A contribution to dog ecology and dog-related zoonoses in Bamako, Mali

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Für meine geliebte Familie

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Summary

Zoonoses are diseases, which are transmitted between vertebrate animals and humans. Neglected zoonoses are often endemic, under-reported, misdiagnosed and affect mostly poor and marginalised populations, which are living in close proximity to their animals. They cause not only enormous human suffering and death but contribute as well to significant economic losses, as they often affect livestock. They do not rank as high priorities of governments and international public health communities, although cures often exist and control in the animal reservoirs is highly cost effective. To date, very little is known about neglected canine zoonoses in West Africa, particularly in Mali. Our work assessed baseline dog population dynamics and the epidemiology of **rabies**, **echinococcosis** and **leishmaniasis** in Bamako, Mali**.** These three diseases were defined by local stakeholders as health priorities within the group of neglected zoonotic diseases (NZD). As a second step the learned knowledge was used to test small scale interventions with two pilot rabies vaccination campaigns offered free of cost for dog owners, which were conducted in Bamako. This work was conducted within the frame of ICONZ (Integrated Control of Neglected Zoonoses in Africa), an EU FP7-funded project with the overall objective to improve human and animal health and alleviate poverty.

We conducted a randomised cross-sectional Knowledge, Attitudes and Practices (KAP) study throughout Bamako. The questionnaires included questions about the compounds, households and animals. Additionally, a blood, faecal and fur sample was collected from every dog older than three months of age. Laboratory analysis included microscopic, immunodiagnostic and molecular methods. Dog-households were followed every six months through visits or phone calls for two years for the successive collection of dog demography data. Following the baseline assessment study, two central point (CP) pilot vaccination campaigns in three different communes of Bamako were conducted.

Rabies is the most important dog-related zoonosis. The investigation of the seroprevalence of post vaccination rabies antibodies showed that although the majority of interviewees were aware of rabies, knew that dogs can transmit the disease and recognised that vaccination of dogs can prevent viral transmission, dogs were inadequately protected against the disease. A main reported reason for non-vaccination of dogs seemed to be the cost of the rabies vaccination.

A very important result is the extremely low dog human ratio of 1:121, which is in clear contrast to other dog ecology studies across Africa and results in an estimated total dog population of 15 000 (95% CI 13 000 – 17 000) dogs in the District of Bamako. A high proportion of young dogs were found as a result of a high turnover rate in the population. The proportion of ownerless dogs is estimated to be below 10%. However, achieved vaccination coverage during both pilot CP vaccination campaigns was far below the World Health Organization (WHO) recommended coverage of 70%. A mixed methods investigation showed that lack of knowledge about the campaign was one of the main reasons for the low attendance.

No cases of leishmaniasis were found, but we report the first known case of canine intestinal *Echinococcus granulosus* (genotype G6) infection in Mali. Several other parasite species were found in the faeces and fur and indicate ongoing transmission of gastrointestinal parasitic infections.

This work contributes vital information towards planning effective and sustainable canine rabies control programs for the district of Bamako. We strongly recommend responsible dog ownership, with frequent rabies vaccination and deworming. Rabies control efforts should be prioritised by Malian ministries, and research findings should be shared with stakeholders in a transdisciplinary way. Rabies vaccination campaigns have to be adapted to the local context and different strategies, like a possible combination of a CP vaccination campaign with a door-to-door vaccination approach, should be evaluated. Dissemination of information on the campaign must be improved using new technologies like announcement beforehand through television and short text messages (SMS). Due to the high turnover rate, we recommend repeated mass-vaccination campaigns of at least 70% dogs in yearly intervals.

Further research must focus on human disease burden, other possible reservoir hosts of *Leishmania sp.,* and the intermediate hosts in the life cycle of *E. granulosus* (genotype G6). The generated knowledge is crucial for planning disease control programmes, mainly for rabies.

Résumé

Les zoonoses sont des maladies qui se transmettent entre les animaux vertébrés et les hommes. Elles sont souvent endémiques, sous-déclarées, mal diagnostiquées et affectent majoritairement les populations pauvres et marginalisées vivant à proximité des animaux. Elles sont responsables non seulement de grandes souffrances et de morts chez les humains, mais contribuent aussi à d'importantes pertes économiques lorsqu'elles touchent le bétail. Malgré l'existence de traitements et de mesures de contrôle rentables visant le réservoir animal, la lutte contre les zoonoses n'est malheureusement pas une priorité pour les gouvernements et la communauté de santé publique internationale. À ce jour, on connaît très peu de choses sur les zoonoses canines négligées en Afrique de l'Ouest et en particulier au Mali. Dans un premier temps, nous avons évalué la dynamique des populations canines et l'épidémiologie de la **rage**, de l'**échinococcose** et de la **leishmaniose** à Bamako (Mali). Ces trois maladies ont été identifiées en tant que zoonose négligée prioritaire par les acteurs locaux. Dans un deuxième temps, les connaissances acquises ont été utilisées pour tester des interventions à petite échelle avec deux campagnes pilotes de vaccination canine antirabique gratuites, à Bamako. Notre travail a été mené dans le cadre du projet européen de lutte intégrée contre les zoonoses négligées en Afrique (ICONZ) qui est financé par le septième programme-cadre de recherché (FP7) et a pour objectif global d'améliorer la santé humaine et animale et de réduire la pauvreté.

Nous avons effectué une étude randomisée transversale CAP (Connaissances, Attitudes, Pratiques) à Bamako. Pour ce faire, nous avons mené des questionnaires ayant trait aux concessions, aux ménages et aux animaux et avons recueilli des échantillons de sang, de matière fécale et de pelage sur chaque chien âgé de moins de 3 mois. Les analyses de laboratoire ont inclus des méthodes microscopiques, immunodiagnostiques et moléculaires. Les ménages en possession d'un chien ont été suivis à 6 mois d'intervalle sur une période de deux ans par des visites ou des appels téléphoniques pour la collecte successive de données liées à la démographie des chiens. Suite à l'étude d'évaluation de base, nous avons mené deux campagnes de vaccination pilote « point central » dans trois communes de Bamako.

La rage est la zoonose canine la plus importante. Bien que la majorité des personnes interrogées connaisse la maladie, sache que les chiens sont des vecteurs et que la vaccination des animaux puisse prévenir la transmission du virus, notre enquête sur la séroprévalence des anticorps antirabiques post-vaccination montre que les chiens étaient insuffisamment protégés contre la maladie. La principale raison reportée pour la non-vaccination des chiens semble être due au prix de la vaccination.

Un résultat très important de notre étude est le très faible rapport observé chien/homme de 1/121. Ce ratio contraste avec d'autres études sur l'écologie des chiens en Afrique et nous permet d'estimer une population canine totale de 15 000 dans le district de Bamako (95% IC : 13 000 – 17 000). Une forte proportion de jeunes chiens a été observée en raison d'une dynamique élevée au sein de la population. La proportion de chiens sans propriétaire a été estimée comme étant inférieure à 10%. Toutefois, le taux de couverture de vaccination pendant les campagnes pilote « point central » a été bien en dessous de la couverture de 70% recommandée de l'Organisation Mondiale de la Santé (OMS). Une investigation de méthodes mixtes a montré qu'un manque de connaissances sur la campagne a été l'une des principales raisons de la faible participation.

Aucun cas de leishmaniose n'a été trouvé. Cependant, nous avons rapporté le premier cas connu d'*Echinococcus* granulosus (génotype G6) intestinale chez le chien au Mali. Plusieurs autres espèces parasitaires ont été identifiées dans les fèces et le pelage des chiens, indiquant une transmission active d'infections parasitaires gastro-intestinales.

Notre travail apporte des informations vitales pour une planification efficace et durable des programmes de contrôle de la rage chez le chien dans le district de Bamako. Nous recommandons fortement aux propriétaires de chiens une attitude responsable comprenant des vaccinations antirabiques et des déparasitages fréquents. Le contrôle de la rage doit être une priorité pour les différents ministères maliens et les résultats des recherches devraient être partagés entre les intervenants afin de favoriser une approche transdisciplinaire. Les campagnes de vaccination antirabique doivent s'adapter aux contextes locaux. Différentes stratégies, comme une combinaison possible d'une campagne de vaccination « point central » avec une approche de vaccination porte-à-porte, devraient être évaluées. La dissémination des informations liées aux campagnes pourrait être améliorée en utilisant des technologies telles que des annonces publicitaires à la télévision et l'utilisation de service de messages courts (SMS). En raison d'une dynamique élevée, nous recommandons de répéter chaque année les campagnes de vaccination canines de masse avec une couverture minimale de 70%.

Des recherches supplémentaires sont nécessaires afin d'étudier le fardeau de la maladie chez l'homme, d'autres hôtes réservoirs possibles de l'espèce *Leishmania* et les hôtes intermédiaires du cycle de vie de *E.* granulosus (génotype G6). Les connaissances générées par le présent travail sont cruciales pour la planification des programmes de lutte contre les zoonoses, en particulier pour la rage.

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AfroREB	The African Expert Bureau
AHCs	Animal Health Clubs
AREB	Asian Rabies Expert Bureau
BSU	Basic Sampling Unit
CE	Cystic Echinococcosis
CL	Cutaneous Leishmaniasis
CNAM	Centre National d'Appui à la lutte contre la Maladie
CNS	Central Nervous System
CP	Central Point
CVL	Central Veterinary Laboratory (Laboratoire Central Vétérinaire)
DALYs	Disability Adjusted Life Years
DNA	Deoxyribonucleic Acid
DNS	National Directorate of Health (Direction Nationale de la Santé)
DPLM	Division of epidemiology, prevention and control of diseases (Division d'épidémiologie de prevention et de lutte contre la maladie)
DRAMR	The regional directorate of rural support (Direction Régionale de l'Appui au Monde Rural)
dRIT	direct Rapid Immunohistochemical Test
ELISA	Enyme-linked Immunosorbent Assay
EU	European Union
FAT	Fluorescent Antibody Test
FAVN	Fluorescent Antibody Virus Neutralization
FP7	Seventh Framework Programme
GARC	Global Alliance for Rabies Control
GPS	Global Positioning System
ICONZ	Integrated Control of Neglected Zoonoses
ICPCs	International Cooperation Partner Countries
KAP	Knowledge Attitudes Practices
LR	Lazaret clinic (La clinique Lazaret des Roches)
LST	Leishmanin Skin Test
MCL	Mucocutaneous Leishmaniasis
MDGs	Millennium Development Goals
MEEREB	Middle East and Eastern Europe Rabies Expert Bureau
NARMP	North American Rabies Management Plan

NTDs	Neglected Tropical Diseases
NZDs	Neglected Zoonotic Diseases
PAIR	Percutaneous puncture
PEP	Postexposure Prophylaxis
PPS	Probability Proportional to Size
REDIPRA	Reunión de los Directores de los Programas Nacionales de Control de Rabia; Rabies control in Latin American countries
SEARG	Southern and Eastern African Rabies Group
SDGs	Sustainable Development Goals
SMS	Short Message Service
Swiss TPH	Swiss Tropical and Public Health Institute
US	Ultrasound
VL	Visceral Leishmaniasis
WHO	World Health Organization
WSPA	World Society for the Protection of Animals

1. Introduction

1.1. Neglected tropical diseases (NTDs)

Neglected tropical diseases (NTDs) are a diverse group of infections caused by viruses, bacteria and helminths. WHO prioritized 18 NTDs, namely Chagas disease, human Af§rican trypanosomiasis, leishmaniases, Buruli ulcer, leprosy, trachoma, yaws, cysticercosis/taeniasis, dracunculiasis, echinococcosis, foodborne trematodiasis, lymphatic filariasis, onchocerciasis, schistosomiasis, soil-transmitted helminthiases, rabies, dengue and chikungunya. These diseases affect more than a billion people in 149 countries worldwide. The NTDs were not specifically mentioned in the United Nations Millennium Development Goals (MDGs) and were thus neglected relative to the 'big three' diseases, namely malaria, tuberculosis and HIV/AIDS, which got the lion's share of global funding (WHO, 2015). The major NTDs, defined by WHO, account for a disease burden of at least 26 million disability adjusted life years (DALYs), which is approximately half the burden of tuberculosis. If other NTDs (cryptosporidiosis, cholera, animal contact (venomous), amebiasis, fungal skin diseases, scabies, trichomoniasis, leprosy) are included, then the burden is more than half of the global burden of malaria and HIV/AIDS (Hotez et al., 2014). Nonetheless, they attract little attention from public health officials, donors and policy makers (Molyneux et al., 2005). They affect mainly the poorest populations and cause enormous human suffering and death. They are additionally a disruptive factor for poverty reduction and overall socioeconomic development. Some of them have zoonotic potential and are classed in the group of neglected zoonotic diseases (NZDs, see chapter 1.1.1.). NTDs are listed in the Sustainable Development Goals (SDGs), which follow the MDGs after 2015. One goal of the SDGs is to achieve health and wellbeing for all people of all ages. Prevention, control, elimination and eradication of NTDs can be achieved through disease management, preventive chemotherapy, management of vector ecology, veterinary public-health services, the provision of safe water, sanitation and hygiene (WHO, 2015).

1.1.1. Neglected zoonotic diseases (NZDs)

Zoonoses are diseases which are transmitted between humans and animals. Almost two-third of all human pathogens are zoonotic and more than 200 different zoonoses are known. Neglected zoonoses, in contrast to newly emerging zoonoses, are often ancient and endemic diseases which affect humans and animals. They affect mostly poor and marginalized populations, which are living in close proximity to animals. NZDs are often under-reported or misdiagnosed, due to low capacity to recognize the agents. They do not rank highly as priorities of governments and or the international public health communities. They generate a significant health burden and economic loss in humans and animals and mostly do not spread rapidly on a global scale. Cures often exist and the control in the animal reservoir is mostly highly cost effective. This fact allows for a double benefit through improved human and animal health. Reflecting the low global assistance for NTDs, NZDs are also known as the 'poor cousins of the poor cousins'. Figure 1 shows the characteristics of NZDs (Fig.1). Good-quality research, involving human, veterinary and environmental health, is required for the sustainable control of NZDs. Their control is part of the Global Plan to fight NTDs (Maudlin et al., 2009; Schelling and Hattendorf, 2015; Welburn et al., 2015; WHO, 2006; WHO, 2015).

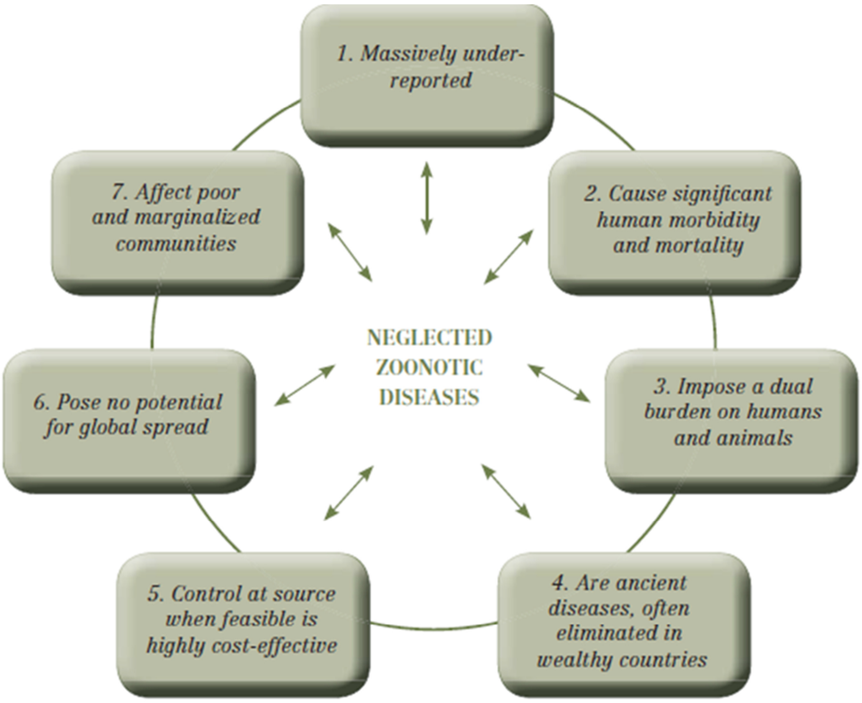

Figure 1: Characteristics of NZDs (WHO, 2010)

African countries are often affected by cutaneous leishmaniasis, zoonotic trypanosomiasis, echinococcosis, porcine cysticercosis and dog rabies. More than 30% of the countries are affected by three or more of the previous mentioned diseases (WHO, 2010). Figure 2 shows a distribution of cutaneous leishmaniasis, zoonotic trypanosomiasis, echinococcosis, cysticercosis (*Taenia solium*) and rabies in Africa (WHO, 2008).

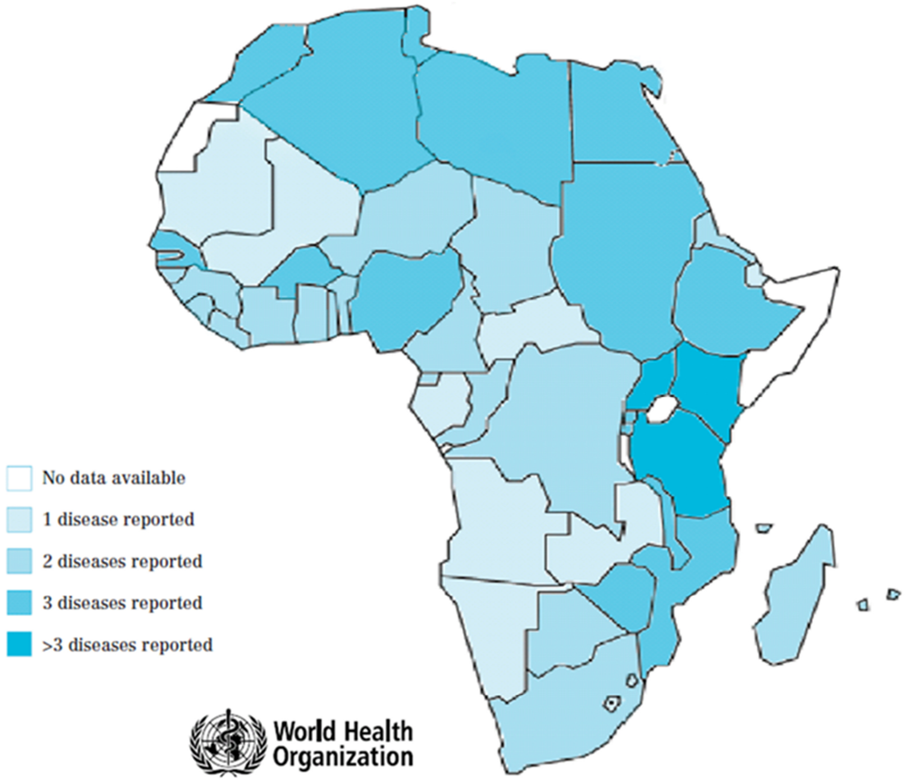

Figure 2: Distribution of selected zoonotic diseases (cutaneous leishmaniasis, zoonotic trypanosomiasis, echinococcosis, cysticercosis (*Taenia solium*), rabies) (WHO, 2008)

I present subsequently only three of many zoonotic disease, which can be transmitted from dogs to humans (WHO, 1988b), on which I did detailed studies for my PhD thesis. The focus is on West Africa and the Maghreb.

Rabies

Rabies is one of the most known and feared zoonoses (WHO, 2006). Hampson et al. (2015) estimated over 3.7 million DALYs lost worldwide each year. It is estimated that 59 000 people die per year due to the disease with the majority of cases in Asia (60%) and Africa (36%) (Hampson et al., 2015). Ninety-nine percent of all human cases occur in the developing world, with children being the most affected group (WHO, 2006). Most of the African countries are high risk areas for contracting rabies (WHO, 2014) (Fig. 3).

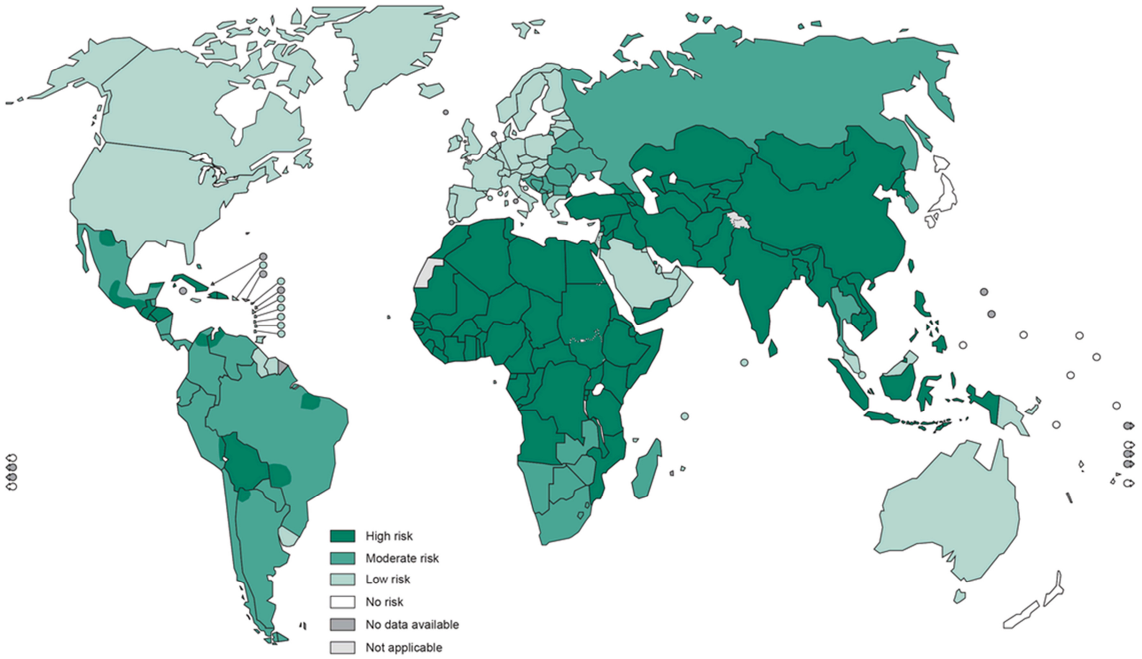

Figure 3: Distribution of risk levels for humans contracting rabies, 2013 (WHO, 2014)

The disease is mainly caused through the canine related classical rabies virus (Family *Rhabdoviridae*, Genus *Lyssavirus*), but also through other rabies related viruses of the same genus circulating in bat species (Lagos bat virus, Mokola virus, Duvenhage virus, European bat lyssavirus-1, European bat lyssavirus-2, Australian bat lyssavirus) (Banyard and Fooks, 2011). In western and central Africa, rabies virus belongs to the lineage 'Africa 2' and in Mali, Group G and H have been identified to date (Talbi et al., 2009).

The majority of cases occur in resource poor countries where the virus is mainly transmitted through dog bites. But transmission is also possible through infected wild animals. After a bite, or contact of a skin lesion or mucous membrane with the virus, the virus migrates via nerve cells to the brain where it causes meningitis with dramatic symptoms and a fatal outcome. Incubation periods can last from a few days to several months depending on the site of virus inoculation. Symptoms in humans and animals come as a result of dysfunction of the brain, and a furious rabies form (madness) and a dumb rabies form (paralytic) are distinguished. Highly effective dog vaccines and post-exposure prophylaxis (PEP) for humans make the disease 100% preventable. PEP consists of local wound care, vaccination and immunoglobulin application. But PEP is often unavailable or too expensive for affected persons in developing countries. Research priorities are the development of thermostable vaccines, easy to use diagnostic tests and methods to provide sufficient immunoglobulin in view of the global shortage (Banyard and Fooks, 2011; Hampson et al., 2015; WHO, 2006; WHO, 2010; WHO, 2015).

Control of the disease still gets a low level of political commitment. Reasons for this are disease under-reporting due to the lack of laboratory confirmation of cases and the fact that many victims are not visiting medical facilities in developing countries (WHO, 2006). Cleaveland et al. (2002) estimated a 10-100 fold higher incidence of human rabies cases, extrapolated from animal bite occurrence, compared to the officially reported human incidence estimated through passive surveillance data. Ninety-nine percent of human rabies cases are likely to be not reported in Tanzania (WHO, 2010). Another problem is misdiagnosis of the disease. In Malawi for example, children with rabies were falsely diagnosed with cerebral malaria (Mallewa et al., 2007). The 'gold standard' for the detection of rabies virus antigen in the brain is the fluorescent antibody test (FAT). The direct rapid immunohistochemical test (dRIT) is a test which can be used under field conditions without the need for an expensive fluorescence microscope (Dürr et al., 2008b; OIE, 2012) .

Fortunately, through some efforts by the NTD community, rabies has gained more global attention in recent years. Some of the global rabies stakeholders are:

GARC (Global Alliance for Rabies Control)

rabiesblueprint

World Rabies Day

Different regional networks

AfroREB (The African Expert Bureau)

AREB (Asian Rabies Expert Bureau)

NARMP (North American Rabies Management Plan)

MEEREB (Middle East and Eastern Europe Rabies Expert Bureau)

REDIPRA (Reunión de los Directores de los Programas Nacionales de Control de Rabia; Rabies control in Latin American countries)

SEARG (Southern and Eastern African Rabies Group)

Malian law determines that dogs must be vaccinated against rabies and that both cats and dogs require an international vaccination certificate prior to export. Further, it is defined that when a rabies case is detected in a certain area, the sequestration of all dogs and cats in the specified area must be maintained for a period of at least two months. During the quarantine, dog owners are not allowed to have their dogs outside their residence and all free-roaming dogs are killed in the defined area. It is also stated that all rabid animals and unvaccinated animals which have been in contact with the infected animal are killed. An exception applies for dogs which have been vaccinated against rabies within the previous year, but they must be revaccinated within seven days following a bite contact with the infected animal. These animals are further kept under quarantine. It is additionally regulated that consumption of meat from infected animals is forbidden. (Law No. 01-022 / of 31 May 2001; Regulating the repression of breaches of the animal health policy in the territory of the Republic of Mali. (Loi N°01-022/du 31 Mai 2001; Regissant la repression des infractions à la police sanitaire des animaux sur le territoire de la republique du Mali.)).

The procedure after an animal bite in Bamako, Mali, is as follows: The bitten person should immediately contact the rabies center of the Division of epidemiology, prevention and control of diseases (DPLM), which is part of the National Directorate of Health (DNS (Direction Nationale de la Santé)) and is responsible for disease surveillance and human PEP (Dao et al., 2006; Maoulid, 2005). Kone (2010) showed that immunoglobulins were not available in urban settings of Mali. Immunoglobulins are, in addition to use for post exposure vaccination, an important preventive measure after a rabies exposure. Suspect human rabies cases are referred to a specialized clinic (Lazaret clinic (LR (La clinique Lazaret des Roches))). In parallel, dog owners are invited by the DPLM to bring dogs which have bitten people to a veterinary clinic for a 15-day observation period. Viral shedding occurs only a few days before death, so an animal that is still healthy after such a period of observation is not rabid. Veterinary clinics are also in charge of preventive dog rabies vaccination. Rabies diagnosis of suspect animals is undertaken by the Central Veterinary Laboratory (CVL). Rabies diagnosis in humans is based soley on a clinical examination due to sociocultural reasons (Dao et al., 2006; Maoulid, 2005). Detection of virus by PCR in a skin biopsy from the neck would be another possibility for disease diagnosis (Dacheux et al., 2008). The disease surveillance is insufficient throughout the entire country, and animal rabies diagnosis can only be conducted at the Central Veterinary Laboratory (CVL) in Bamako on central nervous system (CNS) tissue by means of the FAT. On the basis of positive dog rabies cases detected at the CVL between 2000 and 2011, the relative risk for the whole district in Bamako was estimated (Fig. 4). An incidence of 0.37 human cases per 100 000 inhabitants between 2007 and 2012 was calculated for Bamako (Kone, 2013). Rabid dog incidence was estimated to be 2.2 dogs per 1000 (Traoré, personal communication, 2014). Parenteral vaccination of dogs against rabies is a cost-effective control measure (Zinsstag et al., 2009), and in many African communities at least 60-70% of dogs are accessible for vaccination (WHO, 2005). This percentage corresponds to the recommendation to vaccinate at least 70% of the dog population for efficient rabies control (WHO, 2013). In Mali, rabies vaccination of a dog costs 4 €, in contrast to 83 € for the treatment of an exposed person (Traoré, personal communication, 2014). Seroconversion after vaccination can be evaluated by means of the FAVN (Fluorescent Antibody Virus Neutralizing) test (Cliquet et al., 1998), that is based on the detection of virus neutralization in cell culture, which is the ‘gold standard’ for rabies virus serological assessment but requires infectious virus and specialised laboratory facilities. Another possibility for the detection of rabies antibodies in domestic carnivores is an ELISA (Enyme-linked Immunosorbent Assay) test (Banyard and Fooks, 2011; Wasniewski and Cliquet, 2012).

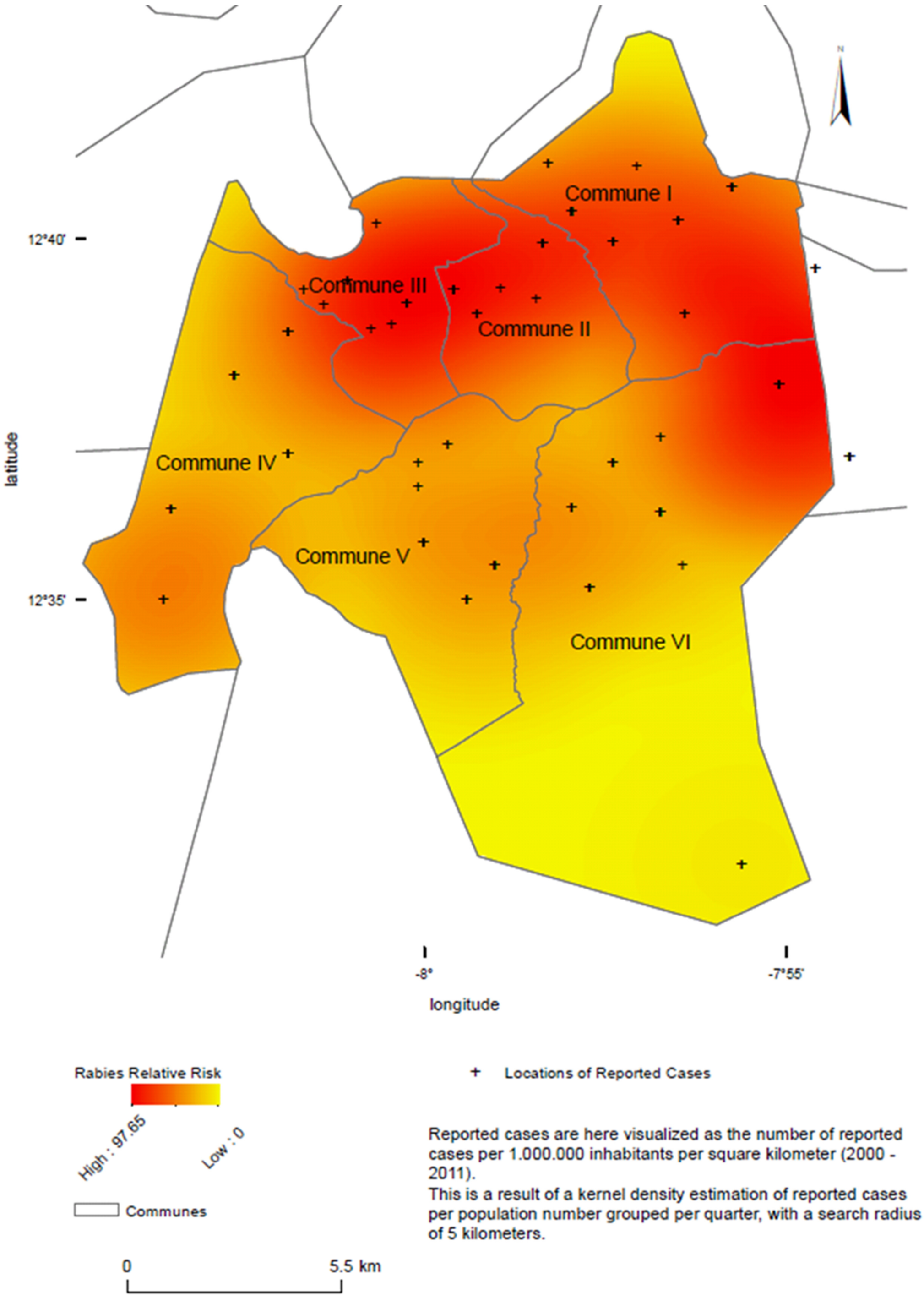

Figure 4: Confirmed dog rabies cases in Bamako between 2000 and 2011 (Data source: CVL; Map production: Ward Bryssinckx, Avia GIS, Belgium)

Cystic Echinococcosis (CE) or hydatid disease

The global burden of cystic and alveolar echinococcosis is estimated at 1.5 million DALYs annually (WHO, 2010). Cystic echinococcosis has high prevalence in North African countries and the Middle East (WHO, 2006). Figure 5 shows the global distribution of zoonotic strains of *Echinococcus granulosus* (Budke et al., 2006). As for all NZD, hydatid disease is often misdiagnosed, in this case as cirrhosis, liver cancer or amoebiasis (WHO, 2010). Cystic echinococcosis (CE) is caused by larval stages of *E. granulosus sensu lato* and is one of the most widespread and important helminth zoonoses worldwide. The lifecycle of the parasite includes carnivores, mainly dogs, as definitive hosts, which harbour adult egg producing stages in their intestines, and intermediate hosts, in which larval stages develop cyst-like structures in different organs (mainly liver and lung) following the ingestion of parasite eggs. Sheep, cattle, horses, camels, pigs and other animals act as intermediate hosts and *E. granulosus* strains are often named after the main intermediate host (sheep strain, horse strain, cattle strain, camel strain, pig strain, cervid strain, and lion strain). Humans are considered to be aberrant intermediate hosts, contracting the infection by ingestion of tapeworm egg contaminated food or water or through handling or petting infected dogs. Incubation period can last many years until symptoms appear, depending on the affected organs. The sheep strain is the most widespread globally. Dogs often become infected by feeding on offal from infected livestock which contains viable cysts (Thompson and McManus, 2001; Torgerson et al., 2011; WHO, 2006). Diagnosis in the final host is done by necropsy, arecoline purgation, coproantigen ELISA or DNA (deoxyribonucleic acid) based tests (e.g. PCR). Diagnosis in the intermediate host is undertaken post mortem. Diagnosis in humans is based on serology and ultrasound examination (US) or other imaging procedures, depending on the location of the cyst. But to date, a 'gold standard' in human disease diagnosis is lacking (Barnes et al., 2012; Torgerson et al., 2011). Treatment in the intermediate and final host is based on chemotherapy, with benzimidazole compounds and praziquantel, respectively. A promising vaccine for the intermediate host, particularly sheep, is currently under development. For humans the disease is life-threatening if untreated and the cure is often surgical, which remains risky and expensive. Non-surgical management, such as percutaneous puncture (PAIR), for inoperable cases is applied. Chemotherapy with benzimidazole compounds is used together with surgery or PAIR. The control and prevention in dogs can be achieved through deworming with praziquantel and prevention from eating, in particular, offal. Effective meat inspection is recommended and contaminated offal should be safely destroyed during slaughtering. 'One Health' education should be considered as an important control focus. Figure 6 shows the transmission cycle of the disease and possible intervention points. In addition to human suffering, the disease causes considerable losses in agricultural productivity (Torgerson et al., 2011; WHO, 2010; WHO, 2015).

Little is known about the disease in West Africa and Mali. Yéna et al. (2002) showed in a retrospective study from 1960 – 2000 in Bamako, 11 cases of pulmonary echinococcosis. Mean age of the patients was 20 years, and women were more affected than men. An abattoir study in dromedaries in northern Mali in 1987 detected *E. granulosus* in one dromedary (Tembely et al., 1992).

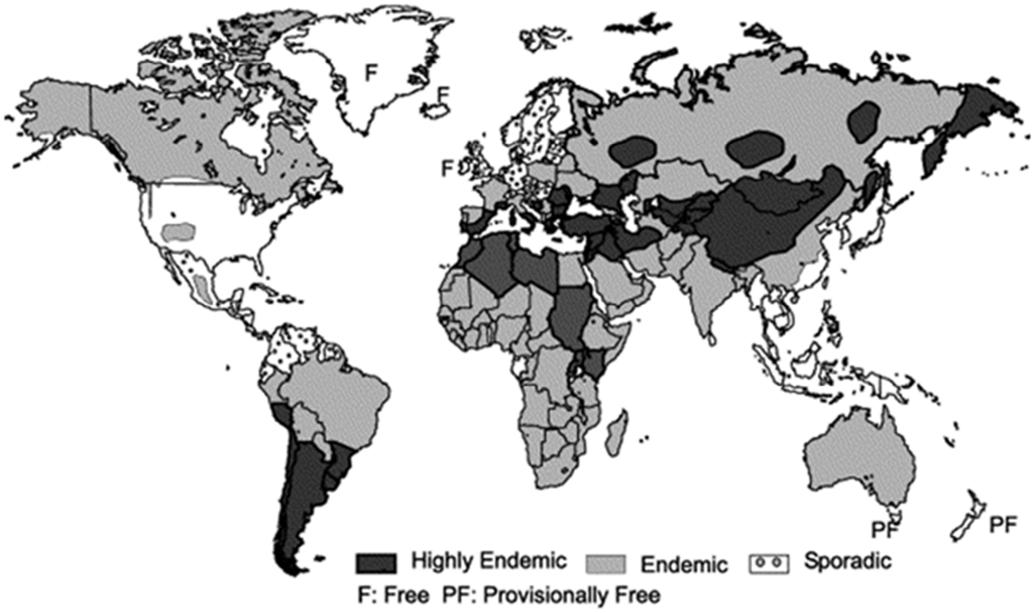

Figure 5: Global distribution of zoonotic strains of *E. granulosus* (Budke et al. 2006)

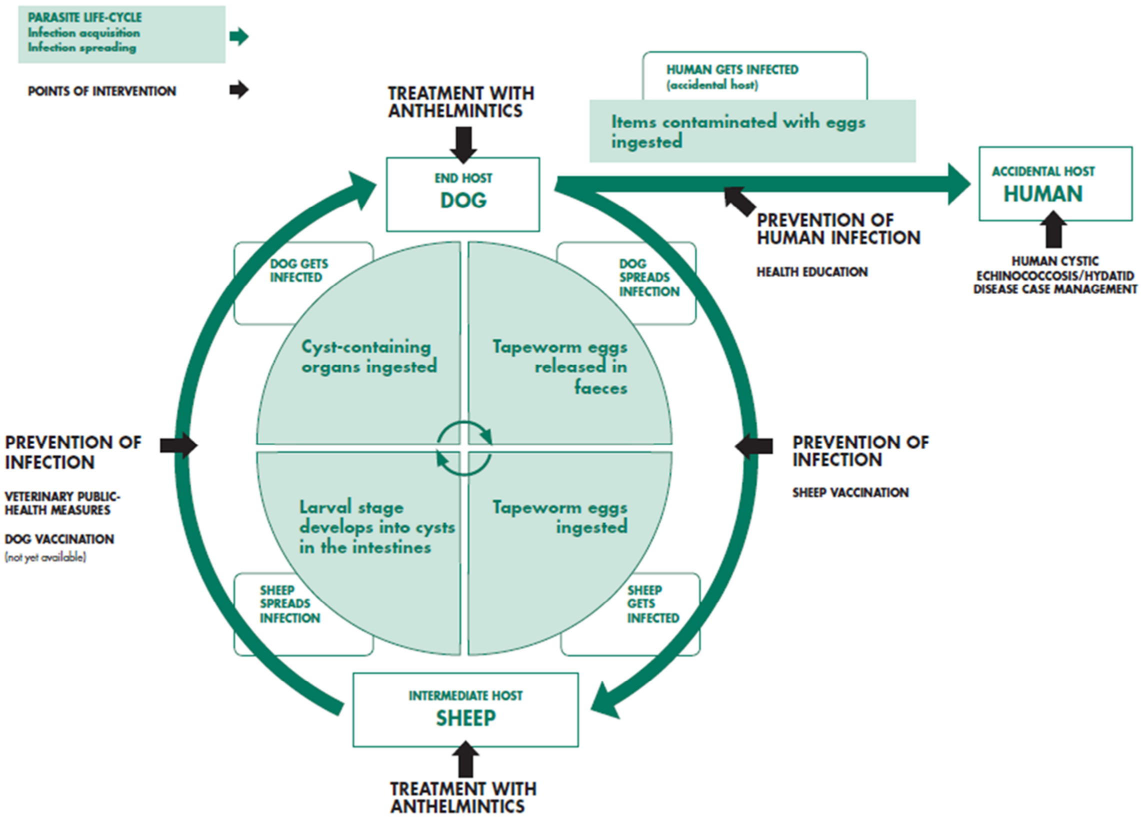

Figure 6: Echinococcus transmission cycle and possible intervention points (WHO, 2015)

Leishmaniasis

Leishmaniasis is a parasitosis which can infect several mammals and is caused by a protozoan of the genus *Leishmania spp.* Fifteen infectious *Leishmania* species exist, and 13 of them have a zoonotic nature. The estimated global human burden is approximately 12 million cases (Gramiccia, 2011). The disease belongs to the group of vector-borne NTDs, often affecting rural populations and exhibiting some degree of sensitivity to climate variability (WHO, 2015). Known vectors of the disease are phlebotomine sand flies and the parasite is alternatively hosted by the sand fly and the mammal host. Transmission takes place through blood meals by the female vector. There are three different forms of the disease in humans, a visceral form called visceral leishmaniasis (VL) or kala-azar, a mucocutaenous form called mucocutaneous leishmaniasis (MCL) and a cutenous form called cutaneous leishmaniasis (CL). CL and MCL are more prevalent than VL. VL is caused by *Leishmania donovani* and *L. infantum* and is the most severe form which is fatal if it is untreated. CL and MCL are caused by a number of different species of *Leishmania* (*L. major, L. mexicana, L. peruviana, L. braziliensis, L.tropica*) and are responsible for considerable morbidity. The most common form in

Africa is CL (Fig. 7), with a high burden particularly in Algeria, but VL also occurs (Fig. 8). In general, all anthrophilic *Leishmania* species can cause a mild localized cutaneous leishmaniasis, which is often a self-healing infection. The true CL lesions are ulcerative or papulo-nodular depending on the involved strain. For *L. major,* which is reported from Mali*,* the 'wet'-ulcerative-type is typical. It is a chronic process with spontaneous cure and a resulting scar which causes social stigmatization associated with the disfigurement. Diagnosis is done by means of parasitological, molecular and serological methods. Confirmation of the disease must be through direct parasitological confirmation (Gramiccia, 2011; Keita S et al., 2003; WHO, 2010; WHO, 2015). Human treatment is based on chemotherapy. For CL the treatment is often topical and physical treatments, like heat-, cryo-, laser- and radiotherapy are also applied (Gramiccia, 2011). Treatment is expensive and toxic. Disease relapses and therapy resistance are not uncommon (Croft et al., 2006). Canine leishmaniasis is based on chemotherapy, but elimination of the parasite is often not achieved (Gramiccia, 2011). Disease control should focus on disease surveillance and identification, treatment, vector control through insecticides (spraying, bed-nets) and control in the animal reservoir (WHO, 2015). Control strategies have been hampered in conflict areas, as has been shown for Syria. Through the movements of infected refugees, it is possible for the disease to spread to new and unaffected countries when the vector is present (Hayani et al., 2015). For canine leishmaniasis, insecticide-based preparations in the form of collars or spot-ons are used for disease prevention (Gramiccia, 2011). Vaccines for dogs are available and show high efficacy (Mutiso et al., 2013). A research priority should be the further development of vaccines for the prevention of the disease in humans (WHO, 2015). Imperato and Diakité (1969) showed that CL is present in Mali. The leishmanin skin test (LST), where a positive reaction is an indurated skin area greater than 5 mm at the injection site, was used for case identification. Paz et al. (2013) showed that *L. major* is possibly the only responsible *Leishmania* species for CL in Mali. In Mali, the Centre National d'Appui à La Lutte Contre La Maladie (CNAM) in Bamako is the only national referral clinic for skin diseases and people suffering from the disease are often not able to travel long distances to the capital. An estimate for the burden of the disease in Mali has not been done to date (Paz et al., 2013). Whereas dogs play an important role as a reservoir host, for example in the Mediterranean for *L. infantum*, it remains unclear if they are involved in the life cycle of the disease in Mali. In the literature, only few observations of *L. major* in dogs were found, for example from dogs in Egypt (Morsy et al., 1987). One known reservoir host for *L. major* is rodents, but no data is available for Mali currently (Gramiccia, 2011; Paz et al., 2011).

Figure 7: Global distribution of cutaneous leishmaniasis (Gramiccia, 2011)

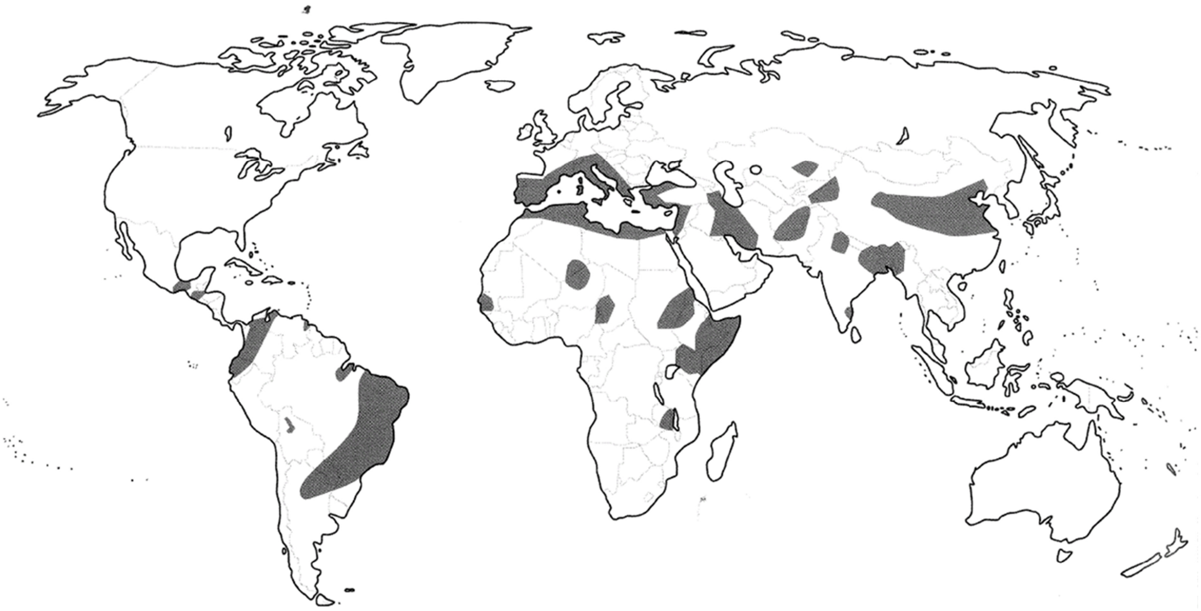

Figure 8: Global distribution of visceral leishmaniasis (Gramiccia, 2011)

1.2. ICONZ (Integrated Control of Neglected Zoonoses in Africa)

This thesis was conducted within the frame of ICONZ (Integrated Control of Neglected Zoonoses in Africa). ICONZ is a large research project funded for a period of six years by the EU under the Seventh Framework Programme (FP7). The overall objective was to improve human and animal health, to alleviate poverty and hence to contribute to the MDGs. Several European and African Partner Institutes, each with own budgets, developed together strategies for an integrated control of eight zoonoses: anthrax, brucellosis, bovine tuberculosis, rabies, cystic echinococcosis, leishmaniasis, *T. solium* cysticersosis/taeniosis and zoonotic trypanosomiasis. These zoonoses are grouped into four clusters (bacterial zoonoses, dog/small ruminant cluster, pig-associated diseases and vector-borne diseases). The study sites were Mali, Morocco, Mozambique, Nigeria, Uganda, the United Republic of Tanzania and Zambia. This work dealt with a multi-disease assessment in the dog population in Mali. The three zoonoses we considered were rabies, cystic echinococcosis and leishmaniasis (Okello et al., 2015; WHO, 2010).

The specific objectives of ICONZ were to map global research on NZDs, to obtain information on disease epidemiology and disease burden, to promote the 'one health' concept (see definition under 1.4.) and intersectoral collaboration, to empower women in decision making relating to the control of zoonoses and to transfer technologies and build capacity in the eight developing international cooperation partner countries (ICPCs) (Welburn, 2010; WHO, 2010).

1.3. The importance of dog demography studies and the role of the dog in West and North Africa

To plan effective dog management and dog-related zoonoses programs, it is crucial to know more about dog populations, notably its size, accessibility, ownership status, density, sex ratio, turnover, growth, function in human societies and ecology (WHO, 1987; WHO, 1988b; WHO, 2005). For example, in rabies control the high proportion of young dogs in the developing world is an indicator for a high turnover rate of dogs by replacement and is often a reason for the collapse of successful dog rabies vaccination campaigns (Hampson et al., 2009).

Frank (1965) published a comprehensive review about the role of the dog in African cultures. In West Africa a big variety in the use and role of dogs is found. In some regions dogs must forage for food and therefore have an important role in cleaning the neighbourhood. They are not mistreated but their existence is characterised by casualness. Dogs are generally used as hunters, guardians of houses, fields and livestock, for breeding purposes and selling and some ethnic groups consume their meat. In some cultural settings, dogs are only given away for free and are not sold. Sometimes they were even valued like human beings and friends and were therefore treated very well. In the review of Frank (1965) in most West- and Central African tribes the dog is very much appreciated. Dogs were spayed or castrated then became fat for consumption or they were roaming less. In some regions, dogs are used to take care of babies (cleaning and for watching over). Dogs can also have significance as a sacrificial animal and mythical creature.

1.4. 'One Health' concept

'One Health' is a scientifically established and validated public health problem-solving concept which results in an added-value as a result of a closer cooperation between human and animal health. The added-value can be defined as health benefits, financial savings or environmental services. The focus was primarily on human and animal health, but contemporary 'One Health' approaches recognise that ecosystems are linked building the animal-human-ecosystem interface (Zinsstag, 2012; Zinsstag et al., 2005). As an example, consider rabies control for most developing countries. In general, the ministry of health is responsible for the prevention of the disease in humans, the ministry of agriculture is in charge of rabies control in animals and the ministry of local government and the ministries of commerce, industry or science and technologies are involved in the rabies vaccine production and imports, dog population management and dog immunisation. Unfortunately the interaction and collaboration between the veterinary sector and the public health departments is often non-existent in these countries, although they are all dealing with the same disease (WHO, 2005). Here a 'One Health' perspective would bring a clear added-value through strengthening the intersectoral cooperation, for example through a better disease surveillance and communication. The approach should always be adapted to the local setting and its human-animal relationship which is governed by the culture and religion (Zinsstag et al., 2015). Another example is a study on zoonoses, where it is much more cost-effective to consider in parallel the medical, veterinary, social and economic aspects of a disease rather than to conduct a separate field study for every aspect (WHO, 2006). A demonstrative example of the 'One Health' approach was the joint human and animal health vaccination campaigns in Chad, where previous research results showed that most animals were vaccinated but no nomadic child was fully immunised. The existing infrastructure of veterinarians was used to simultaneously vaccinate humans and animals (Schelling et al., 2008). Regarding data analysis, a 'One Health' approach implies that data on human and animal health are analysed and interpreted together (Schelling and Hattendorf, 2015).

An approach with similarities to 'One Health' is presented by Ecohealth. Collaborations between the two approaches hold therefore a great potential and could avoid repetition. 'One Health' has its origins in managing diseases which threaten humans and animals. In contrast, Ecohealth has its origin in research aimed at understanding health in ecosystems. Animal health is within its scope, but it is not the focus (Zinsstag, 2012). Climate change, globalisation, urbanisation, deforestation and agricultural intensification globally affect human health and increase the disparity between the rich and the poor. Health problems, often affecting poor and marginalised people, occur from affected and over-exploited ecosystems. The overall aim of Ecohealth is to build healthier communities and environments, given that human health depends on healthy environments. The approach includes systems thinking, which helps in the understanding of health in the context of social-ecological systems. Further included is inter- and transdisiplinary research (section 1.5.), and collaborative stakeholder participation, sustainability, gender and social equity and knowledge to action are a part of it. Different studies from developing regions in the world demonstrated an added value of Ecohealth to health of populations (Charron, 2012; Zinsstag, 2012).

A systemic approach which moves beyond 'One Health' and 'Ecohealth' is health in social-ecological systems (HSES), where interactions and outcomes of human and animal health are incorporated in social-ecological systems. HSES includes social, cultural, economic and political determinants of health. An HSES example is demonstrated by rabies control programmes. Rabies vaccination campaigns, even where the vaccine is highly efficacious, risk failure when they are not adequate and acceptable in different socio-cultural contexts. The vaccine's 'final' effectiveness in a specific setting is determined by different additional parameters, including its availability, accessibility, affordability, adequacy and acceptability (Zinsstag et al., 2011a; Zinsstag et al., 2011b).

1.5. Transdisciplinary Research

Transdisciplinary research tries to manage the mismatch between knowledge produced in academia and the request for specific knowledge to solve a societal problem. It is absolutely crucial that humanities and natural science and other disciplines work closely together and that the users' knowledge and the context of its application is considered. The transdisciplinary form of research involves learning from experience. Knowledge for directly and indirectly affected stakeholders is assessed, which helps in solving practical problems. This knowledge is then transformed to improve existing practices (Hoffmann-Riem et al., 2008).

Transdisciplinary research is an integrated part of 'One Health' and Ecohealth approaches. A further development of 'interdisciplinary' research (Charron, 2012; Zinsstag et al., 2015), it is based on academic and non-academic knowledge for practical problem solving at the human and animal interface (Schelling and Zinsstag, 2015). This approach involves communities, authorities and scientists who work together to find a consensus on, for instance, improvements in disease interventions. In Chad, for example, pastoralist groups did not see a reason for boiling livestock milk as a measure for brucellosis prevention, as they did not know about the risk of disease transmission. So the first approach has to focus therefore on disease education in pastoralists (Schelling et al., 2008).

The ICONZ project as an example for applying transdisciplinary research

Neglected zoonotic diseases are at first societal problems. These diseases were defined from the ICPCs beforehand as health priorities. In a next step, problem solving knowledge was generated, involving communities and authorities, by heterogeneous research groups from different institutions with different backgrounds and in different contexts. The generated knowledge was gained from the disease baseline assessment and was then transformed into disease interventions targeting neglected zoonotic diseases in the ICPCs.

2. Study Rationale and Design

2.1. Research Gaps

Little is known about dog-related zoonoses in Mali and data about the dog population is missing to date. Disease surveillance is insufficient throughout the country and under-reporting is expected regarding leishmaniasis, echinococcosis and rabies. Veterinary laboratory competence for the detection of neglected zoonoses in Mali often does not exist, which further contributes to disease under-reporting. NZDs affect mostly poor marginalised populations, which are living in close proximity to their animals, and generate therefore a significant health burden. In addition, NZDs affect often livestock and also affect people's livelihood. The dog is often a reservoir for the three diseases and information on the dog demography, the epidemiology of dog zoonoses and people's knowledge attitudes and practices (KAP) towards the diseases is absolutely crucial for the planning of disease control programs and to raise international attention for global assistance and funding for health.

2.2. Aims and specific objectives

2.2.1. Aims

The overarching aims were to generate basic knowledge by conducting for the first time a baseline assessment of the epidemiology of the studied neglected dog zoonoses, people's KAP towards the diseases and collection of dog population data. Our results will pave the way for improved and cost-effective control programs and prevention strategies, which result finally in an improvement of human and animal health, poverty alleviation and a contribution to the MDGs.

2.2.2. Specific objectives

Objective 1: Investigation of the dog ecology, demographic structure and population dynamics in Bamako, Mali

Objective 2: Evaluation of the disease prevalence (leishmaniasis, echinococcosis) within the domestic dog population of Bamako, Mali

Objective 3: Typing of the *Leishmania* and *Echinococcus sp*. within the domestic dog population of Bamako, Mali

Objective 4: Estimation of the seroprevalence of rabies antibodies in the domestic dog population of Bamako, Mali

Objective 5: Investigation of people's knowledge, attitudes and practices regarding leishmaniasis, echinococcosis and rabies in Bamako, Mali

Objective 6: Disease burden in humans

- One Health approach
- Simultaneous investigation of human data

Objective 7: A trial of the same approach in a rural setting

- Objective 1-6

Objective 8: Strengthening of laboratory capacity through technology transfer and training of laboratory staff

- If possible, diagnostic methods, used for disease detection in animals, transferred from the collaborating laboratories in Europe to the CVL

Objective 9: Results dissemination to all relevant stakeholders on a national and international level

- Publications of the results in peer-reviewed journals
- Presentation of the results at international conferences
- Applications to donor entities for further research activities

2.3. Study design

2.3.1. Study Site

Mali is a landlocked country in West Africa and is one of the poorest countries in the world. It borders Algeria, Burkina Faso, Côte d'Ivoire, Guinea, Mauritania, Niger and Senegal (Fig. 9). The climate is subtropical to arid, where it is hot and dry between February to June, mild, rainy and humid between June to November and cool and dry between November and February. Three natural zones were distinguished, with a southern cultivated Sudanese, a central semiarid and a northern arid Saharan zone. Several ethnic groups and languages exist, with the majority belonging to the ethnic group of Mande (Bambara, Malinke, Soninke). Other ethnic groups are Peulh, Voltaic, Songhai, Tuareg, among others. The official language is French, but many other national languages (Bambara, Peulh, Dogon, Soninke, Malinke, Sonraï, Minianka, Tamacheq, Sénoufo and others) are spoken. Muslim is the predominant religion with more than 90%, followed by Christian and animist (Central

Intelligence Agency, USA, 2015). A census undertaken in 2009 accounted for about 14.5 million inhabitants (RGPH-2009). The country is divided into eight regions, namely Gao, Kayes, Kidal, Koulikoro, Segou, Sikasso, Toumbouctou, and one capital district, the District de Bamako (Central Intelligence Agency, USA, 2015).

The cross-sectional survey was undertaken in Bamako, the capital, located in the southern part of the country. The city is divided in 6 communes with a total of 67 quarters, and the national census recorded 1.8 million inhabitants (RGPH-2009). The city has a sub-humid savannah climate (Kottek et al., 2006). The total land area is about 267 km^2 (UN-HABITAT, 2010).

Figure 9: Map showing the study site (Source of maps: Central Intelligence Agency, USA, 2015)

2.3.2. Preparatory work and field activities

Field activities took place between 2010 and 2012 in Bamako, Mali. At the beginning of the first field visit in 2010, the responsibilities and accountability of all partners involved in the ICONZ project were clarified through collaborators. Field activities of the PhD student took place in 2010 and 2011. The third field trip of the PhD student was cancelled due to the military coup in 2012 and the Objective 8 (Strengthening of laboratory capacity through technology transfer and training of laboratory staff; see 2.2.2.) was therefore not fully accomplished. A planned field visit of a civil servant during the first follow up survey in 2010 had to be cancelled due to the security situation in the country.

Questionnaire interviews, whenever possible with the head of the household or another adult household member, were conducted in all six communes of Bamako. To obtain a representative sample, and because we did not have a household register, a multi-stage cluster technique was applied (Schelling and Hattendorf, 2015). We randomly selected 32 out of a total of 67 quarters with selection probability proportional to the size (PPS) of the human population in the six communes. We used the commune population, because the reliable data on quarter level was not available during the preparation of the field study. Through the PPS approach, which is a self-weighting procedure, each person in Bamako has subsequently the same probability of being selected (Bennett et al., 1991; Schelling and Hattendorf, 2015). Sample size calculations accounted for potential correlation between individuals in the same cluster and inhomogeneity between the clusters according to Bennett et al. (1991). Samples had to be large enough to be statistically reliable. The intra-cluster correlation coefficient roh (ρ) was assumed to be 0.2 because no comparable study in this setting was available (Schelling and Hattendorf, 2015). Compounds/households were set as the basic sampling unit (BSU) (Bennett et al., 1991). During the first baseline survey in 2010 and the second baseline survey in 2011, households were randomly selected with slightly different procedures. The survey in 2010 was conducted between May and June, while the one in 2011 was between April and May. Beforehand all quarter heads were informed about the study and radio broadcasts were used to inform the population about the survey. Full interviews were conducted in all households in 2010, but only in dog-owning households in 2011. Because the proportion of dog-owning households in 2010 was lower than expected, we decided to conduct a second baseline survey in 2011. During the study in 2010, five field teams, each consisting of one veterinary officer from the CVL and one interviewer, started in a quarter at the household of the quarter chief, then spun a bottle and walked, directed by a Global Positioning System (GPS) device, 200 meters in the indicated direction, where they flipped a coin to select one side of the road for the inclusion of two households in two neighbouring compounds. A compound is defined as all of the houses surrounded by a wall, which could include several households. A household is defined as an individual or a group, related or not, living within the compound under the lead of the household head. It is composed of the head of household, his spouse(s), his unmarried children and possibly other related or unrelated persons. The selection procedure was repeated until at least 38 households per quarter were enrolled and interviewed. The sampling in 2011 was modified, mainly to avoid bias through the 'spin the bottle' technique (Schelling and Hattendorf, 2015). One possible bias is for example sampling along main streets which can effect a so called 'main street bias' (Schelling and Hattendorf, 2015). We decided therefore to use in 2011 as unbiased as possible a sampling procedure using random geo-coordinates (Schelling and Hattendorf, 2015). A sequence of random coordinates was overlaid on a Google Earth map (© 2013 Google Inc.) of Bamako (Fig. 10) using R software. Four field teams started at five randomly selected coordinates, which were located using a GPS device, within the same quarters (Fig. 11). The five lowest numbers of the random points were selected (green pins) and the nearest block was then chosen, where interviews were conducted in households (only one per compound) with the team moving in a clockwise direction until at least 12 households were selected. The location of each household was recorded using a GPS. The project intentionally sought to employ women for the survey and three women interviewers took part in the two baseline surveys. Unfortunately different interviewers were used in 2010 and 2011. In both baseline surveys, one veterinary officer from the National Veterinary Service participated in the survey. The questionnaires included questions about compound and household characteristics (occupation of the head of the household, religion and ethnicity of the interviewee and socioeconomics of the household), questions about KAP regarding zoonoses and control, dog- and non-dog-ownership, total number of dogs, dog movement and dog characteristics (age, sex, breed, function, source, confinement, food source, reproductive history of females) of each owned dog older than three months (Appendix 1). The sample questionnaire from the 'Guidelines for dog population management' from the WHO/WSPA (World Society for the Protection of Animals) (1990) was used as a template for the design of the questionnaire. Some of the KAP questions were designed in the style of Weiss (2001), with open questions in the first phase to evaluate what first comes to mind for the interviewees without prompting specific answers, followed by a list of probed answers that have not been mentioned beforehand. In this way, spontaneous answers have the highest prominence and are used in the design of intervention programs. All questions were, through the aid of local collaborators, adapted for the local context. We define "owned dogs" as dogs that belong to a household. In contrast, "ownerless dogs" are dogs not belonging to a household and living in the street. Prior to the first baseline survey, the questionnaire was pre-tested in a non-selected quarter and the field teams were trained on using a GPS device. The questionnaire was in French and questions were translated into Bambara when necessary. Blood, fur and, in 2011, additionally a faecal sample was collected by the veterinary officer, if possible, from every encountered dog over three months old. Dogs were not sampled if the owner did not give consent or if a dog was uncooperative, aggressive or sick at the time of the survey. Muzzles were used to prevent dog bites. The dog-keeping households were followed, when possible, through telephone interviews every six months for two years (Baseline 2010) or one year (Baseline 2011) for the successive collection of dog demography data (Follow up questionnaires in Appendix 1.). Dogs were recognised with the help of the description (breed, name, age, sex) collected during the baseline surveys. If the household could not be contacted through a call, the household was visited again. Along with the baseline surveys, four follow up surveys were conducted. During the second follow up survey, additional blood and fur samples were possibly collected from the same dogs but also for new dogs in the households. The two baseline surveys and the second follow up survey were conducted on the spot under the lead of the PhD student and our main Malian collaborator. The first, third and fourth follow up survey was conducted by an interviewer, who was familiar with the study through collaboration during the baseline surveys, under the lead of our main Malian collaborator.

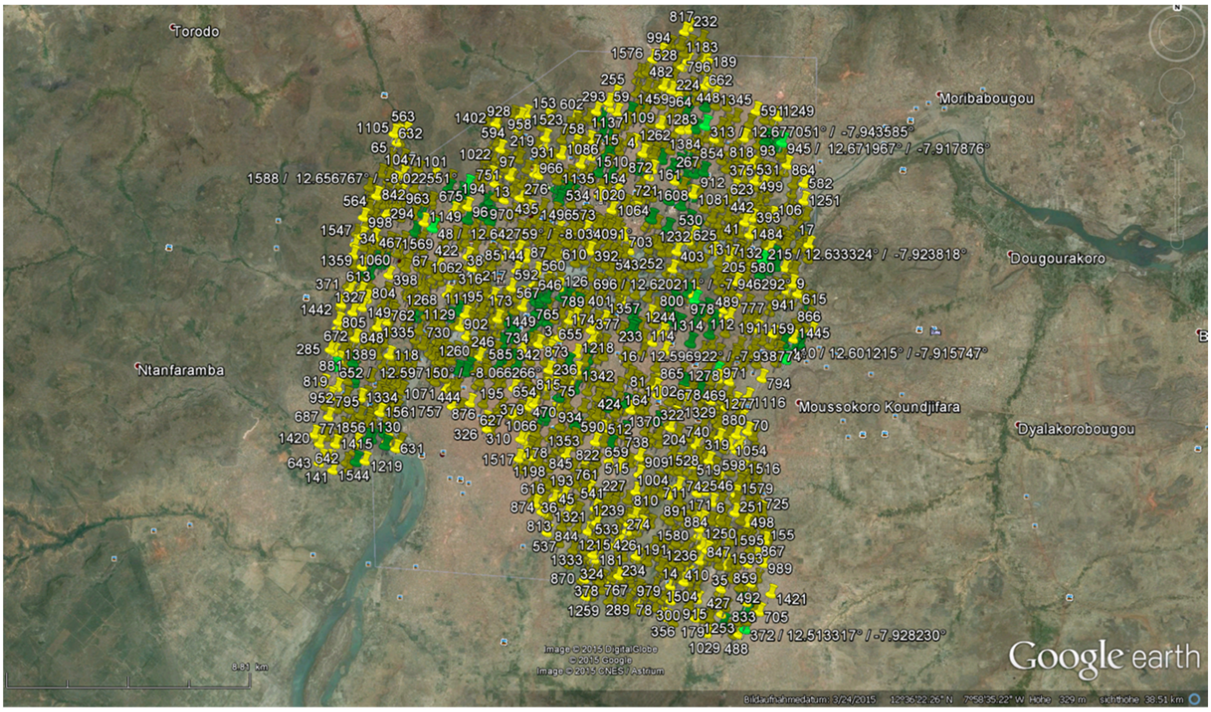

Figure 10: Google Earth map with pins. The green pins were used as starting point during the baseline study in 2011 (Source: Google Earth, 3/24/2015)

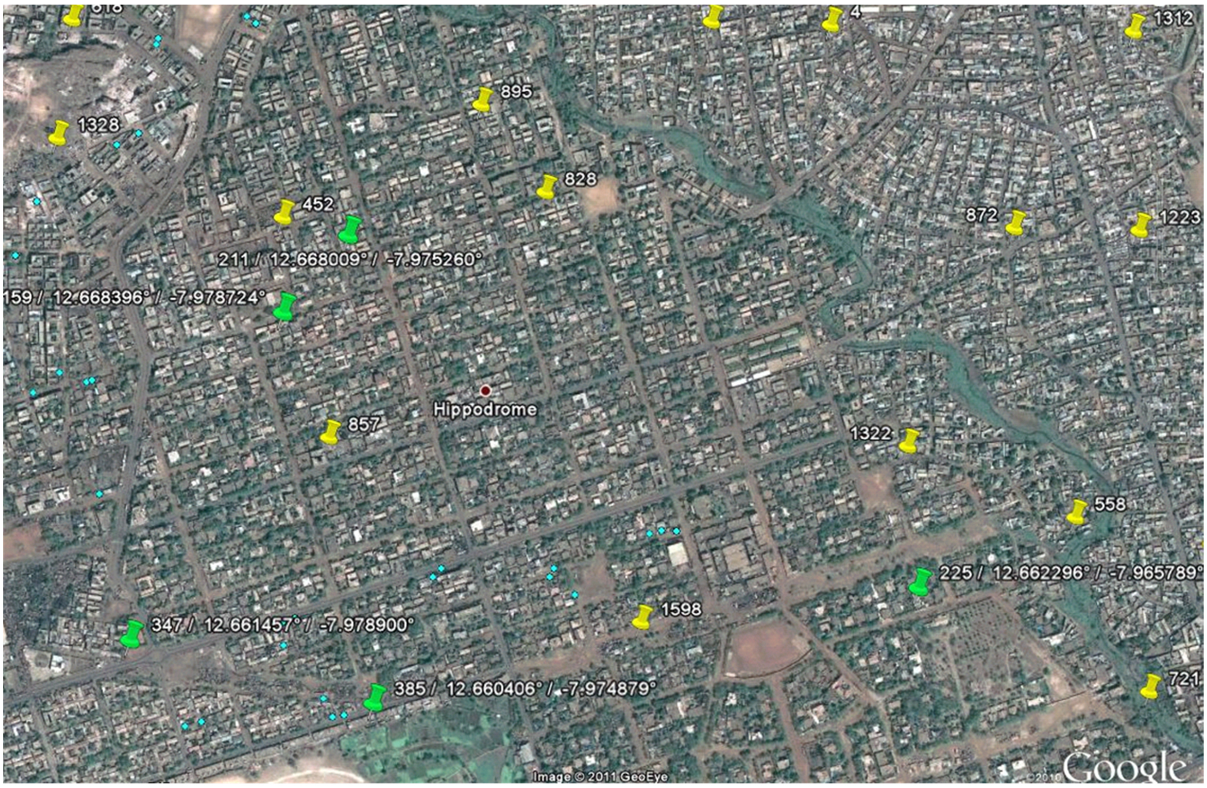

Figure 11: Google Earth map with pins in the quarter Hippodrome. The green pins were used as starting point during the baseline study in 2011. The blue dots represent the visited compounds in 2010. (Source: Google Earth, Image © 2011 GeoEye)

Objective 7 (A trial of the same approach in a rural setting; see 2.2.2.) of this PhD was cancelled due to the insufficient security in the selected areas. Some of the data collection was executed through the Malian collaborators, but these results will be stated elsewhere. Originally it was planned that a Malian doctoral student would conduct in parallel a baseline assessment of the disease burden in humans, but the most important part of the 'One health' approach (Objective 6; see 2.2.2.) was unfortunately cancelled because the formal ethical approval was not available on time.

2.3.3. Laboratory testing

All biological samples (plasma, serum, fur, faecal) were shipped for further examination to specialised laboratories, namely the Anses-Nancy Laboratory for Rabies and Wildlife, the Institute of Parasitology of the Vetsuisse Faculty, which is part of the University of Zurich and the Swiss Tropical and Public Health Institute (Swiss TPH). With the exception of FAVN test, which was conducted in the Anses Nancy Laboratory, the PhD student conducted, with the support of various diagnostic teams, all laboratory testing on her own. Under the principle 'No research without service', dog owners were informed about the results and potential health risk for themselves and infected dogs were appropriately treated. Further details regarding the laboratory analysis are given in the specific chapters.

2.3.4. Data entry and analysis

Data was double entered in Microsoft® Access 2010 by the PhD student and a Master's student and checked for inconsistencies using Epi Info™ 3.5.1. Statistical analysis was done with STATA IC 12. Generally, the analysis took correlation of the outcome within clusters into account. This was done by means of generalized estimating equation models (GEE) (Liang and Zeger, 1986). Data regarding the age and sex of the dog were adjusted if no agreement was obtained between the baseline surveys and follow up surveys.

Further details regarding the statistical analysis are given in the specific chapters.

2.3.5. Ethical considerations

This study did not involve human health data collection and hence did not undergo formal ethical approval. Research approval was granted by Malian state authorities. All data was anonymised and handled confidentially. All involved personnel were vaccinated against rabies. During the surveys, the research team maintained a first-aid kit and stocks of human rabies vaccine to provide PEP to persons exposed to bites from suspected rabid dogs.

2.3.6. Collaborating Partners

This research project was undertaken within the ICONZ project (see 1.2.). Collaborating partners were:

- The Swiss Tropical and Public Health Institute (Swiss TPH), Basel, Switzerland
- Central Veterinary Laboratory (CVL), Bamako, Mali
- The Institute of Parasitology, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland
- Anses-Nancy Laboratory for Rabies and Wildlife, European Union Reference Laboratory for Rabies, WHO Collaborating Centre for Research and Management in Zoonoses Control, OIE Reference Laboratory for Rabies, European Union Reference Laboratory for Rabies Serology, Malzéville, France
- Avia-GIS, Zoersel, Belgium

3. Outline of thesis

This PhD thesis is divided into three main parts. The first part deals, as a preparatory work for dog disease interventions, with a dog ecology study. The second part covers work which was done on rabies and the third part gives attention to dog-related parasites. The three parts are followed by a general discussion and conclusions (Chapter 10).

- Dog ecology
    - First study on domestic dog ecology, demographic structure and dynamics in Bamako, Mali (Chapter 4).
- Rabies
    - Factors associated with dog rabies immunisation status in Bamako, Mali (Chapter 5).
    - Rabies in Mali: molecular characterization of canine rabies virus strains (Chapter 6).
    - Low coverage of central point vaccination against dog rabies in Bamako, Mali (Chapter 7).
    - A mixed methods approach to assess animal vaccination programmes: the case of rabies control in Bamako, Mali (Chapter 8).
- Dog-related parasites
    - First report of *Echinococcus granulosus* (genotype G6) in a dog in Bamako, Mali (Chapter 9).
    - Spectrum of dog parasites found in Bamako, Mali (Chapter 10).

4. First study on domestic dog ecology, demographic structure and dynamics in Bamako, Mali

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Submitted to Preventive Veterinary Medicine

Abstract

Background:

For the planning of an effective dog mass vaccination campaign against rabies in Africa, it is crucial to know more about the dog population. In this paper we describe for the first time the dog ecology, demographic structure and population dynamics of a domestic dog population in Bamako, Mali. In 2010 and 2011, we visited 2956 randomly selected compounds. Questionnaire data was collected on the compound and household level and on each dog individually. Dog-owning households were followed every six months during one (dog-owning households identified in 2011) or two years (dog-owning households identified in 2010) for the successive collection of dog demography data.

Results:

We recorded 379 dogs in 279 compounds. The dog human ratio was estimated at 1:121, and the extrapolation of the domestic dog population in Bamako results in an estimate of 14 906 dogs (95% CI 13 041 - 17 037). The female male ratio was 1:2.8. A high proportion of young dogs was found as a result of a high turnover rate in the population. Mortality/emigration within the first year of life was high, and dogs had a life expectancy at birth of 2.5 years. Although we observed a decrease in the domestic dog population during the observed two years, we estimated an annual dog population growth of 20%, which likely indicates an oscillatory dynamic of the dog population. Christians were more likely to be dog owners than Muslims. Another factor favouring dog ownership was belonging to the ethnic group of *Bobo* or *Malinke*. Dogs were mainly used as watchdogs and fed with household leftovers and garbage. They were most often obtained and given away without remuneration. In addition, dog-owners need to be educated on good dog management. The reduction of dogs through city wide poisoning campaigns is not recommended.

Conclusions:

This work contributes vital information towards planning effective and sustainable dog rabies control programmes for the district of Bamako. Due to the high turnover rate, we recommend repeated mass-vaccination campaigns of at least 70% of the owned dogs at yearly intervals.

Keywords

dog demography, dog ecology, dog population dynamics, rabies, Bamako, Mali

4.1. Introduction

In Africa, information on the domestic dog population relevant for rabies control and other dog-mediated zoonoses has only been recorded in a few countries (Davlin and Vonville, 2012). For the planning of an effective dog mass vaccination campaign against rabies in Africa, where more than 20 000 human deaths per year due to rabies are estimated (Hampson et al., 2015; Knobel et al., 2005), it is crucial to know more about the dog population ecology characteristics such as population size, density, sex ratio, turnover, growth and the role of dogs in human societies (WHO, 1987; WHO, 1988b). The human-dog relationship is highly diverse. Dogs can be kept as pets or working animals (e.g. watchdogs) or can be traded for food. But for cultural and religious reasons they can also be considered as unclean in a religious or hygienic sense, or they can be rejected because of diseases or the risk of bites (Frank, 1965; WHO, 1987). The World Health Organization (WHO) recommends vaccinating at least 70% of the dog population for efficient rabies control (WHO, 2013). In Bamako, Mali, rabid dogs were responsible for the majority of human cases (Dao et al., 2006) but data on dog demographics and ecology is missing to date. Mauti et al. (2015) estimated a seroprevalence of rabies virus antibodies in the examined domestic dog population of 24%, a coverage which is insufficient to interrupt virus transmission (Coleman and Dye, 1996). In a comparable setting in N'Djamena, Chad, it was shown that, on average, below a density of approximately 90 susceptible dogs per km^2 rabies transmission is interrupted (Zinsstag, unpublished data). Kitala et al. (2002) calculated a threshold dog density of 4.5 dogs per km^2 for the interruption of rabies transmission in Machakos District in Kenya. Generally, the proportion of dogs inaccessible for vaccination is low in Africa (WHO, 1988b). In Bamako, the proportion of ownerless dogs was estimated at 8% (Muthiani et al., 2015). Given that dogs are not registered in Bamako, a way to learn more about the domestic dog population and the estimated population size is through questionnaire surveys (WHO, 1987).

In this paper we describe the dog ecology, demographic structure and population dynamics of a domestic dog population in Bamako, Mali. Furthermore, we estimated the dog human ratio and calculated the total domestic dog population in Bamako and investigated reasons for dog- and non-dog-ownership.

4.2. Materials and methods

4.2.1. Study Site

A cross-sectional study was undertaken in Bamako, the capital of Mali, which is located in the southern part of the country. The city is divided in 6 communes with a total of 67 quarters. The national census recorded 1.8 million inhabitants in Bamako (RGPH-2009). The city has a sub-humid savannah climate with an average temperature above 30 °C each month. The total surface area of the district of Bamako is about 267 km^2 (UN-HABITAT, 2010).

4.2.2. Data collection

Questionnaire interviews were conducted, whenever possible with the head of the household, in all six communes of Bamako. We randomly selected 32 out of a total of 67 quarters with selection probability proportional to the size of the human population of the communes. During the first baseline survey in 2010 and the second baseline survey in 2011, compounds were selected by a geographical randomisation procedure. A compound was defined as all of the houses surrounded by a wall, which could include several households. We defined a household as an individual or a group, related or not, living within a compound under the lead of the household head. Most commonly, a household in Bamako is composed of the head of household, his spouse(s), his unmarried children and possibly other related or unrelated persons. The survey in 2010 was conducted between May and June, while the one in 2011 was between April and May. Full interviews were conducted in all households in 2010, but only in dog-owning households in 2011. The sampling method for compounds was based on a random selection approach. During the study in 2010, five field teams, each consisting of one veterinary officer and one interviewer, started in a quarter at the household of the quarter chief, then spun a bottle and walked 200 meters in the indicated direction, where they flipped a coin to select one side of the road for the inclusion of two households in two neighbouring compounds. This selection procedure was repeated until at least 38 households per quarter were enrolled and interviewed. In 2011, four field teams started at five randomly selected coordinates within the same quarters. The nearest block was then chosen, where interviews were conducted in households (only one per compound) with the team moving in a clockwise direction until at least 12 households were selected. The location of each household was recorded using a Global Positioning System (GPS). The questionnaires included questions about household characteristics (number of household members, occupation of the head of the household, religion and ethnicity of the interviewee and socioeconomics of the household), dog- and non-dog-ownership, total number of dogs, dog movement and dog characteristics (age, sex, function, source, confinement, food source, reproductive history of females) of each owned dog older than 3 months. We defined "owned dogs" as dogs that belong to a household. In contrast, "ownerless dogs" are dogs not belonging to a household and living in the street. Prior to the first baseline survey, meetings were held with the local chiefs of each quarter. The questionnaire was tested in a non-selected quarter. The questionnaire was in French, and questions were translated into Bambara when necessary. The dog-keeping households were followed, when possible, through telephone interviews every six months for two years (Baseline 2010) or one year (Baseline 2011) for the successive collection of dog demography data. If the household could not be contacted through a call, the household was visited again personally.

4.2.3. Data entry and statistical analysis

Data was double entered in Microsoft® Access 2010 and checked for inconsistencies using Epi Info™ 3.5.1. Statistical analyses were done with STATA IC 12.

Questionnaire data was analysed with generalized estimating equation models, taking clustering into account. Mean dog age was estimated by fitting the censored data to a gamma distribution using the R package 'fitdistrplus'. Dog densities per km^2 were estimated by dividing the number of dogs extrapolated for Bamako by the total size of the area. The extrapolation of the total domestic dog population of Bamako was done by means of a negative binomial model resulting in a dog per person mean. This number was then multiplied with the total human population counted during the nationwide census in 2009 (RGPH-2009). The building construction of the house roof was used for classification of the socioeconomic status, where a straw roof was considered less wealthy, steel sheet was an indicator for moderately wealthy and a roof out of cement was equated with wealthy.

By means of vertical life tables (Pianka, 1999), several life-history demographic parameters (survival, life expectancy, mortality, fecundity) of our baseline study in 2010 and 2011 could be calculated for age-specific classes (0-1, 1-2, 2-3, 3-4, 4-5, >5). Formulas are provided in this manuscript in Appendix A.

A deterministic demographic dog model was established using Vensim version 6.0 (Ventana systems, Inc., Harvard, MA, USA).

Projection of dog population growth was done by means of an age-structured population projection matrix (Leslie matrix) under the assumption that the environment remained constant and no emigration or immigration took place in the dog population. Female fecundity was in the first row of the matrix and survival (p(x)) was in the subdiagonal of the transition matrix (Vandermeer and Goldberg, 2013).

4.3. Results

4.3.1. Study population

We identified 379 dogs in 279 households/compounds during the two surveys. In total, 2956 households/compounds were visited (Fig. 12). 125 dog- and 1 017 non-dog-households were interrogated in 2010 and 154 dog-households in 2011. For 1 660 non-dog-owning households in 2011, we had only information on the number of persons per compound. Questionnaire data were collected for 311 eligible dogs. Out of these, 26% (81/311) were female and 74% (230/311) were male, resulting in a female male ratio of 1:2.8. Only 5% of the female dogs and 6% of the male dogs were castrated or spayed. From 356 out of 379 dogs we had information on the age (Fig. 13). So a mean age was estimated as 38 months for the whole population, with 42 months for the population in 2010 and 34 months for the population in 2011 (Fig. 14).

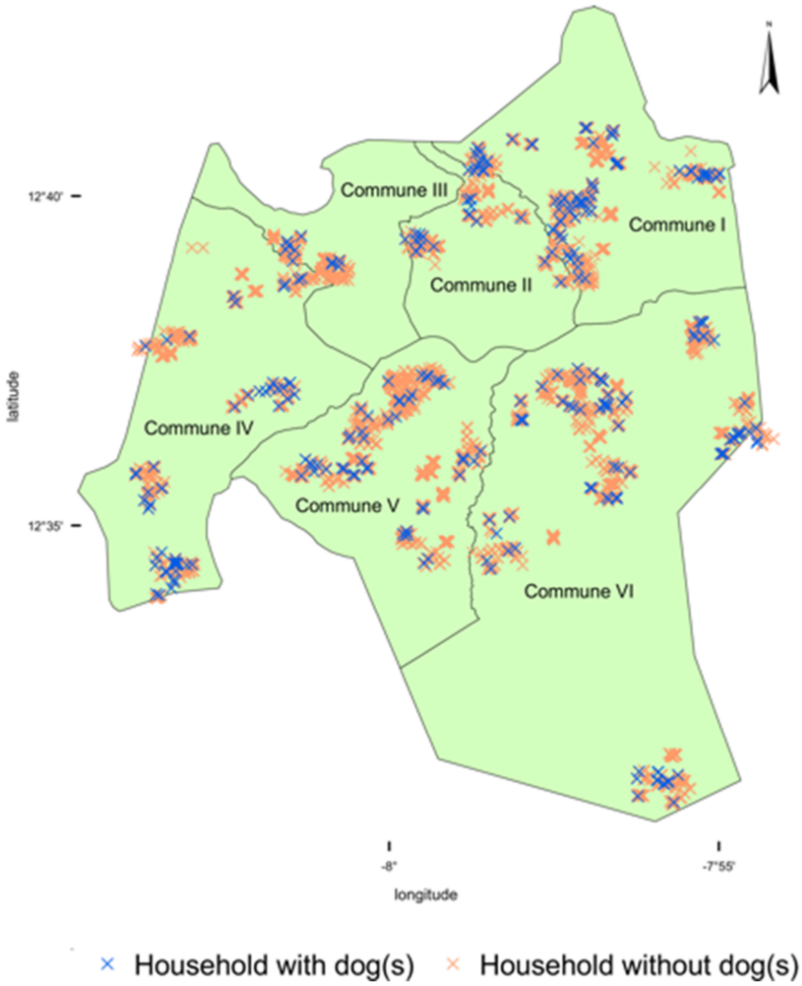

Figure 12: Dog- and non-dog-owning households/compounds in Bamako

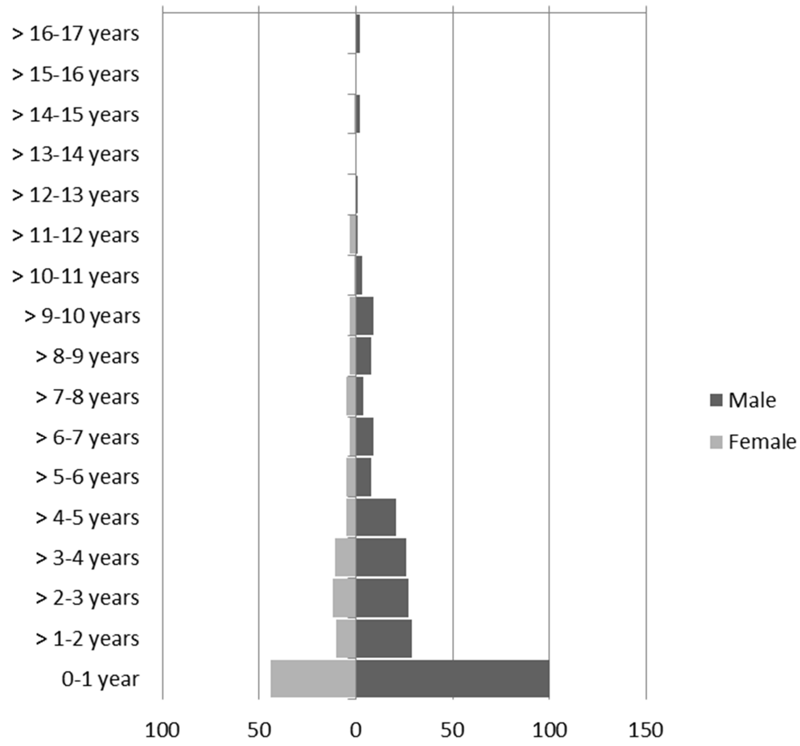

Figure 13: Age distribution of the dog population in 2010 & 2011

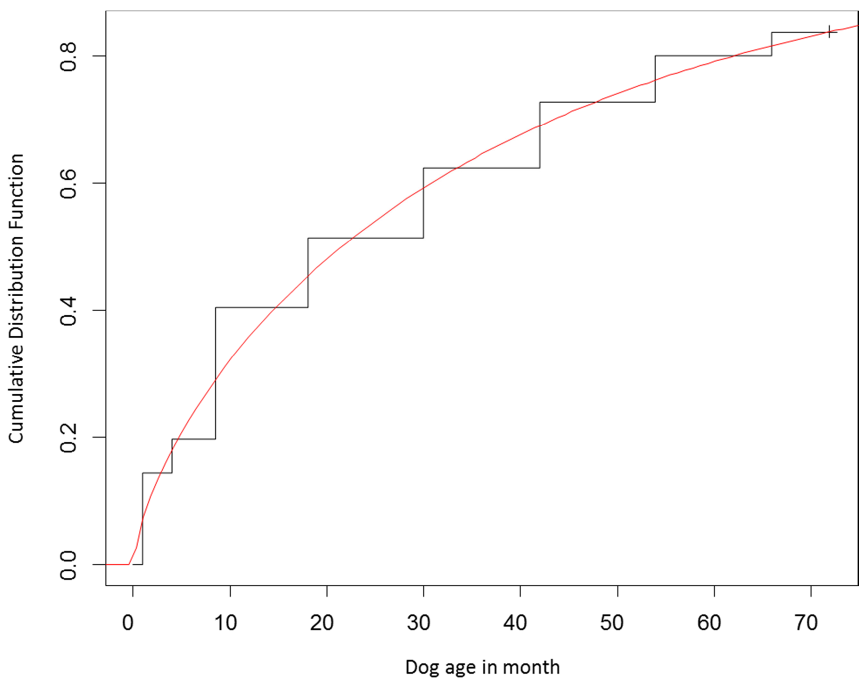

Figure 14: Cumulative distribution of dog age

4.3.2. Extrapolation of the dog population for Bamako

The dog human ratio was estimated at 1:122 in 2010 and 1:120 in 2011, respectively; resulting in a mean ratio of 1:121. We calculated that of all compounds 11% in 2010 and 8% in 2011 had dogs, respectively. Overall, 9% of all compounds had dogs. Overall mean number of dogs per compound was 0.13. When considering only dog-owning compounds, we found 1.3 dogs per compound. On average 17 persons lived in a compound in 2010 and 14 in 2011 respectively; for both baselines together, we calculated a mean of 16 persons per compound (Fig. 15). In 2010 and 2011, 0.8% of persons had a dog (95% CI 0.7% - 0.9%). There was no notable difference between the two years. The estimated dog population of the whole city was estimated at 14 906 dogs (95% CI 13 041 – 17 037) (Tab. 1), resulting in a dog density of 56 dogs/km^2.

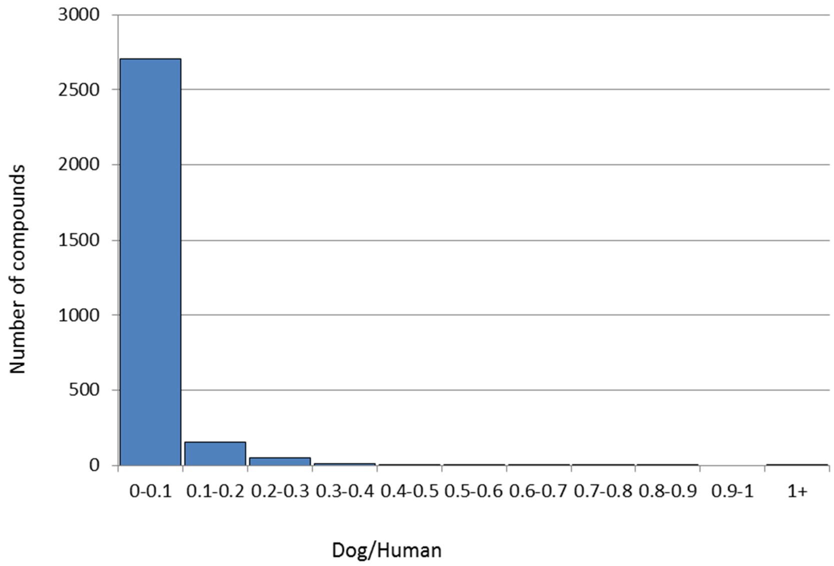

Figure 15: Frequency distribution of the dog density per person in the compounds in 2010 & 2011

Table 1: Extrapolation of the dog population in Bamako

	N	N compounds	Mean	[95% Conf.	Interval]
Dogs	379	2956	0.128	0.112	0.145
Humans	45688	2936	15.561	15.131	15.992
Dog/person			0.008	0.007	0.009
Negative binomial model*					
Dog/person			0.008	0.007	0.009
Extrapolation based on the total human population (1 810 366 inhabitants) estimated in 2009					
Dogs			14'906	13'041	17'037

* adjusted for correlation within quarters

4.3.3. Dog- and non-dog-ownership

Interviewees from dog- and non-dog households were asked about their religion, the ethnic group to which they belong and the occupation of the head of the household. In parallel, the interviewer recorded the construction type of the house for social stratification. Predictors for dog-ownership are listed in table 2.

Table 2: Predictors for dog ownership in 2010

Dog ownership						
Predictor		%-dog-households (n/N)	p-value	OR (95% CI)	p-value	m OR (95% CI)
Occupation (n=1 120)	Private Sector	11% (69/638)		Ref		Ref
	Public Sector	13% (35/264)	0.185	1.3 (0.9-2.1)	0.323	1.3 (0.8-2.0)
	Agriculture	10% (5/52)	0.765	0.9 (0.3-2.2)	0.752	0.9 (0.3-2.3)
	Others	9% (15/166)	0.301	0.7 (0.4-1.4)	0.280	0.7 (0.4-1.3)
Religion (n=1 140)	Muslim	10% (114/1 106)		Ref		Ref
	Christian	37% (11/30)	**< 0.001**	**4.8 (2.2-10.4)**	**< 0.001**	**5.0 (2.3-11.1)**
	Others	0% (0/4)	n.d.	n.d.	n.d.	n.d
Ethnic group (n=1 131)*	Bambara	8% (31/374)		Ref	-	-
	Malinke	14% (25/176)	**0.048**	**1.8 (1.0-3.1)**	**-**	-
	Peulh	11% (20/183)	0.403	1.3 (0.7-2.3)	-	-
	Sarakole/Soninke/Marka	9% (10/117)	0.955	1 (0.5-2.1)	-	-
	Sonraï	7% (3/43)	0.675	0.8 (0.2-2.7)	-	-
	Dogon	11% (5/44)	0.37	1.6 (0.6-4.1)	-	-
	Sénoufo/Minianka	12% (10/82)	0.232	1.6 (0.7-3.3)	-	-
	Bobo	33% (4/12)	**0.018**	**4.7 (1.3-17.0)**	**-**	-
	Others	13% (13/100)	0.195	1.6 (0.8-3.2)	-	-
Building construction (wall) (n=1 140)	Cement	11% (87/806)		Ref	-	-
	Mudbrick	13% (8/64)	0.878	0.9 (0.4-2.2)	-	-
	Improved mudbrick	11% (29/270)	0.554	0.9 (0.5-1.4)	-	-
Building construction (roof) (n=1 138)	Steel sheet	10% (86/821)		Ref		Ref
	Straw	0% (0/3)	n.d.	n.d.	n.d.	n.d
	Cement	12% (38/314)	0.418	1.2 (0.8-1.8)	0.590	1.1-(0.7-1.7)

* excluded from the multivariate analysis due to colinearity

In the univariate analysis, the proportion of dog owners for Christians was 37%, significantly higher compared to the 10% for Muslims (OR: 4.8 (95% CI 2.2-10.4)). *Bobo* and *Malinke* more often owned dogs than those from other ethnic groups. Socioeconomic status had no notable influence on dog ownership. In the multivariate analysis, the odds of having a dog in the household were 5 times higher (95% CI 2.3-11.1) if the interviewee was Christian compared to Muslim. There was no evidence for interaction between the predictor roof and religion (data not shown).

Dogs were mainly kept as guardians (94%; n=311; 95% CI: 88%-97%) and less often as pets or for hunting. There was no notable difference between the two baseline studies.

Dogs were mainly fed with household garbage and leftovers (n=311; 90% (95% CI: 83%-94%)), but also with raw meat and abattoir refuse (14%; 95% CI: 8%-23%) and roasted meat (5%; 95% CI: 2%-10%). Other mentioned food was pasta, street garbage, dog food, milk, bakery goods, fish, vegetables and rice.

Out of 310 dogs, 55% (95% CI: 47%-64%) were permitted to roam freely during day and night, 32% (95% CI: 24%-40%) were confined during the day, 2% (95% CI: 1%-5%) were confined during the night and 10% (95% CI: 6%-18%) were confined day and night. When asked where the dogs mostly spent the day, 84% (95% CI: 79%-89%) indicated the compound, followed by 14% (95% CI: 9%-19%) specified in front of the compound and 4% (95% CI: 1%-13%) were reported as spending most of the time free in the street. A minority of dogs (0.3%; 95% CI: 0.1%-2.1%) spent the day at neighbouring compounds or on the hunting grounds (1%; 95% CI: 0.1%-9.1%). There was a slight difference between the two baselines for spending the day in the compound and in the street with 81% vs. 88% and 5% vs.2% in 2010 and 2011, respectively.

Reasons given for non-dog-ownership for 1 015 interviewees were for 62% (95% CI 54%-69%) no necessity, followed by 12% (95% CI 9%-15%) stating religious reasons. In 6% (95% CI 3%-10%), the reason was the potential danger of dogs and, in another 6% (95% CI 4%-9%), the reason given was because the interviewee was a renter. Other reasons were dislike of dogs, costs, dog bites, the previous dog died, currently planning the acquisition of a dog, because of the interviewee’s custom, the dog’s maintenance, rabies, because of the neighbours, fear, location and other problems caused by dogs.

4.3.4. Life tables and Leslie Matrix

Several life-history parameters were calculated from the combined baseline data from 2010 (n=158) and 2011 (n=198). The results are shown in table 3. Female demography and fecundity is presented in table 4. In order to have a larger female dog sample, data from the baseline surveys and follow up surveys, excluding the follow up survey 1, were combined. The age specific mortality (q(x)) before reaching the next age class was high, with 73% in the less than one year old and 77% in the 4-5 years old (table 3). Life expectancy e(x) at birth was 2.47 years, rising to 5.44 after survival of the first year. The female life table (table 4) showed that 65% of all females between 3-4 years (total 25) of age were breeding. An average number of 5 pups/litter was calculated for all females.

Table 3: Overall population demography

age class	n years	s(x)	% s(x)	l(x)	p(x)	d(x)	q(x)	e(x)
0-1	1	144	40	1.00	0.27	0.00	0.73	2.47
1-2	1	39	11	0.27	1.00	0.73	0.00	5.44
2-3	1	39	11	0.27	0.95	0.73	0.05	4.44
3-4	1	37	10	0.26	0.70	0.74	0.30	3.62
4-5	1	26	7	0.18	0.23	0.82	0.77	3.73
5+	13	71	20	0.01	0.00	0.99	1.00	1.00

age class = age in years; n years = number of years spent in the ageclass; s(x) = number of individuals sampled per ageclass; % s(x) = percentage of sample per ageclass; l(x) = cumulative survival; p(x) = age-specific survival from age x to age x + 1; d(x) = cumulative mortality; q(x) = age-specific mortality from age x to age x + 1; e(x)= age-specific life expectancy.

Table 4: Female demography and fecundity

age class	s(x)	l(x)	d(x)	b(x)	B(x)a	m(x)
0-1	42	1.00	0.00	0.05	2.00	0.05
1-2	45	1.07	-0.07	0.80	4.80	1.92
2-3	24	0.57	0.43	0.42	6.60	1.39
3-4	25	0.60	0.40	0.65	7.20	2.34
4-5	23	0.55	0.45	0.35	4.80	0.84
5+	81	0.08	0.92	0.35	3.60	0.63

age class = age in years; s(x) = number of individuals sampled per ageclass; l(x) = cumulative survival; d(x) = cumulative mortality; b(x) = average proportion of breeding females per year and age-class; B(x) = average number of offspring born in the last litter per female and age-class; m(x) = number of female pups born per female and year

a: Number of pups born was smoothed using a weighted rolling mean with bandwidth of 2 and ends kept original to counteract stochastic effects

Using a Leslie Matrix, we estimated a population growth rate of 20%.

4.3.5. Dog demography model

The cumulated data of our dog cohort was used to establish a dog demography model (Fig. 16). We could not combine the two baseline surveys because of the resulting increased sample size in the second year and used therefore only the dog cohort data from the baseline 2010.

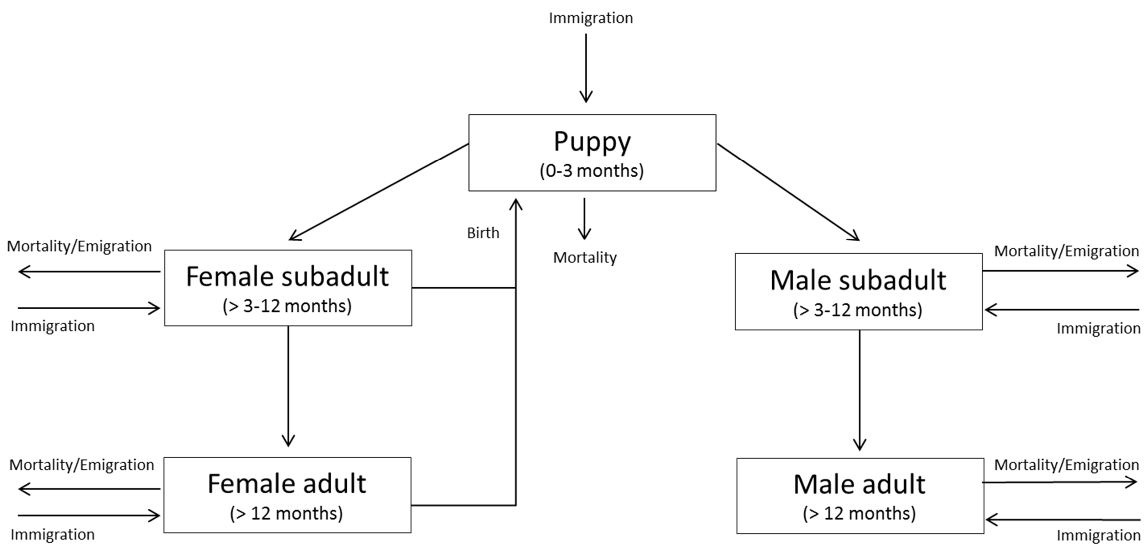

Figure 16: Dog demographic model

The best fit of the model is shown in Figs. 17, 18 and 19. Parameters are described in table 5. The birth rate calculated from the deterministic model was 0.7 compared to the birth rate estimated from the vertical life table which was 1.0 for 6 months.

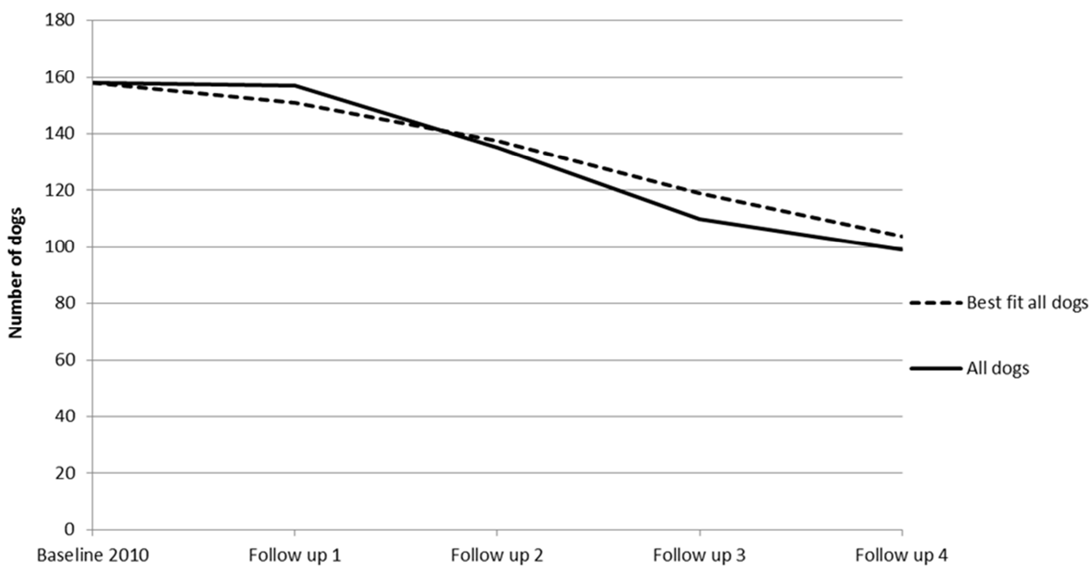

Figure 17: Demographic model fit for all dogs. Interval between the surveys was 6 months.

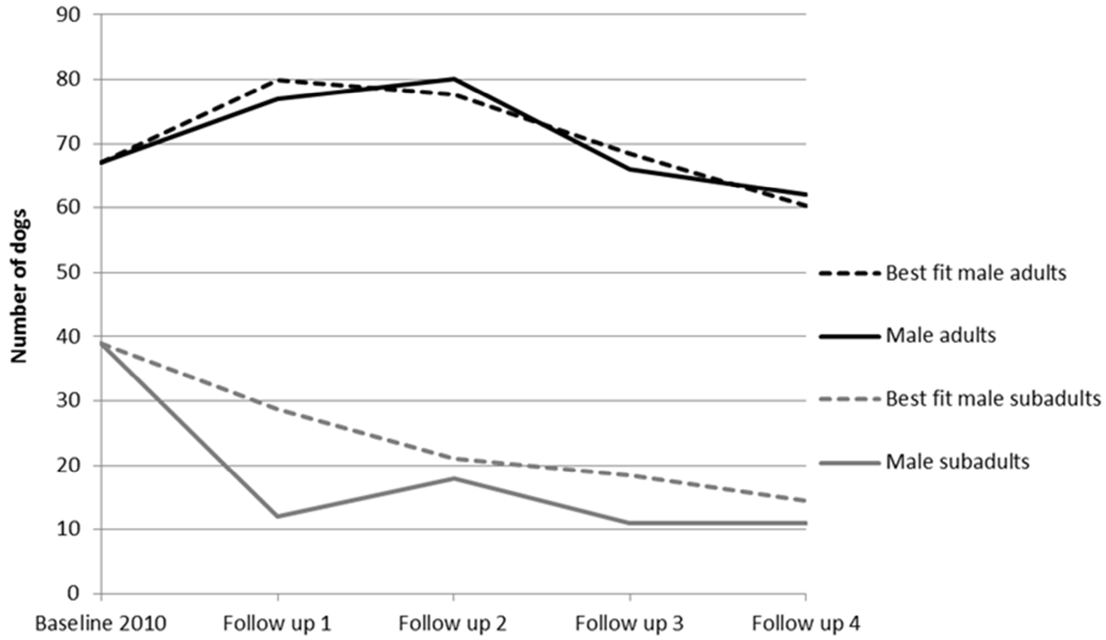

Figure 18: Demographic model fit for male dogs. Interval between the surveys was 6 months.

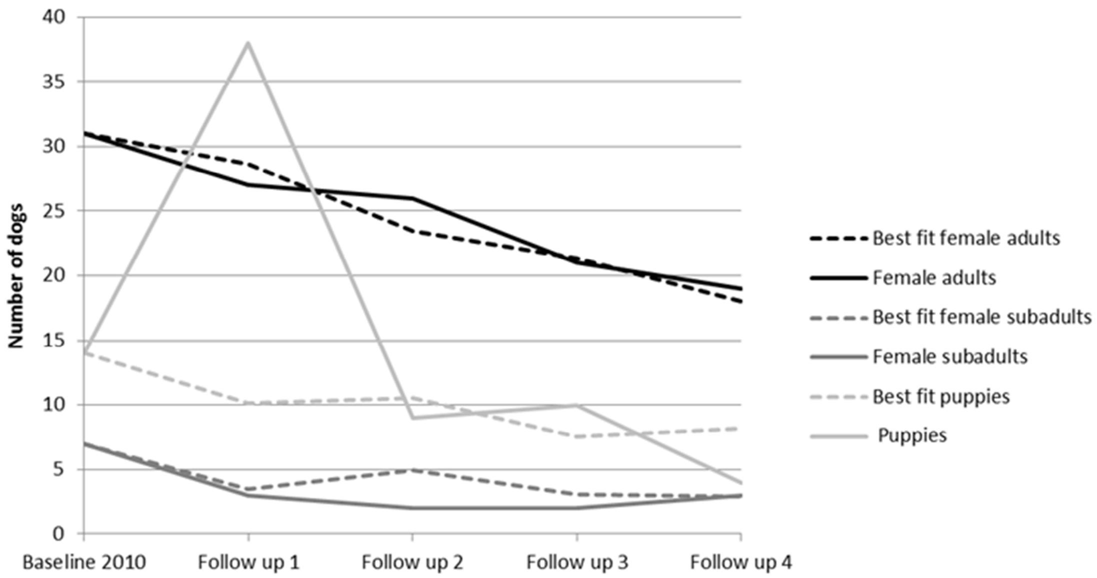

Figure 19: Demographic model fit for female dogs and puppies. Interval between the surveys was 6 months.

Table 5: Best fit parameters for birth rate and mortality per 6 months

	Lower boundary	Best fit	Upper boundary
Puppy birth rate	0.67	0.69	0.71
Puppy mortality rate	0.08	0.14	0.20
Female subadult mortality/emigration rate	1.39	1.51	1.60
Female adult mortality/emigration rate	0.24	0.30	0.37
Male subadult mortality/emigration rate	-0.50	-0.38	-0.22
Male adult mortality/emigration rate	0.33	0.39	0.45

4.3.6. Immigration and emigration of dogs

The investigation of immigration and emigration of dogs in the last six months before the baseline studies in 2010 and 2011 showed that dogs were primarily adopted without remuneration (76 (96%) dogs in 1 243 households) instead of purchased (3 (4%) dogs) and freely given away (67 (61%) dogs). No dog was sold. Only a minority were abandoned (4 (4%) dogs) or killed (8 (7%) dogs). Only three (3%) dogs in 1 260 households disappeared during the last six months, while 28 (25%) died a natural death.

During the baseline study in 2010, 77% (n=147; 95% CI: 68%-85%) of the surveyed owned adult dogs were obtained without cost, often received as a present. An additional 19% (95% CI: 12%-28%) were born in the household, while 4% (95% CI: 1%-11%) were purchased. Similar results were obtained in the baseline study of 2011, where 84% (n=162; 95% CI: 76%-90%) of the dogs were obtained for free, 11% (95% CI: 6%-19%) were born in the household and 4% (95% CI: 2%-8%) were bought. There was only a slight difference between the years considering birth in the household. The majority of dogs were under 2 months old when they were acquired.

4.3.7. Contacts with other dogs

Next to questions concerning the owned dogs, it was also asked if other dogs visited the compound. This was the case in 26% (95% CI: 20%-33%) of the 1 014 non-dog-owning households and 28% (95% CI: 17%-42%) of the 125 dog-owning households in 2010, respectively. In contrast, in 2011 only 13% (95% CI: 1%-21%) of the dog-owning-households had visiting dogs. The difference between the dog-owning households in 2010 and 2011 was statistically significant. In 2010, on average 5 other dogs (n=36) in the dog-owning households and 4 other dogs (n=203) in the non-dog-owning household visited the compound. In the dog-owning households in 2011, 4 other dogs (n=24) on average visited the compound. In 2010, 55% of the interviewees from the dog-owning households (95% CI: 44%-

66%) and 59% of the interviewees from the non-dog-owning households (95% CI: 51%-66%) knew the owners of the visiting dogs.

4.3.8. Follow up survey through telephone interviews

The follow up interviews conducted by mobile phone worked well. We conducted 77% (n=248) of all interviews during follow up 3 using mobile phones.

4.4. Discussion

In this study we showed that the dog human ratio was low with 1:121 and that the domestic dog population in Bamako was estimated at 15 000 dogs. A high proportion of young dogs were found as a result of a high turnover rate in the population. Mortality within the first year of life was high (73%) and dogs had a life expectancy of 2.5 years at birth. Although we recorded a decrease of the domestic dog population during the observed two years, we estimated an annual dog population growth of 20% in the projection matrix. This may indicate an oscillatory dynamic of the dog population. A larger cohort of dogs should be studied for a longer period to further confirm our findings. Dogs were primarily used as working animals and fed with household leftovers and garbage. Dogs were most often acquired and given away with no remuneration.

Table 6 shows a summary of urban dog ecology across Africa (Gsell et al., 2012; Hambolu et al., 2014; Kitala et al., 2001; Léchenne et al., 2016; Mindekem et al., 2005; Morters et al., 2014a, 2014b; Seghaier et al., 1999). This helps to integrate the dog ecology from Bamako into the existing knowledge.

Table 6: Comparison of urban dog ecology across Africa

Location	Dog : human ratio	Dog density (dogs/km2)	Dog-owning households (%)	Dog mean /dog-owning household	Dogs / household	Average dog age	Female : male ratio	0-1 year old (%)	Mortality within the first year of life	Life expectancy at birth	Average number of litters whelped per adult fertile female/year	Average pups born per litter	Population growth	Ref
Bamako, Mali	1:121	56	9%	1.29	0.13	3.2	1:2.8	40%	73%	2.47	0.5	4.8	20%	
Iringa, Tanzania	1:14	334	13%	*2.35*	0.31	2.2	1:1.4	52%	72%	1.76	0.6	5.5	10%	Gsell et al., 2012
Tunis, Tunisia	1:16	916	23%	1.79	*0.42*	-	-	-	-	-	-	-	-	Seghaier et al., 1999
Lagos, Nigeria	1:6	-	95%	2.8	2.7	-	1:0.7	32%	-	-	-	-	-	Hambolu et al., 2014
N' Djamena, Chad	1:22	105	28%	1.25	0.4	3	*1:3.9*	32%	29%	3.11	0.4	4.7	-	Mindekem et al., 2005; Léchenne et al., 2016; Mindekem & Léchenne, unpublished data
Machakos District, Kenya	1:8	13.5	63%	2.1	1.4	1.9	1:1.5	*50%*	39% for ♀	2.9	*0.6*	4.7	9%	Kitala et al., 2001
Zenzele, Johannesburg, South Africa	*1:32*	449	10%	1.3	*0.46*	-	~1:1	*25%*	-	-	-	5.7	-	Morters et al., 2014a, 2014b

Numbers in italics : calculated from the respective Ref

Despite the low dog human ratio and dog density, the dog ecology data from Bamako is similar to that in other African settings. Dog human ratios in urban areas of the developing world range between 1:1.1 in the Philippines and 1:83-214.6 in Tanzania (Davlin and Vonville, 2012). As an example of a developed country, the dog human ratio in the city of Zurich, Switzerland was estimated at 1:60 (Steinbrecher, 2010). Lower dog densities were found in rural than in urban areas. In Kenya, densities in the rural locations were estimated between 6-21 dogs/km^2 (Kitala et al., 2001). Much higher dog densities (> 1000 dogs/km^2) were estimated in different South American countries (Davlin and Vonville, 2012). The mean age in our study was different between the two surveys, with 42 months in 2010 and 34 months in 2011. This could be a result of the variability and the effect of the small sample. Alternatively, this could also have arisen due to the different interview teams used during the two surveys. Muthiani et al. (2015) calculated a mean age of 31 months during a rabies vaccination campaign in Bamako. There is a clear preference of dog owners for male dogs over female dogs in Bamako. This result is similar to the result from N'Djamena (Mindekem, unpublished data). Interestingly, a study conducted in the communal lands in Zimbabwe showed a male female ratio of 1:0.8 (Butler, 1995). The predominance of female dog compared to male dogs in Lagos, Nigeria, was probably a result of the use of dogs for breeding purposes (Hambolu et al., 2014). An average of two years between two litters was estimated for Bamako. For Iringa, Tanzania 1.7 years between two litters was estimated (Gsell et al., 2012). In contrast to Bamako, none of the female dogs less than one year old had whelped in the Kenyan (Kitala et al., 2001) and Chadian (Mindekem, unpublished data) studies.

Using our dog cohort, we could estimate immigration and emigration rates, which was a big advantage compared to static vertical life tables and the Leslie matrix, where survival and mortality could be overestimated due to immigration and emigration of dogs. But while we observed a decline of dogs during the follow up period of two years in the deterministic model, we estimated a population growth of 20% in the Leslie matrix. This likely indicates an oscillatory dynamic of the dog population, which would be in agreement with the synchronous cycles of rabies transmission observed by Hampson et al. (2007). However, our sample size was small and the deterministic model was fitted to a very limited number of time points, limiting the ability to draw an overall conclusion from the calculations. Nevertheless, our questionnaire data underlined emigration and immigration of dogs in Bamako regarding the high proportion of dogs which were freely given away or acquired. Most of the dogs were accepted as a gift when they were less than 2 months old, and this result is in line with those of Kitala et al. (2001) in Kenya and the systematic review of Davlin and Vonville (2012). In rural areas of Mali, dogs are more often purchased than in the capital (Traoré, personal communication). This was also the case for Lagos, Nigeria where most of the dogs were bought

(Hambolu et al., 2014). The high female subadult mortality/emigration rate in the deterministic dog demography model raised suspicion that subadult female dogs are given away more often than male dogs. In contrast a male subadult mortality/emigration rate of -0.4 could be an indicator for increased immigration in that age class. The female male ratio of 1:2.8 supports this observation. Emigration and immigration of dogs in Bamako is therefore an important factor in the population dynamics. We estimated a lower yearly birth rate in the deterministic dog demography (1.4 puppies per female per year) than during the baseline surveys (2 puppies per female per year). The decrease of our dog population in the deterministic dog demography model could have also been affected to a certain extent by the poisoning campaigns which take place annually in Bamako (25 dogs died in commune 4 in 2010 (Traoré, personal communication)). Relative to rabies control, such a practice is unlikely to interrupt disease transmission and should not be continued (Morters et al., 2013; Zinsstag et al., 2009). Morters et al. (2014b) also recorded a decline in the dog cohort over three years for Zenzele, South Africa. Similar to our study, less than 20% of all dogs were born in the household in Zenzele, South Africa (Morters et al., 2014b). In contrast to our study, the proportion of losses due to mortality was much higher in Kenya than other reasons for emigration (Kitala et al., 2001).

In contrast to Tanzania (Gsell et al., 2012), Tunisia (Seghaier et al., 1999) and Nigeria (Hambolu et al., 2014), where 63%, 65% and 64% of investigated dogs were confined at all times, dogs in our study were permitted to roam freely in 55% of all cases and only 10% of dogs were confined during day and night. This result was in contrast to the studies of Muthiani et al. (2015) and Mosimann et al. (2017), where higher levels of confinement were estimated in three communes in Bamako. But results similar to ours were obtained in the study of Mindekem et al. (2005) in Chad and Kitala et al. (2001) in Kenya, where the majority of dogs could freely roam. It is worth noting that most of the dogs in our study nevertheless spent their day in the compound. Partially confined dogs are more likely to be confined during the day than during the night in the Chadian, Kenyan and Malian studies, probably in order to guard the household during the day when people are not in the compound. It is suspected that partially confined and free roaming dogs are foraging for food in the streets (WHO, 1987). Similar to our results, the majority of dogs in N'Djamena spent the day nonetheless within the compound, however, the proportion of dogs which spent the day in the street was at 20%, higher than the 4% we estimated. Regarding dog nutrition, there was agreement between the study from Kenya (Kitala et al., 2001) and our study. The majority of dogs in both studies were fed with household leftovers and waste. In our study some of the animals were also fed with raw meet, which carries the risk of parasitic infection. Morters et al. (2014b) showed that almost all of the identified dogs were owned and regularly fed by their owners. It is suspected that dog populations are regulated by human demand and that environmental resources to support the nutrition of dogs are probably not sufficient. The same is expected for Bamako.

Only a small fraction of dogs were reported to be spayed or castrated in Bamako. In Iringa (Gsell et al., 2012), none of the animals were spayed or castrated, while in Kenya (Kitala et al., 2001), 15% of male dogs had been castrated and none of the female dogs had been spayed.

As with our results, dogs are primarily kept as watchdogs in N'Djamena, Chad (Mindekem et al., 2005), Machakos, Kenya (Kitala et al., 2001) and in general, in urban areas of the developing world (Davlin and Vonville, 2012). This explains the preference for male over female dogs, as there is a belief that male dogs are better guardians and hunters (Kitala et al., 2001). Belonging to the ethnic group of *Bobo* favoured dog ownership in Bamako, which is not surprising given that dog meat consumption is common in this ethnic group. In contrast to our study, where wealth was not statistically significant, the odds of dog ownership was greater in wealthier and bigger households with a better educated head of the household in Tanzania (Knobel et al., 2008). However, only three of the interrogated households belonged to the less wealthy group in Bamako.

Whereas in N'Djamena (Mindekem et al., 2005) being Muslim was the main reason for not keeping a dog, in Bamako non-dog owners mainly did 'not feel the necessity' of having a dog. In both countries, Christians were more likely to be dog owners than Muslims. In Kenya, a common response to non-dog-ownership was that previous dogs had died and a replacement was being sought (Kitala et al., 2001) and similar to our results, only a small fraction answered that they did not like dogs. Interestingly, in contrast to our study, the fear of rabies and costs of post exposure prophylaxis was in 20% a reason for non-dog-ownership in Kenya. The most common answer in Tanzania for not having a dog was that interviewees disliked dogs, followed by lack of time and space. Only 17% did not feel the necessity to have a dog. Similar to Bamako, Muslim households were less likely to own dogs (Knobel et al., 2008). In Senegal, Muslims and Christians owned dogs, and religion was not a reason for not keeping a dog (Nodjimadji, 2008).

The study conducted by Butler in Zimbabwe (Butler, 1995) also reported on other dogs visiting the households in almost 80% of cases. In Bamako, approximately 26% of all compounds were visited by outside dogs. But in both studies the majority of dogs were known to have owners. In Lagos, Nigeria (Hambolu et al., 2014) the majority of interviewees did not allow stray dogs into their compound.

We calculated a mean of 16 persons per compound. This result is slightly above the 13.2 persons per compound which were counted during the census in 2009 (RGPH-2009).

Using our research results and the results obtained from two pilot vaccination campaigns conducted in Bamako, we can now move ahead with planning city wide dog rabies elimination. In reference to dog movement, the reduction of dog contacts to avoid rabies transmission seems to be inappropriate, as does registration of dogs due to the high turnover rate. Dog elimination programs are counter-productive in dog rabies elimination campaigns, as well as being socially unacceptable and should be stopped (WHO, 2013; Zinsstag et al., 2009). Due to the high proportion of young dogs, we recommend establishing yearly rabies mass vaccination campaigns in Bamako. In a next step, a similar study should also be conducted in the rural areas of Mali, where dog ownership is likely to be more common (Traoré, personal communication). Further research should also be conducted on immigration of dogs from outside of Bamako.

4.5. Conclusions

This work contributes vital information towards planning effective and sustainable dog rabies control programmes for the District of Bamako. Due to the high turnover rate, we recommend repeated mass-vaccination campaigns of at least 70% of the owned dogs at yearly intervals. In addition, dog-owners need to be educated on good dog management. The reduction of dogs through city wide poisoning campaigns is not recommended.

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Appendix A. Formulas for the calculation of the life-history parameters of the tables 3. & 4.

l(x): Overall survival from the first age class x1 to a given age class.

$$l(x) = \frac{s(x)}{s(x1)}$$, l(x) is corrected for the number of years taken into account.

p(x): Probability of surviving from a given age class x to the next following age class (x+1)

$$p(x) = \frac{s(x+1)}{s(x)}$$, p(x) is corrected for the number of years taken into account.

d(x): Overall mortality from the first age class x1 to a given age class x

$$d(x) = 1 - l(x)$$

q(x): Age specific mortality before reaching the next following age class

$$q(x) = 1 - p(x)$$

e(x): Age specific life expectancy at a given age class x

$$e(x) = \frac{\sum_{x}^{xn} l(y)}{l(x)}$$, y is summed from age class x to age class xn at the end of life.

e(x) is corrected for the number of years taken into account.

m(x): Average number of female pups born per age class

$$m(x) = B(x) * b(x) * 0.5$$, proportion of female pups was estimated as 0.5

5. Factors associated with dog rabies immunisation status in Bamako, Mali

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Abstract

We conducted a cross-sectional survey in Bamako, Mali, to determine for the first time the seroprevalence of rabies antibodies in the dog population and people's knowledge, attitudes and practices (KAP) towards the disease and its control. Antibody detection was done with the fluorescent antibody virus neutralisation (FAVN) test, with a positivity threshold of 0.25 IU/ml.

We visited 2956 households in 2010 and 2011 and found 379 dogs in 279 households. Data was collected on 279 dog-owning households, on 1 017 non-dog-owning households and on 311 dogs. A serum or plasma sample was collected from 98 dogs. For 26 dogs we had sufficient data to describe the antibody decline over time after rabies vaccination using a quadratic regression.

Ninety percent of interviewed persons (95% CI: 85% - 91%) knew about rabies. The majority of interviewees knew that rabies is transmitted from dogs to humans, and some of the characteristic clinical signs seen in rabid dogs (change of behaviour, biting, salivation) could be listed by the majority. When asked how people behave regarding a rabid dog, killing the animal was the most frequent answer (> 70%). Most (65% of the non-dog-owners and 81% of the dog-owners) were aware that vaccination of dogs can prevent rabies, but only a minority of dog-owners could answer correctly at what age the dog should get a first rabies vaccination (i.e. at 3 months). There was also strong consensus among dog-owners that it is better to protect their dog from becoming rabid by vaccinating it rather than needing to treat a bitten person. Forty-five percent (n=306; 95% CI 38% - 52%) of dogs were reported as vaccinated against rabies at least once, but less than half of these (59/136) had a valid vaccination card. When asked for reasons for non-vaccination, cost was the most frequent reason at 31% (95% CI: 21% - 43%), while general negligence was mentioned by 15% (95% CI: 10% - 24%). Approximately one third of dog-owners would not pay for vaccination. To reach a threshold of 70% of vaccinated owned dogs, vaccination should not cost more than 0.2 € (100 FCFA).

The seroprevalence of rabies virus antibodies in the examined dog population was low: 24% (n=98; 95% CI 15%–36%) with titres ≥0.25 IU/ml and was 46% (n=39; 95% CI 29%–63%) when only including those reported as vaccinated by their owners. A seroprevalence of 59% (n=18; 95% CI 33%–80%) was reached if the analysis included only dogs with a valid vaccination certificate. Interestingly 4/22 dogs showed titres ≥0.25 IU/ml despite being reported as unvaccinated. The Rabisin® vaccine showed generally higher IU titres than the Dog Vac Rabia® vaccine. All animals after booster vaccination had titres ≥0.25 IU/ml which was not the case in primo-vaccinated animals. For the Rabisin® vaccine, a Kaplan Meier estimate suggested that to maintain an antibody titre of ≥0.25 IU/ml for 75% of owned dogs, revaccination should be done after not more than 2.5 years.

This work contributes vital information towards planning an effective dog rabies control programme for the District of Bamako.

Keywords

Rabies, dogs, Bamako, seroprevalence, KAP

5.1. Introduction

Rabies is a neglected zoonosis which, despite the availability of known effective control measures, remains a problem in the developing world (Nel, 2013). It is estimated that 59 000 people die per year due to the disease with the majority of cases in Asia (60%) and Africa (36%) (Hampson et al., 2015), where rabies is transmitted mainly by dogs. In Bamako, the capital of Mali with 1.8 Mio inhabitants, rabid dogs were confirmed to be responsible for the majority of human cases (Dao et al., 2006). Between 1995 and 2011, 68 human rabies cases were registered in Bamako and the virus was always transmitted by dog bites (Kone, personal communication, 2013). Kone (2013) calculated a human incidence of 0.37 cases per 100 000 inhabitants between 2007 and 2012 in Bamako. The mean annual rabid dog incidence for 2001 to 2013 was estimated at 2.2 rabid dogs per 1000 (Traoré, personal communication, 2014). It is important to note that there is significant underestimation of the true human incidence (Cleaveland et al., 2002) because surveillance across the country is insufficient.

In Africa, information on knowledge, attitudes and practices towards rabies has only been recorded in a few countries, for example in Chad (Kayali et al., 2003b; Mindekem et al., 2005) and Zimbabwe (Butler, 1995). Parenteral vaccination of dogs against rabies is a cost-effective control measure (Zinsstag et al., 2009), and in many African communities at least 60-75% of dogs are accessible for vaccination (WHO, 2005). This percentage corresponds also to the WHO recommendation to vaccinate at least 70% of the dog population for efficient rabies control (WHO, 2013).

In Africa, data on seroprevalence surveys and studies addressing the decline of antibodies after vaccination are rare. Millán et al. (2013) found a seroprevalence of 20% in Uganda and Kitala (2001) determined 29% in Kenya. The systematic review of Davlin and Vonville (2012) summarised pre vaccination campaign seroprevalences in Zambia, Chad and Tanzania, where all were below 20%. In Nigeria 43% of dogs had antibody titres exceeding the positive threshold, but this proportion was still far below the required prevalence to stop transmission (Olugasa et al., 2011). Additionally, these studies are difficult to compare since there is heterogeneity among the diagnostic tests, the chosen titre cut-off and the investigated dog population.

This study was undertaken to identify people's knowledge, attitudes and practices with respect to rabies in dogs and its control. We also evaluated the seroprevalence of rabies antibodies in the sampled dog population and used these data to estimate the duration of rabies antibodies in vaccinated dogs in Bamako, Mali.

5.2. Material and methods

5.2.1. Study area

The cross-sectional survey was undertaken in Bamako, the capital of Mali, located in the southern part of the country. The city is divided in 6 communes with a total of 67 quarters, and the national census recorded 1.8 million inhabitants (RGPH-2009). The city has a wet and dry savannah climate with average temperature highs above 30 °C in each month. The total land area is about 267 km^2.

5.2.2. Data and sample collection

Questionnaire interviews, whenever possible with the head of the household, were conducted in all six communes of Bamako. We randomly selected 32 out of a total of 67 quarters with selection probability proportional to the size of the human population of the communes. During the first baseline study in 2010 and the second baseline study in 2011, households were randomly selected with slightly different procedures. The survey in 2010 was conducted between May and June, while the one in 2011 was between April and May. Full interviews were conducted in all households in 2010, but only in dog-owning households in 2011. The sampling method for households was based on a random selection approach. During the study in 2010, five field teams, each consisting of one veterinary officer and one interviewer, started in a quarter at the household of the quarter chief, then spun a bottle and walked 200 meters in the indicated direction, where they flipped a coin to select one side of the road for the inclusion of two households in two neighbouring compounds. A compound was defined as all of the houses surrounded by a wall, which could include several households. A household is defined as an individual or a group, related or not, living within the compound under the lead of the household head. It is composed of the head of household, his spouse(s), his unmarried children and possibly other related or unrelated persons. The selection procedure was repeated until at least 38 households per quarter were enrolled and interviewed. In 2011, four field teams started at five randomly selected coordinates within the same quarters. The nearest block was then chosen, where interviews were conducted in households (only one per compound) with the team moving in a clockwise direction until at least 12 households were selected. The questionnaires included questions about knowledge, attitudes and practices (KAP) regarding rabies and its control by dog- and non-dog-owners as well as basic dog characteristics (age, sex, breed and function) of dogs older than 3 months old. Some of the KAP questions included open questions to record spontaneous answers which were followed by a list of probed answers. Spontaneous answers are more useful to direct the design of future intervention programs. Prior to the first baseline survey, meetings were held with the local chiefs of each quarter. The questionnaire was tested in a non-selected quarter. The questionnaire was in French and questions were translated into Bambara when necessary. The vaccination status of each vaccinated dog was cross-checked with its vaccination card. Blood samples of each encountered owned dog over three months old were collected by the veterinary officer from the saphenous vein into 5 -ml dry tubes and 2 ml EDTA tubes. Muzzles were used to prevent dog bites. Dogs were not sampled if the owner did not give consent or if a dog was uncooperative, aggressive or sick at the time of the survey. All dog-owning households were visited for follow up surveys to collect dog demography data, but these results are published elsewhere. When possible, additional blood samples to estimate the rabies antibody decline were collected one year later during the follow up survey for new dogs in the households.

5.2.3. Laboratory testing

The samples were kept in an icebox and centrifuged within 24 h of collection. Serum and plasma samples were stored at -20°C and shipped on dry ice to the Anses-Nancy Laboratory for Rabies and Wildlife for rabies antibody determination. The samples were analysed using the fluorescent antibody virus neutralization (FAVN) test (Cliquet et al., 1998). The titre of the serum or plasma sample corresponded to the dilution at which 50% of the constant viral dose was neutralised by the antibodies. The titres were expressed in IU/ml by comparison with the standard serum. The threshold of positivity was established at 0.25 IU/ml (Cliquet et al., 1998).

5.2.4. Data entry and statistical analysis

Data was double entered in Microsoft® Access 2010 and checked for inconsistencies using Epi Info™ 3.5.1. Statistical analysis was done with STATA IC 12.

KAP and seroprevalence data were analysed with generalized estimating equation models, taking clustering into account.

A quadratic model was used to analyse antibody decline in vaccinated animals over time. We excluded dogs which were vaccinated within the last 30 days (n=1) or still had high antibody titre five years after vaccination (due to the strong suspicion that not all vaccinations were recorded on the vaccination card) (n=1). For 26 dogs, we had both sufficient epidemiological information on vaccination date and serological result.

For the calculation of the proportion of dogs falling under the protective limit of 0.25 IU/ml after vaccination, a Kaplan-Meier survival estimate was conducted.

5.2.5. Ethical considerations

Research approval was granted by the Ministry of Livestock and Fisheries (Ministère de l'Elevage et de la Pêche; Ordre de mission no. 0382/LCV) and by the national Malian ethics committee of the Ministry of Health (Ministère de la Santé, Comité national d'ethique pour la santé et les sciences de la vie (CNESS); Approbation du CNESS no. 22/MS-SG-CNESS/2011) and is in compliance with the requirements of the EU FP7 project (FP7-KBBE-2007-1-3-09). All involved personnel were vaccinated against rabies following the instructions of the vaccine producer. During the survey, the research team maintained stocks of human rabies vaccines to provide post exposure treatment to persons possibly exposed to suspected rabid dog bites. All data were anonymised and handled confidentially.

5.3. Results

5.3.1. Basic demographics and KAP of dog- and non-dog-owners

We identified 379 dogs in 279 households/compounds during the two surveys. In total, 2956 households/compounds were visited. 125 dog- and 1 017 non-dog-households were interrogated in 2010 and 154 dog-households in 2011. Questionnaire data were collected for 311 eligible dogs.

Overall, nine out of ten interviewed persons in 2010 (total 1142; CI: 85% - 91%) were aware of rabies. There was no noteworthy difference between dog-owners and non-dog-owners in 2010 and no remarkable difference between dog owners in 2010 and 2011.

A total of 1 294 persons were asked during the two surveys which diseases were transmitted from dogs to humans. Dog-owners in 2011 less often spontaneously answered "rabies" than owners in 2010 (41% and 69%, respectively) (Fig. 20).

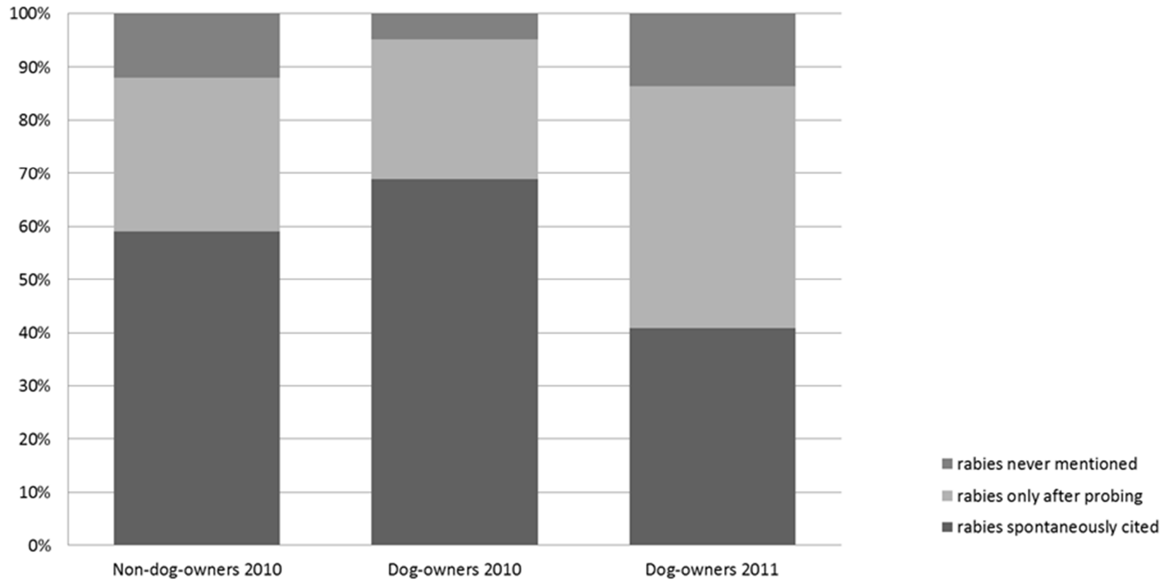

Figure 20: Answer given regarding the question about which diseases were transmitted from dogs to humans

With respect to clinical signs in rabid dogs, change in behaviour and biting were most often cited spontaneously in the three groups (Fig. 21). Other clinical signs like excessive loss of weight, open mouth, conjunctivitis, pendant tongue, paralysis, and hiding were also spontaneously mentioned more than once.

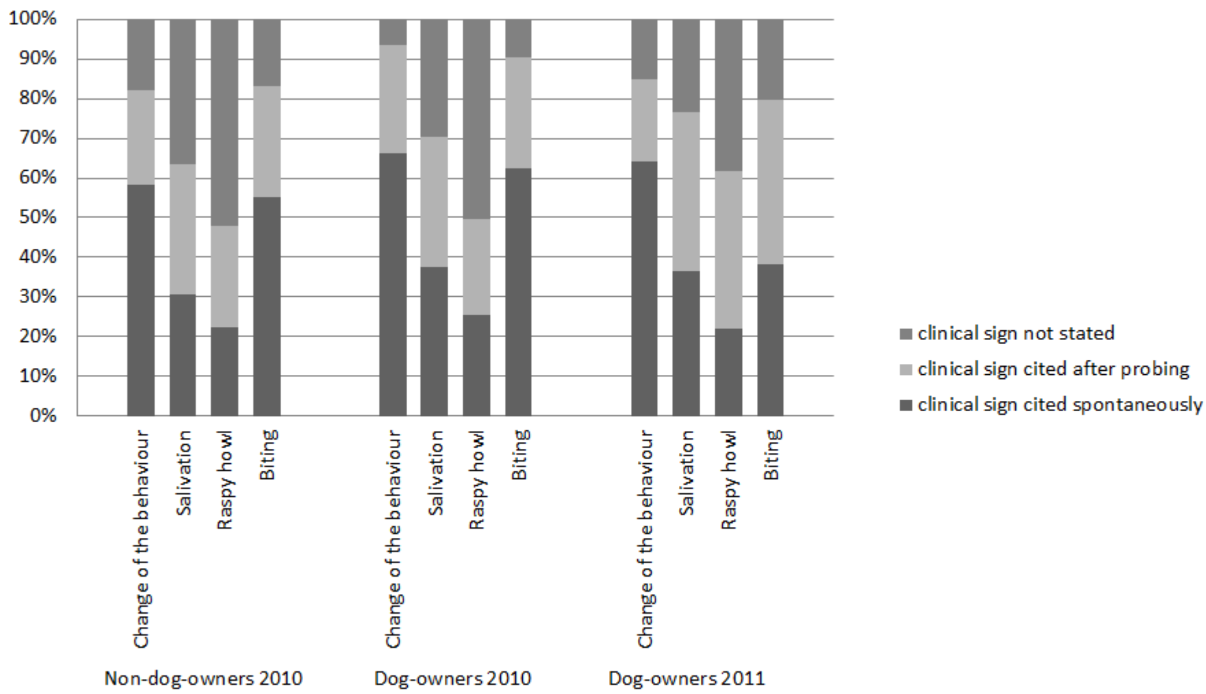

Figure 21: Answer given regarding the question about clinical signs of a rabid dog

We further asked about a person's behaviour regarding a suspicious rabid animal. Killing the dog was by far the most frequent spontaneous answer (Fig. 22). Tethering the dog, informing of responsible persons (owner, authorities, and police), giving the dog away to *Bobo* people (ethnic group who consumes dog meat), safety precautions for oneself and the family and treatment of the suspected animal were also cited.

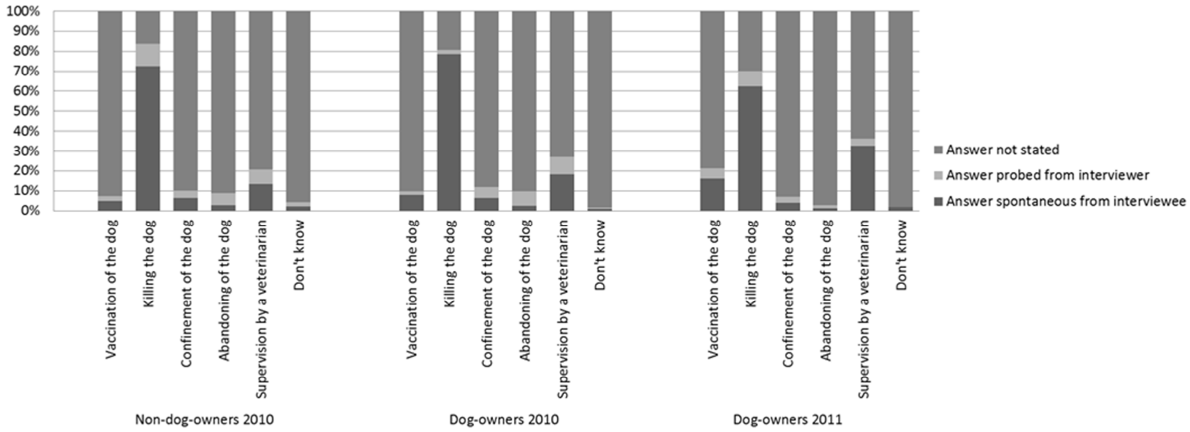

Figure 22: Answer given regarding persons behaviour faced with a dog who is agressive, salivates or changes his behaviour.

To allow a direct comparison we present subsequent only the results from dog- and non-dog-owners in 2010. However, we found no noteworthy difference in dog owners in 2010 and 2011. More than half (65% (CI 57% - 72%) of the non-dog-owners (n=1 015) and 81% (CI 72% - 87%) of the dog-owners (n=125)) were aware that vaccination of the dogs can prevent rabies. Thirty-three percent (CI 26% - 41%) of the non-dog-owners and 16% (CI 11% - 25%) of the dog-owners did not know which measures prevent dog rabies and 5% (CI 3% - 7%) of the non-dog-owners and 6% (CI 4% - 10%) of the dog-owners agreed that confinement of dogs is a method for prevention. Other preventive measures were good care of the dog, traditional medicine, no provocation of the animal, treatment and tethering of the dog.

Although the majority of dog-owners in 2010 and 2011 were aware that vaccination protects dogs from rabies, only 29% (n=276; 95% CI 23% - 35%) were aware of the age at which dogs should get a first rabies vaccination dose. The remaining 70% (n=276; 95% CI 63%-76%) did not know this and 2% (n=276; 95% CI 1% - 5%) were uncertain. However, when probing those who stated they knew the age, 49% (n=78; CI 38% - 60%) answered correctly with 3 months of age. Answers ranged from birth to 12 months old. Dog owners preferred preventive dog rabies vaccination over treatment of a bite victim.

Among the 306 surveyed dogs, 45% (95% CI 38% - 52%), were reported from dog owners as vaccinated against rabies at least once in their life, but only half of these (59/136) had a valid vaccination card.

Most dog owners remembered the date of the vaccination with 90% (53/59) agreement between the reported date and the date in the vaccination card.

The reasons given by dog owners for non-vaccination against rabies are listed in Table 7 for 160 out of 170 non-vaccinated dogs. Costs and general negligence were the most frequent reasons.

Table 7: Reasons for not vaccinating dogs

	Number of dogs	Percent	95% Conf.	Interval
Costs	58	31	21%	43%
Negligence	25	15	10%	24%
Useless	22	14	7%	25%
Lack of knowledge	16	11	6%	20%
No Access	11	8	4%	14%
Don't know	10	6	3%	13%
No time	7	5	2%	10%
Transport	5	3	1%	9%
Animal too young	2	1	0%	8%
Vaccination is planned	2	1	0%	5%
Animal too old	1	1	0%	4%
Planned to get rid of the dog	1	1	0%	4%

A total of 258 dog owners were asked how much they would pay for a rabies vaccination. Thirty-nine persons did not know how much and 132 said they would pay any amount. Figure 23 represents the reverse cumulative percentage of stated values of the 87 remaining dog owners.

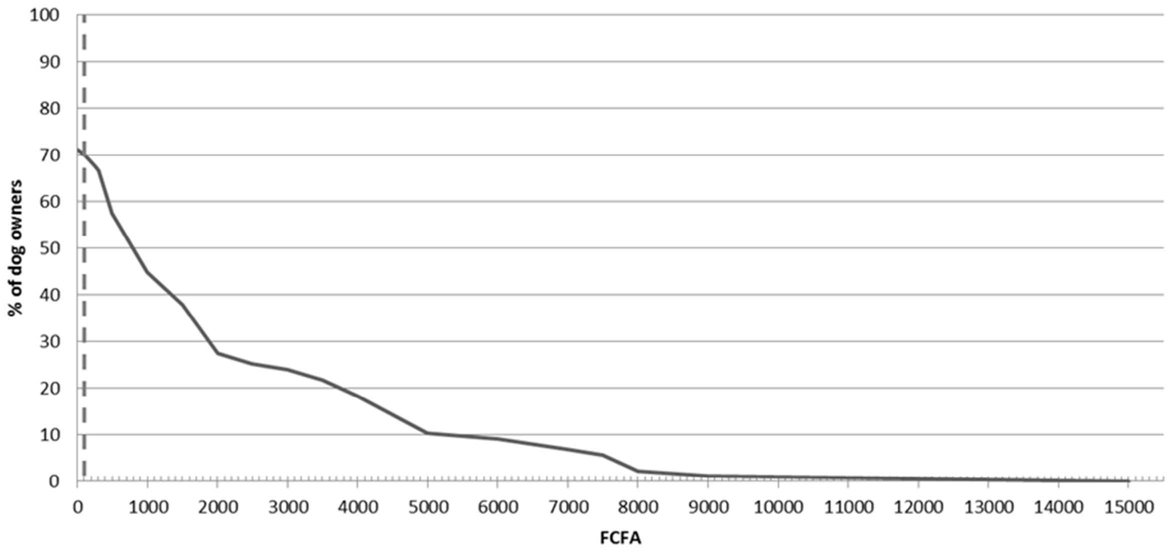

Figure 23: Willingness to pay for dog rabies vaccination in FCFA (1€ = 656 FCFA). Dashed line represents 100 FCFA (0.2 €).

Thirty percent (25/87) of dog owners would not pay for rabies vaccination. When the owners who stated they would pay any amount (n=132) are excluded from the analysis, dog vaccination against rabies should not cost more than 100 FCFA (1€ = 656 FCFA; 0.2 €) to reach a threshold of at least 70% owned vaccinated animals.

The reported costs for each dogs rabies vaccination (n=60) in this survey ranged from 1000 FCFA (2 €) to 10 000 FCFA (15 €) (median: 4525 FCFA (7 €); IQR: 3 000 FCFA (5 €) – 6 000 FCFA (9 €)).

In addition to willingness to pay, we inquired about the time dog owners spent getting their dog vaccinated against rabies by a veterinarian. Two third (82/121) spent less than half an hour, 16% (19/121) spent half an hour to one hour and 14% (17/121) spent more than one hour. In 2% of cases (3/121) the dog owners did not know the response.

5.3.2. Rabies virus antibody titres

Sufficient amount of plasma suitable for the FAVN test was obtained for 32% (98/311) of adult dogs collected during the two baseline surveys, and, of these, 24% (total 22; 95% CI 15% - 36%) showed rabies virus antibody titres ≥ 0.25 IU/ml (CI 15% -36%). There was no difference between the sample collected in 2010 and 2011.

When considering only the serological results of the dogs reported as vaccinated (n=39), 46% (total 18; 95% CI 29% - 63%) showed rabies virus antibody titres ≥ 0.25 IU/ml.

However, when only including dogs which had a valid vaccination card (n=18), 59% (total 11; 95% CI 33% - 80%) showed titres ≥ 0.25 IU/ml, and there was a significant difference between the samples collected in 2010 and 2011 (89% and 33% with titres ≥ 0.25 IU/ml, respectively).

Additionally, 4 out of 22 dogs reported as unvaccinated showed titres ≥ 0.25 IU/ml.

We had a total of 26 dogs (16 dogs from the baseline survey and 10 dogs from the follow up survey) with information on the vaccination date and the IU/ml, allowing for calculation of the vaccination antibody decline over time (full data are provided in this manuscript in the Appendix A). Figure 24 shows the antibody decline for vaccinated dogs. Included vaccines were Rabisin® (Merial, France), Dog Vac Rabia® (Ovejero, Spain) and Hexadog® (Merial, France).

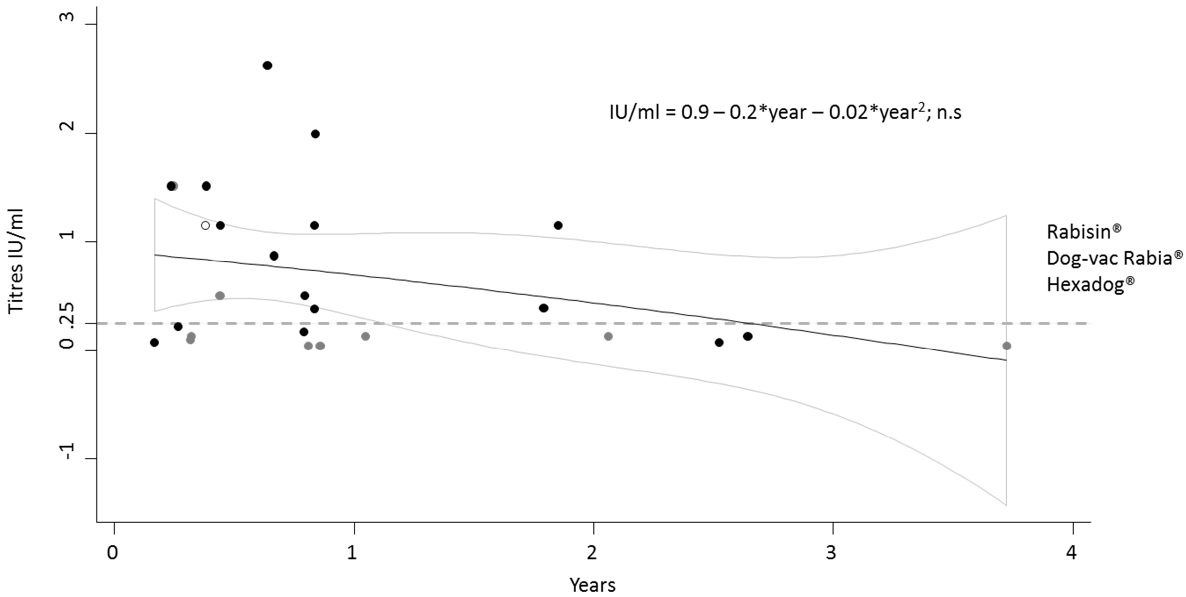

Figure 24: Rabies antibody decline in vaccinated dogs. Dashed line represents the threshold titre 0.25 IU/ml

Dogs vaccinated with Rabisin® (Merial, France) showed generally higher IU titres (n: 16, median: 0.69, IQR 0.2 – 1.33) than dogs vaccinated with Dog Vac Rabia® (Ovejero, Spain) (n: 9, median: 0.13, IQR 0.04 – 0.13). The time interval between vaccination and observation was not notably different in both groups (median: 290 vs 295 days). We did not further stratify according to age and sex or by number of received vaccinations due to small sample size. However, after primary vaccination only one animal (out of 12) had a titre above 0.25 IU/ml, whereas all dogs with a booster shot (4/4) had titres above 0.25 IU/ml (Appendix A).

For dogs vaccinated with Rabisin® (Merial, France), a Kaplan-Meier survival estimate (Fig. 25) revealed that 75% of vaccinated dogs would show positive antibody titres for up to 2.5 years.

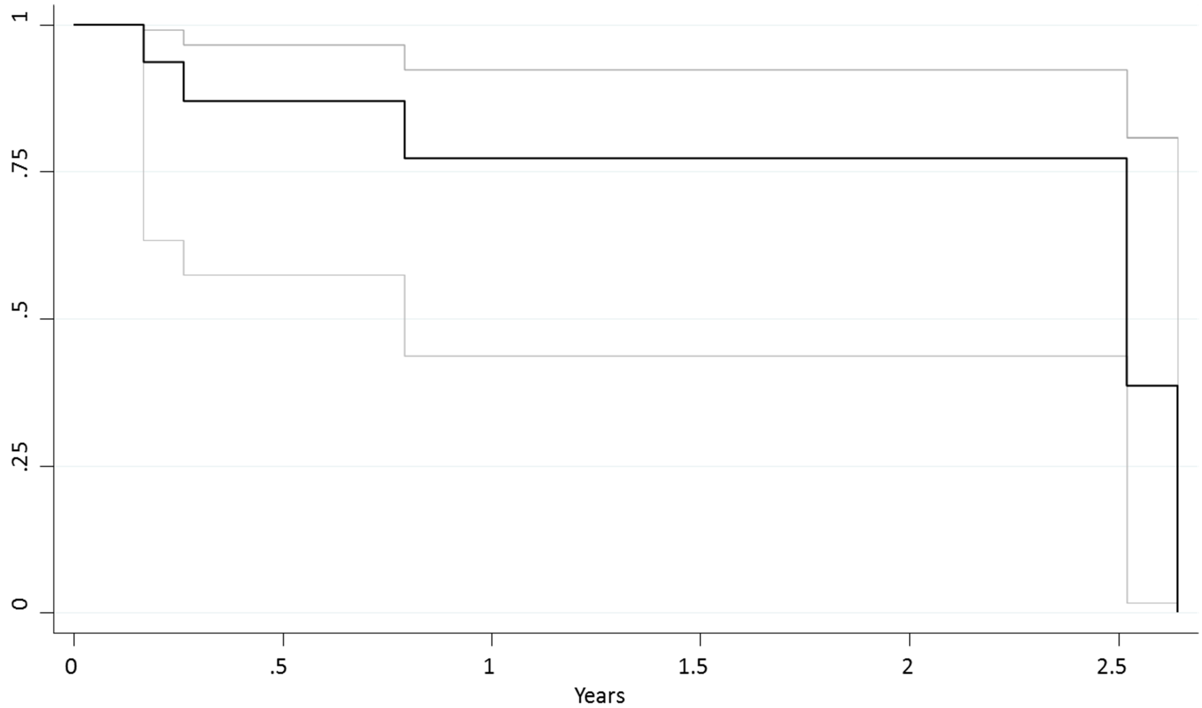

Figure 25: Proportion of dogs dropping below the threshold of 0.25 IU/ml after vaccination with Rabisin®

5.4. Discussion

This study was designed to investigate people's knowledge, attitudes and practices relating to rabies in dogs and its control in Bamako and to evaluate the spontaneous seroprevalence of rabies virus antibodies in the sampled dog population.

We showed that the majority of the interviewees were aware of rabies and knew that dogs can transmit the disease and that vaccination of dogs can prevent the transmission of the virus. However, less than half of the owned dogs were reported as being vaccinated, and dog owners often could not afford dog vaccination. Of all serologically tested dogs, 24% had antibody titres ≥ 0.25 IU/ml. Among dogs vaccinated with Rabisin® (Merial, France), 75% would have antibody titres ≥ 0.25 IU/ml up to 2.5 years after vaccination.

Our results are in line with those of Mindekem et al. (2005) showing that the large majority of dog owners from N'Djaména (Chad) are informed about rabies and three quarters know that the disease can be transmitted from dogs. In a study conducted in Zimbabwe (Butler, 1995), awareness of disease was also high, with 70% of all interrogated households being 'quite clear' about rabies. Among the dog households, almost all interviewees approved of rabies vaccination. Our data showed differences for spontaneous replies in 2010 and 2011. Unfortunately, we utilised different field teams in 2010 than in 2011 which might have contributed to this fact. Similar to our results, some clinical signs of rabies such as salivation, agitation, aggression and a pendant tongue were frequently mentioned in the study of Mindekem et al. (2005). There was also an agreement between our study and that of Mindekem et al. (2005) about reported behaviour when confronted with a suspected rabid dog. More than 70% would kill the dog and fewer would put the dog under veterinary observation or confine it. Some respondents in our study in Bamako would also give away a suspected dog for consumption, which could lead to human infection. We found the same proportion (19%) of vaccinated dogs with a valid vaccination card in Bamako compared to N'Djaména (Mindekem et al., 2005). In Tunisia, 57% of the investigated dogs had vaccination cards (Touihri et al., 2011). In contrast to Mali, mass vaccination campaigns started 30 years ago in Tunisia, and the population seems to be better sensitised.

Reasons for not vaccinating dogs, like lack of money and time, long transport distance or no transportation possibility and that the dog was either too young or too old were also reported in N'Djaména (Kayali et al., 2003b; Mindekem et al., 2005). During the first free pilot vaccination campaign in 2013 in Bamako, where the estimated vaccination coverage was only 18%, lack of money and the dog's age were mentioned, as well as lack of information about the campaign, as reasons for not participating (Muthiani et al., 2015).

It is not surprising that dog-owners preferred prophylactic vaccination of their dog with an injectable vaccine (1350 FCFA (2 €)) over treating an exposed person (54 300 FCFA (83 €)) (Traoré, personal communication, 2015).

To adequately improve the vaccination coverage of owned dogs, dog rabies vaccination should not cost more than 100 FCFA (0.2 €) in Bamako. However, the full cost of dog mass vaccination is more than 2 Euro in a comparable context in N'Djaména (Kayali et al., 2006) and the cost contribution of dog owners would be marginal. Hence, we recommend for logistical reasons that dog mass vaccination in Bamako and other Sahelian cities should be free to the owner (Dürr et al., 2008a). The willingness to pay is not the only barrier of access to dog mass vaccination at high coverage (Muthiani et al., 2015) and further research is needed to understand under which conditions a sufficient coverage to interrupt transmission can be achieved in Bamako (Coleman and Dye, 1996). In addition to the costs of the vaccination, there are indirect costs for the owner such as income loss due to invested time for vaccination even though for 70% this was not more than 30 minutes. Veterinarians or veterinary officers often vaccinate the dogs directly in the household (Sow, personal communication, 2011). Less time was spent on vaccination than in the cost-description of Kayali et al. (2006), where it was assumed that the owner spent one hour of work time to visit the vaccination point during a vaccination campaign.

Similar to our results, Kitala et al. (2001) identified in a Kenyan dog population that only half of dogs reported as vaccinated against rabies had detectable antibodies (≥ 0.2 EU ml^{-1}). The dogs in Kenya were also vaccinated with Rabisin® (Merial, France), but a different antibody detection technique than the FAVN test was used.

We investigated only owned dogs and therefore the observed seroprevalence of rabies antibodies is probably slightly overestimated. However, (Muthiani et al., 2015) estimated the proportion of ownerless dogs at < 10% for Commune 1 in Bamako. The observed average duration of immunity is in the range of the observed life expectancy of dogs in Bamako. Considering the broad confidence limit shown in Figure 25 and the turnover of the dog population, an annual revaccination of dogs in Bamako should be recommended, which is in line with other recommendations (Morters et al., 2014a). This is a shorter interval than the manufacturer' instructions of one of the vaccine used (Rabisin® (Merial, France)), which recommend a booster one year after the primo-vaccination and then triennially.

The four unvaccinated dogs with positive antibody titres are interesting and raise the question about its source. Cleaveland et al. (1999) and Kitala et al. (2001) also demonstrated a proportion of unvaccinated dogs with detectable levels of antibodies. Stated reasons were false positive reactions or following a natural rabies infection. The possibility of vaccination without reporting would be another explanation which could not be excluded in our study.

Among dogs with primary vaccination, only one dog showed antibody titre above 0.25 IU/ml. In contrast all dogs that received booster shots (4/4) had titres above 0.25 IU/ml. These results are consistent with those obtained in vaccinated dogs and cats in France (Cliquet et al., 2003). This study showed also that the humoral response of a primo-vaccinated dog became significantly weaker 5 months after vaccination in contrast to repeatedly vaccinated dogs, in which the antibody titres did not depend on the time elapsed since the last vaccination (Cliquet et al., 2003). The results of our Kaplan Meier survival estimate should be interpreted cautiously. The fact that several dogs showed positive antibody titres up to 2.5 years could indicate that these dogs were vaccinated more than once. In addition the estimate is optimistic since the exact point in time to fall below the threshold of 0.25 IU/ml remains unknown.

Few studies in Africa dealt with the decline of antibodies after rabies vaccination. A study conducted in Tunisia (Seghaier et al., 1999) showed that after the annual vaccination campaign only 36% of vaccinated dogs had positive titres. After a primo-vaccination campaign conducted in Senegal, the mean titre in the dog population after one year was 0.25 EU/ml (analysis performed by ELISA) (Akakpo et al., 1993). Morters et al. (2014a) reported, in a study conducted in Indonesia and South Africa, that dogs with higher post-vaccination antibody levels at day 30 had correspondingly higher titres 360 days after vaccination. Between 20 and 40% of dogs had titres less than 0.5 IU/ml 360 days post-vaccination, and in contrast to our study, post-vaccination titres after three months were higher with some dogs having titres of 3 IU/ml. Our results are analogous to the antibody decline in Kenyan dogs (Kitala et al., 2001), where the mean titre of vaccinated dogs in the previous year was 0.6 EU/ml and in the previous two years 0.4 EU/ml, respectively.

Several factors contribute to differences in antibody responses such as the vaccine brand (Servat et al., 2015), the health and age of the dog, route of vaccination, and whether it was a first or repeated vaccination (Aubert, 1992; Cliquet et al., 2003; Coyne et al., 2001; Morters et al., 2014a).

Besides humoral response, cell mediated immunity plays also an important part in the protection against rabies. Dogs which seroconverted after vaccination are protected even if they no longer have detectable antibodies in the blood at the time of the challenge (Aubert, 1992). The serological monitoring of dogs following parenteral mass vaccination campaigns is no longer recommended by the WHO on a routine basis (WHO, 2013). Rabies virus antibodies show a rapid decrease, particularly in primary vaccinated dogs. Well-designed field seroprevalence studies are now recommended to assess the efficacy of novel vaccines, identify deficiencies during campaigns, such as vaccine potency, cold chain maintenance or vaccination team training, and evaluate post-vaccination seroconversion rates.

Because canine rabies is often a predominantly rural problem (WHO, 1987), a similar study should also be conducted in rural areas of Mali. More dogs should be sampled on the day of the vaccination and again 30 days later.

Considering that the vaccination coverage of owned dogs was too low to interrupt transmission of dog rabies in Bamako, a large-scale vaccination campaign against rabies, which includes puppies which are even less than three month of age, should be repeated annually (Morters et al, 2015; Seghaier et al., 1999; WHO, 2013). It will also be necessary to improve dog registration, awareness campaigns and rabies surveillance both before and after vaccination campaigns.

Conflict of interest

None.

Acknowledgments

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Appendix A.

Table 8: Rabies vaccination history of dogs

ID dog	Date of the last rabies vaccination	Vaccine brand	Vaccination status	Date of blood sample collection	Days after vaccination	Rabies antibody titre (IU/ml)
b175	03.03.2011	Rabisin	primovaccination	03.05.2011	61	0.07
b027	14.03.2010	Rabisin	primovaccination	08.06.2010	86	1.51
b045	21.01.2011	Rabisin	primovaccination	27.04.2011	96	0.22
b485	02.11.2008	Rabisin	primovaccination	11.05.2011	920	0.07
b460	06.09.2008	Rabisin	primovaccination	28.04.2011	964	0.13
b153	01.01.2011	Dog vac rabia	primovaccination	27.04.2011	116	0.1
b614	01.01.2011	Dog vac rabia	primovaccination	27.04.2011	117	0.13
b035	14.07.2010	Dog vac rabia	primovaccination	05.05.2011	295	0.04
b039	12.06.2010	Dog vac rabia	primovaccination	21.04.2011	313	0.04
b459	10.04.2010	Dog vac rabia	primovaccination	28.04.2011	383	0.13
b051	14.04.2009	Dog vac rabia	primovaccination	05.05.2011	751	0.13
b464	20.08.2007	Dog vac rabia	primovaccination	10.05.2011	1359	0.04
b607	08.12.2010	Rabisin	booster	27.04.2011	140	1.51
b331	16.09.2010	Rabisin	booster	17.05.2011	243	0.87
b025	27.06.2009	Rabisin	booster	04.05.2011	676	1.15
b183	10.02.2011	Dog vac rabia	booster	11.05.2011	90	1.51
b001	09.12.2009	Rabisin		20.05.2010	162	1.15
b158	08.09.2010	Rabisin		29.04.2011	233	2.62
b002	08.08.2009	Rabisin		24.05.2010	289	0.17
b003	08.08.2009	Rabisin		24.05.2010	290	0.5
b029	11.08.2009	Rabisin		11.06.2010	305	1.15
b030	11.08.2009	Rabisin		11.06.2010	304	0.38
b031	11.08.2009	Rabisin		11.06.2010	306	1.99
b306	13.07.2009	Rabisin		28.04.2011	654	0.39
b046	23.11.2010	Dog vac rabia		03.05.2011	161	0.5
b007	07.01.2010	Hexadog		26.05.2010	139	1.15

6. Molecular Characterization of Canine Rabies Virus, Mali, 2006–2013

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Abstract

We genetically characterized 32 canine rabies viruses isolated in Mali during 2006–2013 and identified 3 subgroups that belonged to the Africa 2 lineage. We also detected subgroup F rabies virus. This information should be useful for development of mass vaccination campaigns for dogs and eventual large-scale control programs in this country.

Introduction

Rabies causes an estimated 70,000 human deaths annually worldwide, and >99% occur in developing countries, of which ≈43% occur in Africa, where rabies virus circulates in the dog population (WHO, 2013). A person bitten by a rabid dog, if not given postexposure prophylaxis, has an ≈5% (if bitten on the hand) to 70% (if bitten on the face) probability of showing development of clinical rabies (Cleaveland et al., 2002). However, postexposure prophylaxis is often unavailable or unaffordable in many developing countries.

Numerous infectious diseases, including tuberculosis, malaria, dengue fever, and rabies, are present in Mali. The domestic dog is the major reservoir and vector of rabies in this country. Although disease surveillance is insufficient throughout Mali, the level of underreporting of rabies cases is unknown. Animal and human cases are recorded mainly in urban and suburban areas. Surveillance data reflect rabies mainly in Bamako (the capital of Mali; population 1.8 million), where rabies diagnostic testing is available.

A standard procedure is in place in Bamako for reporting of an animal bite. The bitten person should immediately contact the Division of Epidemiology, Prevention and Control of Diseases, which is part of the National Directorate of Health. Persons with suspected cases of rabies are referred to a specialized clinic (Lazaret Clinic) in Bamako. Dog owners are requested by the Division of Epidemiology, Prevention and Control of Diseases to bring their dogs to a veterinary clinic for a 15-day quarantine. Rabies diagnosis of suspect animals is made by the Central Veterinary Laboratory (CVL) in Bamako. A diagnosis of rabies in humans is based only on results of a clinical examination because of sociocultural reasons (Dao et al., 2006). In other cities in Mali, there are reference health centers, hospitals, and veterinary regional services for diagnosis (Mauti et al., 2015).

During 2000–2013, samples from 468 animals showing clinical signs of rabies or to whom humans were exposed were submitted to the CVL for rabies testing by using the fluorescent antibody test (Dean D et al., 1996). Of 468 animals analyzed, 447 (435 dogs, 4 cats, 4 cows, and 4 monkeys) showed positive results for rabies. Twenty-eight human cases of rabies were reported during 2007–2009 in Bamako, which indicated an incidence of 3.3 cases/1,000,000 persons/year despite 141 postexposure prophylaxis vaccinations/1,000,000 persons/year (Mauti et al., 2015). Assuming a dog:human ratio in Bamako of 1:121, the annual incidence of rabies in dogs is ≈2.24 rabid dogs/1,000 dogs during the past 13 years, which is higher than that observed in N'Djaména, Chad (Kayali et al., 2003a), which borders Mali.

A total of 306 (45.0%; 95% CI 38%–52%) of 680 dogs were reported as being vaccinated against rabies at least once. However only 59 (19.3%) of the 306 dogs examined had a valid vaccination certificate (Mauti et al., 2015).

In Bamako, an average of 1,470 persons are bitten by animals each year, of whom 1,427 (97.1%) are bitten by dogs (Dao et al., 2006). A total of 3,544 (60.3%) of 5,870 bitten persons are young adults, including 1,920 (32.73%) children <10 years of age. Men are bitten more often than women.

Four lineages (Africa 1–4) of rabies virus and several subgroups have been detected in Africa. All lineages include classical rabies virus species and vary by geographic area, virus evolution, and reservoir species (De Benedictis et al., 2010; Talbi et al., 2009). The most comprehensive study of western and central African rabies viruses included some isolates from Mali (Talbi et al., 2009). The purpose of our study was to obtain more detailed information on genetic characteristics of rabies virus circulating in Mali and to clarify the geographic distribution and transboundary spread of this virus in the canine population in Mali.

The study

During 2002–2013, a total of 468 specimens were submitted from various regions in Mali to the CVL for rabies diagnosis (Figure 26). Samples were tested by using the fluorescent antibody test (Dean D et al., 1996) and stored at −20°C for further analyses. We selected 100 samples (95 with positive results and 5 with negative results) for further testing on the basis of their geographic origin.

Supernatants (100 µL) of suspensions (10% wt/vol) of dog brains were deposited on test paper cards, which stabilize nucleic acids. Virus RNA was extracted from stabilized samples by using the Iprep PureLink Virus Kit (Invitrogen, Paris, France) and subjected to partial nucleoprotein gene amplification of a conserved sequence (positions 55–660) (Picard-Meyer et al., 2004). Virus RNA was tested by using a hemi-nested reverse transcription PCR (RT-PCR) and a real-time quantitative RT-PCR (Picard-Meyer et al., 2015). After amplification, PCR products were sequenced in both directions by Beckman Coulter Genomics (Takeley, UK) and specific primers. A total of 32 stabilized samples showing positive results by hemi-nested RT-PCR and real-time, quantitative RT-PCR were used for phylogenetic analyses (Table 9).

We constructed a maximum-likelihood phylogenetic tree (Figure 27) that excluded 15 duplicate sequences (Table 10) by using MEGA version 6 software (Tamura et al., 2013). We also constructed a maximum-parsimony haplotype network by using TCS version 1.21 software (Clement et al., 2000).

We analyzed phylogenetic relationships between 18 partial nucleoprotein gene sequences and 31 representative sequences of Africa lineages of rabies virus. This analysis (Technical Appendix; Figure 27) showed that all samples that belonged to the Africa 2 lineage were widely distributed in western and central Africa (Talbi et al., 2009), including Mali and neighboring countries (Mauritania, Guinea, Senegal, Niger, Nigeria, Côte d'Ivoire, and Burkina Faso).

We found <2.1% divergence between all isolate sequences. For 17 haplotypes, 10 sequences were identified as belonging to subgroup G; this subgroup also included 3 sequences from Mali, Mauritania, and Senegal. Seven sequences (forming 6 haplotypes; RV88 was identical to RV90) belonged to subgroup H, which contained representative sequences from Côte d'Ivoire, Mauritania and Mali. One sequence from Mali (isolate RV57) belonged to subgroup F, which was similar to sequences from neighboring countries (Niger and Burkina Faso). Our data indicate that subgroup H might contain 2 distinct groups (Technical Appendix; Figure B)

Analysis of the nucleoprotein gene identified canine rabies subgroups G and H in Mali, as reported (Talbi et al., 2009), and subgroup F, which was found throughout Burkina Faso and Niger (De Benedictis et al., 2010). Subgroup G circulates in Mauritania, Burkina Faso, and Senegal. Subgroup H contains viruses from Mauritania, Mali, Burkina Faso, and Côte d'Ivoire. The RV57 isolate included in subgroup F was isolated from a rabid dog at the border with Niger in 2010. Strong nucleotide identity (99.6%) was shown between RV57 and the strain isolated in Niger in 2010 (Genbank accession no. EU853646).

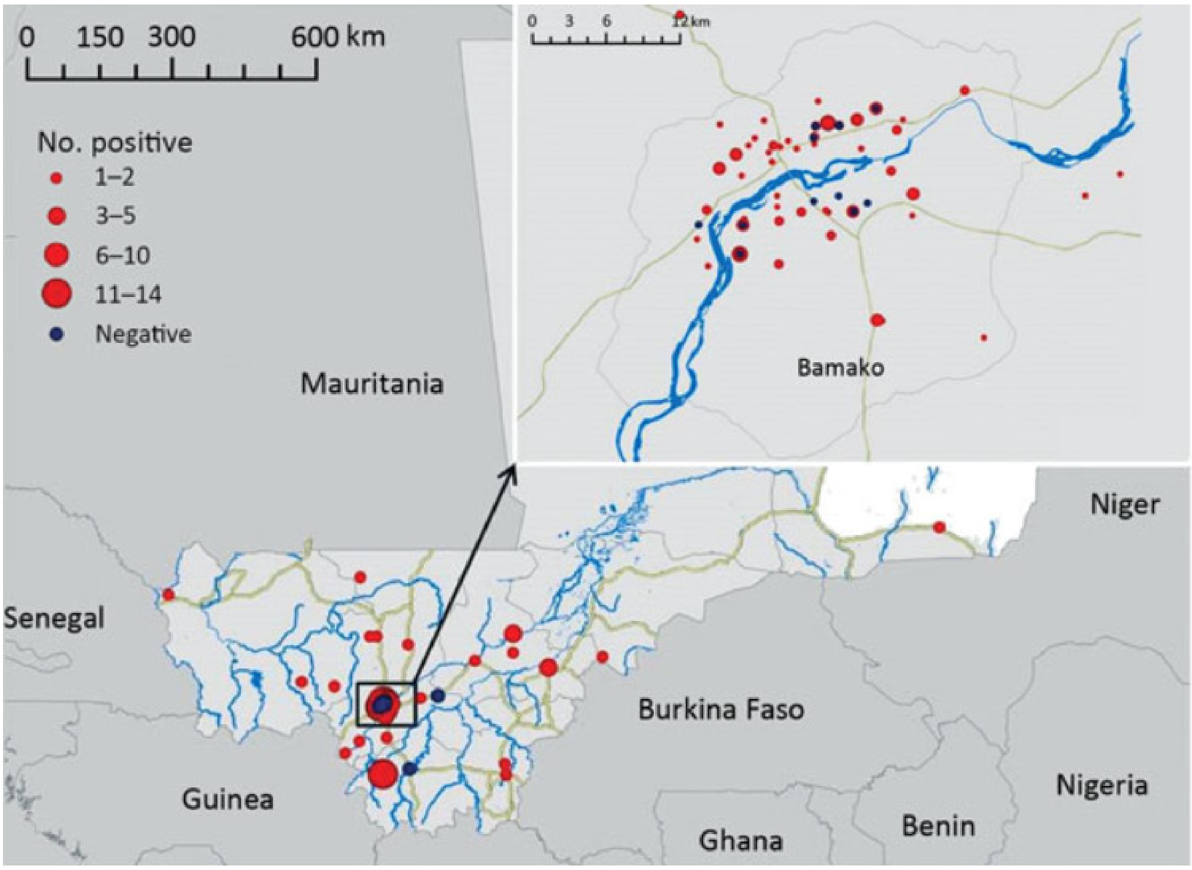

Figure 26: Locations of origin for 100 specimens analyzed in this study (95 with positive results and 5 with negative results) submitted for rabies virus diagnosis, Mali, 2002–2013. Insert shows closer view of the area near the capital city of Bamako.

Table 9: Characteristics of 32 rabies virus samples from dogs, Mali, 2006–2013*

Virus	GenBank accession no.	Sample ID no.	Region	Quantitative RT-PCR C_t	Subgroup of Africa 2 lineage
RV01	KP976113	420/2006	Bamako	28.51	G
RV04	KP976114	345/2007	Bamako	30.19	H
RV05	KP976130	352/2007	Bamako	25.35	G
RV06	KP976125	58/2008	Bamako	24.09	G
RV09	KP976126	146/2008	Bamako	27.51	G
RV10	KP976122	154/2008	Ségou	25.75	G
RV11	KP976124	167/2008	Koulikoro	23.97	G
RV14	NA	259/2008	Bamako	31.59	H
RV15	KP976123	261/2008	Ségou	26.14	G
RV19	NA	530/2008	Bamako	27.85	G
RV20	KP976117	003/2009	Bamako	32.82	H
RV22	NA	69/2009	Bamako	26.15	H
RV27	NA	118/2009	Bamako	32.30	H
RV44	KP976129	587/2009	Bamako	26.22	G
RV50	NA	19/11/2010	Bamako	27.90	G
RV51	NA	42/2010	Bamako	28.59	G
RV56	NA	171/2010	Koulikoro	22.20	G
RV57	KP976121	176/2010	Gao	21.90	F
RV60	NA	221/2010	Bamako	24.60	H
RV67	NA	603/2010	Bamako	21.30	H
RV68	NA	137/2011	Bamako	20.80	H
RV70	KP976119	149/2011	Bamako	21.70	H
RV79	NA	339/2011	Bamako	24.90	G
RV81	KP976127	357/2011	Bamako	34.20	G
RV84	NA	480/2011	Bamako	21.20	G
RV87	KP976116	612/2011	Bamako	22.50	H
RV88	KP976120	628/2011	Koulikoro	21.70	H
RV89	NA	674/2011	Bamako	20.20	H
RV90	KP976118	688/2011	Bamako	30.80	H
RV93	KP976115	223/2012	Bamako	23.20	H
RV95	NA	366/2012	Bamako	21.00	G
RV96	KP976128	100/2013	Bamako	29.00	G

*A fluorescent antibody test was conducted as described by Dean et al. (1996). For each tested sample, test paper was impregnated with 100 µL of 10% brain suspension and subjected to molecular biological analysis. Of 100 samples tested, 32 showed positive results by this test. A conventional hemi-nested reverse transcription PCR (RT-PCR) was performed with rabies virus primers JW12–JW6 as described (Picard-Meyer et al., 2004). All samples showed positive results by this test. A quantitative RT-PCR was performed with rabies primers JW12–N165-146 (Picard-Meyer et al., 2015). This PCR detected >100 RNA copies/µL. The coefficient of determination (R^2) was 0.999, the Y intercept was of 36.65, and efficiency was 99%. Samples in bold (n = 15) had duplicate sequences and were not subjected to phylogenetic analysis. ID, identification; C_t, cycle threshold; NA, not available.

Table 10: Characteristics of representative nucleoprotein gene sequences for rabies virus isolates, Mali, 2006–2013*.

Isolate	Haplotype	Identical sequences (–546 nt of the N gene)	Phylogroup
RV09	2	RV50, RV56, RV51, RV19, RV79	G (Africa 2)
RV96	6	RV84, RV95	G (Africa 2)
RV90	11	RV67, RV60, RV68, RV88, RV89, RV22	H (Africa 2)
RV87	14	RV14, RV27	H (Africa 2)

*All identical sequences have 100% nucleoprotein (N) gene identity on the basis of 546 nt (positions 71–618) compared with the reference isolate. RV, rabies virus.

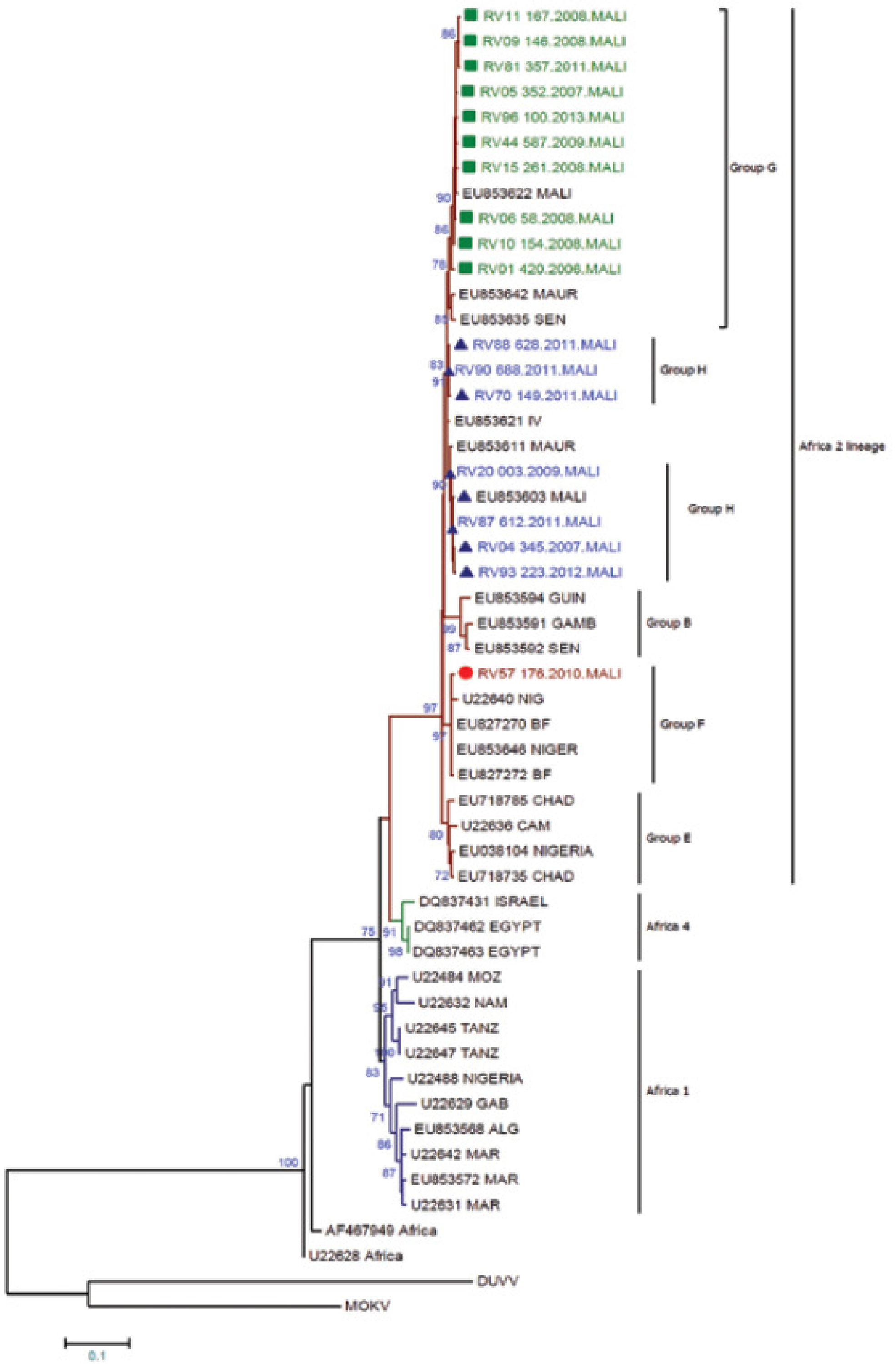

Figure 27: Maximum-likelihood phylogenetic tree based on a 564-nt sequence of nucleoprotein genes of 18 rabies virus sequences from Mali, 2002–2013, and representative sequences from Mali (n = 2), northern Africa (n = 6), South Africa (n = 2), West Africa (n = 32), and central Africa (n = 5). Sequences obtained in this study are identified in green, blue, and red. Green squares indicate genotype G, blue triangles indicate genotype H, and red circles indicate genotype F. The tree is rooted with 2 bat isolates used as outgroups Duvenhage virus (DUVV) (U22848) and Mokola virus (MOKV) (U22843). Bootstrap values (100 replicates) >70% are shown next to nodes. Alg, Algeria; BF, Burkina Faso; Cam, Cameroon; Gab, Gabon; Gamb, Gambia; Guin, Guinea; Maur, Mauritania; Mar, Morocco; Moz, Mozambique; Nig, Niger; Sen, Senegal; Tanz, Tanzania. Scale bar indicates nucleotide substitutions per site.

Conclusions

We identified 3 subgroups of the Africa 2 lineage of rabies virus in Mali. The presence of subgroup F could be explained by the movement of rabid animals across country borders. Previous studies reported rabies virus transmission by human-mediated animal movements (Fèvre et al., 2006; Talbi et al., 2010). The information we obtained in this study should be useful for development of mass vaccination campaigns for dogs and eventual large-scale control programs in this country.

Dr. Abdallah Traoré is a research scientist at the Central Veterinary Laboratory, Bamako, Mali. His primary research interest is diagnosis of viral diseases in livestock and poultry.

Acknowledgement

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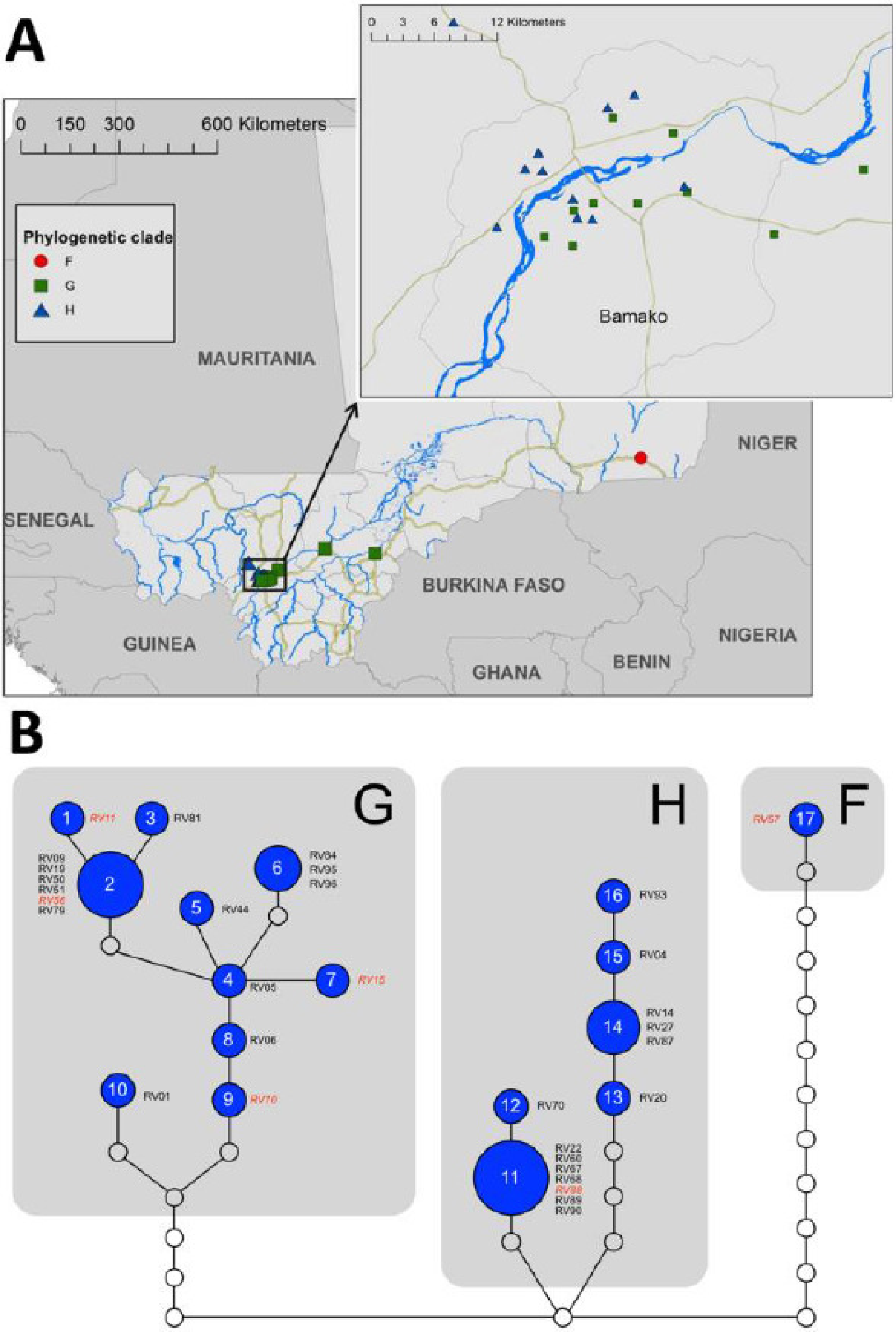
Technical Appendix



Technical Appendix Figure. A) Locations and phylogenetic clades for 32 rabies virus isolates obtained in Mali during 2006–2013. Clades were identified on the basis of results of gene phylogenetic analysis of nucleoprotein gene sequences. B) Maximum-parsimony haplotype network of 32 rabies virus isolates based on 564-nt nucleoprotein gene sequences. Numbers 1–17 indicate haplotype ID, dot size indicates number of samples per haplotype. Each line indicates 1 mutation, empty dots indicate haplotypes not sampled, Sequence codes in red and italics (Table 9 in main text) indicate samples not from the Bamako region.

7. Low coverage of central point vaccination against dog rabies in Bamako, Mali

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Abstract

Canine rabies remains an important public-health problem in Africa. Dog mass vaccination is the recommended method for rabies control and elimination. We report on the first small-scale mass dog vaccination campaign trial in Bamako, Mali. Our objective was to estimate coverage of the vaccination campaign and to quantify determinants of intervention effectiveness. In September 2013, a central point vaccination campaign – free of cost for dog owners – was carried out in 17 posts on three consecutive days within Bamako's Commune 1. Vaccination coverage and the proportion of ownerless dogs were estimated by combining mark-recapture household and transect surveys using Bayesian modeling. The estimated vaccination coverage was 17.6% (95% Credibility Interval, CI: 14.4–22.1%) which is far below the World Health Organization (WHO) recommended vaccination coverage of 70%. The Bayesian estimate for the owned dog population of Commune 1 was 3459 dogs (95% CI: 2786–4131) and the proportion of ownerless dogs was about 8%. The low coverage observed is primarily attributed to low participation by dog owners. Dog owners reported several reasons for not bringing their dogs to the vaccination posts. The most frequently reported reasons for non-attendance were lack of information (25%) and the inability to handle the dog (16%). For 37% of respondents, no clear reason was given for non-vaccination. Despite low coverage, the vaccination campaign in Bamako was relatively easy to implement, both in terms of logistics and organization. Almost half of the participating dog owners brought their pets on the first day of the campaign. Participatory stakeholder processes involving communities and local authorities are needed to identify effective communication channels and locally adapted vaccination strategies, which could include both central-point and door-to-door vaccination.

Keywords

Dog rabies, Mali, vaccination campaign, intervention effectiveness

7.1. Introduction

Canine rabies remains as an important public-health problem with most cases occurring in Asia and Africa. An estimated 55,000 human deaths occur each year (Knobel et al., 2005). Rabies, an important virus in the genus *Lyssavirus*, affects the nervous system, and is transmitted through the bite of a rabid animal with the virus in its saliva. Most human exposures in urban African settings result from the bite of a rabid dog. Immediate wound cleaning and rapid administration of post-exposure prophylaxis (PEP) are required to prevent rabies following a bite incident (Banyard and Fooks, 2011; Rupprecht et al., 2002).

Children are at highest risk of rabies encephalitis, which is an important cause of death among African children (Fèvre et al., 2005; Kayali et al., 2003a; Mallewa et al., 2007). The disease is endemic due to the interplay of social and cultural attitudes toward dogs, weak public- and animal-health systems and poor surveillance systems. In some African countries, there has recently been an increase in incidence of the disease in domestic dogs (Hampson et al., 2007). No country has been reported to be rabies-free in Africa, leading to the formation of international, national, private–public partnerships and networks to raise awareness and funds and evaluate effective rabies-control strategies (Dodet et al., 2008; Taylor, 2013).

7.2. Rabies in Mali

Few studies have quantified the incidence of rabies in Mali. A 4-year retrospective analysis of bites in humans and animals in Bamako district identified 5870 animal bite cases, with 10 human-rabies deaths between 2000 and 2003. From 119 brain specimens tested by direct immunofluorescence, 116 dogs and 1 cow were positive. More than half of the human cases occurred in children under 13 years, with dogs implicated in most bites. The authors further noted that most patients lived on the outskirts of the district and did not receive pre- or post-exposure vaccination against rabies. The single-dose cost (9000 CFA or 15 EUR) was prohibitive for most citizens in Bamako (Dao et al., 2006). Between 2007 and 2012, forty cases of human rabies were reported in Bamako resulting in an incidence of 0.37 per 100,000 person-years (Kone, 2013). The biting animal was almost always a dog (95%). In Mali, rabies was identified as a priority disease within WHO/AFRO's framework of the Integrated Disease Surveillance and Response strategy in 2008, but more needs be done to improve the quality of epidemiological data.

A study on dog ownership in Bamako showed a low dog–human ratio of 1/121 (Mauti, personal communication) which is lower than that reported for Ndjamena 1/20-1/30 (Mindekem et al., 2005). However, many dogs are reported to be vaccinated by owners on their own initiative. In a household survey, 45% of the dogs were reported as vaccinated against rabies, even though only half of the owners presented a vaccination book (Mauti, personal communication). This indicates that dog owners perceive the need for dog rabies vaccination. Because the disease is fatal, it is important to administer PEP as soon after a bite exposure as possible. In settings where PEP is not always available, it is important for the human- and animal-health sectors to implement long-term control-and-prevention programs, such as vaccination of dogs.

Although rabies-control methods vary across different geographic, cultural, and social settings, the most preferred is a combination of measures which includes canine vaccination and reduction of the stray-dog population (Macpherson et al., 2012). Morters et al. (2013) reviewed population density-reduction methods of rabies control such as culling and birth control, which assumes density dependent transmission of rabies and concluded that vaccination is the most effective method in all species (Morters et al., 2013). Currently, dog mass vaccination is the recommended intervention to control and eliminate dog rabies (Cleaveland et al., 2006a; WHO, 2007). Recent research has shown that dog-rabies mass vaccination is a more cost-effective method than human post-exposure treatment in an urban African setting, if sufficient coverage is achieved (Zinsstag et al., 2009). WHO recommends a dog mass-vaccination coverage of 70%, which is supported by evidence from dog–human transmission models where such coverage prevents outbreaks of canine rabies (Coleman and Dye, 1996). In N'Djamena, high coverage was achieved in a campaign that was cost-free to dog owner (Kayali et al., 2003b), whereas coverage was low when owners were required to pay (Durr et al., 2009).

Prior to initiating dog mass vaccination, it is important to know the effectiveness parameters of interventions. Even if a vaccine is highly efficacious, it is important to know whether it can be widely applied in a community. Several effectiveness determinants have been identified for interventions, including vaccine efficacy, accessibility, availability, affordability, adequacy, provider compliance, acceptability, and dog-owner compliance (Obrist et al., 2007; Zinsstag et al., 2011b). In preparation for dog-rabies control and elimination in Bamako, it is important to know what the achievable coverage in the intervention area and level of community participation in the target area are likely to be. The objectives of our study was to estimate the achieved coverage of a mass dog rabies vaccination campaign; to estimate effectiveness parameters, such as community participation; and to assess the feasibility of carrying out a free-to-owner central-point mass vaccination campaign.

7.3. Methods

7.3.1. Study site

Our study took place in Bamako district, Mali in September 2013 (rainy season). The national census recorded 1,800,000 people in 290,000 households in Bamako (R.G.P.H. 2009). The vaccination zone covered Commune 1, which includes 335,000 people living in 9 quartiers (neighborhoods), Banconi, Boulkassombougou, Sikoroni, Djelibougou, Doumanzana, Fadjiguila, Sotuba, Korofina Northand South Korofina, over an area of 35 km2. Bambara is the main language, and most people engage in formal employment, trading, or self-employment. Commune 1 was selected for the small scale trial due to its large population and close proximity to the Central Veterinary Laboratory (Laboratoire Central Veterinaire, LCV). An overview map is provided in Appendix A.

7.3.2. Dog-vaccination campaign

7.3.2.1. Campaign operation

The vaccination campaign was approved by the Ministry of Livestock and Fisheries (Ministre de l'Elevage et de la Pêche), Mali. Approval was granted by the Malian state authority because no animal-ethics committee exists in Mali. Three months before the start of the vaccination campaign, preparatory work involved pre-exposure vaccination of vaccinators and identification and pre-visit of vaccination points within the commune. A stakeholders' meeting with municipal authorities, chiefs of the quartiers, and religious leaders was held three weeks prior to the campaign to discuss the campaign and request their cooperation. During the same time, an information campaign was organized which involved distribution of posters in prominent places (next to schools, houses of local leaders, vaccination points) and radio messages broadcast in the local language.

7.3.2.2. Campaign design

The campaign had 17 teams, each with two veterinary officers from the National Veterinary Services and the Central Veterinary Laboratory. The positions of the vaccination points are provided in Fig. 28. Each team was equipped with chairs, table, vaccination certificates, needles, syringes, registers, animal markers, collars, first-aid kits, disposal bins, scissors, muzzles, and 10 doses of vaccine (Rabisin®, Merial) stored in a cooler box. The chief of the quartier was present at most vaccination points. Most vaccination posts were placed close to the residence of the chief of the quartier, with a few additional posts in front of well-known meeting points and schools. Campaign posters were hung in prominent places near the vaccination posts. Radio announcements in Bambara (local language) about the free vaccination campaign were broadcast from two local radio stations twice daily during the campaign period. Dog owners were invited to bring their dogs between 8 am and 5pm. At midday, supervisors drove between the vaccination posts to receive a progress update, change the cooling agents in the storage boxes, and drop off any additional supplies required by the teams. Most teams took a break to pray and eat.

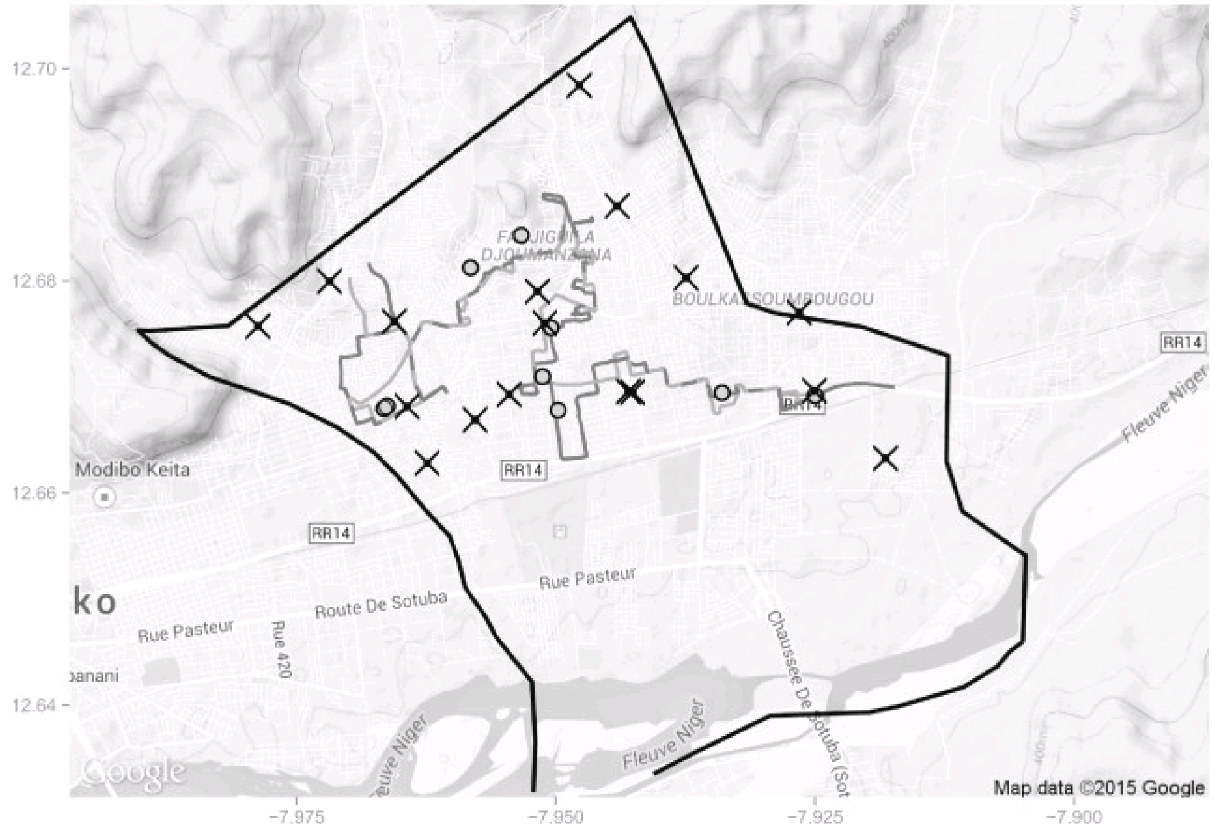

Figure 28: Map of commune 1 in Bamako, Mali. The black line shows the approximate boundary of commune 1. The diagonal crosses indicate the 17 locations of a central-point dog rabies vaccination campaign. The gray dots represent random points which served as starting points a subsequent household survey. Gray lines show two of the transect survey.

7.3.3. Vaccination and marking of dogs

The campaign took place on 3 consecutive days during the school holidays, starting on Friday 6th September with posts positioned in all neighborhoods of Commune 1. Merial (France) provided 3000 doses of Rabisin® vaccine, together with collars and vaccination certificates. Each dog was vaccinated using 1 ml Rabisin®, after which the dog description, age, and owner's address were recorded in the register. Finally, the dog was marked using a green collar (Merial), and a vaccination certificate was issued to the dog owner. The vaccination service was provided free of charge.

7.3.4. Transect survey

Following the campaign, we conducted a transect survey within the vaccination zone. Uniform distributed random points within the vaccination area were generated with Excel using the minimum and maximum coordinates of the vaccination points as boundary (those outside Commune I were discarded). Points were joined together to form a transect route which was followed four times (twice in the morning and twice in the evening on a single day, Fig. 28). The randomly generated points were downloaded into an Android phone with GPS capability, which was used to trace the transect route followed by the car. The length of the transect route was 15.5 km and the single surveys lasted approximately 2 h each. The car was equipped with a team of four persons (2 observers, 1 enumerator and 1 driver). We counted all dogs observed in the street, distinguishing between dogs marked with campaign collars and non-collared dogs.

7.3.5. Household survey

The household survey was conducted simultaneously with the transect study. A recapture sample size of owned dogs in households was calculated using the Petersen–Bailey formula for capture–recapture with direct sampling. The sample size calculation revealed that we would need to observe 420 dogs during the household survey to estimate the owned dog population with a precision in terms of the one side length of the 95% CI of 10% given the known number of dogs vaccinated during the campaign and assumed vaccination coverage of 55%. Households included in the survey owned at least one dog and reported residence in the survey area for more than one year. In our study, a household was defined as an infrastructural unit (a compound of several houses surrounded by a wall) rather than a social unit because several families and dog owners can reside in one household in Bamako. Nine interviewers visited households around nine randomly selected points within the vaccination zone. In each household, after seeking permission from the household head, the interviewers recorded the number of dogs owned, dog vaccination status, reasons for non-vaccination and confinement status of the dogs. In this context, a dog vaccinated by the campaign represents a recaptured dog for the vaccination coverage estimate of the owned dog population. The questionnaire was a shortened version of a questionnaire applied during a previous vaccination campaign. Only questions related to dog confinement and vaccination status and reasons for non-vaccination were kept.

7.3.6. Data management and statistical methods

The main focus of this study was to estimate the vaccination coverage of the total dog population (owned and ownerless). Data from the household survey were entered into Microsoft Access. The total number of vaccinated (marked) dogs, M_v, in the intervention zone is a known value taken from the register of each vaccination point. M_u, the total number of unvaccinated owned dogs, is an unknown value. The total number of owned dogs is defined as $M = M_v + M_u$. Because M_u is unknown, M is estimated by applying the Petersen–Bailey formula to the data collected during the household survey, with point estimate $M = M_v * (n + 1)/(m + 1)$ and variance = $M_v^2 (n + 1)*(n– m)/(m + 1)^2*(m+ 2)$, where n and m are the numbers of all recaptured dogs and recaptured marked (vaccinated) dogs, respectively.

The household and transect-survey data were fitted to a Bayesian model to estimate the owned-dog population and the ratio of ownerless to owned dogs in the study zone to compute the total vaccination coverage (Durr et al., 2009; Kayali et al., 2003b). Bayesian model parameters (see Appendix A) were estimated using Markov Chain Monte Carlo simulation in OpenBUGS (v3.2.3). Prior distributions for the confinement of vaccinated and unvaccinated dogs were estimated from the household survey, accounting for the fact that the dog population is composed of both owned and ownerless dogs. Owned dogs are found in houses or compounds of owners or in the street, depending on their confinement status. Ownerless dogs are estimated as proportion of owned dogs ($P_{tot} = P_{owned} + a*P_{owned}$). The proportion of ownerless dogs (a) is a latent variable which is estimated from the counts of dogs during transects. Dogs counted during the transect study are composed of dogs with campaign collars (owned vaccinated) and dogs without collars (owned unvaccinated and ownerless unvaccinated).

Lastly, an effectiveness-model framework was developed based on the previously described 'access framework' (Obrist et al., 2007; Zinsstag et al., 2011b). The intervention *Effectiveness* is considered as a product term of vaccine efficacy, α, multiplied by the coverage. Vaccination coverage is reduced by numerous factors, $\beta_{(i)}$, which are related to different aspects of provision and access to vaccination services which can often not be directly measured. The individual factors are usually derived from different data sources and are related in a multiplicative way (Eq. (1)), assuming that they are uncorrelated.

$$\text{Effectiveness} = \alpha \prod_{i=1}^{n} \beta_{(i)} \qquad (1)$$

However, in our study, several factors, all related to reasons for non-participation, were assessed simultaneously through an open question in the questionnaire. Because only a single answer was possible, the denominator for calculating the proportions requires adjustment and was calculated as $(N_{quest} – N_{\beta(i)}) * 3/5$. In this adjustment N_{quest} denotes the total number of responses and $N_{\beta(i)}$ the number of responses with reasons related to a certain effectiveness factor. Each reason was converted into effectiveness criteria by calculating one minus the proportion. Barriers (including natural hazards such as floods) and social barriers (like inability to handle aggressive dogs) were considered equal to (1 – campaign accessibility). Similarly, availability = (1 − declared lack of timely information), affordability = (1 − lack of perceived indirect costs), adequacy = (1 − dog and owner present at the time of vaccination), and provider compliance = (1 − vaccination team present at vaccination point). Finally, reasons leading to lack of perceived service needs, such as previous vaccination or advanced dog age, were considered as related to general acceptability. In this category, we included also all persons declaring no specific reason. The overall effectiveness was considered as a product term for all effectiveness factors.

7.4. Results

7.4.1. Vaccination operations and logistics

Central-point vaccination operations related to personnel, logistics and maintenance of the cold chain worked well. All animals brought to the vaccination posts were vaccinated. Children played an important role in bringing most dogs to vaccination posts. The vaccinators were able to handle all dogs brought to the posts, although a few dogs were vaccinated in the houses at the request of dog owners. There were no animal bite incidents reported at the vaccination posts.

7.4.2. Vaccination of dogs

We vaccinated 673 animals, of which 658 were dogs and 15 were cats. The mean age of vaccinated dogs was 31 months; with 21% being female and 79% male. The number of dogs vaccinated at each point varied remarkably between vaccination points (median: 26; range: 7–109). The most dogs were vaccinated on the first day (47%), with only 23% being vaccinated on the third day.

7.4.3. Vaccination coverage

According to the Peterson–Bailey formula, the vaccination coverage in owned dogs was 17.9% (95%CI, 16.6–19.0%), while the owned-dogs population was estimated at 3566 (95% CI, 3211–3922). During the transect study, the number of dogs counted was low, with a total of only 28 dogs in the four transect drives. The Bayesian model estimated the overall vaccination coverage as 17.6% (95% CI, 14.3–22.2%) for Commune 1. The owned-dogs population was estimated slightly lower, at 3459 dogs (95% CI, 2786–4131).The proportion of ownerless dogs was about 8% (see Appendix A for more details).

7.4.4. Household survey: previously vaccinated dogs and reasons for non-vaccination

During the household survey, we visited 356 households with at least one dog. In total, 438 dogs were recorded, with a median of 1 dog per household. Of these, 169 (38.6%) owners reported that the dogs had already been vaccinated prior to the campaign, while 80 dogs (18.3%) were vaccinated in the campaign, of those 28 (6.4%) dogs were vaccinated both previously and again in the campaign. The remaining 217 dogs were of unknown vaccination status. The most common reported primary reasons for not attending the vaccination campaign was the lack of information about the campaign (25.6%) and inability to handle an aggressive dog (16.1%). In 37%, no reason was given or the reason was unclear. Lack of money and time (5–7%) were stated as reasons for non-vaccination. In 5 (3%) cases, the dog owners found no vaccination team at the post (Table 11).

Table 11: Reasons for non-participation given during a household survey following a dog-rabies central-point vaccination campaign in Bamako, Mali (6.–9. September 2003).

Reasons	Dogs (n)	Percent	95% CI
Owner related reasons			
Dog owner absent	14	7.8	4.7-12.6
Lack of information	46	25.6	19.7-32.4
Lack of money	9	5.0	2.6-9.2
Late information	2	1.1	0.3-4.0
Dog related reasons			
Already vaccinated	2	1.1	0.3-4.0
Dog’s age	5	2.8	1.2-6.3
Aggressive dog	29	16.1	11.5-22.2
Others			
Floods	1	0.6	0.1-3.1
Vaccination team absent	5	2.8	1.2-6.3
Unknown reasons			
No reason or unclear reason	67	37.2	30.5-44.5
Total	180	100.0	

7.4.5. Estimates of effectiveness parameters

Coverage factors can be estimated by assessing reasons for non-vaccination directly after a vaccination campaign, as done in this study. In Fig. 29, the multiplicative relationship of different coverage factors is presented as gray bars, with a final effectiveness of 19%. To identify parameters which have the highest leverage on the campaign, we calculated how much the overall effectiveness would increase if a certain factor was increased by 5 percentage-points (truncated at 100%) (Fig. 29, column “sensitivity”). The most important parameter was the general acceptability (1.8 percentage-points), which unfortunately describes a heterogeneous group of reasons. Second most important was low availability, defined as a lack of or not timely information (1.4 percentage-points). Further accessibility was assessed as an important restriction. However, to overcome this limitation a house-to-house campaign should be employed. Affordability was also mentioned by dog owners. This was likely a communication issue because the campaign was free and indicates a lack of information, rather than perceived indirect costs.

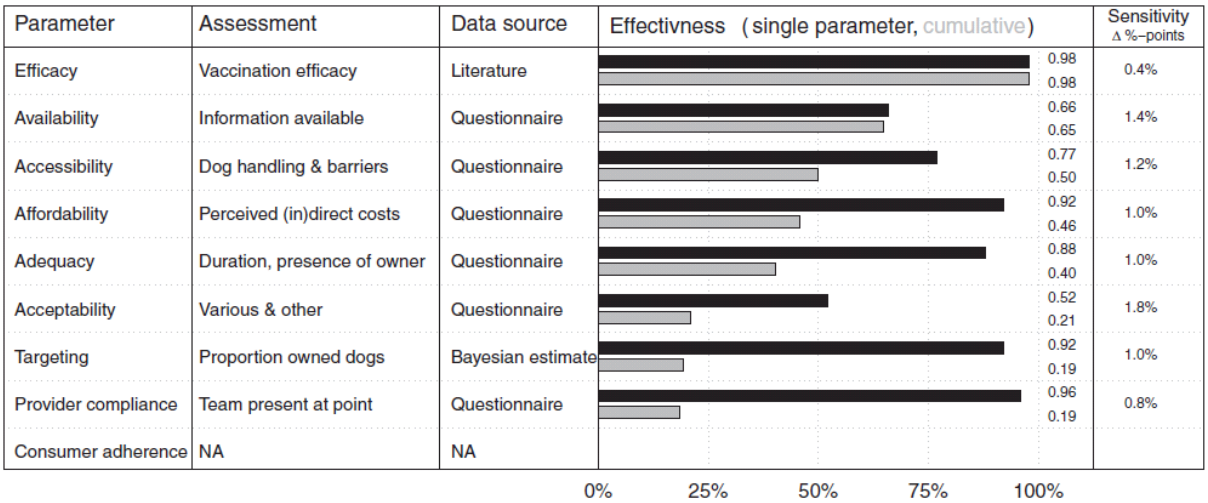

Figure 29: Contribution of parameters determining the effectiveness of a dog rabies vaccination campaign in Bamako, Mali. Individual parameters represent vaccination effectiveness and different aspects of coverage, including determinants of access to the intervention, targeting and provider compliance. Black bars represent the effectiveness associated with a single parameter (e.g., the intervention targeted on owned dogs but an estimated 8% of the total dog population are ownerless resulting in a effectiveness of 92%). Gray bars represent the cumulative effectiveness calculated as the cumulative product. Sensitivity was estimated as the increase in the overall (cumulative) effectiveness if a single parameter would increase by 5 percentage-points.

7.5. Discussion

This was the first attempt of a central-point vaccination campaign in the city of Bamako. The vaccination coverage for the total dog population was 17%, which is below the WHO recommended coverage of 70%. Other campaigns in urban areas have achieved much higher coverage through central-point strategy or a mixture of central-point and house-to-house strategy (De Balogh et al., 1993; Kayali et al., 2003b).

We estimated a relatively high level of confinement for dogs, which was higher for the dogs vaccinated during the campaign than for unvaccinated dogs. The proportion of ownerless dogs was estimated to be about 8%, which is comparable to that found in N'Djamena (Kayali et al., 2003b). We also observed from the household study that less than 13% of the households kept more than one dog, probably because families limit the number of dogs to a manageable number. Additionally, we observed few dogs in the street during the transect drive; this could be attributed to Commune 1 being a predominantly Islamic community which is unlikely to feed ownerless dogs.

A recent study in Mali found that dog owners vaccinate dogs on their own initiative (Mauti, personal communication); therefore, we can assume a level of knowledge about rabies in the community. Dog owners might perceive that keeping dogs confined reduces their risk of acquiring rabies infection from other animals. High confinement of owned dogs may explain low or non-participation, as owners may perceive vaccination of confined dogs is unnecessary. Confined animals have been shown to be more likely to be non-vaccinated (Awoyomi et al., 2008). Although community knowledge was not assessed during this study, misconceptions about rabies transmission and the importance of vaccination can be mitigated by awareness campaigns using radio and television.

Several reasons were cited by respondents for non-vaccination of their dogs. Dog related reasons for non-vaccination, such as inability to handle aggressive dogs, have also been identified elsewhere as deterrents to participation in central point vaccination campaigns. The proposed effectiveness model provides an explanatory framework for the low coverage achieved. Several coverage factors, such as acceptability, are not well known because no further reasons were given by the owners. However, this belongs to the most sensitive parameters for vaccination effectiveness and warrants more in-depth inquiry to identify how a dog rabies vaccination campaign in Bamako could be more effective.

The effectiveness model is a case example and has the potential to be more generally applied in other settings. In addition to the framework described by Obrist, we added accessibility to the animal and perceived adequacy and service need for treating an animal by the owner to reflect the control of zoonosis in an animal reservoir. Further research is needed to specify the role of the animal owner in health care provision for animals.

The variability of the parameters was inferred as absolute value change, thus those closest to zero have the highest impact. However, this revealed that the three most important parameters/variables to determine vaccination effectiveness were unknown reasons, lack of information and dog aggressiveness. Knowledge of these parameters would help to focus and prioritize these critical variables in order to increase the vaccination coverage in Bamako. The first two parameters could be addressed through use of a stakeholder approach where the community is actively involved in planning and implementing the campaign, for example, through educational campaigns, stakeholder meetings and participatory planning. Inability to handle an aggressive dog is the third most sensitive factor and this has also been observed in other central point vaccination campaigns. One way to overcome this challenge could be to carry out a combination of central point and door-to-door campaign where dogs could be sedated or captured using nets and then vaccinated in households.

Overall, the low coverage observed can be attributed to low participation by dog owners. Community engagement and support is necessary for rabies control (Kayali et al., 2003b). Dog-owner related reasons account for approximately 38% of non-vaccination in dogs. As in other African cities, there are many competing interests in regard to community public-health concerns, including an increase in non-communicable diseases contributing to an emphasis on other diseases viewed as more urgent.

There are limitations to our study: Only very few dogs were counted during the transect surveys, leading to an imprecise estimate of the proportion of ownerless dogs. Therefore, we conducted a sensitivity analysis and investigated the estimated the vaccination coverage for a fixed proportion of ownerless dogs of1% and 20%. The coverage estimate changed only slightly by 2 percentage-points. Details are provided in Appendix A. As in all observational studies, there is the possibility that non response might have biased our estimates. It might be even more pronounced in our case, since the study was conducted a predominantly Muslim setting. The reasons given for non-participation were very homogenous, thus it was challenging to identify the specific reason for poor participation. For more than 37% of the dogs, no clear reason was provided for non-vaccination. The respondent's knowledge and perception of rabies was also not investigated. Further studies comparable to (Davlin et al., 2013) could clarify reasons for non-participation and reveal community knowledge gaps for rabies by collecting more data on the socioeconomic situation, knowledge of rabies and detailed dog characteristics. Finally, our campaign was only implemented in one of the six communes in Bamako. Scaling-up might be associated with more public awareness and better mobilization of dog owners.

7.6. Conclusion and future steps

Despite low coverage, we recommend carrying out city-wide campaigns, as most of the reasons given by owners can be mitigated by feasible changes in vaccination campaign design, e.g., to carry out a mixed (door-to-door and fixed point) vaccination strategy for the districts of Bamako.

To increase the community participation, a stakeholder approach should be coupled with more robust communication campaign efforts in the next phase. Developing an effective community-mobilization strategy would further increase participation level. Because children brought most of the dogs (that were presented) to the vaccination points, this could be used as an entry point for advocacy on the importance of rabies prevention in the community. Lastly, more research is needed to validate the effectiveness model, particularly with regard to interactions between effectiveness factors.

Author contribution

SM did the preparatory work on dog ecology in Bamako before the campaign. YM and AT equally contributed to organization of the vaccination campaign and data collection. JZ, JH and YM performed data analysis. YM, AT and JZ wrote the manuscript. All authors approved the final version of the manuscript.

Conflict of interest

The authors declare that they have no financial or professional conflicts of interest relevant to this manuscript.

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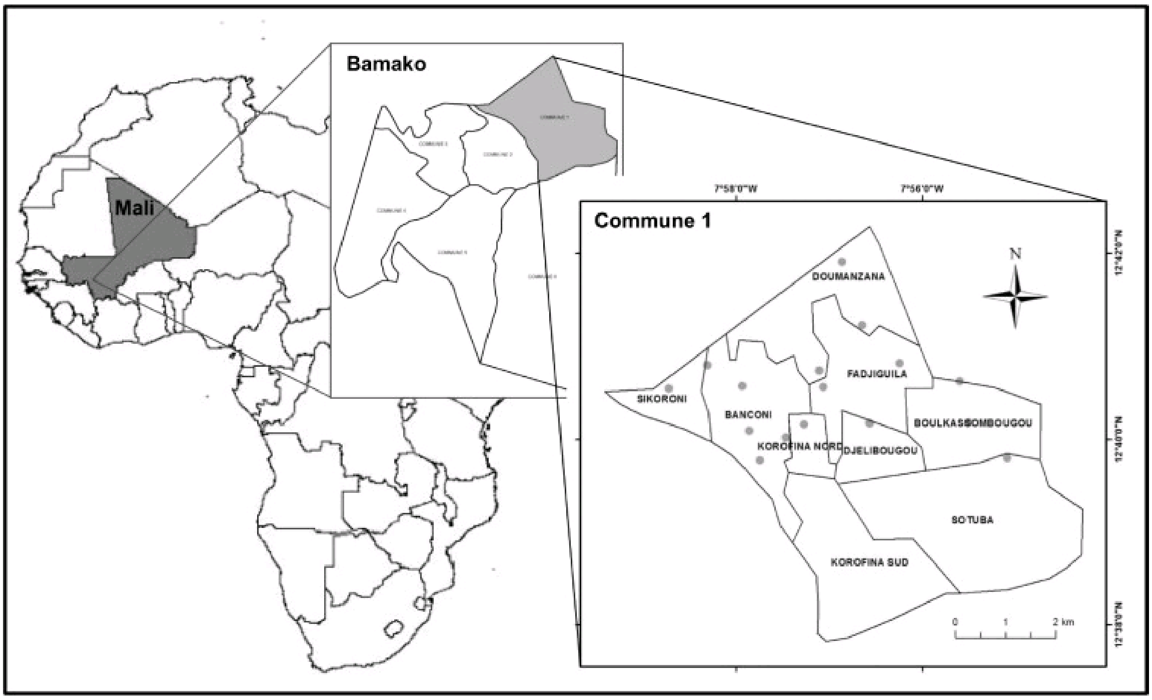
Appendix A



Overview map of study zone.

Appendix 1. OpenBugs code for dog rabies vaccination coverage estimation in commune 1 in Bamako 5-7 September 2013

```
# Estimation of dog rabies vaccination coverage in commune 1 in Bamako, 2013
# Confinement probabilities are recalculated

model {
        for( t in 1 : T ) {                              # Transect data
                x1[t] ~ dbin(p[t], n1)
                z[t] ~ dbin(p[t], n2)
                pp[t]~dunif(pmin,pmax)
                p[t] <- min(pmax,max(pmin,pp[t]))
         }

        n1 <- round((1- c1) * Mv)                 # number of none-confined vaccinated dogs
        n2 <- round((1- c2) * (M-Mv) + a*M)       # number of none-confined unvaccinated dogs

        cover_own <- Mv/M                          # vaccination coverage owned dogs
        cover_tot <- Mv/(M+a*M)                    # vaccination coverage all dogs

        M~dnorm(Mmean,Mtau)                        # number of owned dogs
        Mmean<-Mv*(peter_n+1)/(peter_m+1)
        Mvar<-(pow(Mv,2)*(peter_n+1)*(peter_n-peter_m))/(pow((peter_m+1),2)*(peter_m+2))
        Mtau<-1/Mvar

        c1 ~ dbeta(a1,b1)
        c2 ~ dbeta(a2,b2)
```

```
        a ~ dunif(0.0001,0.2)                    # proportion of ownerless dogs
}

# Data
list(
# Data to estimate vaccination coverage of owned dogs
  # Peterson-Bailey Estimate = M total number of owned dogs (Mmean, Mvar)
  # Data from vaccination posts (total number of vaccinated dogs)
  Mv=658,
  # Data from household survey
  # Number vaccinated (recatupred) dogs (m) and all dogs (n)
  peter_m=80,
  peter_n=438,

# Data to estimate coverage including stray dogs (a: proportion of ownerless dogs)
  # Data from household survey
  # Observed confinement: vac. dogs 47/80, unvac. dogs  143/358 (CIs: 0.48-0.69, 0.35-0.45)
    # Parameters for associated beta distributions were estimated in R
  a1=49.4, b1=34.3,         # vaccinated dogs
  a2=66.3,  b2=99.5,        # unvaccinated dogs
  # Data from transect survey
  T=4,                # Number of transects
  x1=c(1,0,0,0),  # Number of dogs with collars (vaccinated)
  z=c(14,6,1,7),  # Total number of dogs without collars (non-vaccinated owned + ownerless )
# Assumptions: min/max probabilities of recapture probabilities during the transect surveys
pmin=0.001, pmax=0.2 )
```

Parameters were estimated using 2 chains and 40'000 iterations (+20'000 burn in) without thinning. Convergence and model diagnostic were assessed by visual inspection of the parameter density distribution, dynamic trace, and the time series. Considerable uncertainty was observed for the estimate of the proportion of ownerless dogs (a). A sensitivity analysis was done fixing the proportion of ownerless dogs at 1% and 20%. The estimated total coverage changed slightly from 17.6% to 18.3% and 15.6%, respectively. Additional sensitivity analysis revealed that the broader prior distributions for confinement had no noteworthy impact on the estimated parameters. OpenBUGS 3.2.3 and WinBUGS 1.4.3 yielded similar results.

Appendix 2. Bayesian coverage estimates of a central-point rabies vaccination campaign in 2013 in Bamako, Mali.

	2.5 percentile	median	97.5 percentile
Owned dog Population	**2786.0**	**3459.0**	4131.0
Proportion ownerless dogs [%]	0.3	7.8	19.2
Vaccination coverage owned dogs [%]	15.9	19.0	23.6
Vaccination coverage all dogs (incl. ownerless) [%]	14.4	17.6	22.1

8. 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

8.1. 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, 2013). 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 (Shantavasinkul and Wilde, 2011; Shim et al., 2009). 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., 2006a; Lembo et al., 2010; WHO, 2005). 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., 2016). 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 (Kone, 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 (see Section 8.2), 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., 2011a), 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.

8.2. Materials and methods

8.2.1. 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 (Creswell 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).

8.2.2. 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 12).

Table 12: Mixed method assessment of the effectiveness parameters of a dog rabies vaccination campaign (adapted from (Creswell and Clark, 2007; Obrist et al., 2007; Zinsstag et al., 2011a)

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

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 identification of the problem and those 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	– Household survey: Proportion of the concerned households who got the accurate information in time;	– 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 accessible to dog owners (geographical distance, transport, time necessary to get there); owners able to handle their dog for vaccination, otherwise house visits would be necessary; 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; proportion of dog owners which can take their dog to the vaccination point – 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) Indirect costs = transportation, lost time and income, costs for a cord to leash the dog	– Free of charge in this case, otherwise need a willingness to pay study (Durr et al. (2009), Mauti et al. (2015), in press) – Household survey: Proportion of households who think the costs (direct or indirect) are too high	– Semi-structured interviews with dog owners on affordability and their perception of indirect costs: why the effort to get his dog vaccinated is worthwhile or not	Triangulation Convergence Model: Quantitative and qualitative data is collected separately, compared and 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 (n.a. in this study) – 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 (n.a. in this study)	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.	

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 12 provides an overview of available methods to evaluate 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*" (Creswell 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 60% of dog owners 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.

8.2.3. 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 (Fig. 30).

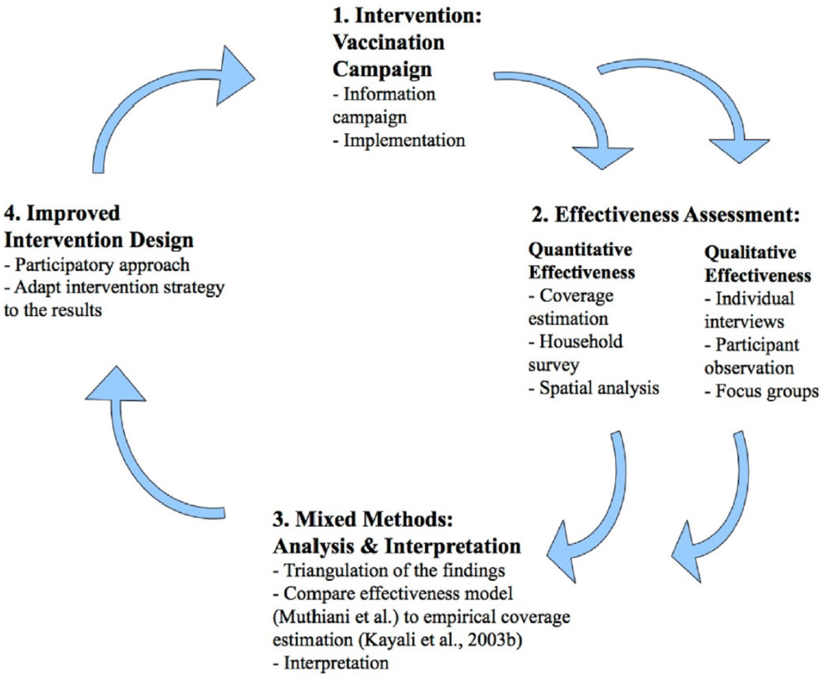

Figure 30: Intervention Effectiveness Optimization Cycle

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 (see 8.2.1) 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).

8.2.4. 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 in Section 8.2 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), 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. (2011a) 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 12). The answers for non-participation were associated to the different effectiveness determinants (see Table 12) 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 (see 8.2.2), which is a limitation of our study.

8.3. Results

8.3.1. 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.

8.3.2. 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 13). In Commune Six (excluding Senou), we vaccinated 784 dogs (Fig. 31). 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.

Table 13: Summary of data collected from the vaccination points, household survey, and transect survey and vaccination coverage estimates of the Bayesian model in Bamako, Mali

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.

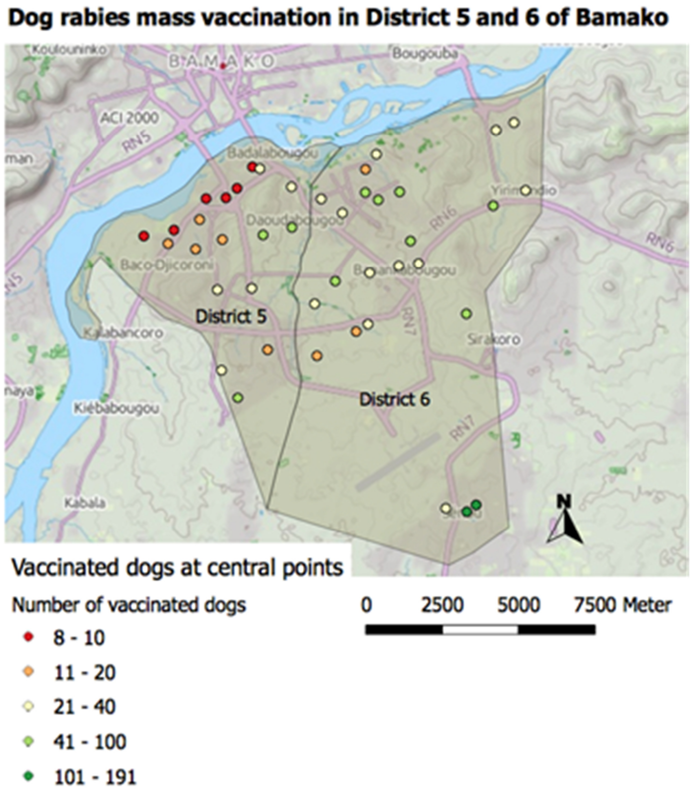

Figure 31: Map of Communes Five and Six of Bamako, Mali. The dots indicate the vaccination points with the respective number of vaccinated dogs.

8.3.3. 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 13). 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.

8.3.4. 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 12). 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 Fig. 32). 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).

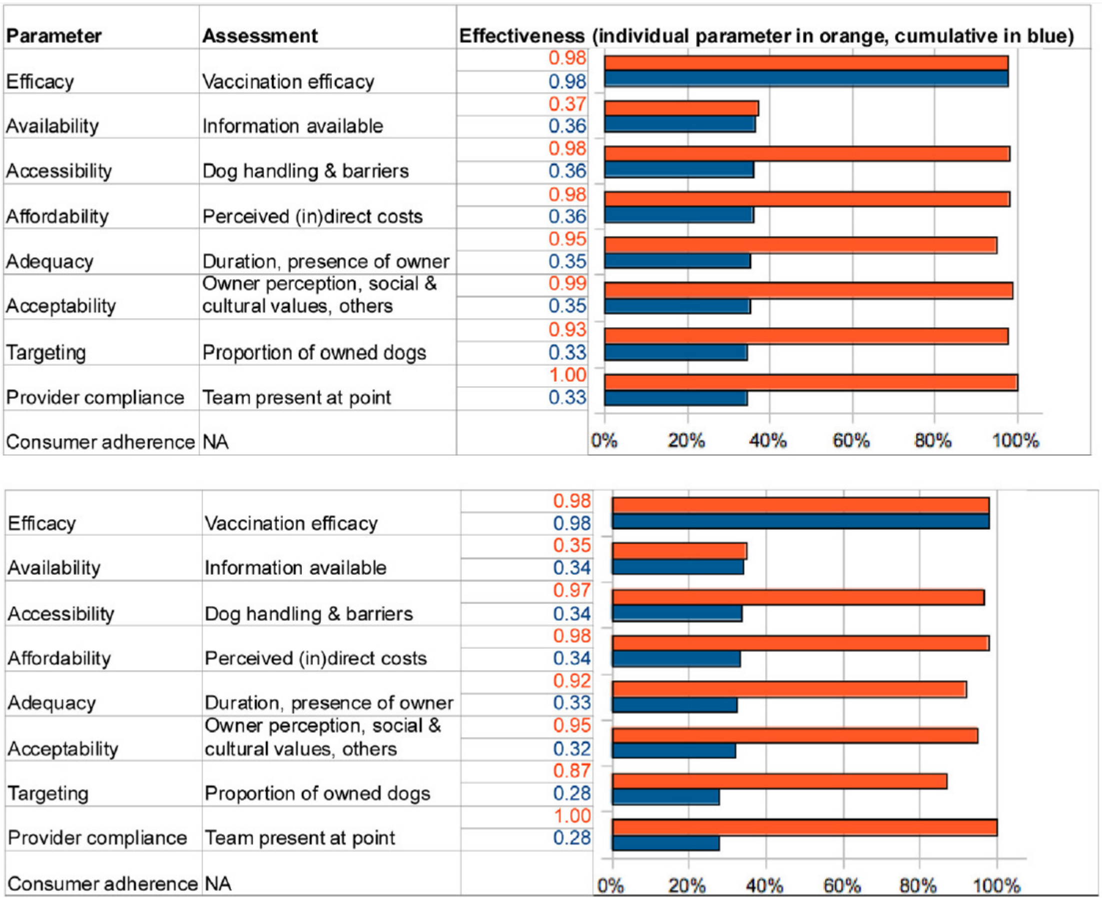

Figure 32: Effectiveness determinants of the dog rabies mass vaccination campaign in Commune 5 (above) and in Commune Six (below) in Bamako, Mali. Orange bars show the extent of each effectiveness determinant, and blue bars the cumulative effectiveness (adapted from Muthiani et al., 2015).

8.3.4.1. Vaccination efficacy

The efficacy of the rabies vaccination for dogs has been tested by the manufacturer. The applied value of 0.98 was assumed from the provider information (Rabisin Merial™).

8.3.4.2. 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 (see 4.1), 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 neighborhoods where a town crier was engaged or the neighborhood chief himself circulated to mobilize his population, and neighborhoods where this was not the case.

8.3.4.3. 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. Fig. 33 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 400 m, while it was about 710 m 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. 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.

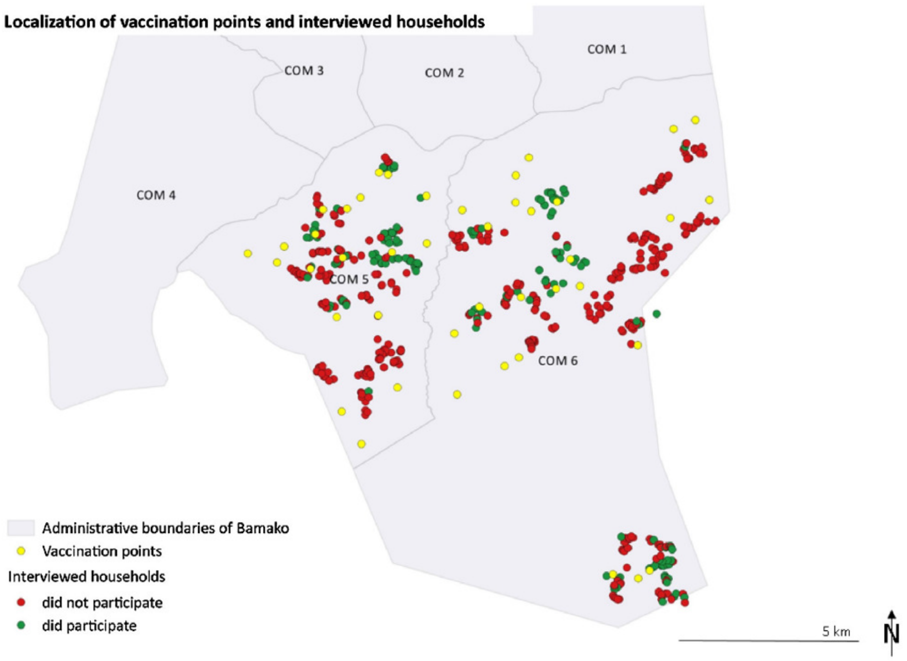

Figure 33: Localization of the vaccination points and the interviewed households.

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.

8.3.4.4. 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.

8.3.4.5. 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 12), 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 interprete 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'Djaména 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.

8.3.4.6. 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.

8.3.4.7. 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.

8.3.4.8. 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.

8.4. 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. There might be a gap between the community awareness of the disease and the awareness of control measures. Therefore, awareness and information campaigns need to clearly explain these measures to overcome mistrust.

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 Ndjamena 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 Ndjamena, 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. On the one hand, it was not mentioned as an argument, and on the other hand, no statistically significant relation could be found out between the religion and the participation rate.

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 (Creswell 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 12), 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 (Fig. 30), 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., 2011a, 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.

8.5. 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. (forthcoming), 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 tonew 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.

Conflict of interest statement

None declared.

Acknowledgments

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9. First report of *Echinococcus granulosus* (genotype G6) in a dog in Bamako, Mali

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Abstract

Cystic echinococcosis is one of the most widespread and important helminthic zoonoses, caused by the larval stage of *Echinococcus granulosus sensu lato*. However, to date there is little information about the disease in West Africa. Faecal and fur samples from 193 dogs, the main final hosts, were collected in 2010 and 2011 in Bamako, Mali. Taeniid eggs were found microscopically in 28/118 (24%) and 80/223 (36%) faecal and fur samples, respectively. One faecal and one fur sample from the same dog were positive for *E*. *granulosus s*. *l*. DNA. In the remaining 27 faecal (96%) and 77 fur samples (96%) only *Taenia* DNA was detected. Three microscopically positive fur samples were negative by PCR. Sequence analysis of part of the NADH dehydrogenase subunit 1 gene identified the parasite as *E*. *granulosus* (genotype G6; *Echinococcus canadensis*). This is the first study to focus on the final host of *E*. *granulosus s. l.* in Mali and the first report of *E*. *canadensis* in Mali.

Keywords

Echinococcus canadensis; *E*. *granulosus* (G6); dog; Mali

9.1. Introduction

Cystic echinococcosis (CE) is caused by larval stages of *Echinococcus granulosus sensu lato* and is one of the most widespread and important helminthic zoonoses worldwide. The lifecycle of the parasite includes carnivores, mainly dogs, as definitive hosts, which harbour adult egg producing stages in their intestines, and intermediate hosts, in which larval stages develop cyst-like structures in different organs (mainly liver and lung) after ingestion of parasite eggs. Sheep, cattle, horses, camels, pigs and other animals act as intermediate hosts, whereas humans are considered to be aberrant intermediate hosts. Dogs often become infected by feeding on offal from infected livestock which contains viable cysts (Torgerson et al., 2011). Parasite eggs are shed in the faeces and disseminated into the environment but may also be found on the fur of the final host (Eckert et al., 2001).

Several host-adapted strains of *E*. *granulosus s*. *l*. are identified. These strains or species exhibit different ecological and biological characteristics but cannot reliably be differentiated morphologically. Species with suspected or proven zoonotic potential are *E*. *granulosus sensu stricto* (including genotypes G1:sheep strain, G2:Tasmanian sheep strain, G3:buffalo strain), *E*. *ortleppi* (G5:cattle strain), *Echinococcus canadensis* (G6:camel strain, G7:pig strain, G8/G10: cervid strain). However, the taxonomy of *E*. *granulosus s*. *l*. continues to be a subject of debate, and some authors consider strains G6 and G7 as *E. intermedius*. Globally, *E*. *granulosus s*. *s*. is the most widespread species and is responsible for the majority of the human CE cases (Nakao *et al*., 2007; Thompson, 2008; Alvarez Rojas *et al*., 2014).

In Africa, *E*. *canadensis* (G6) has been reported in dogs in Kenya, and human cases were described in Egypt, Kenya, Sudan, Mauritania and the Central African Republic. In these countries, camels, pigs, cattle, sheep, and goats were found to be infected with this species. *E*. *canadensis* (G6) was also found in the aforementioned intermediate hosts in Algeria, Tunisia, Ethiopia, and Somalia. (Abdel Aaty *et al*., 2012; Bardonnet *et al*., 2002; Bardonnet *et al*., 2003; Bowles *et al*., 1992; Develoux *et al*., 2011; Dinkel *et al*., 2004; Hailemariam *et al*., 2012; Hüttner *et al*., 2009; M'rad *et al*., 2005; Omer *et al*., 2010; Romig *et al*., 2011; Wachira *et al*., 1993).

Infections with *E*. *granulosus s*. *l*. in dogs have been well documented in many areas of Africa. Local prevalence varies from low, e.g. 0.9% in South Africa (Verster, 1979) and 5% in Egypt (Elshazly et al., 2007), to very high values, such as 66.3% in Uganda (Inangolet et al., 2010) and 85% in Nigeria (Arene, 1984).

However, there is little information about *E*. *granulosus* and CE in Mali. Tembely et al. (1992) found fertile hydatid cysts in the lungs of 1 out of a total of 11 camels in an abattoir survey conducted in northern Mali in 1987. Considering the close contact between camels, their owners and dogs, this finding is of public health importance. Carayon and Robert (1962) described an autochthonous case of CE in a herdsman from Mopti in the Inner Niger Delta region of Mali and Yéna et al. (2002) documented 11 cases of pulmonary echinococcosis in Bamako in a retrospective study from 1960 to 2000.

In this study, fur and faecal samples from owned dogs were examined with microscopic and molecular methods for the presence of *Echinococcus* spp.

9.2. Material and methods

9.2.1. Dogs, faecal and fur samples

From March through July, the hot, dry months, in 2010 and 2011, 118 faecal and 223 fur samples (for some dogs we gathered two fur samples) were collected from 193 owned dogs in Bamako, the capital of Mali. All samples were frozen at −80 °C for 4 days and then stored at −20 °C until examination.

Faecal samples (3.6–110.9 g) were either taken rectally or, if not possible, collected from the ground in the dog owners' courtyard. Samples were microscopically examined for the presence of helminth eggs with a standard flotation technique using 1.45 g/ml (SG 1.45) zinc chloride solution (Deplazes et al., 2013).

The fur samples were collected from the perianal area with scissors and examined for taeniid eggs using a method described by Overgaauw et al. (2009), with slight modification. After constant overnight rotation in 40 ml of phosphate buffer saline (PBS) containing 0.2 ml Tween 20 (Merck, Germany) at 4 °C, the floating hair was removed. The tube was centrifuged at 800 × *g* for 10 min and the supernatant was removed by aspiration. The remaining sediment was poured through a sieve with a mesh size of 100 µm into a new 50 ml tube. The hair which was previously removed was put on the same sieve and rinsed thoroughly with PBS/Tween 20. After centrifugation and washing in PBS as before, the sediment was examined microscopically.

Slides containing taeniid eggs were rinsed with distilled water. The rinsing water and the remaining top 2 ml of the flotation solution (faecal samples) or the rest of the sediment (fur samples) were centrifuged at 10,000 × *g* for 2 min, and the pellet was used for DNA isolation. DNA was isolated using commercial kits (faecal samples: QIAamp DNA Stool Mini Kit; fur samples: QIAamp DNA Mini Kit, Qiagen, Hilden, Germany) after initial alkaline lysis and neutralisation steps (Mathis et al., 1996). Samples were analysed by a multiplex PCR (Trachsel et al., 2007) for the presence of *E*. *granulosus s*. *l.*, *Echinococcus multilocularis* or *Taenia* DNA. For *E*. *granulosus* typing, part of the mitochondrial gene coding for the NADH dehydrogenase subunit 1 (nad1, primers JB11 & JB12, (Bowles and McManus, 1993)) was amplified by PCR. Amplicons were sequenced from both directions (Synergene Biotech GmbH, Zürich, Switzerland) and the consensus sequence was used for BLAST searches.

9.3. Results and discussion

Taeniid eggs were found in 28/118 (24%) and 80/223 (36%) faecal and fur samples, respectively. One faecal and one fur sample from the same dog were positive for *E*. *granulosus s*. *l*. DNA. Three microscopically positive fur samples were negative by PCR. In the remaining 27 faecal (96%) and 77 fur samples (96%), only *Taenia* DNA was detected. The fur sample was collected in 2010 and the faecal sample was collected off of the ground in 2011. *Taenia* DNA was also detected in the fur sample of this dog. The sequence of the nad1 amplicon (430 bp) was identical to the corresponding part of the complete mitochondrial genome of *E*. *canadensis* genotype G6 (GenBank accession number AB208063) whereas there were 4 polymorphic sites as compared to the closely related *E*. *canadensis* genotype G7 (AB235847, complete mitochondrial genome). From nine positive *Taenia* sp. samples, amplification and sequence analysis of the cest3 and cest5 amplicons showed > 99% identity to published sequences of *Taenia hydatigena.*

We report the first known case of an intestinal *E*. *canadensis* (G6) infection in a dog in Mali. This finding implies that the parasite is present in Bamako, and that parasite transmission between intermediate and final hosts does occur. Interestingly, eggs of *E*. *granulosus s*. *l*. were detected, together with *Taenia* eggs, in the fur sample of this dog during the first sampling period one year earlier. Since PCRs used for typing of *E*. *granulosus s*. *l*. will amplify the corresponding targets of both parasite genera, no attempts to further identify the distinct species in this fur sample were carried out. The presence of *Echinococcus* eggs in a fur sample would not automatically prove a current intestinal infection, and the question of whether this dog had an intestinal *Echinococcus* infection at the time of the first sampling period cannot be answered. However, the presence of *Echinococcus* eggs in the fur sample supports the interpretation that infected final hosts of *E*. *granulosus s*. *l*. are present in the area of Bamako and emphasises the importance of fur in the zoonotic transmission of parasite eggs (Aydenizöz-Ozkayhan et al., 2008).

According to our data, intestinal infections with *E*. *granulosus s*.*l*. in dogs from Bamako seem to be rare (1/118 or 0.8%). However, a reliable estimation of the prevalence in dogs is not possible because the sample size was low. Sampling of only owned dogs could represent an underestimation of the true infection rate, as reported by Gusbi (1987), who noted higher infection rates in stray versus domestic dogs in Libya. One explanation for this could be that owned dogs are often kept chained or within walled premises and might, therefore, have different access to offal.

The known intermediate hosts of *E*. *canadensis* are camels, goats, pigs and cattle (Torgerson et al., 2011). As pig keeping is uncommon in the predominately Muslim Bamako area while goats are present in high numbers, they must be primarily considered. However, no data are available on infection rates in potential intermediate hosts to date. Recent data on human cases of CE in Mali are not found in either local or international journals.

Both less favourable climatic conditions (hot and dry) and lack of close contact between dogs and humans in this specific local context would imply that the transmission risk for *Echinococcus* to humans is low. Therefore, we would speculate that numbers of human CE cases would be low in this area.

However, the question of whether or not there is a need for control measures in Bamako, or more broadly in Mali, cannot be answered without a more precise estimate of the infection risk for humans and the losses in husbandry.

Conflict of interest

None declared.

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10. Spectrum of dog parasites in Bamako, Mali

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Working Paper

Abstract

Besides echinococcosis, several other dog parasites are present in Bamako, Mali. The aim of this paper is to describe parasitic infections in dogs in Mali for the first time, as well as people's knowledge and practices towards leishmaniasis and echinococcosis.

We visited 2956 households in 2010 and 2011 and found 379 dogs in 279 households. Data was collected on 279 dog-owning households, on 1017 non-dog-owning households and on 311 dogs. A plasma, faecal and fur sample was collected from each dog older than 3 months of age. For some of the dogs, a second sample was collected during a follow up survey one year later. Laboratory analysis included microscopic, immunodiagnostic and molecular methods.

Less than half of the interviewees were familiar with leishmaniasis and echinococcosis and knew that the disease can be transmitted from dogs to humans. Dog deworming was only reported in 34% (95% CI 26% - 43%) of the examined dogs.

None of the 179 plasma samples harboured anti-*Leishmania* antibodies. Despite some microscopic findings suggestive of *Babesia*, the disease could not be confirmed by PCR. Several parasite eggs/cysts were found in the fur and faecal samples, *Taenia sp. (T. hydatigena), Echinococcus granulosus (genotype G6), Dipylidium caninum, Toxocara sp*., trematode eggs, *Trichuris sp., Spirocerca sp*., other nematode eggs, *Entamoeba sp*. and *Dicrocoelium sp.* and an egg belonging to the family of *Oxyuridae.*

Our findings of multi-parasitism of dogs in Bamako indicate ongoing transmission of gastrointestinal helminths.

10.1. Introduction

To the best of our knowledge, nothing is known to date about parasitic infection in dogs in Mali, and with the exception of echinococcosis little information exists in the literature on intestinal dog parasites in Africa (Arene, 1984; Buishi et al., 2005; Elshazly et al., 2007; Lahmar et al., 2009; Lloyd and Morgan, 2011; Macpherson et al., 1985; Nonaka et al., 2011; Pandey et al., 1987; Wachira et al., 1994). In addition to the recently reported *Echinococcus granulosus* (genotype G6) (Mauti et al., 2016), a variety of other parasites are found in dogs in Bamako, Mali. Some of them have zoonotic potential (*Leishmania sp., Babesia sp., Toxocara sp., Dipylidium caninum*) and often affect livestock, which result in additional economic loss. Leishmaniasis is caused by a protozoan of the genus *Leishmania*. Different species exist, and 13 of them have zoonotic capability. A known vector of the disease is the phlebotomine sand fly, and the parasite is alternatively hosted by the sand fly and the mammal host. Transmission takes place through blood meals of the vector. There are three different forms of the disease in humans, a visceral form called visceral leishmaniasis (VL), a mucocutaenous form called mucocutaneous leishmaniasis (MCL) and a cutaneous form called cutaneous leishmaniasis (CL), with the form dependent on the involved *Leishmania* species (Gramiccia, 2011). *L. major*, which causes CL, is present in Mali (Paz et al., 2013). Dogs with clinical signs are often infected with *L. infantum*, which causes a chronic visceral-cutaneous disease (OIE, 2012). Only few reports of *L. major* in dogs exist, for example from dogs from Egypt (Morsy et al., 1987).

Human toxocariasis is a disease caused by migrating larva of different *Toxocara sp*. The symptoms are related to the larval migration through the liver, lungs, eyes and brain. For neurological toxocariosis, seizures are typical. Clinically apparent canine *Toxocara* infections are common in puppies (Lloyd and Morgan, 2011).

This study was undertaken to assess parasitic infections in dogs and to identify people's knowledge and practices with respect to echinococcosis and leishmaniasis and their control. Blood, faecal and fur samples were collected and examined for parasites by means of microscopic, immunodiagnostic and molecular methods. Fur was included because it has been shown in earlier work that a significant proportion of parasite eggs can be found on the hair coat of dogs; for example, *Toxocara* eggs are known to be very sticky (Eckert et al., 2001; Overgaauw et al., 2009).

10.2. Material and methods

10.2.1. Study area

A cross-sectional survey was undertaken in Bamako, which is the capital of Mali located in the southern part of the country. The city is divided in 6 communes with a total of 67 quarters, and the national census recorded 1.8 million inhabitants (RGPH-2009). The city has a sub-humid savannah climate with average high temperatures above 30 °C every month. The total land area is about 267 km^2.

10.2.2. Data sample collection

Questionnaire interviews, whenever possible with the head of the household, were conducted in all six communes of Bamako. We randomly selected 32 out of a total of 67 quarters with selection probability proportional to the size of the human population of the communes. During the first baseline study in 2010 and the second baseline study in 2011, households were randomly selected with slightly different procedures, as further described below. The survey in 2010 was conducted between May and June, while the one in 2011 was between April and May. Full interviews were conducted in all households in 2010, but only in dog-owning households in 2011. The sampling method for households was based on a random selection approach. During the study in 2010, five field teams, each consisting of one veterinary officer and one interviewer, started in a quarter at the household of the quarter chief, then spun a bottle and walked 200 meters in the indicated direction, where they flipped a coin to select one side of the road for the inclusion of two households in two neighbouring compounds. A compound was defined as all of the houses surrounded by a wall, which could include several households. A household was defined as an individual or a group, related or not, living within the compound under the lead of the household head. It was composed of the head of household, his spouse(s), his unmarried children and possibly other related or unrelated persons. This selection procedure was repeated until at least 38 households per quarter were enrolled and interviewed. In 2011, four field teams started at five randomly selected coordinates within the same quarters. The nearest block was then chosen, where interviews were conducted in households (only one per compound) with the team moving in a clockwise direction until at least 12 households were selected. The questionnaires included questions about knowledge and practices regarding leishmaniasis and echinococcosis and their control by dog- and non-dog-owners. Some of the questions included open questions to record spontaneous answers which were followed by a list of probed answers. Spontaneous answers are more useful to direct the design of future intervention programs. Prior to the first baseline survey, meetings were held with the local chiefs of each quarter. The questionnaire was tested in a non-selected quarter. The questionnaire was in French, and questions were translated into Bambara when necessary. We defined "owned dogs" as dogs that belong to a household. Blood, faecal and fur samples of each encountered owned dog over three months old were collected by the veterinary officer. The blood sample was collected from the saphenous vein into 2 ml EDTA tubes. The faecal sample was either taken rectally or, if not possible, collected from the ground in the dog owners' compound. The fur samples were taken from the perianal area with scissors. Scissors were cleaned carefully and disinfected with alcohol between each sample collection. Muzzles were used to prevent dog bites. Dogs were not sampled if the owner did not give consent or if a dog was uncooperative, aggressive or sick at the time of the survey. All dog-owning households were visited for follow up surveys to collect dog demography data, but these results are published elsewhere. When it was possible, additional blood, fur and faecal samples were collected during the second follow up, in parallel to the second baseline study in 2011.

10.2.3. Laboratory testing

The samples were kept in an icebox and blood samples were centrifuged within 24 h of collection. For the microscopic examination for intra- and extracellular parasitic infection, a blood smear was prepared from each blood sample. Blood smears were fixed with methanol and stained using Giemsa solution. For each sample, the buffy coat was transferred onto blotting paper. All plasma samples were subsequently stored at -20°C. Faecal and fur samples were frozen at -80°C for 4 days and then stored at -20°C until examination. All samples were shipped on dry ice to the Institute of Parasitology of the University of Zurich.

10.2.3.1. Examination of the blood samples

10.2.3.1.1. *Leishmania* ELISA

A well-established antigen-ELISA (Enzyme Linked Immunosorbent Assay) using *L. infantum* antigen was used for the detection of *Leishmania* antibodies (Mettler et al., 2005). The microtiter plates of the ELISA were also coated experimentally with the *L. major* antigen.

10.2.3.1.2. Examination of the blood smears

Due to a possible cross-reaction between leishmaniasis and other diseases (e. g. *Babesia sp., Ehrlichia sp*.), blood smears of all samples were also examined using microscopy.

10.2.3.1.3. DNA isolation and PCR

DNA was isolated using commercial kits (QIAamp DNA Mini Kit, Qiagen, Hilden, Germany). In the case of suspected *Babesia* cases, frozen erythrocytes were used, while buffy coats on blotting paper were used for suspected *Leishmania* cases. The buffy coat blotting papers were snipped into small pieces for the analysis.

For the presence of *Leishmania sp*., samples were analysed by a real-time TaqMan PCR, using the genus specific primers PL-F1 (CCA AAG TGT GGA GAT CGA AGA TG) and PL-R1 (GTG CTG GAC ACA GGG TAA ACC) and the TaqMan probe PL-S2: (CAG GCC GGC CCA TAA GAT CCC).

Specific *B. canis* and *B. gibsoni* primers were used to detect the presence of infection, using primers Bc-F (GTT TCT GAC CCA TCA GCT TGA C) and Bc-R (CAA GAC AMA AGT CTG CTT GAA AC) targeting the 18S rRNA gene of *B. canis* and *B. gibsoni*.

10.2.3.2. Examination of the faecal and fur samples

Faecal samples were microscopically examined for the presence of parasites with a standard flotation technique using 1.45g/ml zinc chloride solution (Deplazes et al., 2013).

Fur samples were examined for parasites eggs using the method described by Overgaauw et al. (2009), with slight modification. After constant overnight rotation in 40 ml of phosphate buffer saline (PBS) containing 0.2 ml Tween 20 (Merck, Germany) at 4°C, the floating hair was removed using tweezers. The tube was then centrifuged at 800 x g for 10 minutes, and the supernatant was removed by aspiration. The remaining sediment was poured through a sieve with a mesh size of 100 um into a new 50 ml tube. The hair which was previously removed was put on the same sieve and rinsed thoroughly with PBS/Tween 20. After centrifugation and washing in PBS as before, the final sediment was transferred to a 1.5 ml Eppendorf tube and examined microscopically.

10.2.3.2.1. DNA isolation, PCR and sequence analysis of taeniid eggs

Slides containing taeniid eggs were rinsed with distilled water. The rinsing water and the remaining top 2 ml of the flotation solution (faecal samples) or the rest of the sediment (fur samples) were centrifuged at 10'000 x g for 2 minutes, and the final pellet was used for DNA isolation. DNA was isolated using commercial kits (faecal samples: QIAamp DNA Stool Mini Kit; fur samples: QIAamp DNA Mini Kit, Qiagen, Hilden, Germany), after initial alkaline lysis and neutralisation steps (Mathis et al., 1996). Samples were analysed by a multiplex PCR (Trachsel et al., 2007) for the presence of *E. granulosus s. l.*, *E. multilocularis* or *Taenia* DNA. Samples which were suspicious for an *Echinococcus* infection were then further analysed by means of a PCR specific for the sheep strain (Stefanić et al., 2004) and for gentotype 6. For *E. granulosus* typing, part of the mitochondrial gene coding for the Cytochrome c oxidase subunit 1 (Bowles et al., 1992) and the NADH dehydrogenase subunit 1 (nad1, primers JB11 & JB12, (Bowles and McManus, 1993)) was amplified by PCR. For *Taenia sp*. typing, Cest3 and Cest5 primers were used (Trachsel et al., 2007). The reaction mixture (50 µl) consisted of 25 µl mastermix (Qiagen multiplex kit, Qiagen, Hilden, Germany), 0.5 µl of each primer (1 mM), 19 µl H2O and 5 µl of template DNA. Amplicons were sequenced from both directions (Synergene Biotech GmbH, Zürich, Switzerland) and the consensus sequence was used for BLAST searches.

The PCR protocol according to Jacobs et al. (1997) was used for further analyses of suspected *Toxocara sp*. eggs.

10.2.4. Data entry and statistical analysis

Data was entered in Microsoft® Access 2010. Statistical analysis was done with STATA IC 12.

Data were analysed with generalized estimating equation models, taking clustering into account.

10.3. Results

10.3.1. Basic demographics and knowledge and practices of dog- and non-dog-owners

We identified 379 dogs in 279 households/compounds (n=2956 households/compounds were visited). In 2010, 125 dog- and 1 017 non-dog-households were interrogated, while 154 dog-households were questioned in 2011. Questionnaire data were collected for 311 eligible dogs.

When dog- and non-dog-owners were asked if they knew about leishmaniasis, 35% (95% CI 23%-49%) and 26% (95% CI 16% - 39%), respectively, answered in the affirmative. There was no notable difference between dog-owners and non-dog-owners in 2010. When asked about echinococcosis, 41% (95% CI 30% - 53%) of the dog-owners and 27% (95% CI 18% - 38%) of the non-dog-owners knew of the disease, and this difference was statistically significant.

A total of 1 293 persons were asked which diseases were transmitted from dogs to humans during the two surveys. The results are shown in Figure 34.

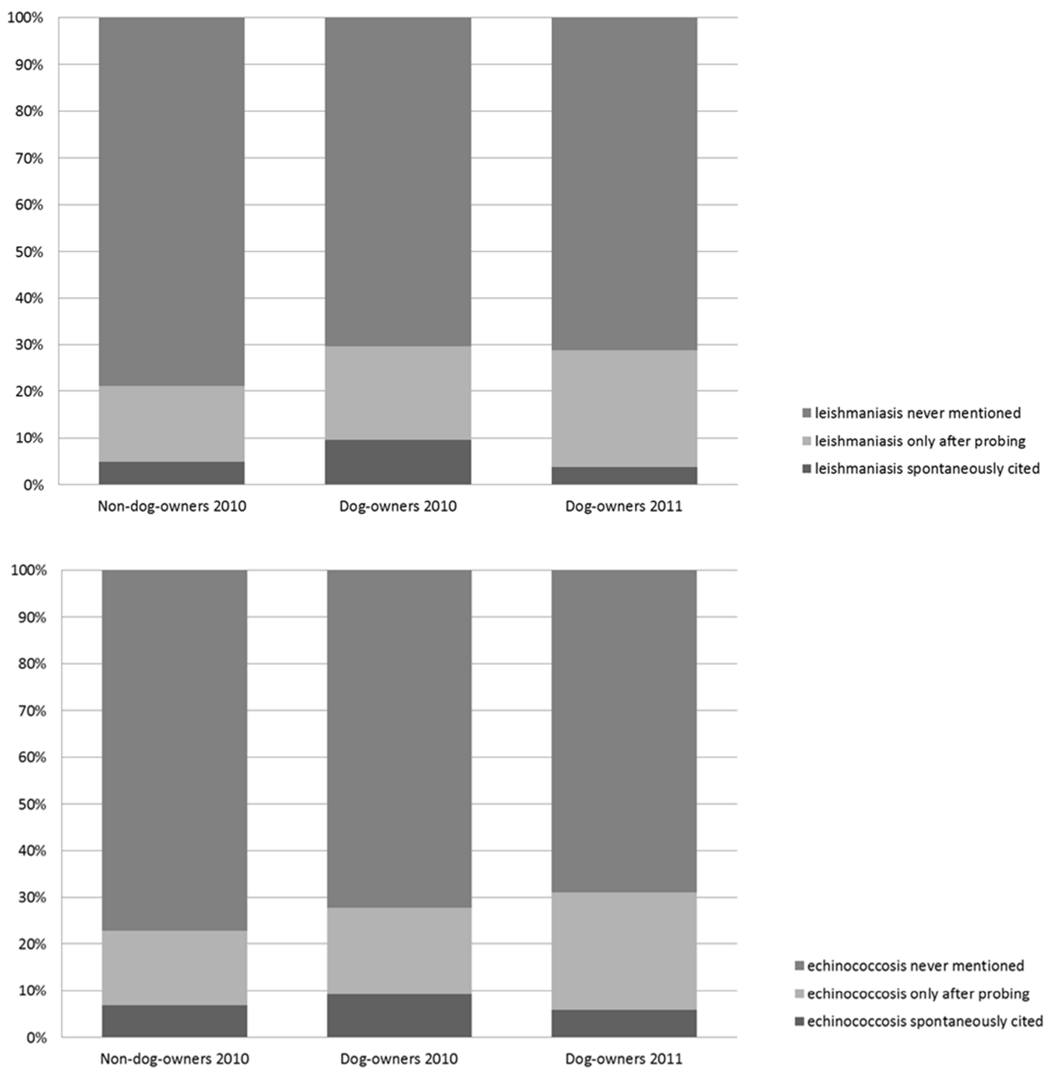

Figure 34: Answer given regarding which diseases were transmitted from dogs to humans

Other diseases or agents mentioned more than once were rabies (result is presented in (Mauti et al., 2015)), scabies, brucellosis, tuberculosis, gonococci infection, respiratory diseases, syphilis, AIDS, fleas and ticks.

Thirty-eight percent of dogs (n=302; 95 CI 30% - 46%) were frequently checked by a veterinarian. In the baseline survey, 34% (n=295; 95% CI 26% - 43%) of dogs were reported as being dewormed in the previous six months. The reported cost for dog deworming ranged from 200 FCFA (0.3 €) to 10 000 FCFA (15.2 €) (median: 1000 FCFA (1.5 €); IQR: 700 FCFA (1.1 €) – 1 500 FCFA (2.3 €)). More than half of the dogs (28/55) which received further veterinary treatment were treated against ectoparasites.

Nine out of ten dogs were reported as being healthy at the time of the survey. Frequently listed conditions for sick dogs were skin changes, parasitic infections or lameness.

10.3.2. Parasitological results of the blood samples

None of the 179 plasma samples yielded anti-*Leishmania* antibodies, either specifically for *L. infantum* antigen or for *L. major* antigen. Five samples which showed slightly higher antibody unit levels were additionally tested using a real-time TaqMan PCR, but the results were all negative.

The poor quality of the blood smears made microscopic examination difficult. Fifty-seven percent (n=182) of the blood smears were morphologically suspicious for *Babesia* infection (*B. canis*, *B. gibsoni*). Twenty-nine randomly selected suspicious cases were further analysed using a PCR but none were positive.

10.3.3. Parasitological result of the faecal and fur samples

Faecal samples (3.6g – 110.9g) were only collected during the baseline survey in 2011 and during the second follow up survey, which took place in 2011 in parallel. Table 14 and Figure 35 show the spectrum of different parasite eggs which were found.

Table 14: Spectrum of different parasites in faeces and fur

	Fur		Faeces	
	Baseline survey (n/179) (%)	Follow up survey (n/44) (%)	Baseline survey (n/79) (%)	Follow up survey (n/39) (%)
Taeniid eggs	59 (32%)	21 (48%)	19 (24%)	9 (23%)
Dipylidium caninum	3 (2%)		4 (5%)	1 (3%)
Toxocara sp.	2 (1%)			
Family *Oxyuridae*	1 (1%)			
Other nematode eggs	2 (1%)	1 (2%)	31 (39%)	22 (56%)
Entamoeba sp.	1 (1%)	1 (2%)		
Dicrocoelium sp.			1 (1%)	
Other trematodes			1 (1%)	
Trichuris sp.			1 (1%)	
Spirocerca sp.				1 (3%)
Total dogs	68	23	57	33

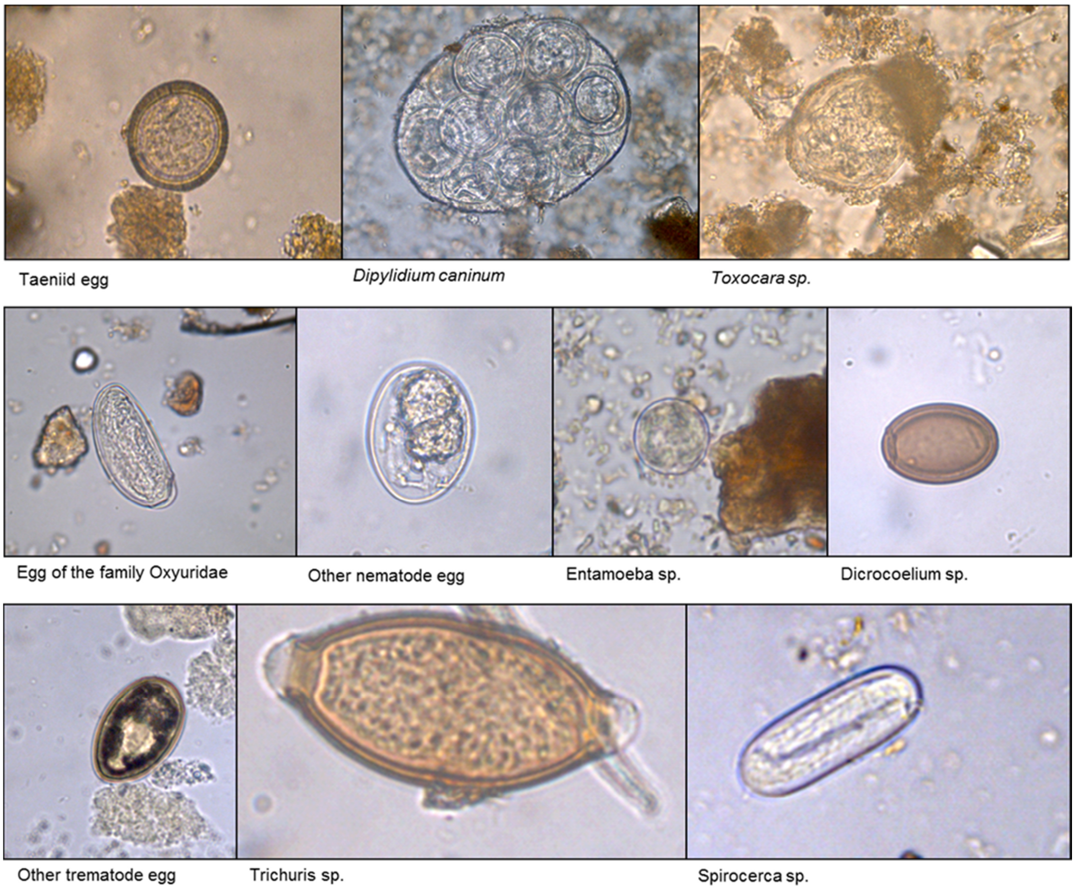

Figure 35: Spectrum of parasites found in fur and faecal samples of dogs in Bamako, Mali. (Photos: S. Mauti)

As already described by (Mauti et al., 2016), sequence analysis of the nad1 amplicon showed an infection in one dog with *E. granulosus* genotype 6/7. The other diagnostic tests described in the methods part relating to *Echinococcus* did not work. In the remaining 27 faecal (96%) and 77 fur samples (96%), only *Taenia* DNA was detected. From nine positive *Taenia sp*. samples, amplification and sequence analysis of the cest3 and cest5 amplicons showed > 99% identity to published sequences of *Taenia hydatigena.* Further analysis of the suspicious fur infections with *Toxocara sp*. were inconclusively positive for *T. canis* and *T. cati* and need to be further analysed.

10.4. Discussion

Less than half of the dog-owners knew about the diseases leishmaniasis and echinococcosis, and only a small proportion of dogs were dewormed in the previous six months. No cases of *Leishmania* and *Babesia* were found, but we could show that a significant proportion of dogs harboured diverse parasite species in the fur and faeces.

The faecal and fur samples were frozen directly after collection at -80°C for 4 days. This was done as a precautionary measure to ensure that any *Echinococcus* eggs would not remain infectious during the analysis. Some parasite eggs were likely to have been destroyed during the freezing, which would mean that our findings probably reveal only a fraction of the actual parasitic burden of dogs in Bamako. The same was expected for the study of (Nonaka et al., 2011), which was conducted in Zambia. Our samples were only examined using the flotation technique, so some parasites eggs, which are only found through other diagnostic tests (e. g. SAF technique), might not have been detected.

Suspicious *Toxocara sp*. eggs were found in the fur samples, and this finding is similar to the study of (Overgaauw et al., 2009). It is known that *T. canis* and *T. cati* have zoonotic potential and cause human toxocariasis (Overgaauw, 1997). Kamuyu et al. (2014) showed that *T. canis* infection is associated with an increased prevalence of epilepsy in sub-Saharan Africa. Fur is an important source of *Toxocara* eggs and poses a risk for human infection (Aydenizöz-Ozkayhan et al., 2008).

Very low infection intensities were found in the fur samples, and often not more than one taeniid egg was found. Despite the low egg quantity, we almost always found a positive result in the multiplex PCR. This finding is similar to that of (Trachsel et al., 2007).

Sequencing of Taenia sp. resulted in identification of *T. hydatigena*. This finding is consistent with the study of (Mauti et al., 2017), where 14% of owned dogs were fed with raw meat and abattoir refuse, and it is suspected that dogs acquire the *Taenia* infection in this manner. Buishi et al. (2005) assumed the same route of infection in a study conducted in dogs in Libya.

Dipylidium caninum has also zoonotic potential. Fleas are the intermediate host. The most affected group in humans are children under 6-12 months of age, who may accidentally ingest infected fleas. So in addition to dog deworming, flea control should be established (Lloyd, 2011).

Considering the fact that dogs are not appropriate hosts for *Dicrocoelium sp*. and *Entamoeba sp.*, we assume, similar to the study of Nonaka et al. (2011), coprophagy of human and animal faeces.

More than half of the faecal samples harboured nematode eggs. As it was not always possible to collect the sample rectally, we sometimes collected faeces from the ground. Further examination of the samples classified 'other nematodes' should be done for possible contamination with soil nematodes.

Another study of Mauti et al. (2015) showed that approximately one third of dog-owners would not pay for rabies vaccination. To reach a threshold of 70% vaccinated owned dogs, rabies vaccination should not cost more than 0.2 € (100 FCFA). In our study, cost for deworming ranged from 0.3 € - 15.2 €, so it would likely be difficult to motivate dog-owners for frequent deworming.

None of the plasma samples harboured anti-*Leishmania* antibodies. So perhaps our sample was too small, dogs are not the reservoir host of L. major or we missed diagnosing some infections by serology. Berrahal et al. (1996), for example, described that asymptomatic dogs are often not detected by serology. In further studies, rodents should be included as a possible *Leishmania* reservoir (Gramiccia, 2011).

10.5. Conclusion

The majority of interviewees did not know about leishmaniasis and echinococcosis, and less than half of the dogs were dewormed. Several parasites were found in our sampled dogs and some of them have zoonotic potential. Further analyses should investigate which *Toxocara sp*. is currently involved in this setting. Disease awareness should be strengthened, and frequent dog deworming should be recommended.

Acknowledgments

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11. General discussion and conclusions

11.1. Significance of research

Very little is known about zoonoses in general, particularly in dogs in Mali (Dao et al., 2006; Konate, 2002; Kone, 2013, 2009; Maoulid, 2005; Paz et al., 2013, 2011; Tembely et al., 1992; Yéna et al., 2002). This PhD project contributed for the first time to the understanding and knowledge of the epidemiology of leishmaniasis, echinococcosis and rabies in the dog population of Bamako, Mali. The work is contextually new and contributes crucially to planning of control and prevention strategies for canine zoonoses in Mali, by better understanding of dog ecology, the dog-human relationship and post-vaccination rabies antibody decline. The results are also of regional importance in view of the global dog rabies elimination strategy by the Global Alliance for Rabies Control (GARC). With the dissemination of our results, we increase the profile of neglected zoonoses in Mali to be a little less neglected and, hopefully, raise interest on a national and international level.

11.2. Research outputs and outcomes

In the following sections, research outputs (New contextual knowledge generated on an issue) and outcomes (The importance assigned to knowledge and its uptake in a local context) are discussed. More detailed information on outputs and outcomes can be found in the Guide for Transboundary Research Partnership (KFPE, 2014). As previously mentioned in section 2.3.2., not all specific research objectives (see 2.2.2.) could be entirely achieved. More detailed explanations are presented in the following sections (11.2.4. & 11.2.5. & 11.2.6.).

11.2.1. Dog ecology in Bamako, Mali

Our findings were generally in line with other studies from Africa. But one important difference was the very low dog human ratio of 1:121 and dog density (see table 6, chapter 4). This is one of the lowest reported in Africa and results in an estimate of a total dog population of 15 000 (95% CI 13 000 – 17 000) dogs in the whole District of Bamako (Gsell et al., 2012; Hambolu et al., 2014; Kitala et al., 1993; Mindekem et al., personal communication, 2014; Seghaier et al., 1999). Our observations of the decline of dogs in the open cohort over the study period and a rather high growth rate in the projection matrix are contrasting but may indicate a cyclical dynamic of the dog population, which would be in agreement with the synchronous cycles of rabies transmission observed by Hampson et al. (2007).

Dogs are used mainly as guardians and not a lot of money is allocated for their maintenance and care.

Despite concerted efforts through a repeat of the baseline survey in 2011, only 379 dogs could be enrolled in the study. The follow up period of two years and the small size of our cohort are insufficient to draw a comprehensive conclusion about the population dynamics and expected population growth. Our study provides a first insight and confirms known reproductive and mortality parameters from similar settings in Tanzania (Gsell et al., 2012), Chad (Mindekem, personal communication, 2014) and Kenya (Kitala et al., 2001). To gain more accurate results, a much bigger cohort needs to be part of the study and it has to be followed over a much longer time period. Similar to human demographic surveillance (Indepth network), the control of zoonoses requires accurate dog and livestock demographic data for effective control. Population dynamic is therefore an important consideration in disease transmission models and helps policy makers to develop strategies for disease control (Schärrer et al., 2014; J. Zinsstag et al., 2005).

The survey teams carefully developed a good relationship with the dog owners during the baseline surveys and explained the importance of the study. This contributed to the high participation in the study with few household losses to follow up in the subsequent two years. During the third follow up survey, dog-owners were contacted in most cases per mobile phone which worked very well. This shows promise for future research projects, as shown for human and animal demographic surveillance in Chad (Jean-Richard et al., 2014), given that the majority of persons have a mobile phone and calls are much cheaper than visits. Recently, 14 613 million mobile phones were registered in Mali (Central Intelligence Agency, 2015) which exceeds the total population of Mali (14 528 662 inhabitants (RGPH-2009)).

The proportion of ownerless dogs is estimated at below 10% (Mosimann et al., 2017; Muthiani et al., 2015), and like other settings, is probably due to scarce food resources (Gsell et al., 2012). This finding is important for future rabies control, allowing mass vaccination campaigns to concentrate on reaching sufficiently high coverage in the owned dog population to interrupt rabies transmission (Kayali et al., 2003b).

In summary, the new knowledge about dog demography and the dogs’ role in this specific local context is fundamental for the design of effective and sustainable dog disease control programmes, for example rabies, in the District of Bamako (Léchenne et al., 2015).

11.2.1.1. The dog-human relationship in Bamako, Mali

The relationship between humans and dogs in Bamako is governed by norms and values determined by the local context (culture, religion) (Zinsstag et al., 2015), with each benefitting from the other in a mutual way. In Bamako dogs are generally kept for a specific purpose, mainly to guard houses and premises. They were often received as a gift and were in the majority of cases given away for free. In Bamako dog breeding for financial incentive was uncommon, whereas in rural areas dog breeding and selling was common (Traoré, personal communication, 2011). In Bamako, very little money was spent on the dogs' maintenance. Regarding religion, certain Qur'anic rules dictate that someone who had physical contact with a dog, mostly through saliva or fur, becomes spiritually unclean. This was especially considered the case preceding prayer (Bardosh et al., 2014). But there is a significant question of interpretation of Islamic tradition and debate on the impurity of dogs and dog-ownership in general. Sometimes only urban dogs are considered unclean because they often consume human garbage (Khaled Abou El Fadl, 2004). Although dogs generally do not appear to be treated badly in Bamako, their care may often be considered insufficient. The reason stated for non-dog-ownership in Bamako was in the majority of cases attributed to "no necessity" (see chapter 4). However, the local belief that dogs are ritually unclean is common in Bamako. Another common belief in Mali is that angels do not enter a house where a dog resides (Sahih Bukhari Volume 4, Book 54, Number 539 - Narrated Abu Talha). The high proportion of Muslims in Bamako may additionally explain the observed comparatively low dog human ratio and provides an example of the need for a contextual analysis considering social, cultural and religious aspects prior to engaging in zoonosis control. Dog rabies vaccination campaigns, for example, will not be successful if dogs, because of sociocultural aspects, are not touched and cannot be handled for vaccination (Léchenne and Zinsstag, 2013).

11.2.2. Seroprevalence of rabies antibodies in the domestic dog population and a synopsis after the two pilot rabies vaccination campaigns in Bamako, Mali

We showed that the majority of interviewees were aware of rabies and knew that dogs can transmit the disease. The possibility to protect dogs by rabies vaccination is also well known and appreciated by the interviewees. Nevertheless, only 24% of dogs showed neutralising rabies antibody titres. One main reason seemed to be the cost of the vaccination. Owners were willing to pay only a fraction (=0.2 €) of the actual price, which is less than the 1.1 €. reported for N'Djamena, Chad (Dürr et al., 2008a). The total cost for rabies vaccination is currently 4 € per dog in Bamako. The existing knowledge of the population about rabies is an important basic requirement for rabies interventions. Dog-owners have to understand why dog rabies vaccination is an important measure against rabies

(Schelling et al., 2008). Based on our results and a previous result from N'Djamena, Chad, where dog-owners were charged for dog rabies vaccination and an insufficient vaccination coverage was achieved (Dürr et al., 2008a), two small scale pilot vaccination campaigns free of cost for the dog owners were organised in Bamako in 2013 and 2014. The aim was to achieve a vaccination coverage of 70% as recommended through WHO (2013). The first free of cost pilot central point vaccination campaign in 2013 in Commune 1 resulted in an estimated coverage of 17.6% (Muthiani et al., 2015). Reasons for non-attendance were lack of information (in 25% of the cases), followed by the inability of dog handling (in 16% of the cases). Muthiani et al. (2015) proposed a transdisciplinary participatory stakeholder approach for the planning and implementing of further campaigns as a suggestion for improvement. This should be implemented at the interface of the society, science and politics. Children should be included in the planning, because they often brought dogs to the vaccination points. A possibility for improving the accessibility parameter would be to add additional door-to-door vaccination approaches along with central point vaccination.
The effectiveness method developed by Muthiani et al. (2015) is an important step to combine the findings of vaccination coverage assessments and to understand the effectiveness parameters, like Efficacy, Availability, Accessibility, Affordability, Adequacy, Acceptability, Targeting and Provider compliance, which are related in a multiplicative way and determine community effectiveness. Community effectiveness gives insight into how, in a specific health and social context, the given efficacy of an intervention, in our case the highly effective rabies dog vaccine, will translate into effectiveness (Zinsstag et al., 2011a).

A second free small scale pilot vaccination campaign, in Commune 5 and 6, was done in 2014 by Mosimann et al. (2017). Again low vaccination coverages were achieved (20-30%). The most sensitive effectiveness parameter was again the lack of knowledge about the campaign. The work of Mosimann et al. (2017) and Muthiani et al. (2015) contributed to the local understanding of cultural practices and social realities in Bamako. These social and cultural determinants are crucial for the effectiveness and sustainability of interventions, as already highlighted by Bardosh et al. (2014). In Bamako, dog owners who did not participate in the campaign responded that radio and television are important channels to inform the community about campaigns. Short message services (SMS) and door-to-door information was also stated as valuable. A questionnaire survey after the vaccination campaign showed that only 20% of dog-owners who had participated in the campaign were informed about it by radio. Thirty-five percent got the information verbally from friends, neighbours and family and 18% from vaccination teams visiting door to door. Similar to the first campaign, dogs were often brought to the vaccination point by children (Mosimann et al., 2017).

Why is there such a big difference in the vaccination coverage between N'Djamena, Chad, (Kayali et al., 2003b; Léchenne, personal communication) and Bamako, despite application of the same approach?

As described by Bardosh et al. (2014), interventions which overlook the social, cultural, political and economic context risk failure. It is absolutely crucial to adapt the intervention to the local context. Whereas in Bamako a reason for not bringing the dog to the vaccination point was intractability of the dog (Muthiani et al., 2015), in another local setting (Tanzania) it was considered embarrassing to bring a dog as this task was viewed as 'a child's duty' (Bardosh et al., 2014). Control programmes cannot be duplicated in a new area without considering the local setting. A similar information campaign as that in N'Djamena, Chad (Kayali et al., 2003b; Léchenne personal communication, 2014), was conducted before the pilot vaccination campaign in Bamako, with the exception that in N'Djamena, local chiefs also went from door-to-door to announce the campaign (Kayali et al., 2003b). In N'Djamena, where vaccination campaigns were first instituted in 2003, the population is probably more sensitized to this topic which might be another reason for the sufficient coverages achieved in recent years. Additionally it should be noted that the proportion of Muslims in Chad is 53%, significantly less than the 90% in Mali (Central Intelligence Agency Mali & Chad, USA, 2015). As previously discussed in section 11.2.1.1, a lack of regular dog handling, due to religious beliefs, could be a reason for the insufficient vaccination coverage. Muthiani et al. (2015) showed that dog handling seemed to be a reason for not bringing a dog to the vaccination point in Bamako and suggest that vaccination coverage might be improved using a combination of central point vaccination campaigns with door-to-door campaigns. Veterinarians or veterinary officers often vaccinate dogs directly in the households in Bamako (Sow, personal communication, 2010), which supports the idea of adding a door-to-door vaccination approach. An effective way of informing dog owners about future vaccination campaigns could be, in addition to television which was already proposed by Mosimann et al. (2017), through SMS. Furthermore, children should be involved in the future planning of campaigns, possibly through education on the importance of rabies prevention (Muthiani et al., 2015). According to Charles Waiswa, children are central for the success of vaccination campaigns and, as such, the Ministry of Education and schools should be engaged in future interventions (WHO, 2010).

11.2.3. Leishmaniasis and echinococcosis within the domestic dog population of Bamako, Mali

No cases of leishmaniasis were found, but we reported the first case of *Echinococcus* infection in a Malian dog. Despite having found such a low prevalence, this finding implies that the parasite is present in this setting. Only a small proportion of dogs were reported as being previously dewormed. Further multi-parasitism in Malian dogs indicates ongoing transmission of gastrointestinal helminths.

Examination of the fur from the perianal region is a good alternative test for parasite egg detection (Eckert et al., 2001; Overgaauw et al., 2009). Nevertheless, faeces should be considered as the diagnostic material of choice, because the presence of parasite eggs on the fur does not prove a current intestinal infection. We did not collect faecal samples in 2010 was because we thought that it would not be possible to collect sufficient quantities (10 g). In this local context, it also seemed inappropriate to ask dog owners to collect fresh faeces from their dogs into plastic bags for transport to the CVL. Nevertheless, we decided to collect faecal samples through rectal sampling during the second Baseline survey in 2011, and, despite low quantities of faeces, we were able to detect parasitic infection. One dog showed *Echinococcus s. l.* eggs in the fur in 2010 (Baseline study) and in the faeces in 2011 (Follow up survey). It was uncertain if this dog had longstanding infection or was newly infected, as worms can persist in dogs for approximately one year (Torgerson, 2003).

Dogs are not adequately dewormed in this setting and harboured multiple parasites. In addition to *Echinococcus,* other intestinal parasites also have zoonotic potential and frequent deworming should be recommended. Deworming should utilize a medication which includes the active component praziquantel. Torgerson (2003) showed that a treatment interval of 3 months reduced disease prevalence significantly. In Bamako, the first choice treatment for dog deworming is Stromiten® Chiens (vétoquinol), with the active ingredients niclosamide and tetramisole. This drug is ineffective against echinococcosis and should be replaced.

A known reservoir host for *Leishmania major* is rodents, but no data is available for Mali to date (Gramiccia, 2011; Paz et al., 2011). We suggest that further work on this disease includes an examination of rodents in this setting. Asymptomatic dogs infected with *L. infantum* were often not detected through diagnostic serology (Berrahal et al., 1996). It must be considered if this could also be true for dogs infected with *L. major.*

11.2.4. Disease burden in humans

As already mentioned in the section 2.3.2., the originally planned study in the human population had to be cancelled because the formal ethical approval was not available on time. This is an important weakness of this study regarding the benefits of a ‘One Health’ approach (See section 1.4.). Joint field teams are often the beginning of a fruitful intersectoral collaboration (Schelling and Hattendorf, 2015). An important lesson is that the agenda must be set collaboratively right from the start, as described in the Guide for Transboundary Research Partnerships (KFPE, 2014). The question ‘who will do what and how’ should be answered in the very beginning. Nevertheless, the dialogue with representatives from the human medical faculty took place, but the formal ethical approval was not available on time.

Regarding the human side of the targeted zoonoses, this work dealt only with people’s knowledge, attitudes and practices (KAP) of the studied diseases. However in this specific case, independent work from Kone (2013), which was also part of the ICONZ project, confirms the importance of rabies as a priority canine zoonotic disease, in Bamako. Both studies were therefore complementary and coherent. In future research projects, fully integrated studies which show an added value of a closer cooperation between human and animal health (see section 1.4.) should be conducted.

11.2.5. Trialling the same approach in the rural setting

The foreseen field trip of the PhD student to the rural setting was cancelled due to insecurity in the area. As already mentioned in section 2.3.2., some of the data and sample collection was conducted by the Malian collaborators, but these results will be analysed in a next step and are stated elsewhere.

11.2.6. Science in a conflict-ridden society

Due to the military coup in 2012, the third field trip of the PhD student was abruptly cancelled while in flight to Bamako, and the technology transfer of the applied diagnostics (Objective 8, section 2.2.2), which was conducted in specialised laboratories in Switzerland and France, unfortunately could not entirely be realised. The original plan was to analyse the biological dog samples from the rural site with the new established diagnostic tests at the CVL. As already described in section 1.1.1., capacity building is a very important point in the fight against NZDs, because they are often under-reported or misdiagnosed due to low diagnostic capabilities.

Fortunately the last follow up survey was conducted, under the lead of our main Malian collaborator, by an interviewer who was already familiar with the study from previous collaboration during the baseline survey. This allowed our research project to be maintained during the crisis (Bonfoh et al., 2011).

11.2.7. Effectiveness of the two different sampling strategies; Baseline study 2010 versus 2011

Currently new and free technologies are available, for example Google Earth (Google Inc.), which represents an clear enhancement for the sampling design of field studies (Kamadjeu, 2009). Urban areas are more eligible than rural areas, considering there is better graphical material for these areas. Researchers are often confronted, as was the case for Bamako, with the inability to find an accurate map of the area for study planning of (Kamadjeu, 2009). The only map which was available for the District of Bamako was not detailed (Appendix 2). In resource poor settings, where we often do not have an existing or adequate internet connection, it is nevertheless often impossible to use tools like Google Earth (Google Inc.).

One reason why we repeated the Baseline survey in 2011 was because of the small dog cohort we acquired during the Baseline survey in 2010. Previous experiences in N'Djamena, Chad (Mindekem et al., 2005), where a large confidence limit of the total dog population was found, prompted us to interrogate more households and to conduct a second baseline. All quarter heads, as already described in section 2.3.2., were informed during the first baseline study about the study. This gave us the opportunity in 2011 not to start with the heads of quarters. It is a long-standing tradition in Bamako that with each study conducted with the CVL, the head of quarters were informed and their households were used as the starting points of the study, thus this was a procedure we were obliged to respect in the planning phase. A year later during the second baseline survey, we implemented a new approach, which is less biased than the previously used 'spin the bottle technique'. We selected more starting points per quarter, which is an appropriate method in larger quarters (Bennett et al., 1991). As described in Bennett et al. (1991), this method does not give the field teams the opportunity to make personal choices and therefore the sample is less biased. The Google Earth maps (Google Inc.) we used in Bamako in 2011 were prepared in Basel, Switzerland, because during the Baseline study in 2010, internet was often not available at the CVL. This is a limiting factor for the development of a suitable and sustainable sampling method in this setting. The survey length between the two surveys cannot be compared, because we did not conduct full questionnaire interviews in non-dog-owning-households in 2011. Five teams conducted the survey in 25 days in 2010 compared to 4 teams in 2011 over 20 days, respectively. The whole survey was completed within one month, in line with the recommendation of WHO (1988a) to ensure that the data is as uniform as possible. As already mentioned in the results part of this thesis (see chapter 4 & 5), different interviewers were used in 2010 and 2011, thus it was not possible to evaluate if differences could have arisen due to the different sampling procedures. Nevertheless an almost identical dog human ratio was found in the two surveys, which represents an additional quality check for the 'spin the bottle' sampling procedure which was used in 2010. A possible bias of the study design in 2010 could have been that the head of each quarter was living in a wealthier neighbourhood; however, this seems to be not true for this local setting, where this traditional function is not depending on wealth (Traoré, personal communication, 2011). An adapted technique for the first baseline survey was proposed by the Swiss collaborators during the field planning session at the CVL: Starting from the household of the quarter head, a randomly chosen cardinal direction was overlaid on Google Map (Google Inc.) maps. Sampling of the households was proposed on roads along this line. This technique was rejected, because it required good graphical pictures which necessitated a strong internet connection and proper map reading skills of the field teams.

Costs of the two surveys were shared between the CVL and the Swiss TPH. A comparison of the costs between the two baseline surveys was not possible. It was discovered during the field work that the translocation of each team by motorbike was a good alternative to transportation by car. In the beginning the teams were taken to the quarters in the morning and picked up in the afternoon/evening. Often an insufficient number of cars were available, and the roads were blocked due to traffic.

During both baseline surveys, the head of household was often absent from compounds. This finding was in line with the study of Sow et al. (1998), where it was reasoned that this was due to the interview time, which took place during the afternoons. This can lead to biased responses, one of the main disadvantages of cross-sectional studies (Levin, 2006). Nonetheless, we considered this study design as the most appropriate, given that it is relatively inexpensive and does not take lots of time (Levin, 2006).

We sampled in total 32 clusters (quarters), which seemed to be appropriate for the District of Bamako considering the project budget and sample size calculation. It is important to remember that more clusters selected increases the study estimates (Turner et al., 1996). Data collection in the Lassa quarter was stopped during the survey in 2010 because all dogs were recently killed due to the annual dog elimination programs undertaken by the regional directorate of the rural support (DRAMR (Direction Régionale de l'Appui au Monde Rural) (Maoulid, 2005; Traoré, personal, communication, 2015).

Similar to the study of Grais et al. (2007), the GPS method used during the second baseline survey was viewed more favourably. Grais et al. (2007) interpreted this finding as due to the new technology. In our survey, the field teams much preferred the procedure in 2011, because the walking distances were notably less than the year before. Whereas the distance was 200 metres after the inclusion of two neighbouring households in two neighbouring compounds in 2010, an "around the block" method was chosen in 2011. The sampling strategy in 2011 was likely more accurate but also more complicated for the field teams to implement.

11.3. Applications of our research

Only one *Echinococcus* and no *Leishmania* cases were found in the examined dog population. Thus, the ICONZ intervention focused only on rabies. The work of Muthiani et al. (2015) and Mosimann et al. (2017) demonstrated well the phase of application and intervention from our scientific results. The dog demography and KAP data was used to inform the planning of these two pilot vaccination campaigns in Bamako.

11.4. General recommendations

Our recommendations are in line with the action points of zoonotic disease control of WHO (2006) (Fig. 36). Intersectoral collaboration should be promoted on all levels (data collection, data evaluation, control). Control strategy should be adapted to the economic, sociological and cultural context (Schelling and Hattendorf, 2015) of Bamako. With the new gained knowledge owing to the effectiveness model developed by Muthiani et al. (2015) and the work of Mosimann et al. (2017), further vaccination campaigns can be improved and adapted to this specific local setting, which should result in an increase of the effectiveness. More details are also provided in section 11.5.

For sustainable effective rabies control programs in Mali, long-term core funding must be generated. The statement from WHO, 'if technical knowledge alone were sufficient, rabies would no longer be a problem in the urban tropics' (WHO, 1988b), bears repeating as it is still true after almost 30 years! It is therefore absolutely crucial, that Malian ministries prioritise rabies control efforts and promote a vertical and horizontal intersectoral collaboration between the Division of epidemiology, prevention and control of diseases (DPLM (Division d'épidémiologie de prevention et de lutte contre la maladie)) which is part of the National Directorate of Health (Direction nationale de la santé), the specialised clinic for human rabies cases (LR (La clinique du Lazaret des Roches), the regional directorate of rural support (DRAMR (La Direction Régionale de l'Appui au Monde Rural)), the Ministry of Livestock and Fisheries (Ministre de l'Elevage et de la Pêche), the veterinary clinics and the CVL. All stakeholders and academia should work together to advocate the importance of rabies and to transform the following recommendations into reality, as suggested by WHO (2015).

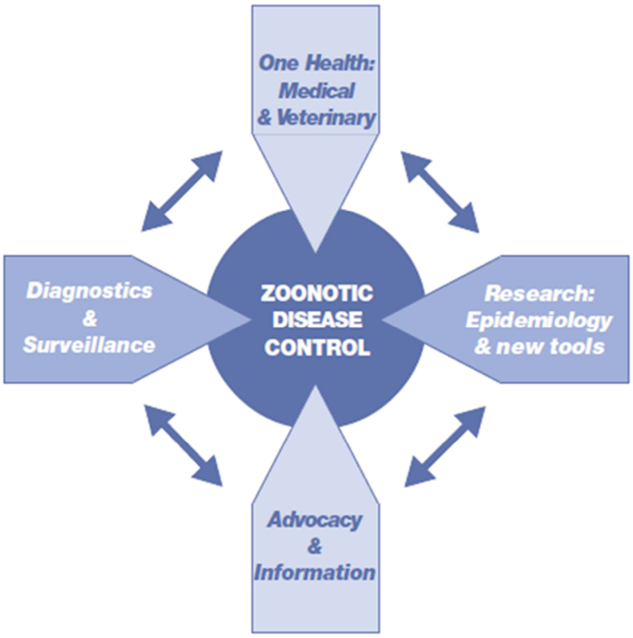

Figure 33: Action points of zoonotic disease control (WHO, 2006)

11.4.1. Integrated dog management policy

To raise disease awareness for prevention and control strategies, research findings must be shared with stakeholders in a transdisciplinary way (see section 1.5) (Welburn et al., 2015). This should be done in a simplified and comprehensible manner. An appropriate way is, for example, through a policy brief which gives a short summary of the findings and implications. The policy brief should address both political and societal issues. Another possibility would be through education and to raise awareness of communities through animal health clubs (AHCs), as established in Sierra Leone. A lack of dog-bite reporting, surveillance of suspected dogs and the absence of PEP for humans in Sierra Leone, contributed to endemic rabies after the civil war. AHCs were developed through a group of university lectures for rabies prevention. AHCs were formed in schools and the lectures taught teachers, students and farmers on different topics, like animal welfare, management, nutrition, disease prevention and control. The information was then spread to communities through AHC members (WHO, 2010).

Our recommendations:

- We strongly recommend responsible dog ownership. This includes frequent dog rabies vaccination and deworming. Deworming has to be done with a medication which includes the active component praziquantel.

- We strongly advise against city wide poisoning campaigns, which are counterproductive and socially unacceptable (WHO, 2013; Zinsstag et al., 2009).

11.5. Outlook and future research needs

- Based on our findings a policy brief for the Ministry of Livestock and Fisheries (Ministère de l'Elevage et de la Pêche, Mali) will be prepared in French.
- We still have more detailed dog economy data for Bamako and questionnaire data from the rural site. These findings give us a deeper insight about dog keeping in this local setting and should be analysed as soon as possible.
- Data on urban livestock keeping was also collected in Bamako and should be analysed and published.
- Biological dog samples from the rural study should be analysed as soon as possible.
- Remaining blood samples could be used for further tests, as dogs are known to be sentinels for a range of diseases (Cleaveland et al., 2006b).
- Rabies
    - Free large scale vaccination campaign should be conducted, considering the important points mentioned in section 11.2.2. It is suggested that a mixed central point and door-to-door vaccination approach be tested in future campaigns and that children should be included in the planning phase. Information about the campaign to the community should be increased. One possibility would be, in addition to announcements through radio, town criers (*crieur public*) and television, the use of SMS before the campaign. Considering that the majority of persons have a mobile phone, this clearly should be further elaborated as an option. As far as I know, SMS to inform dog-owners about a forthcoming vaccination campaign has not used in rabies control so far.
    - For a better estimation of human rabies incidence, mathematical modelling, according to Cleaveland et al. (2002), should be conducted on the basis of dog bite registers from different locations in Mali, as it was done in Chad by Frey et al. (2013). Malian scientists have already collected data on dog bites and human and animal rabies cases. They should be encouraged to publish and disseminate an estimation of human rabies incidences in different urban areas (Dao et al., 2006; Kante, personal communication 2010; Konate, 2002; Kone, 2013, 2010). If we know more about the true public health impact, rabies will garner more political commitment for control.

  - Rabies diagnosis outside of Bamako should be promoted, using for example the dRIT (Dürr et al., 2008b) (See also section 1.1.1., subsection: Rabies)
  - Given that Kone (2010) showed that immunoglobulin was not available in urban settings of Mali, solutions for the provision of immunoglobulin in health centres, as an important preventive measure after a rabies exposure, must be investigated.
- Echinococcosis
  - Slaughterhouse based studies should be conducted to investigate the intermediate host of the parasite. But it has to be recognised that not all animals are slaughtered in slaughterhouses because farmers fear condemnation at abattoirs (Schelling and Hattendorf, 2015). Another point is that slaughtered animals often belong to the same age-group. This could lead to a disease overestimation if older animals are slaughtered or to an underestimation if younger animals are slaughtered (Barnes et al., 2012).
  - Based on gained results about multi-parasitism of dogs and cost of current treatment, free dog deworming should possibly be included in the next rabies vaccination campaign.
  - Estimation of the disease burden in humans should be done
- Leishmaniasis
  - Examination of rodents as possible reservoir host of *Leishmania sp*. in Bamako, Mali
  - Estimation of the disease burden in humans should be done

11.6. Final conclusion

Our societally relevant research results indicate that rabies is the most important dog-related zoonosis. The obtained knowledge on dog ecology, people's knowledge attitudes and practices regarding rabies and the gained experience through the small scale vaccination campaigns will contribute to the policy discussion on rabies control in sub-Saharan African cities. Altogether, this will further pave the way for a scaling up of rabies intervention in the District of Bamako and nationwide.

This PhD was possible through a long-standing North-South research collaboration between different institutions, namely the Swiss TPH, the Institute of Parasitology of the Vetsuisse Faculty of the University of Zurich, the Anses-Nancy laboratory for Rabies and Wildlife and the CVL. For many years, Swiss TPH and CVL have collaborated in a spirit of equity and building mutual trust, which has led to fruitful and effective mutual learning for the improvement of human and animal health.

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Appendix 1: Questionnaires

1. Instructions

Code d'identification pour le questionnaire en général sur le ménage et sur les chiens et le questionnaire individuel chien

On a besoin du code d'identification pour identifier la concession, chien et l'échantillon.

Code d'Identification : |__| |__| |__|__|__| |__|__|__|__|

Commune 1 · Equipe 2 · Ménage 3 · Numéro de chien 4

1 Commune : 1 = Commune 1
2 = Commune 2
3 = Commune 3
4 = Commune 4
5 = Commune 5
6 = Commune 6

2 Euipe : 1 = Namory Kéita
2 = N'Tio Samaké
3 = Abass Diarra
4 = Séman Kanté
5 = Oumar Mangané
6 = Amadou Sery

3 Ménage: (=Concession) Pour chaque équipe, le premier ménage commence par le numéro 001 puis les ménages suivants sont numérotés de 002, 003, 004, 005, 006, 007, …

4 Chiens : Dans chaque ménage, le numéro du premier chien non prélevé commence par la lettre A et le numéro 001 (A001) et les chiens suivants, le numéro A002, A003, ect.

Les chiens à prélever commencent par la lettre B et le numéro de 001 à 750 (B001, B002,….B750). Ces numéros sont identiques pour les tubes de prélèvement de sang et les petits sachets pour la pièce du pelage.

Exemple

- La famille d'Abdullah habite dans la commune 2 à Niarela. Ils ont deux chiens. Cette famille est le premier ménage que l'équipe de Seman Kanté visite.
  Elle aura pour code

  |_2_| |_4_| |_0_|_0_|_1_| |_A_|_0_|_0_|_1_| → pour le premier chien

  |_2_| |_4_| |_0_|_0_|_1_| |_A_|_0_|_0_|_2_| → pour le deuxième chien

- La famille de Mahamat habite dans la commune 1 à Korofina nord. Ils ont un chien. Cette famille et le quatrième ménage que l'équipe de Oumar Mangané visite et elle prélève des échantillons de chien. Le chien aura le numéro 12.
  Elle aura pour code

  |_1_| |_5_| |_0_|_0_|_4_| |_B_|_0_|_1_|_2_|

2. Household Questionnaire

Code d'Identification : |__| |__| |__|__|__|
Commune 1 — Equipe 2 — Ménage 3

Démographie canine à Bamako

Questionnaire sur le ménage et sur les chien(ne)s

Ménage:

Nom de l'enquêteur: ____________________ Code : |__|
Date de l'enquête: |__|__| (jour) |__|__| (mois) |__|__| (année)
Nom Commune : ____________________ Code : |__|
Nom Quartier : ____________________ Code : |__|
Coordonnées du système de localisation GPS: N |__|__||__|__|__|__|__|__|°
W |__|__||__|__|__|__|__|__|°

No Concession : |__|__|__|
Nom du répondant : ____________________

Âge et sexe du répondant : Âge : |__|__| (ans)
Sexe : 1. Homme ☐ 2. Femme ☐

1. **Combien êtes-vous dans votre ménage** |__|__| **concession** |__|__| ?

2. **Quelle est l'occupation principale du chef de ménage?**
   Plusieurs réponses sont possibles
   1. Ménagère, aide familiale ☐
   2. Etudiant, élève ☐
   3. Cultivateur, éleveur ☐
   4. Fonctionnaire ☐
   5. Employé dans le secteur privé ☐
   6. Grand(e) commerçant(e) ☐
   7. Petit(e) commerçant(e) ☐
   8. Sans emploi/ chômeur ☐
   9. Autre, précisez : ____________________

3. **Quelle est votre religion ?**
   1. Musulmane ☐
   2. Chrétienne ☐
   3. Animiste ☐
   4. Sans religion ☐
   5. Autre, précisez : ____________________

4. **Quelle est votre Ethnie (pour les Maliens)/ votre Nationalité (pour les Etrangers) :**

   - Ethnie :
     1. Bambara ☐
     2. Malinke ☐

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Code d'Identification : |__| |__| |__|__|__|
Commune 1 — Equipe 2 — Ménage 3

---

3. Peulh ☐
4. Sarakole/Soninke/Marka ☐
5. Sonraï ☐
6. Dogon ☐
7. Tamachek ☐
8. Sénoufo/ Minianka ☐
9. Bobo ☐
10. Autre, précisez : ____________________

- Nationalité :
  1. Pays CEDEAO ☐
  2. Autres Pays Africains ☐
  3. Autres Nationalité au dehors de l'Afrique ☐

5. Observez le type de l'habitat :

- murs :
  1. Banco ☐
  2. Banco amélioré ☐
  3. Ciment ☐
- toit :
  1. paille ☐
  2. tôle ☐
  3. ciment ☐

6. Avez-vous déjà entendu parler d'une maladie appelée…. ?

- rage : 0. Non ☐ 1. Oui ☐
- leishmaniose/Bouton d'Orient/ Clou de Biskra/ Bouton d'Alep : 0. Non ☐ 1. Oui ☐
- Echinococcose/ Hydatidose : 0. Non ☐ 1. Oui ☐

7. Quels genres d'animaux avez-vous et combien d'entre eux appartiennent à votre concession?

Espèces	Nombre
Chat	
Chèvre	
Mouton	
Porc	
Bovin	
Volaille	
Chameau/ dromadaire	
Cheval/ âne	
Chien	
Autres (à préciser) …………………………………… ……………………………………	

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Code d'Identification : |__| |__| |__|__|__|
Commune 1 — Equipe 2 — Ménage 3

8. Si pas de chien(ne) dans le ménage, les raisons ?
Plusieurs réponses sont possibles

- 1. N'a pas besoin ☐
- 2. Religion ☐
- 3. Coutume ☐
- 4. Locataire (Statut d'occupation du logement) ☐
- 5. Trop cher ☐
- 6. Ne sais pas ☐
- 7. Autre, précisez : ____________________

9. Si trop cher, pourquoi ?
Plusieurs réponses sont possibles

- 1. Prix d'achat ☐
- 2. Coût d'entretien ☐
- 3. Frais ultérieurs (p. ex. d'une personne mordues) pour le propriétaire du (de la) chien(ne) ☐
- 4. Autre, précisez : ____________________

10. Si Prix d'achat, précisez.

	FCFA
1. Chiot	
2. Chienne	
3. Chien	

11. Quelles sont les maladies que les chien(ne)s peuvent transmettre à l'homme ?
Plusieurs réponses sont possibles
Mettez une croix devant la réponse si la personne croit c'est une réponse correct.

	Attendez les réponses de la personne interviewée	Quand la personne a dit pas le réponse spontanée, citez toutes les possibilités
1. Leishmaniose/Bouton d'Orient/ Clou de Biskra/ Bouton d'Alep		
2. Echinococcose/ Hydatidose		
3. Rage		
4. Autres, précisez : ……………………………………………… ………………………………………………		

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Code d'Identification : |___| |___| |___|___|___|
Commune 1 Equipe 2 Ménage 3

12. Quels sont les signes de la rage que le (la) chien(ne) peut présenter ?
Plusieurs réponses sont possibles
Mettez une croix devant la réponse si la personne croit c'est une réponse correct.

	Attendez les réponses de la personne interviewée	Quand la personne a dit pas le réponse spontanée, citez toutes les possibilités
1. Change comportement (par exemple aggression)		
2. Salivation		
3. Hurlement rauque		
4. Mordre		
5. Autres, précisez		

13. Que devez-vous faire quand votre chien(ne) ou un(e) autre de votre entourage devient agressif(-ive), bave ou change de comportement ?
Plusieurs réponses sont possibles
Mettez une croix devant la réponse si la personne croit c'est une réponse correct.

	Attendez les réponses de la personne interviewée	Quand la personne a dit pas le réponse spontanée, citez toutes les possibilités
1. Le (la) vacciner		
2. Le (la) tuer		
3. L'enfermer		
4. L'abandonner		
5. Le (la) mettre sous supervision le centre de rage/ le vétérinaire		
6. Ne sais pas		
7. Autre, précisez :		

14. Que faut-il faire pour éviter qu'un(e) chien(ne) soit atteint(e) de rage ?
Plusieurs réponses sont possibles

- 1. Ne sais pas ☐
- 2. Le (la) vacciner ☐
- 3. L'Enfermer ☐
- 4. Autre, précisez : ______________________________

15. Existe-t-il un vaccin contre la rage du (de la) chien(ne) ?

- 0. Non ☐
- 1. Oui ☐
- 2. Ne sais pas ☐

Code d'Identification : |__| |__| |__|__|__|
Commune 1 Equipe 2 Ménage 3

Chien(ne)s dans votre ménage :

16. Combien de chien(ne)s avez-vous de :

	Nombre
1. Chiots (0 – 3 mois)	
2. Femelles jeunes (4 – 5 mois)	
3. Femelles jeunes (6 – 11 mois)	
4. Femelles adultes (12 - 23 mois)	
5. Femelles adultes (2 – 3 ans)	
6. Femelles adultes (> 3 ans)	
7. Mâles jeunes (4 – 5 mois)	
8. Mâles jeunes (6 – 11 mois)	
9. Mâles adultes (12 - 23 mois)	
10. Mâles adultes (2 – 3 ans)	
11. Mâles adultes (> 3 ans)	
Calcule le total	

17. Au cours des 6 derniers mois, combien de chien(ne)s ou chiots avez-vous….. ?

	Chien(ne)s	Chiots
1. adopté(e)s		
2. acheté(e)s		
3. donné(e)s		
4. vendu(e)s		
5. abandonné(e)s		
6. abattu(e)s/ tué(e)s		

18. Si achetés ou vendus au cours des 6 dernières mois ou avant, quel était le prix/coût pour chaque animal ?

	Chien(ne) ou Chiot (Écrivez le nom du (de la) chien(ne) ou chiot ou si pas un nom, seulement chien(ne) ou chiot devant acheté(e)s)	Coût (FCFA)	Date (mois et année)
- Acheté(e)s			

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Commune 1 Equipe 2 Ménage 3

	Chien(ne) ou Chiot (Écrivez le nom du (de la) chien(ne) ou chiot ou si pas un nom, seulement chien(ne) ou chiot devant vendu(e)s)	**Prix (FCFA)**	**Date (mois et année)**
- Vendu(e)s			

19. Si abattu(e)s/tué(e)s, les raisons ?
Plusieurs réponses sont possibles

	Nombre
1. Rage	
2. Surnombre	
3. Accident de la circulation	
4. Autre maladie	
5. Autre, précisez : ………………………………………………… …………………………………………………	

20. Si abattu(e)s/tué(e)s, les méthodes utilisées et le coût de cette méthode (p. ex. la balle si fusillé) ?

Méthode utilisée	**Coût (FCFA) « si applicable »**

21. Combien de vos chien(ne)s/chiots ont disparu au cours des 6 derniers mois ?

Chiens : |__|__| Chiots : |__|__|

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22. Combien de vos chien(ne)s/chiots sont mort(e)s d'une mort naturelle au cours des 6 derniers mois ?

Chiens : |__|__| Chiots : |__|__|

23. Est-ce que d'autres chien(ne)s viennent dans votre maison/concession ?

- 0. Non ☐
- 1. Oui ☐
- 2. Ne sais pas ☐

24. Si oui, combien ? |__|__|

25. Connaissez-vous les propriétaires de ce(tte)s chien(ne)s ?

- 0. Non ☐
- 1. Oui ☐
- 2. Incertain/e ☐

26. Ces propriétaires sont ils vos voisins ?

- 0. Non ☐
- 1. Oui ☐
- 2. Incertain/e ☐

27. Constatez-vous la présence des chiens sans propriétaires dans votre voisinage ?

- 0. Non ☐
- 1. Oui ☐
- 2. Incertain/e ☐

28. Si oui, combien les estimez-vous ?

- Chien(ne)s : |__|__| Chiots : |__|__|
- Ne sais pas ☐

29. Savez vous à quel âge doit-on administrer la première dose de vaccin contre la rage à son (sa) chien(ne) ?

- 0. Non ☐
- 1. Oui ☐
- 2. Incertain ☐

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30.Si oui ou incertain, à quel âge doit-on administrer la première dose de vaccin ?

- Mois : |__|__|

31. Quelle est votre préférence entre :

- 1. Vacciner votre chien(ne) contre la rage ☐
- 2. Traiter une personne mordue ☐

32. Combien êtes vous prêt à payer pour faire vacciner votre chien(ne) contre la rage ?

|__|__|__|__|__| FCFA

33. Quels sont les coûts pour garder vos chien(ne)s ?
Écrivez les réponses dans la table et complétez les |__|__|

	Coût (FCFA)
Nourriture	/mois
Médicaments	/ au cours des 6 derniers mois
Vaccins	/ au cours des 6 derniers mois
Vétérinaire	/ au cours des 6 derniers mois
Niche	/ au cours de |__|__| dernieres années
Corde	/ au cours de |__|__|__| derniers mois
Frais ultérieurs (si le (la) chien(ne) a mordu quelqu'un)	/ au cours des 6 derniers mois
Autres, précisez :	/ au cours de |__|__|__|derniers mois

34. Le montant des coûts pour tous vos chien(ne)s au cours des derniers 6 mois ?

|__|__|__|__|__|__|__|__|__|__|__|__|__|__|__| FCFA

35. Est-ce que nous pouvons vous rappeler dans 2-3 mois afin de collecter de nouvelles données à propos de vos chien(ne)s?

- Non : ☐
- Oui : Numéro de portable: (+ |__|__|__|) |__|__| |__|__| |__|__| |__|__|

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Commune 1 Equipe 2 Ménage 3

Réservée à l'enquêteur

Est-ce que l'équipe fera des prélèvements ?

- 0. Non ☐
- 1. Échantillon du sang ☐
- 2. Échantillon du pelage ☐

Combien de questionnaires individuels des chiens vous avez fait ?
-Si prélèvement, collez dans la table l'étiquette
-Si pas prélèvement, prenez la lettre A et un numéro continu
-Ecrivez aussi dans le table s'il était pas possible de prendre des prélèvements parce que le (la) chien(ne) était très agressif(-ive) ou pas dans le ménage

Numéro du chien :

3. Dog Questionnaire

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Commune 1 | Equipe 2 | Ménage 3 | Numéro du chien 4

Questionnaire individuel (chien)

Numéro du chien: **(Si prélèvement, collez-ici l'étiquette)**

Nom de l'enquêteur: ______________________________ Code |__|
Date de l'enquête : |__|__| (jour) |__|__| (mois) |__|__| (année)

Chien(ne)

1. Nom du (de la) chien(ne) : ______________________________

2. Si pas un nom, décrivez le(la) chien(ne) (Race, Robe, taille, marques particulières) :

____
____
____
____

3. Sexe du (de la) chien(ne):
1. Mâle non castré ☐
2. Mâle castré ☐
3. Femelle non castrée ☐
4. Femelle castrée ☐

4. Quel est l'âge du (de la) chien(ne) ? |__|__| (jour) |__|__| (mois) |__|__| (an)
Ne sais pas ☐

5. Comment avez-vous acquis le (la) chien(ne) ?
1. Progéniture de chien(ne) du ménage ☐
2. Acheté(e) ☐
3. Reçu(e) comme cadeau ☐
4. Confié(e) ☐
5. Autre, précisez : ______________________________

6. Quel était son âge lors de son acquisition ? |__|__| (jour) |__|__| (mois) |__|__| (an)
Ne sais pas ☐

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Commune 1 | Equipe 2 | Ménage 3 | Numéro du chien 4

7. Pour quelle(s) raison(s) élevez-vous ce(te) chien(ne)?
Plusieurs réponses sont possibles

- 1. Gardien(e) de l'habitation ☐
- 2. Animal de compagnie ☐
- 3. Chasse ☐
- 4. Chien(ne) du troupeau ☐
- 5. Ne sais pas ☐
- 6. Autre, précisez : ____________________

8. Comment le (la) chien(ne) est-il (elle) gardé(e) ?

- 1. Toujours enfermé(e) ☐
- 2. Enfermé(e) pendant la journée ☐
- 3. Enfermé(e) pendant la nuit ☐
- 4. Libre de tout mouvement ☐
- 5. Autre, précisez : ____________________

9. Où passe-t-il (elle) la grande partie de sa journée ?
Au maximum deux réponses sont possibles

- 1. Concession ☐
- 2. Devant la concession ☐
- 3. Chez le voisin ☐
- 4. Dans la rue ☐
- 5. Ne sais pas ☐
- 6. Autre, précisez : ____________________

10. Comment votre chien(ne) se nourrit-il(elle)?
Plusieurs réponses sont possibles

- 1. pâtes ☐
- 2. déchets de cuisine ☐
- 3. déchets de rue/ décharge ☐
- 4. viandes et abats crus ☐
- 5. Ne sais pas ☐
- 6. Autre, précisez: ____________________

11. Quel genre de maladie avez-vous constaté chez votre chien(ne) pendant les 2 derniers semaines?
Plusieurs réponses sont possibles

- 1. En bonne santé ☐
- 2. Grand Changement du comportement (Aggressif, Divauger) ☐

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Commune 1, Equipe 2, Ménage 3, Numéro du chien 4

- 3. Maigrir ☐
- 4. Changement de la peau ☐
- 5. Diarrhée ☐
- 6. Vomissement ☐
- 7. Difficultés urinaires ☐
- 8. Boiterie ☐
- 9. Autre, précisez: ______________________

12. **(Si une chienne)**
Votre chienne a-t-elle mis bas au cours des 6 derniers mois?
- 0. Non ☐
- 1. Oui ☐
- Incertain/e ☐

13. Si oui,

Définir…	Nombre
Nbre chiots au cours des 6 derniers mois	
Nbre chiots vivants par mise bas	
Nbre chiots vivants dans le ménage	
a) Nbre chiots morts de causes naturelles	
b) Nbre chiots abandonnés	
c) Nbre chiots vendus	
d) Nbre chiots donnés	
e) Nbre chiots disparu	
f) Nbre chiots tués	
Sommes des pertes de chiots (a-f)	

14. Si chiots tués, les raisons ?
Plusieurs réponses sont possibles

	Nombre
1. Surnombre	
2. Rage	
3. Autre maladie	
4. Tués par leur mère	
5. Ne sais pas	
6. Autre, précisez : ……………………………………………………….. ………………………………………………………..	

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Commune 1 / Equipe 2 / Ménage 3 / Numéro du chien 4

15. Est-ce que le (la) chien(ne) est suivi(e) par un vétérinaire?

- 0. Non ☐
- 1. Oui ☐
- 2. Incertain/e ☐

16. Est-ce que le (la) chien(ne) a été vermifugé(e) dans les derniers 6 mois?

- 0. Non ☐
- 1. Oui ☐
- 2. Incertain/e ☐

17. Si oui, quel a été le coût ?

|__|__|__|__|__|__|__|__|__|__|__|__| FCFA

18. Est-ce que le chien a eu d'autres soins vétérinaires ou médicaments au cours des 6 derniers mois ?

- 0. Non ☐
- 1. Oui ☐

19. Si oui, demander les détails.

Quoi ?	Coût ? (FCFA)

20. Est-ce que le (la) chien(ne) de votre ménage a été vacciné(e) de sa vie.

0. Non ☐
1. Oui ☐

21. Si oui, contre quoi ?
Plusieurs réponses sont possibles

- Rage : 0. Non ☐ 1. Oui ☐
  → Quand ? ______________________________
  Coût ? |__|__|__|__|__|__|__| FCFA

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Commune 1 — Equipe 2 — Ménage 3 — Numéro du chien 4

Le temps dépense pour aller au service vétérinaire et faire vacciner l'animal ?
- moins d'une ½ heure ☐
- ½ d'heure à 1 heure ☐
- plus d'une heure ☐

- Autres Maladies : 0. Non ☐
  1. Oui ☐ → Précisez : ______________________

Coût ? |__|__|__|__|__|__|__| FCFA

Le temps dépense pour aller au service vétérinaire et faire vacciner l'animal ?
- moins d'une ½ heure ☐
- ½ d'heure à 1 heure ☐
- plus d'une heure ☐

- Ne sais pas ☐

22. Si non vacciné(e) contre la rage, les raisons ?
Plusieurs réponses sont possibles

- 1. Manque de moyens financiers ☐
- 2. Transport ☐
- 3. Pas d'accès ☐
- 4. Inutile ☐
- 5. Autres, précisez : ______________________

23. Avez-vous un carnet de vaccination ?

- 0. Non ☐
- 1. Oui ☐

24. Si oui, vérifier le carnet de vaccination et mentionner la dernière date de vaccination.

|__|__| (jour) |__|__| (mois) |__|__| (année)

25. Donner le nom du (des) vaccin(s) utilisé(és).

Nom : ______________________
Nom : ______________________

4. Follow-up Household Questionnaire

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Commune 1 Equipe 2 Ménage 3

Suivi de la population canine à Bamako

Questionnaire sur le ménage et sur les chien(ne)s

Nom de l'enquêteur: ________________________ Code : |__|

Date de l'enquête: |__|__| (jour) |__|__| (mois) |__|__| (année)

Quartier : ________________________ Code : |__|

Coordonnées du système de localisation GPS: N |__|__|__||__|__|__|__|__|°
W |__|__|__||__|__|__|__|__|°

Nom du répondant : ________________________

Âge et sexe du répondant :Âge : |__|__| (ans)
Sexe : 1. Homme ☐ 2. Femme ☐

1. Combien de chien(ne)s avez-vous de :

	Nombre
1. Chiots (0 – 3 mois)	
2. Femelles jeunes (4 – 5 mois)	
3. Femelles jeunes (6 – 11 mois)	
4. Femelles adultes (12 - 23 mois)	
5. Femelles adultes (2 – 3 ans)	
6. Femelles adultes (≥ 3 ans)	
7. Mâles jeunes (4 – 5 mois)	
8. Mâles jeunes (6 – 11 mois)	
9. Mâles adultes (12 - 23 mois)	
10. Mâles adultes (2 – 3 ans)	
11. Mâles adultes (≥ 3 ans)	
Calcule le total	

2. Au cours des 6 derniers mois, combien de chien(ne)s ou chiots avez-vous….. ?

	Chien(ne)s	Chiots
1. adopté(e)s		
2. acheté(e)s		
3. donné(e)s		
4. vendu(e)s		
5. abandonné(e)s		
6. abattu(e)s/ tué(e)s		

3. Si achetés ou vendus au cours des 6 dernières mois ou avant, quel était le prix/coût pour chaque animal ?

	Chien(ne) ou Chiot (Ecrivez le nom du (de la) chien(ne) ou chiot ou si pas un nom, seulement chien(ne) ou chiot devant acheté(e)s)	**Coût (FCFA)**	**Date (mois et année)**
- Acheté(e)s			

	Chien(ne) ou Chiot (Ecrivez le nom du (de la) chien(ne) ou chiot ou si pas un nom, seulement chien(ne) ou chiot devant vendu(e)s)	**Prix (FCFA)**	**Date (mois et année)**
- Vendu(e)s			

5. Follow-up Dog Questionnaire

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Commune 1 — Equipe 2 — Ménage 3 — Numéro du chien 4

Suivi : Questionnaire chien(ne) individuel

Nom de l'enquêteur: ______________________ Code : |__|__|

Date de l'enquête: |__|__| (jour) |__|__| (mois) |__|__| (année)

Nom du (de la) chien(ne) : ______________________

1. Sexe du (de la) chien(ne):
1. Mâle non castré ☐
2. Mâle castré ☐
3. Femelle non castrée ☐
4. Femelle castrée ☐

2. Quel est l'âge du (de la) chien(ne) ? |__|__| (jour) |__|__| (mois) |__|__| (an)
Ne sais pas ☐

3. Si une chienne
Votre chienne a-t-elle mis bas au cours des 6 derniers mois?

- 0. Non ☐
- 1. Oui ☐
- 2. Incertain/e ☐

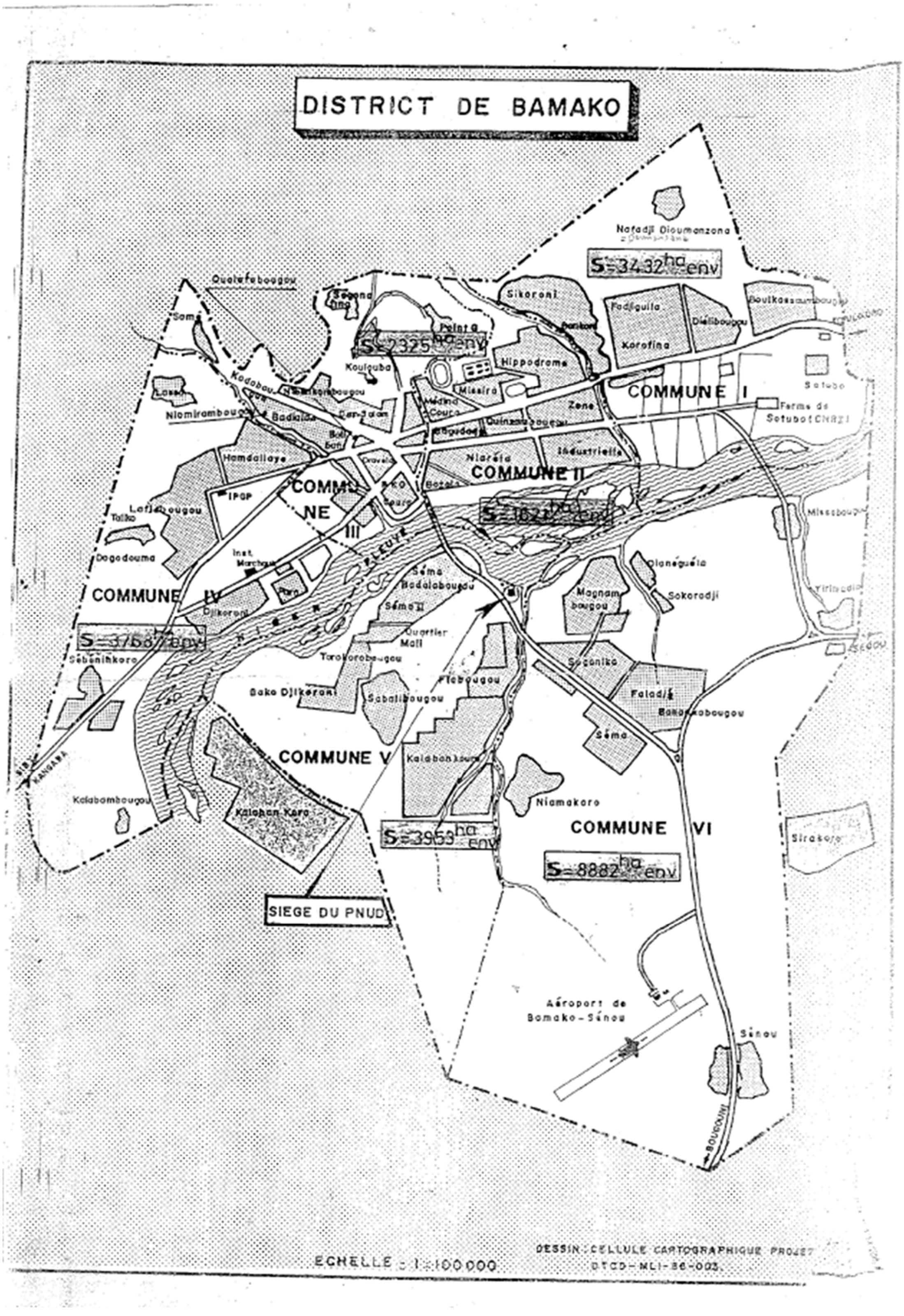
4. Si oui,

Définir…	**Nombre**
Nbre chiots au cours des 6 derniers mois	
Nbre chiots vivants par mise bas	
Nbre chiots vivants dans le ménage	
a) Nbre chiots morts de causes naturelles	
b) Nbre chiots abandonnés	
c) Nbre chiots vendus	
d) Nbre chiots donnés	
e) Nbre chiots disparus	
f) Nbre chiots tués	
Sommes des pertes de chiots (a-f)	

5. Si chiots tués, les raisons ?
Plusieurs réponses sont possibles

	Nombre
1. Surnombre	
2. Rage	
3. Autre maladie	
4. Tués par leur mère	
5. Ne sais pas	
6. Autre, précisez : ………………………………………………………… …………………………………………………………	

Appendix 2: Map of Bamako



Appendix 3: Photos from the field

1. Field preparation (Bamako)


Study planning at the CVL


GPS device training


Pre-test of the questionnaire


Field team 2011

2. Rural field visit (Ségou, San, Tominian)

Planning of the the rural study

Typical Malian compound

Dog seller

3. Field work in Bamako


Each household was recorded using a GPS

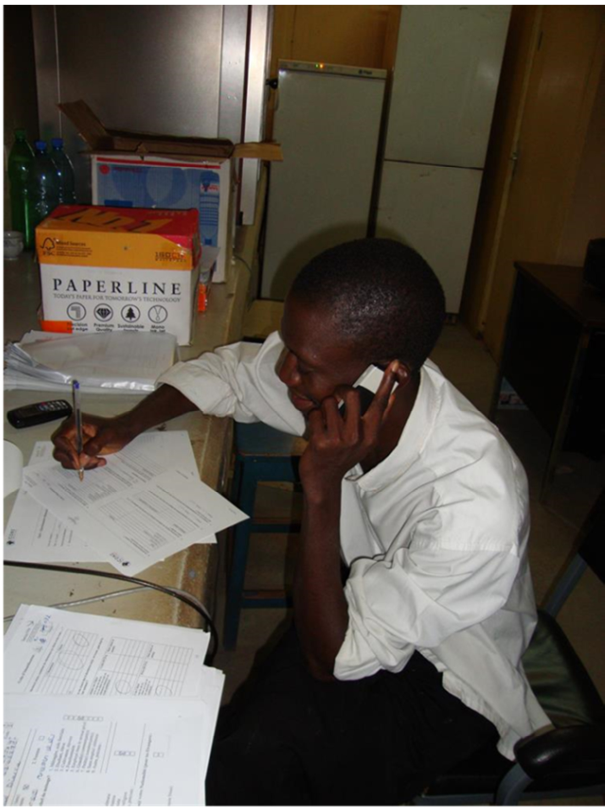

Questionnaire interview


Field team in the streets of a quarter


Blood collection

Lab work

Blood slide preparation



Telephone interview during the Follow-up survey

Appendix 4: Report in the ICONZ Magazine, Issue 4, p. 4-5

Making ICONZ happen

By Stephanie Brickman.
What does One Health mean in practice?
Prof Jakob Zinsstag explains the transition from talk to reality.

"People say they are doing One Health, but if you cannot show added value in terms of human or animal health benefits, cost savings or additional environmental protection from your activity then I wouldn't call it One Health," asserts Prof Zinsstag of the Swiss Tropical and Public Health Institute and leader of Work Package 3.

Work Package 3 sets out to address gaps in current knowledge of disease, a particular problem when trying to assess disease burdens.

"In the first place, of course we need to understand how zoonotic diseases circulate between humans and animals," continues Prof Zinsstag, "but when it comes to intervention the big question is – from what perspective do we analyse our work? That shapes how we intervene."

"Taking the perspective of a Minister of Health, if people get bitten by dogs, you clean the wound and give post-exposure treatment against rabies. It costs money and, worse, you won't eliminate rabies that way. It's not human to human but dog to dog transmission that drives a rabies epidemic."

"Taking the perspective of a Minister of Agriculture, he's interested in cattle not dogs. We need to look at the problem from a cross-sector perspective, more as the Prime Minister would see it. It's when we take this ecological or societal perspective, as we are doing in Mali and Morocco, that we can look at disease transmission, involve all stakeholders and assess the disease risks in humans and animals simultaneously."

Prof Zinsstag and his team have developed a method of "integrated assessment" that shows the comparative cost-effectiveness of different ways of intervening and, crucially, can identify a break-even point in an intervention.

"From an ecological perspective the brucellosis situation is similar to the rabies situation, in that humans are not driving transmission and mass vaccination of animals is required," explains Prof Zinsstag.

"Comparing the cost of livestock vaccination to human health costs, you can see that the public health benefits are maybe only 10-20% of those costs, so the Minister of Health or of Agriculture would say brucellosis control was not profitable. But if you add the benefits to private households from avoided expenditure in health care and coping costs, and the benefits to the agricultural sector in improved livestock productivity, you see that the societal benefits are three times higher than the intervention costs."

The next question when planning an intervention is – who pays? Prof Zinsstag and team have adapted a method called the "separable costs method" in order to answer just this question. If each sector pays a proportion of the disease control costs equivalent to the share of total financial benefits it expects to receive, we can show that such interventions can become financially viable.

This kind of cross sector perspective shows you a profitability that would not be shown if you looked at it only from single sector perspective.

Prof Zinsstag is adamant that research should never be developed from a purely academic point of view. Communities and the authorities should be involved from the outset, defining acceptable interventions, risks and costs.

"We also need to be careful there isn't a north-south division reflected in the work," explains Prof Zinsstag, "with the conceptual analytical work being done in Europe and the field work in Africa. This is why we have invested in Stephanie Mauti, a Swiss PhD student working in Mali. This kind of partnership produces a lot of added value because you mix local knowledge and cutting edge methods and technology."

"One Health has to have practical consequences and in ICONZ we can actually show more and more examples of what happens when human and animal health sectors work closely together."

Rabies control – united we stand.

Bamako, a sprawling city of two million souls, is a stark contrast to the beautifully organised Swiss city of Basel. Yet there is a bridge between these two very different places in the form of a mentoring partnership between two scientists involved in ICONZ.

Dr Abdallah Traoré heads the virology laboratory of the Laboratoire Central Vétérinaire in Mali and is mentor to Stephanie Mauti, a PhD student under the supervision of Prof Jakob Zinsstag. With Dr Traoré's help, Stephanie is writing her thesis on the role of the dog in the transmission of rabies, echinococcosis (hydatid disease) and leishmaniasis.

Stephanie, Dr Traoré and a group of young local science graduates have completed a questionnaire-based survey of dog-keeping Bamako households, to establish some basic canine demography data. The team also took samples of blood and faeces from the dogs to check for echinococcosis, leishmaniasis and also check the rabies antibody titre in the vaccinated dogs.

"We visited almost 3,000 households in 46 days," explains Stephanie, "I don't think we would have been able to conduct the project without Abdallah. He's a really great guy and a passionate scientist. It was so important to have someone with local knowledge and the same aim – eliminating rabies – who I could always ask for help."

Dr Traoré speaks in equally glowing terms about the collaboration with Stephanie. "I am delighted to have been Stephanie's mentor," he exclaims. "She won the hearts of the families involved in the research. It was also very beneficial for the young scientists from Mali working with us."

"Stephanie's project will help my country launch a national campaign against rabies. There is rampant population growth in the cities as dwellings that were temporary become permanent and rabies is a leading public health problem."

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