffered

[19] http://data.worldbank.org/country/Chad
[20] http://hdr.undp.org/en/countries/profiles/TCD
[21] http://data.worldbank.org/country/chad

from a long period of internal conflicts, a part time foreign occupation and the dictatorship of Hissein Habré. In 1990 Idriss Déby took power. Over the 25 years of his presidency, Chad has gained a low level stability and has become a first place of refuge for people escaping from conflict zones in South Sudan, the Central African Republic and, most recently, from Nigeria. Yet, the stability is vulnerable because the current president has not been able to provide social equity for the wider public and construct a political system based on democratic rights. The income from the oil industry, which is the only thriving economy in the country, is unevenly distributed and trickles down only marginally to the greater public. Nonetheless, in parts of N'Djamena a boom is noticeable, for example in the 2nd district around the airport where an economic centre is being built or in Toukra where the new University building has recently been inaugurated. Streets are being paved at a high pace to keep up with the rising number of cars and resulting demand for better traffic capacity. Also private shops and house constructions are numerous. Meanwhile the gap between rich and poor is becoming wider each year and neglected parts of town like the suburbs of the 9th and the 7th districts resemble slum areas.

In 2014, N'Djamena was ranked number 10 of the Mercer's 21st annual Cost of Living Survey[22] for cities. This survey reflects the high living costs and high prices of goods for expatriates. One can only imagine what that means for the local population. Being a landlocked country surrounded by conflict areas, Chad depends on only a few transport links to neighbouring Cameroon, Nigeria and Niger which leads to high prices for imported goods. The high living costs were also reflected in our budget for the vaccination campaign. In addition logistic constraints due to rising traffic volume and poor road conditions in the remote areas of town were challenging. The only climatically favourable window for a vaccination campaign was between October and December when the rainy season was over and mean temperatures were acceptable for work outdoors.

IV.3.2 Program foundation: A decade of rabies research in N'Djamena

In 2000, the Laboratorie et Vétérinaire et Zootechnique de Farcha (LRVZ), now called IRED, approached the Swiss TPH asking for support in the fight against rabies. Collaboration between these institutions had existed since 1997 through a research project on improvement of health services to nomadic pastoralists in Chad (Schelling et al., 2008). The second longstanding local partner in the program is the CSSI, a Chadian Non-Governmental Organization (NGO) closely connected to Swiss TPH.

In a first research phase lasting from 2000 to 2003, basic information on dog demography and public awareness was gathered in N'Djamena. The results were published by Mindekem et al. (Mindekem et al., 2005). Through that work, it was noted that the dog densities varied significantly between different quarters reflecting the cultural and religious background of the inhabitants. Quarters dominated by a

[22] http://www.mercer.com/newsroom/cost-of-living-survey.html

Muslim background had significantly less dogs than quarters with a Christian background. The total population was estimated at 23'560 dogs (955 CI 14'579-37'921), which represented a dog to human ratio of 1:33. Although the majority of dogs were roaming in the street, only about 10% of them were estimated to be feral dogs without any owner. The majority of dogs were kept to guard homes, but only 30% of owners stated that they regularly fed their dogs. Only 19% were vaccinated against rabies, although 80% of respondents stated that they are aware of rabies. Over 80% also knew that the main transmission route was through the domestic dog and that a vaccine was available. However 2/3 thought that rabies could be cured, which highlighted widely present misconceptions.

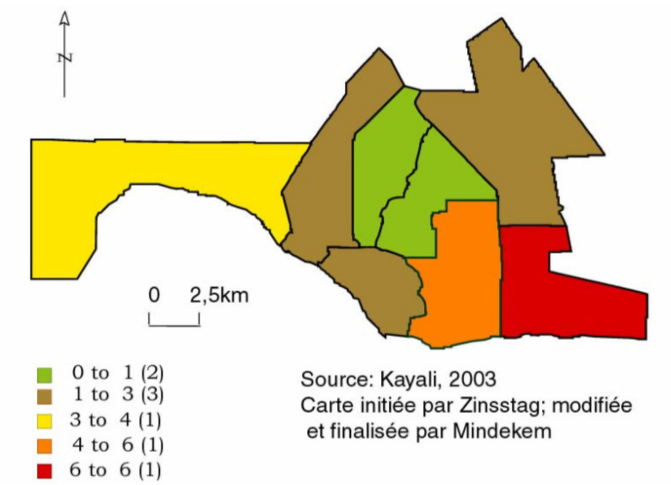

Figure IV.3: Districts of N'Djamena with number of dog rabies cases from December 2000 to June 2001

During the same time period, the rabies laboratory was equipped to perform the Fluorescent Antibody Test (FAT). The subsequent epidemiological study conducted in dogs from December 2000 to June to 2001 reported an incidence of 1.4 ‰ dogs (Kayali et al., 2003a). Most rabies cases were reported from quarters that had the highest dog density identified by the demographic study (Figure IV.3). Another study from September 2005 to November 2006 found a similar incidence of 1.7 ‰ dogs (Dürr et al., 2008a). During the latter epidemiological study the direct rapid immunohistochemical test (DRIT) was established and used in parallel to the FAT test. The study on the validation of the DRIT in developing countries also included a characterization of the viral strains found in N'Djamena and other parts of Chad. All viral specimens found belong to the Group E of the African 2 lineage also circulating in neighbouring Cameroon and Nigeria (Talbi et al., 2009). Specimens found in N'Djamena shared over 98% amino acid identity, whereas a virus sample originating from Sidjeh (around 100km from N'Djamena) shared max. 93.2% nucleotide identity (Dürr et al., 2008a). In 2002, the first pilot vaccination campaign was launched in parts of the 6th and 7th districts of N'Djamena. Results were promising and yielded a coverage rate of 68- 84% (Kayali et al., 2003b). During this first campaign the vaccine was administered free of charge (Kayali et al., 2006). A second campaign conducted in the same area in 2006 charged 3 USD/animal and only achieved vaccination coverage of 24% (Durr et al., 2009), which is insufficient to control transmission. Considering this outcome and also based on the results of a willingness to pay study (Durr et al., 2008b), it was estimated that in order to achieve target coverage of 70% the vaccine costs should not exceed 0.6 Euros (Durr et al., 2009).

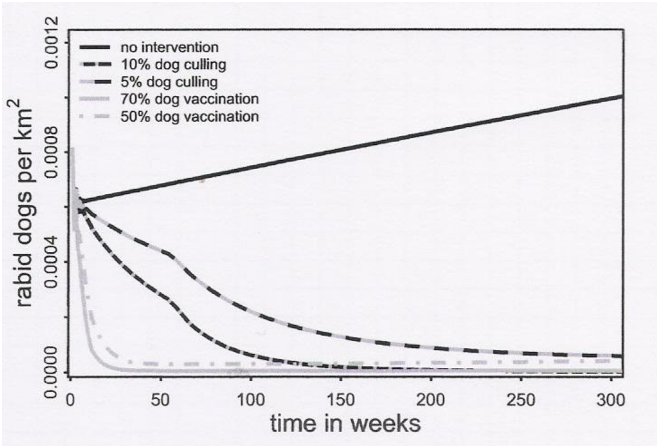

Figure IV.4: Comparison of the impact of different control strategies on rabies epidemiology (Zinsstag et al. 2009)

Using these research results, Zinsstag et al. (2009) developed a dog-human deterministic transmission model with stochastic parameter specification for dog-to-human rabies transmission, providing a tool to assess the comparative cost-effectiveness of different rabies control strategies. Based on the model outputs, it was hypothesised that by a single parenteral mass vaccination campaign of all dogs in N'Djamena the transmission of rabies could be interrupted. In the model, rabies could be eliminated in the city area over a period of about 6 years. In Figure IV.4, the deterministic fit (bold black line) of the transmission model to the occurrence of weekly rabid dogs is depicted. The bold grey line simulates a vaccination campaign with coverage of 70% and predicts the breakdown of transmission for almost five years. All other strategies simulated in the model and depicted in Figure IV.4 demonstrate less efficient effects. In addition, the model predicts a higher cost-effectiveness of the vaccination campaign with 70% coverage including PEP than for PEP alone after 6 years (Figure IV.5).

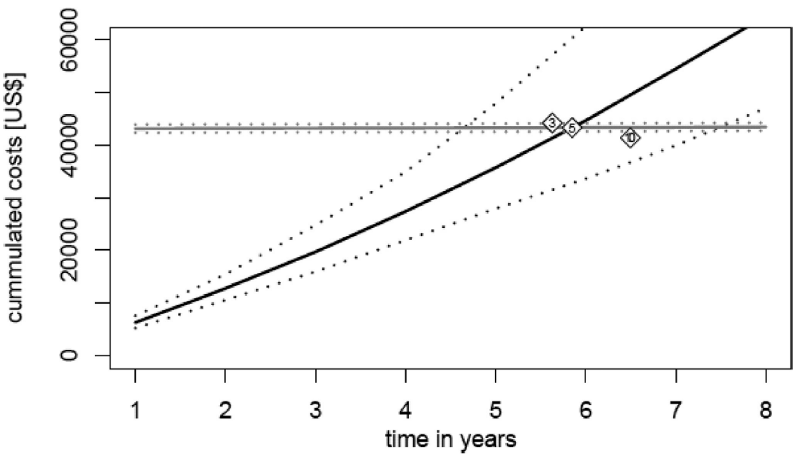

Figure IV.5: Comparative costs of PEP (black line) and dog mass vaccination (grey line) (Zinsstag et al. 2009).

From September 2008 to April 2009 a study based on an animal bite survey in approximately half of all health facilities in N'Djamena estimated that around 7 human rabies deaths (95% CI 4–10 deaths) occur in the city per year. Alarmingly, the study also showed that a considerably high proportion of potentially exposed people (42%) remain without PEP or wound treatment. Given the previously proven feasibility of a free dog mass vaccination campaign and the suggested cost effectiveness of such an intervention, the results on the estimated human burden of rabies in N'Djamena highlighted urgent need for action.

IV.3.3 From Research to action back to Research: The present elimination program

The societal objective of the current control programme is to eliminate human rabies in N'Djamena, Chad as an example for other African cities. As proven by the pilot projects, a successful vaccination campaign in N'Djamena is feasible and 70% coverage can lead to interruption of disease transmission, because the propagation of the virus proceeds on a low and stable endemic level, with an effective reproductive ratio (R_e) of just over 1 (Zinsstag et al, 2009).

The scientific objective of the existing and ongoing work is to compare the actually observed decrease of weekly dog rabies incidence and PEP demand with the model prediction. The main uncertainty is the dynamics of immigration of latent infected dogs which could not be included in the above described model because of lacking data. If immigration of latent infected dogs is low, dog mass vaccination is very likely more cost-effective than PEP alone with a time horizon of 8-10 years. The formal proof of cost-effectiveness of the mass vaccination of dogs is crucial to advocate this strategy in developing countries. However, a validated dog-human transmission model capturing the dynamics of interventions is required for this.

Therefore a new project phase was initiated in 2011 to plan and conduct two consecutive annual parenteral dog mass vaccination campaigns in N'Djamena. The close scientific monitoring of the campaigns are done through a detailed coverage analysis and cost description. The long-term impact of the campaigns is studied through follow up of the incidence of dog rabies before, during and after mass vaccination campaigns by FAT and the monitoring of the dog bite incidences at health structures, similar to Frey et al. (2013).

In line with the previous phases of the control programme, we included a component to enforce the diagnostic capacity of the rabies laboratory by validating a rapid diagnostic test kit for the use in the field. This test has the potential to be performed in peripheral laboratories and hence prepares for the extension of rabies surveillance to the national level. The same intention to expand the control programme to other regions in Chad and eventually to the whole country led to a national demography and Knowledge Attitude and Practice (KAP) survey conducted in 2013. With the help of this survey and the economic analysis of the campaigns in 2012 and 2013 in N'Djamena, a proposed budget for the elimination of canine rabies from Chad was elaborated. Results of this expansion phase are also presented in this thesis. Embedded in the project of the present PhD thesis are the Doctoral thesis of a Chadian geographer, two Master of Science for Chadian students and three Swiss master's students. The main research topics around the vaccination campaigns and their assignment to the two major theses of the project are depicted in Figure IV.6. The master topics of the Chadian students were implanted in the rabies diagnostic part and comprised (1) the extended validation of the rapid diagnostic test on several different RABV lineages; (2) a serological follow up of dogs vaccinated during the campaign to analyse the kinetics of rabies antibodies in dogs of N'Djamena. The Swiss master students worked on (1) the analysis of the demography and KAP survey, (2) the economical exploration of rabies elimination in Chad, and (3) the description of a mixed method approach to determine rabies vaccination effectiveness nurtured by the experiences in N'Djamena and Bamako, Mali.

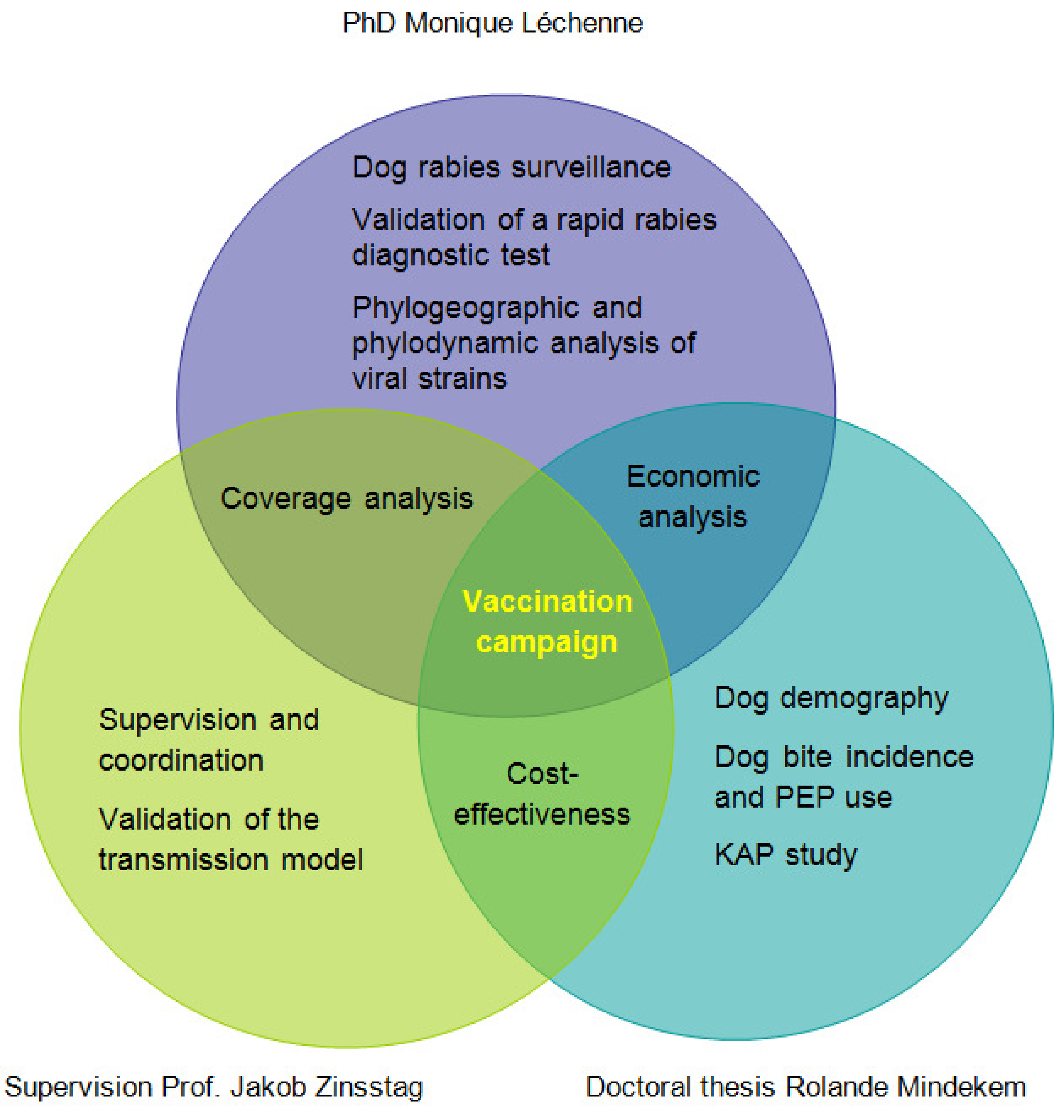

Figure IV.6: Assignment of research topics around the mass vaccination campaign between main study responsibles.

V. AIM AND OBJECTIVES

Aim

The overall aim of the programme is to eliminate rabies from N'Djamena, thereby contributing to the proof of feasibility and effectiveness of dog mass vaccination for rabies control. Along with this goal, public awareness and surveillance is to be enhanced in N'Djamena and its surrounding regions. Experiences in N'Djamena together with data from a nationally representative household survey are further used to prepare a national rabies elimination budget.

Core objectives (identified in the project proposal)

Conduct two consecutive annual dog mass vaccination campaigns covering at least 70% of the total dog population of N'Djamena. (Chapter 1)

Follow up of the dynamics of dog rabies incidence, reported human dog bite injuries and demand for PEP during the intervention period. (Chapter 2)

Validate the Anigen Rapid Rabies diagnostic kit in regard to performance and reliability under limited laboratory conditions. (Chapter 3)

Validation of the dog to human transmission model and prove of interruption of transmission. (Chapter 4)

Assess the rate of reintroduction of rabies virus into N'Djamena by a phylogeographical analysis of rabies isolates collected during routine surveillance. (VI. working paper)

Subsequent objectives (emerged during the project process)

Estimate the national dog to human ratio and assess the knowledge and awareness level of the Chadian population for the preparation of a national dog vaccination campaign. (Chapter 5)

Prepare a tentative budget and present a financing mechanism for a Chadian national dog vaccination campaign. (Chapter 6)

Compare the N'Djamena experience to a similar project site to highlight the need for a mixed methods approach for the evaluation of effectiveness of dog mass vaccination interventions. (Chapter 7)

CHAPTER 1

Operational performance and analysis of two rabies vaccination campaigns in N'Djamena, Chad

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Abstract

Transmission of rabies from animals to people continues despite availability of good vaccines for both human and animal use. The only effective strategy to achieve elimination of dog rabies and the related human exposure is to immunize dogs at high coverage levels. We present the analysis of two consecutive parenteral dog mass vaccination campaigns conducted in N'Djamena in 2012 and 2013 to advocate the feasibility and effectiveness for rabies control through proof of concept. The overall coverage reached by the intervention was >70% in both years. Monthly reported rabies cases in dogs decreased by more than 90% within one year. Key points were a cooperative collaboration between the three partner institutions involved in the control program, sufficient information and communication strategy to access local leaders and the public, careful planning of the practical implementation phase and the effective motivation of staff.

The dynamic and semi to non-restricted nature of dog populations in most rabies endemic areas is often considered to be a major obstacle to achieve sufficient vaccination coverage. However, we show that feasibility of dog mass vaccination is highly dependent on human determinants of dog population accessibility and the disease awareness of dog owners. Consequently, prior evaluation of the human cultural and socio-economic context is an important prerequisite for planning dog rabies vaccination campaigns.

Keywords: rabies, vaccination, dog, coverage, accessibility, One Health

Introduction

More than a hundred years after the invention of the first rabies vaccine by Louis Pasteur, this zoonotic disease still claims an estimated 59,000 lives worldwide each year (Hampson et al., 2015). The onset of rabies in bite victims can be prevented by timely administration of Post Exposure Prophylaxis (PEP), and research regarding new PEP schedules has rendered this intervention in humans less expensive and time consuming in recent years (Warrell, 2012a). Because more than half of all rabies victims are children, the costs of PEP alone per death averted calculated on the basis of Years of Life Lost (YLL) are justifiable (Hampson et al., 2011). But these costs will remain, and potentially rise each year, if transmission between dogs and humans is not interrupted. Vaccination campaigns in dogs are, in the short term, more expensive but will eventually lead to elimination of rabies in the domestic dog population (Zinsstag et al., 2009) and substantially reduce disease burden, as demonstrated in Latin America (Vigilato et al., 2013a). Enhanced advocacy is needed to persuade policy makers and governments to shift the target from trying to minimize the effect of disease in humans to a One Health approach which tackles the problem at its source. In developing countries resources allocated to the health sector are scarce and rabies is one of many other priority diseases, so proof of long term cost-effectiveness is particularly important for advocacy. Short term direct measures of effectiveness are estimated coverage numbers achieved by dog vaccination and the change of rabies incidence dynamics after intervention. Comparable to any other service provision in Health Systems, viability and practical implementation are in the direct causal pathway from the efficiency of a product (in this case rabies vaccine) to the effectiveness outcome in the field (in this case coverage) (Obrist et al., 2007). Logistical constraints, public participation, performance of vaccination teams and the nature of the dog population are all aspects that have an influence on one or several effectiveness parameters, which are defined as availability, accessibility, affordability, acceptability and adequacy. Without proper attention, these factors directly influence cost and reduce coverage of vaccination campaigns in a multiplicative way (Muthiani et al., 2015; Chapter 7, this thesis). Despite the availability of safe and efficacious rabies vaccines, the ultimate effectiveness in the field is a fraction of the potency under perfect conditions (Zinsstag, 2013; Muthiani et al., 2015). In a specific context, an assessment of all these effectiveness components is complex and difficult but urgently needed. It is especially important in light of the trade-off between targeted maximal accessibility and limited financial resources. Practical examples and careful evaluation help to establish a decision framework to balance cost and performance. Intervention effectiveness research on dog rabies vaccination is scarce, particularly in countries with the highest estimated rabies burden (Davlin and Vonville, 2012). Since the year 2000 the Swiss Tropical and Public Health Institute (Swiss TPH) works in close research partnership with two Chadian partner Institutions based in N'Djamena, the Institute de Recherche en Elevage pour le Développement (IRED) and the Centre de Support en Santé International (CSSI). Huge progress has since been made in terms of reinforcement of diagnostic capacities and the proof of feasibility of dog vaccination. The description of the dog demography and two pilot vaccination campaigns (Kayali et al., 2003b, Durr et al., 2009) have shown that a high coverage can be achieved through free immunisation by a fixed post approach. The cost-estimation done with the results of the pilot studies (Kayali et al., 2006, Durr et al., 2009) helped to prepare the citywide campaign and to estimate the cost-effectiveness of such an intervention with a dog to human transmission model (Zinsstag et al., 2009). The thorough analysis of the two consecutive interventions presented here helps to prove the outcome of feasibility and cost-effectiveness.

Materials and Methods

Two campaigns, held in 2012 and 2013, were organized by a coordination committee composed of representatives of IRED, CSSI and Swiss TPH. Finances were shared between IRED (personnel and logistics) and Swiss TPH (vaccine and other material). The operational procedure was based on a Central Point Vaccination (CPV) approach, with the vaccination post installed for the whole vaccination day at a central location, most often in front of a block chief's house. Upon request households with a dog that could not be brought to a post were visited by a mobile vaccinator on a motorbike. Also, in the outskirts of N'Djamena a mobile approach was applied by a vaccination team visiting several villages a day by car. The ten vaccination teams consisted of 3 vaccinators each.

Representatives of the different urban administrative entities participated in preparatory meetings (Plate 2) and were actively involved in the organisational planning, in particular regarding public sensitization and location of posts. The information campaign included use of posters, radio broadcasts, loudspeaker announcements and banners (Plate 3).

Vaccine and materials were procured on the basis of an estimated total dog population of 50,000. Vaccine storage was assured by IRED. In both years, the campaign ran from October to January on Friday to Sunday (13 weeks; 37 days). One week was defined as a sequence and enumerated from 1 to 13. In addition to dogs, vaccine was also administered to all presented cats and primates. All dogs were collared and marked on the trunk with a livestock marker crayon (Plate 4). The operational plan is further described in the supplementary material.

Vaccination sequences and analysis zones were usually comprised of a district (Figure 1.1) and were defined by drawing the circumference around the outermost vaccination posts, with exclusion of peripheral villages. The coverage assessment included a household survey in randomly selected geographical locations within the analysis zone to estimate the proportion of owned vaccinated dogs. In addition, random transect drives (Plate 5) were carried out in the same locations to estimate the proportion of free roaming dogs and the fraction of ownerless dogs (Figure 1.1). Data from both studies were combined in a Bayesian statistical model (see supplementary file).

Information from vaccination posts and daily team performances as well as data collected during the household and transect study were continuously entered in a Microsoft Access database to analyze in real time the achieved coverage per analysis zone using EpiInfo (CDC, 2008) and Winbugs (Lunn et al., 2000). For the preliminary estimate, the number of dogs vaccinated before the analysis in the area was used (Table 1.1). When coverage was < 70% and the coordination committee thought that vaccination of more dogs in the area was achievable, teams were sent back to the area. For the final coverage analysis, the sum of dogs vaccinated before and after analysis in a given zone for a given year was taken into account (Table 1.1).

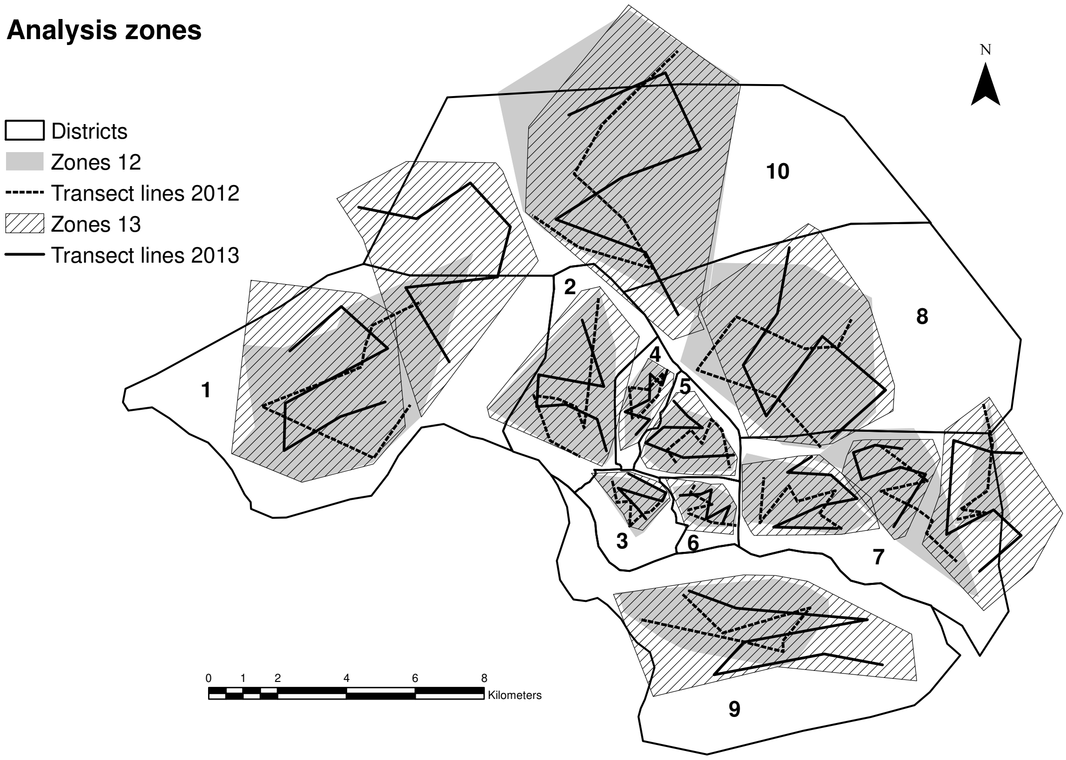

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Figure 1.1: Comparison of analysis zones and transects lines for the coverage survey in 2012 and 2013. Changes in analysis area result from varied locations of vaccination posts and the split of the 1st district in two sequences in 2013. These changes were made to achieve higher coverage.

Dogs vaccinated at posts outside of the analysis zone were excluded from the analysis. Due to the lack of long lasting identification of the dogs and the high rate of loss of certificates it was not possible to register the number of dogs that were initially vaccinated in 2012 and revaccinated in 2013. All expenditures prior to and throughout the campaigns were recorded and analyzed. Incidence data on dog rabies were collected as part of routine diagnostic testing at IRED. Awareness regarding best practice after a dog bite incident and the importance of diagnostic testing was highlighted through a poster campaign in health centers, hospitals and pharmacies prior to the intervention.

Research approval was granted by the Scientific and Technical Research Directorate of the Ministry of Higher Education (Ministère de l'Enseignement Superieur, Direction de la Recherché Scientifique et Technique) in Chad. The study protocol was additionally reviewed by the Ethics Commission of the Cantons of Basel-Stadt and Basel-Land.

Table 1.1: Coverage estimates, number of owned dogs, percentage of ownerless dogs and total dog population by district and vaccination sequence.

District	Sequence (intervention week)	Number of owned dogs	% of ownerless dogs	Total dog population	Vaccinated before analysis[a]	Vaccinated total in analysis area[b]	Coverage	Vaccinated out of analysis area
2012								
1	1	1267	2	1289	834	925	72%	26
2	2	130	57	203	64	64	31%	
3	3	497	4	517	376	376	73%	
4	4	48	30	62	24	24	39%	
5	11	474	1	481	311	311	65%	
6	5	1358	30	1762	793	919	52%	
7	6	4724	19	5617	2775	3342	59%	
7	7	7758	0	7783	5893	6683	86%	
7	8	582	24	721	503	503	70%	100
8	12	862	19	1025	361	413	40%	24
9	9, 10	4648	2	4728	3858	3858	82%	429
10	13	296	21	359	120	120	33%	37
Summary 2012	13	22,643	8	24,547	15,912	17,538	71%	590
2013								
1	6	1582	14	1810	1155	1325	73%	113
1	7	73	68	123	52	62	50%	
2	4	208	4	217	123	123	57%	
3	2	646	25	811	468	468	58%	
4	5	95	85	175	49	67	38%	
5	3	530	9	579	330	330	57%	
6	1	1055	45	1535	722	1044	68%	
7	8	6586	15	7606	4850	5507	72%	
7	9	5573	9	6073	4372	4372	72%	
7	10	3111	16	3594	2660	2699	75%	296
8	12	988	42	1399	691	741	53%	35
9	11	5017	13	5672	4314	4402	78%	415
10	13	348	38	482	200	200	41%	107
Summary 2013	13	25,812	14	30,074	19,986	21,340	71%	966

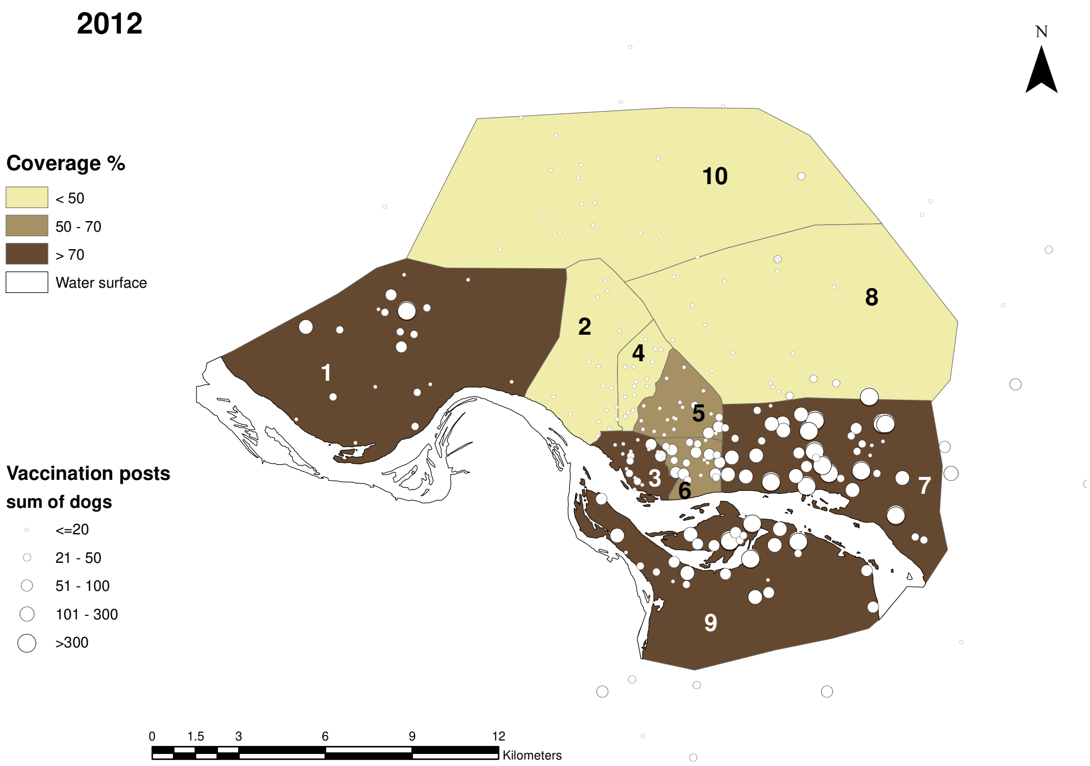

Figure 1.2: Coverage estimates and vaccination posts during the campaign in 2012. The size of the vaccination post bullet indicates the number of dogs vaccinated at the respective post. Vaccination posts outside the borderline of the city include peripheral villages

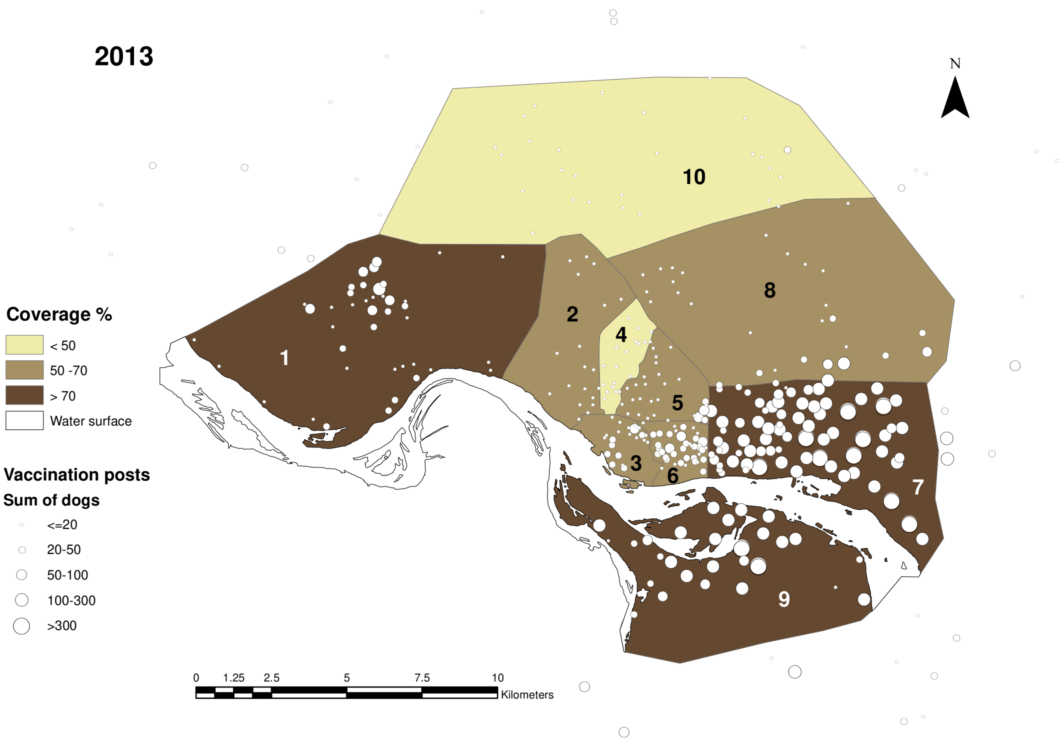

Figure 1.3: Coverage estimates and vaccination posts during the campaign in 2013. The size of the vaccination post bullet indicates the number of dogs vaccinated at the respective post. Vaccination posts outside the borderline of the city include peripheral villages.

Disruption of supply due to logistical challenges regarding traffic only occurred in a few instances and did not greatly impact on the accessibility, because owners were willing to wait at the posts for replenishment. We believe that the cold chain was maintained in the large majority of cases. Preliminary results of follow up vaccination titres show that only 1 of 84 dogs did not have a sufficient titre (>0.5 International Units (IU)) when tested with the FAVN one month after vaccination (Abdelrazakh et al., forthcoming).

The total number of dogs vaccinated was 18,182 in 2012 and 22,306 in 2013 (Table 1.2).

Table 1.2: Summary of animals vaccinated by district in 2012 and 2013

District	Vaccinated 2012			
	Dogs	Cats	Primates	Total district
1	951	135	5	1091
2	64	49	3	116
3	376	79	12	467
4	24	108	2	134
5	311	87	1	399
6	919	80	12	1011
7	10,628	310	41	10,979
8	437	82	3	522
9	4315	372	24	4711
10	157	182	1	340
Total Ndjamena	18,182	1484	104	19,770
District	**Vaccinated 2013**			
	Dogs	Cats	Primates	Total district
1	1500	410	12	1922
2	123	166	1	290
3	468	139	10	617
4	67	265	0	332
5	330	206	0	536
6	1044	154	15	1213
7	12,874	1240	60	14,174
8	774	249	8	1031
9	4828	560	22	5410
10	298	212	2	512
Total Ndjamena	22,306	3601	130	26,037

The Bayesian population model applied (see supplementary file) estimated the total number of dogs for the city to be between 24,500 and 30,000 dogs (Table 1.1), corresponding to a density of about 100 dogs/km^2. This population estimated results in a dog to human ratio of between 1:40 to 1:50, which is similar to ratios reported from an urban location in Zambia (De Balogh et al., 1993) but much lower than the ratio estimated across Africa reported in Knobel et al. (2005). This number is far below the previously estimated total population of 45,000 -50'000 dogs. The discrepancy can be explained by the fact that the demographic study in 2003 (Mindekem et al., 2005) which served as the basis for the preliminary estimate had a high standard error resulting in a low precision of the dog population estimate. Further extrapolation of the ratio resulted in a high overestimation. In addition, we postulate that the dog population numbers did not grow proportionally to the human population. This finding shows that demographic surveys should not be conducted with a long time lag before intervention and are of limited use for precise coverage estimation. Overestimation of the dog population entails the risk of vaccine wastage. In our case the expiration date of the vials purchased in 2012 and the good storage conditions at IRED allowed the use of the vaccine for the two campaigns.

Our results suggest that between 8-14% of the dogs in N'Djamena are actually ownerless (Table 1.1), which is in line with other studies reporting low ownerless dog numbers (Davlin and Vonville, 2012; Jibat et al., 2015). However, this estimate of the Bayesian model is sensitive to one model parameter. Details of the sensitivity analysis are provided in the supplementary appendix. Total vaccination coverage was estimated at 71% in both 2012 (CI 0.68-0.76) and 2013 (CI 0.7 -0.76). However, this number is a mean over all the districts covered. The districts differ greatly in dog density, participation of owners in the campaign and percentage of stray dogs, so coverage data for each district, and even from quarter to quarter, are diverging (Table 1.1). Collar loss, immigration and emigration as well as births and deaths did not presumably greatly influence the results because the coverage survey was done 48 hours after the vaccination in a given zone (see supplementary file). In the household survey in 2013, only 6.8% of dogs were reported to have lost their collars. When observed in transects, this would likely lead to slight underestimation of coverage as well as overestimation of ownerless dogs. Cross-sectional assessments estimate vaccination coverage at a given point in time, but actual herd immunity is dynamic (Townsend et al., 2013a). When a vaccination campaign moves to a new zone, coverage in the previous zone starts to decline due to population turnover and immunity loss. The decision regarding timely progression of campaigns must be a balance between overall achievable coverage which increases with speed and accessibility of dog owners which decreases with faster movement. Daily number of animals vaccinated not only impacts time, and consequently coverage, but also has a high impact on the cost per animal vaccinated. Calculated over the two campaigns the incremental cost per vaccinated animal was 2.1 Euros. In 2012, the operational cost of one vaccination team per day was 109 Euros (71,500 FCFA). One-third of this cost was fixed while 2/3 depended on the length of the campaign. The cost for vaccines and collars was 60 cents /animal. Therefore, each animal on the peak day in 2012 (1 animal out of the total of 325 vaccinated) cost only 90 cents. In turn, if only one animal was vaccinated per day at a post, the cost for this animal in 2012 (1 animal out of the total of 1 vaccinated) was 109.5 Euros.

In 2012 the reason for not bringing a dog for vaccination, as stated by owners of encountered unvaccinated dogs during the household survey (n=1266), was most often lack of information (40%).

With intensified megaphone mobilization around vaccination posts in 2013, lack of information dropped to 31 % (n=365) which would indicate that short term, direct sensitization of the public triggers high response. Preliminary comparison of our campaign with a pilot vaccination in Bamako (coverage < 20%) also indicates that baseline awareness in the community has a considerable impact on coverage (Muthiani et al., 2015). Through several years of work on rabies control in N'Djamena people might have been more informed about rabies than in Bamako where intensified control efforts were only recently implemented. A well informed public is likely to exert societal pressure on dog owners to comply with vaccination. The second most reported cause in both years was inability to handle the dog (25% and 28%, respectively). This reduction in accessibility can be addressed by including a mobile approach, by visiting households on request. Some vaccinators used motorbikes to visit dog owners at their home when a dog could not be brought to a post. This should be carefully implemented and communicated to avoid the expectation that vaccinators will go door to door as in polio vaccination campaigns. In 2013 posts were relocated more quickly when utilization was low, contributing, along with intensified mobilization, to higher performance.

In 2012, 5% of owners stated that their dog was too young to be vaccinated. However, it has been shown that the majority of dogs less than 3 months old still respond positively to vaccination (Seghaier et al., 1999; Morters et al., 2015). In a setting where population turnover is high and a large number of the population is comprised of young dogs, WHO recommends the vaccination of puppies (WHO, 2013). After instructing vaccination teams to encourage vaccination of puppies, age as a cause for non-vaccination dropped to 3% in 2013.

Other reasons, including distance to the post, refusal, neglect and age of the dog, did not exceed 3% of all causes for non-vaccination in both years. Refusal due to infectious disease concerns or fighting due to contact with other dogs as observed in studies in Grenada (Thomas et al., 2013) were not specifically stated by owners. However, some people believe that the vaccine may be harmful or cause the dog to be a less effective guardian (author's observation).

Our team performance indicators show that the highest limitation factors are public accessibility and participation rather than the vaccination capacity of a post. Similar observations were made in Sao Paulo city, Brazil (Polo et al., 2013) where geographical barriers between supply and demand and people's awareness of the benefit of vaccination mostly determined accessibility. The vaccination capacity of more than 200 animals /day and post observed in this study cannot be achieved by a fixed approach in areas where the distance to the post is long, dog population density is low or people are not adequately informed. We found the highest motivation factor for staff is a well visited post, and teams got discouraged when performance was low. Zones with low dog densities, low awareness and accessibility would additionally be impacted by low motivation of vaccinators.

Despite higher overall performance in 2013, the 70% threshold was not achieved in more districts than in 2012 (Figure 1.2 and 1.3). The difference lies mainly in the 2nd and 8th districts, where the 2013 coverage was raised above the threshold of 50%. Districts 4 and 10 were zones that performed weakly (< 50%) in both years. The 4th district houses the central market where ownerless dogs gather. The peripheral district 10 is comprised of low-density settlements where accessibility is challenging due to distance and mobilization. Both districts also have a predominantly Muslim population. In some Hadith verses which accompany the Koran the dog is described as an impure animal (Stilt, 2008). Owned dogs are fewer and potentially less accessible in Muslim communities. Similar observations were made in Tanzania (Cleaveland et al., 2003). The 2nd district, which has the second lowest dog numbers, is dominated by a relatively affluent neighbourhood, which includes the president's mansion and numerous ministries. In wealthy areas dogs are more likely to be confined, and dog owners are less susceptible to social mobilization (Awoyomi et al., 2008). In contrast to Districts 2 and 4, Districts 7 and 9 are dominated by a socio-economically weak, Christian population. Dog density is high and most dwellings are not fenced, so the confinement level is very low. The proportion of ownerless dogs was less than 15% in both years for these districts and coverage was notably high. The performance and background of districts 7 and 9 are comparable to rural agro-pastoralist areas in Tanzania (Kaare et al., 2009). Our results support the finding of Kaare et al. (2009) that there is not one ideal approach for planning dog vaccination campaigns in a diverse context.

After the start of our campaign, the dog rabies incidence calculated based on the population estimates of the campaign dropped from 0.7/1,000 (CI 0.63-0.77) in 2012 to 0.073/1,000 (CI 0.067-0.081) in 2014. We are confident that this decline is not due to a drop in awareness because the rate of negative tested rabies suspicion cases did not change considerably. Since January 2015 cases have risen again in the 9th district which is on the border of the town and thus highly suggestive of reintroduction. Modelling effectiveness of mass vaccination in Bali has shown that even small areas with no or very low coverage can jeopardize the success of a campaign and be the source of continued rabies transmission (Townsend et al., 2013a). Closer investigation through molecular genetic study is currently under way to further detail transmission dynamics.

Whilst financial commitment of Chadian national authorities is lacking for continued citywide vaccination, a small scale containment vaccination in the 9th district and the adjacent villages is funded by donors and planned to take place before end of 2015.

Conclusion

Thorough analysis of the vaccination intervention presented demonstrates the feasibility and cost-effectiveness of dog rabies elimination in an African capital city, supporting the vision of achievable elimination across the continent (Cleaveland et al., 2014b). Identified driving forces of performance are close involvement of local leaders and intense public sensitization. Timely mobilization and adherence of dog owners is crucial to minimize cost and maximize coverage and motivation of vaccinators. Special attention should be given to cultural aspects influencing accessibility of dog populations during the preparation of interventions.

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First and foremost, we thank all the committee members and vaccination teams (Plate 6). They did an incredible job over the three campaign months putting an enormous amount of energy into this project, and without them we could not have succeeded. We also thank Merial for support and generous provision of vaccination certificates. The Direction des Services Vétérinaires (DSV) in Chad, which is part of the Ministère de l'Elevage et de l'Hydraulique helped with their organizational knowledge. Main funding support came from the UBS Optimus Foundation, and the Federal Food Safety and Veterinary Office (FSVO) of Switzerland, the Wolfermann-Nägeli Foundation and the Guggenheim-Schnurr Foundation also contributed important financial resources. We are also grateful for mutual partnership with the Global Alliance for Rabies Control (GARC) and happy to belong to the Partners for Rabies Prevention (PRP) group. Lisa Crump is greatly acknowledged for language editing.

Supplementary file

Organisational plan

The two campaigns were organised by a coordination committee composed of representatives of the three partner institutions, IRED, CSSI and the Swiss TPH, who initiated the intervention together. Among the competences and responsibilities of this group were: decision making on the spatial progress of the campaign and the logistical processes, public information and weekly communication with town authorities. The supervisory committee, composed of the directors of the three institutions or their respective representatives oversaw the funding, personnel issues and relationships with governmental and city authorities.

The city of N'Djamena is composed of 10 districts with several quarters each. The quarters consist of several blocks (neighbourhoods). Block representatives are under supervision of the quarter chiefs who are mentored by the district heads. District and quarter chiefs were invited to a preparatory meeting prior to the campaign during which they took ownership through chairing the discussions.

The educational background of vaccinators was varied. The majority were animal husbandry technicians at the rank of an agent or engineer, but there were also trained veterinarians as well as a human medical student. They were recruited from the staff of IRED, the employees of the national school for livestock technicians (ENATE), the regional livestock delegation of N'Djamena (DREN) and the local urban veterinary clinic (CVUN). All vaccinators were immunised against rabies and participated in a two day training program. The principle topics during this course were the organisational plan, the administrative duties at the vaccination posts (registration of vaccinated dogs, completion of vaccination certificates) and training on how to handle and vaccinate dogs (measures for avoiding bites and scratches of animals, how to use the vaccine). In total 31 vaccinators and 3 supervisors were recruited and trained for the campaign (Plate 6).

Posters were distributed to quarter chiefs during the above mentioned preparatory meetings held in September 2012/2013. The quarter chiefs were requested to share the information and posters with the block representatives, with whom they announced the intervention to the quarter residents.

During the vaccination campaign the organisational committee met with the district chief and the quarter chiefs a few days prior to the scheduled vaccination in a specific area. Together they made a list of locations where vaccination posts were to be installed based on where to expect the highest number of dogs, a central well known location and homogenous distribution of posts. The national radio station and/or different local radio stations with the best acceptance and reception in each zone broadcasted each Thursday to Saturday information about the disease and our campaign. Location of the vaccination posts were announced by megaphone in French, Arabic and Sara/Gambaye every Thursday before the start of vaccination in each zone. Megaphones were also used to inform people around an installed post during the campaign when the post was not well frequented. In 2012 this task was done by vaccinators or block chiefs. Given that in 2012 lack of information was stated as a major cause for non-vaccination, in 2013 special personnel (3) were hired whose sole task was to invite people to participate using a loudspeaker on the day of vaccination.

For the execution of both campaigns the objective was to vaccinate 70% of the total dog population of N'Djamena. We assumed a total of 1 million inhabitants in N'Djamena based on the most recent 2009 population census and an added growth rate of 3%. We applied the dog to human ratio of 1:20 estimated in 2001 (Mindekem et al., 2005) and calculated the total dog population to be between 45-50'000. Rabies vaccine doses, collars, syringes and needles were procured based on this assumed dog population number. The vaccination certificates designed especially for the campaign were donated by Merial Inc. As the estimation of dog numbers was twice as high as figures encountered, this stock lasted also for the campaign in 2013. Other important material comprised 10 registries, 16 cool boxes, 10 banners and 10 stamps. Three four wheel drive pick-up trucks, one per supervisor, were made available for personnel and material transport.

The campaigns were officially launched during the global rabies day, celebrated on September 28th each year. Field work lasted from October to January in both years. One week of campaigning was defined as a sequence and enumerated 1 to 13. The teams proceeded district after district. Vaccination took place only on Friday to Sunday due to availability of staff and best participation of the public during these days as evaluated in previous studies (Kayali et al., 2003b).

The 10 vaccination teams consisted of three vaccinators who shared tasks, usually by one administering the vaccine and two registering the vaccinated animals. Supervisors were in charge of 3 to 4 vaccination teams and their task was to control the quality of the work of the vaccinators and to keep track of the vaccine doses administered and other material used during the day. For this task they received a control sheet for every team which they filled in at drop off time in the morning and in the evening at collection time together with the teams. This document served as data sheet for the numbers of dogs, cats and primates vaccinated per day and team which was later registered in Access. In case of shortage of the vaccine or other material the supervisors were responsible for restocking their teams and they were the primary contact person for block representatives.

Coverage analysis

Because of its size and the extent of the dog population district 7 was divided into 3 zones in both years. In 2013 district 1 was additionally divided into two zones. The coverage analysis perimeters were defined by drawing the circumference around the outermost vaccination posts. Peripheral villages (17 in 2012 and 38 in 2013) were excluded due to logistical and methodological constraints. In general, the accessibility area around a post is circular with a diameter of 1km. Around the edge vaccination posts we created a buffer to avoid counting dogs from outside the vaccination area by not including the whole extent of the circular accessibility area. All dogs with a blue plastic collar and/or coloured mark encountered on the household and transect level were considered as recaptured. Household and Transect data were later combined in a Bayesian statistical model. For final dog density calculation and calculation of posts per km^2 the whole accessibility area was calculated in ArcGIS by creating a buffer of 1km around the polygon areas and subtracting the intersect area between this buffer and the water surface of the Chari River. Preliminary coverage estimates were conducted for each sequence on the Wednesday after the data collection in each of the analysis zones. For this preliminary estimate, the number of dogs vaccinated before the coverage analysis in the vaccination zone, were used. When coverage was under < 70% and the coordination committee believed that vaccination of more dogs in the area was achievable based on the experiences made on vaccination days and the background of the district, a number of teams were sent back to the area. For the final coverage analysis the sums of dogs vaccinated before and after analysis in a given zone were taken into account. Dogs vaccinated at posts out of the analysis zone were excluded from the analysis.

Household survey

A household survey was carried out after every vaccination sequence to recapture a pre-defined proportion of vaccinated owned dogs. This number was calculated by the Peterson-Bailey formula and depended on the number of dogs vaccinated during the vaccination sequence and supposed vaccination coverage of 70% at household level. The information gathered in this survey would later help estimate the ownerless dog population of the zone.

Each Monday following the respective vaccination sequence the interviewers went to the field. Their starting points were predefined as random coordinates inside the vaccination zone (which were at the same time the points of the transect line, see below). These points were created by first defining the north, south, west and east extent of the analysis zone. The coordinates of this extent were then put into excel and using the random function, new coordinates were created within this predefined area. The walking direction from the starting point was chosen by the interviewers, which then visited every household from door to door. For every encountered dog they filled in a questionnaire to register whether it was marked and vaccinated by the campaign, already vaccinated earlier and in possession of a vaccination certificate or unvaccinated. In the latter case, the owner was asked why the dog had not been brought to the vaccination point. Furthermore they noted if it was confined to the compound or not. Positive confinement was defined as the dog was constantly on a leash or the door of the property was always kept closed. Based on this information the vaccination coverage at household level and the confinement probabilities of owned vaccinated and owned unvaccinated dogs could be calculated.

Transect survey

In addition to the household survey, a transect survey was conducted after every vaccination sequence to estimate the number of ownerless dogs and to recapture the vaccinated dogs in the whole dog population. We generated random coordinates inside the vaccination zone (7 points in general). These points were linked such that the resulting line would not cross nor come closer than 50m to the same or a previous transect to minimise the risk of double counting dogs (methodology adapted from Gsell et al., 2012). In addition transects would ideally not be longer than 11km in order to easily cover in a maximum of 2 hours driving time. Dogs are more likely to be in the street in the morning and evening hours when the temperature is not too high. Therefore transects were conducted from 7am to 9am in the morning and from 4pm to 6pm in the evening on Monday and Tuesday, four times in total for each analysis zone. Night transects as described in Gsell et al. (2012) were not undertaken due to security concerns. The transects were driven by car with two dog spotters, one driver and a guide that was responsible for following the transect line as closely as possible on the GPS. Mean speed was not more than 15km/h. Every dog seen in the open street or through the gate of an open household was counted and it was noted whether it was marked (vaccinated) or unmarked (unvaccinated).

Table 1.3 lists the parameters from the household survey that were entered in the Bayesian model. The 95% CI from the reported confinement of vaccinated and unvaccinated dogs were used as beta distributed priors in the Bayesian model.

Table 1.3: Data from the household survey

District	Zone	total recaptured (n)	marked recaptured (m)	unmarked recaptured	confinement vaccinated (%)	confinement unvaccinated (%)
2012						
1	1	392	255	137	33	27
2	2	82	39	43	38	40
3	3	225	171	54	36	24
4	4	30	15	15	87	40
5	11	224	137	87	3	13
6	5	418	244	174	29	27
7	6	799	470	329	19	30
7	7	1222	893	329	14	13
7	8	327	283	44	2	2
8	12	137	58	79	37	39
9	9&10	913	760	153	5	7
10	13	78	32	46	19	31
2013						
1	6	455	332	123	2	2
1	7	41	29	12	1	8
2	4	140	72	68	31	31
3	2	354	256	98	20	20
4	5	67	36	31	28	58
5	3	293	181	112	16	31
6	1	426	291	135	25	14
7	8	1066	785	281	4	7
7	9	919	721	198	2	3
7	10	730	624	106	1	1
8	12	353	247	106	5	9
9	11	905	778	127	1	5
10	13	193	110	83	1	1

Bayesian coverage estimation

A Bayesian model based on earlier work (Kayali et al., 2003b; Gsell et al., 2012) and also applied in Bamako (Muthiani et al., 2015) was fitted to the study data to estimate the vaccination coverage, the total dog population and the ratio of ownerless to owned dogs in each vaccination zone. Bayesian inference takes into account prior information about additional model parameters, e.g. prior probabilities of recapture of marked dogs during transects and confinement probabilities of vaccinated and unvaccinated dogs (Table 1.3). While marked dogs were all vaccinated and owned, the population of unmarked dogs consisted of (i) owned, but not vaccinated dogs, (ii) owned and vaccinated dogs that had lost the collar and (iii) truly ownerless dogs, i.e. dogs that are neither owned by individuals or the community nor actively fed or sheltered.

Based on the number of vaccinated dogs a household recapture sample was calculated according to Peterson-Bailey (Bailey, 2004) assuming a vaccination coverage of 70% and a standard error of 5%. During the household survey, dog owners of vaccinated and unvaccinated dogs were asked about their dog's confinement. A completely confined dog, which could never leave the compound, was assigned with a value of 1, whereas a dog that could leave the household to roam was assigned with a value of 0 (Kayali et al., 2003b). In this way we estimated binomial 50% and 95% quantiles of the confinement of vaccinated c1 and unvaccinated dogs c2, which denote the α and β of the beta distribution of confinement for both groups.

The Bayesian coverage model was modified from Gsell et al. (2012). Briefly, $X_{1t}^{(i)}$and $X_{2t}^{(i)}$ are the number of owned dogs, collared and not-collared, respectively, and $Y_t^{(i)}$ is the number of ownerless (not-collared) dogs recaptured in zone *i* and on transect passage *t* (4 for each zone). As non-vaccinated owned and ownerless dogs cannot be differentiated in the street, we observed only the number of not-collared dogs $\mathrm{Z_t}^{(\mathrm{i})}$, instead of $X_{2t}^{(i)}$ and $Y_t^{(i)}$, where $\mathrm{Z_t}^{(\mathrm{i})} = X_{2t}^{(i)} + Y_t^{(i)}$ and $X_{2t}^{(i)}$ as well as $Y_t^{(i)}$ are latent data. The total number of vaccinated (collared, owned) dogs, $M_v^{(i)}$, in each zone i was known from the vaccination record of each zone.

We assumed that $X_{1t}^{(i)}$, $X_{2t}^{(i)}$ and $\mathrm{Z_t}^{(\mathrm{i})}$ follow binomial distributions with binomial recapture probabilities, $p_{t1}(i)$, $p_{t2}(i)$ and $p_{t3}(i)$, respectively; that is,

$X_{1t}^{(i)} \sim Bn((1 - c_1^{(i)})*M_v^{(i)}, p_{t1}^{(i)})$;

$X_{2t}^{(i)} \sim Bn((1 - c_2^{(i)})*M_u^{(i)}, p_{t2}^{(i)})$ *and*

$Z_t^{(i)} \sim Bn((1 - c_2^{(i)})*M_u^{(i)}\, p_{t2}^{(i)} + N^{(i)}, p_{t3}^{(i)})$,

where $c_1^{(i)}$ and $c_2^{(i)}$ are confinement probabilities related to zone *i* for owned collared and owned not-collared dogs, respectively; $M_u^{(i)}$ is the total number of unvaccinated owned dogs; and $N^{(i)}$ is the total number of ownerless dogs in area *i*. To reduce the number of parameters of the model, we assumed a common recapture probability, $p_t^{(i)}$ for all dogs (collared owned, not-collared owned, and not-collared ownerless), that is, $p_{t1}^{(i)}=p_{t2}^{(i)}= p_{t3}^{(i)} = p_t^{(i)}$.

We estimated the parameters of the model following Bayesian inference implemented by Markov chain Monte Carlo simulation. Prior information about the model parameters was obtained from the analysis of data collected during the household recapture survey. Thus an initial estimate of the total owned dog population $M^{(i)} = M_v^{(i)} + M_u^{(i)}$ in study zone *i* was taken applying the Petersen-Bailey formula for direct sampling on captured (collared) –recaptured data observed during the household survey, that is $M^{(i)} = M_v^{(i)} *(n_i + 1)/m_i + 1$ and standard error $(M^{(i)}) = [M_v^{(i)2}(n_i + 1)(n_i - m_i)]/[(m_i + 1)^2(m_i + 2)]$, where n_i and m_i are the numbers of recaptured dogs and recaptured collared (vaccinated) dogs in the household survey in zone *i*, respectively. These estimates specified the parameters of a normal prior distribution that was adopted for $M^{(i)}$.

The parameter $N^{(i)}$ was expressed as a fraction of the total owned dogs, that is $N^{(i)} = a_i*M^{(i)}$. Uniform prior distributions were assumed for a_i, $a_i \sim$ U(0, 0.2) in every zone. The parameters of the above uniform distribution were chosen by combining the Petersen-Bailey estimate of the owned dogs with a rough estimate of the ownerless dog population per zone obtained from the household questionnaire.

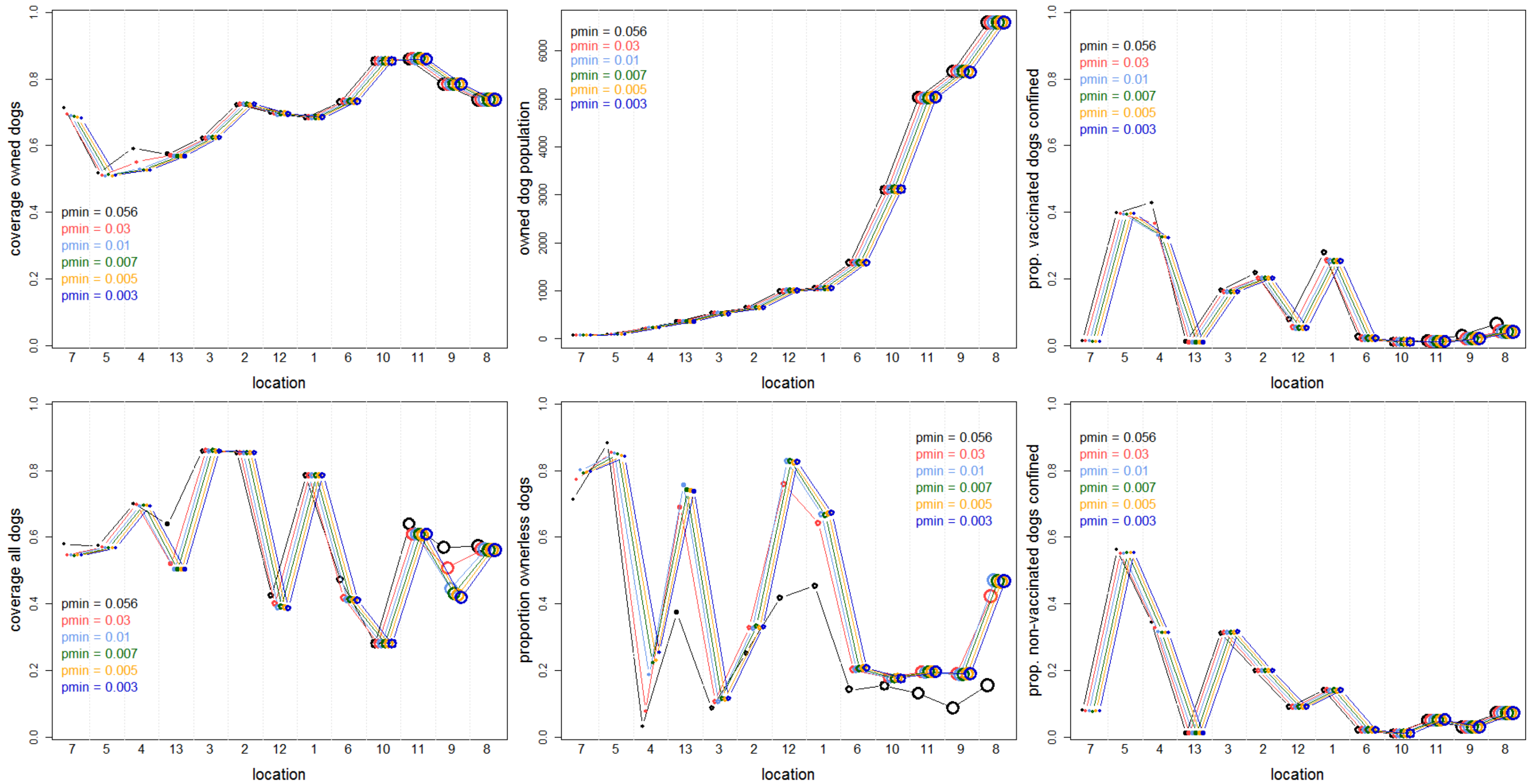
The model code used is as follows:

```
model {
 for (i in 1:zone) {
  for( t in 1 : T ) {
   x1[i,t] ~ dbin(p[i,t],n1[i])
   z[i,t] ~ dbin(p[i,t],n2[i])
   x2[i,t] ~ dbin(p[i,t],n3[i])
   pp[i,t]~dunif(pmin,pmax)
   p[i,t] <- min(pmax,max(pmin,pp[i,t]))
  }
  n1[i]<-round((1-c1[i])*Mv[i])
  n2[i]<-round((1-c2[i])*(M[i]-Mv[i])+aa[i]*M[i])
  n3[i]<-round((1-c2[i])*(M[i]-Mv[i]))
  logit(aa[i])<-a+rand[i]
  c1[i]~dbeta(a1[i],b1[i])
  c2[i]~dbeta(a2[i],b2[i])
  coverage_owned[i]<-Mv[i]/M[i]
  coverage_total[i]<-Mv[i]/(M[i]+aa[i]*M[i])
  M[i]~dnorm(Mmean[i],Mtau[i])
  Mmean[i]<-Mv[i]*(peterson_n[i]+1)/(peterson_m[i]+1)
  Mse[i]<-(pow(Mv[i],2)*(peterson_n[i]+1)*(peterson_n[i]-peterson_m[i]))/
     (pow((peterson_m[i]+1),2)*(peterson_m[i]+2))
  Mtau[i]<-1/(Mse[i])
  rand[i]~dnorm(0,tau)
  }
 tau~dgamma(1,1)
 a~dunif(0.0001,0.2)
}
```

Sensitivity analysis:

We performed a sensitivity analysis and identified the minimum recapture probability (pmin) as the most sensitive parameter which is in agreement with (Muthiani et al., 2015). Unfortunately, values for recapture probabilities are difficult to estimate in larger areas. Therefore, we used as default the values used by Kayali et al. (2003b). Of note, the recapture probabilities have only a negligible impact on the estimate of the owned dog population, vaccination coverage of the owned dog population, and confinement estimates (Figure 1.4). However, the lower boundary of the recapture probabilities has a strong impact on the proportion of ownerless dogs and consequently on the vaccination coverage of all dogs. With decreasing pmin the vaccination coverage of all dogs decreased from 71% to 63%, however, the estimated proportion of ownerless dogs was unrealistically high in several locations.

Figure 1.4: Sensitivity analysis. Change in parameter estimates associated with different values for pmin in 2013. The x-axis shows the sequence number ordered from smallest to highest estimated owned dog population number. The circle area is proportional to the estimated owned dog population. Estimates related to the owned dog population are not sensitive to changes in pmin. However, the proportion of ownerless dogs and therefore the total coverage is affected. Whereby small values of pmin result in unrealistically high estimated proportions of ownerless dogs.



CHAPTER 2

The importance of a participatory and integrated One Health approach for rabies control: the case of N'Djamena, Chad

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Abstract

This study compares data on animal rabies cases from the Chadian national veterinary laboratory with bite case reporting from health facilities. The data collection accompanied a dog mass vaccination intervention over two years in N'Djamena, Chad. This allowed for comparison of the dynamics of the incidence of animal rabies cases, human bite exposure incidence and Post-Exposure Prophylaxis (PEP) demand during a dog rabies elimination attempt. Following the mass vaccination, the monthly animal rabies incidence dropped from 1.1/10'000 dogs observed prior to the campaign in 2012 to 0.061/10'000 dogs in 2014. However, PEP demand was found to be largely unaffected. Suspicion of rabies exposure as reported by health personnel in most cases did not reflect the status of the biting animal but rather the severity of the bite wound, resulting in inappropriate PEP recommendations. In addition, reporting of dead or killed animals to the rabies laboratory was found to be very low. These results reveal a profound lack of communication between health facilities and veterinary structures and absence of an Integrated Bite Case Management (IBCM) approach. Improved communication between human health and veterinary workers is imperative to prevent human rabies deaths through appropriate use of PEP and further translate success in animal rabies control into cost savings for the public health sector through lower PEP demand. Improved training of health and veterinary personnel and sensitisation of the public are needed to achieve good IBCM practice and increase the rate of diagnostic testing, provide adequate and timely PEP and reduce wastage of scarce vaccine resources.

Keywords: Rabies incidence, Post-Exposure Prophylaxis, Integrated Bite Case Management**,** One Health

Introduction

Within a One Health framework, rabies is likely the best documented example for the added value of closer collaboration of human and veterinary medicine for the control of zoonotic diseases (Léchenne et al., 2015). With a few singular exceptions, humans only contract the disease through contact with an infected animal (Jackson, 2013a). The highest disease burden is found in resource poor settings where rabies is endemic in domestic dogs, which are the predominant species causing exposure in humans (Hampson et al., 2015). Poverty negatively impacts access to Post- Exposure Prophylaxis (PEP), which is urgently needed after a bite from a suspected rabid animal. In most rabies endemic countries in Africa the cost for PEP exceeds the monthly income of people living below poverty level (Diop et al., 2007; Hampson et al., 2008; Léchenne et al., 2015). In many remote areas, PEP is not available due to long distances to a health facility or inefficiency of the health system (Tiembré et al., 2010).

In order to achieve the challenging goal of zero human deaths from dog mediated rabies by 2030, as postulated by WHO and partners (WHO, 2015), access to and adequate use of PEP must be implemented. Since only a fraction of all bite cases are inflicted by a rabid animal (Cleaveland et al., 2002; Undurraga et al., 2017) and many countries face a shortage of human vaccine for PEP, an

Integrated Bite Case Management (IBCM) approach is required to guide treatment recommendations in order to save the highest percentage of human lives in the short term. Long term sustainable control of rabies and reduction of human fatalities can only be achieved through interventions which interrupt transmission in the reservoir species. Vaccination of dogs is the only control measure which will lead to elimination of rabies in domestic animals and result in reduction of exposure risk in humans by more than 90% (Hampson et al., 2015).

Dog vaccination reduces the need for PEP and considerably reduces the burden of rabies from premature deaths averting a high number of Years of Life Lost (YLL) (Cleaveland et al., 2003; Tenzin et al., 2012). Therefore, investment in dog vaccination, especially mass vaccination, may be more expensive than prevention in humans in the short term but is advantageous in the long-term, with higher cost-efficiency compared to the cumulative costs of PEP alone (Bogel and Meslin, 1990; Zinsstag et al., 2009; Mindekem et al., 2017). However, reduction of dog rabies incidence does not necessarily translate directly into reduced demand for PEP. Rabies control can even lead to higher PEP demand in the face of decreasing exposure risk, which can be explained by heightened rabies awareness in the community (Mitmoonpitak et al., 1998; Kumarapeli and Awerbuch-Friedlander, 2009; Touihri et al., 2011). To maximise the beneficial financial effects, dog vaccination should be carried out in conjunction with IBCM to prevent overuse of PEP. Identification of bite victims who are not exposed to a suspected animal can be achieved through closer communication of medical staff with veterinary workers who are informed on the status of the respective animal. Ultimately IBCM would also improve surveillance for validation (proof of absence of dog mediated human rabies deaths) and verification (proof of absence of dog rabies) of the 2030 goal.

The present study describes a lack of communication between the human and animal health sectors in the absence of IBCM, as shown by the comparison of laboratory and health centre data. The data collection was done during an epidemiological follow up study on the dynamics of dog rabies incidence, human dog bite incidence and PEP demand during a rabies elimination program in N'Djamena, Chad.

Methods

Background

In 2000, research on rabies control was initiated in N'Djamena, the capital city of Chad. Prior to the intervention, rabies was endemic in the local dog population, circulating at a low and stable level with an R_e of just over 1 (Zinsstag et al., 2009). Pilot vaccination campaigns in 2003 and 2006 validated the feasibility of dog vaccination in the city, showing good participation by dog owners provided the vaccine was offered without charge (Kayali et al., 2003b; Durr et al., 2008b).

Given the promising initial results, the Swiss TPH implemented a large scale mass dog vaccination intervention together with two local partners, the Institut de Recherche en Elevage pour le

Développement (IRED) and the Centre de Support en Santé International (CSSI). In May 2012, community awareness was emphasised to obtain accurate incidence data before the planned vaccination campaigns. Posters in French and Arabic illustrating the best practices after a bite incident were distributed to health centres, hospitals, pharmacies and veterinary facilities throughout N'Djamena. Drawings were used because of the high illiteracy rate in Chad. Information included the importance of washing wounds after a bite, the need to seek medical treatment and the importance of contacting a veterinarian (in the case of a live animal) or bringing the body to IRED (in the case of animal death). Fixed post parenteral vaccination campaigns took place across the entire city from October to December in 2012 and 2013. The organizational details and results of the vaccination campaigns are explained in detail elsewhere (Lechenne et al., 2016a; Mindekem et al., 2017; Zinsstag et al., 2017). Both campaigns reached consecutive vaccination coverage above 70% leading to short term elimination of rabies virus from N'Djamena (Zinsstag et al., 2017). However, PEP demand remained high even when there were no animal rabies cases (January–October 2014) (Mindekem et al., 2017). To investigate why successful rabies control in the animal vector did not translate to beneficial effects in the human health sector through lower demand for PEP, we investigated the available epidemiological follow up data at the laboratory and health facility level.

Laboratory data on suspected and confirmed animal rabies cases

IRED is the only rabies laboratory in Chad equipped to perform the standard Fluorescent Antibody Test (FAT). In addition to FAT, samples at IRED were analyzed with the Rapid Immunodiagnostic Test (RIDT) (Lechenne et al., 2016b). All positive samples were sent to the Pasteur Institute in Paris for PCR virus isolation to confirm test results. Rabies surveillance in Chad is based on passive reporting, thus animals are brought to IRED on a voluntary basis with no active contact tracing. In most cases, the animal dies or is killed before submission to IRED. When an animal is still alive, the rabies laboratory refers the owner to the nearby public veterinary clinic for observation. IRED charges the owner of the animal 5000 CFA (8 USD) for rabies diagnostic testing, and there is no charge for feral dogs. Rabies surveillance in N'Djamena continued before, during and after the vaccination campaigns. Some samples from areas outside of N'Djamena were also sent to IRED for rabies diagnosis. The present analysis included cases reported between June 2012 and the end of December 2014, to mirror the data collection period at the health facility level. Information on the animal (vaccination status, location, symptoms observed, outcome), on the bite victims (number, age, sex, bite location and severity) and the history of the bite (time, place, circumstances) were routinely collected on the diagnostic request sheet. If the test result was positive, victims were advised to initiate PEP at the Mother and Child Hospital (Hôpital Mère et Enfant) where the vaccine was available free of charge to women and children. Adult male victims were referred to the Central National Reference Hospital (Hôpital Centrale de Reference National).

Health facility data on animal bite victims

Data collection on bite cases was done as in a previous study estimating human deaths from animal bite injuries in N'Djamena (Frey et al., 2013). Health facilities for inclusion were identified using a previously generated list (Gami, 2008). However, not all facilities were still operating, and some new structures were identified during exploratory visits in the districts. In total, 91 facilities were contacted and included in the awareness campaign, representing all public health centers in N'Djamena, the most frequented private health structures and hospitals (for profit and non-profit), and major pharmacies which had capability to store rabies vaccine. The largest private veterinary facility was also included. A questionnaire, developed by Frey et al. (2013), was distributed to the facilities, and personnel were asked to complete a form for every bite case presented. The information collected included demographic information about the victim (residence, age, sex), nature of the bite wound (severity, number and location), circumstances of the bite incident (place, provoked/unprovoked, other victims) and the background of the animal (owner status, vaccination status, location, outcome) and contacts with other human health or veterinary structures (referrals from or to other facilities including IRED). The source of information related to the biting animal was sometimes the dog owner but could also be the victim or his representative (especially in the case of feral dogs). Decisions on actions to be taken regarding the animal were primarily made by the owner but could also be the victim in cases of un-owned dogs. The questionnaires were collected biweekly by study personnel, with an incentive of 300 FCFA (0.5 EUR) per completed questionnaire provided to the participating health structure. Health facilities were informed about the study in May 2012, and questionnaires were collected from June 2012 until the end of December 2014. Sixty one facilities responded with at least 1 questionnaire (mean 19.7, median 4, range 1-143) (Table 2.1). This number represents about 30% of the health facilities identified in Gami (2008).

Table 2.1: Number of responding health facilities and questionnaires collected.

Facility Type	Health Facilities		Questionnaires		Quest/Facility
	Count	Percent	Count	Percent	
Pharmacy	33	54%	729	61%	22
Veterinary practice	1	2%	69	6%	69
Hospital (public)	6	10%	210	18%	35
Medical practice	6	10%	33	3%	6
Health center (public)	15	25%	154	13%	10
Missing information	N/A	N/A	4	0%	N/A
Total	**61**	**100%**	**1199**	**100%**	N/A

PEP use and cost

The WHO recommends including rabies immunoglobulin (RIG) in the PEP protocol for cases with category III exposure (transdermal injuries or contact of saliva with mucosa) (WHO, 2013). However, RIG is not available in Chad and therefore PEP only includes active vaccination with cell culture vaccine (CCV) given according to the intramuscular five dose ESSEN scheme (Warrell and Warrell, 2015). The price of one dose of human rabies vaccine ranges between 9’000-12’000 FCFA in N’Djamena, such that a full course of PEP costs 45’000-60’000 CFA (80-100 USD). Additional costs for wound treatment (antiseptic, antibiotics, tetanus vaccine), and private costs of lost work time and transportation to a health facility are estimated to be over 90’000 FCFA (160 USD) (Mindekem et al., 2017). Our study did not include any follow up of bite victims, so data on completion rate and outcome for rabies exposed people is not available.

Data analysis

Questionnaire data was double entered and compared using Epi Info™, then transferred to an Access (Microsoft™) database. Data collected at the rabies laboratory were entered continuously into an Excel spread sheet. For both data sets the analysis was done with Stata/IC 14. Dog rabies incidence was calculated on the basis of the number of positive dog rabies cases observed in N’Djamena over the study period and the dog population estimates for the city obtained during the two vaccination campaigns, ranging from 24’547 in 2012 to 30’074 in 2013 (Lechenne et al., 2016a).The human population census of 2009 served as the basis for the calculation of the bite exposure and PEP incidence (INSEED, 2009). Only bite cases reported from N’Djamena (1143) were included for this calculation. To statistically evaluate differences in incidences (dog rabies cases, dog bites, PEP use) before and after the mass vaccination intervention, paired T-tests were performed to compare the respective monthly incidences observed from June-December 2012 to the monthly incidences of the period of June-December 2013.

For analysis of the health facility data, the vaccination status of biting animals was categorised as “Vaccinated” (date of vaccination reported and less than one year before reported bite incident), “Vaccination unconfirmed” (missing vaccination date or more than one year before bite incident) and “unvaccinated” (vaccination status reported as unvaccinated or unknown). In addition each bite case was attributed a rabies exposure risk variable based on the outcome of the animal as drawn from the information in the questionnaire: “High exposure risk” was attributed to animals reported to have disappeared or been killed after the bite attack; “Moderate exposure risk” was attributed to animals with negative, unclear or out-dated vaccination status which were also put under observation. Vaccinated animal which bit more than two people were defined as moderate exposure risk because data from the laboratory level showed that the number of victims by case is related to a positive test result. In cases of no known owner which were also under observation, exposure was considered to be moderate. “No exposure risk” was attributed when the animal had a confirmed vaccination status, had not bitten more than two people and was placed under observation. The rabies exposure risk categories, derived from the animal status as described above, was compared to the rabies suspicion as noted by the health personnel in the questionnaire (yes, no, do not know). To evaluate parameters influencing PEP recommendation, the respective explanatory variables were coded into categories: severity of bite was coded as WHO category II exposure/WHO category III exposure (WHO category I was not observed in this data); age was coded as adults (>15years)/children (<=15years); number of bites was coded as single bite/multiples bites; number of victims was coded as single victim/multiples victims. Statistical comparison of PEP recommendations per respective categories was done by odds ratio calculation. Regarding the risk of a dog being killed after having inflicted a bite, the ten districts of N'Djamena were assigned an observed predominant cultural background (Christian/Muslim) and differences between the two categories were evaluated by a risk ratio calculation.

Results

Rabies diagnostic results and respective case histories

The awareness campaign before the mass vaccination led to a rapid and considerable increase in number of rabies suspicious animals reported to IRED, from a monthly mean of 1.2 diagnostic requests observed from January to May 2012 (prior to the study period) to 3.6 observed from June to December 2012 (during the study period). Throughout the study period (June 2012 – December 2014) a total of 60 rabies suspect animals were sent to IRED of which 46 originated from N'Djamena, 9 from other areas in Chad (mostly located very close to N'Djamena), 2 from Cameroon (which borders N'Djamena). In 3 cases, the sample origin was unknown. Overall, 32 samples tested positive, 25 were negative and 3 were not testable because of poor sample quality. Table 2.2 shows the distribution of test results by species. In total, 30 (67%) of the submitted dog samples and 2 (33%) of the cat samples were positive. No positive cases were observed in other species.

Table 2.2: Summary of results of samples received for rabies diagnosis at IRED, by species

Species	Negative	Positive	No Result	Total
Dog	13	30	2	45
Cat	3	2	1	6
Monkey	6	0	0	6
Sheep	2	0	0	2
Shrew	1	0	0	1
Total	**25**	**32**	**3**	**60**

Amongst all animals sent to IRED, only 10% (five dogs and one cat) were initially put under observation prior to death and subsequent submission to IRED. In two of those six cases, the animals tested positive for rabies. In most cases, the animal was killed immediately after a bite rather than put under observation (43 cases, 72%). The percentage of positive cases among killed animals was 67% (29 out of 43). The percentage of confirmed rabid among animals that were found dead or died during the observation period was 22% (2 out of 9). In 8 cases, the circumstances of death were not specified.

The majority of animals brought to IRED were owned (72%) but only 2 out of 32 (6%) owned dogs had a valid vaccination (vaccination < 1 year) which was confirmed by a certificate. In one of these two cases, the dog nonetheless tested positive. In six cases, the vaccination status was unconfirmed or out of date and five of these dogs tested positive. Animals other than dogs were all unvaccinated. The most commonly observed symptoms were aggression (88% of cases) and sudden change of behaviour (40% of cases).

On average two (min. 1; max. 6) human bite victims were observed per rabid animal. The proportion of children among bite victims of confirmed rabies cases was 42% (25 of 59). For rabies negative cases, the proportion of children among all victims was only 27% (3 out of 11).

Reported bite cases and related animal history at health facility level

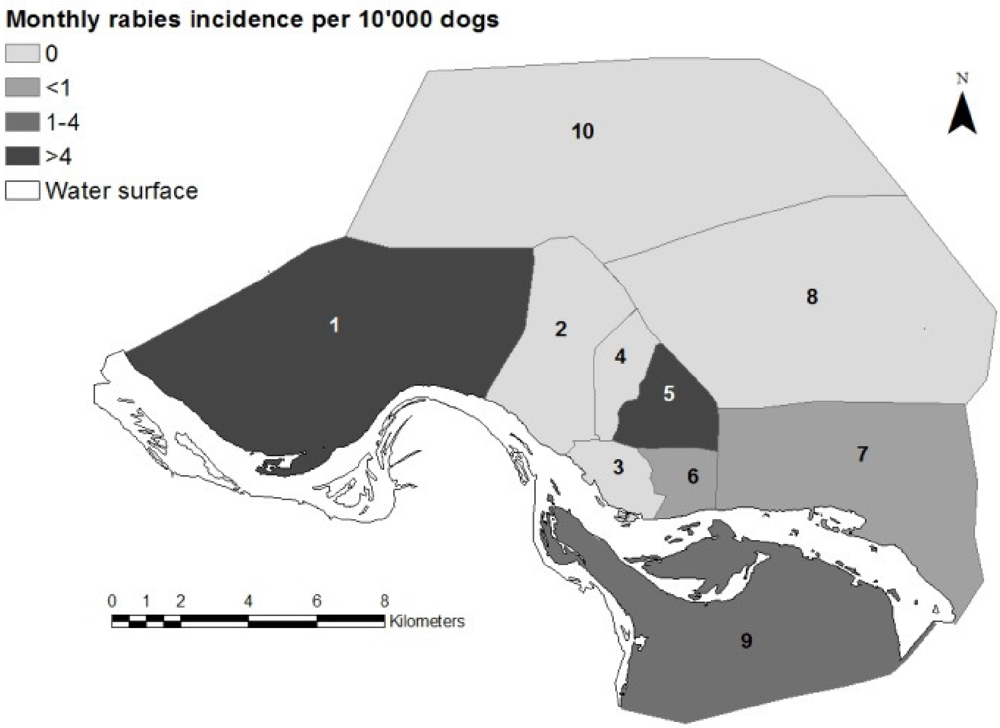
In total, 1203 questionnaires were collected from health facilities during the survey. Three questionnaires were excluded from the data set because the biting animal was a snake and one questionnaire was not completed. The vast majority of the remaining 1199 bite cases were inflicted by dogs (936 cases, 78%), followed by cats (58 cases, 5%) and monkeys (15 cases, 1.5%). For the remaining 16% (190 cases) information on the animal was missing. A high number of reported victims were children ≤13 years of age (42%).

More than 58% of bite exposures took place at the victim's home, while an additional 36% occurred very close to the place of residence. Table 2.3 shows the distribution of reported bite cases and health facilities by district and inhabitants based on the population census of 2009. District 10 is very sparsely represented with only one health facility participating and only 11 bite cases reported from the entire district (Table 2.3).

Table 2.3: Number of questionnaires and participating health facilities per district compared to the population census of 2009.

District Number	Questionnaires (Q)		Health Facilities (HS)		Population (P) 2009		HS/1000P	Q/1000P
	Count	Percent	Count	Percent	Count	Percent		
1	54	5%	8	13%	72,742	8%	0.11	0.73
2	9	1%	3	5%	36,450	4%	0.08	0.25
3	22	2%	6	10%	38,101	4%	0.16	0.58
4	14	1%	3	5%	72,954	8%	0.04	0.19
5	39	3%	3	5%	102,169	11%	0.03	0.38
6	122	11%	6	10%	43,948	5%	0.14	2.64
7	700	61%	17	28%	221,811	23%	0.08	3.08
8	106	9%	12	20%	185,065	20%	0.06	0.53
9	66	6%	2	3%	75,893	8%	0.03	0.86
10	11	1%	1	2%	98,982	10%	0.01	0.10
Total (N'Djaména)	**1143**	**100%**	**61**	**100%**	**948,115**	**100%**	**0.06**	**1.17**

There is low representation of health facilities in this district because of its remote location on the periphery of town. Also a very low dog population density was reported from this district during the vaccination campaigns (Lechenne et al., 2016a). In contrast District 6 and 7 had the highest rate of questionnaires per inhabitants. This is in accordance with the high density of health facilities and very high dog to human ratio found in these two districts (Figure 2.1). District 9 has a similar dog density to district 7, however like District 10 it is an area at the periphery of town were health facilities are not numerous. Figure 2.1 also shows the monthly incidences of dog rabies and bite exposures by district reported before and during the first vaccination round in 2012 (June–December).



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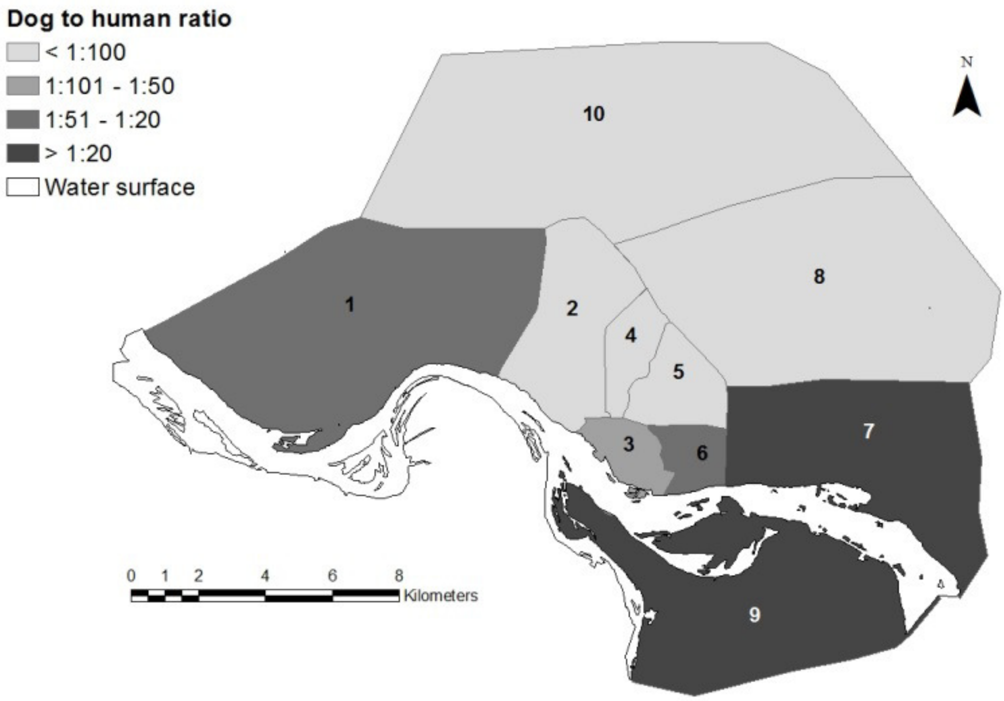

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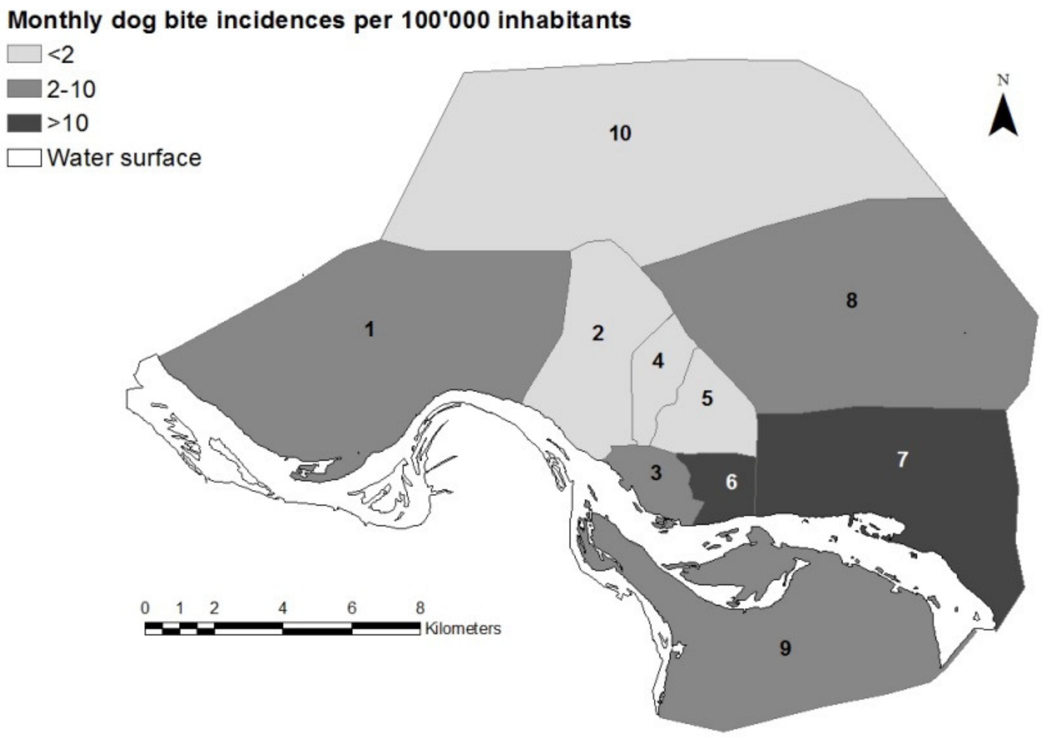

c

Figure 2.1: Maps of N'Djamena depicting monthly dog rabies incidences (a), dog to human ratios (b) and monthly dog bite incidences observed from June to December 2012 (c). Dog population estimated are based on the results of the vaccination coverage analysis in 2012 published in Lechenne et al. (2016a). The human population by district is derived from the population census of 2009 (INSEED). Numbers on the maps indicate the district number.

In over 70% of the bite cases, the animal was reported as put under observation, but in less than 1 in 4 of these cases (23.5%) was it noted that the animal was taken to a veterinary facility. A total of 144 cases (15%) stated that the animal was brought to the rabies laboratory at IRED. However, this number does not correspond to the actual number of animals submitted to the rabies laboratory within the same time period, see above. Moreover in only 2 out of 72 cases where the animal was killed and 1 out of 4 cases where the animal died, was it reported that the carcass was sent to the rabies laboratory. Six dogs were killed despite a reported confirmed vaccination status. For bite cases reported from districts with a predominantly Muslim background the animal was ten times more likely to be killed compared to bite cases occurring in districts with a predominantly Christian background (RR 10.5, 95% CI 6.37 – 17.36, $P < 0.0001$). The mean number of victims per biting animal observed during the health facility survey was 1.5 (max 13). In the majority of cases (82%) only one victim was reported.

The highest number of bite victims was reported from pharmacies. Compared to the total of involved structures per type, hospitals had the highest mean number of reported bite cases followed by pharmacies and health centres (Table 2.1).

In total 161 victims were referred to another facility (Figure 2.2), and pharmacies were the facilities referring the highest number of people to another facility. In 64% of referrals, patients were sent to another human health facility, the majority to hospitals (54%), probably because of shortage of vaccine or for further wound treatment. In only 36% of referrals were the victims sent to a veterinary facility (28.5% to veterinarians, 7.5% to IRED). Hospitals and health centres were the facilities that referred the highest number of cases to veterinary facilities or the rabies lab (83% of overall referred cases). The single veterinary facility that participated referred 74% of bite victims to a hospital or health centre, assumedly because a veterinary facility would not provide human vaccine.

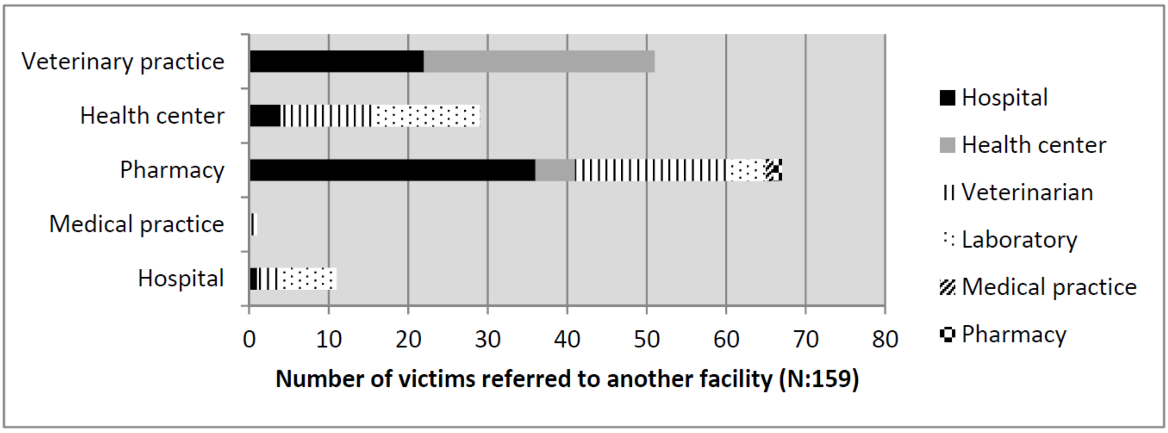

Figure 2.2: Cases of referral of victims to another health facility

PEP treatment decisions on health facility level

PEP treatment recommendation was not always consistent with the rabies suspicion status reported by the health personnel (Figure 2.3). In 27% of bite cases it was noted that the animal was not suspected for rabies but PEP was recommended. In contrast, 56 patients (0.05%) were not recommended to undergo PEP although the animal was reported as a rabies suspect (Figure 2.3).

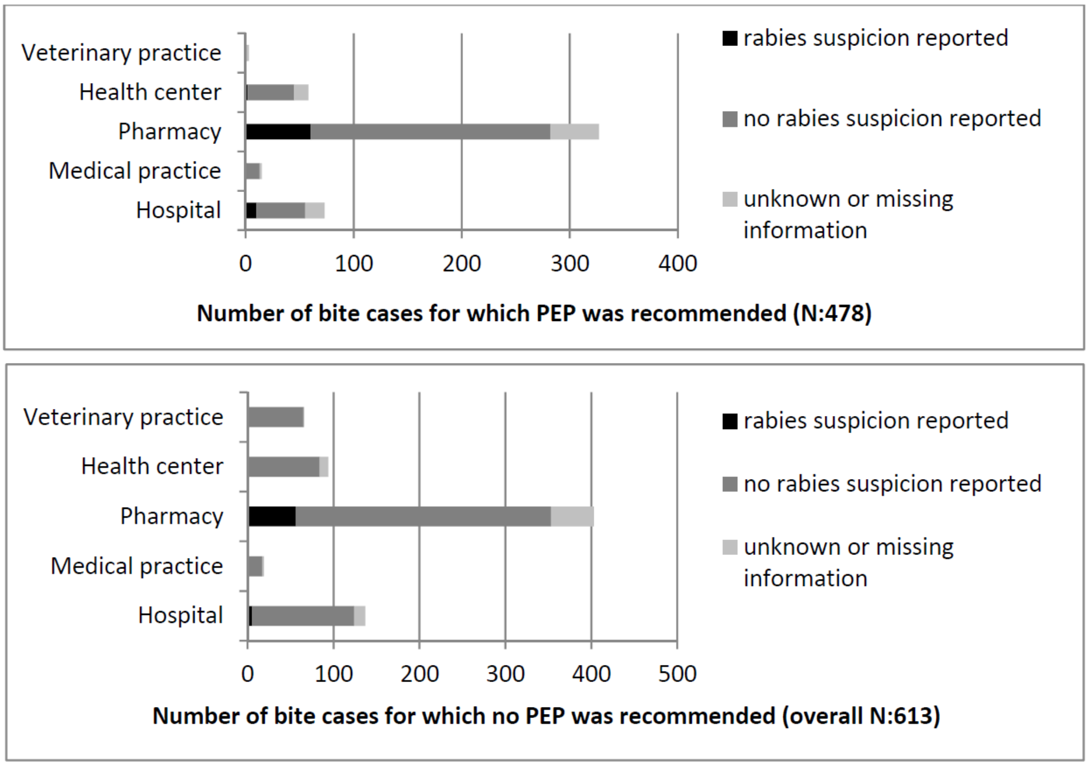

Figure 2.3: Comparison of PEP recommendation and reported rabies suspicion by facility type.

When comparing the rabies exposure risk status defined during analysis (as based on the information available about the biting animal) with the suspicion status reported by the health personnel, we found 40 cases in which no suspicion was reported but a high exposure risk was assessed based on the case history. Similarly when exposure risk was absent as assessed by the case history, 54 cases were nonetheless identified by the health personnel as suspected for exposure to a rabid animal.

The comparison of rabies exposure risk status with PEP recommendation illustrated even higher discrepancies. Of all bite cases, including those reported from outside N'Djamena, with no indication of suspected rabies exposure, 36% (208 out of 577) were recommended to undergo PEP treatment. In 38% (189 out of 487) of the cases where PEP was recommended, the exposure risk was moderate. Finally, in only 17% (81 out of 487) of the cases where PEP was recommended did the history of the animal indicate a high exposure risk. Most alarmingly, in 62% (312 out of 501) of all cases judged to be of moderate exposure risk and in 33% (40 out of 121) of those judged as high exposure risk, the bite victims were not advised to undergo PEP treatment. Detailed results on PEP recommendations by facility type and comparison to the rabies exposure risk are presented in Figure 2.4.

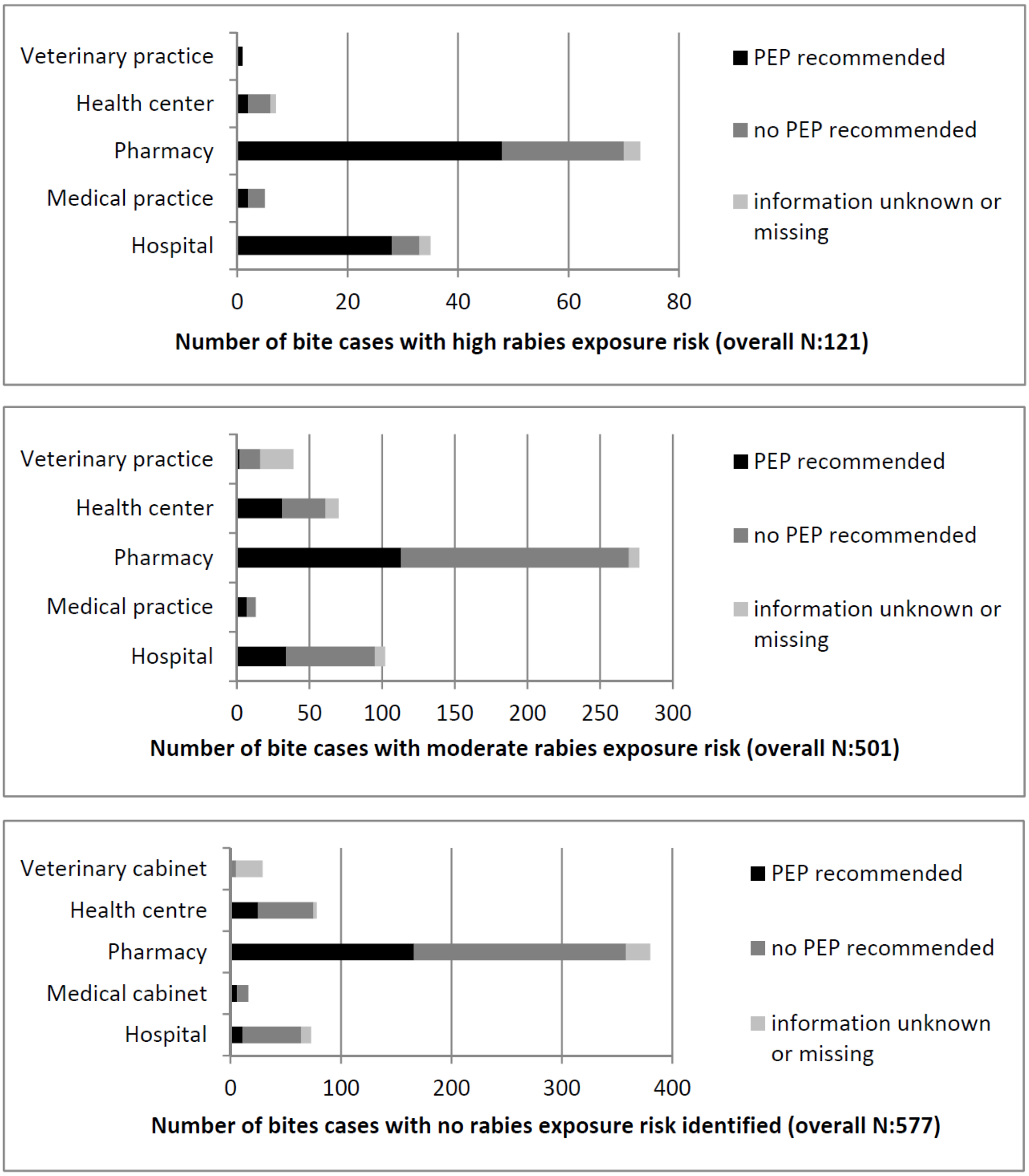

Figure 2.4: Comparison of PEP recommendations made by facility type and rabies exposure risk category (as attributed to bite cases based on animal status).

The inconsistencies in PEP recommendations can be explained by the fact that health personnel were more likely to judge the rabies suspicion according to the severity of the bite inflicted than on the vaccination status of the animal. When the wound was deep or the skin clearly broken, one in five cases was declared suspicious and in 50% of these cases, PEP was recommended. In contrast, when the wound was reported as superficial or only a minor scratch, less than one in twenty were reported suspicious and 28% of these patients were recommended to undergo PEP. PEP was found to be more likely recommended for WHO category III than for category II exposures (OR 0.36, 95% CI 0.28-0.46, P <0.0001). No difference was observed between PEP recommendation for children or adults (OR 1.0065, 95% CI 0.79 – 1.28, P = 0.96) or the number of bites inflicted (single of multiple) (OR 1.17, 95% CI 0.9 – 1.52, P = 0.22). There was also no relation observed between the number of victims reported per animal (single or multiple) and the rabies suspicion status defined by the health personnel (OR 0.81, 95% CI 0.51 – 1.3, P = 0.4).

Impact of mass dog vaccination on dog rabies incidence, animal bite incidence and PEP demand

After the beginning of the vaccination intervention in October 2012, rabies reports from N'Djamena as observed at IRED dropped steadily (Figure 2.5). Following the mass vaccination the monthly animal rabies incidence dropped from 1.1/10'000 dogs observed prior to the campaign in 2012 to 0.12/10'000 dogs in 2013 and only 0.061/10'000 dogs in 2014. This translates as a reduction from 1 rabid dog per week in 2012 to only 2 rabid dogs throughout the whole year in 2014. During the same period of time reporting of rabies cases from areas outside of N'Djamena was steady but remained low (only 11 cases over the study period), mainly due to lower public awareness and logistical challenges. Before the vaccination intervention most rabies cases were reported from the 7th District (Figure 2.1a). After the mass vaccination, rabies cases were absent from districts north of the Chari River for well over a year (February 2013 to October 2014) with the only cases observed during this period coming from District 9 which lies south of the Chari River.

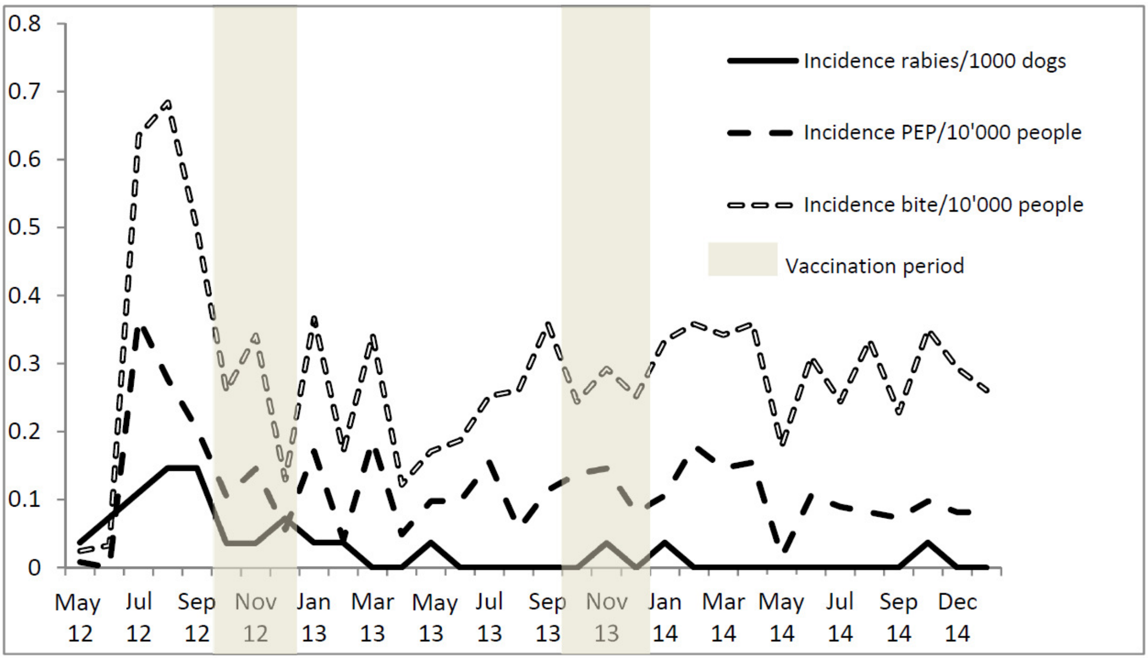

Figure 2.5: Dynamics of monthly animal rabies, human bite exposure and PEP demand incidence rates following dog mass vaccination intervention in N'Djamena

Figure 2.5 shows that incidence of PEP treatment did not decline with decline of animal rabies incidence but was instead closely linked to the overall bite incidence rates observed from the health facility data over the study period. For dog rabies incidence, the paired T-test showed a significant difference between the two periods from June to December in 2012 and 2013 (P=0.0063; t=3.8). Difference in monthly mean PEP incidences over the same period of time was not significant (P = 0.1, t=1.4), nor was the difference observed between monthly mean dog bite incidences (P=0.1, t=1.5).
The proportion of confirmed vaccinated animals among all bite inflicting animals observed on the health facility level only increased by 15%, from 47% observed prior to the start of the vaccination campaign to 62% by end of 2014. Percentage of unvaccinated animals decreased from 23% to 11% and cases with unconfirmed, out of date or unknown vaccination status remained stable at around 30%.

Discussion

The multiple studies undertaken in N'Djamena since 2000 serve as a good example for investigation on feasibility and description of barriers and shortcomings for rabies control in sub-Saharan Africa. The most recent work that included citywide dog mass vaccination and follow-up of the epidemiological impact has demonstrated interruption of rabies transmission (Zinsstag et al., 2017), but highlights the need for better communication between the veterinary and human health sectors to translate success in dog vaccination to beneficial economic effects in public health (Mindekem et al., 2017). In the absence of dog vaccination, the current PEP use in N'Djamena is inadequate to prevent all dog mediated human deaths (Mindekem et al., 2017). In this paper we describe underlying problems which contribute and which should be hypothetically similar in other countries. These factors constitute barriers to the goal of achieving zero human deaths due to rabies and should be addressed early towards the 2030 agenda. The challenges crystallize around implementation of an integrated, community-based One Health approach for efficient rabies surveillance and control. This can only be achieved through high public participation in control measures (vaccination, surveillance), compulsory dog registration and IBCM training for health and veterinary personnel to ensure best practice use of PEP.

Our data illustrate the evident fact that elimination of dog mediated rabies will not lead to considerably lower bite case incidences, as only a very small proportion of bites are caused by rabid animals. For example, in the present study the number of bite victims of truly rabid animals recorded at the laboratory constitute only 5% of overall observed bites in the health facility survey during the same period of time. Therefore it would be an unnecessary burden on public health to recommend PEP for every bite case. Such extensive use of PEP would even be counterproductive given the current shortage of vaccine, as it would lead to insufficient PEP for actual rabies exposed victims.

Our reported numbers for bite incidence are conservative. Not all victims present to a health facility as many use traditional forms of treatment (Mbilo et al., 2016). In addition, this study only covered about one third of the facilities in N'Djamena. Therefore actual numbers of bite exposures are believed to be considerably higher. In general, there are fewer health facilities per inhabitant observed in districts at the periphery of town compared to central districts, which could lead to underrepresentation of cases from peripheral districts. The socio-economic background also differs between the central (wealthier areas) and peripheral (underprivileged areas) districts, poorer communities might be less represented in this study. However, N'Djamena is a relatively small town (292km^2) with adequate road access and public transportation, so geographical distance to a respective health facility should have a minor influence on health seeking and PEP accessibility. Most dogs in N'Djamena are owned, but free roaming, which results in high contact between humans and dogs. Nonetheless, notable differences of bite exposure incidences were observed between different districts. N'Djamena reflects the diverse socio-cultural and socio-economic context of Chad. This background has a significant influence on dog ownership (Lechenne et al., 2016a). Districts with a predominantly Muslim background (2nd, 4th and 10th district) have a much lower dog to human ratio than areas with a Christian context (1st, 7th and 9th district). Similarly dog density is extremely low in the wealthy neighborhoods found in the 2nd district as compared to very high densities found in slum areas at the periphery of town (9th district). Due to this diverse context, dog rabies cases and dog bite exposures are likewise heterogeneously distributed. As dog population estimates are derived from extensive surveys during the mass vaccination campaigns and because the number of health facilities per district does not depend on the district cultural background, we are confident that these differences are not a result of underreporting in predominantly Muslim areas.

The study did not include a follow up of bite patients, and therefore we do not know if victims completed all PEP doses required. Also we do not know how many cases of human rabies occurred during the study period in N'Djamena, because the disease is not notifiable in Chad. In 2008/2009 the annual number of human rabies cases in N'Djamena was estimated to be seven and an extrapolation of the mean number of victims per rabid dog registered revealed a huge proportion of people possibly exposed who did not seek PEP (Frey et al., 2013).

Another study limitation is the lack of follow-up on observation of biting animals, since collection of such data on the veterinary level was not included. Regional governmental veterinary institutions in Chad do maintain registries on animal observations, but the results are not regularly reported to higher national levels. In our study we observed that, although after most exposures the biting animal was reported to be put under observation, a veterinarian was rarely contacted. This indicates that the owner judged his animal to be alive and well, but no action was taken to check the animal for signs of rabies by a veterinarian. No legislation on rabies control exists in Chad other than a short paragraph from 1961 which recommends culling stray dogs. Therefore the 10 day observation period for an animal which has bitten a person cannot be enforced by law. In our study, animals that died were less likely to be tested positive for rabies at the laboratory level. This has important implications for animal welfare, for instance, dogs accustomed to roaming free were reported by owners to have died of strangulation while kept on a leash.

The high number of rabies positive cases among animals with unconfirmed or out of date vaccination status highlights the importance of confirming owner reported vaccination status by certificate. This issue is also highlighted when comparing the proportion of biting animals reported as vaccinated by the health facility survey prior to the mass vaccination campaign, which was 47%, with data from a household survey conducted in 2001 where the proportion of vaccination dogs was only 19% (Mindekem et al., 2005). As the negative financial implications (cost of PEP for all bite victims) and social consequences (responsibility for a human death) for owners of a rabid dog are unbearable, some might not accurately represent the vaccination status of their animal. On the other hand the small increase in proportion of confirmed vaccinated animals observed over all reported bite cases during the dog vaccination intervention is concerning and could be related to the high likelihood of losing paper vaccination certificates (Léchenne et al. 2016a). Therefore, to establish an effective IBCM, a unique identification and registration system for all dogs is necessary.

The positive result found in a dog that was certified as vaccinated highlights the need for an observation period even for vaccinated animals. Vaccine failure due to inappropriate storage conditions or inadequate immune response of the animal can never be excluded. Rabies antibody detection and titration is not yet possible in Chad. Our findings at the laboratory level indicate that the symptoms of rabies and abnormal behavior of animals are generally well interpreted. When there was a suspicion of rabies, especially for aggressive dogs, the animal was killed quickly. However, the focus on aggression could mean that the paralytic form of rabies remains undetected in most cases. IRED is regularly contacted by bite victims seeking PEP treatment who have already killed and disposed of the biting animal. The extremely low number of animals brought to IRED after being killed or found dead shows that the importance of testing is not adequately perceived. One explanation could be the cost of the diagnostic fee, 5000 FCFA (around 10 USD), which is borne by the dog owner. Interaction with victims at IRED indicates that they usually have little doubt about the symptoms observed in the biting animal and whether they have been truly exposed. This could explain a perception that additional laboratory testing is not necessary.

In addition to cases where animals which died or were killed were not submitted for testing, there were several cases where the animal disappeared. This could indicate that the actual number of rabies cases was significantly higher than reported in this study. The observed difference in killing rates between different cultural backgrounds could indicate an underrepresentation of rabies cases from specific areas of town. However, even when simulating different case detection rates using a rabies transmission meta-population model established for N'Djamena, the outcome of the model, which suggested interruption of rabies transmission, remained robust (Laager et al., 2017).

Despite the observed drop in rabies incidence and the absence of animal rabies cases for nine months following the mass vaccination intervention in N'Djamena, PEP demand remained largely unaffected by this epidemiological change. PEP demand is clearly correlated to the overall number of bite exposures observed. The assessment of rabies risk by health personnel was found to be based on the severity of the bite inflicted. However, scratches are listed as WHO category II exposures and would therefore also warrant PEP treatment (WHO, 2013). The influence of the animal status on rabies risk rating was secondary. Moreover, very few patients were referred by health facilities to a veterinarian for animal observation/advice. All these findings clearly indicate that exchange between health personnel and veterinary workers remains inadequate for IBCM and should be improved using inter-disciplinary training and communication platforms.

We only included one veterinary facility and this data is insufficient to compare the performance of veterinary facilities with human health facilities. However the 69 cases of animal bite victims who sought help at the private veterinary clinic likely indicate an understanding amongst the public for the link between human and animal health which provides a basis for scaling up of knowledge.

Similar to other studies, we report a significant number of children among bite victims (Hampson et al., 2008; Tenzin et al., 2011a; Ramos et al., 2015). We found a high number of bite incidents occurred at home. School based education and sensitisation of dog owners regarding rabies and prevention of dog bites should lead to a decline in bite cases. Raising awareness and knowledge about rabies risk in communities is an important element to prevent excessive demand of PEP (Cleaveland et al., 2006; Gautret et al., 2011a) and unnecessary killing of suspected dogs (Sambo et al., 2014; Kabeta et al., 2015) resulting from fear of rabies. A third pillar to establish effective IBCM, along with dog registration and interdisciplinary training, includes community engagement and culturally sensitive education.

Ethical consideration

The dog rabies mass vaccination intervention was approved and co-funded by the government of Chad. The data on rabid animals and human bite exposure was collected on a routine basis by the rabies laboratory at IRED. This study was approved by the ministry for higher education in Chad (Letter N°012/PR/PM/MES/SG/DGESRSFP/DRST/012; Date 31.05.2012). Meetings were held with the mayor of N'Djamena and the district and quarter chiefs in each administrative area who granted permission prior to beginning the study.

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CHAPTER 3

Validation of a rapid rabies diagnostic tool for field surveillance in developing countries

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Abstract

Background: One root cause of the neglect of rabies is the lack of adequate diagnostic tests in the context of low income countries. A rapid, performance friendly and low cost method to detect rabies virus (RABV) in brain samples will contribute positively to surveillance and consequently to accurate data reporting, which is presently missing in the majority of rabies endemic countries.

Methodology/principal findings: We evaluated a Rapid Immunodiagnostic Test (RIDT) in comparison with the standard Fluorescent Antibody Test (FAT) and confirmed the detection of the viral RNA by real time reverse transcription polymerase chain reaction (RT-qPCR). Our analysis is a multicentre approach to validate the performance of the RIDT in both a field laboratory (N'Djamena, Chad) and an international reference laboratory (Institut Pasteur, Paris, France). In the field laboratory, 48 samples from dogs were tested and in the reference laboratory setting, a total of 73 samples was tested, representing a wide diversity of RABV in terms of animal species tested (13 different species), geographical origin of isolates with special emphasis on Africa, and different phylogenetic clades. Under reference laboratory conditions, specificity was 93.3% and sensitivity was 95.3% compared to the gold standard FAT test. Under field laboratory conditions, the RIDT yielded a higher reliability than the FAT test particularly on fresh and decomposed samples. Viral RNA was later extracted directly from the test filter paper and further used successfully for sequencing and genotyping.

Conclusion/significance: The RIDT shows excellent performance qualities both in regard to user friendliness and reliability of the result. In addition, the test cassettes can be used as a vehicle to ship viral RNA to reference laboratories for further laboratory confirmation of the diagnosis and for epidemiological investigations using nucleotide sequencing. The potential for satisfactory use in remote locations is therefore very high to improve the global knowledge of rabies epidemiology. However, we suggest some changes to the protocol, as well as careful further validation, before promotion and wider use.

Author's summary

The high fatality and burden of rabies stands in contrast to the very low performance of laboratory-based surveillance in resource-challenged countries. The absence of reliable human and animal rabies incidence data ultimately result in neglect of disease prevention and control and the perpetuation of RABV transmission despite the existence of powerful management tools. Rapid, easy to perform rabies diagnostic tests that do not require expensive equipment or special storage conditions, which can be reliably performed by trained ordinary veterinary professionals, are needed urgently for use in low income countries. Such novel methods will help to accurately assess the global rabies burden and are necessary to monitor rabies control and elimination. The present study evaluates the performance and reliability of a rapid, easy to use rabies diagnostic tool. Overall, the validated test was in high accordance with the standard reference method for the detection of RABV by immunofluorescence

microscopy and showed even higher reliability when applied in resource poor laboratory conditions. The obtained results support the high potential for the use of this test in the field but suggest a change of the original technical protocol and a need for wider validation.

Introduction

Rabies is a viral zoonotic encephalomyelitis transmitted to humans after exposure to infected mammals, mainly dogs, through bites, scratches or licks on damaged skin or mucous membranes. This disease still continues to represent a public health concern worldwide, with an estimate of 59,000 human deaths per year, mainly in low income countries (Hampson et al., 2015). Because of limited control measures in many countries and a lack of governmental concern, rabies remains a neglected tropical disease. This neglect is especially deplorable given the entirely preventable nature of the disease through vaccination of dogs and timely adherence to Post-Exposure Prophylaxis (PEP) of exposed victims (Jackson, 2013a; Fooks et al., 2014). In this way, human deaths due to this zoonotic disease could be reduced by over 95% (Knobel et al., 2005; Hampson et al., 2015).

Lack of surveillance represents one major element of negligence, leading to missing data on disease incidence, imprecise estimates of the economic impact and a general underestimation of the true worldwide burden of rabies (Nel, 2013; WHO, 2013; Anderson and Shwiff, 2015; Hampson et al., 2015). This means that advocacy for rabies control cannot be supported with solid evidence and the necessity for action is not perceived at the decision maker level, for instance governmental authorities (Lembo et al., 2011). Poor surveillance is primarily a result of lack of political commitment and resource attribution for the control of rabies, and thus a cycle of neglect perpetuates. The vicious cycle is reinforced by the disregard of disease control in domestic dogs, which constitute negligible economic value and the fact that rabies affects largely marginalized communities with difficult access to healthcare. Deficiencies in basic healthcare do not only contribute to hinder access to PEP but also lead to the misdiagnosis of rabies in the face of other causes of encephalitis, such as cerebral malaria, as reported in Malawi (Mallewa et al., 2007). Surveillance is fundamental to accurate burden of disease measures, for advocacy of disease control and also a prerequisite for disease elimination (Klepac et al., 2013; Townsend et al., 2013b). Currently, rabies is believed to be underreported at the extent of 1:60 in humans and this rate could even be much higher for animal rabies incidence (WHO, 2013).

One possible point of leverage to break the cycle of underreporting and neglect is the reinforcement and simplification of diagnostic capacities and tools. Infrastructures required for the current standard diagnostic tests are expensive, and their methodologies and interpretation need thoroughly experienced personnel. Antigen detection of RABV using the direct Fluorescent Antibody Test (FAT) is the World Health Organization (WHO) and World Organisation for Animal Health (OIE) reference test (Bourhy et al., 1989; WHO, 2013, Dean et al. 1996) and is routinely performed in many developed countries. However, it is difficult to establish in developing countries because fluorescence microscopes are expensive and the required maintenance is demanding. Also, the immunofluorescence conjugate necessary for the test is costly and has to be transported and stored refrigerated. Finally, accurate reading of the test needs stringent quality control of the test performance and very experienced personnel. Similar constraints are encountered with the Direct Rapid Immunohistochemical Test (DRIT) (Dürr et al., 2008a; Dyer et al., 2013). Although the DRIT can be read using a light microscope, the test methodology requires a meticulous protocol which currently lacks commercialized biotinylated anti-rabies antibodies and has to be carried out by trained personnel. For rabies diagnosis however, a simpler field test is desirable for various reasons. In the current situation the benefits of rabies diagnosis are not well perceived by the public and rabies suspicious animals are often killed immediately and rapidly disposed (Sambo et al., 2014). Also, a short time lag between suspicion and confirmation of a rabies case is important for early adherence to PEP or the cost savings in case of a negative diagnostic result. Finally, transport of samples over long distances in climatically warm settings increases the risk of poor sample quality, which adversely affects FAT test results (McElhinney et al., 2014). Proximity to the public through decentralized laboratory facilities is therefore vital for good sample quality, as well as rapid detection and response.

A rapid immunodiagnostic test (RIDT) based on the lateral flow principle was first described and evaluated in 2007 on a limited panel of RABV samples (Kang et al., 2007). The same study reported on the detection limit and potential risk of cross reactivity. Further laboratory evaluation was conducted more recently on the use of this RIDT for the detection of RABV circulating in Europe, and extended to the detection of other species of lyssaviruses (Markotter et al., 2009; Servat et al., 2012). Both studies showed positive results regarding sensitivity and specificity of such tests compared to the FAT. To date, only two studies have been conducted under field conditions, both suggesting positive results for the use of RIDT (Voehl and Saturday, 2014; Reta et al., 2013).

In our study, we evaluated the practicability and the performance of this RDIT identified as Anigen Rapid Rabies Test (Anigen test) (Bionote, 2010) in different settings: under field conditions with its application to the surveillance of rabid animals in N'Djamena, Chad and in laboratory settings with a panel of selected RABV isolates. Lastly, we evaluated this tool for a novel application in rabies surveillance, with its use as a vehicle for viral RNA storage and conservation, and demonstrated that recovery and detection of RNA present on the strip of positive samples was possible. The Anigen test appears as a promising tool for the *post-mortem* diagnosis of animal rabies, and the molecular detection and genotyping of positive test strips.

Materials and Methods

Implementation of the Anigen Test in the field

During June 2012, the RIDT Anigen test, a chromatographic immunoassay-based on lateral flow technology manufactured by BioNote, Inc (Gyeongi-do, Republic of Korea) (BioNote, 2010) , was added into the routine diagnostic procedure of the rabies laboratory of the Institut de Recherche en Elevage pour le Développement (IRED) in N'Djamena, Chad (Plate 7). It was utilized in parallel with the FAT test, which had been used since 2001. Rabies-suspect animals were presented to the IRED by their owners or by the bite victim. No active surveillance was initiated throughout the study. However, awareness was intensified prior to the study period, during May 2012, by a poster campaign sensitizing the public in N'Djamena to seek medical treatment after a dog bite and to send the biting animal to the IRED in case of rabies suspicion.

Description of samples and isolates

For the validation of the Anigen test in the field, diagnostic results from June 2012 to February 2015 were included. Only samples originating from dogs were considered for inclusion according to the manufacturer's recommendation (BioNote, 2010). During the 33 months of the study period, a total of 49 rabies -suspect dog heads were submitted to IRED for diagnostic testing. The origin of the samples is detailed in Table 3.1. Most were in fresh condition on arrival and upon testing (85%, n=42). Five of the samples were decomposed, while in one case, the sample quality was not noted. Only one sample was so decomposed that it was impossible to analyse and was excluded from the study. The final sample size of field isolates at IRED was 48 (Table 3.1).

Table 3.1: Description of samples tested in Chad and results obtained after FAT, RIDT and RT-qPCR on FTA papers. All specimens correspond to original brain samples collected from dogs.

Identification	Sampling date	Origin	Condition	FAT Result	RIDT Result	RT-qPCR Result
342	06.06.2012	N'Djamena	Fresh	Neg	Neg	Neg
343	21.06.2012	N'Djamena	Decomposed	Pos	Neg	Neg
344	22.06.2012	N'Djamena	Fresh	Pos	Pos	Pos
345	28.06.2012	N'Djamena	Fresh	Pos	Pos	Pos
346	05.07.2012	N'Djamena	Fresh	Pos	Pos	Pos
347	13.07.2012	Koundoul	Fresh	Pos	Pos	Pos
348	20.07.2012	N'Djamena	Fresh	Pos	Pos	Pos
349	31.07.2012	N'Djamena	Fresh	Pos	Pos	Pos
350	08.08.2012	N'Djamena	Fresh	Pos	Pos	Pos
352	25.08.2012	N'Djamena	Fresh	Pos	Pos	Pos
354	29.08.2012	N'Djamena	Fresh	Pos	Pos	Pos
355	11.09.2012	N'Djamena	Fresh	Pos	Pos	Pos
356	12.09.2012	N'Djamena	Fresh	Pos	Pos	Pos
357	20.09.2012	N'Djamena	Fresh	Pos	Pos	Pos
358	25.09.2012	N'Djamena	Fresh	Pos	Pos	Pos

359	28.09.2012	unknown	Fresh	Pos	Pos	Pos
360	01.10.2012	N'Djamena	Fresh	Neg	Neg	Neg
361	26.10.2012	N'Djamena	Fresh	Pos	Pos	Pos
362	24.12.2024	unknown	Unknown	Pos	Neg	Neg
363	19.11.2012	N'Djamena	Fresh	Pos	Pos	Pos
364	03.12.2012	N'Djamena	Fresh	Pos	Pos	Pos
365	05.12.2012	N'Djamena	Fresh	Neg	Neg	Neg
366	06.12.2012	N'Djamena	Fresh	Pos	Pos	Pos
367	10.01.2013	Moundou	Fresh	Pos	Pos	Pos
368	11.01.2013	N'Djamena	Fresh	Pos	Pos	Pos
369	08.02.2013	N'Djamena	Fresh	Pos	Pos	Pos
371	25.03.2013	Koundoul	Fresh	Pos	Pos	Pos
372	29.05.2013	N'Djamena	Fresh	Pos	Pos	Pos
373	17.06.2013	Koundoul	Fresh	Pos	Pos	Pos
379	12.11.2013	N'Djamena	Fresh	Pos	Pos	Pos
380	12.12.2013	N'Djamena	Fresh	Neg	Neg	Neg
381	31.01.2014	N'Djamena	Fresh	Pos	Pos	Pos
383	16.04.2014	N'Djamena	Fresh	Neg	Neg	ND
384	29.04.2014	N'Djamena	Fresh	Neg	Neg	ND
386	29.04.2014	Guelendeng	Fresh	Neg	Neg	ND
389	28.05.2014	N'Djamena	Decomposed	Imp	Neg	Neg
390	06.06.2014	N'Djamena	Fresh	Neg	Neg	ND
392	23.06.2014	N'Djamena	Decomposed	Neg	Neg	ND
393	14.07.2014	Koundoul	Fresh	Neg	Neg	ND
394	18.08.2014	Loumia	Fresh	Pos	Pos	Pos
395	21.08.2014	Chawaye	Fresh	Pos	Pos	Pos
400	13.10.2014	N'Djamena	Fresh	Neg	Neg	ND
401	14.10.2014	N'Djamena	Fresh	Pos	Pos	Pos
403	09.12.2014	Loumia	Fresh	Pos	Pos	Pos
405	27.01.2015	N'Djamena	Fresh	Pos	Pos	Pos
406	05.02.2015	Djermaya	Decomposed	Pos	Pos	ND
407	11.02.2015	Dourbali	Decomposed	Pos	Pos	Pos
408	13.02.2015	N'Djamena	Fresh	Pos	Pos	Pos

Pos : positive, Neg : negative, Imp : impossible, ND : not done

The Anigen test was further validated at the National Reference Centre (NRC-R) and WHO Collaborating Center for Rabies at the Institut Pasteur in Paris, France, on 73 samples selected from the collections housed in both of these centers, from 12 different species originating from various countries and belonging to different phylogenetic clades (Table S3.1). All these samples were previously analysed by FAT. Thirty of them were negative and the remaining 43 were positive. The positive samples represented a large diversity of RABV. All these 73 samples were stored at -80°C for archive before analysis.

In addition, the limit of detection of the RIDT was evaluated at NRC-R using a panel of 8 different isolates of RABV adapted and amplified on baby hamster kidney cells (BSR cells). Viral suspensions were titrated on the same cells using 5-fold serial dilutions in cell culture medium and expressed as fluorescent focus units per mL (FFU/mL). For the RIDT evaluation, titrated RABV suspensions were first tested at several concentrations using the buffer available from the RIDT kit as a diluent.

Description of the FAT methodology

The FAT, the gold standard technique for *post-mortem* diagnosis of rabies (Dean et al., 1996) was performed at the NRC-R under quality assurance (accreditation ISO/IEC 17025), as previously described (Bourhy et al., 1989). In the rabies laboratory of N'Djamena, the FAT was performed with some deviations regarding the standard procedure: lack of positive and negative control samples inclusion, absence of routine quality assessments, and storage of the immunofluorescent conjugate past the expiration date. In this setting, two microscopic slides were prepared, with two brain impressions per slide. If no viral characteristic fluorescent inclusions were observed on all four impressions, the sample was considered negative. Doubtful results were declared positive due to the potential fatal consequences of a false negative result for the bite victims. However, due to some deviations regarding the standard procedure, it was not possible to consider FAT performed ad IRED as the gold standard for the specificity and sensitivity analysis.

Description of the RIDT Anigen test methodology

The Anigen test is a simple and rapid diagnostic tool, presenting as an all-in-one included kit. Once the brain is extracted, it is used without additional material and equipment except for one dilution step requiring an additional vial of phosphate-buffered saline (PBS) prepared according to the manufacturer's recommendation. However, for our study, we omitted the first dilution step, only using the vial with buffer provided by the kit to simplify the test procedure in view of future application under realistic field conditions. The same procedure was used for the Anigen test at NRC-R and at IRED. If it was possible to anatomically identify the regions of the brain in a sample, the test was performed with a small section of the brainstem (approximately 0.1 g), otherwise the same amount of material was taken from different parts of decomposed brain samples. The brain sample was mixed directly in the tube containing the buffer with the swab, all included in the kit, for about one minute, until most of the brain material was well dissolved and then put on to the test plate using the transfer pipette provided in the kit. Four drops were deposited on the strip (corresponding to nearly 100 µL).

The test could be interpreted when the coloured liquid reached the top of the test and the purple indicator colour had vanished from the filter paper background. As described by the manufacturer, a positive test result was indicated by two purple lines, one in the test zone and the other in the control zone. If a line only appeared in the control zone, the test was considered negative. In cases where only the test line was coloured rather than the control band, the test was declared invalid and was performed once again. The test took approximately 5 to 10 min after deposit of sample and the interpretation was not performed after 10 min, according to manufacturer recommendations. Following these recommendations, the test was suitable for dog, raccoon dog and cattle samples (animals which were used originally in the validation of the method) (Kang et al., 2007), and should be tested immediately after collection. In this study, two different batches were used for the validation of the RIDT assay, with batches n°1801076 and n°1801111 for the field and the laboratory validation, respectively.

Extraction of RNA from Anigen test and FTA Whatman cards

Brain impressions were performed directly on FTA Whatman cards, a support dedicated to the storage and preservation of RNA (Picard-Meyer et al., 2007). Prior to use, the cards were stored at room temperature in a sealed plastic bag in a dry and clean area. The samples were prepared by diluting a small section (approximately 0.1 g) of the brain in 1 ml PBS (10%). After thorough mixing, the brain homogenate was loaded onto the card with a pipette until the sample indicator circle on the filter was covered. The cards were then dried 24 hours at room temperature before being put separately in transparent plastic bags for transportation.

To prepare the samples on FTA Whatman filter paper, 1 cm^2 was cut-off from the area containing the brain impression and incubated during 1 hour in Tri-Reagent LS (Molecular Research Center, Cincinnati, Ohio, USA) or overnight in cell culture medium (DMEM) (Life Technologies, Saint Aubin, France), then placed in Tri-Reagent. To obtain viral RNA directly from the Anigen test strip, the cassettes were opened, the filter paper was removed and the area where the sample was deposited was collected and placed into 1 mL of Tri-Reagent LS. For both FTA and Anigen test supports, total RNA extraction was performed as previously described, following manufacturer recommendations (Dacheux et al., 2008).

Viral RNA detection by RT-qPCR

Viral RNA detection was performed using a one-step dual combine pan-lyssavirus RT-qPCR assay recently described (Dacheux et al., 2016), targeting a conserved region among the polymerase. Briefly, this assay includes a pan-RABV RT-qPCR probe-based technique, able to detect all representatives of the broad genetic diversity of RABV, using two degenerated TaqMan probes. In parallel, a SYBR Green RT-qPCR assay is able to detect all the other lyssaviruses tested, in addition to RABV isolates. Both of these assays, which were optimized to a final reaction volume of 20 µL, were performed using 5 µL of RNA template (previously diluted 1:10 in nuclease-free water). For each assay, appropriate controls were used. Details of the combined pan-lyssavirus RT-qPCR assay are in Table S3.2 and in reference (Dacheux et al., 2016).

Evaluation of viral RNA extracted from Anigen test strips for genotyping

A selected panel of RNA extracts from Anigen test, which were found positive with the dual combine RT-qPCR assay, were evaluated with RT-PCR to generate amplicons suitable for genotyping by sequencing (at least 500 nt in length). Briefly, a volume of 6 µl of total RNA extraction was used for reverse transcription as previously described (Dacheux et al., 2008). RNA was incubated at 65°C for 10 min with 2 µL of pd$(N)_6$ random primers (200 µg/mL; Roche Diagnostics) and 2 µL of sterilized distilled water and then were stored on ice. Each tube was incubated with 200 U of Superscript II RT (Invitrogen), 80 U of RNasin (Promega), and 10 nmol of each nucleotide triphosphate (Eurobio), in a final volume of 30µL for 90 min at 42°C, for reverse transcription. Two microliters of complementary DNA (cDNA) were then amplified by PCR targeting the nucleoprotein gene of RABV, as described in (Bourhy et al., 2016).

Evaluation of the Anigen test in an inter-laboratory trial

The RIDT assay was evaluated by the NRC-R in an inter-laboratory trial organized during 2015 by the European Union reference laboratory for rabies, which is located in Nancy, France (Robardet et al., 2011). The FAT technique was also evaluated in parallel in this trial. The test panel consisted of nine anonymous samples of freeze-dried homogenized brains, either uninfected or infected with various lyssavirus species. Details of this trial have been provided elsewhere (Robardet et al., 2011).

Statistical Analysis

Results obtained with FAT and Anigen techniques were compared using the McNemar and Kappa statistic tests in Stata, and were analyzed to determine the intrinsic parameters of the RIDT assay. However and conversely to the FAT technique done at the NRC-R, the immunofluorescence assay performed at the IRED could not be considered as the reference technique due to several deviations compared to the standard procedure. In case of discrepancy between RIDT and FAT, samples were tested for RNA detection with RT-qPCR assay performed on FTA Whatman cards impregnated with the brain of the corresponding sample. For the determination of the sensitivity and the specificity of RIDT, true positivity and true negativity was defined according to the result that was shared by at least two tests among FAT, RIDT and viral RNA detection.

Results

RIDT test processing and interpretation

For the majority of the total sample size (n=121) tested at IRED and at NRC-R, the RIDT was successfully performed, with the presence of a line clearly visible in the control zone after 5 to 15 min of migration once the sample was deposited (Figure 3.1A and 3.1B). For only a few samples (n<10), the test was repeated, due to abnormal or incomplete migration (absence of the line in the control zone). When they scored positive with RIDT, most samples exhibited a line with strong intensity in the test zone (Figure 3.1B). In a few cases the test bands showed even higher intensity than the control band. Also, for some samples tested at NRC-R, the line in the test area was only faintly visible, despite a strong intensity of the line in the control zone (Figure 3.1A).

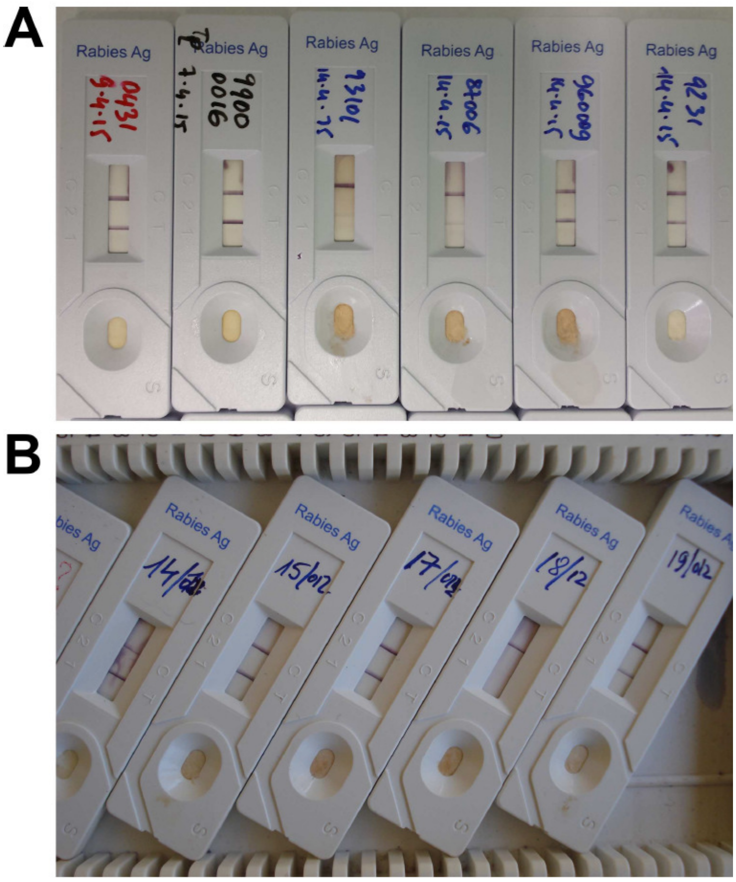

Figure 3.1: Examples of RIDT results. (A) Results from NRC-R, with six positive results including the two middle cassettes (samples 93101 and 87006) demonstrating only a weak test line in the results window. (B) Results from Chad, with one negative result (sample 18/12) and four positive results. The upper line visible in the results window is the control (C) and the lower line represents the test (T). The presence of two lines depicts a positive result, whereas the presence of only the upper line indicates a negative result. The test is considered as invalid when the upper line is not visible (not shown).

Determination of the limit of detection of the RIDT

A total of eight titrated suspensions from different RABV adapted to cell culture was selected to determine the limit of detection of the RIDT (Table 3.2). A volume of 100 µL of each of them, diluted or not, were tested. The lowest number of fluorescent focus-forming units (FFU) detected with this assay was 10^5 FFU, and was obtained for RABV 9704ARG and 04030PHI. Isolates 9147FRA and 9508CZK exhibited a positive signal with 10^6 FFU. Lastly, no positive signal was obtained with the initial viral suspension for virus 8743THA and 9001FRA, indicating that the limit of detection was > 8.1×10^6 FFU and > 2.4×10^5 FFU, respectively.

Table 3.2 : Limit of detection of the RIDT using 8 different titrated rabies virus suspensions.

Virus strain[a]	Original host	Location	Initial virus concentration (FFU/mL)[b]	Limit of detection (FFU)[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	> 8.1×10^6
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	> 2.4×10^5
9704ARG	Bat	Argentina	9.5×10^7	10^5
04030PHI	Human	Philippines	2.5×10^7	10^5

[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

Comparison results between FAT and RIDT

Seventy-three samples from NRC-R, including forty-three positive samples representing a large diversity in term of host species, geographical location and genetic diversity (Table S3.1 and S3.3) were tested. Compared to the gold standard FAT, the RIDT demonstrated an accordance of 95%. The specificity was 93.3% with only two false positive results among the 30 FTA-negative specimens, noticed for samples 150057 and 150125 which were originated from a dog and a cat, respectively (Tables 3.3, S3.1 and S3.3). The sensitivity of the RIDT was 95.3%, with only two false negative results observed for isolates 9217ALL and 9312MAU, a red fox from Germany and a dog from Mauritania, respectively (Tables 3.3, S3.1 and S3.3).

Among the 48 samples included for evaluation at IRED only 3 were not concordant between FAT and RIDT, yielding an accordance of 94% (Table 3.3). Two of the discordant samples (samples 343 and 389) were decomposed and the quality remained unknown for the last one (sample 362) (Table 3.1). The FAT was impossible to perform on one (sample 389) of the 3 and for the two others (samples 343 and 362), the result was positive (Table S3.4). For all these 3 specimens, RIDT tested negative (Table S3.4). In these three cases, where RIDT and FAT did not yield the same result, viral detection performed by RT-qPCR on the FTA Whatman card could not detect viral RNA after multiple attempts confirming the negative RIDT result (Table S3.4). For sensitivity and specificity of RIDT and FAT under field conditions, true positivity and true negativity was defined according to the result that was shared by at least two tests among FAT, RIDT and viral RNA detection on the FTA Whatman card. The RIDT showed a higher specificity (100%) than the FAT (78.5%) at IRED. Accordance with the overall true test results of the 48 samples from IRED was 100% for RIDT and 94% for FAT.

The McNemar test showed no significant difference between the FAT and RIDT (Exact McNemar significance probability = 0.5) on samples test at IRED and the Kappa value was 0.86, indicating excellent agreement between the tests. The exact McNemar significance probability for the comparison of FAT and RIDT performed at NRC-R was 1 and the Kappa value was 0.89. Overall, of the 121 samples analysed at NRC-R and IRED, the McNemar significance probability was found to be 0.45 and the Kappa value was 0.87.

Table 3.3 : Intrinsic parameters of the RIDT after laboratory evaluation (NRC-R), under field conditions (IRED) and when combining both conditions (all).

			FAT results			
			Pos	Neg	Imp	Total
RIDT results	NRC-R	Pos	41	2	0	43
		Neg	2	28	0	30
		Total	43	30	0	73
	IRED	Pos	34	0	0	34
		Neg	2	11	1	14
		Total	36	11	1	48
	All	Pos	75	2	0	77
		Neg	4	39	1	44
		Total	79	41	1	121

Evaluation of viral RNA detection by RT-qPCR using Anigen test strips and comparison with results obtained using FTA Whatman cards

A total of 51 samples were tested at NRC-R for viral RNA detection using RT-qPCR on the Anigen test strip, which were previously found positive for the *post-mortem* diagnosis of rabies (Table 3.4) (Tables S3.1 and S3.4). The FAT scored also positive for all of these specimens. Among them, 32 originating from IRED were used during the field evaluation of the RIDT, whereas 19 were obtained in NRC-R during the laboratory evaluation. Positive detection was obtained for 26 (81.2%), 18 (94.7%) and 44 (86.3%) samples from IRED, NRC-R and the two combined, respectively (Table 3.4). In parallel, detection of viral RNA was also performed at NRC-R on FTA Whatman cards for 31 samples, which were positive after analysis with both FAT and RIDT at IRED (Table S3.4). In this case, a perfect concordance (100%) was noticed. In addition, viral RNA from 2 and 6 samples found previously negative with RIDT were not detected after RT-qPCR analysis performed on the Anigen test strips and FTA Whatman cards, respectively (Table 3.4). When compared to the FTA Whatman card, RT-qPCR performed on the Anigen test strip exhibited a sensitivity of 80.6% (Table 3.5).

Evaluation of the use of viral RNA for genotyping after extraction from Anigen test strips

A limited panel of viral RNA samples extracted from Anigen test strips, which were previously confirmed positive by RT-qPCR, were secondarily tested for genotyping. A total of 14 samples (4 originating from IRED and 10 from NRC-R) were analyzed, among them 13 (93%) provided PCR amplicons (at least 500 nucleotides in length) targeting regions of the nucleoprotein gene commonly used for genotyping after sequencing (Tables S3.3 and S3.4). The only sample found negative (isolate 9702IND) was weakly positive after FAT and RIDT tests, which could then probably explain the absence of PCR amplification.

Table 3.4 : Detection of viral RNA with RT-qPCR on Anigen test strip used in field conditions (IRED), in laboratory conditions (NRC-R) or combined.

		Detection of viral RNA by RT-qPCR on Anigen test strip performed								
		At IRED			At NRC-R			When combined		
		Pos	Neg	Total	Pos	Neg	Total	Pos	Neg	Total
RIDT results	Pos	26	6	32	18	1	19	44	7	51
	Neg	0	3	3	0	0	0	0	3	3
	Total	26	9	35	18	1	19	44	10	54

Table 3.5 : Comparison results of the detection of viral RNA with RT-qPCR performed on FTA Whatman cards and Anigen test strips.

		Anigen test strip		
		Pos	Neg	Total
FTA Whatman card	Pos	25	6	31
	Neg	0	2	2
	Total	25	8	33

Evaluation of the RIDT Anigen test in an inter-laboratory trial

Finally, we evaluated the RIDT Anigen test in an inter-laboratory trial, in parallel of the FAT on nine anonymous samples. The results obtained were concordant with those expected (Table 3.6) (Robardet et al., 2011). In particular, we were able to detect three different RABV isolates (strains CVS27 14-10, GS7 and DR627) constituting the panel, as well as 4 other lyssavirus species, including Duvenhage virus (DUVV), European bat lyssavirus 1 (EBLV-1) and 2 (EBLV-2), and Bokeloh bat lyssavirus (BBLV).

Table 3.6: Evaluation of the RIDT Anigen test in NRC-R in an inter-laboratory trial

Sample	Lyssavirus species[a]	Strain	FAT	RIDT
1	RABV	CVS 27 14-10	Pos	Pos
2	RABV	GS7	Pos	Pos
3	RABV	DR627	Pos	Pos
4	BBLV	127900	Pos	Pos
5	DUVV	DUVV 05-11	Pos	Pos
6	EBLV-1	122938	Pos	Pos
7	EBLV-2	EBL2 RV1787 (1/30)	Pos	Pos
8	Negative	-	Neg	Neg
9	Negative	-	Neg	Neg

[a] RABV: Rabies virus, BBLV: Bokeloh bat lyssavirus, DUVV: Duvenhage virus, EBLV-1: European bat lyssavirus, EBLV-2: European bat lyssavirus 2.

Discussion

The aim of our study was to evaluate both in the laboratory and under field conditions the RIDT Anigen test in comparison with the FAT, to investigate the intrinsic parameters of this rapid technique, as well as the relevance of its application in sub-Saharan Africa, a region with the highest estimated per capita death rate due to rabies (Hampson et al., 2015) but with poor data reporting situation (Taylor et al., 2015).

We first investigated the limit of detection of this RIDT using serial dilutions of different titrated suspensions of RABV. This value varied among the isolates tested but remaining relatively high, ranked from 10^5 to 10^7 FFU.

We then evaluated the use of RIDT for *post-mortem* diagnosis of animal rabies in the laboratory and under field conditions, and compared it to the FAT assay. Our results demonstrate that the lateral flow test performs similar to the FAT. The accordance between RIDT and FAT was high under both conditions (≥94%), with a specificity of the RIDT from 93.3% to 100%. The sensitivity of this technique was also high in laboratory settings, with 95.3%, and approached 100% under field conditions. Our results indicate the high potential of this test for use in the resource challenged African context. Importantly, we show that the intrinsic performance of RIDT under limited laboratory conditions could be higher compared to the FAT test. Conversely to RIDT, several factors could affect negatively FAT results, including storage and quality of the fluorescent conjugate, maintenance of the fluorescence microscope and experience of the reader. However, given the limited sample size, the explanatory power is not overly strong and further evaluation is highly encouraged.

Lastly, we tested the RIDT Anigen test in parallel of the FAT technique in an international inter-laboratory trial organized by the European Union reference laboratory for rabies, Nancy, France and all results were found concordant.

As underlined by the results obtained under field conditions, the advantages of the immunochromatographic test method are manifold. Samples can be analysed one by one proportionate to the diagnostic demand. This is also true for FAT, however, the conjugate used for the FAT can only be stored for a limited time to ensure the quality of the test. Similarly, storage for the reagents is a cause for quality concern for the DRIT. For both DRIT and FAT, negative and positive controls have to be included in the test procedure for standardization, which is not needed for the RIDT (Servat et al., 2012). Storage of the RIDT can be done at room temperature and does not require refrigeration, as is necessary for the conjugates used for the DRIT and FAT test. The tests used at IRED were stored at 20°C in an air conditioned room. There are no data on the reliability of the tests stored at temperatures at above 30°C, as would be encountered in many tropical countries.

The FAT depends heavily on the quality of the immunofluorescent conjugate, the maintenance of the fluorescent microscope and also on an experienced technician reading the microscope slides. In contrast, RIDT is a very easy-to-use kit, which does not require a high level of expertise. This technique is simple to perform and to interpret.

For the dog samples tested in Chad, the test had a specificity and sensitivity of 100%. However, we showed the utility of the Anigen test for many different wild and domestic mammalian species. Our results confirm data obtained from previous studies, and suggest that the spectrum of species which could be tested with this RIDT is larger than recommended by the manufacturer (e.g., dogs, cattle and raccoon dogs) (Markotter et al., 2009; Servat et al., 2012; Reta et al. 2013).

In our study, we mainly focused on RABV species. In particular, we were able to detect RABV isolates belonging to all of the major phylogenetic clades defined previously (Bourhy et al., 2008), with the exception of the Africa 3 clade, which was not tested in our panel. However, positive detection of isolates belonging to this clade has been already demonstrated (Markotter et al., 2009). In addition, we were also able to detect 4 different other lyssavirus species, in addition to RABV, when evaluated this technique in an inter-laboratory trial. These results are concordant with those obtained in two other previous studies using different lyssavirus species including African and bat-related lyssaviruses (including Lagos bat virus, Mokola virus and Australian bat lyssavirus), demonstrating that it can also be applicable for the detection of non-RABV lyssaviruses (Markotter et al., 2009; Servat et al., 2012; Mshelbwala et al., 2015).

Several RIDT kits for RABV detection were evaluated using brain samples (Kang et al., 2007; Nishizono et al., 2008; Markotter et al., 2009; Ahmed et al., 2012; Reta et al. 2013). A very recent study evaluated 8 commercially available RIDT kits in parallel (Eggerbauer et al., 2016). Sensitivity of the Anigen test in that study was observed to be unsatisfactory (Eggerbauer et al., 2016).

We modified the test procedure by omitting a dilution step and placing the brain sample directly into the buffer vial provided by the test. The advantage of this approach is that the test can be used with no additional material other than that provided in the kit. This change in methodology might explain the better sensitivity of the test and the higher intensity of the test band compared to other studies (Servat et al., 2012; Voehl and Saturday, 2014; Sharma et al., 2015), because the RABV antigen level is higher without a second dilution. Sharma et al. (Sharma et al., 2015) found that the intensity of the test band decreases with dilution.

Autolysis of samples is less a concern for sensitivity of the RIDT compared to the FAT and PCR (Servat et al., 2012; Silva et al., 2013; McElhinney et al., 2014), which is illustrated by our results. Sample storage in glycerol was suggested to interfere with the optimal test performance, affecting the intensity of the test line (Servat et al., 2012).

The successful detection of RABV RNA from the Anigen test strip in over 86.3% of samples tested is a promising result and highlights the potential use of the kit as a vehicle for sample submission for further confirmatory diagnostic or genotyping analysis. This potential has also been reported by others (Eggerbauer et al., 2016). However, the sensitivity was lower when compared to the use of the FTA Whatman card, a dedicated support for storage and preservation of nucleic acids.

The price of less than 10 euros is less expensive compared to the cost of performing FAT (Servat et al., 2012; Voehl and Saturday, 2014), but still poses an affordability problem in developing countries.

Further validation has to be conducted with RIDT, especially if the results of this test will guide decision making for PEP. We demonstrated that the sensitivity of RIDT, even high, was not complete compared to FAT. To avoid getting false negative results with this technique, we suggest to confirm all negative results using WHO and OIE reference techniques, such as FAT, before excluding RABV infection in diagnostic samples.

An efficient diagnosis method is just part of the entire process of surveillance and control needed to eliminate rabies, as comparable to translation of efficiency of a vaccine, to the ultimate immunity of the target population (Muthiani et al., 2015). Therefore, all components of the surveillance system, in which the test would be promoted and used, have to be strengthened in parallel (Lembo et al.,, 2012).

Conclusions

Specificity and sensitivity of the evaluated Anigen test are only slightly reduced compared to the known reference tests for rabies virus detection in brain samples. The results are promising for field use, where the test could help to establish rapid preliminary diagnostic results, which would be further confirmed using WHO and OIE recommended tests at central laboratories. However, we suggest important changes to the test protocol: skip the dilution step of brain biopsy in PBS and perform the brain homogenate with the swab directly into the specimen tube containing 1 ml of assay diluent, both provided in the kit. We also recommend to provide a more precise sketch depicting the brain sampling method. Rapid rabies tests cannot substitute for the current reference tests, but are crucial for the success of rabies surveillance systems in developing countries. Further, we demonstrated here that the test cassettes can be used as a vehicle to ship viral RNA to reference laboratories for further laboratory confirmation of the diagnosis and for epidemiological investigations.

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Supporting information

Table S3.1: Description of samples tested at NRC-R, Paris, France.

Identification	Host	Country	Year	Origin[a]	Status[b]	Phylogenetic clade[c]
150007	Cat	France	2015	P	Negative	-
150036	Dog	France	2015	P	Negative	-
150038	Cat	France	2015	P	Negative	-
150041	Cat	France	2015	P	Negative	-
150042	Ferret	France	2015	P	Negative	-
150043	Dog	France	2015	P	Negative	-
150044	Dog	France	2015	P	Negative	-
150049	Dog	France	2015	P	Negative	-
150050	Dog	France	2015	P	Negative	-
150051	Dog	France	2015	P	Negative	-
150052	Cat	France	2015	P	Negative	-
150053	Dog	France	2015	P	Negative	-
150054	Cat	France	2015	P	Negative	-
150055	Red fox	France	2015	P	Negative	-
150056	Cat	France	2015	P	Negative	-
150057	Dog	France	2015	P	Negative	-
150058	Dog	France	2015	P	Negative	-
150059	Dog	France	2015	P	Negative	-
150060	Cat	France	2015	P	Negative	-
150061	Cat	France	2015	P	Negative	-
150062	Cat	France	2015	P	Negative	-
150119	Ferret	France	2015	P	Negative	-

150125	Cat	France	2015	P	Negative	-
150127	Cat	France	2015	P	Negative	-
150129	Cat	France	2015	P	Negative	-
150132	Dog	France	2015	P	Negative	-
150133	Dog	France	2015	P	Negative	-
150134	Red fox	France	2015	P	Negative	-
150148	Horse	France	2015	P	Negative	-
150230	Red fox	France	2015	P	Negative	-
8670NIG	Human	Nigeria	?	M	Positive	Africa 2
8683GRO	Fox	Greenland	1980	P	Positive	Arctic-related
8684GRO	Fox	Greenland	1981	P	Positive	Arctic-related
8692EGY	Human	Egypt	1979	M	Positive	Cosmopolitan (Africa 1 lineage)
8697BEN	Cat	Benin	1986	P	Positive	Africa 2
8706ARS	Fox	Saudi Arabia	1987	P	Positive	Cosmopolitan
8801CAM	Dog	Cameroon	1987	P	Positive	Africa 2
8807ETH	Hyena	Ethiopia	1988	P	Positive	Cosmopolitan (Africa 1 lineage)
8808ETH	Dog	Ethiopia	1987	P	Positive	Cosmopolitan (Africa 1 lineage)
9003CI	Dog	Ivory Coast	1989	P	Positive	Africa 2
9010NIG	Dog	Niger	1990	P	Positive	Africa 2
9021TCH	Dog	Chad	1990	P	Positive	Africa 2
9024GUI	Dog	Guinea	1990	P	Positive	Africa 2
9104USA	Skunk	USA	1991	P	Positive	Arctic-related
9115MEX	Dog	Mexico	1991	P	Positive	Cosmopolitan
9136MAU	Goat	Mauritania	1991	P	Positive	Africa 2
9141RUS	Polar fox	Russia	1988-90	?	Positive	Arctic-related
9217ALL	Red fox	Germany	1991	P	Positive	Cosmopolitan
9218TCH	Dog	Chad	1992	P	Positive	Africa 2
9228CAR	Dog	Central African Republic	1992	P	Positive	Africa 2
9231NAM	Jackal	Namibia	1992	P	Positive	Cosmopolitan (Africa 1)
9302SOM	Dog	Somalia	1993	P	Positive	Cosmopolitan (Africa 1)
9305SEN	Dog	Senegal	1992	P	Positive	Africa 2
9312MAU	Dog	Mauritania	1993	P	Positive	Africa 2
9319IRA	Jackal	Iran	?	P	Positive	Cosmopolitan
9391HON	Fox	Hungary	1993	P	Positive	Cosmopolitan
93101TUR	Fox	Turkey	1993	P	Positive	Cosmopolitan
93105EST	Fox	Estonia	1993	P	Positive	Cosmopolitan
93119ZIM	Dog	Zimbabwe	?	P	Positive	Cosmopolitan (Africa 1)

94289RWA	Dog	Rwanda	1994	P	Positive	Africa 2
9522BRE	Dog	Brazil	1995	P	Positive	Cosmopolitan
9547HAV	Dog	Burkina Faso	1995	P	Positive	Africa 2
9609TCH	Dog	Chad	1996	P	Positive	Africa 2
9613TAN	Dog	Tanzania	1996	P	Positive	Cosmopolitan (Africa 1)
96178POL	Fox	Poland	1994	P	Positive	Cosmopolitan
9702IND	Human	India	1997	P	Positive	Indian subcontinent
9705FRA	Bovine	French Guiana	1997	P	Positive	Bat
9915BIR	Dog	Myanmar	1999	P	Positive	Asia
9916CAM	Dog	Cambodia	1999	P	Positive	Asia
02041CHI	Dog	China	1987	P	Positive	Asia
02052AFG	Dog	Afghanistan	2002	P	Positive	Arctic-related
04031FRA	Dog	France	2004	P	Positive	Cosmopolitan (Africa 1)
04033MAD	Human	Madagascar	2004	NA	Positive	Cosmopolitan (Africa 1)

[a] M : suckling newborn mouse brain sample, P : original primary brain sample, NA: Not available

[b] : Based on FAT results

[c]: According to Bourhy et al., 2008 (Bourhy H, Reynes JM, Dunham EJ, Dacheux L, Larrous F, Huong VT, Xu G, Yan J, Miranda ME, Holmes EC. The origin and phylogeography of dog rabies virus. J Gen Virol. 2008 Nov;89(Pt 11):2673-81)

Table S3.2: Oligonucleotide sequences of primers and probes used in the combo RT-qPCR (combination of pan-RABV and pan-lyssa RT-qPCR assays) and in the internal control eGFP-based RT-qPCR assay.

Application	Reference	Name	Type	Length	Sequence (5'-3')	Sense	Position
Pan-RABV RT-qPCR assay (TaqMan®-based)	This study	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 (SYBR® Green-based)	This study	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 internal control assay	Hoffmann et al., 2006	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 (VIC/TAMRA)	22	AGC ACC CAG TCC GCC CTG AGC A	S	703-724[b]

[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).

The one-step, probe-based real-time RT-PCR assay (pan-RABV RT-qPCR) was performed with the Superscript III Platinum One-Step RT-qPCR kit (Life Technologies, Saint Aubin, France), as recommended by the manufacturer, with only minor modifications. Real-time PCR, which was optimized for a final reaction volume of 20 µL, was performed with 10 µL 2x Reaction Mix, 1.5 µL nuclease-free water, 1 µL of each primer Taq3long and Taq17revlong (10 µM), 0.4 µL SuperScript® III RT/Platinum® Taq Mix, 0.3 µL of each probe RABV4 and RABV5 (10 µM), 0.25 µL MgSO4 (50 mM), 0.2 µL RNasin® recombinant ribonuclease inhibitor (Promega, Charbonnieres, France), 0.05 µL ROXTM reference dye and 5 µL RNA template (previously diluted 1:10 in nuclease-free water). Amplification was carried out according to the following program: 1 cycle of heating at 45°C for 15 min and 95°C for 3 min, followed by 40 cycles of 95°C for 15 s and 61°C for 1 min, during which fluorescence values were recorded.

All reactions were carried out as technical duplicates in Thermo Scientific 96-well plates (Life Technologies, Saint Aubin, France), with an Applied Biosystems 7500 Real-Time PCR System (Life Technologies, Saint Aubin, France).

For each RT-PCR, a quantification cycle number (Cq) was determined as the PCR cycle number at which the fluorescence of the reaction exceeded a value considered to be significantly higher than background by the software associated with the Applied Biosystems 7500 Real-Time PCR System (Life Technologies, Saint Aubin, France). The efficiency (E), slope and correlation coefficient (R2) were also determined with this software. All reactions were carried out as technical duplicates. A cutoff ≥ 38 was defined for negative results.

The pan-lyssavirus RT-qPCR assay was performed with the SuperScript III Platinum SYBR® Green One-Step qRT, as recommended by the manufacturer (Life Technologies, Saint Aubin, France), with the same minor modifications as indicated for the pan-RABV assay. In particular, this real-time PCR was optimized for a final volume of 20 µL, using the same mixture composition and the same amount of diluted sample. The primers used were Taq5long and Taq16revlong and the probes were replaced with nuclease-free water. Amplification was performed on a similar thermocycler, as follows: 15 minutes at 45°C, 3 minutes at 95°C, followed by 40 cycles of 15 seconds at 95°C and 1 minute at 55°C, during which fluorescence values were recorded. After the 40 cycles of amplification, a melting analysis was carried out to check the product amplified by determining its specific melting temperature (increase 0.01°C/s, 55-95°C). As previously indicated, the efficiency (E), slope and correlation coefficient (R^2) were also determined with the software associated with Applied Biosystems 7500 Real-Time PCR System (Life Technologies, Saint Aubin, France). All reactions were carried out as technical duplicates. For this assay, a positive reaction was not based on the Cq value but exclusively on the melting temperature (Tm) value and the shape of the melting curve, both compared to positive and negative controls.

Table S3.3: Comparison results obtained with samples from NRC-R for the *post-mortem* diagnosis of rabies using FAT and RIDT, and for the detection of rabies virus RNA using Anigen test strip as support material.

Sample identification	FAT results	Anigen results	Anigen RNA detection	Anigen RNA genotyping
150007	Neg	Neg	ND	ND
150036	Neg	Neg	ND	ND
150038	Neg	Neg	ND	ND
150041	Neg	Neg	ND	ND
150042	Neg	Neg	ND	ND
150043	Neg	Neg	ND	ND
150044	Neg	Neg	ND	ND
150049	Neg	Neg	ND	ND
150050	Neg	Neg	ND	ND
150051	Neg	Neg	ND	ND
150052	Neg	Neg	ND	ND
150053	Neg	Neg	ND	ND
150054	Neg	Neg	ND	ND
150055	Neg	Neg	ND	ND
150056	Neg	Neg	ND	ND
150057	Neg	Pos	ND	ND
150058	Neg	Neg	ND	ND
150059	Neg	Neg	ND	ND
150060	Neg	Neg	ND	ND
150061	Neg	Neg	ND	ND
150062	Neg	Neg	ND	ND
150119	Neg	Neg	ND	ND
150125	Neg	Pos	ND	ND
150127	Neg	Neg	ND	ND
150129	Neg	Neg	ND	ND
150132	Neg	Neg	ND	ND
150133	Neg	Neg	ND	ND
150134	Neg	Neg	ND	ND
150148	Neg	Neg	ND	ND
150230	Neg	Neg	ND	ND
8670NIG	Pos	Pos	ND	ND
8683GRO	Pos	Pos	ND	ND
8684GRO	Pos	Pos	ND	ND
8692EGY	Pos	Pos	ND	ND
8697BEN	Pos	Pos	Pos	Pos
8706ARS	Pos	Pos	Pos	Pos
8801CAM	Pos	Pos	ND	ND
8807GAB	Pos	Pos	Pos	Pos
8808ETH	Pos	Pos	ND	ND

9003CI	Pos	Pos	Pos	Pos
9010NIG	Pos	Pos	ND	ND
9021TCH	Pos	Pos	Pos	Pos
9024GUI	Pos	Pos	Pos	Pos
9104USA	Pos	Pos	ND	ND
9115MEX	Pos	Pos	ND	ND
9136MAU	Pos	Pos	ND	ND
9141RUS	Pos	Pos	ND	ND
9217ALL	Pos	Neg	ND	ND
9218TCH	Pos	Pos	ND	ND
9228CAR	Pos	Pos	ND	ND
9231NAM	Pos	Pos	Pos	ND
9302SOM	Pos	Pos	ND	ND
9305SEN	Pos	Pos	ND	ND
93101TUR	Pos	Pos	ND	ND
93105EST	Pos	Pos	Pos	ND
93119ZIM	Pos	Pos	ND	ND
9312MAU	Pos	Neg	ND	ND
9319IRA	Pos	Pos	Pos	ND
9391HON	Pos	Pos	Pos	ND
94289RWA	Pos	Pos	ND	ND
9522BRE	Pos	Pos	ND	ND
9547BUR	Pos	Pos	ND	ND
9609TCH	Pos	Pos	ND	ND
9613TAN	Pos	Pos	Pos	ND
96178POL	Pos	Pos	Pos	ND
9702IND	Pos	Pos	Pos	Neg
9705FRA	Pos	Pos	Pos	Pos
9915BIR	Pos	Pos	ND	ND
9916CAM	Pos	Pos	Pos	ND
02041CHI	Pos	Pos	Neg	Pos
02052AFG	Pos	Pos	Pos	ND
04031FRA	Pos	Pos	Pos	Pos
04033MAD	Pos	Pos	Pos	ND

Pos : positive, Neg : negative, ND : not done

Table S3.4: Comparison results obtained with samples from IRED for the *post-mortem* diagnosis of rabies using FAT and RIDT, and for the detection of rabies virus RNA using Anigen test strip and FTA Whatman card as support material.

Sample identification	FAT results	Anigen results	FTA Whatman card RNA detection	Anigen strip RNA detection	Anigen strip RNA genotyping
342	Neg	Neg	Neg	ND	ND
343	Pos	Neg	Neg	Neg	ND
344	Pos	Pos	Pos	Neg	ND
345	Pos	Pos	Pos	Neg	ND
346	Pos	Pos	Pos	Neg	ND
347	Pos	Pos	Pos	Neg	ND
348	Pos	Pos	Pos	Pos	ND
349	Pos	Pos	Pos	Pos	ND
350	Pos	Pos	Pos	Pos	ND
352	Pos	Pos	Pos	Pos	ND
354	Pos	Pos	Pos	Pos	ND
355	Pos	Pos	Pos	Pos	ND
356	Pos	Pos	Pos	Pos	ND
357	Pos	Pos	Pos	Pos	ND
358	Pos	Pos	Pos	Neg	ND
359	Pos	Pos	Pos	Pos	ND
360	Neg	Neg	Neg	ND	ND
361	Pos	Pos	Pos	Pos	ND
362	Pos	Neg	Neg	Neg	ND
363	Pos	Pos	Pos	Pos	ND
364	Pos	Pos	Pos	Pos	ND
365	Neg	Neg	Neg	ND	ND
366	Pos	Pos	Pos	Pos	ND
367	Pos	Pos	Pos	Pos	ND
368	Pos	Pos	Pos	Neg	ND
369	Pos	Pos	Pos	Pos	ND
371	Pos	Pos	Pos	Pos	ND
372	Pos	Pos	Pos	Pos	ND
373	Pos	Pos	Pos	Pos	ND
379	Pos	Pos	Pos	Pos	Pos
380	Neg	Neg	Neg	ND	ND
381	Pos	Pos	Pos	Pos	Pos
383	Neg	Neg	ND	ND	ND
384	Neg	Neg	ND	ND	ND
386	Neg	Neg	ND	ND	ND
389	Imp	Neg	Neg	Neg	ND
390	Neg	Neg	ND	ND	ND
392	Neg	Neg	ND	ND	ND
393	Neg	Neg	ND	ND	ND

394	Pos	Pos	Pos	Pos	Pos
395	Pos	Pos	Pos	Pos	Pos
400	Neg	Neg	ND	ND	ND
401	Pos	Pos	Pos	Pos	ND
403	Pos	Pos	Pos	Pos	ND
405	Pos	Pos	Pos	Pos	ND
406	Pos	Pos	ND	Pos	ND
407	Pos	Pos	Pos	Pos	ND
408	Pos	Pos	Pos	Pos	ND

Pos : positive, Neg : negative, Imp : impossible, ND : not done

CHAPTER 4

Mass dog vaccination rapidly interrupts rabies transmission and human exposure in an African city

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One sentence summary: Mass dog vaccination is feasible and rapidly interrupts rabies transmission and reduces human exposure in an African city.

Abstract

After polio, dog transmitted rabies is one of the most promising viral diseases that can be targeted for elimination. Two consecutive dog mass vaccination campaigns, co-funded by the Chadian government and external donors in 2012 (dog vaccination coverage: 72%) and 2013 (coverage: 70%) interrupted transmission for nine months in N'Djamena, the capital city of Chad. A deterministic dog-human rabies transmission model, fitted to weekly incidence data of rabid dogs and exposed human cases showed that the effective reproductive number fell below one through November 2014. The incidence of human rabies exposure fell to less than one per million per year. A phylodynamic estimation of the reproductive number from 29 dog related rabies virus genetic sequences of the N-protein confirmed the results of the deterministic transmission model, implying that rabies transmission was interrupted after the vaccination campaign. However, new cases appeared earlier than the transmission and phylodynamic models predicted, which we suspect is due to the continuous movement of rabies-exposed dogs into N'Djamena from adjacent rural areas. Our results show that dog rabies transmission can be interrupted in African cities with currently available dog vaccines, provided the vaccination area includes larger adjacent areas and communities are well informed and engaged.

Introduction

Dog rabies has been eliminated in large parts of the industrialized countries in Europe and North America. In the last few decades, a concerted effort of South and Central American countries has strongly reduced dog rabies transmission close to elimination (Hampson et al., 2007). Despite the existence of effective vaccines for dogs, dog transmitted human rabies persists and has even re-emerged in Asia and Africa where still more than 59,000 people die annually from this preventable disease. The largest part of the burden is borne by India followed by Africa, China and South East Asian countries (Hampson et al., 2015). It appears feasible to eliminate dog mediated human rabies because of its low reproductive number through the mass vaccination of dogs (Hampson et al., 2009; Cleaveland et al., 2014a). However, reaching this goal, in partnership with the World Health Organization (WHO), the Food and Agriculture Organization of the United Nations (FAO), the World Organization for Animal Health (OIE) and the Global Alliance for Rabies Control (GARC, www.rabiesalliance.org), requires a rigorous scientific approach (Zinsstag, 2013).

Reaching sufficient coverage to interrupt dog rabies virus transmission and prevent re-introduction requires an in-depth understanding of dog ecology, the dog-human relationship, and the social and cultural determinants of vaccine acceptability; as well as the effective deployment of vaccines with a highly sensitive surveillance system (Kayali et al., 2003b; Obrist et al., 2007; Dürr et al., 2008b;

Hampson et al., 2009; Talbi et al., 2010). A science of rabies elimination will have to fill the gap between knowledge and effective action by addressing the social, political, economic and psychological complexity of effective rabies control interventions by a systemic approach to health in social-ecological systems (Zinsstag et al., 2011a). It requires scientists to closely collaborate with authorities and communities as partners in a transdisciplinary way between human and animal health (Hirsch Hadorn et al., 2008; Charron, 2012). Concomitant mathematical and economic frameworks yield new insights into fundamental properties of pathogen transmission (Townsend et al., 2013a) and comparative cost-effectiveness (Zinsstag et al., 2009) but do not explain sufficiently how the necessary effectiveness can be achieved (Klepac et al., 2013).

Dog rabies control in African cities is not easy, but small scale studies showed its feasibility (Kayali et al., 2003b) given the low cost of 2-3 USD per vaccinated dog in 2003 (Kayali et al., 2006). However, in most African countries dog owners cannot afford dog rabies vaccination and depend on mass vaccination campaigns that are free to the owner (Dürr et al., 2008b; Jibat et al., 2015). Analysis of the pre- and post-vaccination rabies case data and economic analysis showed that a single campaign was able to interrupt transmission and that mass vaccination of dogs was less costly than human post-exposure prophylaxis (PEP) (Zinsstag et al., 2009). A proof of the feasibility of dog rabies elimination in African cities and adjacent areas would have far reaching consequences for a regionally concerted elimination of dog rabies in Africa. In this study we demonstrate the operational and technical feasibility of interrupting transmission of dog rabies through mass vaccination campaigns in N'Djamena, Chad, which is a key step towards rabies elimination.

Extensive preparatory research in rabies surveillance (Kayali et al., 2003a; Durr et al., 2008a), dog ecology (Mindekem et al., 2005), small scale mass vaccination campaigns (Dürr et al., 2008b; Durr et al., 2009), human dog bite exposure (Frey et al., 2013) and the simulation of dog mediated human rabies elimination (Zinsstag et al., 2009) preceded the current study. In 2012, among 1.1 million humans, a medium sized dog population of 35,000 dogs lived in N'Djamena, together with a few thousand cats and less than a thousand monkeys. The rate of importation of dogs in the city is not known. However, the dog/human ratio has remained stable at approximately one dog per 33 inhabitants since 2001 (Mindekem et al., 2005; Léchenne et al., 2016a). Dogs are mainly kept as watch dogs but an unknown proportion is also consumed as a source of food (Mindekem et al., 2005).

A city-wide dog rabies mass vaccination campaign was set up in partnership with the Chadian authorities, the Institut de Recherches en Elevage pour le Developpement (IRED), the Centre de Support en Santé Internationale (CSSI) and the Swiss Tropical and Public Health Institute (Swiss TPH). The Chadian government paid for the costs of personnel and logistics and a philanthropic donor paid for the costs of dog vaccines and research. The campaign operations are described in more detail in a previous publication (Léchenne et al., 2016a) but we summarise the results here. Participatory stakeholder processes with the city authorities, quarter chiefs and communities ensured the acceptance and ownership of the dog mass vaccination campaign, with additional poster and radio campaigns informing the broader public. Ten vaccination teams of three persons and three supervisors mass vaccinated dogs, cats and monkeys from October to December in 2012 and 2013 in twelve and thirteen weekly intervals (sequences). A fixed point vaccination approach was used, shifting vaccination points as soon as no more dogs were brought for vaccination (Léchenne et al., 2016a). Vaccination coverage surveys followed each sequence to assess the achieved coverage and the deficit to reach 70% target coverage (Léchenne et al., 2016a). In 2012, 72% of all dogs were vaccinated (95% confidence interval 69-76%) and in 2013 70% were vaccinated (95% confidence interval 69-75%)) Passive dog rabies and human exposure surveillance started before the campaigns and is still ongoing. We analyse this surveillance data using mathematical transmission models and phylodynamic analyses of dog related rabies virus (RABV) to investigate the impact of the vaccination campaigns in interrupting transmission and their potential for maintaining elimination.

Results

Field Data

The results are based on weekly incidence of rabid dogs (Figure 4.1A) and the incidence of related human exposure (Figure 4.1B), collected through passive surveillance at the rabies laboratory in N'Djamena and RABV strains collected from the rabid dogs. Recorded numbers of vaccinated dogs were used for the estimation of the vaccination coverage (Léchenne et al., 2016a). This data was used to estimate the effective reproductive number R_e (the number of new rabid dogs infected by one rabid dog at any time, accounting for immunity and interventions, estimated from the transmission model) and the threshold population density of susceptible dogs using mathematical models. The data suggests that mass dog vaccination campaigns in 2012 and 2013 reached sufficient coverage to interrupt transmission from January 2014 to October 2014. Dog rabies incidence, estimated from passive surveillance, dropped from 0.33 rabid dogs / (10 000*week) prior to the mass vaccination campaign to 0.016 rabid dogs / (10 000*week) in 2014 (Figure 4.1A). Similarly, the incidence of human exposure to rabid dogs, estimated from passive surveillance, dropped from 1 rabies exposed human / (1 000 000*week) prior to the mass vaccination to less than 0.002 / (1 000 000*week) in 2014, which is less than one in a year (Figure 4.1B).

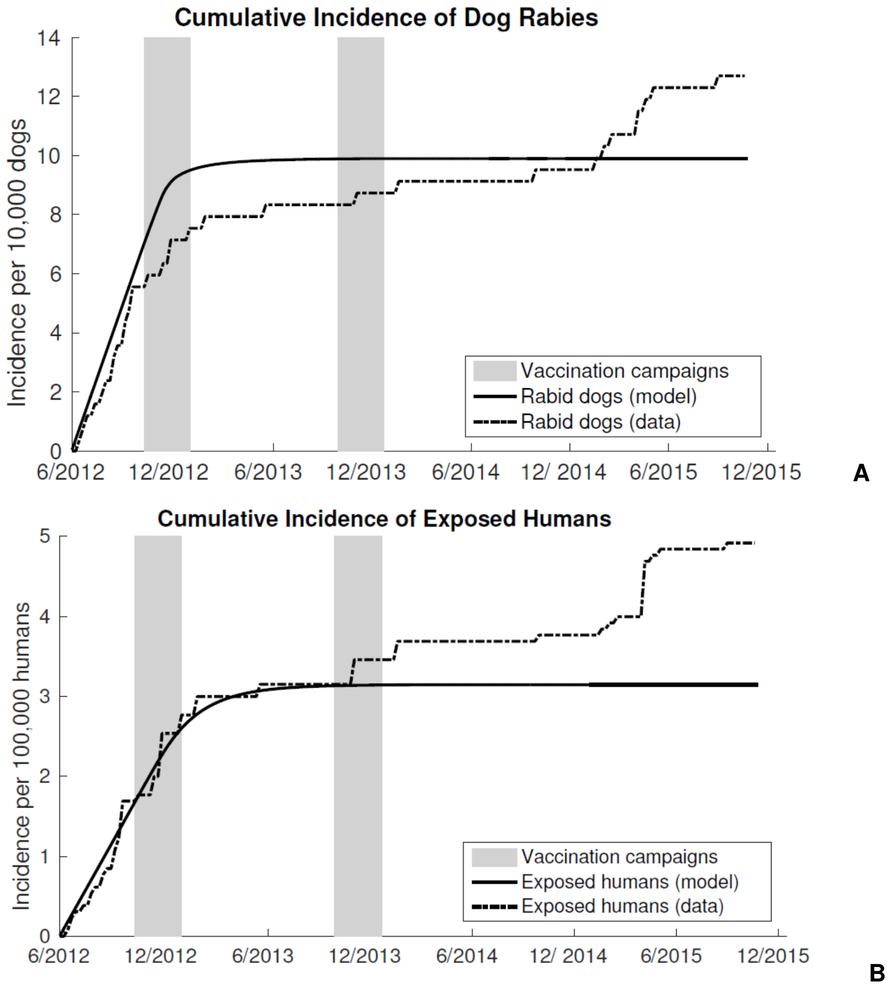
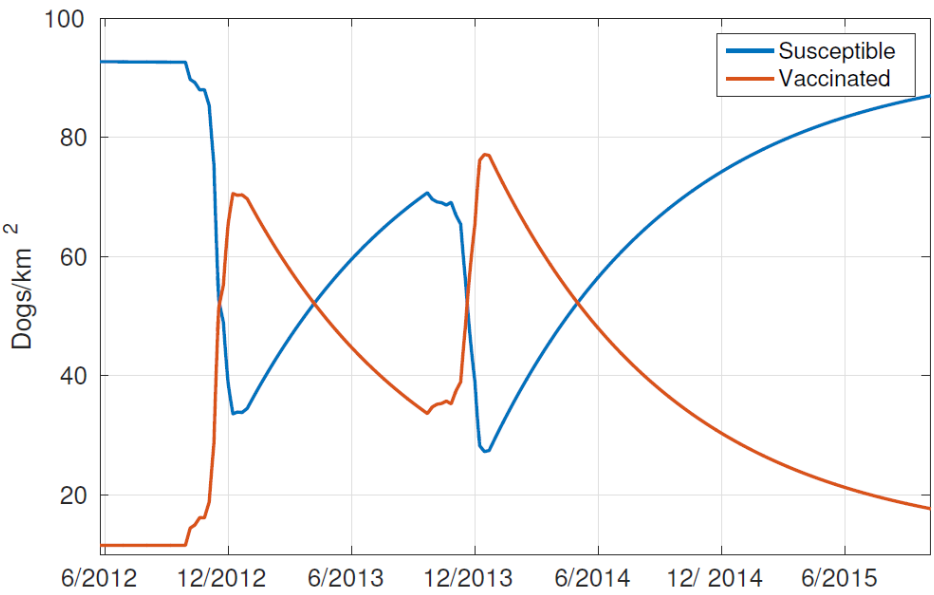


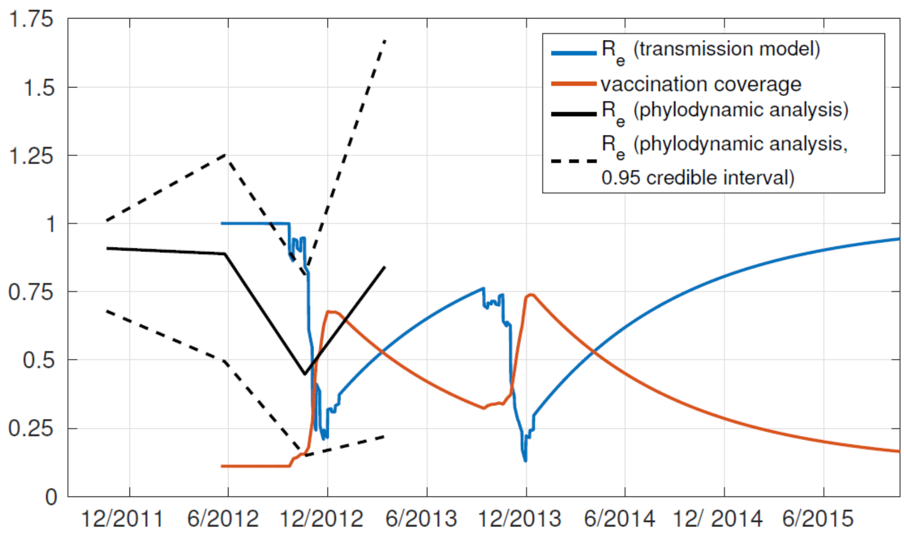
Figure 4.1: (A) Cumulative incidence of recorded cases of dog rabies (infectious dogs) and simulated incidence of dog rabies in N'Djamena from 6 June 2012 to the end of October 2015. (B) Cumulative incidence of recorded human exposure to rabid dogs and simulated incidence of human exposure to rabid dogs in N'Djamena from 6 June 2012 to the end of October 2015.

Transmission model

The transmission model showed that between the two campaigns in 2012 and 2013, effective recorded vaccination coverage decreased from a peak of 67% (December 2012) (Léchenne et al. 2016a) to a trough of 33% (October 2013), assuming an exponential distribution for the persistence of immunity, which was estimated from 105 immunized dogs with repeated serological measurements. This represents a 51% relative coverage loss (Figure 4.2A). The model suggests that population replacement by the birth of susceptible dogs accounted for 29% of the relative coverage loss, whereas individual dog immunity loss accounted for 22% of this relative coverage loss. The effective reproductive number, R_e, decreased from the equilibrium value of 1 from the start of the first vaccination campaign and remained below 1 through November 2014, implying that the conditions for rabies virus persistence were not maintained since the start of the vaccination campaigns. Simulations of a deterministic ordinary differential equation (ODE) model (Figure 4.2B), fit to rabies case data from N'Djamena, and a stochastic extension (Figure 4.3A&B) suggested that transmission was interrupted by early 2013 onwards. Sensitivity analysis showed that simulation results were robust to our estimates of parameter values (Figure 4.4) and that under reporting of cases was unlikely to have a substantial effect on our results (Figure 4.5).



A



B

Figure 4.2: (A) Density of susceptible (blue lines) and vaccinated (red lines) dogs against time since 6 June 2012. The solid lines show the simulated values from an ordinary differential equation (ODE) transmission model from June 2012 to October 2015. (B) The effective reproductive number, R_e, and vaccination coverage against time. The solid red line shows the vaccination coverage and the solid blue line shows the effective reproductive number – both estimated from the ODE transmission model. The black line is the median R_e obtained from the phylogenetic sequencing data, with upper and lower 95% credible intervals as black dashed lines.

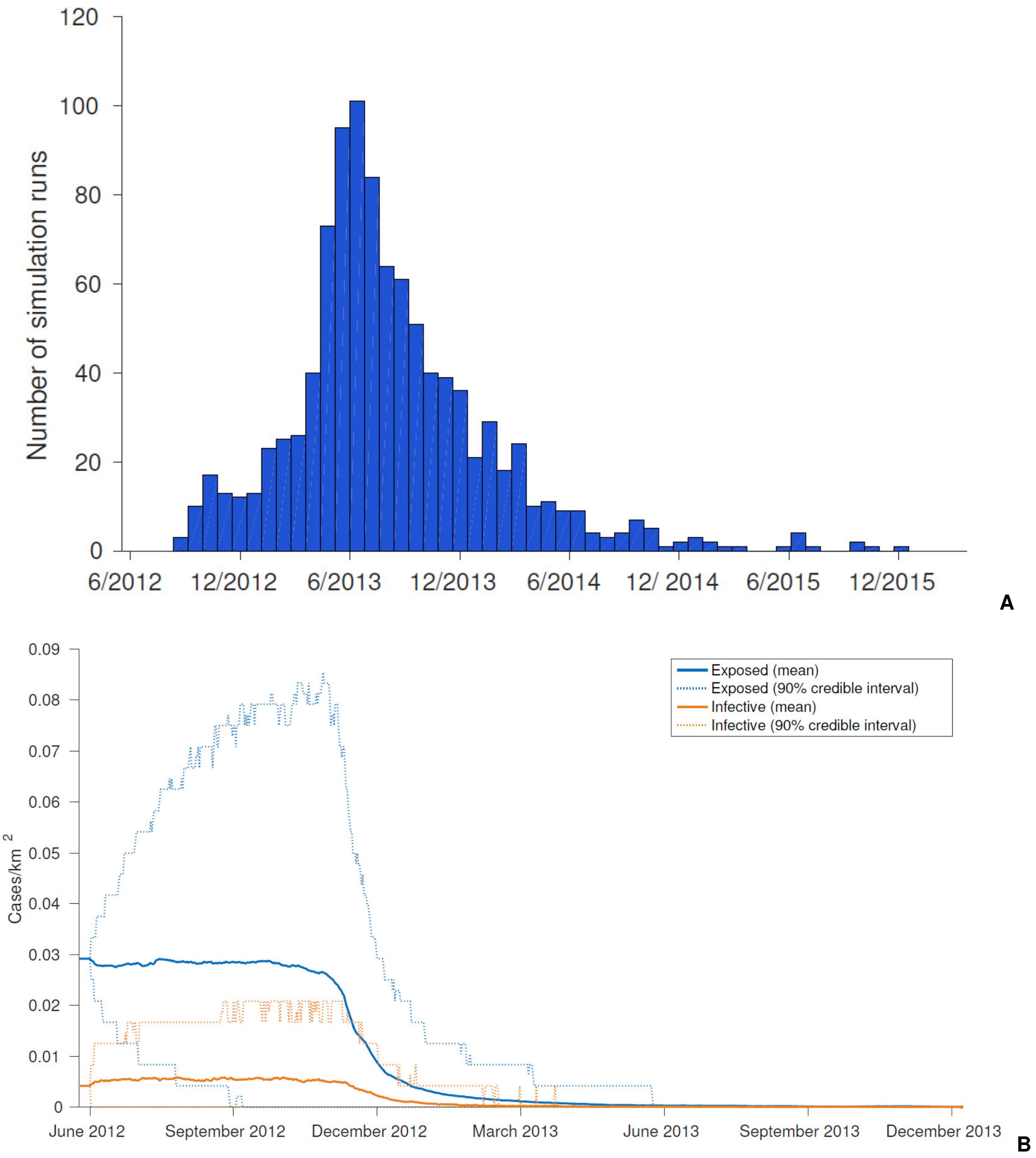


Figure 4.3: (A) Distribution of the simulated expected date of interruption of transmission from 1000 simulation runs of the stochastic model of dog rabies transmission. (B) Mean and 90% credible interval for exposed and infectious dogs from 500 runs of the stochastic model.

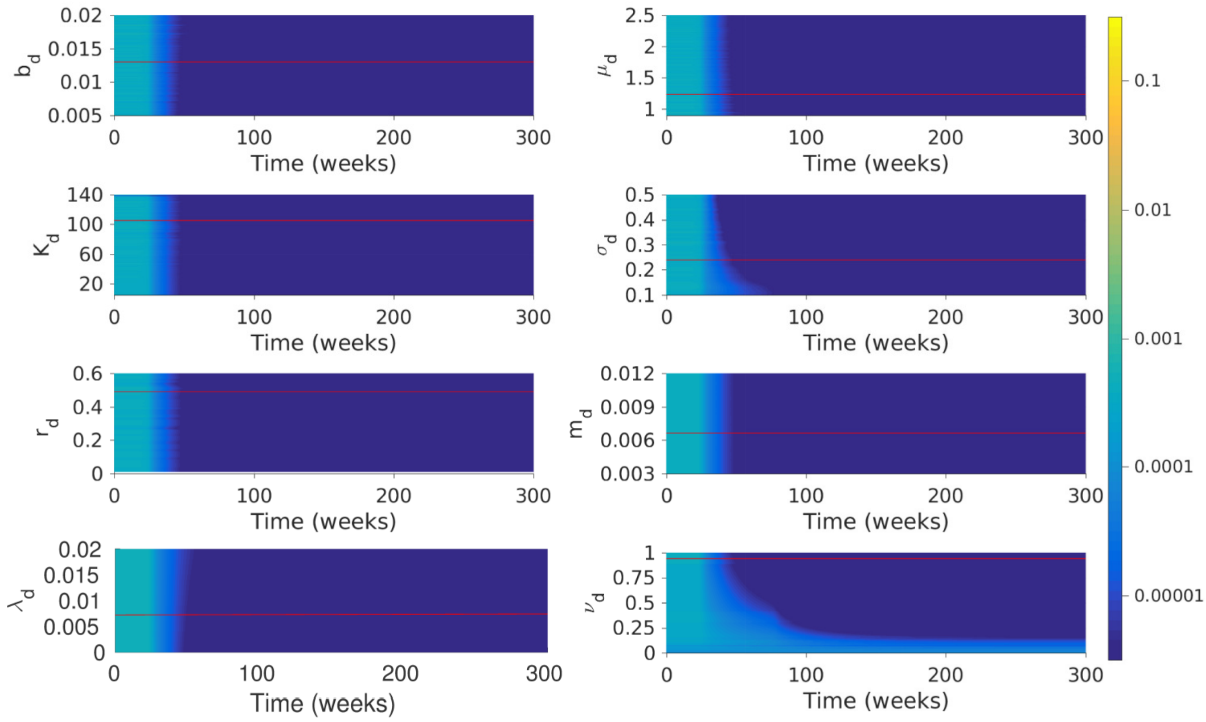


Figure 4.4: One-dimensional sensitivity analysis of simulation results on parameter values. The plots show simulations of the density of infectious dogs over 6 years (300 weeks) where all parameters are fixed at values described in Table 4.3 except for the parameter being varied and β_{dd}. The x-axis shows the time in weeks and y-axis shows the value of the parameter (in its corresponding units). The colour of each pixel represents the density of infectious dogs. The horizontal red lines correspond to the parameter values in Table 4.3 and the solution plotted in Figure 4.1A and Figure 4.2A.

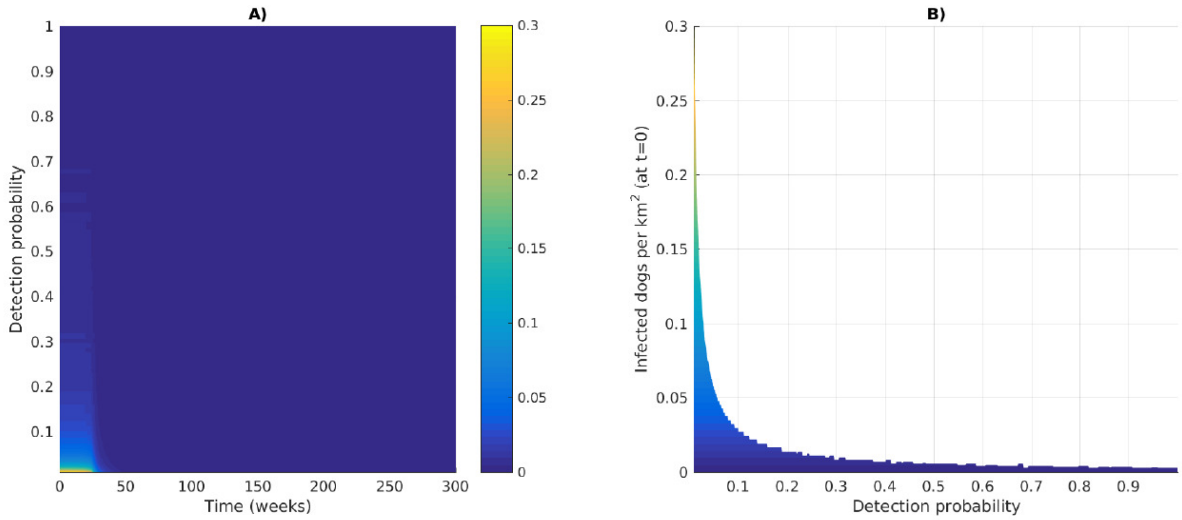


Figure 4.5: Sensitivity analysis of the simulation results on the probability of detecting rabid dogs. (A) The simulated density of infectious dogs depending on the detection probability of rabid dogs, p_d, over time. The x-axis corresponds to time (measured in weeks) and the y-axis to the detection probability. The colour of each pixel corresponds to the density of infectious dogs. (B) The endemic equilibrium value for density of infectious dogs (in the absence of vaccination campaigns) depending on p_d. The x-axis corresponds to to the detection probability, p_d and the y-axis (and colour of the pixel) correspond to the endemic equilibrium density of infectious dogs.

Phylodynamic Analysis

Phylogenies of 29 RAV genetic sequences of the N-protein collected between August 2011 and June 2013 and obtained as previously described (Talbi et al., 2009; Talbi et al., 2010) were analysed with Beast v2 (Bouckaert et al., 2014). Although the 95% highest posterior density intervals are wide because of the small number of sequences, the effective reproductive number estimated from the genetic data shows the same pattern as the R_e estimated from the incidence data (Figure 4.2B).

Discussion

The models and data presented here show that the period with no rabies transmission in N'Djamena is much longer after mass vaccination campaigns than without such campaigns, demonstrating that dog rabies virus transmission in an African city can be interrupted and consequently human rabies exposure can be prevented through mass vaccination campaigns of dogs. However, the duration of interruption of transmission in our study is shorter than our model predictions here and in earlier work (Zinsstag et al., 2009) indicating that there was likely to be a re-introduction of infection from wildlife or latent infected dogs from the adjacent rural areas, similar to a recent study on rabies transmission in Bangui (Bourhy et al., 2016). Our study suggests that urban centres are not hotspots of dog rabies transmission leading to spill over cases in rural areas as previously thought, but rather that the reverse is more likely to be true. Dog rabies transmission is ongoing in rural African areas and is likely to be continuously transmitted into urban areas through human mediated transport of dogs (Talbi et al., 2010). Sustainable elimination of dog rabies therefore requires action over a much larger geographical area and we have proposed a Development Impact Bond (DIB) financing scheme for dog rabies elimination in the entire country of Chad (Anyiam et al., 2016).

Our result was possible because a team of vaccinators reached the necessary coverage of dogs in two consecutive rounds of a mass vaccination free to dog owners (Dürr et al., 2008b). Costs were shared between the Chadian state and a private donor. A team of 33 vaccinating staff was sufficient to vaccinate up to 22,000 dogs in three months, although extensive information campaigns and the involvement of municipal authorities were additional key elements for successful implementation.

The effectively vaccinated surface area in our campaign of 240 km^2 (2012) was much lower than the 770 km^2 assumed in an earlier simulation (Zinsstag et al., 2009). The parameter estimates and threshold density of susceptible dogs based on the empirical data from this study are more realistic and provide an estimate for the basic reproductive number of $R_0 = 1.14$, instead of $R_0 = 1.01$.

There is still considerable uncertainty surrounding the role of density and spatial heterogeneity and external re-introduction in the transmission of dog rabies (Morters et al., 2013). A meta-population or contact network modelling approach may better represent the observed heterogeneity of the dog population in N'Djamena (Figure 4.6). Further research is needed to assess how dog density and the spatial heterogeneity of dog populations influences the dynamics of dog rabies elimination (Begon et al., 2002; Morters et al., 2013).

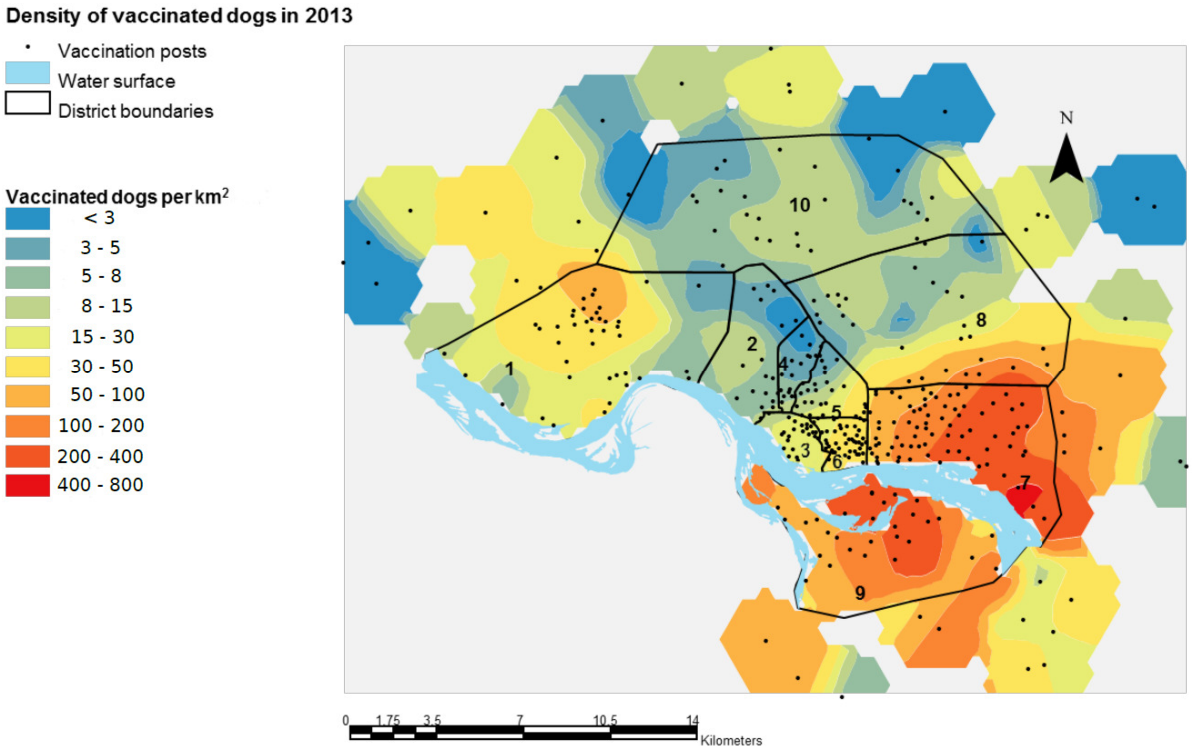


Figure 4.6: Density of vaccinated dogs in N'Djamena in 2013 calculated based on the data presented in Léchenne et al. 2016a. Black dots indicate the locations of the fixed vaccination posts. It is assumed that dogs diffuse from these locations after vaccination in a homogeneous way. We used a diffusion kernel prediction map with a bandwidth of 1040 m (which is the diameter of a circle of 0.86 km^2, the area per post of 331 posts in a total area of 285km^2). The water surface was included as a barrier function.

The results of our sensitivity analysis suggest that underreporting of rabies cases does not play a large role in the persistence of transmission. Even accounting for heterogeneity in underreporting, it is unlikely that unreported transmission persisted for nine months and more likely that a re-introduction occurred, either from wildlife or dogs from ongoing transmission outside the city of N'Djamena. Although our model did not include importation of infections, we hypothesise that the dog rabies cases seen in 2015 (Figure 4.1A) are due to imported cases (with subsequent local transmission) rather than sustained ongoing transmission from 2014. We suspect that domestic dogs from surrounding rural areas are the more likely source of reinfection than wildlife, because in a previous phylogenetic analysis of rabies strains in N'Djamena (Dürr et al., 2008a) only dog related strains of the lineage Africa 2 and no wildlife related strains were found.

Determining the optimal timing of vaccination campaigns in N'Djamena to maintain elimination would require better knowledge of the rabies importation rate into the city. Our results are in line with a recent study from Bangui (Central Africa) showing that rabies is continuously re-introduced in town by human related transport of dogs from surrounding rural areas (Bourhy et al., 2016) and rapidly dispersed between cities (Talbi et al, 2010). Therefore, dog rabies control in African cities should be planned for larger areas including suburban and rural areas and be coordinated regionally between neighbouring countries for an effective elimination of dog rabies in Africa (Hampson et al., 2007). In particular, movement of dogs with or without their owners should be restricted to limit the rapid dispersal of RABV. Dog mass vaccination campaigns should also be complemented by affordable compulsory dog registration. This study further supports the need for an improvement and a reinforcement of rabies surveillance in rural and more remote areas and therefore the development of rapid tests that could be used in a decentralized manner. The potential for satisfactory use of some of these tests in remote locations is very high to improve the global knowledge of rabies epidemiology (Lechenne et al., 2016b).

Contrary to a previous editorial (Durrheim et al., 2015), this study suggests that mass vaccination of dogs, coupled with Post-Exposure Prophylaxis, can be sufficient to eliminate rabies transmission in an African city – both in humans and dogs if it is done in a larger area. In the long term, eliminating the infectious reservoir in dogs will be more cost-effective than perpetual Post- or Pre-Exposure Prophylaxis in humans. Clearly this will require a regional approach similar to the well-coordinated dog rabies control efforts between Latin American countries (Hampson et al., 2007). The recent creation of the Pan African Rabies Control Network (PaRaCON, paracon.rabiesalliance.org) is thus an important step towards the goal of dog rabies elimination in Africa by 2030.

Materials and Methods

Surveillance of dog rabies and human exposure

Passive routine dog rabies surveillance started on 4 June 2012 and is currently ongoing in N'Djamena at IRED by standard immunofluorescence as described in (Kayali et al., 2003a; Zinsstag et al., 2009). Prior to the mass vaccination campaign, the average weekly incidence of dog rabies was of 0.33 dogs per 10,000. For every laboratory-confirmed rabid dog, on average 1.6 humans were reported to be exposed (from questioning the dog owner) leading to a weekly incidence of 0.11 per 100,000 people.

Dog rabies mass vaccination campaign 2012 and 2013

A citywide mass dog vaccination campaign including all 10 districts of N'Djamena took place in 2012 and was repeated in 2013. In both campaigns, the objective was to vaccinate 70% of the total dog population of N'Djamena with the dog rabies vaccine rabisin^{tm} (Merial Inc. Lyon, France). The vaccination campaigns began in the first week of October 2012 and 2013 and lasted for a total of 13 weeks until the first week of January of the next year. Vaccination took place only on Friday to Sunday due to availability of staff and participation of the public during these days (as evaluated in previous studies) (Kayali et al., 2003b). Every Friday to Sunday, ten fixed post vaccination teams were set up in one of 12 (13 in 2013) areas of the city corresponding to administrative boundaries. Over the three day period, these teams vaccinated on average 1,433 (min. 24; max. 6,460) dogs in 2012 and 1,709 dogs (min. 67; max. 4,591) dogs in 2013, depending on the socio-cultural and ecological context of the city district (Table 4.1). Details of the operational performance and the results of the vaccination campaigns are published elsewhere (Léchenne et al., 2016a).

Table 4.1: Number of dogs vaccinated in each week of the vaccination campaigns. The campaign in 2012 started on 8 October 2012 (week 19) and in 2013 started on 30 September 2013 (week 70), as described in (Léchenne et al., 2016a).

Vaccination Week	Vaccinated dogs (2012)	Vaccinated dogs (2013)
1	834	722
2	181	468
3	376	330
4	24	434
5	793	67
6	2901	1173
7	6460	928
8	1393	4215
9	3074	4372
10	1698	3424
11	311	4591
12	385	979
13	209	525

Coverage assessment

A coverage assessment was carried out each week after vaccination in the previously vaccinated area. Vaccination zones and their analysis perimeter corresponded in most cases to a district. The coverage assessment was composed of a household survey in randomly selected geographical locations within the analysis perimeter to estimate the proportion of owned vaccinated dogs. In addition random transects drives were carried out in the same zone to estimate the dog density in the street and the proportion of ownerless dogs. Data from both studies were then combined in one Bayesian statistical model as reported elsewhere (Léchenne et al., 2016a).

Description of mathematical model of dog-dog and dog-human transmission

We use a deterministic population based model of ordinary differential equations (odes) extended from a previously published model for dog to dog rabies transmission (Zinsstag et al., 2009),

$$\frac{DS_D(t)}{Dt} = B_D N_D(T) + \Lambda_D V_D(T) - R_D B_{Dd} S_D(T) I_D(T) - \left(N_D A_D(T) + M_D + \Gamma_D N_D(T)\right) S_D(T), \quad (1a)$$

$$\frac{DE_D(t)}{Dt} = R_D B_{Dd} S_D(T) I_D(T) - \left(\Sigma_D + N_D A_D(T) + M_D + \Gamma_D N_D(T)\right) E_D(T), \quad (1b)$$

$$\frac{DI_D(t)}{Dt} = \Sigma_D E_D(T) - \left(M_D + M_D + \Gamma_D N_D(T)\right) I_D(T), \quad (1c)$$

$$\frac{DV_D(t)}{Dt} = N_D A_D(T)\left(S_D(T) + E_D(T)\right) - \left(\Lambda_D + M_D + \Gamma_D N_D(T)\right) V_D(T), \quad (1d)$$

Where the state variables and parameters are defined in Tables 4.2 and 4.3 respectively.

The total dog population size is,

$$N_D(T) = S_D(T) + E_D(T) + I_D(T) + V_D(T), \quad (2)$$

And the density dependent death rate is,

$$\Gamma_D = \frac{B_D - M_D}{K_D}, \quad (3)$$

Where K_D Is described in Table 4.3 and B_D Is required to be greater than M_D. We note here that we assume density-dependent transmission and that in general, (1) is a non-autonomous model where $A_D(t)$ varies with time,

$$A_D(T) = A_D^* + A_0^{(I)}(T) + A_1^{(I)}(T)E^{-\varphi t}, \quad (4)$$

Where A_D^* Is the (assumed) constant background vaccination rate, $A_0^{(i)}(t)$ and $A_1^{(i)}(t)$ are campaign-dependent vaccination values for the i^{th} week, and φ is a saturation parameter. Outside of the campaigns, $A_D(t) = A_D^*$. We further restrict the values of $A_0^{(i)}$ And $A_1^{(i)}$ To ensure that $A_D(t)$ is continuous so that the system for rabies transmission (1) has a unique solution that exists for all time.

We similarly use an ODE model for dog to human transmission based on (Zinsstag et al., 2009) ,

$$\frac{DS_H(t)}{Dt} = B_H N_H(T) - B_{Hd} S_H(T) I_D(T) + A_H E_H(T) - M_H S_H(T), \quad (5a)$$

$$\frac{DE_H(t)}{Dt} = B_{Hd} S_H(T) I_D(T) - (A_H + \Sigma_H + M_H) E_H(T), \quad (5b)$$

$$\frac{DI_H(t)}{Dt} = \Sigma_H E_H(T) - (M_H + M_H) I_H(T), \quad (5c)$$

Where the total human population size is,

$$N_H(T) = S_H(T) + E_H(T) + I_H(T), \quad (6)$$

Σ_H Is the rate of progression from the exposed to the infectious state depending on the site of the bite,

$$\Sigma_H = \frac{P_2 P_6}{I_{Head}} + \frac{P_3 P_7}{I_{Arm}} + \frac{P_4 P_8}{I_{Trunc}} + \frac{P_5 P_9}{I_{Leg}}, \quad (7)$$

A_H Is the abortive rate of progression from the exposed back to the susceptible state,

$$A_H = \frac{P_2(1-P_6)}{I_{Head}} + \frac{P_3(1-P_7)}{I_{Arm}} + \frac{P_4(1-P_8)}{I_{Trunc}} + \frac{P_5(1-P_9)}{I_{Leg}}, \quad (8)$$

And the probabilities of biting different parts of the body (P_2 Through P_5), the probabilities of subsequent progression to rabies (P_6 Through P_9), and the average time to do so ($1/I_\Xi$ Where ξ is head, arm, trunk or leg), are described in more detail in the previous formulation of the model (Zinsstag et al., 2009) .

Figure 4.7 shows a schematic of the model system. We note that the dynamics for rabies transmission in humans is dependent on rabies transmission in dogs but the transmission in dogs is independent of transmission in humans.

Table 4.2: State variables of dog rabies transmission model.

$S_d(t)$:	Susceptible dog density at time t
$E_d(t)$:	Exposed dog density at time t
$I_d(t)$:	Rabid dog density at time t
$V_d(t)$:	Vaccinated dog density at time t
$S_h(t)$:	Susceptible human density at time t
$E_h(t)$:	Exposed human density at time t
$I_h(t)$:	Rabid human density at time t

Table 4.3: Parameters of the rabies transmission model with estimated values and sources. Most parameters have the same value as in the previous model (Zinsstag et al., 2009), but some have been updated from more recent publications or from new data from the current study (as described in the section on parameter estimation).

Symbol	Description	Source	Value	Unit
b_d	Dog birth rate	Household survey	0.013	week^{-1}
m_d	Dog natural death rate	Household survey	6.64×10^{-3}	week^{-1}
K_d	Dog carrying capacity	Estimated from dog population size and area from coverage survey 2012	105	animals km^2
β_{dd}	Dog to dog transmission rate	Estimated for this study	0.0292	km^2 week^{-1} animals^{-1}
σ_d	Dog rate of progression from exposed to infectious state	Previous model (Zinsstag et al., 2009)	0.239	week^{-1}
r_d	Probability of exposed dog developing rabies	Previous model and (Hampson et al., 2009)	0.49	1
μ_d	Death rate dues to rabies in dogs	Previous model	1.23	week^{-1}
α_d^*	Background dog vaccination rate	Estimated for this study	2.96×10^{-3}	week^{-1}

ν_d	Efficacy of vaccine in dogs	Previous model	0.94	1
λ_d	Rate of loss of vaccine efficacy in dogs	Estimated assuming exponential decay based on data from 105 dogs vaccinated during the campaign in 2012.	5.5×10^{-3}	week^{-1}
b_h	Human birth rate	Previous model	$7.6 \times 10 - 4$	week^{-1}
m_h	Human mortality rate	Previous model	2.9×10^{-4}	week^{-1}
β_{hd}	Dog to human transmission rate	Estimated for this study	2.34×10^{-5}	week^{-1} animals^{-1}
σ_h	Human rate of progression to rabies	Calculated with parameter values from the probability tree of the location of human exposure (Zinsstag et al., 2009) using equation (7)	0.0251	week^{-1}
a_h	Human abortive rate of progression back to susceptible	Calculated with parameter values from the probability tree of the location of human exposure (Zinsstag et al., 2009) using equation (8)	0.0967	week^{-1}
μ_h	Death rate due to rabies in humans	Previous model	1	week^{-1}

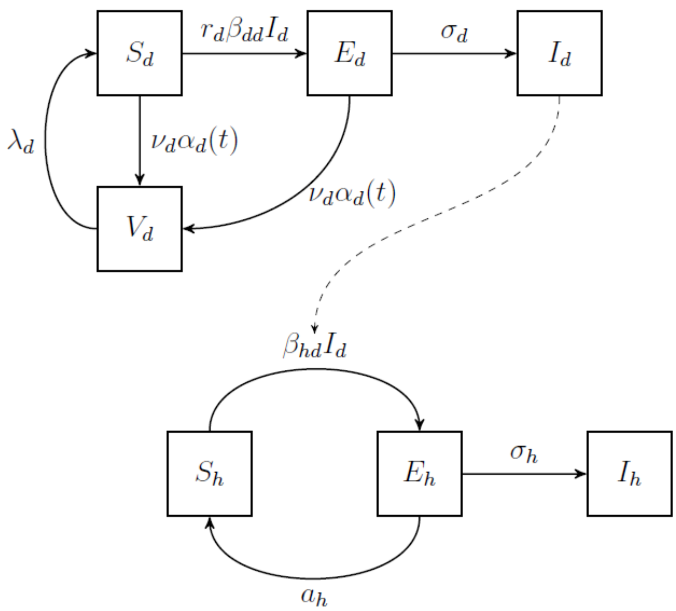


Figure 4.7: Schematic of mathematical model of rabies. Birth and death rates of humans and dogs are not shown.

Mathematical analysis

In the absence of vaccination campaigns ($A_D(t) = A_D^*$), the autonomous mathematical model for rabies transmission in dogs (1) has a trivial disease-free equilibrium point,

$$S_D = \frac{(B_D + \Lambda_D)K_D}{B_D + \Lambda_D + N_D A_D^*}, \quad (9a)$$

$$E_D = 0, \quad (9b)$$

$$I_D = 0, \quad (9c)$$

$$V_D = \frac{N_D A_D^* K_D}{B_D + \Lambda_D + N_D A_D^*}. \quad (9d)$$

The control reproductive number for the dog rabies model is the number of dogs that one newly introduced rabid dog would infect, assuming no disease in the population (with only background vaccination),

$$R_C = \frac{R_D B_{Dd} \Sigma_D K_D}{(\Sigma_D + N_D A_D^* + B_D)(M_D + B_D)}. \quad (10)$$

We omit the mathematical details here but can show that with only background vaccination (no vaccination campaigns) when $R_C < 1$, the disease-free equilibrium point (9) is locally asymptotically stable and when $R_C > 1$, the disease-free equilibrium point is unstable and there exists a locally asymptotically stable endemic equilibrium point where rabies persists in the population. Additionally, if there is no background vaccination, the control reproductive number reduces to the basic reproductive number,

$$R_0 = \frac{R_D B_{Dd} \Sigma_D K_D}{(\Sigma_D + B_D)(M_D + B_D)}.$$

At any time, t, allowing for vaccination campaigns, the effective reproductive number, $R_E(t)$, represents the expected number of new infections caused by one infectious dog,

$$R_E(t) = \frac{R_D B_{Dd} \Sigma_D S_D(t)}{(\Sigma_D + N_D A_D(t) + M_D + \Gamma_D N_D(t))(M_D + M_D + \Gamma_D N_D(t))}. \quad (11)$$

If we assume that the total dog population is at carrying capacity (which is reasonable because the density of rabid dogs is low so has a minimal impact on the population density of dogs), the effective reproductive number simplifies to,

$$R_E(t) = \frac{R_D B_{Dd} \Sigma_D S_D(t)}{(\Sigma_D + N_D A_D(t) + B_D)(M_D + B_D)}. \quad (12)$$

The threshold density of susceptible dogs at which transmission occurs, S_D^* Is the density at which $R_E = 1$. From (12), outside of vaccination campaigns this is,

$$S_D^* = \frac{(\Sigma_D + N_D A_D^* + B_D)(M_D + B_D)}{R_D B_{Dd} \Sigma_D}. \quad (13)$$

The threshold vaccination coverage reached in a campaign to eliminate transmission, Ψ^*, is given by,

$$\Psi^* = 1 - \frac{1}{R_C}, \quad (14)$$

When background vaccination takes place outside the campaign. Equivalently this is,

$$\Psi^* = \frac{K_D - S_D^*}{K_D}. \tag{15}$$

After the vaccination campaigns, the coverage of protected dogs decreases exponentially due to population loss of susceptible dogs (proportionally $B_D/(\Lambda_D + B_D)$: 57% for parameter values in Table 4.3) and due to loss of vaccine efficacy (proportionally $\Lambda_D/(\Lambda_D + B_D)$: 43% for parameter values in Table 4.3).

Parameter estimation

The values for most parameters are taken from the previous model (Zinsstag et al., 2009) except where new published results or new data have allowed for revised values. The parameter values and their sources are summarised in Table 4.3. The birth and death rates of dogs were calculated as in previous work but the carrying capacity of dogs was revised to reflect a total population of 25,103 dogs in an area of 240km^2 as estimated in the 2012 coverage assessment. The vaccination rate of dogs and the transmission rates from dogs to dogs and dogs to humans were estimated as described below.

Dog vaccination rate

The baseline study found that 12% of all owned dogs had antibodies (so could be considered to be effectively vaccinated) implying that there was some ongoing background vaccination outside of the two campaigns conducted in 2012 and 2013. The coverage assessment estimated that for every 10 owned dogs, there was one unowned dog. Assuming that the background vaccination rate was constant and the proportion of vaccinated dogs was at equilibrium (9), with demographic and other vaccination parameters as in Table 4.3, the per capita background vaccination rate was 2.96×10^{-3}/week.

The number of dogs vaccinated (and marked as vaccinated) in each campaign is shown in Table 4.1. We estimated the vaccination rate parameters, $A_0^{(i)}$ And $A_1^{(i)}$ Using a simple model of vaccination for each campaign,

$$\frac{DU^{(i)}}{Dt} = B_D\left(K_D - U^{(i)}\right) - \overline{A}_D^{(I)}(T)U^{(I)}, \tag{16a}$$

$$\frac{DV^{(i)}}{Dt} = \overline{A}_D^{(I)}(T)U^{(I)}, \tag{16b}$$

Where $U^{(i)}$ Is the density of all unmarked dogs, $V^{(i)}$ Is the density of dogs marked in campaign week i, and $\overline{A}_D^{(i)}(t)$ is the rate of marking dogs during campaign week i. We define time, t, as varying from 0 at the start of each campaign week to 1 at the end of each campaign week.

The coverage assessment could only determine whether dogs were marked as vaccinated or not and did not determine the immune status of dogs. We therefore ignore the efficacy of the vaccination and do not consider background vaccination because these dogs would not be marked as campaign-vaccinated dogs, so

$$\overline{A}_D^{(I)}(T) = A_0^{(I)}(T) + A_1^{(I)}(T)E^{-\varphi t}. \quad (17)$$

For simplicity we ignore rabies transmission, assume the dog population is at carrying capacity and ignore the death of marked dogs or the loss of marking collars during the campaign week. Since the coverage assessment was conducted within three days of the vaccination campaign, these assumptions are reasonable. We assume that the markings from the 2012 campaign do not last until 2013 so for both campaigns, the initial density of unmarked dogs is equal to the carrying capacity,

$$U^{(1)}(0) = K_D, \quad (18a)$$

And from continuity,

$$U^{(i)}(0) = U^{(i-1)}(1) \quad \text{for } i > 1. \quad (18b)$$

The initial density of dogs marked during a campaign week is zero,

$$V^{(I)}(0) = 0 \quad \text{for } i \geq 1. \quad (18c)$$

We fix $\varphi = 100$. To ensure that $A_D(t)$ is continuous, we set

$$A_0^{(1)} + A_1^{(1)} = 0, \quad (19a)$$

$$A_0^{(i)} + A_1^{(i)} = A_0^{(i-1)} + A_1^{(i-1)}E^{-\varphi} \quad \text{for } i > 1. \quad (19b)$$

The final density of dogs marked in a campaign week, $V^{(i)}(1)$ is set equal to the number of marked dogs estimated from the coverage assessment for that week (Table 4.1) divided by the campaign area for that year (240km^2 in 2012 and 285km^2 in 2013). Condition (19) and the odes for the vaccination model (16) with its boundary conditions provide two sets of equations for each campaign week. For other parameter values as provided in Table 4.3, we numerically simulate the vaccination model using an adaptive step-size Runge-Kutta method (ode 45) and then use a root-finding algorithm (fzero) to calculate $A_0^{(i)}$ And $A_1^{(i)}$ (in MATLAB, version 8.5) for each campaign week. Figure 4.8 shows the final estimated vaccination rates during the two campaigns.

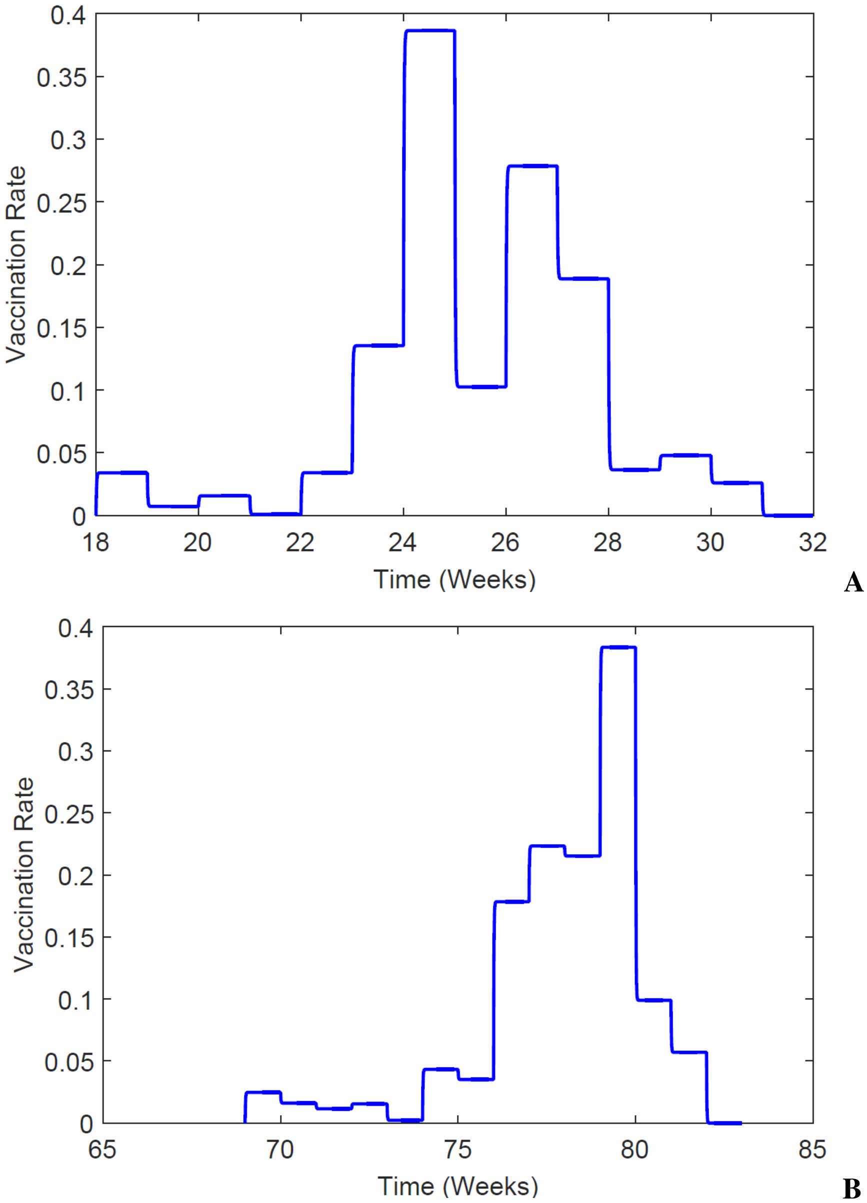


Figure 4.8: Vaccination rates during the two campaigns in N'Djamena, Chad A) in 2012 and B) in 2013. The weeks are labelled starting from 4 June 2012.

Rate of loss of vaccine immunity

In 2012, before the vaccination campaigns were conducted, a total of 105 dogs in N'Djamena were tested for antibody titers, vaccinated and then followed up over a period of one year. Of these dogs, 58 had initial antibody titers that showed no previous vaccination and were successfully followed up over the entire year. After one year, 44 dogs had antibody titers above 0.5 IU, which, as a conservative estimated, we considered as protective (Moore and Hanlon, 2010). We calculated the rate of loss of vaccine decay, Λ_D, assuming exponential decay and a relative value of 0.76 after 52 weeks.

Rabies transmission rates

The number of rabid dogs and exposed humans recorded per week since 4 June 2012 are shown in the additional file rabiesdata1.txt. Recording of both human and dog cases is ongoing but the analysis only included cases until the end of October 2015. We divide the numbers of dogs and humans by the area estimated in the coverage assessment of the 2012 vaccination campaign (240 km^2) to provide the densities of rabid dogs and exposed humans.

We first fit the dog to dog transmission rate, B_{Dd}, for the model with transmission only in dogs (1) with the data for the number of rabid dogs with other parameter values as described in Table 4.3 and the vaccination rate as described above. We then use the this value for B_{Dd} To estimate the dog to human transmission rate, B_{Hd} , for the full model with transmission between dogs (1) and to humans (5) with the data for number of exposed humans.

To fit B_{Dd}, we numerically simulate (1) using an adaptive step-size Runge-Kutta method and minimise the Euclidean distance between the simulated incidence of infectious dogs (from the first term of the right hand side of (1c)), and the observed weekly incidence of infectious dogs in MATLAB. We assume that the probability of detecting a rabid dog, $P_D = 0.5$ so that on average there were twice as many rabid dogs as those detected. There is little data on this parameter but our sensitivity analysis showed that unless P_D Is very low, the estimated values for B_{Dd} Did not change much (Figure 4.5). We assume that the initial condition for the odes in June 2012 is at the unique endemic equilibrium (with $A_D(t) = A_D^*$) and the dog density is at carrying capacity.

We similarly fit B_{Hd} By numerically simulating (1) and (5) and minimising the Euclidean distance between the simulated density of exposed humans, $E_H(t)$, and the observed density of exposed humans on a weekly time step in MATLAB. Here we assume perfect detection of exposed humans ($P_H = 1$) and that the initial condition in June 2012 is at the unique endemic equilibrium with a population density of humans of 4833 humans/km^2 (from a total population size of 1.16 million in 2012 estimated from the 2011 population size of 1.079 million using a growth rate of 7.5%) (INSEED, 2009; Indexmundi, 2015).

Sensitivity analysis

We conducted local and global sensitivity analysis of the control reproductive number, R_C, (10) to the model parameters (Supplementary Figure S4.1). We used the normalized forward sensitivity index for the local analysis (Chitnis et al., 2008; Arriola, 2009) at the parameter values defined in Table 4.3 and the partial rank correlation coefficients for the global analysis (Wu et al., 2013), assuming all parameters were uniformly distributed in the intervals: $R_D \in [0.049,1]$, $B_{Dd} \in [0.00292,0.0614]$, $K_D \in [10.5,221]$, $\Sigma_D \in [0.0239,0.504]$, $B_D \in [0.0013,0.0273]$, $M_D \in [0.123,2.59]$, $N_D \in [0.094,1]$ and $A_D^* \in [0.000296,0.00622]$. Both the local and global analysis showed that the probability of developing rabies, R_D, the transmission rate, B_{Dd}, the carrying capacity, K_D And the rabies induced mortality rate, M_D, had a strong impact on the threshold for sustained transmission, R_C, while the other parameters had minimal impact.

Figure 4.4 shows simulation results of the density of infectious dogs over 6 years allowing for uncertainty in each of the parameter values (varied one at a time). In each simulation run, the dog transmission rate, B_{Dd}, was refitted for that set of parameter values. The results were robust to uncertainty in the parameter values and except for low vaccine efficacy values, the simulations predicted that transmission would be interrupted after the first campaign.

Supplementary Figure S4.2 shows a similar sensitivity analysis of the simulated number of infectious dogs but with a fixed value for the dog transmission rate, B_{Dd}, estimated from the baseline set of parameter values (Table 4.3). The ranges of the parameter values are greater than in Figure 4.4 and the results show the importance of that parameter on the expected number of rabid dogs over time. Most parameters have little effect, but similar to the sensitivity analysis for R_C, high values for the carrying capacity of dogs and the probability of an exposed dog developing rabies and low values for the rabies induced death rate lead to a high number of infectious dogs.

Figure 4.5 shows the simulated densities of infectious dogs and exposed humans depending on the probability of detection of infectious dogs, P_D, and of exposed humans, P_H, used to fit B_{Dd} And B_{Hd}, respectively. Low values of these detection probabilities result high in higher numbers of infectious dogs and exposed humans, leading to higher estimates for the dog to dog, B_{Dd}, and dog to human, B_{Hd}, transmission parameters. The results indicate that the simulation results are robust to these detection probabilities unless the probabilities are very low.

Stochastic model simulations

We derived and numerically simulated a stochastic dog to dog transmission model based on (1), with the master equation,

$$\frac{Dp(n,t)}{Dt} = \sum_{i}[W_I(n|M_I)P(M_I,t) - W_I(M_I|n)P(n,t)], \qquad (20)$$

Where n is any state of the system at time t and W_I Are the transmission rates deduced from the parameters in Table 4.3, using the Gillespie algorithm with the tau-leaping simulation method (Gillespie, 1977; Cao, 2006). Supplementary Figure S4.3 shows a sample stochastic simulation of the density of exposed and infectious dogs with the corresponding simulation of the deterministic model and the underlying data for the number of infectious dogs. Figure 4.3B shows that the mean of 500 simulation runs of the stochastic model declines after the first vaccination campaign in a similar manner to the deterministic model.

Phylogenetic analysis

The 29 sequences of rabies viruses from dogs, collected between August 2011 and June 2013 were analysed with Beast v2 (Bouckaert et al., 2014). We chose a Hasegawa-Kishino-Yano (HKY) model for substitutions with a relaxed log-normal clock (Drummond et al., 2006). We assumed an exponential (0.001) prior for the mean rate, an exponential (0.3333) prior for the standard deviation, and a log-normal $(1,1.25)$ prior for kappa.

For the epidemiological model, we chose the birth-death skyline model (Stadler et al., 2013). We used a log normal $(0,1)$ prior for the effective reproductive number, R_E, and allowed R_E To change in January 2013, August 2012, and April 2012, i.e. Every 4.8 months prior to the last sample in June 2013. We assumed a uniform prior on interval $(9.44,9.5)$ for the removal rate (corresponding to an expected infected time (exposed and active rabies) between $(1/9.5,1/9.44)$ years, which is about 1.1 months. The sampling probability of a rabid dog was assumed to be 0 prior to the first sample, and uniform on $(0.4,0.6)$ between the first and last sample. The time of the initial case in that transmission chain was assumed to be a uniform prior on $(0,20)$, prior to the most recent sample. We ran the Markov chain Monte Carlo (MCMC) simulations for 10^9 Steps. We neglected the first 10% of the states as a burn-in period. The effective sample size of all parameters was 350 or higher, implying that we obtained very good mixing.

In order to investigate sensitivity towards our assumption of a constant sampling proportion, we performed a second analysis allowing the sampling proportion to change at the same time points as when the R_E Changes. As above, sampling was assumed to be 0 prior to the oldest sample. Further sampling was assumed to be uniform on $(0.2,0.6)$ in each interval compared to uniform on $(0.4,0.6)$ above. As shown in Supplementary Figure S4.4 the results do not change qualitatively.

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Supplementary material

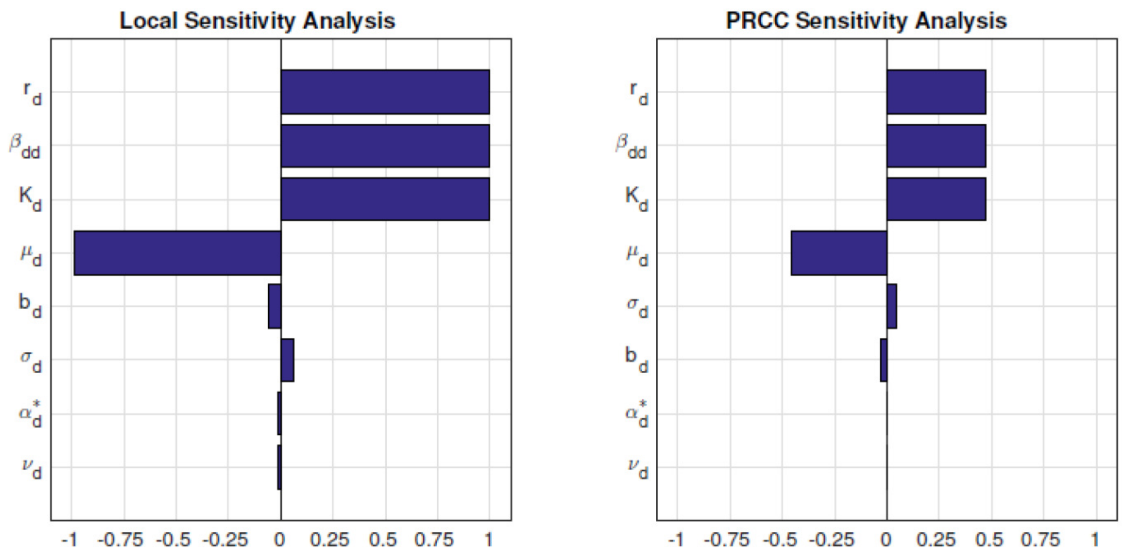

Figure S4.1: Local and global sensitivity indices of the control reproductive number, R_c, to the model parameters.

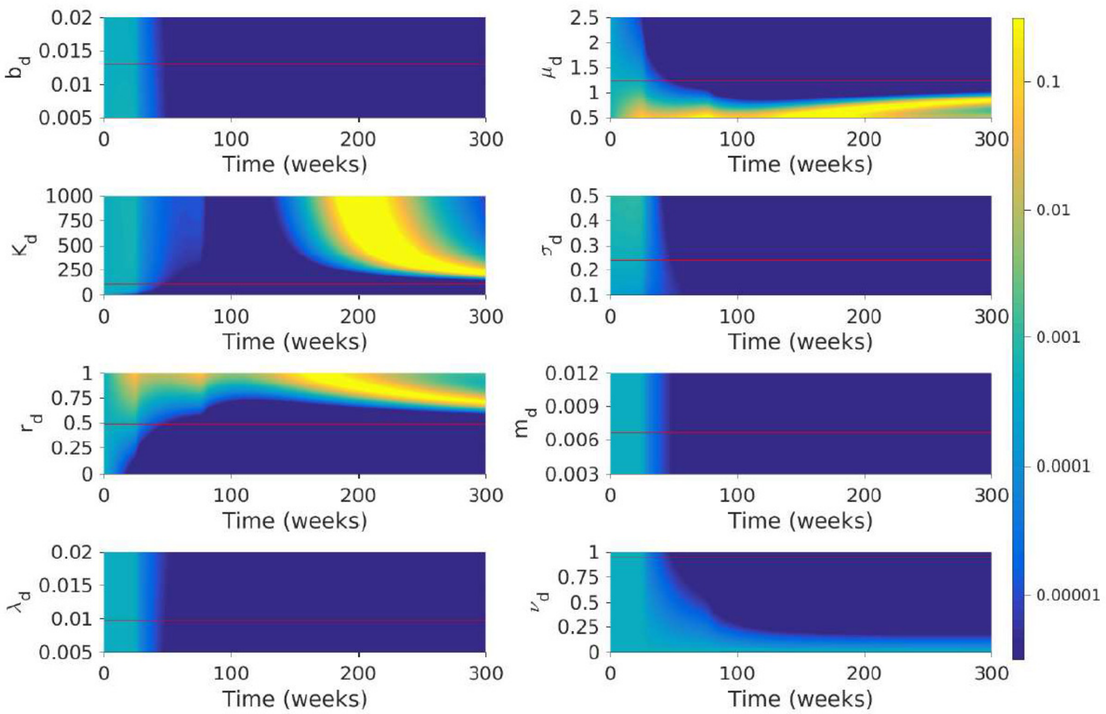

Figure S4.2: One-dimensional sensitivity analysis of simulation results on parameter values. The plots show simulations of the density of infectious dogs over 6 years (300 weeks) where all parameters are fixed at values described in Table 4.3 except for the parameter being varied (β_dd is fixed at 0.0292). The x-axis shows the time in weeks and yaxis shows the value of the parameter (in its corresponding units). The colour of each pixel represents the density of infectious dogs. The horizontal red lines correspond to the parameter values in Table 4.3 and the solution plotted in Figure 4.1A and Figure 4.2A.

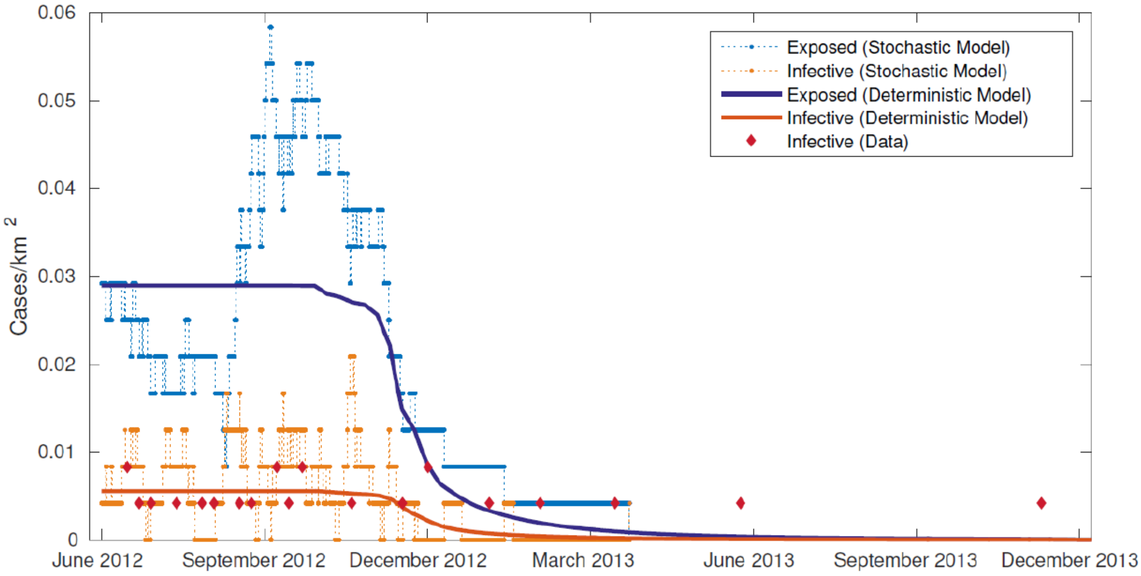

Figure S4.3: Sample simulation of the stochastic model showing the density of exposed and infectious dogs with the simulation results of the deterministic model and the observed number of infectious dogs.

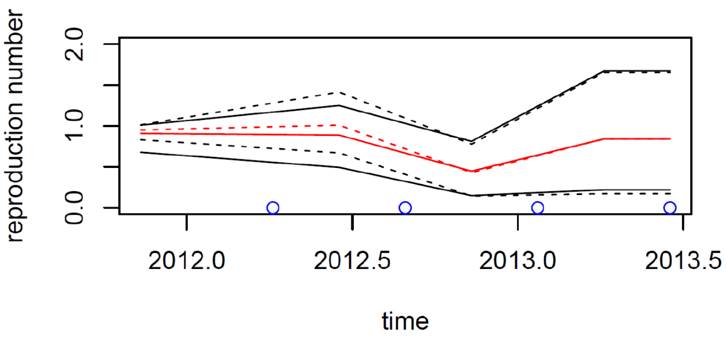

Figure S4.4: Results of the phylodynamic analysis showing median (red) and 95% HPD interval (black) for R_e through time. Solid lines correspond to the constant sampling proportion assumption, and dashed lines to the changing sampling proportion assumption. Blue points indicate the change of R_e and sampling proportion. We plot the R_e estimate for each interval at the midpoint of the interval, and interpolate linearly in between.

CHAPTER 5

Rabies awareness and dog ownership among rural northern and southern Chadian communities - Analysis of a community-based, cross-sectional household survey

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Abstract

Canine rabies represents a major – but preventable – public health threat in Chad. In preparation for a nation-wide canine parenteral mass vaccination campaign, we conducted a community-based, cross-sectional multi-stage cluster survey in 40 villages in two southern and two northern regions of Chad. Our objective was to investigate rabies awareness and dog-ownership among the rural population.

Almost half of the households (45%) owned dogs, with an overall dog:human ratio of 1:7.8. Southern households owned almost two thirds (701/918) of all dogs and the number of dogs per household was twice as high when compared to the north (2.7 vs. 1.3, respectively). This translates into a dog:human ratio of 1:5.2 in the south and 1:16.4 in the north. Only 76% of the respondents had heard of rabies. Respondents who (1) were male, (2) >19 years, (3) had primary education or higher and (4) were of Christian or "other" (Animist, no religion or undefined religion) faith were more likely to have heard of rabies. A high level of knowledge was positively associated with (1) southern residence, (2) any kind of education and (3) Christian or "other" religions. In contrast to rabies awareness, high level of knowledge was negatively associated with increasing age.

Eleven % of respondents reported that at least one family member had been bitten by a dog in the past year and half of these bite victims were children. Thirty-one % of respondents knew someone who had died of rabies and twice as many (58%) reported having encountered a rabid animal. Most of the respondents could identify classical rabies symptoms, however they lacked knowledge about rabies prevention and appropriate wound management. Only 2 out of 963 (0.5%) reported to have vaccinated their dog.

A major proportion of our study population is at great risk of rabies due to lack of awareness of the disease, inappropriate post-bite treatment and insufficient knowledge about preventive measures. This reflects the urgent need for advocacy programs to raise rabies awareness among the community. Close intersectoral collaboration between the public health and veterinary sector and integration of local authorities is a key element in the fight against rabies

Keywords: rabies, awareness, dog:human ratio, Chad, rural, community

Introduction

Although canine rabies has been eliminated in vast parts of the western world, it remains endemic and neglected in resource-limited countries. An estimated 59'000 people die of rabies every year with the majority of victims occurring in Asia (59.6%) and Africa (36.4%) (Hampson et al., 2015). Rabies in humans (Cleaveland et al., 2002) and dogs (Kitala et al., 2000) is greatly under-reported in Africa. In a study conducted by Cleaveland et al. (2002) in Tanzania, the true number of human rabies cases was 10 to 100 times greater than officially reported.

To date, no effective therapy to cure patients who have developed clinical symptoms has been established (Jackson, 2013a) and recovery after treatment of clinical rabies occurs only in very rare cases, usually with permanent severe neurological damage (Madhusudana et al., 2002; Willoughby et al., 2005). The administration of immediate post-exposure prophylaxis (PEP) – consisting of bite wound cleansing and injection of rabies vaccine and immunoglobulins - after exposure to rabies can prevent the onset of the disease (WHO, 2013). More than 15 million people require PEP annually (WHO, 2013). However, in many African countries the availability of PEP is limited and often not affordable for a large part of the population (Dodet et al., 2008). Moreover, administration of PEP alone is insufficient to interrupt the transmission cycle between animals and humans. Only interventions targeted at the host species will eliminate rabies in the dog population and eventually stop transmission to humans. Zinsstag et al. (2009) concluded that the most cost-effective intervention strategy to eradicate rabies is the combination of parenteral dog mass vaccination campaigns and PEP. The authors have used a dog-human mathematical transmission model to show that after 5 years a break-even in costs could be achieved compared with costs of PEP alone.

In Chad, rabies is an endemic disease and, thus represents a major – but preventable – public health threat. The human rabies mortality is estimated to be 7 per million per year in N'Djamena, the capital of Chad, alone (Frey. et al. 2013). A partnership between the Chadian authorities and the Swiss Tropical and Public Health Institute (Swiss TPH) established in the year 2000 is working towards the goal of canine rabies control in N'Djamena. Over the ten years of rabies research, incidence in dogs was reported to be stable (1.4-1.7/1000 dogs) (Kayali et al., 2003; Dürr et al., 2008) with a reproductive number R0 of just above 1 (Zinsstag et al., 2009). One rabid dog is exposing on average 2.3 humans (Kayali et al., 2003a).

In 2012 a parenteral dog mass vaccination campaign covering the whole town of N'Djamena was conducted and repeated in 2013. Both interventions reached a coverage of over >70% (Léchenne et al., 2016a), exceeding the WHO-recommended required immunization coverage to interrupt the transmission cycle of the rabies virus (Coleman and Dye, 1996). The interventions led to a reduction of dog rabies cases of over 90% (from 19 cases in 2012 to 2 cases in 2013) and are proof of the feasibility of dog vaccination in the region.

The long-term target in the fight against rabies in Chad is a nation–wide canine parenteral mass vaccination campaign. To plan the campaign Chad's cultural diversity needs to be taken into account. The most widely practiced religions in Chad are Islam and Christianity (INSEED, 2009). Muslims predominantly inhabit northern Chad, whereas Christians primarily live in southern regions. A previous study conducted by Mindekem et al. (2005) in N'Djamena showed that Muslims tend to keep fewer dogs than Christians and during the vaccination campaigns in N'Djamena it was observed that accessibility of dogs was lower in districts inhabited by a Muslim majority (Léchenne et al., 2016a).

Dog:human ratios and the local community's rabies awareness are not only expected to vary within these different contexts but also at the national level and geographic region.

Surveys focusing on knowledge, attitude and practices (KAP) are widely used to plan efficient veterinary public health interventions, to take an in-depth look at local health behaviour and to identify knowledge gaps that might affect appropriate practices (WHO, 2008). Additional information, such as socio-demographic characteristics, is usually collected during KAP surveys. Rabies-related studies conducted so far in Chad were limited to the urban settings of N'Djamena (Kayali, 2003a&b; Mindekem et al., 2005; Dürr et al., 2008a&b; Zinsstag et al., 2009).

Between February and March 2014 we conducted a community-based, cross-sectional multi stage cluster survey in 40 villages in rural Chad. The aim of this study was (1) to assess differences in rabies awareness, knowledge, prevention and health-seeking behaviour in northern and southern Chad, (2) to estimate the dog rabies vaccination coverage, (3) to estimate the dog:human ratio and the dog-population density, (4) to collect and compare data on socio-demographic characteristics between northern and southern households

Material and Methods

Study area

Chad is a landlocked country located in Central Africa spreading over 1.284 million km2. Geographically, Chad is divided into three distinct regions; the Sahara Desert in the north bordering Libya and the Sudanese savannah in the south bordering Central African Republic. The Sahelian belt lies in the centre of Chad bordering Niger, Nigeria and Cameroon in the west and Sudan in the east. As diverse as the geographical setting is Chad's cultural background with over 200 distinct ethnic groups. Vast parts of the northern regions are part of the Sahara desert with only a few mobile pastoralist communities inhabiting the area. A hotspot of mobile pastoralist and sedentary agricultural communities can be found around Lake Chad, the most important water body of the Sahel. The more densely populated southern regions of Chad are primarily inhabited by sedentary farmers. According to the last official population census conducted in 2009 the country counts about 11'176'000 inhabitants of which 78% live in rural and 22% in urban areas (INSEED, 2009).

Administratively, Chad is divided into 23 regions and each region is headed by a governor. Regions are divided into 61 departments each lead by a prefect. The departments are again divided into sub-prefectures (200 in total) comprising each a number of different cantons (over 400 in total) led by a chief of canton. The smallest administrative entity in a rural setting is the village with its village chief.

58% of Chadians are Muslim (with the vast majority being Sunni) predominantly inhabiting northern Chad. Christians (19% Catholics, 16% Protestants), Animists (4%) and others (3%) primarily live in southern regions (INSEED, 2009).

Sampling procedure

We divided Chad into north and south on regions level and employed stratified multi level sampling. Two northern regions and two southern regions (4 regions in total) were randomly selected, with selection probability proportional to the size of the population. In each region, one department was selected proportional to population size (4 departments in total) and in each department we randomly selected 10 villages (40 villages in total). As data on the population on village level was not available this sampling was done by simple random sampling. The departments sampled were Kouh Ouest in the region of Logone Oriental, Grand Sido in Moyen Chari, Dar Tam in the region Wadi Fira and Guera in the region Guera (Figure 5.1). Some key characteristics of the regions sampled, based on the 2009 national census, are presented in Table 5.1.

Data collection

A structured questionnaire in French was developed by the authors to gather information on (i) the characteristics of the person interviewed, (ii) household demographics and (iii) dog owner's rabies awareness, knowledge and prevention. The questionnaire consisted of closed- and open-ended questions. For some questions (rabies symptoms in humans and dogs, reason for non-vaccination of dogs and treatment of dog bites) we recorded spontaneous and probed answers (Weiss, 2001). Interviews started always with open questions to avoid biased responses as much as possible. Open questions are formulated in a way that expected answers cannot be anticipated. I.e. We asked: "Can you tell us about issues between dogs and humans?" and not: "Do you know rabies?". Only after answers on open questions were completed, we probed on further issues. Limitations of this approach may be that open questions are formulated in a too broad way for the respondent to understand. Probed questions reflect only what the interviewer has in mind.

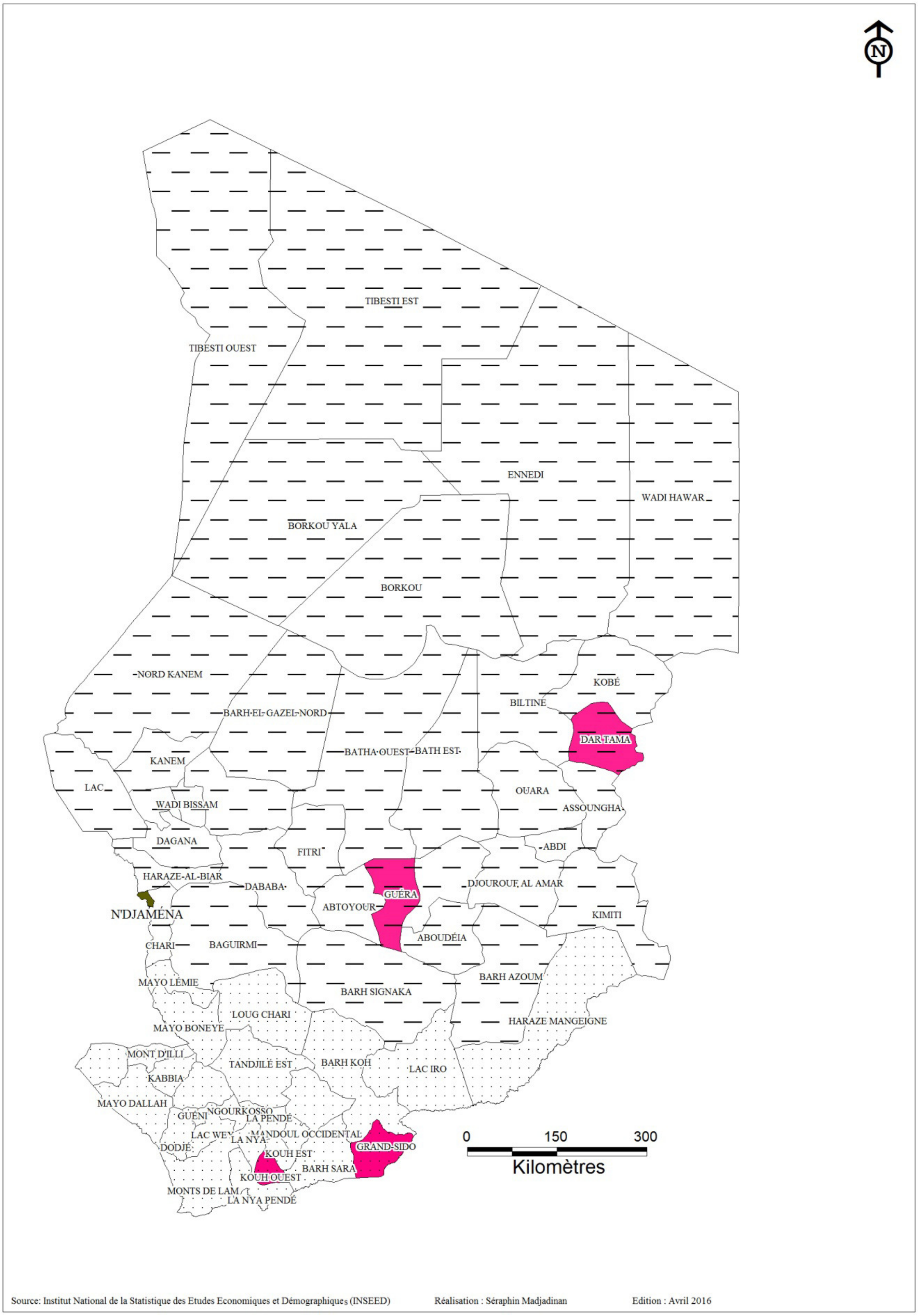

Figure 5.1. Departments visited during the national household survey

Table 5.1. Key characteristics of the regions sampled

Characteristics	**North**		**South**	
Region	Wadi Fira	Guera	Moyen Chari	Logone Orientale
Total population	494’993	553’795	598’284	796’453
Total households	74’448	106’348	102’245	144’857
Population density (per km^2)	9.7	8.9	14.6	33.2
Religion (%)				
Muslim	99.3	95.6	25.4	8.6
Christian	0.4	2.7	60.0	90.0
Animist	0.2	1.3	6.4	0.5
Others	0.1	0.4	8.2	0.9
Urbanization (%)				
Urban	13.6	14.3	22.4	11.7
Rural	86.4	85.7	77.6	88.3
Sedentary (%)	96.8	97.2	96.9	99.9
Mobile (%)	3.2	2.8	3.1	0.1
Ecological region	Sahara	Sahelian belt	Savannah	Savannah
Department surveyed	Dar Tama	Guera	Grande Sido	Kouh Ouest

[1]Based on national population and housing census 2009 (INSEED, 2009)

Interviewers were recruited from the Centre de Support en Santé International (CSSI) and trained in a one-day course. The training consisted of perusal of the questionnaire, mock interviews between course members in French, Arab and Gambaye as well as testing of the questionnaire in 30 households in a rural area of the 9th district of N’Djamena. The final 8 interviewers were chosen according to their performance during this course. After pre-testing minor changes were made to the layout and the questions for better understanding. Two teams consisting of a supervisor, four interviewers and one driver collected data on household level. One team with interviewers speaking French and Gambaye went south to the departments Grand Sido and Kouh Ouest. For the northern departments Guera and Dar Tama interviewers speaking Arab and French were chosen. Based on the preliminary field experience data collection performance of 24 households (6 questionnaires per interviewer) in one village per day was deemed achievable. We calculated a timeline of 28 days (including travel time) for each team to complete the mission. Despite the differences in road conditions, distance to villages and geography of the area both teams completed the survey within 24 days (12th February to 7th March 2014). All questionnaires were checked and approved by the respective supervisor the day after collection.

Local authorities and military officials were a big support for the accomplishment of the study, specifically in areas with difficult security status such as the region of Wadi Fira near the border to Sudan and around the town of Sido near the border to Central African Republic. Visa were obtained from the respective governor of the regions as well as from the prefects of the departments before working and after completion of the study in a respective area. Accommodation for the survey team was often provided by the chief of canton. Before starting data collection in a village, the village chief was informed and asked for consent. The village was then divided into four equal parts based on the knowledge of the village chief and each part was attributed to an interviewer. The sampling interval between households (range 1 to 5) was calculated by dividing the estimated total number of households by 24. Local guides designated by the village chief accompanied the interviewers to the households. For the selection of interviewees a household was chosen as a starting point. The next household was selected following the sampling interval of 1-5 households after spinning a pen for the direction of search. In a selected household, we asked after the head of the household or his or her representative. Upon informed consent we interviewed the person.

The size of villages encountered in the field varied greatly. In 2 villages we found less than 24 households. Hence, the number of households to be sampled was complemented in the neighbouring village. In the south, additional to the official villages, we encountered informal nomad settlements. To take these settlements into account we sent one Arab-speaking interviewer to the nomad camps whenever there was one close-by the village to be sampled. In the north, two villages on the sampling list, Alland in the canton Abassié and Dirames Dougous in the canton of Misserié Oyo, did not exist. These villages were replaced by two additional villages randomly selected in the same region.

Data entry and analysis

For quality control all data of the household were double-entered into Epi Info (Epi InfoTM CDC, Atlanta, GA) by 4 interviewers (in groups of 2) who had participated in the data collection. The data was then checked data for consistency and completeness in close contact with the field supervisors. Descriptive and analytical statistics was performed using SPSS version 22 (SPSS Inc. Chicago, IL) and STATA version 13 (StataCorp LP,TE). We applied generalised estimating equation models for the statistical analysis to account for potential correlation within villages due to multi-stage cluster sampling. For binary outcome variables, i.e. rabies awareness (having heard vs. having not heard of rabies) and rabies knowledge (high vs. low), generalised estimating equations for binomial distributed outcomes and logit link functions were applied. Estimates are presented as odds ratios (OR) and their corresponding 95% confidence interval. The dog:human ratio was estimated using generalised estimating equations for negative binomial distributed outcomes (dog counts per households). The point estimate and confidence limits were divided by the mean number of persons per household. Associations with explanatory variables (age, gender, education status, religion, dog ownership, bite history in family, respondent is head of household, residence) were analysed using univariable and multivariable analysis. All explanatory variables with p-values <0.2 in univariable analysis were included in the multivariable model.

Knowledge scores were only calculated for households that had heard of rabies (n=736) based on the following 4 questions: knowledge of rabies symptoms in both humans (1) and dogs (2), rabies prevention in humans and dogs (3), treatment possibility of a person already displaying rabies symptoms (4). To reduce the influence of the relatively large number of clinical symptoms compared to other parameters on the total knowledge score, a score of 1 was assigned when one or more symptoms were identified spontaneously. A score of 0.5 was given, when the answers had to be probed. In this way we distinguish between knowledge that can spontaneously be expressed and more passive knowledge that can be stated after being asked about it. Table 5.2 shows the scores assigned to correct answers. A maximum of 5 scores could be achieved. The most frequent scores were two and four (30% and 24%, respectively) and further visual inspection of the frequency distribution revealed that a score >= 3 would be a good cut-off value to distinct between low and high knowledge.

Table 5.2. Knowledge parameters and scores assigned to correct answers

Knowledge parameter	**Scores assigned**	
Symptoms of rabies in humans[1]	Spontaneous	1
	Probed	0.5
	Do not know	0
Symptoms of rabies in dogs[1]	Spontaneous	1
	Probed	0.5
	Do not know	0
Can a person already displaying rabies symptoms be cured?	No	1
	Yes	0
How can rabies be prevented?[2]	Vaccination of dogs	2
	Vaccination of humans	1
	Restraining of dogs	1
	Do not know	0

[1]Maximum score of 1 per household

[2]Maximum score of 2 per household

Results

Socio-demographic characteristics of the study population

A total of 963 households, comprising 480 households in the north and 483 households in the south, completed the survey. Only one household refused to participate yielding a response rate of almost 100%. The mean household size was about 7.5 in both areas. The households covered a total population of 7221 people (range: 1-39 persons per households) and 67% of the interviews were conducted with the head of household. The majority of head of households were male (80%). Table 5.3 presents socio-demographic characteristics of the study population.

About half of the respondents were females. The proportion was higher in the Muslim-oriented north (54%) compared to the Christian-oriented south (42%). The mean age of the respondents was 38 years and did not differ between the two areas. Only 16% of respondents in the north received formal education (defined as Primary, Secondary or Tertiary education) whereas in the south two thirds of the respondents received primary or higher education. In general, the proportion of female respondents with formal education was lower (North 12%, South 15%). Out of the 103 interviewees (11%) who received secondary or higher education, the majority were male (86%).

Dog-ownership and dog:human ratio

Out of 963 households almost half (45%) owned at least one dog with a total number of 918 dogs recorded during the survey. Southern households owned more than two thirds (701) of all dogs (Table 5.4). Only one third (37%) of the northern households owned dogs which was significantly lower compared to 54% of southern households (odds ratio [OR]: 0.45; 95% CI: 0.27-0.73; P=0.001). This translates into a dog:human ratio of 1:16.4 and 1:5.2, respectively. In addition, the number of dogs per dog owning-household in the south was twice as high compared to the north (2.7 vs. 1.3). The estimated overall dog:human ratio was 1:7.8. However, we observed notable differences in dog:human ratios among the surveyed regions, which was especially pronounced in the north. Within Moyen Chari – the only region with some religious heterogeneity – the number of dogs per household was similar among religions (1.1 vs. 1.2), although the sample size of Muslim households was small (n=21).

Based on an extrapolation of the respective dog:human ratio, we estimated the number of the rural owned dog population. Our estimates predicted a total of 57’000 owned dogs for the two northern regions Wadi Fira and Guera. The estimated rural owned dog population in the two southern regions, Moyen Chari and Logone Oriental, was four times higher (231’000). Table 5.5 gives more detailed data on dog numbers and dog densities.

Table 5.3. Socio-demographic characteristics of the 963 surveyed households by geographical area

Characteristics	All households (n=963) (%)	North (n=480) (%)	South (n=483) (%)
Gender of respondent			
Female	48	54	42
Age groups of respondent (Years)			
Range (Min.-Max.)	15-88	15-88	17-80
Median age	35	33	36
<19	3.1	4.6	1.7
20-30	36	39	33
31-40	27	25	30
>41	33	32	35
Education status of respondent			
No formal education	40	50	31
Primary	31	11	50
Secondary	10	4.6	16
Tertiary	0.7	1	0.4
Koranic school	18	34	3.1
Religion of respondent			
Muslim	52	98	6.4
Christian	44	1.3	87
Others [1]	33.5	0.2	6.6
Respondent is head of household			
Yes	67	69	73
Gender of head of household[2]			
Male	80	79	81
Number of household members			
Mean (SD)[3]	7.5 (3.9)	7.44 (3.5)	7.56 (4.3)

[1]Others includes traditional religion/Animists (1/963), no religion (30/963), undefined religion (2/963)

[2]Data of one person is missing (n=962)

[3]Standard deviation

Table 5.4. Estimated dog:human ratio in the study population and different subpopulations.

	Households	Dogs	Persons	Dog:human ratio	95% CI[1]
Total	963	918	7221	1:7.8	1:9.7 – 1:6.3
North	480	217	3569	1:16.4	1:23.0 - 1:11.8
Wadi Fira	240	160	1603	1:10.0	1:13.8 - 1: 7.2
Guera	240	57	1966	1:34.5	1:49.3 - 1:24.1
South	483	701	3652	1:5.2	1:6.4 - 1:4.1
Moyen Chari	242	264	1782	1:6.7	1:8.1 - 1:5.6
Logone Oriental	241	437	1870	1:4.3	1:5.9-1:3.2
Muslims	504	249	3755	1:14.0	1:18.8 - 1:10.4
Christian	426	641	3223	1:5.1	1:6.5 - 1: 4.0

[1]Confidence intervals were constructed via generalized estimating equations to account for cluster sampling

Table 5.5: Linear extrapolation of the rural owned dog population and dog density on region level[1]

Region	Rural population in 1'000 (%)	Total rural dogs in 1'000	95% CI	Area in 1'000 km^2	Dogs km^2
North					
Wadi Fira	428 (86%)	43	31 - 59	51	0.9
Guera	475 (86%)	14	10 - 20	62	0.2
South					
Moyen Chari	464 (78%)	69	57 - 83	41	1.7
Logone Oriental	703 (88%)	162	119 - 221	24	6.8

[1]based on data from census 2009 (INSEED, 2009)

The most common stated reason for keeping a dog in both northern and southern households (61% and 60% respectively) was for security (Figure 5.2).

Out of 963 study participants, 107 (11%) reported that at least one family member had been bitten by a dog in the past 12 months and half of these bite victims were children. Bite incidents occurred significantly more often in the south (14%) compared to the North (7%) (OR= 2.0, 95% CI: 1.2-3.4, P=0.008). No difference was observed between dog owning and non-dog owning households with respect to bite incidence (both 11%).

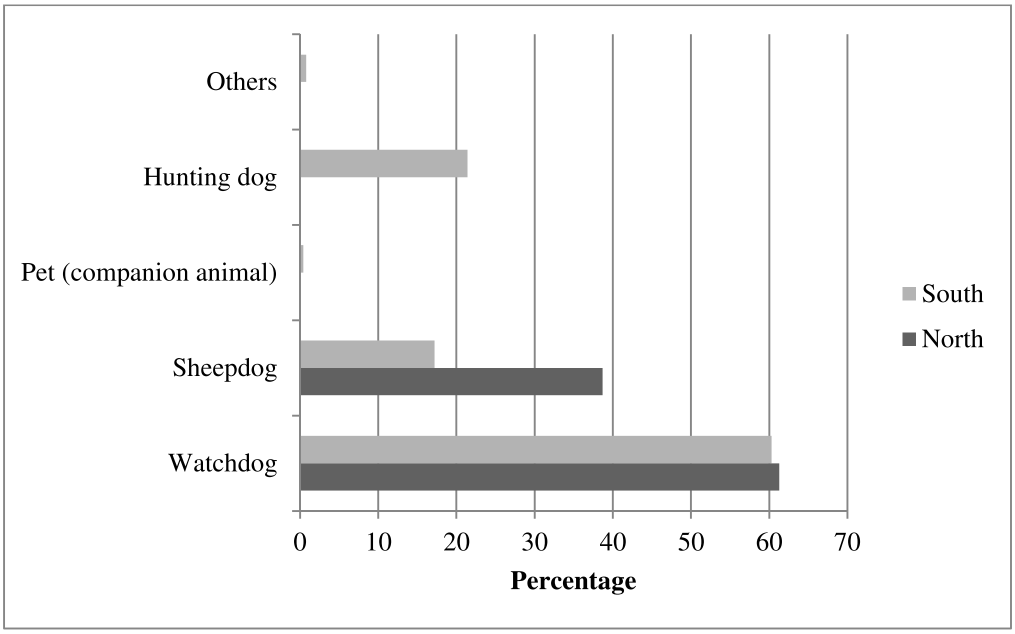

Figure 5.2. Reasons for keeping a dog

Factors associated with rabies awareness

Only 76% of the respondents had formerly heard of rabies and thus were considered as being aware of the disease. The proportions were similar in the northern and southern area (77% vs. 76%). Rabies awareness differed strongly among departments. Dar Tama was the department with the highest percentage of household respondents (42%) who were not familiar with rabies, followed by Kouh Ouest (27%), Grande Sido (25%) and Guera (6%). Within departments the proportion of households that had heard about rabies in a village was independent from the proportion of dog owning households (Appendix I).

Male respondents were more likely to have heard of rabies (84% vs. 68%; adjOR: 1.8; 95% CI: 1.2-2.6). The proportion of respondents aware of rabies rose significantly with increasing age (Table 5.6). Not surprisingly, education level was positively associated with awareness, whereas we did not observe a difference between no education and Koranic school (71% vs. 76%; adjOR: 0.9; 95% CI: 0.6-1.4). Household heads were more often aware of rabies but this association disappeared in the multivariable model likely due to a higher education level. Interestingly, we found no association with dog ownership or previous bite history. Although we found no association of rabies awareness with residence, we observed an association with religion. The raw proportions differed only slightly between Christians and Muslims but the multivariable model suggests that Christian respondents were less likely to have heard of rabies keeping other socio-demographic characteristics constant (adjOR 0.5; 95% CI: 0.3-0.8). “Other” religions were less likely to have heard of rabies but the number of interviewed households was small (adjOR 0.2; 95% CI: 0.1-0.6).

Community knowledge

Only study participants who had heard of rabies (n=736) were further queried. When asked about zoonotic diseases transmitted by dogs, the majority of respondents (81%) mentioned rabies followed by skin infections (2%) often referred to as fungus but most likely caused by ectoparasites. Respondents were asked to identify symptoms corresponding with rabies first spontaneously and then by probing from a list of symptoms in the questionnaire. In humans, agitation (79%) and anorexia (58%) and in dogs, aggressiveness (94%) and salivation (88%) were the most frequent symptoms identified. Only a fraction mentioned paralysis as a symptom of rabies in humans or dogs (17% and 19%, respectively). Figure 5.3 gives detailed information on proportions of symptoms identified by the study population.

Out of 736 respondents 230 (31%) knew someone who had died of rabies. The majority of the respondents (222/230) were able to provide plausible description of these cases. The most common symptoms seen in these persons were aggressive behaviour (21%), barking like a dog (11%), agitation (10%), screaming and shouting (5%) and hydrophobia (4%). 3 out of 736 study participants reported that they had seen paralysis or weakness as a rabies symptom. Interestingly, one person stated that the symptoms of rabies resembled those of malaria.

More than half of the study population (58%) reported having encountered a rabid animal at least once in their lifetime. When asked about symptoms seen in these animals, the most frequently mentioned were aggressiveness (47%), agitation (15%) and salivation (12%).

Table 5.6: Univariable and multivariable generalized estimating equation models for binomial outcome variables to determine factors associated with rabies awareness. The analysis accounts for potential correlation within villages due to cluster sampling.

Characteristic	N	% (Npos)	OR (95%CI)	adjOR (95%CI)
Residence				
North	480	77% (371)	reference	
South	483	76% (365)	0.90 (0.5-1.6)	
Gender of respondent				
Female	462	68% (314)	reference	reference
Male	501	84% (422)	2.4 (1.8-3.3)***	1.8 (1.2-2.6) **
Age of respondent				
≤19	30	53% (16)	reference	reference
20-30	348	72% (249)	2.3 (1.1-4.8)*	2.4 (1.1-5.1) *
31-40	263	79% (209)	4.0 (1.9-8.4)***	4.0 (1.8-9.0) ***
≥41	322	81% (262)	4.4 (2.1-9.3)***	4.1 (1.8-9.3) ***
Education status of respondent				
No formal education	388	71% (276)	reference	reference
Primary	295	79% (233)	1.4 (1.0-2.0)	1.6 (1.1-2.4) *
Secondary or higher	103	89% (92)	3.1 (1.6-5.9)***	3.0 (1.5-6.1) **
Koranic school	177	76% (135)	1.2 (0.8-1.8)	0.9 (0.6-1.4)
Respondent is head of household				
No	322	67% (322)	reference	reference
Yes	641	81% (520)	2.1 (1.5-2.8)***	1.3 (0.8-1.8)
Dog-ownership				
No	532	78% (416)	reference	
Yes	431	74% (320)	1.0 (0.7-1.3)	
Bite history in family				
No	856	75% (648)	reference	reference
Yes	107	82% (88)	1.4 (0.9-2.4)	1.5 (0.9-2.5)
Religion of respondent				
Muslim	504	78% (393)	reference	reference
Christian	426	76% (322)	0.7 (0.5-1.2)	0.5 (0.3-0.8) **
Others[1]	33	64% (21)	0.4 (0.2-1.0)*	0.2 (0.1-0.6) ***

Significance levels: *** p: <0.001, ** p: <0.01, * p<0.05

[1]Others includes Animists (1/963), no religion (30/963), undefined religion (2/963)

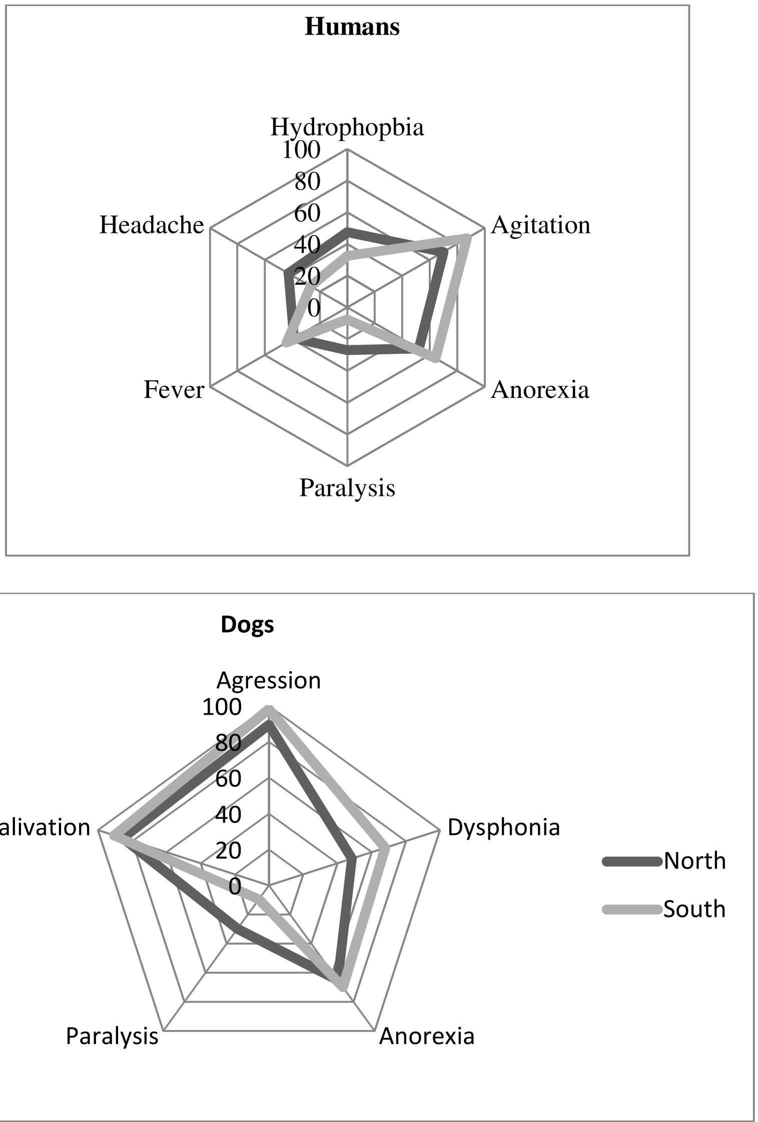

Figure 5.3. Probed answers of rabies symptoms in humans and dogs in northern and southern households.

Determinants of rabies knowledge

The mean knowledge score was 2.5 (SD:1.3, median: 1), with a minimum of 0 and a maximum of 5 scores. Using the cut-off score of >=3 to classify high or low level of rabies knowledge, 41% of the households were classified as having a high level of knowledge. High level of knowledge was positively associated with (i) southern residence, (ii) any kind of education and (iii) Christian or “other” religions (Table 5.7). In contrast to rabies awareness – which rose with increasing age – the proportion of households with high knowledge scores decreased with increasing age. Similar to rabies awareness there was no evidence of an association with household head, dog ownership or previous bite history. The northern departments Dar Tama and Guera had the highest amount of households with a low level of knowledge (88% and 68% respectively).

Table 5.7. Univariable and multivariable Generalized Estimating Equations models for binomial outcome variables to determine factors associated with knowledge of rabies (high vs. low). The analysis accounts for potential correlation within villages due to cluster sampling.

Characteristic	N	% (Npos)	OR (95%CI)	adjOR (95%CI)
Residence				
North	371	24% (90)	reference	Reference
South	365	59% (215)	4.7 (3.0-7.2) ***	3.5 (2.3-5.4) ***
Gender of respondent				
Female	314	36% (113)	reference	Reference
Male	422	46% (192)	1.3 (1.0-1.7)	0.9 (0.6-1.4)
Age of respondent				
≤19	16	56% (6)	reference	Reference
20-30	249	45% (113)	0.6 (0.2-1.5)	0.4 (0.1-1.2)
31-40	209	41% (86)	0.4 (0.2-1.1)	0.3 (0.1-0.9) *
≥41	262	37% (97)	0.4 (0.1-1.0) *	0.3 (0.1-0.8) *
Education status of respondent				
No formal education	276	24% (67)	reference	Reference
Primary	233	58% (134)	3.1 (2.1-4.5) ***	2.6 (1.7-4.0) ***
Secondary or higher	92	72% (66)	5.8 (3.5-9.8) ***	5.2 (2.9-9.3) ***
Koranic school	135	28% (38)	1.3 (0.8-2.0)	1.7 (1.0-2.9) *
Respondent is head of household				
No	216	36% (77)	reference	Reference
Yes	520	44% (228)	1.3 (0.9-1.7)	1.5 (0.9-2.4)
Dog ownership				
No	416	39% (164)	reference	
Yes	320	44% (141)	1.1 (0.8-1.4)	
Bite history in family				
No	648	40% (262)	reference	
Yes	88	49% (43)	1.1 (0.7-1.8)	
Religion of respondent [a]				
Muslim	393	25% (97)	reference	NA
Christian	322	60% (192)	10.5 (3.6-30.5) ***	NA
Others[1]	21	76% (16)	4.3 (2.9-6.5) ***	NA

[a]Excluded from multivariable model due to correlation with Residence

Significance levels: *** p: <0.001, ** P: <0.01, * p<0.05

[1]Others includes Animists (1/963), no religion (30/963), not defined religion (2/963)

Rabies prevention

Investigation of knowledge of rabies prevention revealed that 57% of the respondents did not know how rabies could be prevented. One third (34%) proposed dog vaccination as an adequate measure to prevent rabies infection in humans and dogs and only 0.3% mentioned vaccination of humans. Among the respondents, 5% spontaneously stated that they would immediately kill a rabid dog. 15 respondents out of 736 (2%) believed that feeding an appropriate diet could prevent dogs from developing clinical rabies symptoms.

Almost half of the respondents (44%) were aware of the fact that once clinical symptoms are apparent rabies is incurable. However, 15% believed that rabies is curable. The rest of the respondents (41%) did not know any answer to this question.

Dog vaccination coverage

When asked whether their dog was vaccinated or not only 2 out of 736 (0.5%) reported that their dog had been vaccinated against rabies. However, none of them could prove that vaccination was up-to-date by providing a vaccination certificate. We probed 5 reasons for non-vaccination. Overall, the most common reason why a dog lacked vaccination was that the respondent did not know where to find the vaccine (77%) followed by lack of information (70%), distance to veterinary clinic (33%) and cost of vaccine (28%). One fifth (22%) of the respondents reported that they would not be able to handle their dog appropriately to bring them to vaccination and 12% feared that their dog might change its behaviour after being vaccinated. Reasons for non-vaccination among northern and southern households are shown in Figure 5.4.

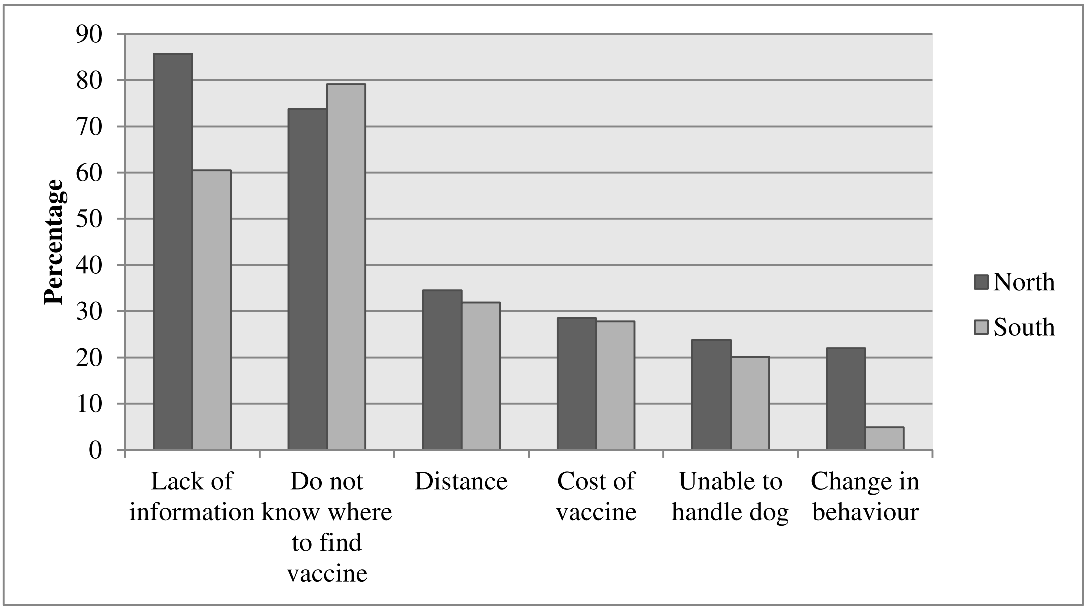

Figure 5.4. Reasons for non-vaccination of dogs among northern and southern households

Health-seeking behaviour

As a first aid measure in the event of a bite, 53% of the respondents would apply a traditional medicine to the wound, 37% would seek care from a doctor and 27% would visit a traditional healer. Only 19% spontaneously reported that they would wash the wound with water. When probed, however, the majority (69%) agreed with wound washing. Other sporadic responses were burning the wound or visit a veterinarian. More northern residents (56%) would seek help from a traditional healer compared to only 20% of southern residents. However, only about half (53%) of the respondents in the south would wash a wound in the event of a bite compared to 82% of respondents in the north. Differences in health seeking behaviour between northern and southern residents are shown in Figure 5.5.

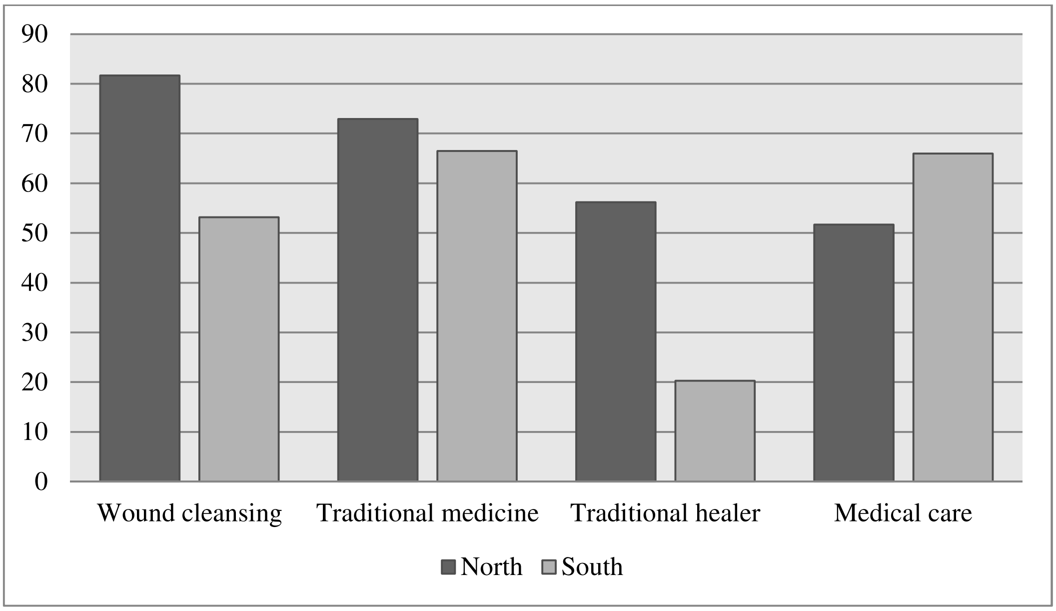

Figure 5.5. Differences in health-seeking behaviour between northern and southern residents (probed answers).

Discussion

Dog ownership and dog:human ratio

In our study, almost half (45%) of all households owned at least one dog, which is comparable to other studies carried out in rural African areas. In semi-rural Zambia (De Balogh et al., 1993) 42% of households kept dogs and in Machakos District, Kenya (Kitala et al., 2001), dog ownership varied between 53% and 81%. However, these proportions of dog owning households deviate from findings obtained in urban African settings, where dog ownership seems to be less widespread. For instance, in N’Djamena, Mindekem et al. (2005) observed a proportion of dog ownership of 28% and Gsell et al. (2012) reported 14% dog owning households in Iringa, Tanzania. The average estimated dog:human ratio of 1:7.8 is consistent with findings from other rural African areas (De Balogh et al., 1993; Kitala et al., 2001) and is on good agreement with a meta-analysis estimating the ratio at 7.4 in rural Africa

(Knobel et al., 2005). As expected, dog:human ratio was much higher in southern Chad (1:5.2) compared to a dog:human ratio of 1:16.4 in northern Chad. Similarly, we estimated a higher dog:human ratio for Christian households (1:5.1) compared to Muslim households (1:14), which has already been observed in N'Djamena (Mindekem et al., 2005). In Tanzania (Knobel et al., 2008) and in Ethiopia (Kabeta et al., 2015) Muslim households were less likely to own dogs than other households.

The estimated dog:human ratios give us an idea of the owned rural dog population. Assuming that our sample is representative for whole rural Chad, we would expect a total owned rural dog population of about 1'360'000 dogs (weighted by religion).

11% of respondents reported that at least one family member had been bitten by a dog in the past 12 months. This is consistent with a study on dog bite incidents in South Africa (Hergert and Nel, 2013) where 13% of study participants reported of dog bite injuries in the family. In our study, half of the bite victims were children. This is a higher proportion than recently reported by Frey et al. (2013), where 37% of all bite victims in N'Djamena were children. Children are at greater risk of rabies encephalitis since they are more likely to suffer dangerous bites to the neck or arms (Hattwick and Gregg, 1975), which have the shortest incubation period (Jackson, 2013b).

Dog bite injuries can be used to indirectly estimate human rabies incidence (Cleaveland et al., 2002). To implement such a study recording of bite incidents and further investigations, such as identification of the offending dog and follow-up of treatment, are currently underway.

Rabies awareness

Unlike reports from surveys conducted in Africa and Asia, rabies awareness among our study population was considerably low. Of the 963 respondents, only 76% reported having heard of a disease called rabies. In Tanzania (Sambo et al., 2014), for example, over 95% of study participants and in Ethiopia (Jemberu et al., 2013) even a higher percentage (98%) had heard of rabies. Studies conducted in Asia also observed higher awareness rates; Thailand 93% (Kongkaew et al., 2004), Cambodia 93% (Lunney et al., 2012) or Philippines 94% (Davlin et al., 2014). However, our finding is similar to the work conducted in N'Djamena by Mindekem et al. (2005) who reported that only 80% of study participants were aware of the disease.

Christian households were less likely to have heard of rabies than Muslim households. However, high knowledge of rabies was positively associated with southern residence. These findings could be rooted in language: in southern Chad there are a lot of different languages and the interviewers might not have used the locally appropriate expression for rabies, whereas in the north rabies is uniquely called "Al Jame".

Dar Tama was the department with the highest percentage of respondents who had not heard of rabies. This maybe explained with the fact that it is one of the most remote and least accessible departments of Chad. Vast parts are deserted and the nearest school or health care centres are often miles away. Obtaining information on diseases like rabies might be very difficult. Detailed examination of sources of rabies information might reveal deficiencies in knowledge transfer but also indicate appropriate means to disseminate information about rabies among the community.

It is necessary to increase awareness among the local population. This could be achieved through implementation of public educational programs and broadcasting messages about rabies in radio or television. From our own experience with large scale mass vaccination in N'Djamena, active involvement of community and religious leaders is essential to disseminate information to the public. Moreover, initiatives, such as the global World Rabies Day (28th September) created by the Global Alliance for Rabies Control (GARC) are great tools to raise public attention and actively involve the community in the fight against rabies (GARC, 2015).

Rabies knowledge

Most of the respondents were able to identify classical rabies symptoms, such as salivation or agitation, for both humans and animals. 31% of respondents knew someone who had died of rabies and twice as many (58%) reported having encountered a rabid animal. Plausible description of rabies symptoms in the cases reported by the study population strongly indicates that rabies really exists in the study area. However, the minority of respondents mentioned paralysis as a possible sign of rabies, which is typical for the "dumb" or paralytic form of rabies. Interestingly, one respondent stated that the symptoms of rabies resembled those of malaria. A study by Mallewa et al. (2007) in Malaria-endemic Malawi found that 11.5% of fatal cases originally attributed to cerebral malaria were indeed caused by rabies. Lack of awareness of atypical manifestation may result in cases going undetected and, thus untreated.

The World Health Organization recommends immediate washing of bite wounds as a crucial first aid measure after the bite of a suspected rabid animal (WHO, 2013). Thorough wound cleansing helps to remove animal saliva from the bite site and reduce the viral load (WHO, 2013). We found that the majority of respondents were unaware of this simple preventive measure, which is consistent with several other studies (Frey et al., 2013; Hergert and Nel, 2013; Sambo et al., 2014; Mindekem et al., 2005). Hergert and Nel (2013) suggested that lack of fresh water in the house might negatively influence appropriate wound management. Assuming that flush toilets indicate indoor plumbing (Hergert and Nel, 2013) more than 85% of the households in our study did not dispose of running water to wash a potential bite injury. This warrants attention and shows the urgent need to increase knowledge about the importance of appropriate wound management – prompt wound washing and application of disinfectants - as a simple but essential first aid measure after exposure to a bite.

Rabies prevention and dog vaccination coverage

We found that almost two thirds (57%) of the respondents were unaware of any preventive rabies measure and only 34% knew of the need for dog vaccination. Almost none (0.3%) mentioned vaccination of humans. In contrast, in Tanzania (Sambo et al., 2014) or N'Djamena (Mindekem et al., 2005) the majority of respondents knew of dog vaccination. Dog mass vaccination is the most effective method to control dog rabies and to reduce the number of human deaths from rabies (WHO, 2013). A vaccination coverage of 70 % is recommended by the WHO to interrupt the transmission cycle within the dog population (Coleman and Dye, 1996). Our study revealed that dog vaccination was basically non-existent among our study population. This is alarming and shows the great need for public health interventions.

Reasons for non-vaccination were similar to those reported from Ethiopia (Jemberu et al., 2013); lack of awareness about dog vaccination, lack of access and cost of vaccine. In order to improve vaccination coverage rates, access to and affordability of vaccine needs to be increased, but most importantly community sensitization for the importance of vaccination needs to be increased.

Knowledge of the accessibility of dogs for vaccination is crucial for the planning of feasible rabies elimination campaigns. A recent study conducted in Bamako by Muthiani et al. (2015) revealed that the inability to handle aggressive dogs was an important reason (16%) for non-participation in a central point vaccination campaign. During the two vaccination campaigns in N'Djamena (Léchenne et al., 2016a) inability to handle a dog was the second most stated reason (25-28%) for non-vaccination following lack of information. This is similar to our finding (22%). The inability to handle aggressive dogs is a constraint and could negatively affect participation in vaccination campaigns resulting in low vaccination coverage rates. Muthiani et al. (2015) suggest door-to-door vaccination campaigns to tackle this issue; however this would have cost implications. Dogs feel more comfortable in familiar environments resulting in easier handling of the animal. Furthermore, aggressive dogs could be given oral vaccination or sedation before vaccination. Thomas et al. (2013) propose engagement of community volunteers who help bringing dogs to vaccination points or veterinary clinics. The idea behind this is that involving community volunteers could help increasing social pressure for vaccination. Other studies however do not see inability to handle dogs as a constraint in achieving high vaccination coverage rates (Lembo et al., 2010).

A limitation of this study is that we did not further investigate the willingness to pay for vaccination since the fees charged for vaccination are likely to influence the achievable vaccination coverage. A willingness to pay study conducted in N'Djamena revealed that vaccination coverage dropped to 25%, when owners had to pay for vaccination, compared to previous free vaccinations where a vaccination coverage rate of 64-87% was achieved (Dürr et al., 2009).

Health-seeking behaviour

The proportion of respondents who would seek care from a doctor was quite low in our study (37%), compared to findings in South Africa where more than 80% of bite victims reported clinic visits (Hergert and Nel, 2013). Moreover, we found that 57% of our study population would apply a traditional herbal treatment to the wound and almost one third (27%) would visit a traditional healer, which differs from South Africa where less than 2% of bite victims sought help of traditional practitioners. In contrast, a study conducted in Ethiopia found that more than 84% of study participants relied on traditional medicine (Jemberu et al., 2013). In northern Chadian regions reliance on traditional medicine was more prevalent. This might be explained by the great distances to the next health care facility and the long traveling hours due to bad road conditions, compared to higher rabies knowledge and easier access to health care facilities in southern Chad.

Conclusions

Rabies poses a major public health threat in rural Chad. Our findings on variation in dog:human ratio and dog population density in regard to geographical area and religion combined with the experience gathered in N'Djamena help to implement a nation-wide dog mass vaccination campaign in Chad. A major proportion of our study population is at great risk of rabies due to lack of awareness of the disease, inappropriate post-bite treatment and insufficient knowledge about preventive measures. This reflects the urgent need for advocacy programs to raise rabies awareness among the community in order to prevent exposure and to promote appropriate wound management and PEP. Moreover, policy-makers need to be informed about the burden of the disease in order to assure political commitment. It is widely recognized that close intersectoral collaboration between the public health and veterinary sector and integration of local authorities is a key element in the fight against rabies (Lembo et al., 2010; Partners for Rabies Prevention, 2010).

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Appendix

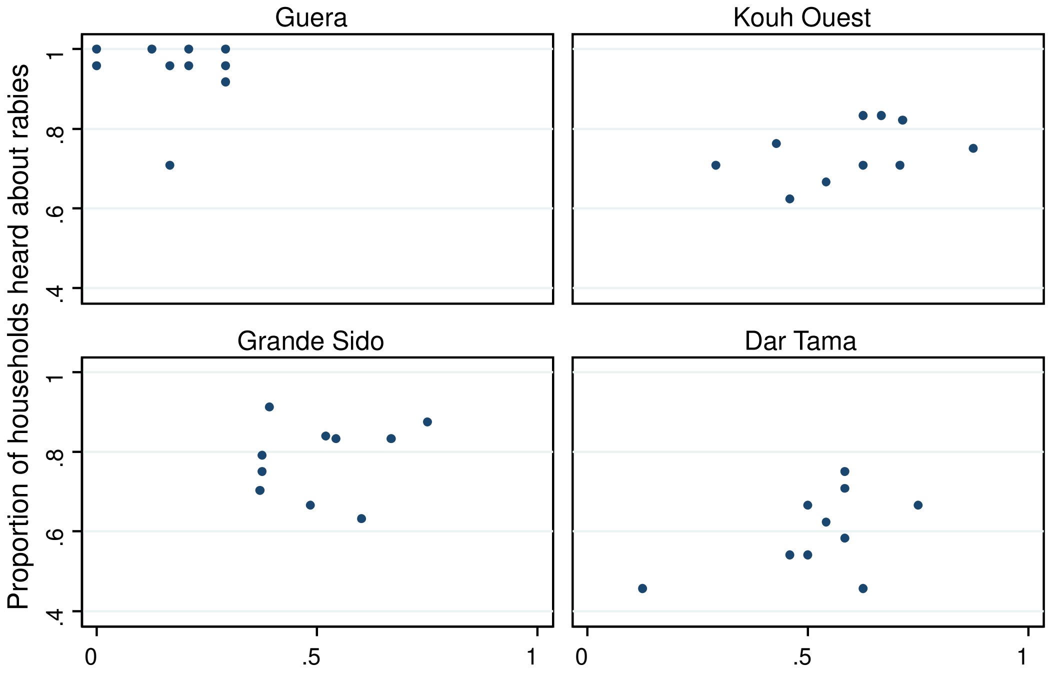

Figure S5.1: Proportion of dog owning households

CHAPTER 6

Cost estimate of canine rabies elimination by parenteral mass vaccination in Chad

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Abstract

More than 59,000 humans die of rabies each year, most of them in Africa and Asia. Clinical rabies can be prevented by post-exposure prophylaxis (PEP). However, often PEP is either not available or not affordable in developing countries. Another strategy besides treating exposed humans is the vaccination of vector species. In developing countries, the most important vector is the domestic canine, which, once infected, is a serious threat to humans. After a successful mass vaccination of 70% of the dogs in N'Djamena, we report here a cost-estimate for a national rabies elimination campaign for Chad. In a cross-sectional survey in four rural zones of Chad, we established the canine: human ratio at household level. Based on human census data and the prevailing socio-cultural composition of rural zones of Chad, the total canine population was estimated at 1'205'361 dogs (95% Confidence interval 1'128'008 –1'736'774 dogs). Cost data was collected from government sources and the recent canine mass vaccination campaign in N'Djamena. A Monte Carlo simulation was used for the simulation of the average cost and its variability, using probability distributions for dog numbers and cost items. Assuming the vaccination of 100 dogs on average per vaccination post and a duration of one year, the total cost for the vaccination of the national Chadian canine population is estimated at 2'716'359 Euros (95% CI 2'417'353 – 3'035'081 Euros) for one vaccination round. A development impact bond (DIB) organizational structure and cash flow scenario were then developed for the elimination of canine rabies in Chad. Cumulative discounted cost of 28.3 million Euros over ten years would be shared between the government of Chad, private investors and institutional donors as outcome funders. In this way, the risk of the investment could be shared and the necessary investment could be made available upfront - a key element for the elimination of canine rabies in Chad.

Keywords

Canine rabies, Chad, Cost estimate, Development Impact Bond, mass vaccination, Monte Carlo simulation.

Introduction

The most recent analysis estimates that annually worldwide, canine rabies causes 59,000 human deaths, over 3.7 million disability-adjusted life years (DALYs) and 8.6 billion USD economic loss (Hampson et al., 2015). Clinical rabies can be prevented by Post-Exposure Prophylaxis (PEP). However, often PEP is either not available or not affordable in developing countries. Even if PEP were subsidized, out-of-pocket expenses remain a huge cost burden to affected families, exceeding 2-3 months wages (Zinsstag et al., 2009; Frey et al., 2013). Clearly any human exposed to a suspected rabid animal bite must receive PEP. However, the use of PEP alone will never interrupt rabies transmission. In developing countries, the most important vector is the domestic dog, which once infected, is a serious threat to humans (Wandeler et al., 1993). Only mass vaccination of dogs can eventually interrupt transmission and lead to the elimination of the disease.

Boegel and Meslin (1990) showed that after 15 years a canine rabies control program consisting of a combination of PEP and canine vaccination becomes more cost effective than PEP alone (Boegel and Meslin, 1990). This is because the use of PEP alone never interrupts human exposure. A recent simulation of canine rabies mass vaccination in an African city showed that the break-even of the cost of canine rabies vaccination and PEP can occur already after six years (Zinsstag et al., 2009). Because of the low reproductive number of canine rabies transmission, the World Health Organization (WHO) estimates that the immunization of 70% of the canine population could interrupt transmission and prevent the virus from spreading and, thus, rabies could be eliminated (WHO, 1987). The cost of canine mass vaccination should eventually break even with the cumulated cost of human PEP. However, dog owners in West and Central Africa are often not able to pay for the vaccination of their dogs. In Chad fewer than 19% of the dogs are vaccinated by their owners. In willingness to pay study that followed a mass vaccination campaign paid by the owner we could show that the reported willingness to pay corresponded well with the actually achieved vaccination coverage (Dürr et al., 2008b; Durr et al., 2009). In order to reach a sufficiently high coverage in West and Central Africa, canine mass vaccination campaigns should therefore be free to the owner and freedom from canine rabies should be recognized as a public good. The Global Alliance for Rabies Control works towards worldwide canine rabies elimination by 2030. After the eradication of Poliomyelitis, and compared to ongoing elimination efforts like Guinea worm, malaria and tuberculosis, canine transmitted human rabies is one of the most promising next candidate for global elimination because of its low reproductive number of less than 2. To achieve the elimination of canine transmitted rabies, interventions must be directed towards the domestic dog. This requires a close cooperation between public health and animal health, called “One Health” (Zinsstag, 2015a). Latin American countries have made enormous progress in this regard and strong efforts are underway in South-East Asia (Vigilato et al, 2013a; Hampson et al., 2007). In July 2015, the Pan African Rabies Control Network (PaRaCon) was founded with the clear aim of worldwide canine rabies elimination. The elimination of an infectious disease such as rabies requires a highly-coordinated and concerted effort to reach all affected areas of a country in a short time frame. Considerable upfront funding is required to start the mass vaccination and reach a high enough coverage to interrupt transmission. A city-wide canine rabies elimination campaign in N’Djamena at a scale of 35’000 dogs and a human population of 1.1 million was able to do this with the engagement of the Chadian state and private donors (Lechenne et al., 2016a). For the mobilization of the necessary funding for a country-wide mass vaccination campaign, alternative financing models are necessary, as neither national states, nor institutional donors are able to provide large amounts upfront. There is a growing interest among development donors and impact investors in the use of so-called “Development Impact Bonds” (DIB): Private investment provides upfront capital for development programs, only calling on donor funding to repay capital and a potential return based on achieved results (Hughes, 2014; Welburn and Coleman, 2015).

The present study intends to contribute to the efforts of PaRaCON by estimating the cost of canine rabies elimination on a national scale for an African country by providing a comprehensive cost estimate and a proposal for a DIB for the elimination of canine rabies in Chad. A cost estimate for Chad will allow the extrapolation of the costs of campaigns in other countries of the Sahel (Dürr et al., 2008; Durr et al., 2009; Zinsstag et al., 2009).

Materials and Methods

Study area, sampling procedure and data collection

Details on the characteristics of the studied regions, the sampling procedure and the data collection on household level are provided in the companion paper on rabies awareness and dog ownership by Mbilo et al., 2016 (chapter 5, this thesis).

For a household study of the canine: human population ratio, the Sahara area of Borkou-Ennedi-Tibesti (BET) was omitted, having an eligible canine population. The remainder of Chad was divided into north (predominantly Sahelian) and south (predominantly Savannah) regions and was subjected to stratified multilevel sampling (Mbilo et al., 2016). Two northern regions and two southern regions (4 regions in total) were randomly selected, with selection probability proportional to the size of the population. In each region, one department was selected proportional to size (4 departments in total) and in each department 10 villages were randomly selected (40 villages in total). As data on the population at village level was not available, simple random sampling was used. The departments sampled were Kouh Ouest in the region of Logone Oriental, Grand Sido in Moyen Chari, Dar Tam in the region Wadi Fira and Guera in the region Guera. Details of the characteristics of the studied regions are provided in the companion paper on rabies awareness and canine ownership (Mbilo et al., 2016). The household study was approved by the ethical review board of the cantons of Basel, Switzerland (Ethik Kommission beider Basel, EKBBref. 168/13, 29 July 2013) and authorized by the Chadian public health authorities.

Dog / human ratio

The estimation of the canine: human ratio is based on results of the data collection as well as recommendations by Chadian experts at the Institut de Recherches en Elevage pour le Developpement (IRED). It was assessed for every administrative region of Chad. The canine : human ratio is largely determined by the socio-cultural and religious composition of a region. Essentially, there are more dogs per humans in Christian/Animist communities than in Muslim communities (Mbilo et al., 2016). Hence empirically observed canine: human ratios were assigned to the relative numbers of Christians/Animists and to Muslims (Table 6.1). For every region the overall canine population was summed up. The overall total canine population of Chad was then obtained from the sum of canines in all regions. For the variability of the canine population we took the overall variation of the canine: human ratio of 1:6.3 to 1:9.7 from Mbilo's study (Mbilo et al., 2016).

Organization of the vaccination campaign

Decentralized operations are important for the administration of a nationwide vaccination campaign. The 61 departments of Chad were chosen as the administrative unit suitable for the organization of the campaign. Each of the 61 departments has several veterinary posts and at least one health centre, which can provide infrastructure, equipment, and professionally trained manpower. Local staff needs to be recruited in every department. Training of vaccinators must take place in each department. Chadian veterinary staff regularly vaccinates large cattle populations against Anthrax and other livestock diseases. With support from the Pan-African Rinderpest Campaign (PARC), Chad successfully eliminated Rinderpest (FAO, 2012). In 2012 and 2013 a canine rabies mass vaccination campaign reached 70% of the canine population, hence we can be confident, that with sufficient training, canine rabies mass vaccination campaigns can also be operated in rural Chadian areas (Lechenne et al., 2016a).

Public engagement

Information can be spread nation-wide via posters displayed in public places, radio broadcasts, and megaphone announcements of the on-going campaign. Informing the population efficiently reduces unnecessary waiting time at the vaccination points (VP), thus reducing the cost of the campaign. Prior to the vaccination campaign, an information day for the governors of the 22 regions should be held in N'Djamena, the capital and largest city of Chad, to inform them about the campaign. The information will then be passed to smaller government units and from them to the leaders of smaller settlements, who are connected to the local population. In this way information on the mass vaccination will be conveyed in a contextually adapted way to all concerned.

Table 6.1: Canine : human ratios and estimated dog population in the respective study zones

Region	Christians (catholics and protestants) and animists in %	Muslims %	Total number of Christians / Animists	Canine : human ratio among Christians / Animists	Estimated dog number among Christians / Animists	Total number of Muslims	Canine : human ratio among Muslims	Estimated dog number among Muslims	Total dog estimate	Source of data	Total human population (Census 2009)
Batha	1.2	98.8	5,861	1:5.1	1,149	482,597	1:14.0	34,471	35,620	2	488,458
Borkou	0.7	99.3	655	1:5.1	128	92,929	1:14.0	6,638	6,766	2	93,584
Chari Baguirmi	15.9	84.1	91,970	1:5.1	18,033	486,455	1:14.0	34,747	52,780	2	578,425
Guéra	4.4	95.6	23,688	01:10	2,369	514,671	1.34.5	14,918	17,287	1	538,359
Hadjer Lamis	1.8	98.2	10,203	1:5.1	2,001	556,655	1:14.0	39,761	41,762	2	566,858
Kanem	0.6	99.4	2,000	1:5.1	392	331,387	1:14.0	23,670	24,063	2	333,387
Lac	1.2	98.8	5,205	1:5.1	1,021	428,585	1:14.0	30,613	31,634	2	433,790
Logone Occidental rural	92.3	7.7	497,431	1:5.1	97,536	41,498	1:14.0	2,964	100,500	2	538,929
Logone Occidental urbain (Moundou)	92.3	7.7	138,556	01:14	9,897	11,559	1:220	53	9,949	3	150,115
Logone Oriental rural	91.4	8.6	615,064	1:4.3	143,038	57,872	1:14.0	4,134	147,172	1	672,936
Logone Oriental urbain (Doba)	91.4	8.6	97,252	01:14	6,947	9,151	1:220	42	6,988	3	106,403
Mandoul	91.3	8.7	573,423	1:5.1	112,436	54,642	1:14.0	3,903	116,339	2	628,065
Mayo Kebbi Est	82.1	17.9	636,096	1:5.1	124,725	138,686	1:14.0	9,906	134,631	2	774,782
Mayo Kebbi Ouest	88.9	11.1	501,814	1:5.1	98,395	62,656	1:14.0	4,475	102,870	2	564,470
Moyen Chari rural	74.6	25.4	340,877	1:6.7	50,877	116,063	1:34.5	3,364	54,241	1	456,940
Moyen Chari urbain (Sarh)	74.6	25.4	97,777	01:14	6,984	33,291	1:220	151	7,135	2	131,068
Ouaddaï rural	1.2	98.8	6,990	1:5.1	1,371	575,492	1:14.0	41,107	42,477	2	582,482
Ouaddaï urbain (Abéché)	1.2	98.8	1,664	01:14	119	137,020	1:220	623	742	3	138,684
Salamat	1.4	98.6	4,232	1:5.1	830	298,069	1:14.0	21,291	22,120	2	302,301
Tandjilé	91.6	8.4	606,306	1:5.1	118,884	55,600	1:14.0	3,971	122,855	2	661,906
Wadi Fira	0.7	99.3	3,559	01:10	356	504,824	01:10	50,482	50,838	1	508,383
N'Djaména	29.3	70.7	278,765	01:14	19,912	672,653	1:220	3,058	22,969	3	951,418
Barh El Gazal	0.7	99.3	1,801	1:5.1	353	255,466	1:14.0	18,248	18,601	2	257,267
Ennedi	0.6	99.4	1,008	1:5.1	198	166,911	1:14.0	11,922	12,120	2	167,919
Sila	1	99	2,935	1:5.1	575	290,516	1:14.0	20,751	21,326	2	293,450
Tibesti	2	98	426	1:5.1	84	20,877	1:14.0	1,491	1,575	2	21,303
Total	41.54	58.46	4,545,559	1:5.3	818,607	6,396,123	1:14.6	386,754	1,205,361		10,941,682

Source of data: 1 = national household survey data; 2 = extrapolated from national household survey results; 3 = vaccination campaign data 2013.

Vaccination campaign

The nationwide vaccination campaign must be coordinated carefully with respect to seasonal meteorological dynamics. During the rainy season (June to September) vaccination can only be performed in central and northern regions. The vaccination campaign in N'Djamena showed that one VP with three vaccinators can vaccinate 50 to 200 canines a day. In a nationwide campaign, we assume that, on average, 100 canines can be vaccinated by one VP per day.

Table 6.2: Scenarios used for the cost calculations

	Scenario 1	Scenario 2	Scenario 3	Scenario 4
Duration of 1 round (in years)	3	3	1	1
Dogs vaccinated/day/VP	100	50	100	50

For the feasibility and budget planning of the campaign, we examined four different scenarios to be able to assess how different cost items influence the overall costs of a campaign (Table 6.2). Scenario 1, for example presumes that the whole canine population is vaccinated within three years (936 working days), at a rate of 100 canines per VP per day. In the case of scenario 1, 6 VP and thus 18 vaccinators are needed at a time. At least 2 cars, each with 1 driver and 1 supervisor, are needed to coordinate the teams and supply the VP with material. One additional manned car will be needed to visit the settlements scheduled next. Several villages, small towns, or districts of bigger towns will be vaccinated simultaneously.

Cost description

The costs of a pilot vaccination campaign in N'Djamena in 2005 were split into public and private costs (Kayali et al., 2006):

Public costs = Marginal vaccination cost + Equipment of VP + Staff allowance + Information + Transportation

Private costs = Lost work time+ Transportation

Societal costs = Public costs + private costs (Kayali et al., 2006)

Public cost: Marginal vaccination costs per canine include 1 syringe and needle, 1 dose of vaccine, and 1 vaccination certificate. The prices of the vaccine are quoted by the local importer; syringes and needles are bought in Chad. Equipment for VP and vehicles will be bought locally. Vaccination cards will be printed locally. Megaphones and first-aid kits will be bought in Switzerland. Staff working with canines must be protected from rabies exposure by Pre-Exposure Prophylaxis (PreP). The local importer will determine the prices of the human vaccine.

All people involved in the vaccination campaign will be paid a daily allowance. The prices of the equipment, consumables and personnel cost are derived from the vaccination campaign in N'Djamena in 2012 and 2013 (Lechenne et al., 2016a). A detailed cost analysis of this campaign will be published shortly.

Information and transportation costs: Posters will be printed locally. Radio spots on Radiodiffusion Nationale Tchadienne (RNT) will be broadcast twice a week; spots on local channels twice a day. We assessed the distances covered during the whole campaign by one car at 75,000 km and the fuel consumption per car at 7,500 liters.

Private costs consist of the loss of salary due to lost work time and transportation costs arising from taking an animal to the VP. We expect transportation costs to be insignificant because vaccination posts will be positioned close to settlements and moved frequently. The average per capita income in Chad is 52,325 FCFA (80 Euros) per month, i.e. 327 FCFA (0.50 Euros) per hour (IBRD, 2015) (International Bank for Reconstruction and Development; data.worldbank.org/country/chad?view=chart, accessed 8 October 2016, 1 USD = 0.91 Euro).

Assuming the process of vaccinating one dog vaccinated lasts 90 minutes on average, each dog owner incurs the costs of the lost work time equal to 490 FCFA (0.75 Euros) per dog (opportunity cost of labor).

Societal cost: The societal cost of rabies mass vaccination is the sum of the public cost and the private cost incurred to the dog owner. In the present calculation we assume that the private dog owners will bear the opportunity cost of bringing their dog to the vaccination cost and do not include it in the further assessment which considers only the social costs.

Consideration of uncertainty

Our assumptions and estimations are variable and we took into consideration the uncertainty of the involved parameters via Monte Carlo simulation. The cost analysis was implemented in Microsoft Excel using a Monte Carlo simulation add-on (Ersatz, Epigear.com) using 50’000 iterations. Canine : human ratios and cost items were expressed as probability distributions (Table 6.3).

Surveillance

In addition to the costs of a vaccination campaign, surveillance has been taken into account in the budget planning. Given the geographical size and limited transport infrastructure of Chad, at least one laboratory with an optical microscope should be established in each regional capital of the 22 regions assuming that the Direct Rapid Immunehistochemical Test (DRIT) could be used, combined with confirmation by the Fluorescence Antibody test (FAT), which is the current gold standard (Durr et al., 2008). Chad has currently only one fluorescence microscope in N’Djamena, the capital city. In addition, rapid laboratory diagnosis at the 108 veterinary posts (peripheral laboratories) should be established. This is, however, pending the successful validation of the rapid laboratory diagnosis. Only rabies diagnostics have been considered in these budget calculations. However, these same facilities can be used in the future for the diagnosis of other animal diseases (Table 6.4)

Table 6.3: Model parameters and their distributions

Item	Most likely dog numbers/ Most likely prices applied in scenarios	Min.	Max.	Probability distribution	Unit
Dog population	1,205,361	1,128,008	1,736,774	Pert	dogs
Canine : human ratio	1 : 9.07	1 : 9.7	1 : 6.3		
Doses of vaccine	0.20	n/a	n/a	n/a	Euro
Syringes and Needles	0.04	n/a	n/a	n/a	Euro
Vaccination Certificates	0.08	n/a	n/a	n/a	Euro
Human vaccine	76.34	n/a	n/a	n/a	Euro
Tables	0	0	0	n/a	Euro
Chairs	0	0	0	n/a	Euro
Registers	15.27	12.21	18.32	Pert	Euro
Red Pens	0.23	0.15	0.31	Pert	Euro
Blue Pens	0.23	0.15	0.31	Pert	Euro
Markers	0.76	0.61	0.92	Pert	Euro
Stamps	15.27	13.74	16.79	Pert	Euro
Muzzles	22.90	15.27	30.53	Pert	Euro
Cooling Boxes	45.80	38.17	53.44	Pert	Euro
Cooling Elements	1.53	0.76	2.29	Pert	Euro
First Aid Kits	49.62	22.90	76.34	Pert	Euro
Megaphones	68.70	22.90	114.50	Pert	Euro
					Euro
Posters	0.76	0.76	2.29	Pert	Euro
Radio Broadcasts (national)	45.80	38.17	53.44	Pert	Euro
Radio Broadcasts (local)	38.17	30.53	45.80	Pert	Euro
Cars	21374.05	18320.61	24427.48	Pert	Euro
Fuel	0.92	0.76	0.99	Pert	Euro
Supervisors (training)	30.53	22.90	45.80	Pert	Euro
Supervisors (vaccination)	45.80	22.90	45.80	Pert	Euro
Supervisors (information)	45.80	22.90	45.80	Pert	Euro
Drivers	30.53	7.63	30.53	Pert	Euro
Vaccinators (training)	15.27	7.63	30.53	Pert	Euro
Vaccinators (vaccination)	22.90	7.63	30.53	Pert	Euro
Veterinarians	30.53	22.90	45.80	Pert	Euro
Health workers	30.53	22.90	45.80	Pert	Euro
Delegate of the ministry of animal husbandry	61.07	30.53	61.07	Pert	Euro
Governors of regions	45.80	15.27	45.80	Pert	Euro

Table 6.4: Budget calculations for surveillance laboratories

	Price per Unit (Euro)	Units	Euro
Budget calculations for veterinary posts			
Equipment			
Fridge	305	1	305
Cool-boxes	17	2	34
Generator	214	1	214
Fuel for generator	46	36	1,649
Water & electricity	46	36	1,649
Cellular phone	26	1	26
Credit for cell phone	31	36	1,099
Total Equipment			4,976
Reagents			
Rabies antigene test	5	150	750
Total Reagents			750
Transportation			
Scooter	1,527	1	1,527
Fuel	8	180	1,374
Total Transportation			2,901
Subtotal			8,626
Management fees		0.13	1,121
Total			9,748
108 veterinary posts			1,052,758
Budget calculations for central laboratories			
Reagents			
Rabies antigene test	5	450	2,250
Diagnostic consumables			6,412
Sampling material			4,580
Total reagents			13,242
Transportation			
Vehicles with drivers	107	180	19,237
Fuel	15	180	2,748
Total transportation			21,985
Training			
Taining of veterinarians	76	10	763
Intersectorial workshop			3,053
Total training			3,817
Subtotal			39,044
Management fees		0.13	5,076
Total			44,120
22 central laboratories			970,631

The investment scenario

A DIB proposal is composed of an organizational and a financial plan. The essential components of the organizational plan (Figure 6.1) are investors who are willing to make available the required funds upfront to a rabies elimination authority. The rabies elimination authority is a special purpose vehicle in charge of the implementation of the suppression and maintenance phase but also for the One Health campaign effectiveness (Muthiani et al., 2015) and for surveillance of elimination. Its aim is to reach a recognized freedom of rabies status for Chad. Based on the effective reduction of rabies transmission, to be verified independently by the rabies elimination authority, the outcome funder, (an institutional donor, such as the European Union or other development agencies) refunds the investor with a previously-determined profit based on the result. In this way the risk of the investment is shared between the investors, the outcome funders and the national government. National governments cover the cost of the surveillance system and inform the outcome funder of the surveillance results independently of the outcome verification. In this way the outcome is verified by two independent sources. The supervisory board is composed of all involved stakeholders and directly supervises the rabies elimination authority. The rabies elimination campaign is composed of four stages:

1) During the pre-implementation phase, the detailed design of the campaign is prepared, surveillance and effectiveness tools are established in the central and peripheral laboratories; the state to cover the surveillance cost (Figure 6.1).

2) During the suppression phase, the first round of mass vaccination takes place within one year to not more than 18 months. The second round of mass vaccination requires preparation and would take place in the third year and fourth year.

3) During the consolidation phase, the basis for a third round would be prepared in order to protect borders and areas where rabies re-emerges in years five to eight.

4) Finally, during the post-elimination maintenance phase, active surveillance is sustained and fully financed by the government of Chad.

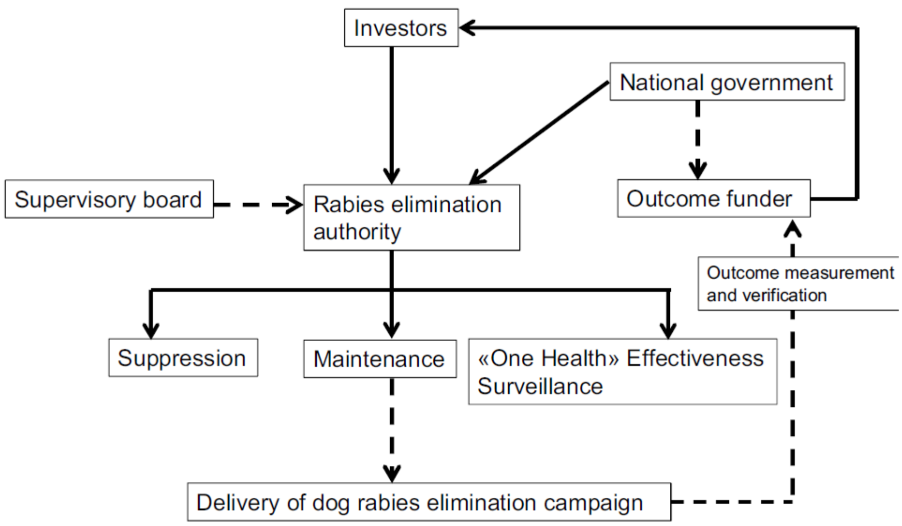

Figure 6.1: Proposed development impact bond organizational structure.

Result-based return on investment

The return to investors was divided into two components (1) a return of the capital outlaid to fund operational activities in the four different phases, and (2) premium payments, over and above the return of capital, based on the successful reduction in rabies transmission from the start of the consolidation phase onwards. The division of the payments reflects the risks being transferred to the investors; risk of operational delivery failure and epidemiological risk that following the successful operational delivery the expected reduction in disease transmission is not achieved. For the capital repayments, we assume cash is returned to the investors in year 3, at the end of the mass vaccinations in the suppression phase, year 6, midway through the consolidation phase, year 8, the end of the consolidation phase, and year 10. For the premium payments, reflecting a reward for a successful reduction in rabies transmission, we assume annual payments from year 4, the start of the consolidation phase, onwards. Different thresholds of operational delivery could be set to determine what fraction of outstanding capital is returned at different phases. Similarly, the premium payments can be explicitly linked with a measure of reduction in rabies transmission or incidence of cases. Depending on the investor/donor negotiations, refunding schemes could be adapted to regional outcome tar-gets. The selection of these thresholds can be used to incentivize investors to ensure the long-term reduction in transmission e.g.back-loading return of capital and premium payments. However, there are cost implications to the donors, as the longer the investor capital is not repaid the higher the premium will be sort by investors and so the more expensive, in absolute terms, the DIB. Here, we examine the trade-offs in structuring payments to investors under three main payment scenarios:

1 back-loaded – in which payments (capital return and premium) are weighted towards later years

2 front-loaded – with payments weighted towards earlier years

3 even – with payment spread more evenly across the different years.

The total premium paid by the donors was varied from zero to50% of the total capital investment made by the investors, and the internal rate of return (IRR) received by the investors calculated under the three different scenarios.

Results

Canine population

Based on Mbilo's household study (Mbilo et al., 2016) and the regional breakdown of the human population by prevailing social-cultural and religious composition, the canine population of Chad is estimated at 1,205,361 canines (95% confidence limits:1,128,008–1,736,774 canines).

Campaign costs and campaign scenarios

The average cost of the different scenarios is between 1.9 and 4.7million Euros depending on the number of canines vaccinated per day per vaccination post and the overall duration of one round of country-wide vaccination between 1 and 3 years. Using an estimated canine population of 1.2 million dogs the social cost per vaccinated dog varies between 1,047-2,575 FCFA (1.5-3.9 Euros, 1Euros = 655 FCFA) (Table 6.4). The high turnover of the canine populations in Chad, with an average live-span of not more than three years (Mindekem et al., 2005), indicates that scenarios favouring the vaccination of the total Chadian canine population should be prioritized. Thus the following sensitivity analyses and DIB investment calculation is focused on scenarios 3 and 4 (Table 6.5).

Table 6.5: Output of Monte Carlo simulations of the cost estimate of different scenarios of nation-wide canine mass vaccination in Chad.

Scenario	Median Euro	Mean Euro	StDev Euro	Low Euro	High Euro	LCI 95% Euro	HCI 95% Euro
(1) 100 dogs/3 years	1,919,999	1,928,120	126,111	1,528,017	2,471,773	1,705,622	2,191,253
(2) 50 dogs/3 years	3,036,124	3,051,286	245,936	2,341,363	4,044,089	2,619,973	3,564,933
(3) 100 dogs/1year	2,712,426	2,716,359	159,042	2,217,077	3,342,698	2,417,353	3,035,081
(4) 50 dogs/1 year	4,733,178	4,739,432	316,036	3,657,035	5,891,900	4,140,898	5,370,532

Table 6.6a: DIB financing overview and repayment scenarios.

Cash flow profile		a) Undiscounted cost					Outcome Founder		Front-loaded Scenario		Even Scenario	
Year	Effective Reproductive Number	Government Surveillance Euro	Investor Effectiveness Euro	Investor Mass vaccination Euro	Investor Total annual cost Euro	Total Annual cost Euro	Outcome Funder % Outstanding Capital	% Total Premium	Outcome Funder % Outstanding Capital	% Total Premium	Outcome Funder % Outstanding Capital	% Total Premium
−1	1.5	1,000,000	1,100,000		1,100,000	2,100,000						
0	1.5	1,000,000	1,100,000	3,000,000	4,100,000	5,100,000						
1	0.7	1,000,000	1,100,000		1,100,000	2,100,000						
2	0.5	1,000,000	1,100,000	3,000,000	4,100,000	5,100,000						
3	0.3	1,000,000	1,100,000		1,100,000	2,100,000	0.5		1		0.8	
4	0.15	1,000,000	1,100,000	1,000,000	2,100,000	3,100,000		0.05		0.25		0.142857
5	0.075	1,000,000	1,100,000	1,000,000	2,100,000	3,100,000		0.05		0.2		0.142857
6	0.0375	1,500,000	600,000	1,000,000	1,600,000	3,100,000	0.75	0.1	1	0.2	0.8	0.142857
7	0.017	1,500,000	600,000	500,000	1,100,000	2,600,000		0.1		0.1		0.142857
8	0.0085	1,500,000	600,000	500,000	1,100,000	2,600,000	0.9	0.15	1	0.1	0.8	0.142857
9	0.004	1,500,000	600,000	500,000	1,100,000	2,600,000		0.15		0.1		0.142857
10	0.002	1,500,000	600,000		600,000	2,100,000	1	0.4	1	0.05	1	0.142857
		14,500,000	10,700,000	10,500,000	21,200,000	35,700,000						

Table 6.6b: Example for the Even Scenario and a Total Premium of 5 million Euro giving an IRR to the investors of 9%

Year	Effective Reproductive Number	a) Undiscounted cost Government Surveillance Euro	Investor Effectiveness Euro	Investor Mass vaccination Euro	Investor Total annual cost Euro	Total Annual cost Euro	Outcome Founder Outcome Funder Capital Euro	Premium Euro	Net Cash Flow Total Euro	Investor Annual Euro
−1	1.5	1,000,000	1,100,000		1,100,000	2,100,000				−1,100,000
0	1.5	1,000,000	1,100,000	3,000,000	4,100,000	5,100,000				−4,100,000
1	0.7	1,000,000	1,100,000		1,100,000	2,100,000				−1,100,000
2	0.5	1,000,000	1,100,000	3,000,000	4,100,000	5,100,000				−4,100,000
3	0.3	1,000,000	1,100,000		1,100,000	2,100,000	9,200,000		9,200,000	8,100,000
4	0.15	1,000,000	1,100,000	1,000,000	2,100,000	3,100,000		714,286	714,286	−1,385,714
5	0.075	1,000,000	1,100,000	1,000,000	2,100,000	3,100,000		714,286	714,286	−1,385,714
6	0.0375	1,500,000	600,000	1,000,000	1,600,000	3,100,000	6,480,000	714,286	7,194,286	5,594,286
7	0.017	1,500,000	600,000	500,000	1,100,000	2,600,000		714,286	714,286	−385,714
8	0.0085	1,500,000	600,000	500,000	1,100,000	2,600,000	3,056,000	714,286	3,770,286	2,670,286
9	0.004	1,500,000	600,000	500,000	1,100,000	2,600,000		714,286	714,286	−385,714
10	0.002	1,500,000	600,000		600,000	2,100,000	2,464,000	714,286	3,178,286	2,578,286
		14,500,000	10,700,000	10,500,000	21,200,000	35,700,000	21,200,000	5,000,000	26,200,000	5,000,000
										23.6%
										9.0%

Sensitivity analysis

A sensitivity analysis of the Monte Carlo simulation using the Spearman correlation coefficient (SCC) related the cost estimate with the variability of all input variables (Table 6.3). In scenario 3and 4, the most sensitive variables are the human vaccination cost, the vaccinator numbers and vaccinator allowances with SCC values above 0.2. Canine population numbers were less sensitive with SCC values below 0.1.

Development impact bond financing

Based on the laboratory cost for surveillance and effectiveness (Table 6.4) and the cost of the mass vaccination scenarios 3 and 4 (Table 6.5), a cash flow profile for a DIB for the nation-wide elimination of canine rabies in Chad was developed. Table 6.6a presents the undiscounted contributions of the Chadian Government for the surveillance and those of the investor for the effectiveness monitoring and the mass vaccination. The outcome funder starts paying back the investor from year four onwards. Over a period of ten years, the Chadian government would contribute 14.5 million Euros and the investor 21.2 million Euros, which are refunded successively upon the achievement of operational delivery in the different phases and elimination result, expressed as the effective reproductive number R_e tending towards zero. The percentage of capital and premium payments back to the investors (assuming all output and outcome targets are met) for the three different scenarios are also shown. Table 6.6b gives an example for the "Even" repayment scenario and a total premium paid by the donors of 5 million Euros for successful operational and dis-ease reduction delivery. The annual net cash to the investors gives an IRR of 9%. Figure 6.2 explores how the IRR varies as a function of the total premium donors are prepared to pay for success under the different scenarios of weighting payments by time. To give a target IRR that is acceptable to investors will cost more the more back-loaded the repayments are. Back-loading payments is important to ensure payments equate to long-term successfully delivered outcomes. However, this comes at a cost in terms of total premiums paid. In reality, the details of the payment triggers and levels of returns will require extensive negotiations between investors and payers. The more complex the DIB financing structure is conceived, the higher are the transactions costs, which are an additional hidden cost not included here.

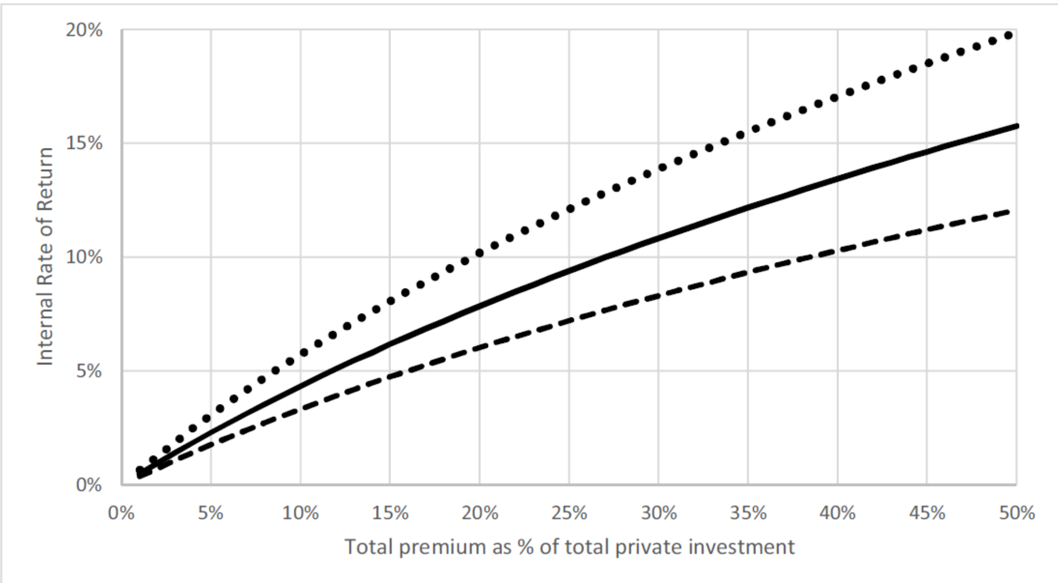

Figure 6.2: Internal Rate of Return (IRR) under the three different payment scenarios for different total premiums: straight line = even; dotted line = front-loaded; dashed line = back-loaded.

Discussion

We used data from a representative national household study on the canine: human ratio and the last population census of Chad for the estimation of the total canine population of Chad (Mbilo et al., 2016). Further, the vaccination costs of a mass vaccination campaign in N'Djamena, Chad of 18,000 canines in 2012 and 22,000 canines in 2013 were used for a nation-wide extrapolation (Lechenne et al., 2016a). The estimated total costs are based on the effective cost of materials, means of informing the population, salaries, methods of vaccinating, and time factor of the process of vaccinating canines. The costs of coordinating and performing the campaign, and the distances to be travelled during the campaign were also estimated. There are few studies estimating the costs per canine vaccinated in a parenteral mass vaccination campaign in Africa. A representative study was performed by Bögel et al. in 1990, which assumes vaccination costs of 1.18 Euros per canine. A smaller study was performed in N'Djamena in 2005, when 3000 canines were vaccinated in a parenteral vaccination campaign. The societal costs per canine vaccinated added up to FCFA 1,610 (2.46 Euros) per canine and marginal costs for every additional canine were FCFA 1,036 (1.58Euros) (Kayali et al., 2006). A recent study in South-Eastern Tanzania reports cost per vaccinated dog between 2.5 USD and 22.49 USD (2.26–20.4 Euros) (Hatch et al., 2016) Our estimates of the scenario 3 and 4 of 1,476 (2.25) and 2,575 FCFA (3.93 Euros) are slightly higher than earlier campaigns in Chad, which is largely due to increasing personnel costs, reflecting the economic development in Chad over the past ten years. As the sensitivity analysis shows, vaccinator salaries paid during a nationwide parenteral vaccination campaign is the cost item with the greatest impact on the budget calculations, followed by the cost of human vaccination, depending on the number required. This is the first DIB cash flow profile estimate for a nation-wide canine rabies elimination campaign in Africa. As the basic concept of a DIB, the overall risk is shared between investors, outcome funders and national governments. As the refunding of the investor is result- based, the cost of surveillance and campaign effective-ness is substantial. However, this investment in animal disease surveillance will be of considerable benefit to the surveillance of other animal diseases in Chad, such as foot and mouth disease (FMD) or contagious bovine pleuro-pneumonia (CBPP). Overall this analysis shows that a DIB is beneficial to all parties: The nation of Chad becomes free of canine rabies and gains a functional animal disease surveillance system; the investor benefits reasonably from his/her investment, and the outcome funder obtains a tangibleresult for result-based refunding of the primary investment. A successful result requires a well-functioning organizational structure with effective communication between the supervisory board, the rabies elimination authority, the outcome funder, and the investor (Figure 6.1).

Conclusion & Recommendations

Eliminating canine rabies by vaccinating the primary vector species has been successful in many countries in Europe and in North America (Freuling et al., 2013). Canine mass vaccination campaigns can interrupt canine-to-canine transmission of the virus, upon which the incidence of canine rabies significantly depends. Vaccination coverage of at least 70% is expected to stop canine-to-canine transmission completely. Provided the experience with the previous canine mass-vaccination campaigns in N'Djamena in 2012 and 2013 are applicable to other areas of Chad, our results indicate that a parenteral canine rabies mass-vaccination campaign provided free-of-charge, repeated one or two times, and covering the whole of Chad each time is a feasible and cost-effective way to eliminate rabies nationwide. We recommend testing campaigns in rural areas on a small scale to validate our assumption of the number of dogs that can be vaccinated daily in rural areas of Chad. This study serves as a preparatory step for a DIB-funding request to several governments and private investors. DIBs are an extension of Social Impact Bonds (Hughes, 2014) and are based on the principle of sharing risk between donors and a stringent result-based pay-back regime. Thereby private investors make available the needed funds upfront and are refunded by an institutional donor i.e. a development agency based on the effective results of the operation. In this way all participants take a more active role towards a successful operation. This approach could become an instrument for a global subsidiary principle of zoonoses elimination, which is currently not part of the Global Fund portfolio (Zinsstag et al., 2007).

Ethical consideration

This study was approved by the ethical review board of the cantons of Basel, Switzerland (Ethik Kommission beider Basel, EKBB ref. 168/13, 29 July 2013) and authorized by the Chadian public health authorities.

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CHAPTER 7

A mixed methods approach to assess animal vaccination programmes: the case of rabies control in Bamako, Mali

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Abstract

In the framework of the research network on integrated control of zoonoses in Africa (ICONZ) a dog rabies mass vaccination campaign was carried out in two communes of Bamako (Mali) in September 2014. A mixed method approach, combining quantitative and qualitative tools, was developed to evaluate the effectiveness of the intervention towards optimization for future scale-up. Actions to control rabies occur on one level in households when individuals take the decision to vaccinate their dogs. However, control also depends on provision of vaccination services and community participation at the intermediate level of social resilience. Mixed methods seem necessary as the problem-driven transdisciplinary project includes epidemiological components in addition to social dynamics and cultural, political and institutional issues. Adapting earlier effectiveness models for health intervention to rabies control, we propose a mixed method assessment of individual effectiveness parameters like availability, affordability, accessibility, adequacy or acceptability. Triangulation of quantitative methods (household survey, empirical coverage estimation and spatial analysis) with qualitative findings (participant observation, focus group discussions) facilitate a better understanding of the weight of each effectiveness determinant, and the underlying reasons embedded in the local understandings, cultural practices, and social and political realities of the setting. Using this method, a final effectiveness of 33% for commune Five and 28% for commune Six was estimated, with vaccination coverage of 27% and 20%, respectively. Availability was identified as the most sensitive effectiveness parameter, attributed to lack of information about the campaign.

We propose a mixed methods approach to optimize intervention design, using an “intervention effectiveness optimization cycle” with the aim of maximizing effectiveness. Empirical vaccination coverage estimation is compared to the effectiveness model with its determinants. In addition, qualitative data provide an explanatory framework for deeper insight, validation and interpretation of results which should improve the intervention design while involving all stakeholders and increasing community participation. This work contributes vital information for the optimization and scale-up of future vaccination campaigns in Bamako, Mali. The proposed mixed method, although incompletely applied in this case study, should be applicable to similar rabies interventions targeting elimination in other settings.

Keywords

Mixed methods, mass vaccination, effectiveness, rabies, Mali

Introduction

With an estimated 60,000 human deaths caused annually, rabies constitutes an important public health problem, especially in Asia and Africa (Hampson et al. 2015). The virus is transmitted by animal bites, mostly from dogs, causing infection of the nervous system that is inevitably fatal after symptoms appear (Jackson, 2013a). The high cost of Post-Exposure Prophylaxis (PEP) in addition to delivery and availability problems with vaccines and immunoglobulins intensify the burden of the disease in resource-poor countries (Shim et al., 2009; Shantavasinkul and Wilde, 2011). In Africa and Asia, human rabies is nearly always due to dog bites, and children are disproportionally exposed (Kayali et al., 2003a; Sambo et al., 2013).

Dog mass vaccination campaigns are the most effective intervention strategy for rabies control in settings where the disease is endemic (Cleaveland et al., 2006; Lembo et al., 2010; WHO, 2013). To interrupt transmission and eliminate the disease, a vaccination coverage of 70% or higher is recommended (Coleman and Dye, 1996).

In N'Djamena, Chad, a mass vaccination campaign for domestic dogs took place in 2012 and was repeated in 2013. Dog owners were encouraged to bring their animals to fixed vaccination points, set up in all urban districts, where dogs received free rabies vaccination. Vaccination coverage of 71% was achieved in both years (Léchenne et al., 2016a). However, significant variability of the coverage and participation was observed between vaccination zones.

Canine rabies is an important public health issue in Mali. The incidence of human rabies in the capital city of Bamako was 0.37 cases per 100’000 persons per year (Koné, 2013). Due to insufficient disease surveillance throughout Mali, we assume there is a substantial underestimation of the human incidence. Cleaveland et al. (2002) evaluated disease underreporting of ten- to one hundredfold in Tanzania. On the basis of a Knowledge, Attitudes and Practices (KAP) study of people in Bamako about rabies and its control (Mauti et al., 2015), the first pilot mass vaccination campaign took place in 2013 in one of the six communes of Bamako. Although the intervention strategy and the setting were comparable to those in N'Djamena, the estimated vaccination coverage in Mali was only 18% (Muthiani et al., 2015). In order to evaluate more precisely the reasons for such low participation of dog owners, a second pilot mass vaccination campaign was conducted in 2014 in Communes Five and Six of Bamako.

Rabies vaccination campaigns are problem-driven and transdisciplinary by nature, being at the interface of society, science and politics. Several cross-cutting issues interfere, such as participation, values and uncertainties, which can be both epidemiological and sociocultural (Hirsch Hadorn et al., 2008). While their effectiveness has been mostly evaluated using quantitative data, a recent anthropological study was conducted in Southern Tanzania to explore community perceptions and responses to a dog rabies vaccination program in the region, showing the importance of social and cultural determinants of the effectiveness of interventions (Bardosh et al., 2014). For example, such determinants might be local understandings of the disease and experiences of rabies cases, ideas of social responsibilities, or livelihood patterns and the linked cultural practices, including religion.

Access is a key issue for the success of any health intervention which depends on a series of factors. Obrist et al. (2007) developed an analytical, action-oriented framework to evaluate health care access in poor countries. This framework, developed specifically for Africa, “combines health service and health-seeking approaches and situates access to health care in a broader context of livelihood insecurity” (Obrist et al., 2007, p.1584). The five dimensions of access identified are availability, accessibility, affordability, adequacy and acceptability. Based on this framework, an “effectiveness model for health interventions” has been developed by Zinsstag et al. (2011b), integrating the five factors of access and adding the efficacy of the vaccine, diagnostic accuracy, provider compliance and consumer adherence. Effectiveness is then calculated as the product of the efficacy of the vaccine and the coverage, the latter being reduced by the determinants of access named above. It is important to understand as many elements as possible influencing a given health and social systems context to identify where and why an intervention loses effectiveness. This model corresponds precisely to the case of rabies control programs, which are strongly embedded in a larger socio-cultural, political and institutional context and depend on the participation of the community and all involved stakeholders.

To understand the meaning and importance of the different effectiveness parameters, a deeper qualitative assessment is required to account for the cultural, social, political and institutional context as well as the social dynamics of the setting. Since interventions like rabies mass vaccination campaigns exceed the individual participation of dog owners, it is important to consider the concept of social resilience. “Resilience refers to an ability, capability or capacity of individuals, social groups and even social-ecological systems to live with disturbances, adversities or disasters“ (Obrist et al., 2010, p.286). While rabies control programs intervene at the household level, where individuals take the decision to vaccinate their dog or not, their success depends on a higher level of social resilience to deal with the threat of rabies. Effective cooperation and collaboration of and between all stakeholders, institutions, political authorities, social groups and communities are necessary on the intermediate (community) level to build social resilience.

To monitor the recent campaign in Bamako, we used a mixed methods approach. We applied the effectiveness model of health interventions (Zinsstag et al., 2011b), adapted to the context of rabies control (Muthiani et al., 2015), and we used a triangulation method for each effectiveness parameter. We further applied an integrated mixed methodology for the intervention level allowing for consideration of cultural, social, political and institutional aspects. The pilot dog rabies mass vaccination campaign in two of Bamako's communes served as a case study where the methodology was partially applied to evaluate strengths and weaknesses and potential replicability in other settings. Along with the effectiveness analysis, a coverage estimation based on a Bayesian model was done, and the two results compared in order to validate the analysis.

The aim of this study was to elaborate a mixed methods approach providing key epidemiological and sociocultural data in order to improve and optimize future intervention designs through better evaluation of the key effectiveness determinants in rabies control and better integration of local understandings and enhanced community participation. This article presents the methodology and its application to the small scale vaccination campaign in Bamako in September 2014, subsequent to the first pilot mass vaccination campaign conducted one year earlier (Muthiani et al., 2015). The Ministry of Livestock and Fisheries (Ministère de l'Elevage et de la Pêche, Mali) approved the study which was conducted by the Central Veterinary Laboratory of Bamako (Laboratoire Central Vétérinaire, LCV) and the governmental veterinary services, in collaboration with the Swiss Tropical and Public Health Institute (Swiss TPH). The campaign was funded by the Swiss Agency for Development and Cooperation (SDC) in Mali and the EU FP7 project ICONZ supported the operational research.

Materials and Methods

Effectiveness evaluation of a rabies vaccination campaign

A mixed methods approach was developed to determine a) the effectiveness of a free-central-point rabies vaccination campaign and b) its optimization at an intermediate (community) level. The effectiveness of an intervention has both quantitative and qualitative components requiring a mixed methods approach within or between phases of the research process (Creshwell and Clark, 2007). In the case of rabies control and its multidimensionality, a mixed methods approach appears particularly adequate, as emphasized also by Bardosh et al. (2014). Rabies control has an important quantitative component consisting of the epidemiological characteristics, the vaccination coverage estimation and the participation rate in the intervention. At the same time, it has also a qualitative component that includes reasons for the participation at the household level, the involvement of each stakeholder as well as various social, cultural and political factors affecting the effectiveness parameters of the intervention. Mixed methods research relies on these different perspectives, data collection, analysis, and inference techniques, "and is cognizant, appreciative, and inclusive of local and broader sociopolitical realities, resources, and needs" (Johnson et al., 2007, p.129). Moreover, rabies control shows some characteristics of a "wicked problem" which " [involves] multiple interacting systems, [is] replete with social and institutional uncertainties, and for which only imperfect knowledge about [its] nature and solutions exist." (Mertens, 2015, p.3)

Assessment of effectiveness parameters

For each effectiveness parameter that is significant in the context of a dog rabies vaccination campaign, qualitative and quantitative methods are suggested to evaluate their dimensions, and a way of triangulation of both types of findings is proposed (Table 7.1).

Apart from vaccine efficacy and consumer adherence, all effectiveness parameters play a direct and significant role in the achievable coverage of dog rabies control interventions. For the assessment of the different effectiveness parameters we propose a triangulation of mixed methods.

Table 7.1 provides an overview on the evaluation of targeting accuracy, affordability, acceptability and provider compliance using the triangulation convergence model. In this way "quantitative and qualitative data is collected separately on the same phenomenon and then the different results are converged (by comparing and contrasting the different results) during the interpretation" (Creshwell and Clark, 2007, p.64). While the quantitative part allows for calculating the extent of these effectiveness parameters (how much), the qualitative approach contributes to find the reasons (why). For example a proportion of 60% of dog owner had no access to vaccination (quantitative, how much) because they should have crossed a busy road (qualitative, why). For the quantitative part, a household survey is conducted, while the qualitative data is collected by means of in-depth interviews with dog owners, focus group discussions and participant observation. Both types of data assessment take place during the same phase, and the findings are weighted equally.

An explanatory design is proposed to evaluate availability and adequacy of the intervention. Qualitative data are collected before the implementation of the vaccination campaign, through focus group discussions with dog owners about their needs, the feasibility of the operation strategy and timing as well as their perception of adequacy. Those findings are followed by a quantitative household survey after implementation of the campaign, which allows measuring the real extent of these effectiveness parameters. Participant observation during the sensitization and vaccination campaign and semi-structured interviews with dog owners provide an explanatory framework for the understanding of factors influencing availability and adequacy.

Finally, accessibility is analysed using a triangulation validating quantitative data model. Quantitative findings, arising from a household survey and a spatial analysis of the geographical coordinates of the vaccination points and the randomly selected households with dogs, are validated and expanded with qualitative data as an add-on. Qualitative data are collected by means of participant observation during the campaign and open-ended questions included in the household survey questionnaire to reveal reasons for lack of access.

Table 7.1: Mixed method assessment of the effectiveness parameters of a dog rabies vaccination campaign (adapted from Creshwell and Clark, 2007; Obrist et al., 2007; Zinsstag et al., 2011b)

Effectiveness parameter	**Description**	**Quantitative assessment**	**Qualitative assessment**	**Analysis: Mixed Method Design**
Vaccine efficacy	Biological feature of the vaccine	- Require vaccine trials, not assessed in this study	N.a.	Compare perceived efficacy with results from a vaccine trial
Targeting accuracy	How well health care providers and stakeholders identify the true health problem; Correct problem and most at risk	- Quantitative preparatory study about dog demography and rabies prevalence in the area - Household survey: Proportion of households who consider rabies as a problem and priority to treat	- Semi-structured interviews and participant observation to examine involvement and commitment of all stakeholders (authorities, services, traditional leaders, community, dog owners…) - Evaluation of the participatory approach of the elaboration of the campaign strategy and its implementation	Triangulation Convergence Model: Quantitative and qualitative data is collected separately, compared and contrasted, and then interpreted
Availability	Service meets clients' needs: - Household got information about the campaign - Dog handling possible, otherwise house visits would be necessary	- Household survey: Proportion of the concerned households who got the accurate information in time; proportion of dog owners which can take their dog to the vaccination point	- Focus group discussion with dog owners about their needs - Participant observation of the communication campaign preceding the intervention	Exploratory Design: Validation of qualitative data collected before the intervention by means of quantitative data collected after the intervention
Accessibility	Locations of the vaccination points are in line with the location of dog owners (geographical distance, transport, time necessary to get there); no physical barriers (river, main road) or social barriers (security, gender issues, dog handling)	- Household survey: Proportion of interviewees declaring the possibility to reach the vaccination point without difficulties - Spatial analysis of the distribution of vaccination points and households with dogs (geographical coordinates)	- Participant observation during the campaign: surroundings of vaccination points, categories of people coming to the vaccination points - Semi-structured interviews with dog owners to find out reasons for the lack of access or the "sociocultural distance" to particular places (vaccination points)	Triangulation Validating Quantitative Data Model: Validation of quantitative results with qualitative data, collected during the same phase
Affordability	Willingness and possibility to pay and perception of indirect costs: Direct costs = n.a. (vaccination is free of charge)	- Free of charge in this case, otherwise need a willingness to pay study (Durr et al., 2009; Mauti et al., 2015)	- Semi-structured interviews with dog owners on affordability and their perception of indirect costs: why the effort to get his dog vaccinated is worthwhile	Triangulation Convergence Model: Quantitative and qualitative data is collected separately, compared and

Effectiveness parameter	Description	Quantitative assessment	Qualitative assessment	Analysis: Mixed Method Design
	Indirect costs = transportation, lost time and income, costs for a cord to leash the dog	- Household survey: Proportion of households who think the costs (direct or indirect) are too high	or not	contrasted, and then interpreted
Adequacy	The organization of the intervention meets the clients' expectations: Campaign design and implementation; open hours of the vaccination points (school time, work time, week end), enough days; time of the year (school holidays); Intervention is socio-culturally adequate	- Household survey: Proportion of interviewees perceiving the service is adequate considering the campaign strategy, timing, dog management; proportion of dog owners which are present at the time of vaccination	- Focus group discussion on socio-cultural and religious issues influencing their participation, the implementation strategy and timing of the intervention - Semi-structured interviews with dog owners about their perception of the adequacy of the intervention and, if so, of reasons why the intervention is not adequate	Exploratory Design: Qualitative data collected before the intervention by means of quantitative data collected after the intervention; Qualitative assessment after the intervention to provide an explanatory framework for the understanding of factors influencing adequacy
Acceptability	Dog owners' and community's perception: Intervention is worthwhile and necessary (for dog or human health); Characteristics of providers match with those of the clients, including social and cultural values, religion, gender issues, level of awareness about rabies which is underpinned by experiences (individual, community) of human cases	- Household survey: Proportion of dog owners who think the intervention is worthwhile and necessary	- Focus group discussion on perceived service needs and community public health concerns, as well as reasons which can lead to non-acceptability of the intervention, such as cultural and social reasons or values, religion, gender issues, lack of awareness, perception of dogs' status in the society	Triangulation Convergence Model: Qualitative assessment provides an explanatory framework for deeper understanding of acceptability and underlying reasons of its lack
Provider compliance	How well the provider initiates the correct procedure for the intervention: enough doses and staff at the vaccination points	- Household survey: Proportion of dog owners which encounter a vaccination point without staff or vaccine	- Participant observation during the intervention of the campaign to detect logistical problems	Triangulation Convergence Design: Complementary data on the same issue; Qualitative assessment provides information about the origin of logistical problems
Consumer adherence	How well the recipient follows the medical advice given	n.a.	n.a.	

Intervention Effectiveness Optimization

After analyzing each effectiveness parameter individually and describing the specific mixed method design, we propose a cyclical process for the optimization of the effectiveness of the intervention. Four different phases of effectiveness optimization of an intervention can be distinguished and visualized in the form of a cycle (Figure 7.1).

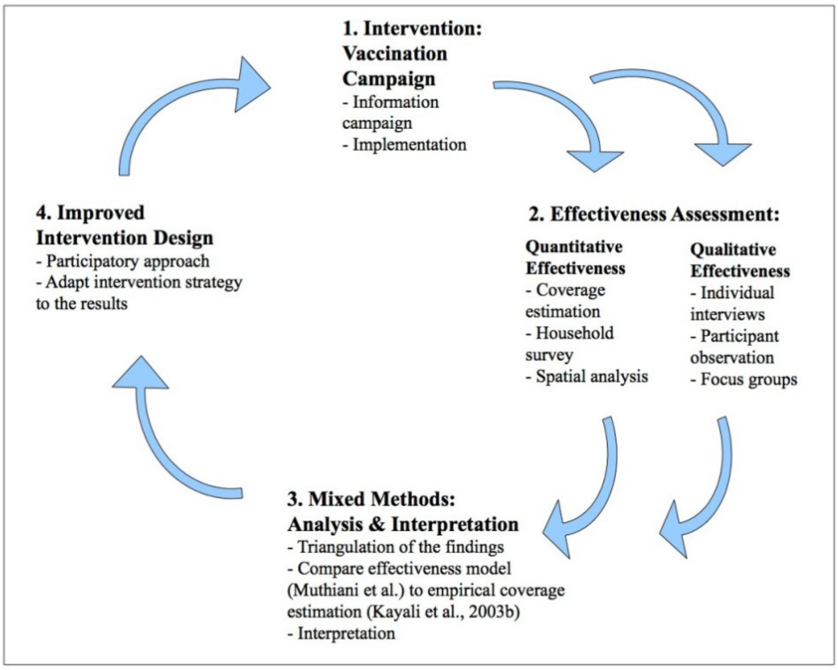


Figure 7.1: Intervention Effectiveness Optimization Cycle

The elaboration and implementation of the mass vaccination campaign constitutes the first phase. Then, its effectiveness is evaluated by means of the mixed-methods design for every effectiveness determinant to evaluate the most sensitive ones and their underlying reasons, as well as the cumulative effectiveness of the parameters. In parallel, the vaccination coverage is empirically estimated using a capture-mark-recapture approach (Gsell et al., 2012; Kayali et al., 2003b; Muthiani et al., 2015). In a third phase, the mixed method design aids in analysis and comparison of results. The estimated vaccination coverage is compared to the effectiveness model, and qualitative data serve as an explanatory framework for deeper insight, validation and interpretation of the results. Finally, the intervention design and implementation strategy is improved and adapted to the local realities, needs and understandings, and the cycle starts again. Ideally, a participatory stakeholder meeting involving communities, authorities and scientists is held to find consensus on proposed improvements of intervention designs (Schelling et al., 2008).

Testing of the method

To evaluate the pilot dog rabies mass vaccination campaign which took place in September 2014 in Communes Five and Six in Bamako, the mixed methods approach developed is partially applied to evaluate the effectiveness of the intervention and to determine the key parameter for the further steps in rabies control in this specific context. In both communes, central vaccination points, where dog owners could bring their animals for free rabies vaccination, were placed at strategic places in all neighborhoods for five consecutive days. In Commune Six, 24 central points were identified in the ten neighborhoods by their chiefs, while in Commune Five, 19 central points were determined for seven neighbourhoods.

Coverage assessment of the campaign was conducted using the capture-mark-recapture approach analogous to Gsell et al. (2012), Kayali et al. (2003b) and Muthiani et al. (2015). Briefly, vaccinated dogs, tagged with a polyethylene collar, were counted along four random transects through each commune (Commune Five and Commune Six without Senou) on the two subsequent days in the morning and in the evening using a GPS receiver. In addition, a household survey was conducted starting from 15 random points in each commune. For the analysis of the vaccination coverage and the effectiveness, the neighborhood of Senou in Commune Six, which is situated south of the airport and thereby isolated from the contiguous built surface of the city, was separately analyzed. In Senou, no transect study was done. On the basis of the Peterson-Bailey formula, the recapture sample size of owned dogs in households could be determined. Depending on the number of initially vaccinated dogs during the campaign, this statistical formula identifies the number of dogs to be recaptured during the household survey. Hence, visited households numbered 364 in Commune Five, 591 in Commune Six (excluding Senou), and 314 in Senou. The data about the vaccinated and unvaccinated dogs in the streets and in the households, the number of initially marked dogs and the rate of confinement were used to estimate the vaccination coverage of the total dog population (owned and ownerless) by means of a Bayesian model (Supplementary material). Confinement was defined as a dog enclosed in a compound and unable to get out on the street. For further clarification on the Bayesian model see Muthiani et al. (2015).

As a second approach, the effectiveness of the campaign was assessed extending the method proposed by Muthiani and colleagues, who applied the effectiveness model of Zinsstag et al. (2011b) to rabies control. The model shows the multiplicative relationship of the different coverage factors, confirming the observed vaccination coverage by the final effectiveness of a similar range (Muthiani et al., 2015). To assess the different effectiveness parameters, an in-depth questionnaire was conducted with dog owners within the vaccination zone. Starting from 54 randomly defined points, the interviewers walked systematically around the squares looking for families with dogs. In total, 604 households were visited and questioned in this way after the vaccination campaign – 283 in Commune Five, 247 in Commune Six (excluding Senou) and 74 in Senou. This second questionnaire aimed to collect data to quantify the different effectiveness parameters by asking open questions about their reasons of (non-) participation at the campaign, their perception of the risk of rabies, and the socio-economic and cultural background of the dog owners.

In addition, a spatial analysis of the participation was done, relating distance to the vaccination post and access to participate in the campaign. The coordinates of the vaccination points and the coordinates of the visited households were recorded with a GPS receiver. Distances could be quantified and a spatial circular model created, evaluating the participation around each fixed point vaccination post, indicating the spatial dimension of accessibility (see Table 7.1). The answers for non-participation were associated to the different effectiveness determinants (see Table 7.1) and their individual effectiveness was calculated as the proportion out of the non-participating households, in other words, '1 – the calculated proportion'. As these parameters are linked in a hierarchic and multiplicative way, the final effectiveness of the intervention can be evaluated as the product term of all the proportions. Specifically the values of all effectiveness determinants were multiplied. The results of both approaches (effectiveness model and empirical coverage estimation) are compared, and they should coincide.

During the preparation of the campaign and its implementation, as well as in parallel to the quantitative assessment of the effectiveness, qualitative data was collected. We first used participant observation during the preparation and the execution of the intervention. Observations from meetings with the authorities, neighborhood chiefs and other stakeholders were used for the information campaign and to determine the location of the vaccination posts. During the information campaign and the vaccination campaign, reactions of the community members, dog owners, vaccinator staff and other stakeholders were registered and some informal discussions were conducted, which also form an important qualitative data source. Some open questions were integrated in the in-depth questionnaire administered with the 604 dog owners. The whole qualitative part provides an explanatory framework for understanding the extent of effectiveness parameters of the dog rabies mass vaccination campaign and supplies critical information about social, cultural, political and institutional specificities of rabies control in urban and peri-urban Bamako.

Both types of findings were first analyzed separately. For example, the quantitative targeting accuracy can be expressed as a proportion of households which consider rabies as a priority intervention. The qualitative assessment of the targeting accuracy provides the spectrum of stakeholders committed to rabies control and provides interpretations of the achieved targeting accuracy. The findings of both methods were compared for their consistency and their contribution to a converging understanding. For example if in the quantitative assessments a large proportion would not have vaccinated their dog for financial reasons, but in the qualitative survey, financial reasons would not have been stated as a problem, there would have been a lack of consistency between the two methods. In this way information from the quantitative and qualitative assessments contribute to a convergent understanding with high validity for future interventions and their optimization, particularly in view of a scale-up to the city level. Similarly the availability of services can be quantified as proportion of households declaring that the service was available to them. Reasons for the lack of availability can be interpreted from qualitative statements.

Due to constraints in time and resources, it was not possible to conduct focus group discussions as proposed by the assessment framework of the effectiveness parameters, which is a limitation of our study.

Results

Information campaign

Before the start of the intervention, different information channels were used to communicate the upcoming free dog rabies mass vaccination campaign. Two radio stations (one commercial and one community-based) broadcast five times an announcement in both French and Bambara with general information about rabies and its risks and prevention, with the aim to raise community awareness. In a second step, during the week before the vaccination days in each commune, a communication explaining the exact location of all vaccination points was broadcast on a total of six radio stations (five commercial and one community-based). The message broadcasts were in French and Bambara approximately 20 times each week at different times of day. Neighborhood chiefs were asked to engage a town crier (crieur public) and compensated for the cost. In some neighborhoods this seemed to be effective. In some mosques, the message was communicated after prayer times. Every neighborhood chief, the town halls of the two communes and governmental veterinary services got an information poster to display. Although originally planned, no information was broadcast on television.

Vaccination campaign

During the five vaccination days in Commune Five, 429 dogs were vaccinated, with a minimum of 8 dogs and a maximum of 63 dogs per vaccination point (Table 7.2). In Commune Six (excluding Senou), we vaccinated 784 dogs. The minimum number of vaccinated dogs at one point was 11 dogs, the maximum was 80 dogs. In Senou, the situation was quite different. Only three vaccination teams were installed in this extensive neighbourhood, but they nonetheless vaccinated 410 dogs. One of the vaccination points vaccinated 191 dogs. A total of 97 cats, 4 monkeys and a jackal were brought and also vaccinated. When the attendance flagged on the second or third day, some vaccination teams initiated house-to-house vaccination around their points.

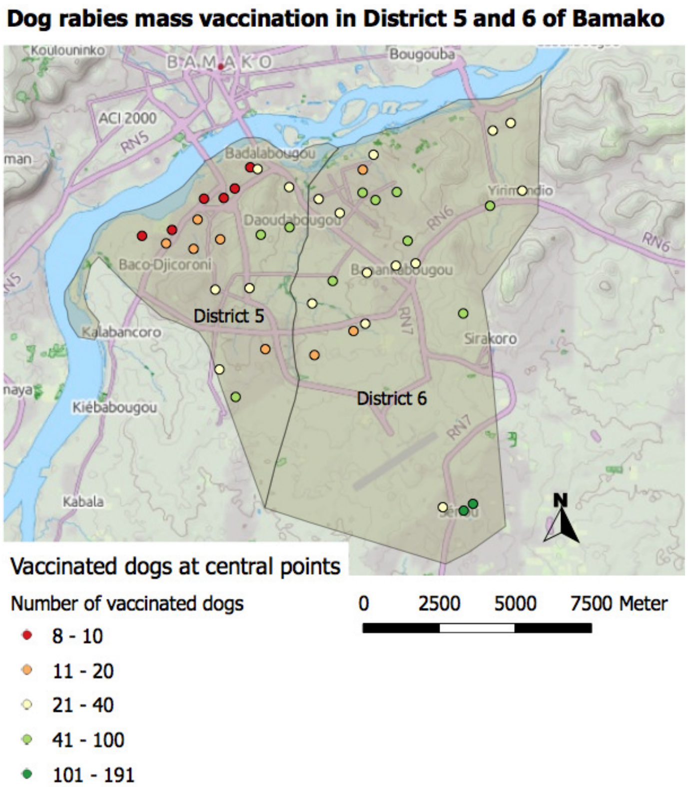


Figure 7.2: Map of Communes Five and Six of Bamako, Mali. The dots indicate the vaccination points with the respective number of vaccinated dogs.

Vaccination coverage

As described in Muthiani et al. (2015), vaccination coverage was estimated using a capture-mark-recapture procedure and a Bayesian model.

For Commune Five, the owned dog population was estimated at 1531 animals and the vaccination coverage of owned dogs at 28%. The proportion of ownerless dogs was estimated at 2%, which results in an overall coverage of 27% (Table 7.2). For Commune Six, 3510 owned dogs were estimated, with vaccination coverage of 22%. The proportion of the ownerless dog population was 8% and the overall vaccination coverage 20%. The confinement of vaccinated dogs was about 34% in Commune Five and 51% in Commune Six, and for unvaccinated dogs 45% and 43%, respectively. For Senou, the approximate vaccination coverage in the owned dog population could be estimated on the basis of the household survey. Of the 314 recaptured dogs, 152 were vaccinated, which resulted in a vaccination coverage of 48% (the vaccination status of one dog was unknown). Likewise, the proportion of confined dogs differed, with only 6% in Senou in contrast to the other areas.

Table 7.2: Summary of data collected from the vaccination points, household survey, and transect survey and vaccination coverage estimates of the Bayesian model in Bamako, Mali

	Commune 5	Commune 6[a]	Selou
	N or % (n/N)	N or % (n/N)	N or % (n/N)
Data from vaccination points			
Area[b] [km2]	19.7	32.3	nd
Number of vaccination posts	19	24	3
Number of dogs vaccinated	429	784	410
Data from household survey			
N dog identified during households survey	364	591	314
Vaccinated dogs identified during household survey	26% (93/364)	22% (131/591)	48% (152/314)
Confined vaccinated dogs	34% (32/93)	51% (67/131)	9% (13/152)
Confined non vaccinated dogs	45% (121/270)	43% (197/457)	3% (5/161)
Data from transect survey			
Average transect length [km]	9.2	11.1	na
Dogs identified during transect survey[c]	29	74	na
Dogs with collars identified during transect survey[c]	21% (6/29)	8% (6/74)	na
Recapture probability [d] [range]	0.007-0.5	0.005-0.5	na
Bayesian estimates	**Median (IQR)**	**Median (IQR)**	
Owned dog population [in 1000]	1.5 (1.3-1.8)	3.5 (3.0-4.0)	nd
Ownerless dogs [%]	1.6 (0.0-18.9)	7.6 (0.0-42.6)	nd
Vaccination coverage owned dogs [%]	27.8 (23.5-33.7)	22.3 (19.4-26.2)	nd
Vaccination coverage all dogs [%]	26.8 (22.5-32.5)	20.4 (16.2-24.4)	nd

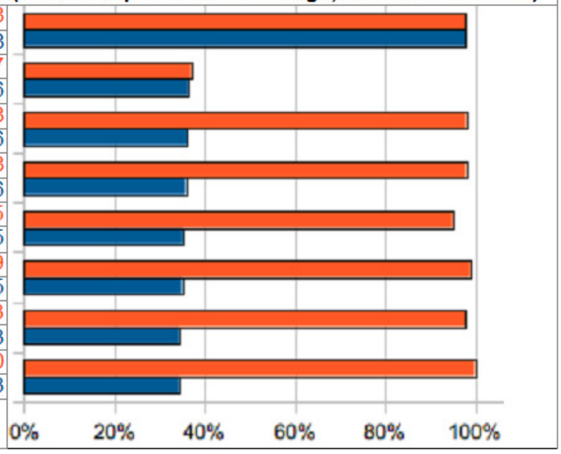
[a] excluding Senou
[b] area within polygon of outer vaccination posts
[c] total of 4 transects
[d] Used in the Bayesian model. Calculated as described by Kayali et al. 2003b but minimum divided by 2 to account for area calculation

Assessment of effectiveness parameters

Reasons for non-participation in the vaccination campaign by the interviewed dog owners were categorized to attribute them to the different effectiveness parameters (see Table 7.1). The most stated categories (all zones mixed) were “not informed” (90%), “dog owner not at home” (3%), “aggressive dog” (1%), “vaccination useless” (1%), “lack of time” (1%), “difficult to get there” (0.5%) and “didn't know where” (0.5%). Other stated reasons were “vaccination dangerous for the dog”, “dog not at home” and “other things to do”. In 10 cases (2.5%), the reason was not known.

Assuming that there was one dominating reason for non-vaccination of a dog, the mutually excluding reasons were categorized and assigned to the different effectiveness parameters (see Figure 7.3). The final effectiveness was estimated at 33% for Commune Five and at 28% for Commune Six. Coverage estimation and questionnaire based effectiveness assessment are similar. Thereby, effectiveness assessment of the second small scale rabies mass vaccination campaign in Bamako validates the effectiveness model developed by Muthiani et al. (2015).

Parameter	Assessment	Effectiveness (individual parameter in orange, cumulative in blue)
Efficacy	Vaccination efficacy	0.98 / 0.98
Availability	Information available	0.37 / 0.36
Accessibility	Dog handling & barriers	0.98 / 0.36
Affordability	Perceived (in)direct costs	0.98 / 0.36
Adequacy	Duration, presence of owner	0.95 / 0.35
Acceptability	Owner perception, social & cultural values, others	0.99 / 0.35
Targeting	Proportion of owned dogs	0.93 / 0.33
Provider compliance	Team present at point	1.00 / 0.33
Consumer adherence	NA	



Efficacy	Vaccination efficacy	0.98 / 0.98
Availability	Information available	0.35 / 0.34
Accessibility	Dog handling & barriers	0.97 / 0.34
Affordability	Perceived (in)direct costs	0.98 / 0.34
Adequacy	Duration, presence of owner	0.92 / 0.33
Acceptability	Owner perception, social & cultural values, others	0.95 / 0.32
Targeting	Proportion of owned dogs	0.87 / 0.28
Provider compliance	Team present at point	1.00 / 0.28
Consumer adherence	NA	

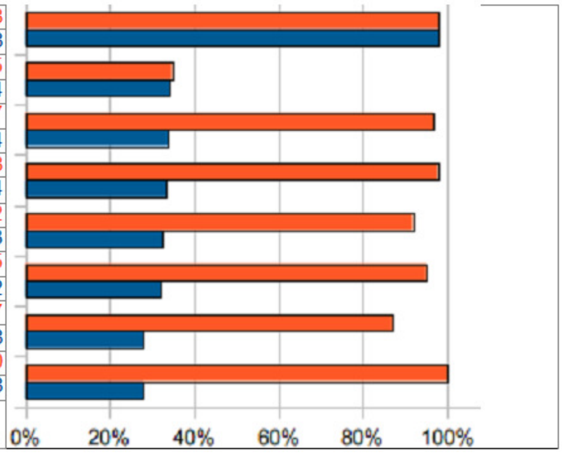


Figure 7.3: Effectiveness determinants of the dog rabies mass vaccination campaign in Commune 5 (above) and in Commune Six (below) in Bamako, Mali. Orange bars show the extent of each effectiveness determinant, and blue bars the cumulative effectiveness (adapted from Muthiani et al., 2015).

Vaccination efficacy

The efficacy of the rabies vaccination for dogs has been tested in the laboratory. The applied value of 0.98 was assumed from the provider (Rabisin MerialTM).

Availability

In both communes, availability appeared to be the key parameter. Lack of information among the population explains the important decrease of effectiveness: while the other parameters all showed high individual effectiveness of between 92% and 100%, the value for the parameter of availability attained only 37% and 35% respectively in Commune Five and Commune Six. Availability implies that the households got all necessary information about the campaign to find the vaccination point and go there in time. Even though an information campaign took place before the start of the vaccination, this was the key issue contributing to a low final effectiveness of the intervention.

The majority of the interviewed dog owners which did not participate in the vaccination campaign thought that television and radio were important channels to inform people (61% and 48%). 21%

mentioned town criers, while 16% mentioned posters. About 6% stated either the neighborhood chief or mosques and churches as important channels. Other answers were short text message (SMS) sent by the two national telecom companies (Malitel and OrangeTM) and door-to-door awareness campaign.
Dog owners who did participate in the vaccination campaign were asked how they were informed. 35% mentioned neighbors, friends or family members, 20% heard from radio, and 18% by the vaccination team coming to the door. 8% heard the message from a town crier and 6% from their neighborhood chief. The sharing of information about the campaign to family and friends and motivating others to bring their dogs for vaccination constitutes a capacity to deal with the threat of rabies. One third of the participating households got the information from another person, which constitutes social resilience at the intermediate level of the community.
Thus, in terms of the applied triangulation methodology (exploratory design), the quantitative data could validate qualitative findings from the participant observation of the communication campaign (focus group discussions unfortunately missing). Differences of the information and participation rates were noted between neighbourhoods where a town crier was engaged or the neighbourhood chief himself circulated to mobilize his population, and neighbourhoods where this was not the case.

Accessibility

Dog handling was not a problem for the majority and only some isolated dog owners said it was difficult to get to the vaccination point. Therefore, accessibility reached a good score of about 97% in both communes. No major physical barriers were mentioned, despite the campaign taking place during the rainy season.
An important factor of accessibility was the physical distance between the vaccination points and the dog keeping households. Figure 7.4 shows the localization of vaccination points (yellow dots) and interviewed households for the questionnaire study. The green dots represent the interviewed households which did participate in the campaign, while red dots represent non-participating households. In general, households close to the vaccination point were more likely to participate. Spatial analysis of all interviewed households and the nearest vaccination point provided the following results: the average distance between households who did participate and vaccination point was nearly 400m, while it was about 710m for non-participating households. A possible explanation is that it was easier for them to bring the dog to the vaccination point, but additionally, the probability that they were informed about the intervention was likely also higher. The cluster of red dots on the eastern side of Commune Six illustrates this fact, as no vaccination point was situated in this area. Therefore, it might be of crucial importance to have as many vaccination points as possible dispersed in the territory.

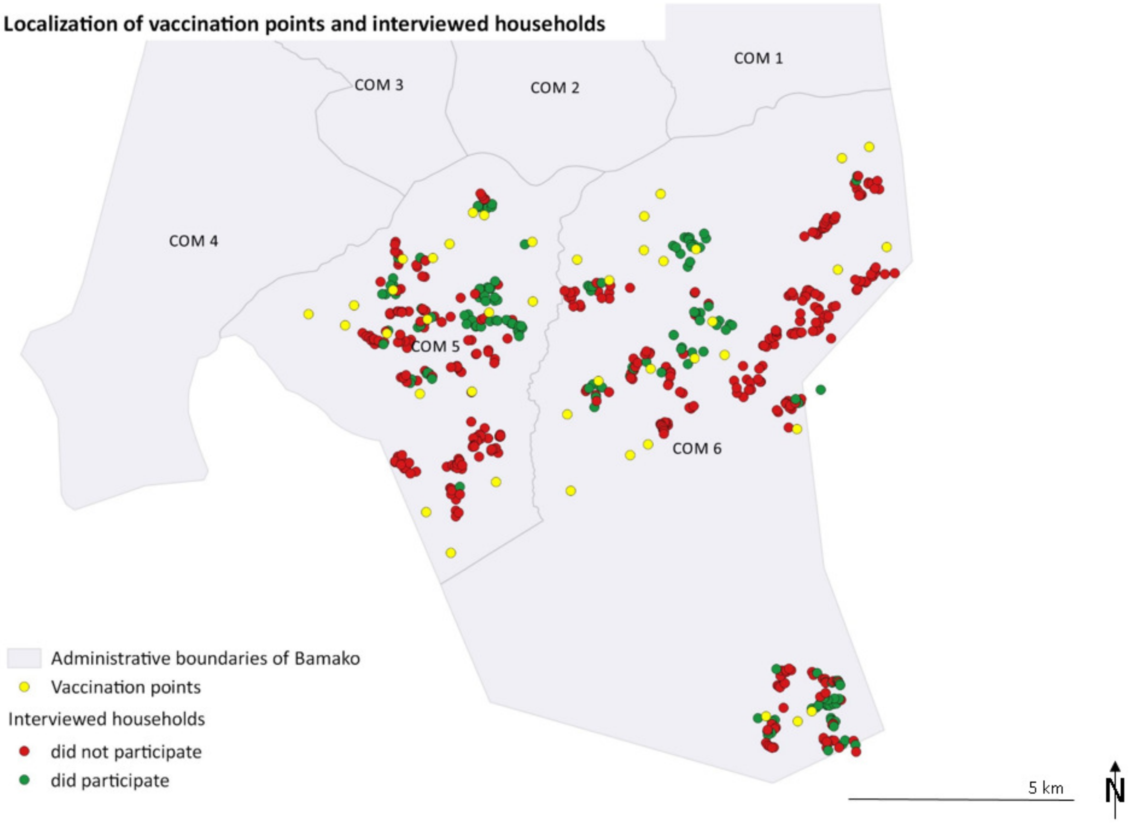


Figure 7.4: Localization of the vaccination points and the interviewed households

Dog handling is also a crucial component of accessibility, as a vaccination campaign by central vaccination points is not accessible for households who cannot lead their dogs. Only 1% stated "aggressive dog" as reason for non-participation.

In 37% of the cases, dogs were brought to the vaccination point by children. In 9% each, it was the household chief or another adult person, while in 2% it was the wife who brought the dog. For 39% of the vaccinated dogs, the vaccination team came directly to the household. This was not originally, but implemented spontaneously by vaccination teams rather than waiting when owners were not bringing dogs.

Affordability

Individual effectiveness of this parameter was estimated at 98%. As the vaccination was free of charge for the dog owners, direct costs were not an issue, explaining the high effectiveness score of this determinant. However, the time necessary to get to the vaccination point might potentially imply loss of income and can therefore be considered as indirect costs. Only a few dog owners mentioned that it took too much time to bring the dog to the vaccination point, with 1% stating that they had other things to do. According to the triangulation convergence model proposed for the analysis of this parameter, these quantitative data were then compared to the qualitative statements on affordability and general financial issues around dog vaccination of the in-depht questionnaire and participant observation. Certainly, it is difficult to evaluate the perception of indirect costs in a simple questionnaire study. Nonetheless, the answers of the dog owners allow for estimating the range of the parameter. Expenses for transportation would cause other indirect costs but this was not mentioned, as almost all dogs were brought to the vaccination point on foot.

Adequacy

The effectiveness parameter of adequacy was slightly lower, with a score of 95% for Commune Five and 92% for Commune Six. The decision to take a dog for vaccination is taken by its owner. A few dogs were not vaccinated because of the absence of the owner. With the exception of these owners, duration was judged to be sufficient with five consecutive vaccination days per commune. Since many dogs were brought to the vaccination points by children, the timing of the intervention was adapted to the school holidays. In terms of the triangulation methodology that was applied, which is the exploratory design (see Table 7.1), we compared the qualitative data collected beforehand by Mauti et al. (2015) and Muthiani et al. (2015) with our quantitative findings. Qualitative information issue of the participant observation during the vaccination campaign helped to interpret the findings on the adequacy, especially timeliness, of the campaign organization. In other terms, former experiences showed that the timing of the intervention (during school holidays and weekend) were judged to be a good timing. Quantitative findings presented above could be confirmed by the qualitative statements of dog owners about the timing of the intervention. Respondents mentioned mainly timeliness aspects; we could also expect qualitative statements on cultural adequacy. For example in N'Djamena we experienced that placing a vaccination post in front of houses from some religious groups were not considered adequate. In this way, such qualitative statements contribute to the interpretation of quantitative data on adequacy.

Acceptability

At 99% (Commune Five) and 95% (Commune Six), acceptability was higher than evaluated by Muthiani and colleagues for the pilot dog rabies mass vaccination campaign in Commune One in 2013 (Muthiani et al., 2015). Very few people showed a negative attitude towards the vaccination campaign of dogs, which was generally linked to lack of knowledge about the disease. A few people thought that the vaccination was pointless or dangerous for the dog or mentioned that the vaccine was not of good quality because it was free of charge offered by a foreign (European) institution.

At the same time, participating households stated great satisfaction about the campaign. Reasons given for participation were mostly linked to safety of the family members and neighbors, the dog's well-being, and prevention of problems in case of a dog bite. The general level of community awareness about rabies and its risks seemed to be quite high. Dog owners were also aware of potential problems if the dog bites somebody, such as the high costs for PEP treatment and conflicts with the neighbors.

Therefore, people were wary when interviewers asked them if they had a dog. Triangulated with the quantitative findings mentioned above, these qualitative data provide an explanatory framework for better understanding of this effectiveness parameter and underlying reasons of the lack of accessibility.

Targeting accuracy

This parameter was quantified using Bayesian estimation of the ownerless dog population, as the chosen intervention strategy with fixed vaccination points can only reach the owned dog population. Targeting accuracy was evaluated at 98% for Commune Five and 92% for Commune Six. The work of Mauti et al. (2015) and the pilot vaccination campaign in 2013 (Muthiani et al., 2015) served as foundations for the present project guiding the strategy to implement by identifying important information about dog demography in Bamako and rabies awareness of Bamako's population. Communes Five and Six are areas with a higher rabies incidence (together with Commune One), so these zones were chosen for the pilot campaigns (Traoré, Personal communication, 2014). The target goal of 70% vaccinated dogs in the overall population could theoretically be reached through exclusively vaccinating the domestic dogs.

Provider compliance

No shortage of vaccine doses or absences of vaccination staff were reported by dog owners in the questionnaire study, and observations during the vaccination days concurred. Thus, effectiveness of provider compliance was evaluated at 100% in both communes.

Discussion

Rabies control is a complex undertaking due to its multidimensionality (epidemiological components, along with cultural, social, political and institutional aspects) and the diversity of stakeholders involved. These issues all affect the social resilience of the community and, thereby, influence community participation and effectiveness of an intervention. This article proposes a mixed methodology to evaluate effectiveness of a rabies intervention, developed on a small scale dog rabies mass vaccination campaign carried out in two Bamako communes.

Although the vaccination coverage in Communes Five and Six was slightly higher, 27% and 20%, than previously in Commune One, 18%, both were far below the WHO recommendation of 70%. The proportion of ownerless dogs was estimated at 2% and 8%, but few dogs were observed in the streets during the transect study. A confidence interval for the ownerless dog population was considered, but this had a limited impact on total vaccination coverage. It is important to note that sensitivity analysis of the Bayesian model revealed the minimum recapture probability as the most sensitive parameter. However, only the proportion of ownerless dogs was sensitive to this parameter, whereas the proportion of vaccinated owned dogs remained relatively stable.

Effectiveness assessment achieved a similar range as vaccination coverage, with a final effectiveness of 33% for Commune Five and 28% for Commune Six. Effectiveness assessment based on the household survey showed similar results, thus confirming them. Vaccine efficacy was not a factor here, but for other diseases, it could be important to compare vaccine trial results and user perception. For example in the case of cattle vaccination against anthrax in Chad, livestock owners perceived a loss of vaccination quality which led to the discovery of a contaminated vaccine (Schelling, personal communication). Availability, essentially lack of information, stood out as the key effectiveness parameter, similar to the earlier campaign in Commune One (Muthiani et al. 2015). Despite the preceding communication campaign using radio as main channel, many dog owners stated that they did not get the information. Theoretically, this could be an excuse for not attending vaccination. However, we don't think that people were not telling the truth, but rather that they did not get all necessary information to take action. Missing background information and knowledge about why exactly dogs are vaccinated may lead to lacking attention given to the communications about the campaign, resulting that people say they had not been informed.

Television announcements might have reached additional households and should certainly be included in future. Furthermore, as participation rate was higher in neighbourhoods where town criers had been engaged, this communication channel or loudspeakers should be better used in a next campaign. Hanging up public posters at strategic places, such as health centres, schools or market places could be increased (more than one poster per neighborhood). Most often children brought the dog to the vaccination point. Some of the vaccinator teams initiated house-to-house vaccination, which functioned to increase the vaccination coverage and effectiveness.

Inspite of a similar procedure as the one applied for the dog rabies mass vaccination campaigns in N’Djamena where vaccination coverage exceeded 70%, vaccination coverage of the pilot campaign in Bamako was again very low. Missing information and awareness were the primary reason. Compared to N’Djamena, neighborhood chiefs were less comitted and did not push dog owners in an autoritative way to participate. Religion did not turn out to be a reason for people to participate or not.

Although coverage estimation and assessment of effectiveness parameters using quantitative methods allows for evaluation of the final effectiveness level of the intervention, no insight is provided for underlying reasons. That component requires a qualitative assessment to provide an intersubjective explanatory framework to understand factors which influence, in one way or another, effectiveness. A mixed-research design provides the methodological foundation to integrate different methods and findings at the level of each effectiveness parameter and at the intervention level, contributing to optimization of intervention design. Moreover, mixed-methods research is particularly useful in low- and middle-income countries to assess health systems performance and social, economic and cultural context (Ozawa and Pongpirul, 2013). This approach allows researchers with different backgrounds to collaborate across disciplinary and academic boundaries and work with non-academic stakeholders, whichstrengthens the transdisciplinary research (Creshwell and Clark, 2007). Such broader understanding of the societal components is necessary for a problem-driven study oriented toward practical and implementable results to improve intervention strategy in the future. The mixed-method approach proposed reflects such qualities, bringing together diverse researchers and integrating, via qualitative methods, the voices of the different actors. Using a triangulation method reduces the bias of each data source because they are joined to others, so “the result will be a convergence upon the truth about some social phenomenon” (Denzin, 1978, in Johnson et al., 2007, p.115).

Depending on the effectiveness determinants, different forms of triangulation methods are proposed (see Table 7.1), with changes in the modalities of timing of qualitative and quantitative methods, their respective weighting, and the way to integrate findings for the analysis. Careful consideration of the type of assessment allows better understanding of each effectiveness parameter individually, before addressing effectiveness at the intervention level. The proposed methodology, presented in form of a cycle (Figure 7.1), aims to guide methodological considerations allowing for continuous intervention optimization. Since rabies control interventions, such as a dog mass vaccination campaign, occur at the community level of social resilience, a holistic viewpoint is essential. The mixed approach provides the required data – quantitative and qualitative – and its consolidation, which is necessary to better understand and explain intervention effectiveness and social resilience on an intermediate level.

However, a mixed research design still has drawbacks. More time and resources are necessary to conduct different methods in the same study. Also, it is challenging to articulate and integrate both types of data findings, and the analysis is more time-consuming. Attention is required to maintain transparency on methods applied. If discrepancies arise between the different data findings, further explanations need to be considered, often as the subject of a future study, i.e. a second iteration of the proposed cyclical approach.

Regarding the quantitative methods, we used the same approach as Muthiani et al. (2015), including coverage estimation by a capture-mark-recapture procedure and effectiveness model – complemented by an in-depth questionnaire. These methods are well established and simple to carry out but do require logistics, time and software. Some biases of the coverage and effectiveness estimation are possible, such as the low number of dogs counted during the transect routes, non-neutral answers given during the household survey, or the selection of survey questions to investigate effectiveness parameters.

The qualitative methods used are limited to participant observation and informal discussions. Even though these are appropriate methods to collect valuable data about social, cultural, political and institutional aspects influencing effectiveness, focus group discussions and semi-structured interviews with dog owners would have generated deeper insight into underlying reasons for participation at the vaccination campaign. Furthermore, communities may generate explanations by themselves, in a constructivist way. “Sociocultural aspects, such as actors’ perceptions of risk or the identification of particular risk groups, are essential for the development of successful interventions.” (Zinsstag et al., 2011b, p.627) It would be useful to conduct focus group discussions or semi-structured interviews with key informants to collect more detailed information on enabling factors, local capacities and building resilience, as proposed by Bardosh et al. (2014). The introduction of the concept of social resilience in the research of effectiveness in rabies control is novel and, by its nature, needs qualitative research. The strength is a focus not only on the individual level, but also on the community level which is crucial in rabies control interventions. Yet, the interpretation warrants intersubjective convergence and the involvement of diverse stakeholders. Rabies control and research on intervention effectiveness is a significant example illustrating interlinked qualitative and quantitative inquiry and the interface between paradigms of humanities and natural science. The methodology proposed in this article could be usefully applied to similar health interventions in other settings.

The present study validates the effectiveness assessment of Muthiani et al. (2015). In addition, with the mixed-methods approach developed in this paper we establish further information on underlying reasons of dog owners to participate in such a campaign, community awareness and stakeholder collaboration, and other social, political, cultural and institutional factors which may influence the effectiveness of rabies control interventions. Because of qualitative data collection before, during and after the campaign, the quantitative effectiveness parameters can be interpreted in a more precise way.

Conclusions

Further research with other disease systems is needed to validate and generalize the proposed mixed methods approach. Independent teams should separately apply the qualitative and quantitative methods and subsequently compare them.

Considering the low vaccination coverage and effectiveness of the second small scale dog rabies mass vaccination campaign in Bamako, the intervention design should be adapted in the future steps. We identified availability as key effectiveness parameter, which is strongly linked to lack of information about the campaign. Therefore, a future intervention should concentrate on a strong information campaign adding television as communication channel along with more information posters and town criers (griots) in the concerned neighborhoods. As stated by Muthiani et al. (2015) and Léchenne et al. (2016a), children are important regarding the transfer of information. Communication campaigns in schools could be an efficient strategy to increase community awareness.

Communes Five and Six are both large areas and the number of vaccination teams was limited to cover the whole territory. For further interventions, it could be useful to stay fewer days at the same vaccination point, moving more quickly to new points. A combination with a house-to-house strategy is probably necessary to achieve high vaccination coverage.

The proposed mixed-methods approach helped to better understand the effectiveness parameters. Focus group discussions should be integrated in future. Importantly, the proposed methodology can be adapted to other settings and interventions.

Acknowledgments

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VI. WORKING PAPER

Molecular dynamics of classical rabies virus during a mass dog vaccination campaign

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Introduction

Classical rabies virus (RABV) consists of a tightly encapsidated negative stranded RNA genome, which encodes for five different proteins: the Nucleoprotein (N), the Glycoprotein (G), the Matrixprotein (M), the Phosphoprotein (P) and the Polymerase (L for large protein) (Schnell et al., 2010). The Nucleoprotein regulates transcription and replication. Is has been shown to trigger the innate immune system and can be used as an Adjuvans in vaccine development (Koser et al., 2004). As the Glycoprotein is the surface protein of the virus capsule, it is the key player for infectivity and pathogenicity. Virus neutralizing antibodies are specifically directed against the G protein (Schnell et al., 2010).

The epidemiology of the classical rabies virus RBV is strongly linked to canid species, first and foremost the domestic dog in developing countries but also to foxes (Mørk and Prestrud, 2004; Aikimbayev et al., 2013), jackals (Bingham et al., 1999) and the raccoon dog (Bourhy et al., 1999).

From these reservoir host species the virus can spill over to individuals of other mammalian families in the area (Randall et al., 2004; Prager et al., 2012).

On the African continent there are 2 widespread canine lineages described. Africa 1 is more similar to the European and Asian lineage and is found in Central, East and Southern Africa. African lineage 2 is found in Central to West Africa. In general the proposed introduction time of these lineages and their putative spread coincides with the time of colonization of the respective areas and the rise of urbanization in the 19th century. A further lineage, Africa 3 is seen in South Africa in mongoose species and represents the adaption of rabies to a new host reservoir family (Talbi et al., 2009).

The evolution of dogs is closely linked to the human since the day of the domestication of the wolf and therefore rabies too depends on and takes advantage of human behaviour. From the dispersal of the disease from Europe to Africa by colonization (McElhinney et al., 2008) until nowadays, rabies spread is heavily influenced by human travel patterns. Human mediated movement of dogs can transmit the disease over large distances and are frequently the source of remerging cases in Europe (Gautret et al., 2011). Phylogenetic studies in dogs in North Africa showed that rabies strain spread was best explained by road distances instead of actual geographical distances (Talbi et al., 2010). Not only human-mediated expansion into new areas are advantageous for RABV epidemiology but also human-made favourable conditions for holding up an endemic situation in a dog population help rabies to persist. Poverty is a root cause for poor supervision of dogs in developing countries. The majority of people in Africa do not have a budget for their dog and therefore the majority is unvaccinated and not regularly fed. In consequence dogs search for additional food in the street. Poverty influences not only dog vaccination status, but also the overall health status of the dog population, which again influences population turnover. High birth and death rates, which lead to a high percentage of young dogs and rapid decline of immunisation coverage on the population level is a major obstacle to the interruption of rabies transmission by vaccination (Zinsstag et al., 2017; Chapter 4, this thesis). In addition different human contexts, be it religious, socio-economical or educational, influence behaviour of the domestic dog and their accessibility for vaccination (Cleaveland et al., 2003; Léchenne et al. 2016a).

In N'Djamena, Chad two successful mass dog vaccination campaigns have been conducted in the years 2012 and 2013. Coverage was consecutively estimated to have reached over 70% of the cities dog population (Léchenne et al. 2016a). In a previous phylogenetic study a virus specimen originating from Lake Chad (Sidje, 150km from N'Djamena) was not belonging to the same cluster than the predominant circulating clusters of Africa-2 in N'Djamena (Dürr et al., 2008a). In contrast all virus isolates found in N'Djamena were found to be very homogenous sharing 98.1–100% nucleotide identity. This finding gave rise to the hypothesis that the success of a control intervention can be followed by the continuous phylogenetic analysis of viral samples during a vaccination campaign.

Traditional methods to study disease spread are based on incidence data and demographic information. However such data are not always reliable and often incomplete in a setting with poor health and laboratory infrastructure coverage (du Plessis and Stadler, 2015). Such circumstances are common for rabies, where surveillance is generally poor over most parts of Africa (Dodet, 2009; Nel, 2013). In recent years genomic epidemiology has emerged as a new promising method to analyse disease outbreaks and patterns. With phylodynamic modelling of the Ebola outbreak it was possible to estimate the reproductive ratio and the number of unreported cases from genetic data (Gire et al., 2014; du Plessis and Stadler, 2015).

RNA viruses usually show high genomic variation and indeed when passaged in vitro rabies virulence changes as a result of substitutions in the G Protein, which indicates positive selection (Morimoto et al., 1998). In nature however the rabies virus is very constraint. Somehow a strong selective pressure is hindering occurring mutations to evolve into new virus types. One hypothesis for this fact is that rabies has to keep the ability to infect many different species as well as many different cell types (muscular, neuronal and salivary gland tissue) (Holmes et al., 2002; Jenkins et al., 2002). Our study explores if phylodynamic modelling methods are valid to describe disease spread for an extremely constraint pathogen and concentrated on a small area. With a phylogeographical study of viral specimens found in N'Djamena and the surrounding region during the intervention period we intend to confirm the elimination of the RABV strain circulating in the city before the vaccination and to characterize potential re-emerging strains. The gained epidemiological knowledge will guide future strategies towards reactive or proactive-vaccination approaches (Stark et al., 2006).

Results and Discussion

The results of the two vaccination campaigns conducted in N'Djamena in the year 2012 and 2013, show a strong decline of rabies incidence. The mean number of rabid dogs began to drop during the first campaign in 2012 and continued to decline towards zero (Léchenne et al., 2017; Zinsstag et al. 2017; Chapter 2&4 of this thesis). Animal rabies incidence dropped by 90% (Léchenne et al. 2017; Chapter 2, this thesis) until the end of 2014. However, single cases were still observed from time to time in the 9th district of town (Walia) and since January 2015 cases began to rise in this area again reaching 7 positive cases up to September 2015. Modelling of the disease dynamics over the study period show that the coverage achieved was sufficient to interrupt transmission from October 2012 to November 2014 (Chapter 4, this thesis; Zinsstag et al. 2017). In theory elimination of the rabies from N'Djamena should be achieved, yet there is a wide heterogeneity of dog density and coverage over the whole area. There is therefore the possibility of continued transmission in isolated patches. Townsend reported that even small areas of low coverage had a negative impact on the long term effect of a vaccination campaign in Bali (Townsend et al., 2013a). However we hypothesise that cases observed in 2014 and 2015 are rather cases of reintroduction. Walia is a district of very high dog density and with a poor socio-economic context. Dogs are for the majority owned but are to over 90% free roaming. Coverage was exceeding 80% in both years in this area, which should be more than sufficient to interrupt the transmission of rabies (Coleman and Dye, 1996). Therefore we heavily suspect reintroduction of the virus from outside the city area. Sequencing of the virus samples to support this hypothesis is ongoing. Preliminary results on 20 obtained virus sequences show a difference between samples originating north of the river before the vaccination campaign and samples originating from south of the river after the vaccination campaign (Figure VI.1). Furthermore, the virus samples from Walia obtained after vaccination seem to be closer related to with virus samples from Koundoul, than with samples from the city centre. Koundoul is a village very close to Walia that was not included in the vaccination campaign. Geographical boundaries and immunological status play a significant role in rabies transmission as quick spread in previously naïve racoon populations have shown (Real et al., 2005).

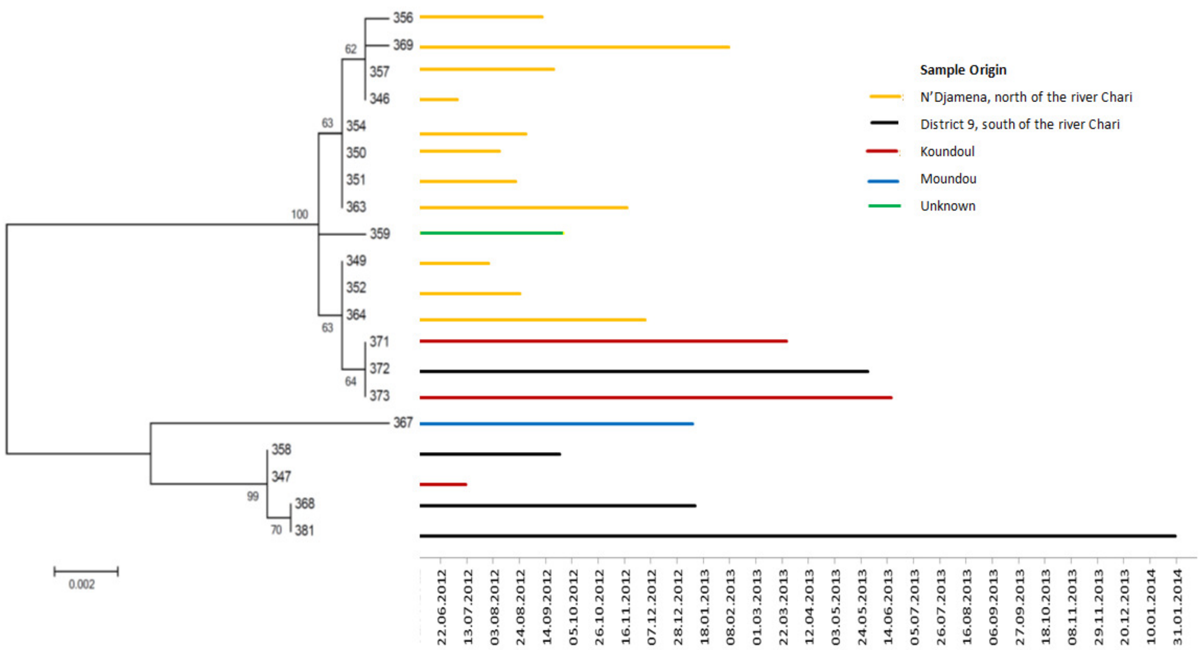

Figure VI.1: Phylogeographical time axis of RABV specimens found in N'Djamena and the surrounding area June 2012 to January 2014.

In the study of Dürr et al. (2008a) differences between viral isolates from N'Djamena and other parts of Chad, as well as neighbouring Cameroon and Nigeria, were easily observed. We hope to strengthen this evidence by detecting similar differences between the rabies specimens found during the study period (2011-2012) in and around N'Djamena, which would help to identify virus reintroduction. Our hypothesis of interrupted transmission would therefore be confirmed if the positive cases observed in 2014 and 2015 as well as future cases genetically differ from the strains observed in 2011/2012 before the vaccination campaign. On the contrary, if the samples are genetically very similar we would have to conclude that transmission of the same strain still persist despite our intervention.

If the genetic analysis of all 55 virus isolates to be included in the study provides sufficient evidence that the most recent strains are genetically different from those observed prior to the mass vaccination campaign, cases of this most recent strains can also be used to estimate the immigration parameter of dogs that are incubating rabies.

Similarly we expect to model the dynamics of the basic reproductive ratio (R_0) from phylogenomic data as done for the 2014 West African Ebola virus epidemic (du Plessis and Stadler, 2015). Preliminary analysis on the basis of the 20 sequenced rabies isolates suggest that R_0 dropped below the threshold of 1 between July and September 2012 (Figure VI.2). As mass vaccination was not started before October 2012 this result does not reflect the circumstances in the live setting. Also, the phylogenetic tree derived from a second methodology using the same priors as for the calculation of R_0 turned out very different from the first (Figure VI.3). This second tree cannot be interpreted in regard to the spatio-temporal study background in the same way as the one in (Figure VI.1). The results of phylodynamic methods are highly dependent on assumptions about the model priors. Therefore they have to be validated against the plausibility and likelihood of the modelled scenario compared to observation in reality. Jenkins et al. (2002) reports a general lack of a molecular clock for RNA viruses and therefore recommends caution for using gene sequences to estimate divergence times. But the most important limiting factor is the small sample size in this preliminary analysis. We are hoping to be able to refine our models with an additional 32 virus isolates.

Mostly genomic data is used to analyse outbreaks retrospectively. In future it could be used to assess the real-time spread of diseases and become an integral part of control of infections, as documented for health care associated MDR cases on hospital level (Tang and Gardy, 2014).

Also the dynamics and the spread of rabies in the dog population on the national level during a countrywide elimination campaign can be followed in real time and the optimal progress of vaccination in time and space be guided with such data.

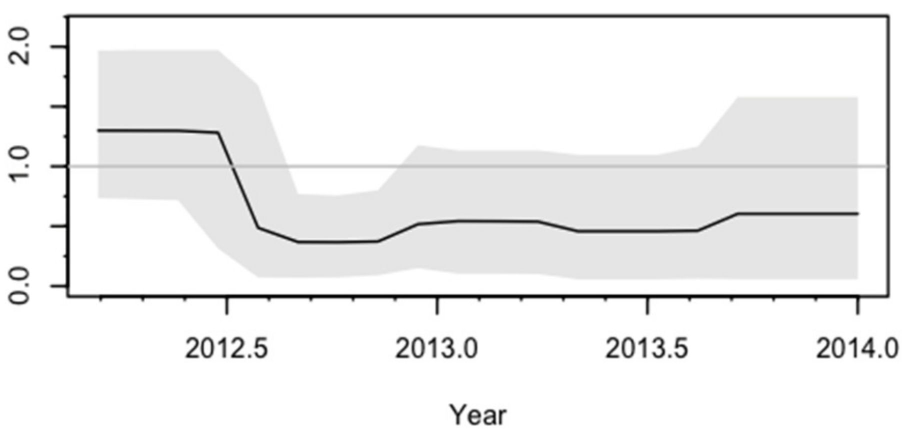

Figure VI.2: Dynamics of R_0 during the study period as modelled from the phylogenomic data of 20 virus isolates found June 2012 to January 2014

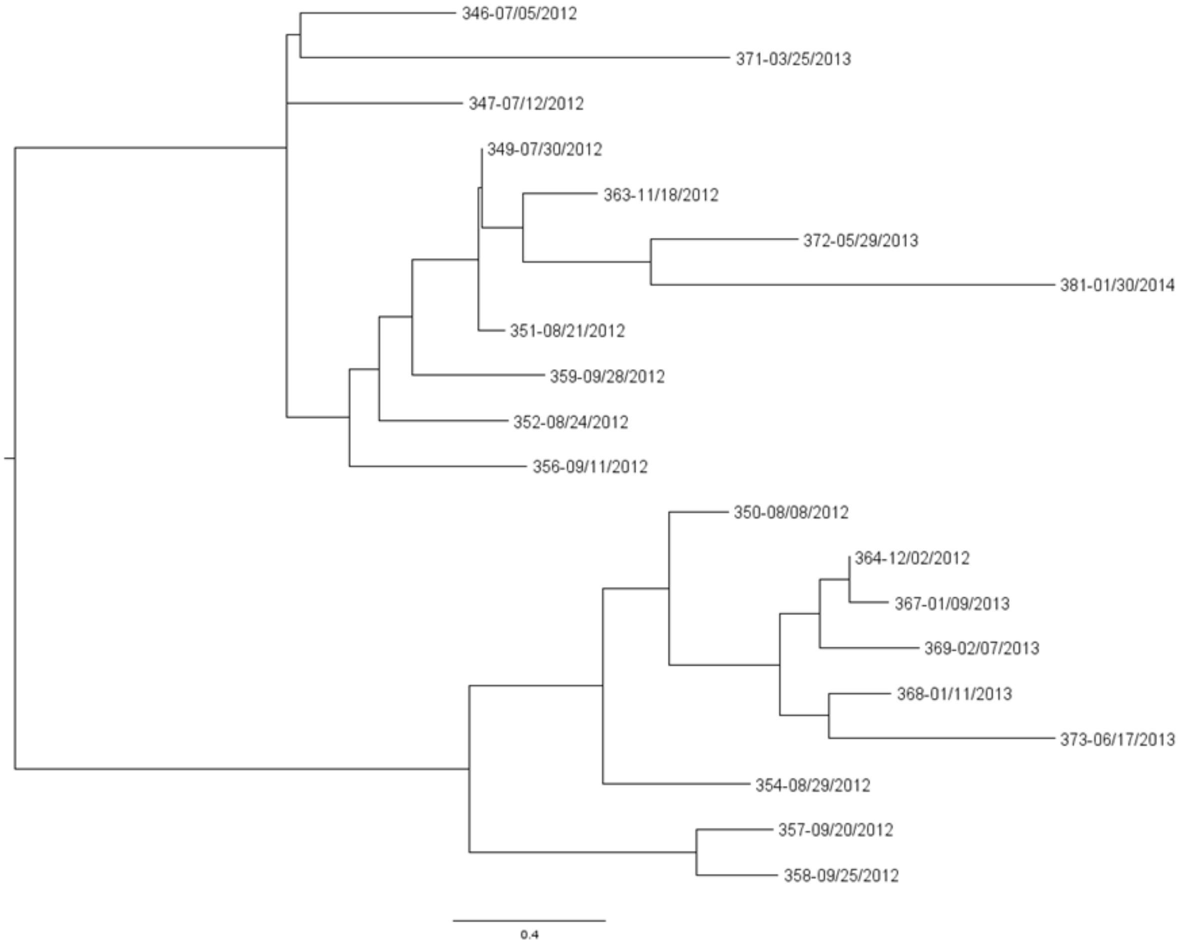

Figure VI.3: Phylogenetic tree of the 20 rabies samples as calculated by the second method with Beast V2

Materials and Methods

Study location

N'Djamena lies in the south east of Chad, very close to the border of Nigeria and directly adjoining Cameroon to the South. Maps of the town can be found in Chapter 1&2 of this thesis (Figure 1.2; 1.3 & 2.1). Walia is situated on the extreme south-east border of N'Djamena and is very close to a village called Koundoul that was not integrated in the vaccination campaign. The main transport link to the southern part of Chad is passing through this village and Walia before crossing the river Chari. Only two bridges cross this river and therefore the Chari could be a natural barrier to the spread of rabies.

Modelling

The 20 sequences of rabies from dogs, collected between Mai 2012 and January 2014 were analysed with two different approaches to calculate a phylogenetic tree. To build the phylogenetic tree in figure VI.1 we used Beast v1 and applied a relaxed uncorrelated Log-normal clock model and the Yule model as tree prior that is appropriate for analysing sequences from different species, in our case dogs and cats. The length of chain in the MCMC prior was set to 800’000, the screen log was set to 10’000 and the file log to 200. The phylogenetic tree in figure VI.3 was calculated with Beast v2 (Bouckaert et al., 2014). We chose a GTR model for substitutions with a relaxed log-normal clock (Drummond et al., 2006). The prior for the substitution rates were used as default in Beauty. For the epidemiological model, we chose the birth-death skyline model (Stadler et al., 2013). We used a log normal (0,1) prior for the reproductive number R_0, and assumed 5 equidistant intervals for R_0 over the time of the tree. We assumed a Uniform prior on interval (9.5,9.7) for the removal rate (corresponding to an expected infected time (exposed + active rabies) between (1/9.5,1/9.7) years, which is about 1.2 months. The sampling probability of a rabies dog was assumed to be Uniform on (0.4,0.6). The time of the initial case in that transmission chain was assumed to be a Uniform prior on (0,20), prior to the most recent sample. We ran the MCMC for 10’000’000 steps.

Sample collection

Rabies isolates are obtained from positive samples found during routine diagnostic at the rabies laboratory of IRED in N’Djamena. PCR and sequencing was done in collaboration with the rabies reference laboratory at the Insitute Pasteur in Paris. The diagnostic procedures are described in detail in Chapter 3 of this thesis. Currently the complete N gene sequence could be obtained from 20 samples. We will include another 32 samples in the final study (Table VI.1)

Table VI.1: Positive rabies cases and their origin observed at the rabies laboratory of IRED 2011-September 2015.

year	N'Djamena	other Chad	unknown	Total
2011	10	1	1	12
2012	18	1	1	20
2013	4	3	0	7
2014	3	3	0	6
2015	5	2	0	7
Total	**40**	**10**	**2**	**52**

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VII. Book Chapters

VII.1 Integrated Rabies Control

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Introduction

Rabies is a classic zoonotic disease, infecting all mammal species. It is generally transmitted through an invasive manner from saliva to a bite wound, leading to encephalitis with distinct, severe symptoms followed by death. Since the earliest descriptions of this ancient disease, animals, and especially dogs, have been recognized as the source and cause of rabies in humans (Rosset, 1985). To this day, rabies provides an exemplar of a One Health problem requiring an understanding of the linkages between human and animals and an integrated approach to disease control.

In 1882, shortly before discovering the first rabies vaccine for humans, aided by experiments on rabbits and dogs, Louis Pasteur wrote in his third correspondence to the Academy of Science:

…l'homme ne contractant jamais la rage qu'à la suite d'une morsure par un animal enragé, il suffira de trouver une méthode propre à s'opposer à la rage du chien pour préserver l'humanité du terrible fléau. [People contract rabies only after a bite of a rabid animal; it would be enough to find a proper method to fight rabies in the dog to protect humanity from this terrible scourge.][1]

Although this statement by Pasteur simplifies the epidemiology of rabies by ignoring sylvatic rabies in wildlife reservoirs and lyssavirus transmission by bats, it describes the very essence of the prevention of rabies in humans. Even now, the domestic dog is the main vector for transmission of rabies to people, being responsible for more than nine out of ten cases worldwide. An estimated 7 million people per year come into contact with a rabid dog (Knobel *et al*., 2005) and should receive post-exposure prophylaxis (PEP). This treatment is the only measure available to prevent onset of the disease, but it is often inaccessible for various reasons, including lack of knowledge about where to seek help, lack of money to pay for it or simply lack of the vaccine itself in local health facilities.

Despite exploration of different protocols, no consistently effective treatment exists against rabies encephalitis and the disease is almost always fatal (Jackson, 2013a). Although PEP is highly effective in terms of prevention, many hundreds of thousands of people across Africa and Asia do not have access to prompt and appropriate PEP. As a result, it is estimated that at least 55,000 people die of rabies each year, which represents an under-reporting of human rabies cases by a factor of from 20 (Asia) to 160 times (Africa) (Knobel *et al*., 2005).

Rabies can also be effectively prevented in both human and animal hosts through pre-exposure vaccination, with several highly immunogenic and effective vaccines available. The availability of effective vaccines raises the prospect for effective control and elimination of rabies, and several other features of rabies further meet the criteria for a disease that can be eliminated (Klepac *et al*., 2013). The virus cannot persist in the environment, no carrier state has been identified, and the infectious period lasts only a few days until the host invariably dies (Warrell and Warrell, 2004). Furthermore, the basic reproductive ratio (R_0) of canine rabies transmission is consistently below 2, regardless of dog density and demographic setting (Hampson *et al*., 2009; Morters *et al*., 2013), which suggests that elimination should be epidemiologically feasible. This is supported by empirical evidence demonstrating the success of canine rabies elimination in Europe (Aikimbayev *et al*., 2013), North America and recently in Latin America, where human and dog rabies cases have declined considerably following dog mass vaccination campaigns (Streicker *et al*., 2010; Vigilato *et al*., 2013a).

The main burden from this disease is now found in Asia and Africa, where rabies continues to be neglected in many regions, and too often its public health impact is overshadowed by other priority diseases like HIV/AIDS, malaria and avian influenza (Knobel *et al*., 2005; Shwiff *et al*., 2013). This situation typifies the inequities in health investments that are directed to the prevention of emerging zoonoses (perceived as a threat to high-income countries) in comparison to the prevention and control of neglected endemic zoonoses (predominantly affecting low-income communities) (De Balogh *et al*., 2013; Zinsstag, 2013). Although the number of lives lost and the estimated costs (Shwiff *et al*., 2013) may be viewed as less compelling than other public health priorities, several studies have demonstrated the cost-effectiveness of canine rabies control for preventing human rabies deaths (Zinsstag *et al,* 2011a; Fitzpatrick *et al*., 2014). The threshold immunization coverage of a reservoir species required to interrupt transmission has been estimated at 70% (Coleman and Dye, 1996). For canine rabies, vaccination campaigns have successfully achieved this level (Kayali *et al*., 2003b; Kaare *et al*., 2009), but challenges remain for reaching and maintaining sufficient coverage in some rural and urban low-income settings, where dog populations are both dynamic and poorly supervised. Awareness is also growing about the importance of ensuring completeness of vaccination campaigns among communities, in order to prevent gaps in coverage which can severely jeopardize control efforts (Townsend *et al*., 2013a). For human rabies prevention, poor access to pre- and post-exposure vaccines remains a problem for remote and marginalized communities (Warrell, 2003; Hampson *et al*., 2011). A major challenge also relates to surveillance systems for both human and animal rabies, systems which are very poor or non-existent in many parts of Africa and Asia (Banyard *et al*., 2013; Nel, 2013).

The described obstacles to rabies control can be addressed by an integrated approach based on the 'One Medicine' concept, which is extended to One Health and to a broader systemic understanding of ecological (ecohealth) and social systems (health in social-ecological systems, HSES; (Zinsstag *et al*., 2011a). The resulting 'equity effectiveness' approach aims towards an approach for the control of dog rabies that considers disadvantaged groups in order to reach the whole population equitably (Zinsstag et al. 2011b). Even when a vaccine is highly effective, as is the case for dog rabies vaccine, use in the field is often limited by a number of factors in a multiplicative way. As a result, the effectiveness of an intervention, assessed here as the proportion of dogs protected from transmission, may be well below the actual biological efficacy of the vaccine. A vaccine's effectiveness is determined, among other factors, by availability, accessibility and affordability (Zinsstag et al., 2011b). To understand better these determinants of intervention effectiveness, we move from 'One Medicine' to a HSES approach, and discuss in detail their involvement in a sustainable, cost-effective elimination of rabies in domestic animals.

One Medicine

More than a century ago, Austin Peters, veterinarian and contemporary to Louis Pasteur, addressed the following words on the subject of combatting rabies to the Cattle Commission of the United States (Peters, 1891):

I merely offer the suggestion, would it not be better to merge matters pertaining to the public health in one bureau, this board not only to do what the present Cattle Commission does, but also to act in a broader scope, considering contagious animal diseases in their relation to the public health, as well as a menace to our live-stock interests. (…) Before we can ever have a system of protection to the public health approaching perfection, it will be necessary to place the contagious and infectious diseases of animals in the same category with those of man, and have the same authorities exercise supervision over both.

Such a system would truly be preferable for the surveillance of rabies. Reliable incidence data on animal and human rabies, brought together in one shared database, would significantly enhance communication with decision makers on the different national and international levels, as well as with the public as a whole (Lembo *et al*., 2011; Banyard *et al*., 2013; Meslin and Briggs, 2013; Taylor et al., 2013). In reality, even separate reliable surveillance systems do not currently exist for either the veterinary or the public health sector. An online database created by WHO, the Rabnet website, was discontinued due to inconsistent reporting and poor response (Nel, 2013). In many countries, rabies is not even included as a reportable disease (Nel, 2013). This dire situation is best illustrated by the low figure of financial resources allocated to rabies diagnostics and the very small percentage of animal tests performed, compared to PEP numbers (Townsend *et al*., 2013b). In Bhutan over 10,000 PEP treatments were reported between 2001 and 2008. In the same period only a little over 200 animals were tested by a laboratory (Tenzin *et al*., 2012), which means that the infectious status of the source was ascertained for less than 2% of all exposed cases. Lack of knowledge about rabies leads to extreme under-reporting of cases both in animals and humans, and can also lead to misdiagnosis in humans as another encephalitic infection, particularly malaria (Mallewa *et al*., 2007). This might be due to differential diagnosis of rabies being considered only when typical symptoms, hydrophobia in humans and aggressive behaviour in animals, are present. Therefore other, less frequent syndromes, for example paralytic progression, might not be thoroughly investigated particularly in situations with a lack of history of an animal bite.

Good surveillance is not only an important prompt for the international community to recognize rabies as a public health tragedy, but is also indispensable for control and especially elimination attempts (Klepac *et al*., 2013). Diagnostic capacity and surveillance are essential to promote vaccination campaigns and to demonstrate the effectiveness of interventions. During and after mass vaccination campaigns, surveillance sensitivity must increase considerably in order to continue detecting cases once they become rare (Klepac *et al*., 2013; Townsend *et al*., 2013b).

Such an improvement can be achieved by close communication between animal- and human-health workers (Meslin and Briggs, 2013). The advantage of sharing information is clear: related to each human rabies case, there is an animal rabies case; connected to each animal rabies case, there are possible human exposures. From information about incidence of bites to humans, the veterinary sector can draw conclusions about rabies incidence in animals. Conversely, starting from a known rabid animal, human exposure can be explored by a contact tracing approach (Hampson *et al*., 2009; Banyard *et al*., 2013). Such a tracking method is in line with the concept of risk-based surveillance, which is economically advantageous for both sectors (Stark *et al*., 2006). In Bohol in the Philippines, contact tracing has been successfully applied (Lapiz *et al*., 2012).

Because a common sign of rabies in dogs is unusual aggressiveness, one rabid individual can expose several victims. The average number of bite victims per rabid animal from 2011 to 2014 was 2.5, as derived from the database of the rabies laboratory in N'Djamena, Chad. A retrospective study in Senegal on human rabies cases revealed that for each victim who died at a hospital, there were four non-reported people exposed (Diop *et al*., 2007).

Arguably the greatest advantage of close cooperation of human and animal health services is the avoidance of unnecessary, costly PEP delivery resulting from the unknown status of a biting animal and uncertainty about the epidemiological situation in an area. Examples of over-use of vaccine are frequent, and are partly a direct consequence of poor cooperation but also a result of the understandable anxiety associated with consequences of mistakenly withholding PEP. In Bhutan, a whole region continued PEP treatment to bite-patients despite elimination of rabies from this particular district because in the southern part of the country frequent introduction of rabies from India still occurred. Due to these introductions, Bhutan did not acquire rabies-free status from WHO (Tenzin *et al*., 2011b, 2012a). Similarly in Tunisia, Thailand and Sri Lanka, the demand for PEP increased after dog vaccination campaigns, presumably due to increasing awareness of rabies, but contrary to the expectations (Mitmoonpitak *et al*., 1998; Kumarapeli and Awerbuch-Friedlander, 2009; Touihri *et al*., 2011).

For India, Dr M.J. Mahendra described a phenomenon that grew among people with public awareness that he called the 'hydrophobia phobia', and Cleaveland *et al.* (2006) found that in Tanzania rabies was more feared than malaria, despite being less prevalent. In France where the rabies-free status is repeatedly threatened by imported rabid dogs from endemic countries, media warnings of such a reintroduction increase the demand for PEP and rabies immunoglobulin (RIG) (Lardon *et al*., 2010; Gautret *et al*., 2011a).

Wasting valuable products, which are indispensable in the event of an actual exposure, can lead to shortages as described in Europe and the USA (Bourhy *et al*., 2009). In low-income countries, where post-exposure vaccines are rare and RIG is virtually non-existent, each injudiciously used dose can potentially result in a fatality for another exposed rabies victim. Clearly, it is not ethical to deny treatment to those with uncertain exposure history because the consequences could be grave, but this uncertainty could in many cases be avoided through 'One Medicine' thinking and close collaboration between physicians and veterinarians. Simple questions about the circumstances of the bite incident or potential exposure (e.g. provoked or unprovoked attack), the whereabouts of an aggressive animal (free-roaming or confined), ownership status (owner known or not known), animal vaccination and health status (symptoms of rabies observed) and, most importantly, its fate (dead or missing versus alive and well) can guide the initial inquiry step. Only when an animal has a valid immunization certificate and can be clearly identified, is it possible to know with certainty that it has been correctly immunized. But often this information is not available. In Turkey, only 17% of suspect dogs which were investigated after a bite had a valid vaccination certificate (Kilic *et al*., 2006). Compulsory dog registration would facilitate this identification and decrease the amount of PEP due to uncertain vaccination status. Where doubt exists, veterinary services can observe and follow up animals that inflicted a bite for 2 weeks. If the animal is still alive after that period of time, there is no risk of rabies and PEP for the respective victim can be discontinued. This simple but evidence-based method is still used in many regions, particularly where diagnostic facilities are not in place (Mitmoonpitak *et al*., 1998).

To prevent unnecessary, expensive PEP after bites from negative animals or undetected exposures from a truly rabid case, each human presenting at any health facility for the treatment of a bite wound should trigger a contact to the veterinary service to notify the case. Thus, information on vaccination status of the animal and the result of diagnostic tests, if performed, can be shared and an investigation on the occurrence of other exposures (human or animal) or additional cases in the same area can jointly be undertaken.

Ideally, as suggested by Peters in 1891, a One Health rabies surveillance system should automatically involve such direct communication between public and animal health sectors and involve well-trained specialized personnel who are able to engage in timely dog rabies diagnosis and human PEP. Even if human and animal health facilities remain separated, scarce infrastructure and equipment, such as microscopes and refrigerators, as well as resources such as electricity, could potentially be shared for rabies surveillance and control (Schelling *et al*., 2005). Savings from such sharing of resources in developing countries should not be underestimated, where the most basic infrastructure can be hard to find, especially for public institutions.

A recent study in Tanzania showed that even if only 1% of all PEP administered are given to people truly exposed, it remains cost effective (Hampson *et al*., 2011). Critics might therefore argue why bother to put in place a common surveillance system, when the prevention of human cases can be cost effectively achieved by extensive, widely available PEP treatment alone? Such reasoning ignores the fact that concentrating on human PEP will never interrupt transmission. Ultimately, only an intervention in the reservoir host can lead to dog rabies elimination, and this approach will be more cost-effective than human PEP (Zinsstag et al., 2015b). The next step to sustainable control should be a joint effort of veterinary and human medicine, enhancing inter-sectoral communication and controlling rabies at its animal source. The detailed benefits of such a One Health approach are discussed in the following section.

One Health

While ‘One Medicine’ has a clinical and curative connotation, the term One Health emphasizes the added value of preventive action from closer cooperation between public and animal health (Zinsstag et al., 2015a & c). The WHO’s recommended and well-proven threshold to interrupt rabies transmission in a given population is 70%, owing to the generally low reproductive number being close to 1 (Coleman and Dye, 1996), irrespective of dog density (Hampson *et al*., 2009). In low-income countries, this coverage can often only be achieved by providing the vaccine free of charge (Durr *et al*., 2008b and 2009). It is usually recommended that freedom from dog rabies is considered a public good, and that in low-income settings, dog rabies vaccination should be free of charge to the owner. Comparative cost-effectiveness analyses of dog mass vaccination compared to human PEP alone informs authorities and decision makers considering whether to engage in dog rabies mass vaccination. While government veterinary services remain committed to mechanisms for cost recovery, it is unlikely that any fees recoverable during a mass dog vaccination campaign would offset the additional costs involved (i.e. an extra person involved in handling cash during vaccination campaigns). There are many reasons why charging a fee at the point of vaccine delivery could be counter-productive, for example, if vaccine is refused to dogs brought by children who are without the means to pay for vaccination. None the less, other mechanisms may exist for supporting dog vaccination campaigns through owner payments, for example through charging dog registration fees, as has been introduced successfully in the Philippines, or through establishing community insurance schemes.

WHO promotes mass canine vaccination as part of a cost-effective approach to human rabies prevention, and estimates that in areas where the dog population is the only driver of epidemiology, this approach becomes more cost effective than PEP alone after a period of 15 years (Bogel and Meslin, 1990). Many successful dog vaccination campaigns have been undertaken in the last decades, and led to the control of rabies and marked declines of human rabies in Latin America and several regional settings in Africa and Asia (Belotto *et al.*, 2005; Lucas *et al.*, 2008; Davlin and Vonville, 2012). The cost-effectiveness of such campaigns has occasionally been assessed (Cleaveland *et al.*, 2006; Zinsstag *et al.*, 2009; Tenzin *et al.*, 2012). In N'Djamena, Chad, the cost of dog vaccination compared to PEP alone breaks even after 5 years, provided there is no reintroduction after the successful elimination occurs (Zinsstag *et al.*, 2009; Zinsstag et al. 2015a). An equal time period to achieve similar cost-effectiveness has been reported for vaccination campaigns in Bhutan (Tenzin *et al.*, 2012).

This clear advantage of canine vaccination is due to the very high costs of human PEP vaccine and immunoglobulin. Vaccinated, rabies-free domestic dog populations can considerably lower the demand for PEP, but as discussed in the previous section, this does not always occur. Figure VII.1 shows a projection of possible worst and best case scenarios for the progress of PEP demand after a vaccination campaign. The rise of awareness for rabies in the course of interventions is closely linked to a rise in PEP numbers. Closer contact between veterinary and human medicine can buffer this impact. In parallel, studies to find new, less expensive vaccines and regimens demanding smaller and less frequent vaccine doses, like the recent WHO accepted intradermal administration, must be maintained to constantly improve cost-effectiveness of PEP itself (Verma *et al.*, 2011; Warrell, 2012b).

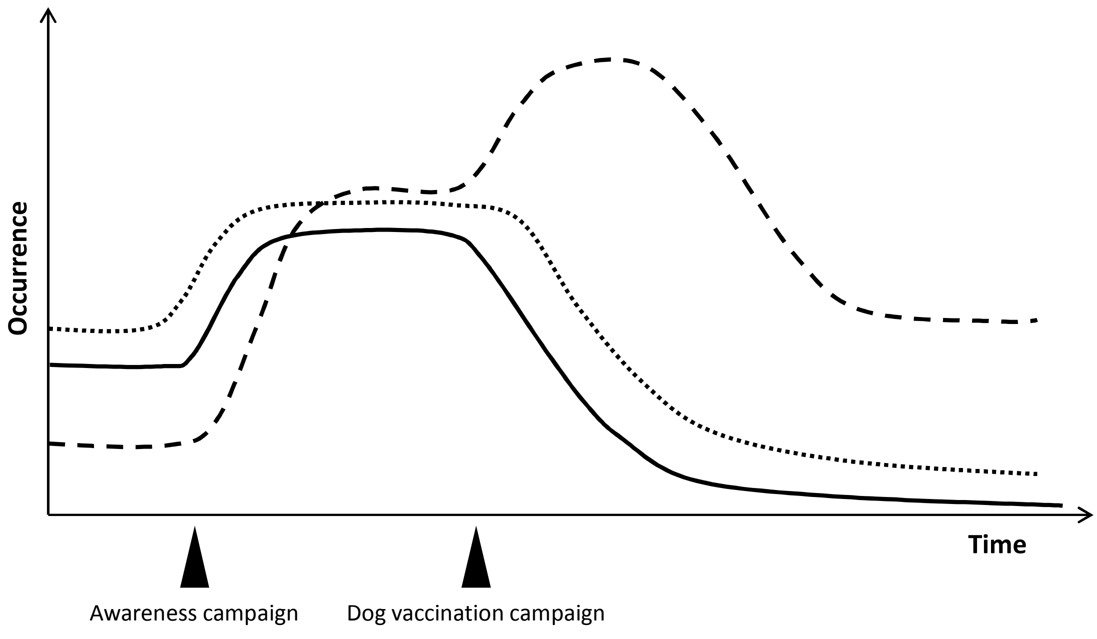

Figure VII.1 Scenarios for the influence of dog vaccination campaigns on the demand for human Post Exposure Prophylaxis (PEP): Trend of rabies incidence (continuous line); possible rise in PEP due to elevated rabies awareness (broken line) and the preferred decline of PEP following the decrease of rabies risk (dotted line).

If one area has successfully eradicated rabies, there will always be a danger of reintroduction so long as the disease persists in other regions. Even if natural barriers block the free movement of dogs, human behaviour can transmit the disease over long distances (Talbi *et al*., 2010). In the same speech cited above, Austin Peters also pointed out the necessity for the local authorities to enforce the law and supress outbreaks of the disease, but regretted that neighbouring authorities do not cooperate together well enough (Peters, 1891):

…last summer the town of Framingham ordered that all dogs within its limits must be muzzled. Now the town of Brookline orders all dogs muzzled or chained for sixty days, while most of our cities and towns take no action whatever in regard to the matter; but it is very doubtful if such erratic and independent action has any marked influence upon the prevalence of the disease.

For successful elimination of rabies, concerted joint measures and common efforts are needed across many different administrative zones within a country, and even between countries. Unfortunately, the dog falls into an administrative gap in developing countries. The veterinary sector is focused on livestock health, and companion animals are ignored because they lack value for the economy, whereas most public health ministries will only deal with human aspects, rarely being motivated (or trained) to tackle problems in other species.

Meanwhile, a simple calculation illustrates financial advantages for actions at the source of rabies transmission. If an unvaccinated dog contracts rabies and bites two people, these two victims each have to take three to five doses, depending on the post-exposure regimen applied. If treatment is not sought, not available or properly administered, each victim faces a 20% probability of death from rabies (Shim *et al*., 2009). As a practical example, dog owners in N'Djamena are shown the advantage of vaccination with the help of very clear figures: it is their choice to spend the equivalent of US$5 for dog vaccination now, to spend US$100 per victim in the event that their dog becomes rabid and bites, or to pay for the death of a person when no PEP is administered. What is evident in this micro-relationship from case to case also holds as a broad picture for the macro-level of rabies economics. If money is not allocated to prevention at the source of infection, the spending that occurs farther on for PEP treatment of all possible victims to prevent human rabies is considerably higher (Figure VII.2). Ultimately, the loss of lives, if sufficient resources for good access to PEP are lacking, will cost the economy of a country 100 times more than an investment in dog vaccination. Millions of dollars are therefore lost worldwide. Expressed in disability adjusted life years (DALYs), rabies accounts for 1.74 million life years lost, a figure that considers the number of deaths but also the fact that most victims are children, resulting in the lost potential of tens of thousands of productive years (Knobel *et al*., 2005).

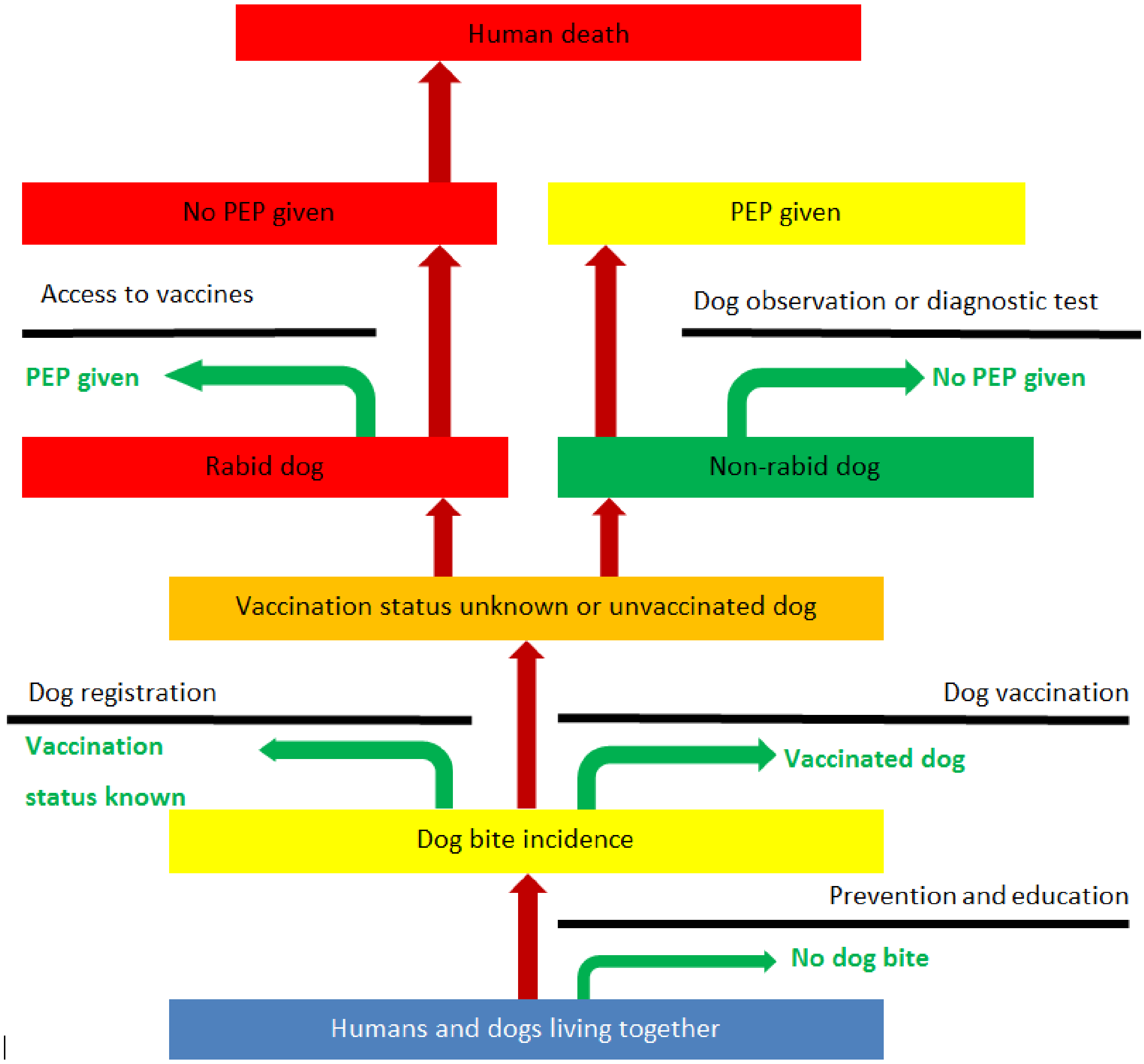

Figure VII.2: Possible progress from a dog bite to Post Exposure Prophylaxis (PEP) or human death. Black bars show intervention possibilities to prevent further economical impact and harm, green arrows points to positive effect of the intervention, red arrows point to further step of aggravation of the situation.

A substantial part of the economic burden to a country can also be livestock losses due to rabies, especially in Asia where the highest numbers are reported (Shwiff *et al.*, 2013). This provides an additional incentive to take action against dog rabies. In some areas, e.g. Botswana, cattle are the species for which most rabies cases are reported. Because they are kept on farms far from settled areas, it is suspected that jackals rather than dogs could be the vector for rabies transmission to livestock (Moagabo *et al.*, 2010). Such additional disease reservoirs might only become apparent during the last phase of disease elimination (Klepac *et al.*, 2013). Others are already identified and may undermine attempts to control rabies by repeatedly re-infecting previously rabies-free domestic populations. The ecological perspective of rabies control and the problem of complex epidemiological settings for the elimination of rabies will be discussed in the next section.

Ecosystem Approaches to Health

One Health focuses on the closer cooperation of human and animal health. As such it is embedded in the broader concept of ecosystem approaches to health (Zinsstag, 2012). Because in the Americas the majority of domestic animals are vaccinated, bats became apparent as the second most important source of rabies in humans (Belotto *et al*., 2005). Particularly in the USA, where rabies has been eliminated in domestic animals, the epidemiology has shifted to wildlife species like foxes, raccoons and skunks (Rupprecht *et al*., 1995). A very valuable source of information to establish the background relationships in rabies epidemiology in a given region is the molecular study of identified viral strains. With this method, it has been shown that mongoose rabies in Southern Africa forms an independent cycle (Nel *et al*., 2005). The same reservoir function is being disputed for the bat-eared fox (Nel, 1993; Sabeta *et al*., 2007). These examples show that in certain environments the control of rabies through dog vaccination should be complemented by the control of rabies in wildlife reservoirs (Muller *et al*., 2012). Close communication among biologists and wildlife veterinarians is required to detect these changes and patterns.

As rabies leads inevitably to death, outbreaks of the disease in small, vulnerable species populations can potentially lead to extinction. In the 1980s, domestic dogs were most probably responsible for an outbreak of rabies in the African wild dog (*Lycaon pictus*) (Gascoyne *et al*., 1993), listed as an endangered species. Domestic dogs are also blamed for repeated outbreaks of rabies in the Ethiopian wolf (*Canis simensis*) (Mebatsion *et al*., 1992), a distinct canid species, endemic only to the Ethiopian Highlands. These examples show how rabies and other diseases transmitted from an abundant vector species like the domestic dog can have devastating impacts on small, endangered populations. This kind of local or worldwide extinction is not only an irrevocable loss for biodiversity, but often also results in a downgrading of the affected national parks and conservation areas (Cumming et al., 2015 a and b).

An example where the epidemiology of rabies is driven by a domestic reservoir is the Serengeti and Ngorongoro region (Lembo *et al*., 2008), where dog vaccination can be of great value to wildlife and ecosystems. Mass dog vaccination campaigns conducted in a *cordon sanitaire* around the Serengeti National Park (Kaare *et al*., 2009) have resulted in the elimination of canine rabies from pastoral and wildlife-protected areas of the ecosystem, despite the presence of abundant and diverse wildlife populations (Lembo *et al*., 2010). While much emphasis has been given to achieving sufficient levels of vaccination coverage, with 70% coverage considered a critical threshold, recent studies have also highlighted the importance of 'completeness' of coverage. Even small areas of low coverage, involving <0.5% of dog population, could have a significant impact on the effectiveness of rabies control and time to elimination as shown for Bali (Townsend *et al*., 2013a).

Health in Social-Ecological Systems

Finally, the One Health idea can be extended to systemic approaches of health and well-being known as health in social-ecological systems (HSES) (Zinsstag *et al*., 2011b). In complement to ecological determinants, systemic social determinants are emphasized, such as the functioning of health systems and, arguably the most important actor in the fight against rabies, the dog owners. In addition to affordability, other critical determinants of a successful dog vaccination campaign must be considered: accessibility, adequacy, acceptability and adherence. These factors are related in a multiplicative way and depend on the social and cultural context. Lack of understanding of these effectiveness factors prevents mass vaccination campaigns from reaching sufficient coverage (Matter *et al*., 2000; Kaare *et al*., 2009; Thomas *et al*., 2013). Even when vaccination teams work well and the logistics are guaranteed, if there is low accessibility of vaccination posts or facilities, performance will be low. Polo *et al*. (2013) defines accessibility in this context as the sum of: (i) supply (vaccination sites); (ii) demand (dog densities); (iii) geographical barriers between supply and demand; and (iv) people's awareness of the benefit. The first three points are closely linked: the locations of vaccination points should be carefully chosen based on dog density, as determined by prior dog demographic studies, or as estimated on the basis of human density and dog to human ratio as well as geographical distances and barriers. These location choices can only be optimized with the help of geographers and local people who know the current setting well. In special cases where there are only a few small far apart settlements or where mobile populations are involved, mobile vaccination teams might be a better option than poorly performing fixed posts (Kaare *et al*., 2009).

Stakeholders, including dog owners, municipal authorities, community health and veterinary workers, should be involved in planning interventions from the beginning. Community ownership and participation are part of a trans-disciplinary approach (Matter *et al*., 2000; Catley and Leyland, 2001; Lapiz *et al*., 2012; Schelling and Zinsstag, 2015). In Grenada, willingness to bring a dog to vaccination facilities was low, because people feared that their animal might be infected by ectoparasites from other animals (Thomas *et al*., 2013). In such cases, rabies vaccination could be combined with a treatment against parasites or hygiene measures, to increase the perceived benefits of participation (Catley and Leyland, 2001; Kaare *et al*., 2009; Thomas *et al*., 2013). Another cause for low participation can be misconception about the vaccine itself (Belsare and Gompper, 2013). For instance, fear that it might cause rabies, weakness or aggressive behaviour or that the vaccine is a poison (Léchenne, personal observation, Chad). A recent dog mass vaccination campaign in N'Djamena showed that dog owner participation markedly affects cost per vaccinated dog. The cost per vaccinated dog varied between US$0.6 and US$130 per dog, depending on whether 300 dogs or only one were vaccinated per day.

A further aspect of effectiveness is the willingness of the dogs to be handled (Plate 8 A and B). The human–dog relationship is determined by socio-cultural factors, with differences in the tameness of dogs observed between different religious backgrounds in Chad and in Tanzania; dogs in predominantly Moslem communities were more difficult to handle than in others (Cleaveland *et al*., 2003), whereas in a Buddhist setting, it may be possible to handle even stray dogs (Bogel and Joshi, 1990). In certain contexts, dogs in wealthy households are more likely to be vaccinated (Awoyomi *et al*., 2008). Consideration of social and ecological determinants provides the key for locally adapted and effective approaches towards dog rabies elimination in a given context. Such systemic approaches contribute to a science of dog rabies elimination (Zinsstag, 2013).

Cultural practices and low income may lead to poor supervision of dogs in developing countries. The majority of people in Africa for example do not allocate resources for their dogs. Dogs are fed with leftovers from the table and left to forage when the quantity is insufficient. A false conclusion that the majority of dogs roaming in the street are ownerless is often drawn in low income countries. This false supposition is then followed by another wrong conclusion that getting rid of these ownerless dogs equals getting rid of rabies. Despite substantial scientific evidence against the culling of dogs as a control for rabies (Windiyaningsih *et al*., 2004; Morters *et al*., 2013; Putra *et al*., 2013), the practice continues. There are several reasons for failure of this method. Among others, due to fear of culling, people may relocate dogs to an area where rabies is not currently prevalent or may seek a replacement dog from a rabies-prevalent area and reintroduce the disease (Davlin and Vonville, 2012). But one of the most important undesirable effects of culling interventions is that the fight against rabies is negatively perceived in society, and results in lack of community support.

To confront the problems of low awareness, low motivation and low possibility of handling dogs, participatory community engagement, information, education and communication are central elements of successful rabies control. Education can help to prevent dog bites and human rabies exposure. Children, the most vulnerable group for exposure, can be taught right from the beginning how to behave to avoid conflicts with animals (Mitmoonpitak *et al*., 2000; Kilic *et al*., 2006). In addition, by teaching owners responsible dog ownership, a healthier more stable dog population could be attained – one that is less susceptible to rabies (Davlin and Vonville, 2012). In the Bohol Provincial Rabies Elimination Programme, the Department of Education is mandated to integrate lessons on responsible pet ownership, and rabies and its prevention into the elementary school curriculum. During a 2-year adaptation process, teaching modules on rabies were incorporated into diverse subjects, including mathematics, science, health, social science, English and Filipino. The key to this successful assimilation was the involvement and ownership of Bohol teachers, educators and provincial officials from the Department of Education, and their work in adapting the national prototype teacher's manual on rabies curriculum integration (Lapiz *et al*., 2012). Initially, round-table discussions with teachers, intensive planning and workshops to develop lesson plans, and orientation and training of the teachers who would use the tool were conducted. A pre-test of the first developed lesson plan was carried out for 6 months in one municipal school in Corella. The following school year, the teacher's textbook was published and distributed to all 982 public elementary schools in the province, with the target that every teacher should have a personal copy. The orientation and training of the teachers were done by those who had pre-tested the curriculum in the previous school year. The province-wide rollout by 2009 reached over 182,000 children between 5 and 12 years old, representing about 20% of the provincial population. A complement to this effort was the creation of Rabies Scouts. These were boy and girl scouts who had successfully completed a rabies and responsible pet ownership training programme. They were engaged as peer advocates and served as examples of positive action for other children. Throughout the school year, fun educational events for children were also undertaken, such as poster-making contests and pet shows to celebrate the bond between children and pets. The main programme limitation is that the intervention only reaches children who are enrolled in school, and does not include those who are likely at higher risk of being exposed to rabid dogs. Nevertheless, this education component of a comprehensive One Health approach is important to long-term sustainability of the programme, since the children continue to have higher awareness and are a source of accurate information about the disease, its prevention and responsible pet ownership.

Conclusion

Integrated approaches to rabies control based on One Health thinking have clear advantages towards the elimination of dog rabies. For example, integrated surveillance of dog rabies and human exposure, by closer cooperation between public and animal health, increases the sensitivity of surveillance and should avoid unnecessary or overuse of PEP. One Health approaches further provide the evidence for:

1. The feasibility of elimination of dog rabies by mass vaccination at high coverage which cannot be achieved by dog culling or the prevention in humans alone.
2. A higher cost-effectiveness of dog mass vaccination and PEP versus PEP alone, after 5 to 15 years, and the interruption of dog rabies transmission.
3. The importance of understanding rabies ecology, and community engagement, for the development of locally effective and equitable dog mass vaccination campaigns.

To reach the goal of elimination of rabies by the year 2025, set by the Global Alliance for Rabies Control (GARC) (Lembo *et al*., 2011), we will have to reach further than veterinary and human medicine and also include biologists, cultural scientists, sociologists and geographers. Some might say that the goal of elimination is a far reach, but more than 100 years ago in his letter to the Academy of Science, Pasteur continued the above cited text regarding the control of rabies:

Ce but est encore éloigné, mais, en présence de faits qui précédent, n'est–il pas permit d'espérer que les efforts de la science actuelle l'atteindront un jour? [This goal is still far away, but in light of the facts that precede, is it not permitted to hope that the efforts of modern science will achieve it one day?]…

VII.2 Rabies in East and Southeast Asia - a mirror of the global situation

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Introduction

The history of rabies is closely intertwined with the Asian region. One possible scenario for the evolution of rabies suggests the Indian sub-continent was the place where the classical canine rabies strain first emerged. From there, it putatively spread to Europe and, mediated by human movement during the colonial era, to Africa (Bourhy et al., 2008). This theory however can be disputed because a disease very similar to rabies was already described in ancient Greece (King, 2004). Certainly, the first overseas Pasteur Institute was founded by Albert Calmette in Saigon, former French Indochina, today Vietnam, in 1891 (Hawgood, 1999). Soon after, and exactly ten years from the first administration of the newly invented rabies vaccine on Joseph Meister, the second Asian Pasteur Institute was established in 1895 by Alexandre Yersin in Nah Trang, Vietnam (Hawgood, 2008). Both Calmette and Yersin must have administered the vaccine to numerous victims of rabid dogs during their stay in these former French colonies. The vaccine was far from being safe in those days, but the alternative was to take one's chances in the face of probable death. At last, there is hope to overcome a disease most feared by men since ancient times. Louis Pasteur was confident in the final years of his life that the methods of modern science would one day eliminate rabies in domestic animals (Rosset, 1985). But from his time up until now, domestic dogs remain the most significant source for transmission of rabies to humans. Despite the fact that Pasteur's pioneer work translated over time into effective and safe vaccines, for both animals and humans, still more than 60'000 people die from rabies every year (Knobel et al., 2005). The disease affects mainly under-privileged communities in developing countries which have limited healthcare access (Meslin and Briggs, 2013). The Post-Exposure Prophylaxis (PEP) that should be administered after a bite from a rabies suspect animal is usually too expensive or not available. Many bite victims are informed about this crucial treatment and rely on ineffective traditional methods. Not all countries list rabies as a notifiable disease, and even where rabies reporting is compulsory, inadequate surveillance is common in remote areas die at home, because diagnostic possibilities are limited, misdiagnosis is frequent and often people die at home (Banyard et al., 2013). All these factors lead to a continual state of neglect at every level of disease control: missing perception of the true worldwide burden, absent international and governmental commitment and low public awareness (Bourhy et al., 2010; Banyard et al., 2013).

East and Southeast Asia are no exception in this vicious circle of neglect. Asia carries the biggest continental burden associated with the disease, with over 50% of all worldwide human rabies deaths and more than 80% of the global monetary expenses (Anderson and Shwiff, 2015). The detailed picture for East and Southeast Asian countries is very heterogeneous and reflects the worldwide situation. Timor Leste and Singapore have been historically free of canine rabies, like New Zealand and Australia (Tenzin and Ward, 2012). Other countries have successfully eliminated the disease and implemented strict control measures to prevent reintroductions, for instance, Japan and Taiwan which mirror several European countries such as the United Kingdom and Switzerland (Rupprecht et al., 2004; Takahashi-Omoe et al., 2008; Liu, 2013). In South Korea, rabies remains prevalent in racoon dog populations near the border with North Korea and challenges for control are similar to the ones in the United States, where rabies research is driven by prevention of wildlife rabies (Rupprecht et al., 2004; Cheong et al., 2014). Among the regions that still face endemic rabies in domestic animals and, consequently, considerable numbers of human rabies fatalities, different priorities between the known measures to prevent animal to human transmission can be observed. Cambodia, Laos and Myanmar are the countries farthest from elimination. They compare with many countries on the African continent where accessibility to PEP, adequate surveillance and sustainable dog rabies control plans has are yet to be established nationally (Nel, 2013). In many countries like China and Vietnam, the focus is on PEP administration to bite victims. This is reflected in the large amount of funds allocated to human vaccination compared to other prevention measures. Over 80% of the expenses are allocated to PEP, with much less to dog vaccination (15%) and diagnostics (0.03%) (Shwiff et al., 2013). In consequence, the number of reported PEP administrations exceeds a thousand fold the number of confirmed animal rabies cases in many countries (Ly et al., 2009; Tenzin et al., 2012; Song et al., 2014) and dog rabies control remains insufficient to interrupt transmission. Despite dog vaccination campaigns being undertaken in many areas, they remain patchy, and often do not reach a sufficient coverage or have the impact evaluated (Davlin and Vonville, 2012). Unsustainable culling interventions to control stray dog populations are utilized less often, being replaced by the Animal Birth Control Approach (ABC) (Reece, 2007). Shifting resources from the human to the diagnostic and animal sector could, however, effectively reduce overall costs from rabies and is the only way to fight the disease at its source. PEP, dog vaccination and population control jointly implemented and balanced in a One Health framework (Box 1) can successfully lead to the elimination of dog rabies, as has been shown in Latin America (Vigilato et al., 2013b). This approach has also been proven to be cost effective in several different settings (Bogel and Meslin, 1990; Zinsstag et al., 2009; Tenzin et al., 2012; Fitzpatrick et al., 2014).

Disease management tools to formulate and implement One Health strategies for successful rabies control are available, and momentum has increased in recent years towards integrated rabies control targeting elimination. The Food and Agriculture Organization (FAO), the World Organization for Animal Health (OIE) and the World Health Organization (WHO) have established a tripartite coordination mechanism to harmonize technical cooperation at the regional and country level for prevention and control of zoonoses, including rabies (OIE, WHO, FAO, 2010). The Member States of the Association for Southeast Asian Nations (ASEAN) and the South Asian Association for Regional Cooperation (SAARC) have also identified rabies as a priority public health problem, and have set a target of elimination of canine-mediated rabies by 2020 (OIE, 2013). The goal of this roadmap is to control and eliminate dog-mediated rabies and protect and maintain rabies-free areas in South and Southeast Asia. The objective is to strengthen the regional and national coordination as well as technical and institutional capacities to manage dog rabies elimination programs. While One Health is becoming established at national levels, many communities have not yet been integrated in the One Health framework. By implementing community-based activities, these populations will also have access to other public health and veterinary services. Local examples of community integrated One Health rabies elimination are the programs in Bohol, the Philippines and in Bali, Indonesia (Lapiz et al., 2012; Townsend et al., 2013a). We will introduce these programs in this chapter as well as highlight all other stages of rabies control in East and Southeast Asia which, combined, mirror the global rabies situation.

Box 1:

> The One Health framework is an integrated approach of cooperation between human and animal health using the synergistic potential. Thereby public and animal health create an added value by establishing close communication, coordination and action plans for joint surveillance and control of dog rabies (Zinsstag et al. 2015a)

Challenging neglect: Lack of surveillance and effective control

Rabies, a zoonosis with a companion animal reservoir, often falls between the gap of public health interventions on the human side and animal disease control programs, which target the economically important livestock sector. In addition, it predominantly affects poor people in remote areas who have limited access to healthcare, with the result that most human and animal rabies cases go unnoticed, so the burden of the disease is significantly underestimated. Taylor et al. (2015) illustrates the lack of surveillance, particularly in Asia and Africa. In Asia, five countries do not list rabies as a notifiable disease: India, Pakistan, Indonesia, Myanmar and Bangladesh. Even in countries where rabies is notifiable the surveillance system is ineffective and not existent nationwide, notably in Cambodia. This also reflects the situation in Africa, where rabies is notifiable in about half of the countries, but only in a handful of these countries is an effective surveillance system in place. The identified shortcomings include lack of case definition, specific legislation and dissemination of data. These facts highlight the need for enforcement of legislation and guidance on national levels, including training of laboratory and health personnel. In Vietnam, a survey among human and animal health workers showed that knowledge of rabies reservoirs, wound management and guidance on PEP was unsatisfactory (Nguyen et al., to be published). Despite the fact that rabies is feared by many and generally known to be deadly, the knowledge about prevention measures which should be taken following an animal bite or control measures for disease prevention in dogs has been shown to be limited in several local Knowledge Attitude Practice (KAP) surveys, e.g., in Cambodia (Lunney et al., 2012), Bohol (Davlin et al., 2014) and India (Ichhpujani et al., 2006). This lack of awareness, together with misconceptions, leads to delayed or inadequate health seeking behaviour after a dog bite. Too often, traditional health practices are either preferred or even the only available option (Sudarshan et al., 2006)

The problems facing rabies control are symptomatic of the general problem of access to healthcare for marginalized, poor communities in predominantly rural settings (Obrist et al., 2007). The goal of any rabies control program must, therefore, be to contribute to strengthening the overall access to health care rather than only focusing on elimination of rabies. Interventions, although vertically targeting rabies, should always also be transferable to a horizontal approach and conducted with local support to guarantee sustainability (Ollila, 2005). As a zoonotic disease requiring close collaboration between human and animal health services for effective control, rabies has great potential to build up One Health thinking and partnerships. One Health is an answer to the neglect described above, from which other zoonosis would also benefit; however, although this approach is more often discussed, only a few countries have taken specific action. In theory, cooperation between the Ministries of Health and Agriculture and joint Technical Working Groups for the prevention and control of zoonoses are in place for avian influenza, rabies, anthrax and leptospirosis in several Asian countries, including Vietnam, Indonesia, Cambodia and the Philippines. The need for practical implementation persists, particularly in Cambodia, Laos and Myanmar. Although joint regional disease control activities are already on-going in the larger Mekong basin area, rabies has not yet been prioritized. The rabies control programs of the Mekong countries are striving to keep up with the pace of the regional efforts for disease elimination, although no comprehensive national rabies programs have yet been established.

Control measures are currently being implemented in Lao PDR with vaccination against canine rabies, but vaccination coverage remains very low. There has been no active surveillance program for canine rabies, and human rabies cases are reported only through event-based surveillance (OIE, 2013).

The disease is thought to be widespread in Cambodia, though surveillance is limited, and PEP is available only at the Pasteur Institute in Phnom Penh and Angkor Hospital for Children in Siem Reap (Ly et al., 2009). Consequently, 95% of the reported PEP administrations are given to Phnom Pen residents and people from the surrounding provinces (Ly et al., 2009). On the basis of incidence numbers in the Phnom Penh area, Ly et al. (2009) modelled the overall human rabies incidence for the whole country to be 5.8/100’000 people. This figure is 15 times higher than the official national reports and one of the highest rates in Asia. Reasons are the striking lack of nationwide access to PEP and the high dog ownership rate, with a dog to human ratio of 1:3 particularly in rural areas.

For Myanmar, the Ministry of Health estimates over 600’000 dog bites and 1000 human deaths due to rabies per year (Gongal and Wright, 2011).These numbers remain putative as rabies is not notifiable, and no national program for prevention and control has been implemented (AREB, 2011).

When vaccinations are undertaken, they often fail to reach the set target of 70% coverage necessary to interrupt disease transmission (Coleman and Dye, 1996). There is a strong need for interventional research in Asian countries including dog demography, population characteristics and best vaccination strategy to achieve sufficient coverage. Average dog to human ratios in Asia are comparable to Europe and are estimated to be 1:7.5 in urban and 1:14 in rural areas (Knobel et al., 2005). Therefore, it is not the overall dog number but rather the characteristics of the dog populations which make them vulnerable to be rabies reservoirs (Kitala et al., 2001; Morters et al., 2013). On the one hand, the poor health status of many dogs in poverty settings leads to a high population turnover. On the other hand, dog owners with little means do not feed their animals regularly and to the animals scavenge additional food in the streets. Cultural and religious backgrounds have also been identified as influencing dog populations and accessibility of dogs to vaccination. In Muslim communities, dog to human ratios are very low and the animals are less likely to be brought to fixed vaccination posts because dogs are viewed as unclean (Mindekem, personal communication). In contrast in Hindu and Buddhist communities, dogs are more likely to be stray or community owned and are fed in the street, but access for parenteral vaccination might still be adequate (Bogel and Joshi, 1990; Totton et al., 2010).

Such factors require thorough investigation and must be taken into account in planning interventions in dogs, but until the problem of rabies in dogs is addressed even countries with good access to PEP will continue to invest considerable resources into prevention of human rabies fatalities.

Control without elimination: The focus on PEP

China administers the highest numbers of vaccine doses worldwide (12 - 15 million/year) at an estimated cost of 1 billion US dollars. Nonetheless, 19,222 human rabies deaths were reported from 2005–2012 (Yin et al., 2013; Song et al., 2014). More than 50% of these cases occurred in rural areas of southern China (e.g., Guizhou, Guangxi, Hunan, Guangdong and Yunnan) (Si et al., 2008; Wu et al., 2009; Tu, 2011; Guo et al., 2013). In 2005, a national rabies surveillance program was introduced to investigate the outbreak in terms of vaccination coverage, PEP treatment and geographic and social composition. Although the number of human rabies cases decreased from 2007 to 2012, the geographic distribution of cases has increased, with a number of counties that were previously rabies free reporting human fatalities in recent years (Guo et al., 2013). The failure to receive PEP is attributed to the fiscal decentralization and privatization of the health care system, leading to lower access to health care services for many poor and rural families. Also the replacement of concentrated vaccines with more expensive purified cell-culture vaccines contributed to a lower accessibility of marginalized communities to PEP (Si et al., 2008; Hu et al., 2009). Another cause for the re-emergence of rabies in China is believed to be the high dog population growth and low vaccination coverage. In addition, the development of the transportation network increased the movement of people with their dogs, particularly in rural areas.

As with access to PEP, dog vaccination coverage also shows a striking disparity between wealthy and rural provinces. Over 90% of dogs are vaccinated against rabies in Shanghai, as opposed to less than 10% in neglected rural areas. In south China, half of all cities register vaccination rates below 70% (Song et al., 2009).

After the re-emergence of rabies in China, thousands of dogs were destroyed in an effort to control the rabies epidemic (Associated Press, 2006; Chinadaily, 2009; WeirdAsiaNews, 2009). Mass elimination of dogs is no longer recommended to reduce populations and control rabies because it increases population turnover while decreasing herd immunity, and the public opposition to dog removal can lead to the failure of rabies control programs (Morters et al., 2013). Recently, the approach has shifted focus towards dog registration and compulsory vaccination (Xinhuanet, 2009; Tu, 2011; Wang et al., 2011). The interdisciplinary Rabies Advisory and Technical Board is meeting twice a year to move forward rabies prevention and control in China. Rabies research from Chinese scientists has also increased in the last decade, but Yin et al. (2013) identified a lack of studies regarding intervention, policies and surveillance compared to laboratory based publications. Such a gap is also observed on the international level (Zinsstag, 2013).

A similar picture to China can be observed in Vietnam. Most cases are reported in rural areas where about 80% of the overall 7 million dogs are found (WHO, 1996; Hanh, 2011b). The majority of rabies cases occur in the northern mountainous provinces which do not have adequate access to information and health services. In 1987, a vaccine card and rabies vaccination register were introduced in all provinces/cities in the northern part of Vietnam to measure vaccine usage and record the number of deaths from rabies. During 1992 and 1995, Vietnam recorded 414 human rabies deaths and 345,000 people received PEP per year (WHO, 1996).

Rabies prevention and control programs included both mass dog vaccination and intensified human PEP. With the increased rate of PEP, reaching as high as 790/100,000 population in 2004, the rabies death rate in humans has decreased drastically from 0.71/100,000 population in 1994 to 0.1/100,000 population in 2011 (Xuyen, 2008; Hien, 2009; OIE, 2013). However, in 2007 a temporal peak of 131 deaths (12/100,000 population) was observed (Thanhniennews., 2010; Hanh, 2011b). Besides dog bites, butchering of dogs and cats for human consumption has been identified to account for 1.6% of human rabies deaths (Hanh, 2011b). The country spends at least US$ 15.4 million on PEP against rabies annually due to the high commitment of the Vietnamese government for rabies prevention and control programs (Dodet and AREB, 2009). Vietnam has 936 rabies vaccination posts in the whole country for rabies consultation and prophylaxis, and about 300,000 people received rabies vaccine each year during the period from 2008 to 2010 (Hanh, 2011a).

Nationwide rabies vaccination campaigns were conducted for dogs and cats, but the coverage was low, at approximately 35−50% (Hien, 2009; Hanh, 2011b). In light of this fundamental problem, the Ministry of Agriculture and Rural Development (MARD) endorsed a US$ 7.5 million project to implement the National program on rabies control and elimination for the period 2011-2015. In the same year, the Ministry of Health (MOH) also allocated US$ 15 million for communication, training, workshops, vaccination and monitoring of rabies control and prevention activities (OIE, 2013). Recently, the MOH and MARD jointly signed the Circular 16 dated May 27, 2013 providing guidelines for coordinated prevention and control of key zoonotic diseases including rabies through a One Health approach (Xinhuanet, 2013). In December 2012, Vietnam also received 200,000 doses of rabies vaccine from the OIE Rabies Regional Vaccine Bank funded by the European Union (HPED programme) (OIE, 2013). These coordinated approaches represent a big step towards eliminating rabies by 2020.

The advantage of dog rabies vaccination

China and Vietnam have in recent years made an important turnaround and are thriving with their policy decisions pointed in the right direction. Vaccination of dogs is the best way to eliminate rabies in the animal reservoir and to prevent infection in humans (Bogel and Meslin, 1990; Cleaveland et al., 2006; Zinsstag et al., 2009). There are many field examples in which vaccination coverage ranging from 60 to 87% resulted in significant decrease in incidence of dog rabies and human exposures. The oldest are dating as far back as the 1950s, such as in Memphis, Tennessee in the USA (Steele and Tierkel, 1949). With the success in Latin America, an entire continent previously endemic for dog rabies has nearly succeeded in elimination (Vigilato et al., 2013b). In Asia, the Phetchabun province in Thailand (Kamoltham et al., 2003), the cities of Jaipur and Jhodpur in India (Reece and Chawla, 2006) and the Bohol province in Philippines (Lapiz et al., 2012) have, among others, set a successful example. The recent incursion of rabies in Bali in Indonesia was first targeted with mass elimination of dogs which failed to control rabies, and later a mass dog vaccination campaign was initiated. Following the dog vaccination campaign, the incidence of rabies declined significantly in both dogs and humans (Putra et al., 2013).

Regions and countries that are free from canine rabies enjoy long-term cost savings by discontinuing animal and human rabies prevention and control programs (Fishbein et al., 1991; Cleaveland et al., 2006; Zinsstag et al., 2009; Shwiff et al., 2013). The average cost of dog vaccination varies in a range of different rural and urban settings depending on the accessibility of dogs and the level of community participation during the campaigns. Direct costs per dog vaccinated were estimated to be on average US$ 1.30 in Asia and Africa (Bogel and Meslin, 1990; Knobel et al., 2005). In contrast, the cost of human PEP is very high, with an average estimated cost (both direct and indirect) of US$ 40-55 per person (Bogel and Meslin, 1990; Knobel et al., 2005). In addition, the human diploid cell vaccine (HDCV) or equine rabies immunoglobulin (ERIG) would cost about US$ 110 or US$ 25 per person, respectively (Knobel et al., 2005). It has been estimated that globally more than 15 million people receive rabies prophylaxis annually, with the majority of them living in China and India (WHO, 2013).

In Africa and Asia, the current estimated annual cost of rabies is above US$ 500 million, excluding the cost of Years of Life Lost (YLL) (Shwiff et al., 2013).

Detailed field studies confirm broad scale economic evaluations and the concept of advantageous One Health interventions in Asia and Africa (Sambo et al., 2013). A study using Indonesian Bali rabies control program data indicates that a comprehensive high dog vaccination coverage campaign would likely result in elimination, saving 550 human lives and US$15 million in human rabies prophylaxis costs over the next ten years (Townsend et al., 2013a). Similarly, a cost estimation of rabies control in Flores Island, Indonesia also indicated that providing PEP for humans is costly for government and cannot provide a permanent solution to prevent rabies (Wera et al., 2013). Dog mass vaccination, in contrast, is promising in regard to its potential for elimination of rabies. Yet, because dog populations in developing countries are highly dynamic and not closely supervised, vaccination intervention must go hand in hand with dog population control and promotion of responsible dog ownership in order to become sustainable. The island of Bohol and the Phetchabun Province in Thailand have implemented control programs with a holistic systems approach which integrates many different aspects of rabies control and the interdependencies and serves as an example for other control projects.

Community empowerment: Integrated One Health elimination programs

In 2007, the National Rabies Law was enacted in the Philippines and a national rabies elimination strategy was developed to implement local rabies elimination activities and to establish and maintain rabies-free zones. The 'Bohol model' initiated in the same year has proven that, with the leadership and commitment of the local government and adequate funding and approaches, human rabies will decrease and eventually disappear. In addition to dog rabies vaccination, education of children, improvement of surveillance and empowerment of the local community have been included in the program. This was done through motivating communities to establish their own rabies control program. Giving the community the key role ensured their full awareness on the rabies situation and prevention measures as well as the participation of the public during the intervention. The community-based approach fostered empowerment and integrated the One Health approach to expand ownership of the program by involving other sectors beyond animal and human health staff. The education sector, with teachers and students, led the integration of rabies prevention and responsible pet ownership in the primary and secondary school curriculum. Police and customs officials, women's cooperatives, religious and other civil society leaders, rural development agencies and business groups were also partners. Establishment of rabies-free zones contributed to safe tourism activities bringing more income to the communities. With committed, but limited, local resources, the local government was keen to sustain the programs and advance towards dog rabies elimination.

A similar program was implemented in Phetchabun Province, Thailand to prevent human deaths from rabies. This five-year rabies control project was initiated in March 1993, with the specific aim of eliminating human rabies throughout the province by 2000. The program had several strategies including: increasing the accessibility of post-exposure treatment for humans exposed to animals potentially or confirmed as rabid; increasing coverage of post-exposure treatment in humans; increasing awareness of rabies through advocacy in provincial schools, television programs, and newspapers; reducing canine rabies by monitoring the dog population and implementing vaccination and sterilization programs; increasing cooperation between the Ministries of Public Health, Agriculture, and Education on a provincial level; and finally assessing the impact of the program through intensified follow-up of patients exposed to both suspected and laboratory-confirmed rabid animals. Increased use of PEP in humans was achieved by expanding the use of the intradermal Thai Red Cross regimen. By the third year of the program, no further human rabies death was reported, which proved the success of the chosen approach (Kamoltham et al., 2003).

Many people, and especially disadvantaged groups, lack knowledge about rabies and may engage in high-risk practices, e.g., having stray dogs in their villages, eating dog meat, preferring traditional healers for bite wound treatment. Therefore, a risk communication campaign adapted for a given local background and a well-designed Information Education Communication (IEC) plan is important for any such integrated approach. A KAP study undertaken after the implementation of the Bohol control program indicates that the Bohol population today have a good knowledge of the danger of rabies and understand the importance of Responsible Pet Ownership, registration and vaccination of dogs (Davlin et al., 2014). Investigations into the dog vaccination coverage on the household level showed that over 60% of dogs had been vaccinated by the program although the vaccination and registration was not free of charge (Davlin et al., 2013).

The majority of dog bite victims are children (Sriaroon et al., 2006). Education targeting children has proven to be very successful and sustainable in pilot areas in Thailand, the Philippines and Indonesia. Most local government units have limited funds to conduct the needed community-based activities, and they have limited number of staff to carry-out field activities. Therefore many local programs in a limited geographic area depend on NGO or private funding.

On constant watch: Dog rabies free countries and areas

There are only very few historically rabies free countries in East and Southeast Asia. Brunei and Timor-Leste have not reported any occurrence of animal rabies and have specific surveillance, vaccination, quarantine and precautions instituted at borders. Other countries have successfully eliminated rabies over the last 70 years and stayed rabies free for over 2 years. These countries are therefore eligible to qualify for disease freedom in accordance with the provisions of the OIE Terrestrial Animal Health Code. After the development of the first rabies vaccine in 1885 by Louis Pasteur, Japan initiated the first urban dog vaccination program in the world in 1921 and eliminated rabies in 1954 through vaccinating dogs and reducing the urban dog population (Tamashiro et al., 2007; Takahashi-Omoe et al., 2008). Also in the 1950s, several other Asian countries and areas carried out rabies elimination programs through mass dog vaccination and destruction of dogs, with canine rabies being eliminated from Malaysia in 1954 (Wells, 1954, WHO 1987), Taiwan in 1961 (Weng et al., 2010; Liu, 2013) and Singapore in 1953 (OIE, 2013). South Korea eliminated rabies in domestic dogs in 1985 and stayed rabies free until 1993, when cases re-emerged in the form of wildlife rabies (Kim et al., 2006; Joo et al., 2011). The cycle seems to be maintained in raccoon dogs with spillovers to cattle and domestic dogs (Kim et al., 2005). The problem is believed to have emerged from the demilitarized zone bordering North Korea, and efforts are undertaken to understand transmission dynamics and to control the disease via oral vaccination of wildlife and dog vaccination (Kim et al., 2006; Oem et al., 2013). The problem of rabies in wildlife also exists in China where rabies in ferret badgers has become an increasing concern (Yu et al., 2012).This shows that after elimination of rabies in domestic dogs, persistent wildlife rabies cycles will remain as a risk for reintroduction of the disease into dog populations and for direct transmission from wildlife to humans. Direct transmission from wild animals to humans becomes more apparent after elimination of rabies in domestic animals, as observed for skunks and raccoons in the United States (Rupprecht et al., 1995) and vampire bats in Latin America (Schneider et al., 2009). However, wildlife rabies is currently contributing very little to the overall burden of rabies on public health in Asia and worldwide.

Rabies free countries have a strict quarantine and regulatory system for the import of dogs and cats from rabies endemic countries. They are also carrying out risk assessment for re-introduction of rabies through the importation of dog and cats from overseas and making policy changes to reduce the risk (Wilsmore, 2006; Tamashiro et al., 2007; McQuiston et al., 2008; Kamakawa et al., 2009; Weng et al., 2010; Sparkes et al., 2014). For example, with widespread rabies outbreaks and dissemination in Indonesia, the risk of rabies entering northern Australia is real (Sparkes et al., 2014). Despite stringent regulations, there have been a number of well documented cases importation of rabid dogs recorded in rabies free countries through failure of border controls or ignorance of importation rules (Fooks et al., 2008; Gautret et al., 2011b). Low awareness by travellers, illegal pet trade and animal rescue organizations are potential sources for reintroduction.

In addition to imported dog rabies, 60 imported human rabies cases were recorded globally from 1990 to 2012 (Carrara et al., 2013b). All these imported cases, both in dogs and in humans involve financial cost. Illegal or accidental importation of rabid dogs into rabies-free countries have led in the past to resource-intensive, costly public health responses (McQuiston et al., 2008; Gautret et al., 2011a; Johnson et al., 2011; Mailles et al., 2011). Costs involved with the importation of rabid dogs include expenses for diagnostic tests, as well as disease investigation, prevention and control, posing unnecessary strain on health systems. For example, importation of rabid dogs from Morocco to France in 2004 and 2008 resulted in a high demand of PEP in humans (Lardon et al., 2010; Gautret et al., 2011a).

The emergence and spread of rabies from endemic foci to rabies free areas poses an even higher risk than importation. A notable example is rabies incursion into Bali Island in Indonesia during 2008. More than US$ 17 million has been spent to eliminate rabies in Bali, but the disease has not been completely eliminated (Putra et al., 2013). The re-emergence of rabies in previously free areas has also been reported in Bhutan (Tenzin et al., 2011b). Nationwide intensive mass dog vaccination, as well as administration of PEP to dog bite victims, continues although only sporadic cases of rabies are reported in the south where there are shared borders with India. Consequently high costs for rabies control are observed in Bhutan, and the trend of PEP and the cost of the treatment are escalating each year (Tenzin et al., 2012).

The impact on global trade due to endemic rabies in Asia is unknown. Although companion animals are increasingly being imported for a thriving commercial pet trade by industrialized countries in Europe and the USA, there is scant data on the number of dogs imported from Asian countries. Nonetheless, illegal trading between Asian countries of thousands of live dogs for meat consumption is believed to be occurring and has become a concern. A ban on these movements would be beneficial for animal welfare and rabies control. The international trading of dog and cat fur from Asian countries to the USA and many European countries existed until the mid-2000s but has subsequently been banned on animal welfare grounds.

The proposed elimination of rabies by the year 2020 in Asia and worldwide in 2025, will also lead to financial savings in rabies free countries. Not only would the costs described above due to rabies importation or re-emergence be alleviated, but also the costs for prophylactic pre-exposure vaccination of veterinarians and travellers would avoid. As long as dog rabies endemic areas exist, all people remain at risk of rabies infection in our globalized world, and rabies awareness will have to be maintained in all countries.

Rabies in a globalized world: Rabies awareness and risk in travellers

Every year, millions of people travel around the world for various purposes, putting themselves at risk of being exposed to rabid animals in rabies endemic countries. Southeast Asia is one of the most popular tourist destinations for travellers worldwide, and rabies is a recurring public health concern in this area (Wilde et al., 2005; Piyaphanee et al., 2012) A similar concern exists in North Africa for the many European visitors (Gautret et al., 2011b). Several studies have been conducted to assess the risk of rabies exposure among travellers, as well as their treatment seeking behaviour, attitudes and practices related to rabies. Potential rabies exposure of travellers by bites or licking is fairly common, while only a low percentage (<20%) received pre-exposure vaccination before travel (Phanuphak et al., 1993; Pandey et al., 2002; Piyaphanee et al., 2010). In a study from Thailand, more than half

(54%) of all exposures occurred in the first 10 days after arrival in Southeast Asia (Piyaphanee et al., 2010), contrasted with the advice of travel medicine guides which recommend pre-exposure vaccination only when people stay in an endemic area for longer periods of time.

The most recent survey conducted among foreign travellers in Thailand indicated that the risk of being bitten was 1.11 per 100 travellers per month and the risk of being licked was 3.12 per 100 travellers per month. Among those who were bitten, only 37% went to a hospital to get PEP. While almost 60% sought health information before their trip, only about 1 in 10 participants had completed rabies pre-exposure prophylaxis (Piyaphanee et al., 2012).

A study to examine the characteristics of rabies PEP in returned travellers from France, Australia and New Zealand who were victims of an animal-related injury in Africa, Southeast Asia or the Pacific region highlighted important deficiencies in rabies PEP for travellers in rabies-endemic countries, with the majority not receiving adequate PEP or experiencing substantial delay before treatment (Gautret et al., 2008). The rabies risk perception among the travellers was shown to be largely unknown. Travel-associated rabies risk awareness needs to be improved, particularly regarding the prevention of animal bites, post-bite measures and the urgency for obtaining PEP (Hamer and Connor, 2004; Toovey et al., 2004; Wilder and Smith et al., 2004; Altmann et al., 2009). This is due to the general public from rabies free countries having little knowledge about rabies, even when living in proximity to an endemic country. For instance, a study among tour leaders in Taiwan showed that they had a positive attitude toward rabies vaccination but a relatively low level of knowledge about rabies regarding clinical manifestations, rabies-endemic areas, prevention and management (Huang et al., 2014).

As a sad consequence of the lack of awareness, the inadequacy in the use of pre-travel immunization and the access to PEP, cases do occur where travellers are bitten by rabid dogs in rabies endemic countries and later diagnosed with rabies after returning home. Amongst them, people traveling for tourism, business or expatriates from high income countries accounted for 57% of the cases, while migrants originating from low income countries accounted for 43% of cases. The vast majority (85%) of the cases resulted from exposure to rabid dogs and were due to failure to seek post exposure treatment or inadequate treatment (Gautret et al., 2011b; Malerczyk et al., 2011; Carrara et al., 2013a). This reinforces the point that travellers should be adequately counselled about animal-associated injuries and rabies risk when visiting rabies-infected countries.

There is no clear data to indicate that endemic rabies in Asia has any negative effect on the tourism industry since millions of people travel to Asian countries each year. For example, China and Thailand were ranked 4th and 10th, respectively, among the top 10 international tourism destinations in 2013. China recorded 55.7 million international arrivals in 2012 and 2013, whereas Thailand recorded 22.4 million international arrivals in 2012 and increased to 26.5 million arrivals in 2013. The tourism industry accounts for billions of dollars in these countries (UNWTO, 2014). For the whole East Asia region (northern and southern part), the international arrivals increased from 47.6 million people in

1990 to 220 million in 2013, demonstrating a significantly increasing trend of international arrivals (UNWTO, 2014) apparently unaffected by the rabies endemic state. Bali Island, for example, reported a major rabies outbreak in 2008, which continued to report rabies cases in both humans and animals until the mass dog vaccination program described earlier controlled the outbreak and significantly reduced rabies incidence (Putra et al., 2013). Although there were reported incidences of dog bites among travellers in Bali during this time, it has not deterred the travellers nor affected the tourism industry. On the contrary, numbers of international arrivals in Bali have steadily increased from 1.96 million people in 2008 to 3.27 million in 2013 (BGTO, 2013). This data indicate that neither endemic rabies nor on-going rabies outbreaks affected the tourism industry in Asia.

The way forward to rabies elimination by 2020

Rabies is one of the few communicable diseases which can possibly be eliminated using the currently available tools for veterinary and public health interventions. Still, more than a billion people in Asia are at potential risk of contracting rabies. While the burden of rabies is primarily on human health, the disease control has to target the animal source. Cost-effectiveness studies of rabies control have demonstrated that dog rabies vaccination is more economical and ethical than the intensified use of PEP in humans alone. The World Animal Health Organization (OIE) has stated that just 10% of the costs currently used to treat people bitten by potentially rabid dogs would be sufficient to eradicate dog rabies worldwide and thereby prevent almost all human rabies cases (Vallat, 2011).

Asian nations have set a regional goal to control and eliminate dog-mediated rabies by 2020 and protect and maintain existing and future rabies-free areas. With the objective to strengthen regional and national coordination as well as the technical and institutional capacities to manage dog rabies elimination programs, a regional road map is being developed that aims to integrate country level rabies elimination efforts. There are many challenges that need to be addressed to reach elimination in Asia and worldwide. More work is needed to improve diagnosis and increase the coverage of laboratory-based surveillance to break the cycle of neglect. Especially in rural areas, rabies awareness, access to timely PEP, sufficient dog vaccination coverage and effective implementation of dog population control have yet to be established. Priority has to be given to physical, financial and human resource to build more capacity. There is also the constant need to assess the epidemiologic situation, considering the on-going rabies control activities. There are many possible risk factors that may further amplify and disseminate the disease in the at-risk areas. South-south collaboration should be encouraged by twinning countries with successful local programs currently undertaking community-based action against rabies. Such programs use a highly successful bottom-up approach that entails collaboration across various sectors – animal and human health, education, trade, local media, community-based NGOs and local government units at provincial, commune and village levels. The partnerships established during experience with avian influenza could be extended to work on rabies using the One Health approach. These partnerships will encourage an exchange of best practices and joint training.

With a comprehensive and integrated approach, it is expected that dog rabies will be eliminated, and there will be eventual decline and disappearance of human rabies cases. Countries that are still endemic for dog rabies, as well as those that are rabies free, will gain from elimination, and the resilience of communities in confronting the threat of other zoonotic diseases will be strengthened.

VIII. Discussion

The discussion evaluates the contribution of the many axes of the PhD thesis to rabies control, critically analyses the project implementation in regard to the research approaches and provides an outlook on future research and continued actions.

V.III.1 Did we turn the wheels?[23]

Surveillance wheel: Training and Innovation

The positive validation of the Anigen Rapid Rabies Test® by our study gives a push to the surveillance wheel. We hope that the momentum will be taken up by experts in the rabies field and further extensive validations will eventually lead to an uptake of the RIDT methodology into WHO and OIE recommended procedures for rabies diagnosis.

The requirements for a new test are high. Because rabies is a fatal disease, sensitivity has to be close to 100% to exclude with certainty false negative results that would have potentially devastating consequences for true exposure victims. Also specificity should be high due to the high costs of PEP vaccine which can exceed a month's wages in a low income setting. Nonetheless there is no alternative to such an innovation. Rabies diagnostic tests which can be performed in field laboratories under harsh climatic conditions with rudimentary means are imperatively needed. Once such a test is widely established, it will directly contribute to the collection of valuable data and thereby constitutes a window to overcome the neglect on the political level.

In addition timely investigation into rabies suspected cases and early case reporting is of utmost importance for PEP. Ideally the first vaccine dose is given the same day the bite incident occurred (day 0). A second dose is due on day 3 for most WHO approved regimens (Hampson et al., 2011). Diagnostic results should therefore be obtained in less than 3 days, which is impossible to deliver when distance to a diagnostic facility is too long or accurate transport options are scarce. Adequate and efficient surveillance positively directly influences the practitioner's level in guiding best practice decisions and indirectly influences the awareness and support of the public. As active participation of dog owners and bite victims is an important criterion for reporting, an effective surveillance system has to be rapid and close to communities in need. Delayed laboratory response causes decreased perception of the benefit of testing and leads in consequence to reporting deficiencies.

Finally, surveillance is also a prerequisite to determine the disease distribution, to drive vaccination policy decisions and to guide control measures during rabies elimination attempts, and it, therefore, influences the intervention wheel (Townsend et al., 2013b; MacNeil et al., 2014).

[23] Relates to Figure IV.1 and is a metaphor for progressing the cause of rabies control at all different levels.

Validation and adoption of potential rapid and affordable tests for developing countries can only be done through a North-South research partnership and networks linking focal national laboratories with international reference laboratories (Fooks et al., 2009; Dacheux et al., 2010).

Our study gives a positive example of such a partnership leading to study results with a direct link to applicability in the field.

North-South partnerships based on the 11 principles of transboundary research partnerships[24] ought to have a strong impact on capacity building and mutual learning. We have addressed this in our project by facilitating the master studies of 2 Chadian students in the field of laboratory diagnostics. In addition a workshop was held in N'Djamena during which 30 veterinary workers, representing regional livestock delegations distributed over the whole country, were instructed on the sampling of brain material from suspected animals and the use of the Direct Rapid Immunodiagnostic Test (DRIT). Rabies diagnostics based on DRIT will form an integral part of the nationwide surveillance system that will be piloted soon in selected regions of Chad (see VIII.3). The results on the cost estimation for a surveillance system covering the whole country presented in chapter 6 of this thesis serve as a template for a detailed operational plan and budget to be submitted to involved ministries.

Intervention wheel: Facilitated access to vaccination

This is the wheel we have turned most strongly during our project. Much of the success of the mass vaccination campaign can be attributed to the good fortune of having the right people at the right time in the right place. The involvement of the Chadian government through its financial support was pivotal for the operational success of the campaign. During our intervention, however, we encountered settings where the highest motivation of personnel and the resources allocated became secondary factors for reaching target coverage. No-charge fixed post mass vaccination alone is therefore not the magic gun (meaning the perfect approach) matching the magic bullet (meaning the potent vaccines available). While the intervention has reached the average effectiveness goal of 70% vaccination coverage over the whole city, we have not achieved equity effectiveness defined as equal effectiveness across all social groups (Zinsstag et al., 2011b) because coverage remained heterogeneous. Effectiveness was observed to be influenced by two main sociological factors: economic status and religious affiliation. Dog ownership is not viewed favourably in some Muslim communities because of factors well described by Stilt (2008), so owners are reluctant to be seen in the street with their animal. In addition, dogs reared in a Muslim background are less likely to be tame, as observed in our study and in pastoralist communities in Tanzania (Cleaveland et al., 2003). A mobile door to door approach would likely yield a higher effectiveness in such a background than a fixed post approach (Plate 9 A and B).

[24]www.naturalsciences.ch/organisations/kfpe/11_principles_7_questions?_ga=1.52298218.2144634779.1436390257

While dogs in wealthy households are more likely to be vaccinated by owner-funded, routine veterinary care (Awoyomi et al., 2008) it can be observed that socioeconomically higher classes are less accessible to free vaccination. This is illustrated with the results of our campaign in the 6th district of N'Djamena. In this central district the participation of dog owners was very high and consequently coverage achieved within a week was between 65-71% in 2003, with only lack of vaccine preventing an even higher coverage (Kayali et al., 2003b). During the recent intervention coverage in this very same small area was 52% in 2012 and 68% in 2013. These coverage numbers were only achieved by adding to the number of dogs vaccinated through deploying selected teams again in this district for a second week. The striking decline in participation is most probably a result of the higher socio-economic standard of this district compared to 2003. Over 50% of owners that did not vaccinate their dog in District 6 stated that they were not informed of the campaign. In 2003 the main cause for non-vaccination, other than the lack of vaccine, was the inability to handle the dog. Dwellings of higher socio-economic status are usually surrounded by high walls and therefore potentially less accessible to loudspeaker announcements, which were identified to be a main driver of sensitisation in most other parts of N'Djamena. Results from Bamako suggest that radio and television are better channels to reach wealthier neighbourhoods (Mosimann et al., 2016; Chapter 7, this thesis).

To turn the wheels equally in all different contexts and reach equity effectiveness, a qualitative research component with a sociological perspective is called for, as described in chapter 7 of this thesis. Insights into social determinants of access of the dog to veterinary care can then be incorporated in the planning of campaigns to apply the approach with the maximum likelihood of success in a given background.

Nonetheless it can be argued that equity effectiveness in regard to dog vaccination has to be exploited to its largest extent for the control of rabies. Our results show that even with a heterogeneous distribution of coverage, transmission of the disease was interrupted in the larger part of town (Zinsstag et al., 2017; Chapter 4, this thesis). This is partly due to the fact that within a Muslim context the dog to human ratio, and consequently dog densities, are much lower than in a Christian/traditional religious context (Léchenne et al. 2016a; Mbilo et al., 2016; Chapter 1&5, this thesis). Provided that a threshold density of susceptible dogs exists below which rabies fails to be transmitted in a population, it might not be necessary to reach high vaccination coverage in a sub-population (Zinsstag et al., 2017, Laager et al., upcoming). Ultimate equity effectiveness is achieved by the elimination of disease (Dowdle and Cochi, 2011).

The planning and implementation of our project provides a good example for intercultural and transdisciplinary partnership (discussed also in VIII.2) which could be applied in other settings. However the detailed logistical approach and the public sensitisation campaign are heavily context dependent and have to be adapted accordingly. The scaling up of our results to other African towns must therefore be considered with caution.

Policy wheel: Political commitment

Rabies control policy is in itself a wheelwork with specificities at the community, regional, national and international level. This complexity and also the generally inert nature of political decision processes make this wheel the hardest to turn. Although we knocked on numerous doors during the project process, we have not yet achieved a legislation change on national level in Chad towards compulsory vaccination and registration of dogs. Despite the close interaction with local political stakeholders we were only able to gain their short term support. We have not yet reached sustainable commitment or translation of the inclusion process into long-term allocation of financial resources to rabies control. However political advocacy is a multifaceted process achieved jointly with all stakeholders to be successful. While academia contributes expert opinion to problem solving, problem identification has to be rooted in a public need and be translated as a bottom up community pressure on decision makers. Further, expert opinion is taken up by international public, private and NGO institutions resulting in superordinate regulations and top down recommendations to member states. Through our close cooperation with GARC and membership in PRP our findings are contributing to this international advocacy process (Lembo et al., 2011). As described in the introduction (IV.1.3), GARC has produced a number of leading documents on many aspects of rabies control. One tool especially noteworthy for its potential to positively trigger political commitment on the national level is the SARE framework (Figure VIII.1 and section IV.1.3). Evaluation of the current stage of rabies control in countries and disclosure of these reports during international meetings is likely to generate positive competition. It is easy to believe that we have the nicest flowers in our garden if we are not looking across the fence onto our neighbours' property. Likewise we can gain the impression that with our project Chad became the most advanced country in the Sahel region in regard to rabies control. But, Chad falls between stage 1 and 2 of the SARE framework because the intervention was based on a short term local action plan. The most important requirement to reach stage 2, the introduction of a legislation that makes rabies notifiable in Chad, is not yet met.

Similar to triggering ambition through competition on the international level, bottom up pressure from affected communities is likely to be effective by targeting self-esteem of local political leaders. A study comparing financial with non-financial incentives found that extrinsic rewards improve the performance of workers in public services (Ashraf et al., 2014). Motivation to commit is therefore not only based on financial remuneration but first and foremost on improving the social status in a community. Consequently the vital importance of close collaboration with local authorities lies not only in the improvement of the short term impact of a project but also in the provision of a platform that brings authorities and the public closer together to work sustainably towards a common goal. The role of the block representative during the vaccination campaign was to provide furniture for the vaccination post and guarantee safety around the work place. In addition to this passive contribution, many also were helping actively by sensitizing the public via loudspeaker and putting collars on the dog. The created sense of collectiveness and ownership will hopefully resonate into other aspects of daily community practice.

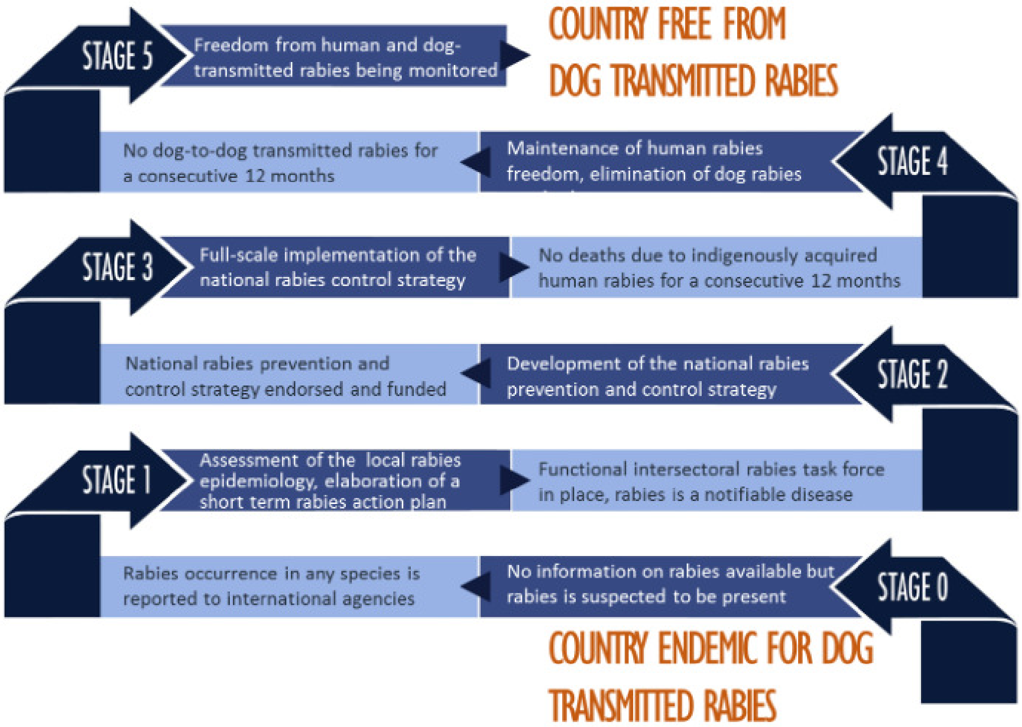

Figure VIII.1: The different stages of the Stepwise Approach for Rabies Elimination Framework derived from the blueprint for rabies control[25]

Public wheel: Raising awareness

Awareness is the first step on the path leading to knowledge creation and finally to change of practice. A project can contribute to the sensitization of the public with a well-designed Information, Education and Communication (IEC) concept. Raising awareness in our project was largely based on passive information via poster, radio and television. Nevertheless even passive input has the potential to lead to a partly self-sustaining momentum that turns the wheel of public engagement for a certain time. Once a sufficient proportion of a society is aware of a danger and starts to change their practice, they potentially influence in turn the practices of the society as a whole. We observed, for example, the building of a societal pressure to have one's dog vaccinated. This was indicated by the rumour that began to circulate in N'Djamena that dogs without collars will be killed after the campaign, which was not true but helped the cause of achieving even higher participation. We also noticed that the blue collar as a visible sign of vaccination was very important to people. Even though we explained that it is for study reasons that we put the collar on dogs, people also wanted their cats and primates to be collared to show their neighbours that their animal is vaccinated.

[25] http://caninerabiesblueprint.org/IMG/pdf/stepwise_approach_toward_rabies_elimination_sept_2014.pdf

Communication was implemented on the local stakeholder level and on a limited scale with the public via the telephone number provided on posters and leaflets. This communication should be expanded to an active participatory process leading to true local ownership (see VIII.2). Without this active component information, communication will always be dependent on constant outside input. Comparison of the success of the N'Djamena vaccination campaign with the mass vaccination in Bamako indicates that long-term repeated sensitization might play a crucial role for elevated baseline awareness. Also, the oscillatory nature of reported animal rabies cases at IRED that, since 2001, rises and falls with the intensity of research activities highlights the importance of continued information and communication. Reporting and high level surveillance is crucial in the years to come in order to monitor the epidemiological situation in N'Djamena after the mass vaccination intervention. Awareness must be boosted at regular intervals. A good platform for information and communication is provided by the international World Rabies Day (WRD) celebrated each year on the 28thSeptember (Plate 10) (section IV.1.3).

Our data from the national household survey show that education determines awareness (Mbilo et al., 2016; Chapter 5, this thesis). Although we have initiated contact to the Chadian national curricula we were unfortunately not able to include an educational aspect in the current project. Education is a core concern of GARC and a large pool of educational material especially for schools is available on their website. However, documents should always be adopted carefully across different settings. Similar to the design of a vaccination campaign, the design of an IEC concept has to be adapted to the specific local context. Accessibility to information, adequacy of the communication and education provided, and adherence to practices are heavily dependent on sociological determinants of a community.

The target of education is not only rabies prevention but also bite prevention and the promotion of responsible pet ownership (RPO) defined by OIE as a "…*situation whereby a person accepts and commits to perform various duties according to the legislation in place and focused on the satisfaction of the behavioural, environmental and physical needs of a dog and to the prevention of risks (aggression, disease transmission or injuries) that the dog may pose to the community, other animals or the environment.*"[26]

Supposedly, the adoption of RPO can significantly reduce the numbers of stray dogs and positively influence rabies control through dog population management. However RPO has to be evaluated in light of the financial resilience and social values within a respective community. We have seen that dog owners overwhelmingly take responsibility to vaccinate their dog within the frame of a free vaccination campaign. The fact that participation declines in owner charged campaigns gives rise to the assumption that only low value is attributed to the dog in the African society. Another example is the finding, reported in Chapter 2 of this thesis, that dogs are quickly killed when there is a rabies suspicion. Again this shows that owners take responsibility but could also lead to the conclusion that a

[26] http://www.oie.int/doc/ged/D9926.PDF

dog's life is not valued. However during several encounters with dog owners at work or privately I noticed that people were reluctant to euthanize dogs, because of religious values, even when they were critically ill. In the same line of reasoning, surplus puppies are put into the street to have a chance to survive rather than to actively determine their fate by culling.

These examples show that significant differences between values exist across cultures. We therefore have to be careful to impose an educational concept based on European centred values which is prone to fail.

Application of culturally sensitive and appropriate IEC concepts calls for a new research approach to reach beyond the measuring of knowledge and the description of attitudes and practices. It should rather try to understand the mechanisms leading to change of the observed current practices to a practice that is favourable to rabies control while at the same time meeting the needs of the people and fitting into the respective context of cultural values.

Practitioner's wheel: One health communication

Veterinary and human health services are wheels that have to be aligned in the same direction in order to move forward for rabies control. While our project has a strong One Health component on the research level, we have unfortunately not successfully managed to put the different actors together in practice. Chapter 2 illustrates the huge deficiencies of communication between veterinarians and human health workers, leading to misinterpretations and inaccurate decisions both in regard to treatment and concerning actions on the animal side. These findings serve as strong argument for the need of a joint interdisciplinary working committee to elaborate an action plan for rabies control across all target groups.

The danger of a "vertical program", which targets one specific disease, is interference with good general primary health care. To prevent such unfavourable effects, we have to envisage a communication platform that contributes to the control of zoonotic diseases in general. A well planned One Health network will guarantee collaboration on many other aspects aside from rabies control, for which close contact between veterinarians and human doctors are favourable. This can only be achieved if participation of practitioners in a specific disease program is leading to active ownership and eventually to a joint community of practice with elevated general resilience, capacity and flexibility.

Another danger of mass programs that provide a free service for a limited time is interference with private baseline services. Although very few dogs were vaccinated by a private veterinarian prior to the mass vaccination campaign (Mindekem et al., 2005) this service was in fact available. After the mass vaccination campaign in 2014 the IRED was contacted by veterinarians that were in need of rabies vaccine, because it was not available anymore at the main local supplier for veterinary products. We suspect that the offering free vaccination interfered with the local market, making the selling of rabies vaccine a losing proposition. In future such market mechanisms should be taken into account in the planning of interventions. As the ultimate goal is sustainability of vaccination, private veterinary services should be reinforced rather than undercut. This can be prevented by the active inclusion of private veterinarians in the planning of an intervention. Although we embedded local veterinarians as vaccinators within our campaign they were not involved in the development of the operational plan. Retrospectively, we should have planned a round table with veterinary practitioners and local vaccine suppliers to jointly discuss the possible impact of the project and insure a positive effect on their practice.

Veterinary services in Africa are still heavily focused on livestock. Through the experience veterinarians gained during the vaccination campaign they were sensitized for the importance of prevention of zoonotic diseases also in small domestic animals like dogs and cats. In addition we provided a short-term platform for closer contact between pet owners and veterinarians. This potential should be further exploited. Research into the determinants of access of small domestic animals to basic veterinary services is needed on the technical, the economic and the social level. Once these mechanisms are better understood, the findings can guide a program strategy that will ultimately lead to elevated general veterinary care for dogs and cats.

VIII.2 Have we met our ideals?

Transdisciplinary research

As our project was guided by the 11 principles for transboundary research partnership the implementation of these principles are evaluated in detail in Table VIII.1. A self-rating is applied to each principle and the results of scores are presented in Figure VIII.2. In general, all principles were addressed by the project. High scores are given for transboundary management issues like agenda setting and clarification of responsibilities. The project has also contributed well to enhancement of capacities, sharing of data and dissemination of results. Room for improvement is identified regarding aspects of communication to a larger audience (accounting to beneficiaries and mutual learning) and in terms of result application and securing of outcomes.

Overall our research activity generated sound knowledge, further strengthened the existent environment of mutual trust and mutual learning across the partners and shared ownership of results within the tripartite. However, we have to improve in future to synthesise knowledge beyond the partnership on the political and public level. Transboundary and intercultural research is a continuous process. We have to continuously fuel this process to achieve sustainability of the project, not only with the tripartite but also involving all stakeholders.

If we look at the list of cross cutting issues relevant to transdisciplinary research (TR) presented in the introduction of this thesis (IV.2.2), our project ticks many boxes. Our results provide a case study of relevance to other settings and to expert colleagues in rabies control and from other fields. As discussed above the obstacles encountered during the study have shown that rabies control is greatly affected by values and uncertainties that have to be addressed in joint research with the humanities. In terms of TR management we have maximised synergies across the Tripartite and developed skills of all partners. However this process can be strengthened with a stronger self-reflexive component and in-depth discussion of needs and interests of all participants. Education and mutual learning is a strong pillar of our collaboration with the Chadian partners. As discussed above, it should be extended from the academic level to reach out to the practitioner's level and to the wider public. Similarly the integration of the concept of TR and systems thinking/analysis that has become a strong foundation of our intercultural research team is waiting to grow strong roots in academic institutions in Chad.

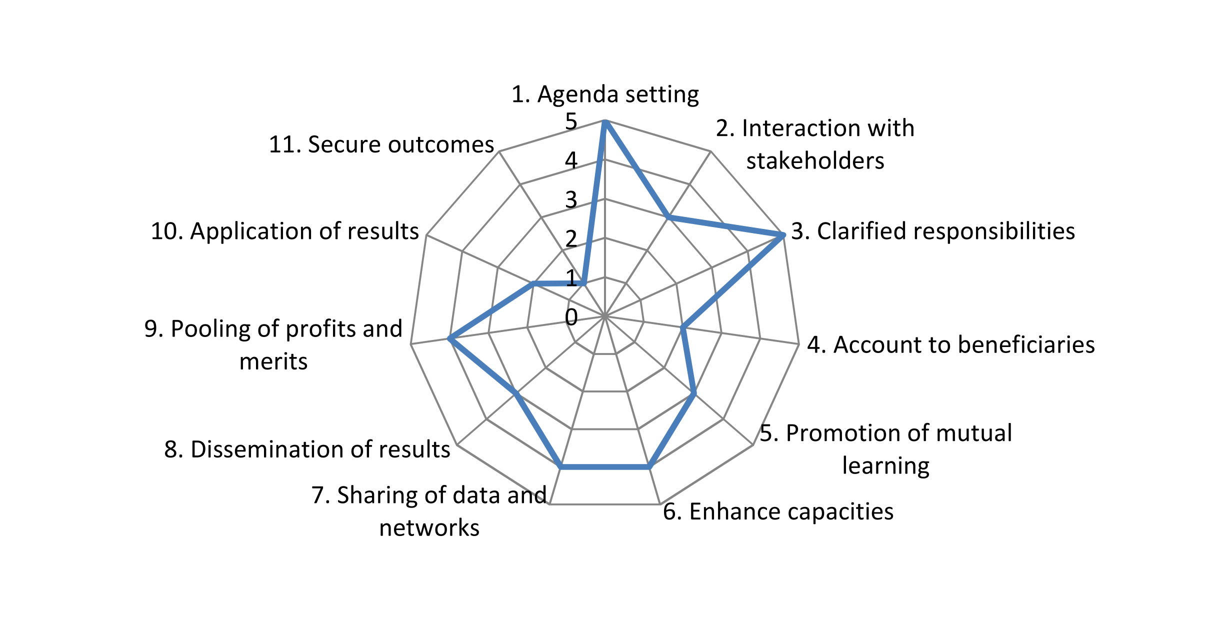

Figure VIII.2: Spider graph of the project evaluation along the 11 principles of transboundary research partnerships. Rating score: 5 close to ideal practice, 4 good application, 3 applied in principle, 2 major advances still needed, 1 unsatisfactory, 0 not considered.

Table VIII.1: Evaluation of the project along the 11 principles for transboundary research partnership. Rating score: 5 close to ideal practice, 4 good application, 3 applied in principle, 2 major advances still needed, 1 unsatisfactory, 0 not considered

	Praise	Critics	Rating
1. Agenda setting	Long term research collaboration Agenda based on previous joint experience Partners involvement defined in a tripartite contract Research need initially identified by local partners Joint funding Swiss TPH and IRED	Research questions largely identified by Swiss TPH Research methods defined by Swiss TPH	5
2. Interaction with stakeholders	Meetings with political stakeholders* early on in 2011 Yearly meeting with stakeholders during world rabies day Town authorities involved in public sensitisation and placing of vaccination posts Voluntary participation of quarter and block chiefs during the campaign	Low involvement in research process and capitalization One sided initiation of contact and passive role of stakeholders Erroneous perception of stakeholders in regard to rabies control Output and outcomes defined by project No immediate communication of results	3
3. Clarified responsibilities	Roles and responsibilities defined in a tripartite contract Shared competencies: IRED – personnel, infrastructure and logistics CSSI - administration, infrastructure logistics and data management Swiss TPH - research expertise, international network Long term experience with problem solving in case of conflict Clear assignment of duties within organisational committee and between research fellows	Rigid compliance to hierarchical structures poses a risk to optimal workflow	5
4. Account to beneficiaries	Reporting to funder with clearly defined indicators and milestones Registration and certification of vaccinated dogs Response to concerns and questions of the public directly during the campaign and through telephone contact specified on posters and leaflets Certified diagnostic results Results shared with a wide research community through conference participation and scientific articles	Reporting exclusively done by Swiss TPH Campaign outcome not communicated with the greater public No follow up on reported cases of paralysis after vaccination Research results not translated into a clear message to stakeholders and the public No accounting to health workers and local veterinarians	2
5. Promotion of mutual learning	Direct exchange of results and discussion of analysis during the vaccination campaign within the organisational committee Insights and experiences shared with other researchers Long-term evolved learning culture between the three institutions A stakeholder workshop on the information on research results is planned for January 2016 (atelier de restitution)	Local stakeholders not included in capitalization of lessons learnt. Learning targets not clearly defined within the project Focus is higher on individual than on institutional training and learning Educational component of the project not yet implemented	3

6. Enhance capacities	Enhanced diagnostic capacities Trained master and doctoral students Strengthened organisational capacities of partner institutions Enhanced scientific capacities as benefit to all institutions Strengthened communication between the three institutions Experience of local veterinarians in mass interventions Training of veterinarians in rabies diagnostics	Long-term effect of capacity enhancement remains to be evaluated in the years to come Long-term local political support not generated No long term local funding or core funding secured	4
7. Sharing of data and networks	All data and budget are shared between the tripartite All partners are involved in networking activities Long-term partnership creates confidence and trust	Expectations, interests and concerns of partners should be more openly discussed Language barriers of local partners for international networking	4
8. Dissemination of results	High scientific output and clearly defined publication pipeline Publication in English and French Sharing of results with local stakeholders and international community on the occasion of the rabies day Conference and meeting participations	No communication of results to local community to date. Dissemination heavily based on peer review publications	3
9. Pooling of profits and merits	Shared authorship Clearly defined article pipeline Full transparency and close communication on dissemination issues	Language barrier to first authorship for Chadian colleagues	4
10. Application of results	Changes to the operational plan in 2013 based on the experiences in 2012 Anigen Rapid Rabies Test will be applied in selected provinces Dialogue with stakeholders during rabies day Clear plan of scaling up to national level	No legislation changes achieved Weakening of vaccination of dogs by private veterinarians Lacking sustainability Long term impact of the project remains to be evaluated No translation of dog vaccination into less demand for PEP	2
11. Secure outcomes	Contract renewed between partner institutions Personal career planning continued for Swiss TPH and local Chadian staff Follow-up project secured	No local financial resources and long-term political commitment secured Weak policy dialogue Reintroduction of rabies virus not prevented No One Health dialogue established on the practitioners level yet. Momentum remains heavily dependent from Swiss TPH Fragile independent local ownership	1

*Ministry of Public Health; Ministry of Livestock Production; Town Major; Representatives of WHO, OIE and FAO.

Community Participation

To evaluate the project in regard to its participatory inclusion of the community the ladder of Pretty is applied (Table VI.1, section IV.2.3). Because the public was only informed about the project and asked to comply with vaccination without being consulted and having the opportunity to share an opinion, the project did not reach beyond step 3 (participation by information). The possibility to ask questions was provided through the contact phone numbers on leaflets and posters. This contact was quite regularly used and also the frequency of visits of dog owners and bite victims increased at IRED. In addition questions and concerns were also directly answered by the vaccination team members at vaccination posts.

Unfortunately we were not able to further investigate cases of paralysis reported after vaccination in two young dogs. In all probability, canine distemper virus (CDV), which like rabies affects the nervous system, is circulating in the dog population in N'Djamena. CDV diagnosis is not yet established in Chad and the incidence of this disease is unknown. A vaccination campaign bringing together many dogs at one place might facilitate the spread of CDV. This case shows the importance of a feedback mechanism to detect potentially problematic impacts of a program that should be addressed and if possible prevented in the future. The previously mentioned rumour about culling of dogs without a collar is another example of the advantages of communication. Also, some dog owners feared that the vaccine could be harmful to the dog or render the dog less able to guard the house. Such concerns were also reported from Bamako (Mosimann et al., 2016; Chapter 7, this thesis). Whether based on facts or not, information is always shared and therefore the project and the correct information have to be clearly and repeatedly shared with the public. Negative effects from misunderstandings and misconceptions have the potential to seriously interfere with program success.

Assessing the participation of local authorities who are selective members of a community we can put our project on step 5 (functional participation). District, quarter and block chiefs were involved in decision-making, development and execution of the vaccination campaign. Responsibility, however, remained in the hands of the tripartite and participation was not interactive.

To explore the CP component further the project was evaluated with the assessment framework proposed by Rifkin et al. (1988). The criteria and background for the rating are described in Table VIII.2 and Figure VIII.3 depicts the evaluation result in a spidergram. Rifkin et al. (1988) designed the framework to assess a program in its process. In order to do so, the program should be evaluated, ideally by community members, at different time points during its application.

The evaluation presented here is a personal view over the whole time period of the project. However it would be favourable to have the project also evaluated by members of the tripartite and local stakeholders. Such an evaluation repeated annually would guide the community involvement process and help to self-reflect on the outcomes achieved.

Table VIII.2: Evaluation of the rabies control program in N'Djamena with the help of the assessment factors published in Rifkin et al. 1988.

Assessment factors	**Range** (derived from Rfikin et al. 1988)	**Commentary**	**Project rating**
Leadership	Minority - Variety of interests	In the hands of the Tripartite, that only partly represents variety of interest of the community; small involvement of local authorities	4
Organisation	Created by planners - community organisation	planning imposed by Tripartite, but community and local leaders became active	3
Resource Mobilisation	Small commitment and limited control - good commitment and committed control	Campaign financed to 50% by local ministry and expenditures controlled by IRED. No resources raised in the public community.	4
Management	Professional induced - community interests	Project solely managed by Tripartite	2
Needs Assessment	Professional view - community involvement	Need for rabies control is professional's point of view; community point of view passively assumed but not actively assessed	1

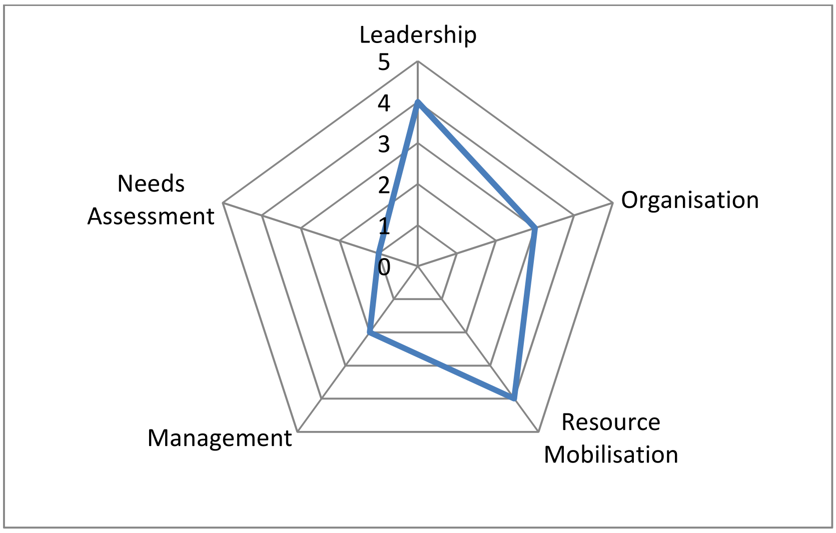

Figure VIII.3: Spidergram of the CP assessment of the rabies control program in N'Djamena.

In general our project has incorporated community member in leadership and organisation. We were also able to mobilize resources in the form of personnel, infrastructural and local financial support. However needs assessment and management have a very strong top-down approach. That rabies control is of strong interest and represents a priority to the wider public is only assumed but has not been investigated. In a study in Colombia, rabies was stated by veterinary and health workers as being a priority zoonotic disease (Cediel et al., 2013). Such a survey could also be undertaken in Chad. From the national household survey data analysed in Chapter 5, it can be interpreted that rabies is one of the most often stated health concerns related to dogs. Other identified problems are parasitic diseases that are believed to be transmitted from dogs to humans causing skin disorders. An active dialogue with dog owners and the uptake of other perceived problems related to the dog other than rabies will strengthen community participation.

Lastly we reported in Chapter 2 that only a fraction of dogs suspicious of rabies are reported to a veterinary structure or to the IRED. Our findings are similar to results from a household survey in Tanzania where people prefer to kill an animal than to report to animal health services (Sambo et al., 2014). At the same time the symptoms of rabies are reasonably well known in the public (Mbilo et al., 2016; Chapter 5, this thesis; Sambo et al. 2014). This implies that we are missing out on the most valuable source for rabies surveillance, highlighting the necessity to better communicate the benefits of reporting. As discussed in De Balogh et al. (2013) a benefit is not always measured through financial savings or gains. Communication in the form of rapid feedback can be in itself an incentive and is likely to trigger continued participation and support of the community.

VIII.3 Short and long term prospects for rabies control in Chad

The immediate next steps

In the coming months, dissemination of results and extensive valorisation of our findings are priorities. One aspect that has not been part of this thesis but has also led to a peer reviewed publication is the detailed economic analysis of the two interventions (Mindekem et al., 2017). Aspects that will be discussed are the trade-off between accessibility and cost: Door to door vaccination may increase the accessibility but will also lead to higher cost. In turn, a stringent fixed vaccination post is extremely costly when calculated on the basis of invested resources per animal vaccinated when there is low participation.

As described in the working paper on the phylodynamic modelling (Chapter VI), all positive rabies cases reported from N'Djamena in 2015 originated from District 9 at the periphery of N'Djamena. From January to September 2015, seven cases were found in this district which has a population of 4728 to 5672 dogs (Chapter 1, this thesis). If there is continued reporting of an average of one rabies case per month, the incidence projection for District 9 in 2015 is alarmingly high (2.11-2.54/1000 dogs). We are therefore planning to re-vaccinate District 9 before end of 2015 and also integrate the neighbouring village of Koundoul in the vaccination zone.

To detect cases of re-emergence in the future intensified surveillance must be guaranteed in the years to come. One prerequisite is the continuation of the information and communication strategy. Very soon we will launch an education campaign based on school visits. A rabies prevention guide is being prepared together with the national curricula and the ministry of education. Through this guide pupils will learn how to behave and interact with a dog to avoid bites, how to properly take care of a dog to decrease rabies infection risk and what to do in case of a bite. This guide is not only addressed to children but includes also information for teachers and parents.

At the laboratory level, IRED will continue to be in close contact with the Insitute Pasteur in Paris as initiated during the study. Such a north-south partnership can improve the quality of the diagnostic procedure in Chad and in turn provide fertile soil for innovation. The introduction of the PCR method is one of the next objectives. A PCR detection machine is already available at IRED and used for detection of other pathogens, for instance, TB. The head of the rabies laboratory has been trained in this method at Institut Pasteur and a laminar-flow work station has just been inaugurated at the rabies laboratory in N'Djamena. Another test expected to be applied is the ELISA kit for rabies antibody detection in sera. Trained personnel and the equipment needed are also already available.

A two year, GAVI funded study that will start in 2016 has the objective to estimate the burden of rabies and the demand for PEP across the whole country. To do so we combine retrospective data on bite exposure, collected with a household survey, with data on reported bite victims from the health facility level which will be continuously collected. We plan to include two rural and two urban sites to accurately extrapolate our data to the national level. Parallel to bite exposure data, rabies diagnostic testing will be established and animal rabies incidence surveyed in the selected study sites. The same study will also be conducted in Côte d'Ivoire and Mali, so the combined results will contribute valuable data to the description of rabies transmission and prevention in West and Central Africa.

Medium to long term projects

For the coming two years diverse research objectives will be addressed. On the disease modelling side the estimation of the threshold population size for persistence of dog rabies transmission in an African city will be further refined. The dynamics of protection from rabies and its subsequent elimination is determined by mechanisms at population and individual levels. The contribution of population vaccination coverage and the vaccine immunity of individual dogs will be comparatively assessed in regard to their role in disease transmission. Further a metapopulation model will extend the current model in Chapter 4 into 4-5 interconnected sub-models. When dog populations are unevenly distributed, as observed in N'Djamena, this heterogeneity should be considered in the model methodology. Our unique empirical data set collected during the past four years will be used to test a compartment model approach for the description of epidemiological dynamics. Thirdly, dog to dog and dog to human contact network data will be collected by observational studies for the establishment of the contact network structure of dog populations. A contact network model will extrapolate individual dog contact networks to a citywide contact network as a backbone for the transmission of rabies. The three different models will be tested for their capacity to optimize the interval between campaigns, to simulate different levels of vaccination coverage and to estimate the relative contribution of population dynamics and individual protective antibody kinetics to sustain an immune population. This research will generate new knowledge on optimized dog rabies vaccination schemes in terms of coverage and campaign interval.

To date, the rabies control program is limited to the capital city. We don't have any data on the incidence in the rest of the country due to the lack of diagnostic structures in the provinces and the absence of a transport network to send samples to the rabies laboratory in N'Djamena. We plan to initiate a pilot extension of rabies surveillance to five selected provinces of Chad. In these provinces the regional livestock delegations of the national surveillance network for animal diseases (REPIMAT) (Ouagal et al., 2012) will be equipped with the DRIT. In addition, the Anigen Rapid Rabies Test® will be distributed in selected peripheral veterinary posts.

Our long term vision is to establish an effective "one health" animal and human rabies surveillance system capable of detecting at least 10% of all rabies cases in Chad. It will be based on direct contact between veterinary and public health workers to obtain reliable data on rabies incidence for Chad and to trace the epidemiology of rabies in the country to guide a national elimination program.

The network is depicted in Figure VIII.4. Briefly, all 23 administrative regions of Chad will have a peripheral laboratory for preliminary, fast rabies diagnostics. They will be in close contact with health centres in the same regions, so that all registered bite cases are followed by an investigation of the aggressive animal. On the other hand, each rabies suspicious animal brought to a veterinary post should trigger a search for possible bite victims. This first step of peripheral rabies surveillance has to be strongly connected with a central reporting and communication entity for rabies suspicions on the one hand and with a reference laboratory on the other hand to confirm preliminary cases by the OIE standard diagnostic procedure.

Our project to reinforce rabies surveillance will be built on these infrastructures in place and envisages integrating them together with the human health structures into a mobile phone based joint animal and human rabies surveillance network. This network will be animated by a centre established in N'Djamena and coordinated by our partner NGO, the CSSI. This structure will centralise all information on rabies suspected animal bite cases, human rabies cases and suspected rabid animals. It will also offer a communication service for the members of the network and the public.

A functional and effective national surveillance network is a prerequisite for the envisioned elimination of rabies from Chad through a national mass dog vaccination campaign.

Towards a national Chadian dog rabies elimination programme

Based on the first rural assessment of the dog-human ratio and the analysis of the cost structure of dog rabies mass vaccination in Chad an estimation of the cost of a national dog rabies elimination programme has been elaborated (Anyiam et al., 2016; Chapter 6, this thesis). This forms the basis for the preparation of a business case for dog rabies elimination using the new concept of Development Impact Bonds (DIB) which are inspired from social impact bonds[27]. A DIB involves a private-public partnership in which a private investor provides the necessary funds upfront and is refunded by an institutional donor (i.e. a development agency) on a results basis. The DIB concept is promising; however, currently institutional donors remain more reluctant to engage than private donors. Moreover, the Chadian government's priorities are currently oriented more towards the control of highly contagious livestock diseases like Foot and Mouth Disease. There might be potential for an integrative approach to the control of viral disease, combining several diseases at once.

[27] J. Hughes, and Scherer, J., , "Foundations for Social Impact Bonds," (Social Finance, Boston, MA, USA, 2014).

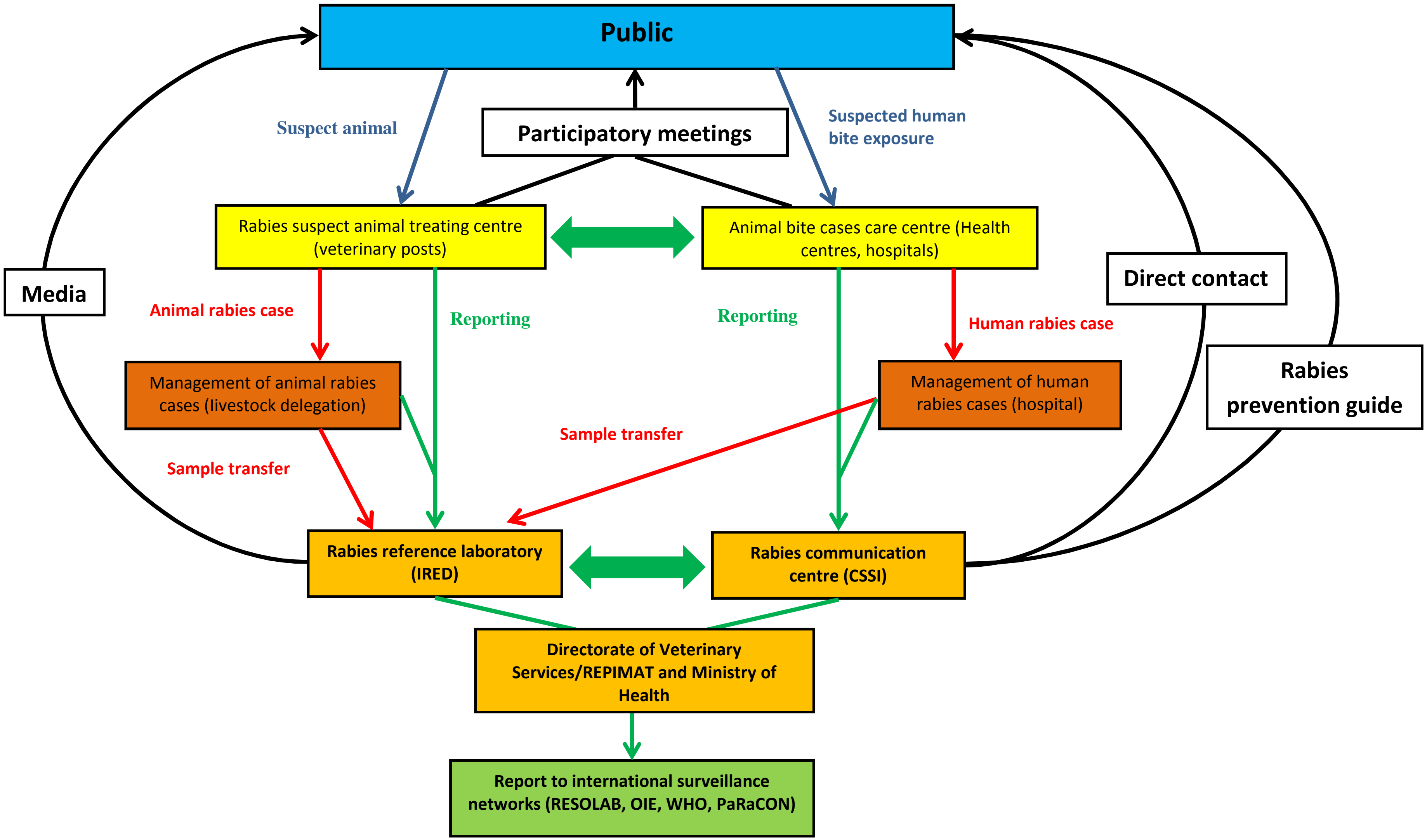

Figure VIII.4: Outline of the information pathways and services linked to rabies in a One Health surveillance network for Chad. Green line: information sharing, reporting system. Blue line: First contact in case of rabies suspicion or possible contact. Red line: Rabies cases, sample transfer. Black line: public sensitisation pathways. Yellow box: peripheral level. Orange box: Central level (N'Djamena). Broun box: rabies cases management level.

IX. CONCLUSION

Considering all the current evidence at hand, canine rabies has a great potential to be eliminated as with rinderpest and smallpox. Our project is part of the worldwide effort to control this disease and created further evidence of the feasibility of rabies control through immunisation of dogs in Africa.

The obstacles encountered emphasize the need for transdisciplinary thinking to achieve elimination.

To combat this virus, the many aspects contributing to its transmission dynamics and its successful prevention have to be pinpointed with the help of diverse research approaches: (1) Rabies detection has to be facilitated by technical innovation on the laboratory level; (2) The detailed epidemiology under elimination pressure has to be understood with the help of mathematical and phylogenetic disease modelling; (3) The accessibility of dogs and the underlying human social determinants have to be investigated by social science disciplines; (4) evidence of the benefit of elimination to facilitate advocacy has to be created through health economic analysis.

All this research however is futile if the findings are not applied in real life within a true, active public participatory framework.

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XI. PLATES

Plate 1: Children bringing a dog to be vaccinated against rabies.

Plate 2: Local leaders with rabies leaflet

Plate 3: The banner put up at each vaccination post.

Plate 4: Dogs being registered after vaccination.

Plate 5: Counting dogs on a transect drive

Plate 6: The vaccination team in 2012

Plate 7:Enos Madaye and myself preparing to perform rabies diagnostic tests at IRED

A B

Plate 8 : Differences in the ability of dog handling ; A very difficult dog to handle brought to the vaccination post in a mosquito net; B very tame dog

A B

Plate 9: A Dog owners waiting in line for vaccination in densely populated area; B mobile teams in the outskirts of N'Djamena

Plate 10: Information caravan in the streets of N'Djamena on world rabies day