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Studies on designing effective and efficient canine vaccination program for rabies control in Zambia

(ザンビア共和国における狂犬病制御のための効果的・効率的な犬のワクチン接種計画の立案に関する研究)

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Notes

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ABBREVIATIONS

BHK	baby hamster kidney
CI	confidence interval
CRI	credible interval
CVS	challenge virus standard
dpv	days post vaccination
DVO	district veterinary office
EPI	expanded program on immunization
FAVN	fluorescent antibody virus neutralization
GMT	geometric mean titer
GPS	Global Positioning System
IU	international units
OIE	World Organisation for Animal Health
PEP	post-exposure prophylaxis
TCID ₅₀	50% tissue culture infective dose
USD	United States dollar
WHO	World Health Organization
ZMW	Zambian kwacha

GENERAL INTRODUCTION

Rabies is one of the most feared, fatal zoonotic diseases in the world; it causes approximately 59,000 human deaths worldwide each year, with over 95% of cases occurring in Asian and African countries (Hampson et al., 2015). Although rabies may affect all species of warm-blooded animals, the large majority of human rabies cases are intermediated by dogs in Asia and Africa (Lembo et al., 2010). Therefore, in addition to providing human post-exposure prophylaxis (PEP), canine vaccination is a key measure to control dog-mediated human rabies (Cleaveland et al., 2007, 2003; Kaare et al., 2009; World Health Organization [WHO], 2018). Despite the presence of established control measures, rabies remains endemic in over 100 countries and territories (WHO, 2018) because of the low public awareness of rabies (Dodet et al., 2008), low prioritization of rabies control (Lembo et al., 2010), poor responsibility of dog owners and insufficient management of free-roaming dogs (Taylor et al., 2017; Tenzin et al., 2015a), unavailability of high-quality animal vaccines (Hu et al., 2008; WHO, 2018), lack of resources required to implement control programs (Lembo et al., 2010), and the presence of wild animals that share rabies infections (Nel et al., 2005). Given the situation, rabies is defined as one of the neglected tropical diseases (WHO, 2010), coupled with the fact that whole picture of the situation surrounding rabies and the size of the disease burden in endemic countries, particularly in Asia and Africa, is not well understood because of inadequate surveillance, underreporting, frequent misdiagnosis, and a lack of coordination between human and animal health sectors (Broban et al., 2018; Nel, 2013; WHO, 2018).

To prevent a rabies outbreak in a dog population, 20–45% of the dog population must always be immune; this threshold is recognized as the critical vaccination coverage

of rabies (Hampson et al., 2009). This is calculated from the basic reproductive number of rabies, which is estimated to be between 1 and 2 around the world (Hampson et al., 2009). Canine mass vaccination campaigns are commonly implemented to immunize dogs in rabies endemic countries, particularly in Asia and Africa. To maintain herd immunity beyond the aforementioned critical threshold coverage in the interval between vaccination campaigns, a higher vaccination coverage must be achieved in the dog population during those campaigns (Hampson et al., 2009). This high coverage must be achieved owing to the rapid decline in herd immunity due to the death of immunized dogs, the birth and immigration of susceptible dogs (Conan et al., 2015), and the loss of individual immunity (Morters et al., 2014a). Therefore, the actual vaccination coverage that should be achieved during one campaign depends on the dog population dynamics, the duration of vaccine-induced immunity, and the interval between campaigns. Empirically, a vaccination coverage of at least 70% of the dog population has been recognized as the coverage required in mass vaccination campaigns that are generally conducted annually (Coleman and Dye, 1996; WHO, 2018). Recently, this empirically derived consensus was verified by a study that used retrospectively collected dog demographic data in Tanzania (Hampson et al., 2009) and in studies that used prospectively collected cohort data in South Africa and Indonesia (Conan et al., 2015; Morters et al., 2014b). These studies estimated that a target vaccination coverage of 60–70% is sufficient to avoid coverage falling below the critical threshold of 20–45% in those studied dog populations through annual mass vaccination campaigns. However, the actually observed levels of coverage that have successfully controlled rabies vary according to the circumstances (Eng et al., 1993; Lee et al., 2001). Vaccination campaigns that do not reach 70% of the dog population can sometimes be effective, but they often fail to prevent rabies outbreaks, which are primarily affected by the dog demographic

characteristics (such as rapid turnover) of each population that contribute to the decline of coverage (Hampson et al., 2009). Thus, understanding the dynamics and demographics of dog populations in rabies endemic countries can help design strategies for controlling rabies in dogs and humans. Furthermore, it can also help conduct canine mass vaccination campaigns effectively to utilize limited resources.

Some studies focusing on dog demographics and/or vaccination coverage achieved through canine vaccination campaigns/programs in African countries were reported from Tanzania (Cleaveland et al., 2003; Czupryna et al., 2016; Gsell et al., 2012; Kaare et al., 2009), Kenya (Kitala et al., 2001), Chad (Durr et al., 2009; Kayali et al., 2003), Malawi (Gibson et al., 2016), Mali (Mauti et al., 2017; Muthiani et al., 2015) South Africa (Conan et al., 2015; Morters et al., 2014a, 2014b; van Sittert et al., 2010), Nigeria (Olugasa et al., 2011) and so on. This thesis focused on the Republic of Zambia, which detailed dog demographics, potential vaccination coverage attainable through a mass vaccination campaign, and effectiveness of vaccines used in the field had not been reported for, to pile up new knowledge adding to the existing limited reports. Outcome of this study will contribute to increasing information that is necessary to develop not only effective canine vaccination programs in Zambia but also transnationally coordinated control programs in Africa, as it is known that successful control of dog-mediated human rabies in endemic countries, which is sustained in the long term, needs region- and continent-wide coordination in the execution of control programs (Hampson et al., 2007; Vigilato et al., 2013).

Rabies is considered endemic in all regions of Zambia that report several clinically diagnosed human cases, several dozen more cases in animals (suspected and diagnosed), and several hundred to thousands of dog bite cases annually (Babaniyi et al., 2016; Muleya et al., 2019, 2012; Munang'andu et al., 2011; Southern and Eastern African

Rabies Group, 2013). Moreover, the costs of PEP and rabies immunoglobulin disbursement coupled with mortality place a huge burden on the public health sector (Southern and Eastern African Rabies Group, 2013). However, rabies reporting in Zambia has been inconsistent, with various studies reporting different figures (Beyene et al., 2017; Munang'andu et al., 2011; Southern and Eastern African Rabies Group, 2013); this is possibly attributed to poor surveillance and a lack of collaboration and communication between human and animal health sectors (Nel, 2013). Hence, considering common situations in rabies endemic countries such as inadequate laboratory and transport infrastructure in addition to the aforementioned situations (Banyard et al., 2013; Cleaveland et al., 2002), the number of rabies cases reported in Zambia is very likely underestimated, and the actual disease burden of rabies could be much higher.

Rabies control in Zambia through canine vaccination has been enforced similarly to that in other African countries. The dog population in Zambia was reported to be 968,372 between 2017 and 2018 by the Department of Veterinary Services, Zambia. In Zambia, dogs are sometimes confined to their houses or premises, which are surrounded by fences and block walls in urban settings, such as the capital city of Lusaka. Conversely, in rural areas, dogs are mostly allowed to freely roam and kept without confinement using chains or collars. Although several reports on the rabies situation in Zambia have been published (Berentsen et al., 2013; Muleya et al., 2019, 2012; Munang'andu et al., 2011; Röttcher and Sawchuk, 1978), only a few papers have discussed vaccination coverage in dog populations (de Balogh et al., 1993; Mulipukwa et al., 2017).

Mulipukwa et al. reported the vaccination status among dogs in Nyimba District, which is a rural district located in Eastern Province (Mulipukwa et al., 2017). However, no information regarding vaccination coverage attainable through central-point mass vaccination in the rural areas of Zambia is available; central-point mass vaccination,

organizing mobile vaccination teams and setting up temporary vaccination posts in well-recognized locations in communities, is considered the most cost-effective vaccination strategy in particularly rural communities of resource-limited countries (Kaare et al., 2009). Similarly, no previous studies have reported on the dog demographics or dynamics in rural settings of Zambia even though such information is crucial for designing and planning effective vaccination strategies matched to the target dog community.

With regard to urban settings de Balogh et al. reported the vaccination status and vaccination coverage that was attained through central-point mass vaccination among dogs in a low-income, densely populated residential area in Lusaka District (de Balogh et al., 1993). Because spatial heterogeneity in vaccination coverage in highly dense, large, and connected dog population, such as urban dog population, allows rabies transmission to be sustained (Ferguson et al., 2015; Knobel et al., 2020; Townsend et al., 2013), comprehensive vaccination programs targeting entire population and its success are needed in urban settings. However, no information is available on vaccination coverage and current attainment of programs targeting the whole dog population in Lusaka District.

This thesis aimed to assess the current attainment of rabies control programs targeting dogs in rural and urban areas of Zambia and to investigate key factors to enhance effective and efficient rabies control programs in dogs. In the first chapter, cross-sectional survey was conducted to investigate domestic dog demographics and population of ownerless dogs in a rural area of Zambia. In addition, the potential for attainment of a 70% vaccination coverage among overall dog population through a central-point mass vaccination campaign targeting domestic dogs in a rural area of Zambia was evaluated through conducting free mass vaccination campaigns, transect survey, and household survey. In the second chapter, immunization coverage was assessed through measuring rabies virus-neutralizing antibody titers targeting domestic dog population in Lusaka,

which is the capital city of Zambia. Moreover, the time-series trend of neutralizing antibodies against rabies in vaccinated dogs was evaluated.

CHAPTER I

Domestic dog demographics and estimates of canine vaccination coverage in a rural area of Zambia

1 INTRODUCTION

Despite the presence of established control measures (i.e., canine vaccination and human PEP), rabies remains endemic in over 100 countries and territories (WHO, 2018) owing to the low public awareness of rabies (Dodet et al., 2008), low prioritization of rabies control (Lembo et al., 2010), poor responsibility of dog owners and insufficient management of free-roaming dogs (Taylor et al., 2017; Tenzin et al., 2015a), unavailability of high-quality animal vaccines (Hu et al., 2008; WHO, 2018), lack of resources required to implement control programs (Lembo et al., 2010), and the presence of wild animals that share rabies infections (Nel et al., 2005). Furthermore, particularly in rural communities, the low availability of rapid and appropriate PEP makes controlling human rabies cases difficult (Cleaveland et al., 2007). Based on the estimate that over 75% of human rabies cases in Africa occur in rural settings (Knobel et al., 2005), it is thus important to establish sustainable and suitable control measures in rural settings in an effort to effectively control rabies.

Although the importance of rabies control in rural settings is highlighted, no information regarding vaccination coverage attainable through central-point mass vaccination in the rural areas of Zambia is available. A substantial number of mass vaccination campaigns in dogs have been conducted in Zambia, but the attained coverage has been poorly investigated and assessed, including the factors that likely influence the success of campaigns such as owners' willingness to pay for vaccination, household density, or campaign styles matched to lifestyle and land-use, etc. (Durr et al., 2009; Fitzpatrick et al., 2014; Kaare et al., 2009). Moreover, no information on the dog demographics or dynamics in rural settings of Zambia is available despite importance of such information for designing and planning effective vaccination strategies.

This chapter aimed to elucidate dog demographics and assess the vaccination coverage achievable through a canine mass vaccination campaign in rural settings of Zambia with an eventual goal of verifying the feasibility of eliminating rabies from dogs in Zambia. To attain the above aims, this chapter involved (1) estimating the ownerless dog populations, (2) investigating the demographics of the domestic dog population, (3) conducting mass vaccination campaigns (the first mass vaccination and the follow-up mass vaccination), (4) estimating the vaccination coverage attained through the campaigns, and (5) revealing the owners' knowledge, attitude, and practices for rabies and its control, which influences achievable vaccination coverage, in a rural setting of Zambia.

2 MATERIALS AND METHODS

2.1 Ethical approval

Ethical approval for this study was obtained from the Ministry of Fisheries and Livestock of the Government of the Republic of Zambia. This study was conducted under the monitoring project of the Ministry of Fisheries and Livestock, therefore, this study was not categorized as animal experiments. For human participant, verbal formal consent was obtained from each participant.

2.2 Study area

Two canine mass rabies vaccination campaigns and a survey on canine demographic characteristics and vaccination coverage estimates were conducted in Kalambabakali in Mazabuka District of Zambia. Mazabuka District (15.86°S, 27.76°E),

which is located in Southern Province, has a total human population of 230,972 individuals (2010 census), approximately 22,764 dogs (in 2017–2018; according to Department of Veterinary Services), and an area of 6,242 km² (Banda et al., 2015) (A part of Mazabuka District has been separated and is known as Chikankata District in 2011). Several hundred dog bite cases are recorded in this region annually, with a total of 61–360 cases reported annually between 2010 and 2015 (Annual Reports, Department of Veterinary Services). In 2014, one canine and one bovine suspected rabies case tested positive, and an additional 147 suspected canine cases were reported (Annual Reports, Department of Veterinary Services). The study area consists of four continuous zones. Zones A and D correspond to the administratively subdivided areas of village 2 and the Mukuyu area, respectively, in Kalambabakali. Zones B and C correspond to village 3 and village 4, respectively, in the same region (Figure 1). All zones were well defined by administrative boundaries. The total area of the study zones was approximately 30.6 km². Zones A, B, C, and D have areas of 4.6, 7.0, 8.0, and 11.0 km², respectively. These zones are located in rural areas, approximately 17 km from central Mazabuka. The main agricultural activities in this region include maize and cotton cultivation and livestock rearing.

2.3 The first canine mass vaccination (capture)

Two mass vaccination campaigns were conducted in this study: the first mass vaccination campaign (capture) and the follow-up mass vaccination (described in the latter section). The first mass vaccination campaign was subsequently followed by the transect survey and household survey while the follow-up mass vaccination campaign was conducted three weeks after the first mass vaccination campaign. Mazabuka district veterinary office (DVO) staff distributed posters announcing the vaccination campaign a

week prior in the target zones. The posters were written in both English and the local language and displayed in front of schools, clinics, and houses belonging to the local chiefs, where people commonly gather. Additionally, Mazabuka DVO staff traveled by motorcycles in the target zones to publicly use loudspeakers to advertise the upcoming campaign several times during the week before the vaccination campaign.

A central-point canine vaccination campaign was conducted from 9:00 to 13:00 in zone B and from 14:00 to 18:00 in zone A on May 21, 2016 (Saturday). Vaccinations were held from 9:00 to 13:00 in zone D and from 14:00 to 18:00 in zone C on May 22, 2016 (Sunday). Each zone had one vaccination spot: the dip tank site in zone A, the school in zone B and D, and the local chief's premise in zone C. Four veterinary assistant officers from the DVO administered the vaccines and issued the vaccination certificates. One local livestock officer was also present during the campaign. Human PEP anti-rabies vaccines (Verorab; Sanofi Pasteur, Lyon, France) and disinfectants were also provided in case of any dog bites. The dogs were vaccinated subcutaneously with 2 ml of Rabies Alum Adjuvant Vaccine (Central Veterinary Research Institute, Lusaka, Zambia) using a single syringe and needle for each animal. The vaccine used was a locally produced rabies vaccine that is commonly provided and used by the DVO in the target zones. The vaccines were distributed free of charge. Dogs aged less than three months and those that were obviously unhealthy were not vaccinated as per the rabies vaccination guidelines of the vaccine manufacturer and the "Protocol on Rabies Disease Control in Zambia" based on the Control of Dogs Act, Cap 247 of the Laws of Zambia. A strict cold chain was observed, and the vaccinated dogs were labeled with color spray on their bodies and issued a Government of the Republic of Zambia rabies vaccination certificate. Additionally, information about the owners' names and addresses, and the dogs' names, age, sex, color, markings, and vaccination history were recorded.

2.4 Transect survey (the first recapture)

Vaccination coverage was assessed in the target zones using the capture-recapture method described earlier by Kayali et al. (Kayali et al., 2003). Two transect teams comprising four observers who each counted all dogs encountered in the transect lines were organized. Dogs labeled with the color spray (vaccinated) were distinguished from unlabeled dogs (unvaccinated). The two teams used cars traveling at 15 to 20 km/h for the transect survey to avoid accidental bite injuries. The transect survey in each zone was conducted in the morning on the first day following the mass vaccination campaign (May 23, 2016). We conducted one transect survey in each zone, although this survey should ideally be conducted several times per area to avoid biased observations. Only one survey was performed because of the difficulty to adjust the schedule of the DVO staff due to other administrative affairs. The main roads were selected as the transect lines in each target zone. Additionally, a 50-m wide buffer around the boundary of each zone was established to avoid counting migrating (even temporarily) dogs from outside the zone in our survey. During the transect survey, we carried Global Positioning System (GPS) tracking devices to record log of our movement. The length of the transect lines were measured via Google Earth Pro software (2015 Google) with the record of the GPS log. The total length of the transect lines in each zone were as follows: 5.12 km in zone A, 8.91 km in zone B, 7.80 km in zone C, and 8.08 km in zone D.

2.5 Household survey (the second recapture)

Household surveys were conducted on day 5 after the mass vaccination campaign and were continued for another five days. The household survey targeted all households in the study area regardless of whether they owned dogs for assessing the number of owned dogs and humans and owned dog demographic characteristics. Each

household in the study area was visited during this time, and the heads of the households were interviewed. If the head of the household was absent, a suitable substitute was chosen for the interview. All respondents were told the purpose of the study, and their consents for participation were obtained. The questionnaire was written in English, but the interview was performed in the local language if necessary. Information on the number of dogs in the household and the presence of spray-marks on any dogs were collected, as were each dog's age and sex. Each dog's previous vaccination history and its validity were also assessed. Dog owners were asked to provide their reasons for not participating in the first mass vaccination campaign, when applicable. The owners were also asked about their knowledge of rabies to assess whether they had accurate information on rabies. Furthermore, they were asked about the affordability of the canine rabies vaccination (willingness to pay) and what they had actually paid for the vaccination (actual cost). The confinement probability was estimated by confirming whether each dog was confined to each household's premises (e.g., by chain or cage).

2.6 Follow-up mass vaccination

A follow-up mass vaccination campaign was provided three weeks after the first mass vaccination campaign for owners who missed the first campaign. The follow-up campaign was held at the same locations as the original campaign. Flyers were distributed to each household during the household survey described above to advertise the follow-up campaign. The other conditions of the follow-up mass vaccination campaign were the same as those in the first campaign.

2.7 Data analysis to estimate the ownerless dog population and total vaccination coverage

The Bayesian model modified from Kayali et al. was used in this study (Kayali et al., 2003). In each study zone i ($i = 1, 2, 3$, and 4 corresponding to zones A, B, C, and D, respectively), all vaccinated dogs were labeled with color spray during the mass vaccination campaign.

We modelled the sampling process of the capture-recapture study. First, we defined an owned dog as a dog kept by a human and belonging to a household. We also defined an ownerless dog as a dog that is not kept by a human and does not belong to a household. During the transect survey, dogs were distinguished by whether they were marked or unmarked. Since there was no way to determine whether an unmarked dog was owned, the number of unmarked dogs observed in study zone i , Z_i , can be written as follows:

$$Z_i = X_{2,i} + Y_i, \quad (1)$$

in which $X_{2,i}$ and Y_i denote the number of unmarked dogs that were owned and the number of unmarked dogs that were ownerless and were recaptured during the transect survey in a given zone i , respectively. All of the marked dogs were owned dogs since ownerless dogs were not brought to the mass vaccination campaign. $X_{1,i}$ represents the number of marked, owned dogs that were recaptured during the transect survey in a given zone i .

The recapture process in our capture-recapture survey was assumed to follow a binomial sampling process with a recapture probability that is equal among all dogs (marked owned, unmarked owned, and ownerless) but differed by zone. Hereafter, we refer to the recapture probability in zone i as p_i . The probability of the number of marked and unmarked dogs recaptured in a given study zone i , $X_{1,i}$ and Z_i , can be written as follows:

$$X_{1,i} \sim \text{Bin}\left((1 - c_{1,i})M_{v,i}, p_i\right), \quad (2)$$

$$Z_i \sim \text{Bin}\left((1 - c_{2,i})M_{u,i} + N_i, p_i\right), \quad (3)$$

in which *Bin* denotes binomial distribution, $c_{1,i}$ and $c_{2,i}$ are confinement probabilities related to zone i for owned marked and owned unmarked dogs, respectively; $M_{u,i}$ is the total number of unvaccinated owned dogs; and N_i is the total number of ownerless dogs in zone i . The total number of vaccinated (marked and owned) dogs in zone i , $M_{v,i}$, was obtained from the registration at the vaccination point. A description of each parameter is listed in Table 1.

The model parameters were estimated using the Markov chain Monte Carlo simulations in the OpenBUGS software (version 3.2.3 rev 1012).

Likelihood was determined as the product of probability mass functions for the observed data of the marked and unmarked dogs during the transect survey as follows:

$$\text{Likelihood} = \text{pmf}(X_{1,i}, X_{1,i}^{obs}) \text{pmf}(Z_i, Z_i^{obs}), \quad (4)$$

in which $\text{pmf}(x,y)$ denotes the probability mass function describing the probability of observing y with a distribution x .

The total number of owned dogs in each study zone was initially estimated using the Chapman estimate formula (Chapman, 1951; Seber, 1970; Tenzin et al., 2015b) via data collected from the household survey:

$$M_i = \left\lceil \frac{(M_{v,i} + 1)(n_i + 1)}{(m_i + 1)} \right\rceil - 1 \quad (5)$$

and variance:

$$\text{var}(M_i) = \frac{(M_{v,i} + 1)(n_i + 1)(M_{v,i} - m_i)(n_i - m_i)}{(m_i + 1)^2(m_i + 2)}, \quad (6)$$

in which n_i and m_i are the numbers of recaptured dogs and recaptured marked (vaccinated) dogs in the household survey in zone i , respectively. These estimates specify the

parameters of a normal prior distribution that was adopted for M_i . The other prior distributions were also obtained from data collected during the household survey (Table 2). Vaccination coverage was calculated as the proportion of actual vaccinated dogs during each of the first and follow-up mass vaccination campaigns in the owned and overall dog populations estimated via Bayesian modeling. Sensitivity analysis was performed by varying the confinement probabilities (0–0.8), recapture probability (0.2–0.8), or ratio of ownerless dogs to owned dogs (0.2–0.8) while keeping the values of the other parameters fixed.

2.8 Dog demographics and projection of dog population growth

A static life table and a female fecundity table (Caughley, 1977; Pianka, 1999) were constructed based on dog information collected during the household survey. The collected information included: (i) the number of dogs currently owned, (ii) the sex and age of all dogs, and (iii) the reproductive history of female dogs (the number of litters in a lifetime and within the last 12 months and the size of the most recent litter). Static life tables can be calculated directly from a stationary age distribution only when the frequency of each age class x is equal to or greater than that of $x + 1$ (Caughley, 1977). To construct a static life table, the observed dog frequency in each age class was smoothed by fitting the data of the age distribution of dogs with a statistical model describing age structure (Caughley, 1977) as follows:

$$\log(n_a) = \alpha + \beta a + \gamma a^2, \quad (7)$$

in which, n_a denotes the number of dogs aged a . The parameters α , β , and γ were estimated by a nonlinear least squares regression with the model as above. By substituting estimated values of α , β , and γ , we obtained the smoothed number of dogs per age and completed the static life table. The data on age and sex of 861 of 872 dogs was converted into a static

life table after excluding the data of 11 dogs whose age was unidentified (Table 3). The information obtained from 334 female dogs, excluding females whose fecundity data were not complete among the total females ($n = 374$), was used for constructing a female fecundity table (Table 4). The formulas used to construct the static life table and female fecundity table are provided in Table 5. The population growth was projected by means of an age-structured, population projection matrix (Leslie matrix) (Pianka, 1999), under the assumption that the environment remained constant and no emigration or immigration occurred in the dog population. An elasticity analysis was performed to assess the relative contributions of survival and fecundity in different age classes. In the elasticity analysis, elasticity (e) was determined for each element at different age class; all elasticities sum to unity, and thus represent the proportional contributions of each element to the dominant eigenvalue (λ) representing population growth rate (Benton and Grant, 1999; de Kroon et al., 1986). These analyses relating to the Leslie matrix were performed using the R package “demogR” in R version 3.6.3 (R Core Team, 2020).

2.9 Statistical analysis

According to the dog owners’ willingness to pay for a vaccine, we calculated reverse cumulative probability of vaccination with respect to the vaccination cost to evaluate the expected vaccination coverage, which relied on owners’ willingness. This indicated how much owners’ willingness to pay for a vaccine reduced with the increase of the cost. The probability started from 1.0, which substitutionally represented vaccination coverage, and gradually decreased as the cost increased, showing survival of expected vaccination coverage at the specific cost of vaccine. We also calculated reverse cumulative proportion of how much owners actually paid according to what they had actually paid for a single canine rabies vaccination before. Furthermore, the difference

between the owners' willingness to pay and the actual cost of vaccine was depicted as the difference of the survival curves. It was regarded that difference between the falling process of the expected probability of vaccination and the falling process of the actual cost indicated discordance between owners' affordability and actual cost of the vaccination. Therefore, a log-rank test was performed on the reverse cumulative probability of vaccination (willingness to pay) and reverse cumulative proportion of how much owners actually paid for a canine rabies vaccination (actual cost) to detect discordance between owners' willingness and actual cost, using the R package "survival" in R version 3.6.3 (R Core Team, 2020). A *p*-value of < 0.05 was considered statistically significant.

3 Results

3.1 Household and dog population characteristics

During the household survey, we visited 333 households that owned at least one dog and 177 households that did not own any dogs. In total, 510 households were visited (Table 6). In the study area, 3.6% of households were missed because the residents were absent or simply because the house owners refused to participate in the survey. A total of 3,882 people were covered by the survey, and the mean number of persons per household was 7.6 (8.6 among the dog-owning household group), except for two households whose data were unavailable. In total, 872 of the owned dogs were covered in the household survey. The characteristics of the dog population are exhibited in Table 7. A total of 29% of dogs in the study area were young dogs (under one year old) based on the information from the household survey. Of these dogs, 57.7% were less than three months old and

thus were ineligible for vaccination according to the vaccine manufacturer and the “Protocol on Rabies Disease Control in Zambia.” The owners reported various reasons for owning their dogs ($n = 333$; because of multiple answers, a total of 379 answers were reported including four unavailable answers) such as for guarding (98.2%), hunting (13.5%), as a pet (0.6%), and for breeding (0.3%).

3.2 Demographics and population growth in the owned dog population

Age-specific mortality was highest in the dogs under one year old (47%) according to the static life table (Table 3). The life expectancy at birth was 3.17 years. The sex-specific static life tables indicated tendencies showing that the age-specific survival (particularly in the reproductive age class) and the age-specific life expectancy in female dogs were lower than those in male dogs (Table 8). Females began breeding aged 0.75 years as observed in the survey. Their reproductive period continued up to the age of 14 years on the basis of owners’ reports. The mean litter size was 4.3 puppies (95% confidence interval [CI]: 4.0–4.6). Female fecundity is summarized in Table 4.

The dog population growth projection from the Leslie matrix is described as follows. Population growth (λ) was estimated at 1.15. The net reproductive rate (R_0), which is defined as the mean number of female offspring that a female produces during her lifetime, was 1.93. The generation time, which is defined as the mean parental age at which all offspring are born, was estimated at 4.6 years. The intrinsic growth rate (r), which is a measure of the instantaneous rate of change of population size per individual, was 0.14. The elasticity analysis of the Leslie matrix identified the survival of dogs under one year old to have the greatest proportional contribution on the change of the dominant eigenvalue λ , accounting for 0.23 of the elasticity (e). Survival of the age class 1–2 ($e = 0.20$), followed by survival of the age classes 2–3 ($e = 0.13$) and 3–4 ($e = 0.09$) also

influenced population growth.

3.3 Ownerless dog population and vaccination coverage estimates

A total of 392 dogs were vaccinated at the four vaccination points during the first mass vaccination campaign in the study zones (74 in zone A, 146 in zone B, 74 in zone C, and 69 in zone D, including 29 dogs from outside the zones). Three hundred dogs were vaccinated in the follow-up mass vaccination campaign (55 in zone A, 89 in zone B, 9 in zone C, and 122 in zone D, including 25 dogs from outside the zones). The median ownerless dog population was estimated at 11 (95% credible interval [CRI]: 0–40) in zone A, 5 (95% CRI: 0–29) in zone B, 2 (95% CRI: 0–10) in zone C, and 15 (95% CRI: 0–76) in zone D. The ratio of ownerless to owned dogs was 0.06 (95% CRI: 0.00–0.23), 0.02 (95% CRI: 0.00–0.10), 0.01 (95% CRI: 0.00–0.08), and 0.05 (95% CRI: 0.00–0.23) in zones A, B, C, and D, respectively. Vaccination coverage in the owned dog population attained through the first mass vaccination campaign was estimated at 20.9–52.6% in the four zones and was almost similar to the coverage among the overall dog population because there were so few ownerless dogs (Table 9). Vaccination coverage attained through the follow-up mass vaccination campaign was increased to 57.9–77.8% in owned dogs (Table 10). Table 11 shows the posterior distributions. The sensitivity analysis showed that the posterior median of the ownerless dogs in zone A was comparatively sensitive to the fluctuation of the confinement probabilities or the recapture probability, although the range of change was small. Except for this note, no remarkable impact on the estimated ownerless dog population was found in the sensitivity analysis even though each estimated parameter slightly fluctuated according to change of the prior distributions (Table 12).

3.4 Reasons for non-participation in the first mass vaccination

A total of 152 owners participated in the first mass vaccination campaign out of the 333 dog-owning households visited during the household survey. The owners who did not participate in the first mass vaccination campaign were asked why they did not participate (Table 13). The most common reason was that the owner had not been informed about the mass vaccination beforehand (32.0%). The second and third most common reasons were that the owner was not available at the time of the campaign (26.5%) and that owner failed to restrain his/her dog(s) (23.8%) (Table 13).

3.5 Rabies knowledge in dog owners

In the household survey, 75.4% ($n = 333$; including five unavailable answers) of dog owners answered that he/she was knowledgeable about “rabies.” The main sources of their knowledge on rabies were from their family, relatives/neighbors, and through their experiences from keeping dogs (Table 14). Despite this knowledge, most of those who answered that they were knowledgeable on rabies (70.5%, $n = 251$) were unable to list the symptoms of rabies in humans. The remaining 29.5% of owners answered that they could describe the characteristic symptoms of rabies in humans. Most of the symptoms listed by the respondents as the typical symptoms of human rabies were in fact satisfactory as answers indicating actual symptoms of human rabies (Table 15). The owners who answered that they were knowledgeable about rabies were also asked about the transmission mode of rabies to humans. A total of 34.7% ($n = 251$, including one unavailable answer) of owners did not know how rabies is transmitted to humans, while 63.4% mentioned “dog bite” as a transmission mode. The remaining (approximately 1.6%) gave other answers, such as “through poison” or “by witchcraft.”

3.6 Affordability of canine rabies vaccination and owners' practices of dog vaccination

A total of 32.0% of owners desired free canine rabies vaccination, and the median amount they were willing to pay for a canine rabies vaccination was ZMW 5.00. However, 30.9% of owners had never vaccinated their dogs before, and the median amount actually paid was ZMW 10.00 (Table 16). The log-rank test detected significant difference between the decreases in the willingness to pay (reverse cumulative probability of vaccination) and the actual amount paid (reverse cumulative proportion of how much owners actually paid), which were calculated based on the aforementioned data ($p < 0.05$, Figure 2). Regarding the owners' practices of vaccinating their dogs, 86.8% of dog owners who had their dogs vaccinated in the past ($n = 234$, including 16 unavailable answers) only did so when the veterinary officers came to their villages. A total of 3.4% of owners said that they vaccinated their dogs at home while another 1.7% and 0.9% vaccinated their dogs at the DVO and during mass vaccination campaigns, respectively.

4 DISCUSSION

This chapter describes a canine mass rabies vaccination campaign in the rural parts of Mazabuka District in Zambia and how such a program can lead to success. This is the first report estimating vaccination coverage after a mass vaccination campaign in rural Zambia.

The present study provides information on the local dog population and its demographics in the chosen study area. In agreement with earlier studies from other rabies endemic countries in Africa and Asia (Czupryna et al., 2016; Durr et al., 2009; Estrada et

al., 2001; Gsell et al., 2012; Kitale et al., 2001; Matter et al., 2000; Mauti et al., 2017; Morters et al., 2014a), the studied dog population in the rural part of Mazabuka was young and male-biased. This male-biased sex ratio may be a result of the owners' preference of male dogs for various roles (e.g., better guard dogs) (Morters et al., 2014b) and the higher mortality rate in female dogs (Conan et al., 2015). In accordance with the relatively low survivorship in female dogs reported frequently (Czupryna et al., 2016; Kitale et al., 2001), this study also supported the tendency of lower survival in females than in males. These characteristics such as male-skewed population (owners' preference of male dogs) and lower survival in female may be partly attributed to difference in degree of treatment/care between male and female dogs. This may further bring implication of influence on owners' decision to vaccinate either male or female dog with a limited budget, such as giving priority to male dogs. Almost all dogs in the study area were kept for the purposes of security as guard dogs, followed by hunting purposes. Because our study area is located comparatively near national parks and game management areas in the Kafue flats, it is highly possible that hunting dogs frequently come into contact with wild animals.

The human-to-dog ratio was determined to be 4.45:1 in the study area. Earlier studies reported the human-to-dog ratio in Zambia to be 45:1 in the urban Lusaka District (de Balogh et al., 1993), 6.7:1 in a semi-rural setting in Chongwe District, Lusaka Province (de Balogh et al., 1993), and 3.0:1 in Nyimba District, a rural setting (Mulipukwa et al., 2017). It is generally understood that the human-to-dog ratios in rural settings are lower than those in urban settings (Czupryna et al., 2016; Gibson et al., 2016; Gsell et al., 2012; Kaare et al., 2009; Kitale et al., 2001; Mauti et al., 2017). This is possibly associated with the fact that dog density in rural settings is generally lower than that in urban settings given the tendency for rural settings to allow residents to have more dogs. Focusing on the human-to-dog ratio in rural settings, the ratios recorded in rural

Zambia (the present study and the study in Nyimba District [Mulipukwa et al., 2017]) are lower than the ratios recorded in other rural settings of African countries (Czupryna et al., 2016; Kaare et al., 2009; Kitala et al., 2001). Although the factors contributing to this lower human-to-dog ratio in rural Zambia have not been clarified, this simply signifies that the dog population per human population tends to be larger in rural Zambia compared with other rural settings of African countries. This implies that opportunities to contact dogs per person might increase in rural Zambia. In addition, this may result in increase of dog bite cases in rural Zambia, which further highlights the needs for provision of PEP and education of proper dog-handling skills.

The population of ownerless dogs in the study area was estimated to be very low compared with the population of owned dogs. This suggests that rabies control in humans and dogs is feasible through mass vaccination campaigns targeting owned dogs. The overall vaccination coverage achieved in the first mass vaccination campaign was estimated to range between 19.8% and 51.6% in each targeted dog population (in Zones A, B, C, and D). Vaccination coverage of the owned dog population of 20.9% to 52.6% were attained in the four study zones. These figures are still lower than the 70% vaccination coverage recommended by WHO that should be achieved in mass vaccination campaigns (Coleman and Dye, 1996; Bögel and WHO, 1987; WHO, 2018); it is also below the vaccination coverage reported earlier through free mass vaccination campaigns conducted in other African countries: in urban settings in N'Djaména, Chad (Kayali et al., 2003), and Iringa, Tanzania (Gsell et al., 2012), and in rural settings in the Serengeti (Kaare et al., 2009) and the Mara Region (Cleaveland et al., 2003) in Tanzania. As for Zambia, reports showing that sufficient vaccination coverage could be attained through central-point mass vaccination in rural areas are lacking. Mulipukwa et al. reported a vaccination coverage of 8.7% based on a household survey among owned dogs in Nyimba

District, Eastern Province (Mulipukwa et al., 2017). This figure was similar to, but still higher than, the pre-coverage figure before our mass vaccination, which was roughly estimated based on the data of our household survey (3.9% in zone A, 4.0% in zone B, 0% in zone C, and 7.5% in zone D). This finding implies that vaccination coverage without any interventions in dog populations of Zambia, particularly in rural settings, is considerably less than the critical threshold coverage of 20–45% required to interrupt rabies transmission in a dog population (Hampson et al., 2009).

The following three major reasons were given for non-participation in the first mass vaccination campaign: lack of information, owners' unavailability, and owners being unable to handle their dogs. Despite putting up posters at major gathering points where they could be seen by the public one week before the day of the first mass vaccination, and traveling by motorcycles with loudspeakers in the target zones several times during the week prior to the vaccination, almost one-third of non-participating owners stated that they were not informed about the campaign. First, this simply indicates that such advertisements were not sufficient to reach all dog owners. This was likely because of the increased numbers of posters that are usually displayed in public places advertising all sorts of things that might not be appealing to all community members and because of the limited timing and frequency of the publicity by motorcycles using loudspeakers. Zone D had a much larger area and more spread out houses (i.e., not along main streets) than the other zones, and this could have reduced the probability for dog owners and other members of the community to read and spread the information on the rabies vaccination campaign, ultimately resulting in a notably lower coverage. Secondly, dog owners frequently reported that they had not been informed about the mass vaccination (Durr et al., 2009; Muthiani et al., 2015), and this may be the easiest answer to provide without admitting their actual reasons for non-participation. Thirdly, the

coverage after the follow-up mass vaccination campaign also failed to reach 70%, as recommended by WHO, in zones C and D even though all households involved in the household survey had received the flyers. A possible influencing factor was the day on which the mass vaccination campaign was conducted in zones C and D (Sunday), although the actual reasons for owners' non-participation and the relationship between choice of day and owners' non-participation were unclear. This, however, implies the limit of enhancing vaccination coverage in the current conditions (e.g., arrangement of day and time and the owners' low prioritization of vaccination). Furthermore, this may be related to the reason of "owners' unavailability," which was the second major reason for non-participation, and it indicates that owner-related scheduling conflicts limit the amount of vaccination coverage achievable. Fundamentally increasing dog owners' awareness of the importance of canine rabies vaccination, which is also related to the owners' knowledge on rabies mentioned in the latter paragraph, is necessary to obtain the maximum outcome of mass vaccination campaigns. Additionally, promoting community support involving other stakeholders such as local chiefs, local veterinarians and human doctors, and local teachers at schools is essential to achieve a successful mass vaccination campaign. Dog handling difficulties was the third major reason owners cited for not participating in the mass vaccination. Most of the owned dogs in the target zones were allowed to roam freely, as is common in most other African countries. Our findings were similar to earlier reports on free mass vaccinations in other African countries (Kayali et al., 2003; Muthiani et al., 2015) and indicate that improvement in owners' dog handling skills, general dog training knowledge, and proper equipment use (e.g., collar and chain) are still required. An alternative vaccine delivery strategy of house-to-house vaccination has been recommended in cases where dogs are difficult to handle. In this program, the owners do not have to take their dogs to long-distance vaccination sites, albeit this approach involves

substantial labor and capital investments (Kaare et al., 2009). This strategy is also applicable in extremely remote communities, as discussed later. Another potential alternative strategy is oral rabies vaccination; this is a complementary measure to increase the vaccination coverage in mass parenteral dog vaccination campaigns, wherein unrestricted dogs that cannot be vaccinated parenterally under normal conditions hamper reaching 70% vaccination coverage (WHO, 2018). However, regulatory authorities of different countries need to assess the suitability and necessity of the application of oral rabies vaccination for dogs considering both the benefits and the potential risks of oral vaccine-associated adverse events (particularly the limited efficacy in comparison with the parenteral vaccines, along with safety in humans and other species in cases of unintentional exposure, or release of genetically modified/self-replicating organism into the environment) (WHO, 2018).

There are other possible reasons that our mass vaccination campaigns did not reach the 70% vaccination coverage. First, puppies younger than three months old are not eligible for rabies vaccination in Zambia. However, puppies younger than three months old comprised 16.7% of the surveyed dog population in our study. Therefore, we propose including puppies below three months old as subjects for rabies vaccination despite the high mortality of this age class because vaccination of puppies with high-quality vaccine is strongly recommended and regarded as a cost-effective approach to maintain herd immunity (Anderson et al., 2019; Morters et al., 2015; WHO, 2018). Second, the comparatively large area of zone D may have reduced the owners' motivation to take their dogs to the vaccination sites. It has previously been reported that vaccination coverage decreased as household distance from the vaccination site increased (Kaare et al., 2009), but this early study noted that the coverage was generally greater than 70% even at 5 km from the vaccination sites. In such cases, house-to-house vaccination combined with

central-point mass vaccination will be applicable although it requires substantial investment in labor and capital and is operationally difficult (Kaare et al., 2009). However, the continuous shortage of veterinary field staff and resources for rabies vaccination in Zambia (Mulipukwa et al., 2017) are obstacles to overcome for the application of house-to-house vaccination.

Although most of the dog owners considered themselves knowledgeable about rabies, the majority did not in fact know the symptoms of rabies in humans. Moreover, approximately one-third of them did not know the transmission mode of rabies. These data imply that dog owners may not have sufficiently accurate knowledge on rabies, even if they have heard the term before. More official education about rabies from relevant authorities (e.g., government, medical hospitals, and veterinary clinics) could be utilized to acquire correct knowledge. This would enhance the public awareness of rabies, which could lead to a better understanding of responsible dog ownership coupled with the importance of canine vaccination. The official education from experts could also provide people with proper skills to better handle their dogs. These are all steps that could help increase vaccination coverage to a point that can be effective in controlling or avoiding rabies outbreaks.

Dog owners' willingness to pay for the rabies vaccine is another consideration when promoting canine vaccination. Our data show that the median price of rabies vaccines in rural Mazabuka District was ZMW 10.00, but owners felt burdened paying that much for the vaccination. Free vaccination will be necessary to attain vaccination coverage of 70% or higher. In our study area, canine vaccination is commonly distributed by personnel from the DVO by visiting villages. This visiting-community campaign method is thought to be appropriate for remote rural areas far from veterinary clinics or DVO headquarters (Kaare et al., 2009). However, there is evidence that the pre-

vaccination coverage in our study area was roughly 0–7.5% according to the results from our household survey. This may indicate the owners' reluctance to vaccinate, or it may have been caused by a variety of other factors, such as the owners not being provided enough chances to receive vaccination, which could have been caused by a lack of resources (Mulipukwa et al., 2017). By ensuring opportunities for owners to have their dogs vaccinated based on regular enforcement by administering and providing free vaccinations, vaccination coverage could be improved, resulting in enhancing public health and maintaining herd immunity.

According to the Leslie matrix, the dog population growth rate was estimated at 15% per annum ($\lambda = 1.15$). The instantaneous rate of increase, r , was calculated as 0.14. These values, which indicate high population growth, are similar to other reports demonstrating the growth of dog populations in African countries (Czupryna et al., 2016; Gsell et al., 2012; Kitale et al., 2001; Mauti et al., 2017). The main determinants of population growth were the survival of younger age classes. Although this dog population had a high mortality of almost 50% in dogs under one year old, this mortality was lower compared with those in earlier reports conducted in Iringa of Tanzania (72%) (Gsell et al., 2012) and Bamako of Mali (73%) (Mauti et al., 2017). Assuming a vaccination coverage of 70% attained at the start of the year, the data obtained from our survey indicated that the coverage would decrease to 43.7% in one year because of the death of vaccinated dogs and the birth of naïve juveniles under this level of population growth. Based on the critical vaccination threshold of 20–45% that should be maintained to prevent rabies outbreaks (Hampson et al., 2009), annual vaccination campaigns might be sufficient in this dog population if 70% of the population is vaccinated at the start of a year. As mentioned above, information on dog demographics provides beneficial parameters for designing and planning canine rabies mass vaccinations. The present study highlighted

some parameters for evaluating population demographics and growth projections of dog populations in rural Zambia; these parameters can be utilized for designing and planning canine mass vaccinations. However, the static life table and the Leslie matrix used in the present study are limited because they do not take migration or density effects into account. Recently, longitudinal cohort studies have revealed that no population growth was observed in domestic dog populations in rabies endemic countries (Conan et al., 2015; Morters et al., 2014b). Conversely, a decline in population was observed in some areas in previous studies (Conan et al., 2015; Morters et al., 2014b). These earlier studies demonstrated that the high birth and death rates resulting in high turnover of the population rather than net population growth led to the decline of vaccination coverage in the dog populations in rabies endemic countries (Conan et al., 2015; Morters et al., 2014b). The present study did not perform longitudinal monitoring of the population dynamics that can be used to investigate birth and death rates and dog migrations. This is a limitation of our study because of its cross-sectional nature. To obtain more realistic evaluations and projections, cohort studies that take dog migration (movement of dogs by humans) that consists of a substantial fraction of a dog population into account must be conducted (Conan et al., 2015; Mauti et al., 2017; Morters et al., 2014b). From the viewpoints of designing and implementing effective canine mass vaccinations in Zambia, as we revealed in our survey, we propose performing annual canine rabies mass vaccinations and including puppies below three months old in the vaccination campaign to attain the 70% threshold coverage in a dog population.

5 SUMMARY

An estimated 75% or more of the human rabies cases in Africa occur in rural settings, which underscores the importance of rabies control in these areas. Understanding dog demographics can help design strategies for rabies control and plan and conduct canine mass vaccination campaigns effectively in African countries. A cross-sectional survey was conducted to investigate domestic dog demographics in Kalambabakali, in the rural Mazabuka District of Zambia. The population of ownerless dogs and the total achievable vaccination coverage among the total dog population was estimated using the capture-recapture-based Bayesian model by conducting a canine mass vaccination campaign. This study revealed that 29% of the domestic dog population was under one year old, and 57.7% of those were under three months old and thus were not eligible for the canine rabies vaccination in Zambia. The population growth was estimated at 15% per annum based on the cross-sectional household survey. The population of ownerless dogs was estimated to be small, with an ownerless-to-owned-dog ratio of 0.01–0.06 in the target zones. The achieved overall vaccination coverage from the first mass vaccination was estimated 19.8–51.6%. This low coverage was principally attributed to the owners' lack of information, unavailability, and dog-handling difficulties. The follow-up mass vaccination campaign achieved an overall coverage of 54.8–76.2%. This chapter indicates the potential for controlling canine rabies through mass vaccination in rural Zambia. Rabies education and responsible dog ownership are required to achieve high and sustainable vaccination coverage. Our findings also propose including puppies below three months old in the target population for rabies vaccination and emphasize that securing an annual enforcement of canine mass vaccination that reaches 70% coverage in the dog population is necessary to maintain protective herd immunity.

Table 1. Parameters used in the model.

Parameter	Description	Source
M_i	The total number of owned dogs in zone i	estimated
$M_{v,i}$	The total number of vaccinated (marked and owned) dogs during the mass vaccination in zone i . This was obtained from the registration at the vaccination point	observed
m_i	Number of recaptured marked (vaccinated) dogs in the household survey in zone i	observed
n_i	Number of recaptured dogs in the household survey in zone i	observed
N_i	Total number of ownerless dogs in zone i	estimated
a_i	Ratio of ownerless dogs to owned dogs in zone i , written as $N_i = a_i * M_i$	estimated
p_i	Recapture probability, written as $p_i = C_i * E_i * R_i$	estimated
C_i	Coverage stands for the area covered by the transect line	observed
E_i	Probability of encountering a specific dog given the area	observed
R_i	Recording probability of the observer actually recording an encountered dog	observed
$c_{1,i}$	Confinement probability for owned marked dogs	estimated
$c_{2,i}$	Confinement probability for owned unmarked dogs	estimated
$X_{1,i}$	Number of marked dogs observed during the transect survey in zone i	observed
Z_i	Number of unmarked dogs observed during the transect survey in zone i	observed

Table 2. Prior distributions.

	Zone A	Zone B	Zone C	Zone D
Recapture $p_i^\dagger$				
Uniform (range)	0.035–0.357	0.040–0.256	0.031–0.460	0.023–0.308
Coverage (C_i)	0.056–0.401	0.064–0.287	0.049–0.516	0.037–0.346
Encountering (E_i)	0.70–0.90	0.70–0.90	0.70–0.90	0.70–0.90
Recording (R_i)	0.90–0.99	0.90–0.99	0.90–0.99	0.90–0.99
Confinement $c_{1,i}$				
Beta (α, β)				
α	5.908	NIL [‡]	0.985	0.983
β	58.092	NIL [‡]	65.015	57.017
Confinement $c_{2,i}$				
Beta (α, β)				
α	5.936	NIL [‡]	NIL [‡]	NIL [‡]
β	87.064	NIL [‡]	NIL [‡]	NIL [‡]

[†] $p_i = C_i * E_i * R_i$

[‡] No confinement was observed in the zone.

Table 3. Overall population demographics (Static life table).

Age class	n years	Smoothed frequency $s(x)$	% $s(x)$	$l(x)$	$p(x)$	$d(x)$	$q(x)$	$e(x)$
0–1	1	253	31.55	1.00	0.53	0.00	0.47	3.17
1–2	1	133	16.58	0.53	0.81	0.47	0.19	4.13
2–3	1	108	13.47	0.43	0.80	0.57	0.20	3.85
3–4	1	86	10.72	0.34	0.77	0.66	0.23	3.58
4–5	1	66	8.23	0.26	0.76	0.74	0.24	3.36
5–6	1	50	6.23	0.20	0.74	0.80	0.26	3.12
6–7	1	37	4.61	0.15	0.70	0.85	0.30	2.86
7–8	1	26	3.24	0.10	0.69	0.90	0.31	2.65
8–9	1	18	2.24	0.07	0.67	0.93	0.33	2.39
9–10	1	12	1.50	0.05	0.67	0.95	0.33	2.08
10–11	1	8	1.00	0.03	0.63	0.97	0.38	1.63
11+	12	5	0.62	0.02	0.00	0.98	1.00	1.00

Age class: age in years

n year: number of years spent in the age class

$s(x)$: smoothed number of individuals sampled per age class

$s(x)$ %: percentage of sample per age class

$l(x)$: 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 fecundity.

Age class	Smoothed frequency $s(x)$	$b(x)$	$B(x)$	$m(x)$
0–1	114	0.00	0.00	0.00
1–2	64	0.34	4.08	0.70
2–3	44	0.58	4.03	1.17
3–4	30	0.53	4.27	1.14
4–5	21	0.76	4.59	1.74
5–6	14	0.81	4.67	1.89
6–7	10	0.40	3.60	0.72
7–8	7	1.00	3.50	1.75
8–9	5	0.83	4.33	1.81
9–10	3	0.50	4.00	1.00
10–11	2	0.75	4.00	1.50
11+	2	0.50	2.50	0.63

Age class: age in years

$s(x)$: smoothed number of individuals sampled per age class

$b(x)$: mean proportion of breeding females per year and age class

$B(x)$: mean number of offspring born in the last litter per female and age class

$m(x)$: number of female pups born per female and year

Table 5. Formulas for calculating the life history parameters.

Parameter	Definition	Formula
$S(x)$	Number of individuals sampled per age class x , which was smoothed by mean of equation 7 in the main text.	
$l(x)$	Cumulative survival from the first age class $x1$ to a given age class x .	$l(x) = \frac{s(x)}{s(x1)}$
$p(x)$	Probability of surviving from a given age class x to the next following age class $(x + 1)$.	$p(x) = \frac{s(x + 1)}{s(x)}$
$d(x)$	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 .	$e(x) = \frac{\sum_{y=x}^{xn} l(y)}{l(x)}$
	y is summed from age class x to age class xn at the end of life.	
$m(x)$	Mean number of female pups born per age class.	$m(x) = B(x) * b(x) * 0.5$
	proportion of female pups was estimated at 0.5.	

Table 6. Number of households involved in the study.

	Zone A	Zone B	Zone C	Zone D	Total in the study area
Total number of households	89	176	100	145	510
Number of dog-owning households among total number of households	66	115	51	101	333

Table 7. Characteristics of the studied dog population.

Total number of dogs involved in the survey		872
Human-to-dog ratio		4.45:1
Male-to-female ratio in dogs (except for 15 dogs whose sex was not identified)		1.27:1
Number of dogs in a dog-owning household	Mean	2.6
	Median	2
Age (except for 11 dogs whose age was not identified)	Mean (years old)	2.7
	Median (years old)	2

Table 8. Sex-specific population demographics (Static life tables).**a. Static life table of male dogs.**

Age class	<i>n</i> years	Smoothed frequency <i>s</i> (<i>x</i>)	% <i>s</i> (<i>x</i>)	<i>l</i> (<i>x</i>)	<i>p</i> (<i>x</i>)	<i>d</i> (<i>x</i>)	<i>q</i> (<i>x</i>)	<i>e</i> (<i>x</i>)
0–1	1	120	27.76	1.00	0.54	0.00	0.46	3.60
1–2	1	65	15.01	0.54	0.88	0.46	0.12	4.81
2–3	1	57	13.18	0.47	0.85	0.53	0.15	4.34
3–4	1	48	11.17	0.40	0.82	0.60	0.18	3.94
4–5	1	39	9.14	0.33	0.79	0.67	0.21	3.60
5–6	1	31	7.22	0.26	0.76	0.74	0.24	3.29
6–7	1	24	5.50	0.20	0.74	0.80	0.26	3.01
7–8	1	17	4.05	0.15	0.71	0.85	0.29	2.73
8–9	1	12	2.87	0.10	0.69	0.90	0.31	2.43
9–10	1	9	1.97	0.07	0.66	0.93	0.34	2.08
10–11	1	6	1.30	0.05	0.64	0.95	0.36	1.64
11+	12	4	0.83	0.03	0.00	0.97	1.00	1.00

b. Static life table of female dogs.

Age class	<i>n</i> years	Smoothed frequency <i>s</i> (<i>x</i>)	% <i>s</i> (<i>x</i>)	<i>l</i> (<i>x</i>)	<i>p</i> (<i>x</i>)	<i>d</i> (<i>x</i>)	<i>q</i> (<i>x</i>)	<i>e</i> (<i>x</i>)
0–1	1	118	33.57	1.00	0.59	0.00	0.41	2.98
1–2	1	69	19.70	0.59	0.73	0.41	0.27	3.37
2–3	1	50	14.30	0.43	0.72	0.57	0.28	3.27
3–4	1	36	10.26	0.31	0.71	0.69	0.29	3.16
4–5	1	26	7.27	0.22	0.70	0.78	0.30	3.05
5–6	1	18	5.09	0.15	0.69	0.85	0.31	2.93
6–7	1	12	3.52	0.10	0.68	0.90	0.32	2.79
7–8	1	8	2.40	0.07	0.67	0.93	0.33	2.61
8–9	1	6	1.62	0.05	0.67	0.95	0.33	2.39
9–10	1	4	1.08	0.03	0.66	0.97	0.34	2.09
10–11	1	3	0.71	0.02	0.65	0.98	0.35	1.65
11+	5	2	0.46	0.01	0.00	0.99	1.00	1.00

Age class: age in year

n year: number of years spent in the age class*s*(*x*): number of individuals sampled per age class*s*(*x*) %: percentage of sample per age class*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 9. Estimated vaccination coverage in owned and overall dog populations through the first mass vaccination campaign.

	Zone A	Zone B	Zone C	Zone D
Vaccination coverage in the owned dog population (%)	41.3 (38.9–44.1)	48.3 (46.9–49.8)	52.6 (50.0–55.4)	20.9 (19.3–22.8)
Overall vaccination coverage (%)	38.7 (33.7–42.3)	47.3 (43.9–49.2)	51.6 (48.2–54.7)	19.8 (16.8–22.1)

Values in parentheses are 95% credible intervals.

Table 10. Estimated vaccination coverage in owned and overall dog populations through the follow-up mass vaccination campaign.

	Zone A	Zone B	Zone C	Zone D
Vaccination coverage in the owned dog population (%)	72.0 (67.8–76.8)	77.8 (75.5–80.2)	59.0 (56.1–62.2)	57.9 (53.5–63.1)
Overall vaccination coverage (%)	67.4 (58.7–73.7)	76.2 (70.6–79.3)	57.9 (54.0–61.3)	54.8 (46.4–61.1)

Values in parentheses are 95% credible intervals.

Table 11. Posterior distributions.

	Zone A	Zone B	Zone C	Zone D
Estimated owned dog population (M_i)	179 (168–190)	302 (292–311)	140 (133–148)	329 (302–357)
Ratio of ownerless to owned dogs (a_i)	0.06 (0.00–0.23)	0.02 (0.00–0.10)	0.01 (0.00–0.08)	0.05 (0.00–0.23)
Probability to recapture dogs (p_i)	0.34 (0.29–0.36)	0.23 (0.19–0.25)	0.44 (0.39–0.46)	0.17 (0.13–0.22)
Confinement probability for owned marked dogs ($c_{1,i}$)	0.07 (0.03–0.13)	NIL [‡]	0.00 (0.00–0.03)	0.01 (0.00–0.05)
Confinement probability for owned unmarked dogs ($c_{2,i}$)	0.06 (0.03–0.13)	NIL [‡]	NIL [‡]	NIL [‡]

[‡] No confinement was observed in the zone.

Values in parentheses are 95% credible intervals.

The number of digits after the decimal point was truncated in the number of dogs.

Table 12. Summary of the sensitivity analysis.

Changed parameter	Changed values of the prior distributions	Posterior median of the ownerless dog population			
		Zone A	Zone B	Zone C	Zone D
Mean [standard error] of confinement probabilities	0 [0]	9 (0–38)	5* (0–29)	2 (0–10)	15 (0–77)
	0.1 [0.05]	13 (0–42)	6 (0–31)	NA	14 (0–73)
	0.2 [0.05]	NA	6 (0–32)	NA	12 (0–66)
	0.4 [0.05]	NA	NA	NA	12 (0–56)
	0.8 [0.05]	NA	NA	NA	NA
Recapture probability	0.01–0.2	NA	8 (0–40)	NA	16 (0–78)
	0.01–0.4	7 (0–33)	5 (0–27)	2 (0–13)	15 (0–76)
	0.01–0.8	5 (0–27)	5 (0–27)	1 (0–8)	15 (0–76)
Ratio of ownerless dogs to owned dogs	0–0.2	10 (0–32)	5 (0–29)	2 (0–10)	14 (0–58)
	0–0.4	11 (0–42)	5 (0–29)	2 (0–10)	16 (0–85)
	0–0.8	11 (0–43)	5 (0–29)	2 (0–10)	16 (0–91)

Values in parentheses are 95% credible intervals.

For the posterior median of the ownerless dog population, the digits after the decimal point were truncated.

NA stands for that Markov chain Monte Carlo sampling cannot be completed.

* Same condition with the original results.

Table 12. Summary of the sensitivity analysis (continued).

Changed parameter	Changed values of the prior distributions	Vaccination coverage in owned dogs through the first mass vaccination (%)			
		Zone A	Zone B	Zone C	Zone D
Mean [standard error] of confinement probabilities	0 [0]	41.5 (39.0–44.2)	48.3* (46.9–49.8)	52.5 (50.0–55.4)	20.9 (19.3–22.8)
	0.1 [0.05]	41.2 (38.8–44.0)	48.2 (46.8–49.7)	NA	20.9 (19.3–22.7)
	0.2 [0.05]	NA	48.2 (46.8–49.7)	NA	20.9 (19.3–22.7)
	0.4 [0.05]	NA	NA	NA	20.8 (19.2–22.6)
	0.8 [0.05]	NA	NA	NA	NA
Recapture probability	0.01–0.2	NA	48.2 (46.8–49.7)	NA	20.9 (19.3–22.7)
	0.01–0.4	41.6 (39.1–44.3)	48.4 (47.0–49.9)	52.1 (49.6–54.9)	20.9 (19.3–22.8)
	0.01–0.8	41.9 (39.4–44.9)	48.4 (47.0–49.9)	53.2 (50.5–56.2)	20.9 (19.3–22.8)
Ratio of ownerless dogs to owned dogs	0–0.2	41.3 (38.9–44.0)	48.3 (46.9–49.8)	52.5 (49.9–55.4)	20.9 (19.3–22.8)
	0–0.4	41.3 (38.9–44.1)	48.3 (46.9–49.8)	52.6 (50.0–55.4)	20.9 (19.3–22.8)
	0–0.8	41.3 (38.9–44.1)	48.4 (46.9–49.8)	52.5 (50.0–55.4)	20.9 (19.3–22.8)

Values in parentheses are 95% credible intervals.

NA stands for that Markov chain Monte Carlo sampling cannot be completed.

* Same condition with the original results.

Table 12. Summary of the sensitivity analysis (continued).

Changed parameter	Changed values of the prior distributions	Overall vaccination coverage through the first mass vaccination (%)			
		Zone A	Zone B	Zone C	Zone D
Mean [standard error] of confinement probabilities	0 [0]	39.1 (34.0–42.6)	47.3* (43.9–49.2)	51.6 (48.1–54.6)	19.8 (16.7–22.1)
	0.1 [0.05]	38.2 (33.3–42.0)	47.1 (43.6–49.1)	NA	19.8 (16.9–22.0)
	0.2 [0.05]	NA	47.0 (43.4–49.0)	NA	20.0 (17.1–22.1)
	0.4 [0.05]	NA	NA	NA	19.9 (17.5–21.9)
	0.8 [0.05]	NA	NA	NA	NA
Recapture probability	0.01–0.2	NA	46.8 (42.4–48.9)	NA	19.7 (16.7–21.9)
	0.01–0.4	39.6 (34.9–42.9)	47.4 (44.1–49.3)	50.9 (47.1–54.1)	19.8 (16.8–22.1)
	0.01–0.8	40.5 (35.9–43.9)	47.4 (44.1–49.3)	52.4 (49.1–55.6)	19.8 (16.8–22.1)
Ratio of ownerless dogs to owned dogs	0–0.2	38.8 (34.7–42.3)	47.3 (43.8–49.2)	51.6 (48.1–54.7)	19.9 (17.4–22.1)
	0–0.4	38.6 (33.4–42.3)	47.3 (43.9–49.2)	51.6 (48.2–54.7)	19.8 (16.4–22.0)
	0–0.8	38.6 (33.4–42.3)	47.3 (43.9–49.2)	51.6 (48.1–54.7)	19.8 (16.2–22.1)

Values in parentheses are 95% credible intervals.

NA stands for that Markov chain Monte Carlo sampling cannot be completed.

* Same condition with the original results.

Table 12. Summary of the sensitivity analysis (continued).

Changed parameter	Changed values of the prior distributions	Vaccination coverage in owned dogs through the follow-up mass vaccination (%)			
		Zone A	Zone B	Zone C	Zone D
Mean [standard error] of confinement probabilities	0 [0]	72.3 (68.0–77.1)	77.8* (75.5–80.2)	58.9 (56.1–62.1)	57.9 (53.5–63.0)
	0.1 [0.05]	71.9 (67.7–76.7)	77.6 (75.4–80.0)	NA	57.8 (53.4–63.0)
	0.2 [0.05]	NA	77.6 (75.3–79.9)	NA	57.8 (53.4–62.9)
	0.4 [0.05]	NA	NA	NA	57.5 (53.2–62.5)
	0.8 [0.05]	NA	NA	NA	NA
Recapture probability	0.01–0.2	NA	77.6 (75.3–80.0)	NA	57.8 (53.4–62.9)
	0.01–0.4	72.5 (68.1–77.3)	77.9 (75.6–80.3)	58.5 (55.7–61.6)	57.9 (53.5–63.1)
	0.01–0.8	73.1 (68.6–78.2)	77.9 (75.6–80.3)	59.7 (56.6–63.1)	57.9 (53.5–63.1)
Ratio of ownerless dogs to owned dogs	0–0.2	72.0 (67.8–76.7)	77.8 (75.5–80.2)	58.9 (56.0–62.1)	57.9 (53.5–63.1)
	0–0.4	72.1 (67.8–76.8)	77.8 (75.6–80.2)	59.0 (56.0–62.1)	57.9 (53.5–63.1)
	0–0.8	72.0 (67.8–76.9)	77.8 (75.6–80.2)	58.9 (56.0–62.1)	57.9 (53.5–63.1)

Values in parentheses are 95% credible intervals.

NA stands for that Markov chain Monte Carlo sampling cannot be completed.

* Same condition with the original results.

Table 12. Summary of the sensitivity analysis (continued).

Changed parameter	Changed values of the prior distributions	Overall vaccination coverage through the follow-up mass vaccination (%)			
		Zone A	Zone B	Zone C	Zone D
Mean [standard error] of confinement probabilities	0 [0]	68.1 (59.3–74.2)	76.2* (70.6–79.3)	57.8 (54.0–61.3)	54.8 (46.3–61.1)
	0.1 [0.05]	66.7 (58.0–73.3)	75.9 (70.2–79.0)	NA	54.9 (46.8–61.0)
	0.2 [0.05]	NA	75.6 (69.8–78.9)	NA	55.2 (47.4–61.2)
	0.4 [0.05]	NA	NA	NA	55.1 (48.5–60.6)
	0.8 [0.05]	NA	NA	NA	NA
Recapture probability	0.01–0.2	NA	75.2 (68.2–78.8)	NA	54.6 (46.2–60.7)
	0.01–0.4	69.0 (60.8–74.7)	76.4 (71.0–79.4)	57.1 (52.8–60.6)	54.8 (46.4–61.1)
	0.01–0.8	70.5 (62.6–76.5)	76.4 (71.0–79.4)	58.8 (55.1–62.4)	54.8 (46.4–61.1)
Ratio of ownerless dogs to owned dogs	0–0.2	67.6 (60.5–73.7)	76.1 (70.6–79.3)	57.8 (54.0–61.3)	55.0 (48.2–61.2)
	0–0.4	67.4 (58.3–73.7)	76.2 (70.6–79.3)	57.9 (54.0–61.3)	54.8 (45.5–61.0)
	0–0.8	67.4 (58.2–73.7)	76.2 (70.6–79.3)	57.9 (54.0–61.3)	54.8 (44.9–61.1)

Values in parentheses are 95% credible intervals.

NA stands for that Markov chain Monte Carlo sampling cannot be completed.

* Same condition with the original results.

Table 13. Reasons for non-participation in the first mass vaccination campaign.

Reason	Answers (n)	%
Not informed	58	32.0
Owner's unavailability (Owner went to church/work/funeral/hospital etc.)	48	26.5
Owner failed to restrain his/her dog(s)	43	23.8
Mistime/misunderstood the venue	9	5.0
Vaccine was still valid	5	2.8
Dog was too young for vaccination	4	2.2
Owner was reluctant	2	1.1
Owner was sick	2	1.1
Owner misunderstood that his/her dogs had valid certificates	1	0.6
Owner doubted if it was free vaccination or not	1	0.6
Owner's house was far from the vaccine site	1	0.6
Owner had not yet got dog at the time of the vaccination	1	0.6
Unavailable answers	6	3.3
Total number of respondents	181	100

Table 14. Sources of information about rabies (multiple answers).

Reason	Answers (n)	%
Through relatives/neighbors	83	33.1
Through experience from keeping dogs/saw a rabid dog	83	33.1
Through family	80	31.9
Through TV/radio	33	13.1
Through doctors/hospitals	21	8.4
Through veterinarians/vet clinics	21	8.4
At school	12	4.8
Saw a rabid human	2	0.8
Others	3	1.2
Unavailable answers	3	1.2
Total number of answers	341	
Total number of respondents	251	

Table 15. Answers about symptoms of rabies in humans (multiple answers).

Reason	Answers (n)	%
Salivation	43	58.1
Barking like a dog	22	29.7
Getting mad (insanity)	14	18.9
Behavior change	12	16.2
Die	8	10.8
Fighting (violent)	6	8.1
Restlessness	6	8.1
Moving about	5	6.8
Mental disturbance/disorder	5	6.8
Hyperactivity	4	5.4
Biting	2	2.7
Hydrophobia	2	2.7
Crying	2	2.7
Failure eating	2	2.7
Others	5	6.8
Unavailable answers	4	5.4
Total number of answers	142	
Total number of respondents	74	

Table 16. Affordability of canine rabies vaccination.

(ZMW)	Number of responses (%)	
	Willingness to pay	Actual cost
0 (or never vaccinated before)	105 (32.0)	99 (30.9)
0.50–5.00	174 (53.0)	8 (2.5)
10.00	46 (14.0)	198 (61.9)
15.00	2 (0.6)	14 (4.4)
20.00	1 (0.3)	1 (0.3)
Total number of valid responses	328	320
Unavailable answers	5	13

ZMW (Zambian kwacha): 1 USD was equivalent to ZMW 10.36 on May 27, 2016.

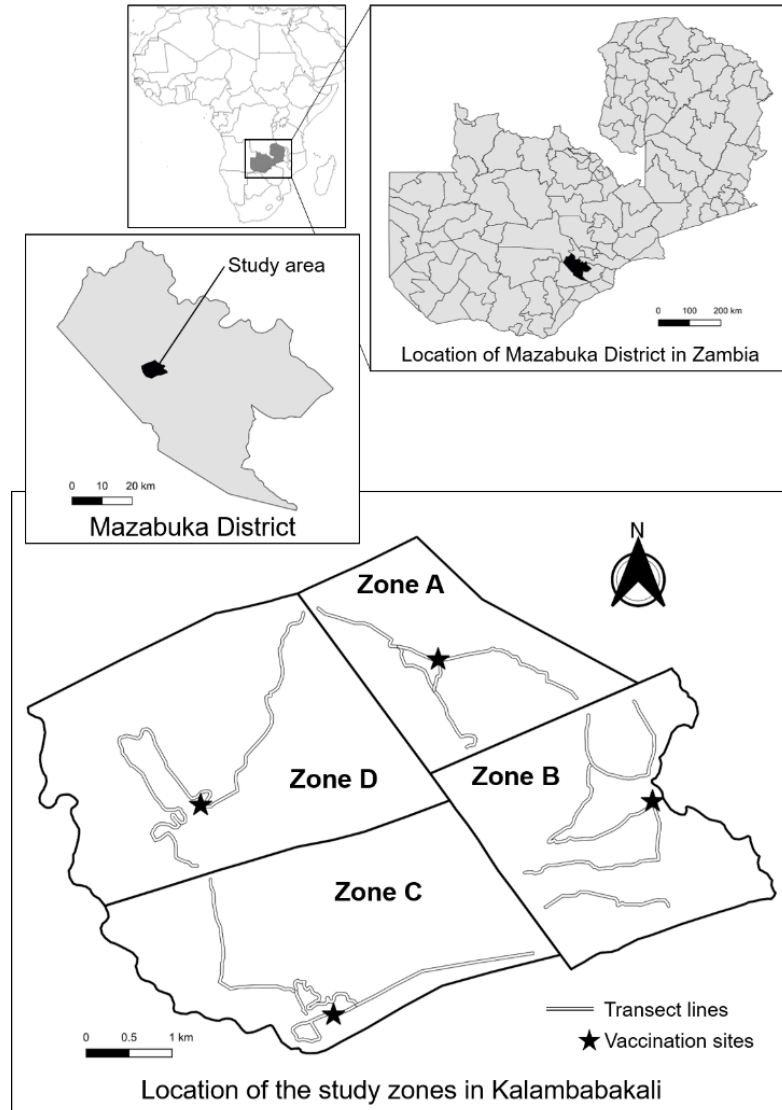


Figure 1. Location of the study area in Kalambabakali, Mazabuka District of Zambia. Study area consists of four continuous zones (Zone A, B, C and D) in Kalambabakali. Map of the African Continent was obtained from the Natural Earth (<https://www.naturalearthdata.com/>). Map of Zambia was downloaded from the Humanitarian Data Exchange (<https://data.humdata.org/dataset/zambia-administrative-boundaries-level-1-provinces-and-level-2-districts-with-census-2010-population>), which is shared under Creative Commons Attribution for Intergovernmental Organizations license (<https://creativecommons.org/licenses/by/3.0/igo/legalcode>). The shapefiles provided under this license themselves were not modified, but the shapefiles originally created for representing study area were overlaid on the shapefiles corresponding to Mazabuka District. Maps were created using the QGIS 3.10 software (<https://qgis.org/en/site/>).

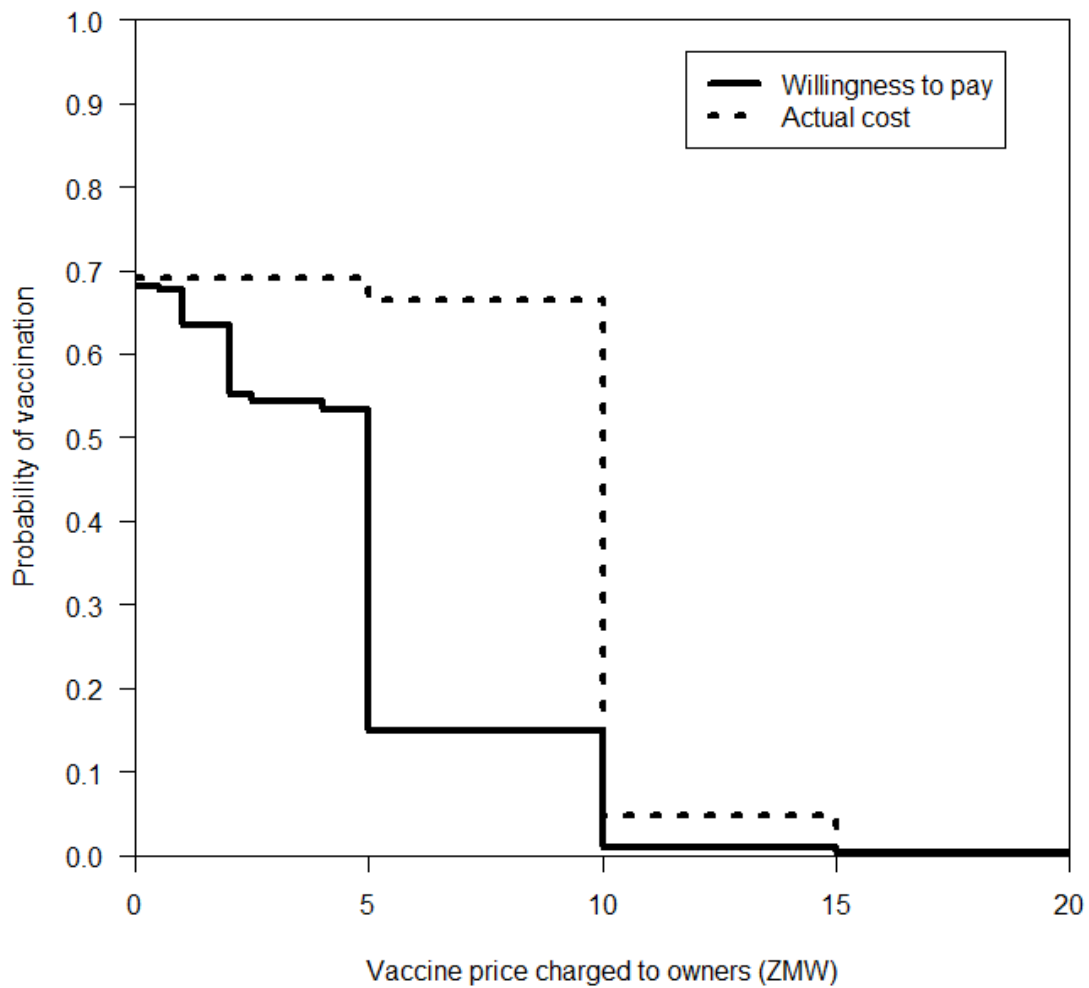


Figure 2. Expected probability of vaccination based on the vaccine price. The solid line shows the reverse cumulative probability of vaccination based on the amount that owners are willing to pay for a single canine rabies vaccination. The broken line shows the reverse cumulative proportion for the amount that owners have actually paid for a single canine rabies vaccination. These are based on data collected from the household survey.

CHAPTER II

Immunization coverage and antibody retention against rabies in domestic dogs in Lusaka District, Zambia

1 INTRODUCTION

Canine rabies vaccination has been conducted to maintain herd immunity in dog populations. It is known that 20–45% of dogs must always be immune to interrupt the rabies transmission in a dog population, and this coverage is recognized as the critical vaccination coverage of rabies (Hampson et al., 2009). During canine mass vaccination campaigns, which are usually conducted annually in resource-limited countries, it is well understood that 70% vaccination coverage must be attained in a campaign (Coleman and Dye, 1996; Hampson et al., 2009; WHO, 2018). This coverage, which is higher than the abovementioned critical threshold (i.e., 20–45%), is required to prevent the decline of herd immunity below the critical threshold during the intervals between vaccination campaigns (Conan et al., 2015; Hampson et al., 2009; Morders et al., 2014b). Particularly in highly dense, large, and connected dog populations, spatial heterogeneity in vaccination coverage allows rabies transmission to be sustained (Ferguson et al., 2015; Knobel et al., 2020; Townsend et al., 2013). Although there is no evidence that rabies virus transmission depends on the dog population density (Hampson et al., 2009; Morders et al., 2013), epidemics likely continue for longer durations, with more cases in larger and higher-density populations (Knobel et al., 2020). Therefore, rabies control programs need to include comprehensive canine vaccination across dog populations, particularly in urban settings.

Dog owners' accessibility to canine rabies vaccines is considered to be better in urban than in rural settings (Cleaveland et al., 2007; Sambo et al., 2013; Wallace et al., 2017). Furthermore, dog owners who reside in high-income residential areas are likely to intentionally vaccinate their dogs (de Balogh et al., 1993; Olugasa et al., 2011). Therefore, in urban settings, a combination of mass vaccination campaigns in low-income residential

areas and vaccination in veterinary clinics in high-income residential areas could effectively enhance and maintain the canine herd immunity against rabies in urban settings (de Balogh et al., 1993). In such situations, household surveys are necessary to assess the vaccination coverage achieved in urban settings because low- and high-income residential areas, which are probably covered by mass vaccination campaigns and owners' voluntary vaccination, are sometimes intermingled. However, owners' improper maintenance of vaccination certificates makes an assessment more difficult in household surveys. WHO states that routine serological monitoring after canine mass vaccination campaigns is unnecessary if the following criteria are observed: (1) high-quality vaccines manufactured according to international standards have been used; (2) vaccinators have been trained in the proper administration and handling of vaccines as well as of dogs; and (3) the cold chain has been maintained throughout (WHO, 2018). However, in cases where vaccination certificates are unavailable, serological evaluation will provide helpful information to assess the actual immunization coverage, defined as the proportion of dogs that retain protective antibody titers in a dog population.

Rabies control programs have been promoted in Lusaka District of Zambia, and a considerable number of canine rabies vaccinations have been implemented during mass vaccination campaigns and at veterinary clinics. A household survey conducted in a low-income, densely populated area of Lusaka in the early 1990s demonstrated a canine vaccination coverage of 16% (26/160 dogs) based on the vaccination status (de Balogh et al., 1993). Although mass vaccinations have been conducted in many parts of Lusaka District, particularly in populated residential areas, the vaccination coverage of the domestic dog population in Lusaka District has never been estimated despite the continued presence of rabies in both humans and animals (Hamoonga, 2018; Muleya et al., 2019, 2012). Therefore, this chapter aimed to estimate the "vaccination coverage"

based on vaccination certificates and the “actual immunization coverage” based on the seropositivity in the owned dog population of Lusaka District and to retrospectively evaluate antibody decline in vaccinated dogs by measuring antibody titers with reference to the dates of vaccination.

2 MATERIALS AND METHODS

2.1 Ethical approval

Ethical approval for this study was obtained from the Ministry of Fisheries and Livestock of the Government of the Republic of Zambia. This study was conducted under the monitoring project of the Ministry of Fisheries and Livestock, and thus was not categorized as a study involving animal experiments.

2.2 Study area

The study was conducted in Lusaka District, located in Lusaka Province, in the central part of Zambia (Figure 3). The district covers 360 km² with a total human population of 1,747,152 individuals according to the 2010 census (Banda et al., 2015). The dog population was estimated at 44,054 dogs between 2017 and 2018 by the Department of Veterinary Services, Zambia.

2.3 Cluster survey method

Sampling was conducted from March 23, 2015 to April 17, 2015 following the expanded program on immunization (EPI) cluster survey, with modification (WHO, 2005, 2008). The sampling in this study aimed to estimate the immunization coverage within a

$\pm 10\%$ desired precision, with a 95% CI. This survey consisted of a two-stage cluster sampling. In the first stage, 20 of the wards were sampled as clusters with a probability proportionate to the households' size in the wards (Table 17). The selected clusters are shown in Figure 3. In the second stage, at least ten households that owned dogs were selected within each cluster. The subjects were chosen by selecting a household randomly, and every eligible subject in the household was included in the sampling (Hoshaw-Woodard and WHO, 2001), with a few exceptions mentioned in the next paragraph.

The survey was accompanied by one veterinary assistant officer from the Lusaka DVO. In the dog-owning households selected, the purpose of the study was explained to the head of the household or suitable representatives, and their verbal consent for participation was obtained. All dogs in the households selected were included in the survey for blood sample collection and determination of previous vaccination certificates. However, in situations where the dog was too vicious, could not be restrained by the owner, or was less than 3 months of age, the dog was not sampled. Information on the dog(s), way of keeping dog(s) in the household, previous vaccination, product name of the previous vaccine, manufacturer, lot number, and validity of the vaccination was collected in each household.

This study designated the proportion of dogs that had valid vaccination certificates among the targeted dog population as "vaccination coverage." To estimate the vaccination coverage based on the information in the previous vaccination certificates, we followed the criteria as follows: (i) the vaccination certificate was valid for 6 months (180 days) and 1 year (365 days) in case of the first vaccination and from the second vaccination, respectively; and (ii) dogs whose vaccination history was unclear without a previous vaccination certificate and dogs whose vaccination certificates had expired were regarded as unvaccinated. These criteria correspond to the "Protocol on Rabies Disease

Control in Zambia,” as stipulated in the Control of Dogs Act, Cap 247 of the Laws of Zambia.

2.4 Blood sample collection from owned dogs

Blood samples were collected to measure the neutralizing antibody titers against the rabies virus. This was done to estimate the proportion of seropositive dogs among the targeted dog population, which was defined as the “actual immunization coverage,” and to assess the antibody decline over time in the vaccinated dogs. To estimate the actual immunization coverage, 251 dogs were sampled according to the EPI cluster survey. To assess the antibody decline over time, 27 additional blood samples were collected in the same period from Lusaka District, in addition to the 251 samples. Of the 278 samples, 37 samples were obtained from dogs that had received a single vaccination using Rabisin (Merial, Lyon, France), Rabigen-mono (Virbac, Carros, France), or Rabies Vet (Bio-Med, Ghaziabad, India) before our sampling. Similarly, 39 samples were obtained from the dogs that had been vaccinated multiple times with the aforementioned rabies vaccine products before our sampling. This information was obtained from vaccination certificates.

The cephalic vein on the foreleg was used to collect blood. Briefly, 2 mL of blood from each dog was collected in sterile tubes and allowed to settle at room temperature for 1 hour to promote coagulation. Blood samples were subsequently stored at 4°C overnight to exude serum. Afterward, sera were collected into new tubes, and the samples were stored at –80°C until being shipped to Japan for subsequent laboratory analyses. According to the Protocol on Rabies Disease Control in Zambia, puppies aged below 3 months were not sampled as they are ineligible for the rabies vaccination; unhealthy dogs were also not sampled.

2.5 Measurement of antibody titer against rabies

Antibodies against the rabies virus in the serum samples were measured using the fluorescent antibody virus neutralization (FAVN) test at the Hokkaido University, International Institute for Zoonosis Control, Japan, according to the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2013 (Cliquet et al., 1998) released by the World Organisation for Animal Health (OIE). Briefly, the rabies virus challenge virus standard (CVS) strain and BHK-21 C13 cells (ATCC CCL-10) were used for the FAVN test. The serum samples were first heat-treated at 56°C for 30 min to inactivate the complements and serially diluted in 96-well plates. The diluted serum samples were incubated with 100 TCID₅₀ (50% tissue culture infective dose) of CVS in 50 µL. Any un-neutralized CVS could replicate on BHK-21 C13 cells and be detected by fixation with 10% formalin and staining with fluorescein isothiocyanate anti-rabies monoclonal globulin (Fujirebio Diagnostics, Malvern, PA, USA). The stained cells were evaluated qualitatively by fluorescent microscopy. The Spearman–Kärber method (WHO, 1996) was used to calculate the 50% endpoint titers of the serum, and the titers were converted into international units (IU/mL) by comparison with the OIE-positive standard serum (ANSES, Maisons-Alfort, France) with a known neutralizing titer. Following the WHO recommendations, a neutralizing antibody titer ≥ 0.5 IU/mL was regarded as positive (WHO, 2013; WHO, 1992), which is a criterion required for international dog movement (OIE, 2013). Furthermore, another threshold of 0.2 IU/mL was adopted as the “minimum” titer that was considered adequate to protect host dogs from the rabies virus infection, which was studied by Bunn et al. between 1983 and 1984 (cited in Aubert, 1992). For every sample whose titer could not be measured at a certain value because of the limit of detection, particularly in the range less than 0.1 IU/mL, we assigned an arbitrary value corresponding to the maximum possible value to be able to perform the analysis, for

example, ≤ 0.042 , ≤ 0.056 , ≤ 0.073 , and ≤ 0.096 IU/mL of the actual detected values were regarded as 0.042, 0.056, 0.073, and 0.096 IU/mL, respectively.

2.6 Data and statistical analyses

Excel 2016 was used for data input. Subsequently, the vaccination and actual immunization coverage according to the results of the FAVN test were calculated. Data analyses of the antibody titers were performed using R version 3.6.3 (R Core Team, 2020).

The association between the “vaccination status,” represented by the validity of the vaccination certificate, and “seropositivity,” represented by antibody retention at thresholds of ≥ 0.5 IU/mL, and the association between the vaccination status/seropositivity and dog sex were tested using Fisher’s exact test and the R package “fmsb” in R version 3.6.3. A p -value < 0.05 was considered statistically significant. The p -values in the multiple tests were adjusted using the Benjamini–Hochberg method (Benjamini and Hochberg, 1995).

3 RESULTS

3.1 Dog population characteristics

Two hundred dog-owning households were selected for participation in this study. The mean number of dogs per dog-owning household was 1.8 (median 1, minimum 1, and maximum 7). Of the 366 dogs owned in the surveyed households, blood samples were collected successfully from 251 dogs for the EPI cluster survey. The male-to-female ratio in the sampled dogs was 1.04:1. The mean age of the sampled dogs was 1.2 years (median: 1.3 years). The age distribution of the sampled dogs is shown in Table 18. A

total of 62.9% of the sampled dogs (158/251) were allowed to roam freely, 22.3% (56/251) were kept as free-range only within the fenced premises according to the owners' reports, 4.4% (11/251) were reported to be confined in cages or kept by chains, and 10.4% (26/251) were reported to be kept using a mixed style of free-range inside the premises and confinement, depending on the time and situation.

3.2 Rabies vaccination and immunization coverage in dogs

A total of 19.9% of the sampled dogs (50/251) had valid vaccination certificates (Table 19). In contrast to this certificate-based vaccination coverage, 42.2% (106/251) had sufficiently high levels of rabies virus–neutralizing antibodies (i.e., ≥ 0.5 IU/mL) (Table 19a). When a value of 0.2 IU/mL was adopted as the threshold titer, 52.6% (132/251) had the minimum protective levels of the antibodies at the sampling time (Table 19b). For a conservative estimate of the vaccination coverage among the entire owned dog population in Lusaka District, the 115 dogs excluded from the study were added to the denominator, with the assumption that all of them had never been vaccinated; minimum vaccination coverage of 13.7% (50/366; 95% CI: 8.7–18.6) was obtained for the owned dog population in Lusaka District based on the EPI cluster survey estimates. In the same manner, minimum immunization coverage, defined as the minimum proportion of seropositive dogs among the total owned dog population in Lusaka District, was also estimated, and the results are presented in Table 20. The geometric mean titer (GMT) of 251 serum samples was 0.43 IU/mL (95% CI: 0.33–0.55; minimum: 0.042 IU/mL; median: 0.22 IU/mL; maximum: 159.9 IU/mL).

Dogs that had valid vaccination certificates were significantly seropositive, with a 0.5 IU/mL threshold titer, compared with dogs whose status was uncertain/expired or had never been vaccinated (p -values < 0.01). Dog sex was neither associated with

vaccination status (p -values > 0.5) nor seropositivity with 0.5 IU/mL of the threshold ($p = 0.16$).

3.3 Antibody decline in vaccinated dogs

The association of antibody titers in single-vaccinated dogs ($n = 37$) with days post vaccination (dpv) is presented in Figure 4. In the tested dogs, the probabilities of vaccination success within 180 dpv, applying the thresholds of 0.5 and 0.2 IU/mL were 78.6% (95% CI: 49.2–95.3; $n = 14$) and 85.7% (95% CI: 57.2–98.2), respectively. The GMT among the single-vaccinated dogs ($n = 37$; minimum dpv: 18; maximum dpv: 1,117) was 0.81 IU/mL (95% CI: 0.44–1.48), whereas the GMT in those within 180 dpv ($n = 14$) was 1.53 IU/mL (95% CI: 0.49–4.79).

The time-series trend of antibody titer in multiple-vaccinated dogs ($n = 39$) is presented in Figure 5. In these dogs, the probabilities of vaccination success within 365 dpv, with the thresholds of 0.5 and 0.2 IU/mL, were 89.3% (95% CI: 71.8–97.7; $n = 28$) and 96.4% (95% CI: 81.7–99.9), respectively. The GMT among multiple-vaccinated dogs ($n = 39$; minimum dpv: 18; maximum dpv: 1,323) was 3.34 IU/mL (95% CI: 1.90–5.86), whereas the GMT in those within 365 dpv ($n = 28$) was 4.49 IU/mL (95% CI: 2.23–9.03).

4 DISCUSSION

This chapter estimated the immunization coverage and demonstrated the antibody decline over time in vaccinated dogs in Lusaka District of Zambia. This is the first report describing the actual immunization coverage against rabies, represented by a proportion of seropositive dogs in the owned dog population in the capital city of Lusaka,

Zambia.

Even though vaccination certificates had expired or were uncertain in nearly half of the dogs (119/251), over 50% of such dogs (62/119) had rabies virus–neutralizing antibodies with titers ≥ 0.5 IU/mL. Over 60% of those (77/119) dogs had antibody titers ≥ 0.2 IU/mL (Table 19). Therefore, the immunization coverage, defined as the proportion of dogs that had actual protective levels of the antibody, was not extremely low as a whole, even though one-third of the dogs had never been vaccinated, based on their owners' statements. The measurement of antibody titer is unnecessary to evaluate immunization coverage after a mass vaccination campaign if certified vaccines are used and the vaccinators are well trained to conduct the vaccination (WHO, 2018). However, it is difficult to assess the immunization coverage if owners do not properly preserve the vaccination certificates. Indeed, 14.6% of the dogs (12/82 dogs that had never been vaccinated) in this study had antibody titers ≥ 0.2 IU/mL, although they were declared as never been vaccinated by their owners. However, it should be noted that these antibodies against the rabies virus may come from nonlethal exposure to antigens, for instance, through the consumption of carcasses that have died of rabies or another lyssavirus infection (Berentsen et al., 2013; Deem et al., 2004; Shipley et al., 2019) in addition to the possibility of owners' lapses of memory for the vaccination status. As one-third of dogs could not be designated as vaccinated or not, the further necessity of improving dog-owner responsibility, such as good conduct of vaccination and proper preservation of vaccination certificates, is emphasized to enhance rabies control in dogs. Regarding the level of herd immunity, the immunization coverage was 52.6% among the dogs tested and the minimum immunization coverage was estimated at 36.1% in Lusaka District, when 0.2 IU/mL of titer was adopted as the threshold. These values would be moderately sufficient to protect the dog population from a rabies outbreak compared to the critical

vaccination coverage of 20–45% that is required to interrupt rabies transmission in a dog population (Hampson et al., 2009). However, it should be noted that the immunization coverage demonstrated in this study targeted the owned dog population in Lusaka District without involving the ownerless dog population. It should be reminded that herd immunity needs to be maintained in the total dog population, including both owned and ownerless dogs, although the ownerless dog population in Lusaka District seemed to be very low (de Balogh et al., 1993), in addition to the increasing evidence that most free-roaming dogs in rabies-endemic countries are owned (Gsell et al., 2012; Kayali et al., 2003; Morters et al., 2014b; demonstrated in chapter I).

The vaccination coverage observed in this study was lower than that in earlier studies conducted in other rabies-endemic countries, such as 85% in Santa Cruz de la Sierra, Bolivia (Suzuki et al., 2008), and 70% in Thungsong District, Thailand (Kongkaew et al., 2004). Alternatively, the actual immunization coverage observed in this study was similar to or slightly less than the seropositivity-based immunization coverage, with 0.5 IU/mL of threshold titer recorded in other African countries, such as a 42.6% immunization coverage in Ilorin city, Nigeria, by stratified random sampling (Olugasa et al., 2011). That earlier study mentioned both a lack of stable rabies vaccination programs in the city and vaccination failure that were common in Nigeria (Adeyemi and Zessin, 2000; Adeyemi et al., 1993) as factors contributing to the immunization coverage observed (Olugasa et al., 2011). In Gaborone, Botswana, a 54% seropositivity in dogs sampled in animal clinics was reported (Sebunya et al., 2007). Moreover, in Emalahleni in the Eastern Cape Province of South Africa, immunization coverage of 32% was reported, with vaccination coverage of 56% among a randomly sampled dog population (Van Sittert et al., 2010).

The immunization/vaccination coverage in African urban settings described

above is remarkably higher than the vaccination coverage of below 10% without any interventions in rural Zambia reported previously (Mulipukwa et al., 2017) and in chapter I. This could be attributed to the differences in urban and rural settings regarding the availability of vaccine products and the dog owners' accessibility to the vaccine, affordability of the canine rabies vaccine, and so on (Wallace et al., 2017). As demonstrated in rabies-endemic African countries, free rabies mass vaccination campaigns are capable of achieving the WHO-recommended vaccination coverage of 70% (Coleman and Dye, 1996; WHO, 2018), whereas owner-charged vaccination campaigns achieve a vaccination coverage that is insufficient to prevent the transmission of rabies (Durr et al., 2009; Jibat et al., 2015; Kayali et al., 2003). Although dog owners, particularly in rural settings, need free rabies mass vaccination to achieve 70% vaccination coverage in a campaign, a certain number of dog owners in urban settings may be capable of paying for regular canine vaccination. Therefore, it is possible to maintain the critical threshold coverage in urban settings with a combination of mass vaccination campaigns and veterinary clinic-based vaccination unless the supply of high-quality vaccine products is unstable. As there is a tendency for higher seropositivity in high-income residential areas and lower seropositivity in low-income residential areas in urban settings (Olugasa et al., 2011), differences in the owners' income level and the affordability of the canine rabies vaccine could be factors influencing the immunization coverage and heterogeneity in the dog population in urban settings, where the residents' characteristics may be more varied than those in rural areas. This study did not analyze the differences in vaccination coverage and actual immunization coverage among the selected wards by the income level of the dog owners. However, this should be considered when making policies aimed at improving vaccination coverage with a combination of owner-charged rabies vaccination and free rabies mass vaccination to raise the

vaccination coverage in the entire city of Lusaka.

This study demonstrated that the antibody declines over time among vaccinated dogs in Lusaka District, Zambia. It retrospectively verified that a single vaccination with certified vaccines could have acceptably induced and retained protective antibodies for at least 180 dpv, as the certificate for the first vaccination is regarded as valid for 180 days. However, the peak titer has been reported to influence prolonged antibody retention after vaccination (Morters et al., 2014a). The higher the peak titer, the longer the antibody titer remains potent enough to protect the host animal (Morters et al., 2014a). In contrast, if the peak titer is low, the antibody titer will decline to below the protective level even within the period of vaccination validity (Morters et al., 2014a). This highlights the possibility of the rapid decline in antibody titers among the dog population studied even if they had a protective level of antibody titers at the time of sampling because the titers, which would be considered peak titers 3–6 weeks after vaccination (Morters et al., 2014a; Pimburage et al., 2017; Sugiyama et al., 1997; WHO, 2018), were not high in some individuals in this study. Here only three dogs retrospectively corresponded to the duration approximately 3–6 weeks after the first vaccination. Antibody titers for these dogs were 23.4, 13.5, and 0.29 IU/mL after 18, 32, and 42 dpv, respectively. A field trial showed a GMT of 14.8 IU/mL as a peak titer at 30 dpv, which declined to 0.81 IU/mL at 180 dpv in a rabies-endemic African country (Morters et al., 2014a). We could not determine whether the aforementioned titers observed between 3 and 6 weeks after vaccination would be retained at the level of ≥ 0.2 or ≥ 0.5 IU/mL until 180 dpv, because we did not prospectively assess the kinetics of the antibody titers in individuals. However, it should be emphasized that declines in individual antibody titers must be considered during the planning of rabies mass vaccinations with the aim of maintaining herd immunity.

Nevertheless, the probability of vaccination success was 78.6% in the single-vaccinated dogs and 89.3% in the multiple-vaccinated dogs with the threshold of 0.5 IU/mL, and 85.7% in the single-vaccinated dogs and 96.4% in the multiple-vaccinated dogs with the threshold of 0.2 IU/mL. Other field studies demonstrated a seroconversion of 83% in field dogs in South Africa (Van Sittert et al., 2010) and 87.2–93.7% seroconversion and antibody retention at ≥ 0.5 IU/mL until 180 dpv from single-vaccinated dogs in Sri Lanka (Pimburage et al., 2017), both using commercial rabies vaccine products. Our findings are similar to those reported in these abovementioned studies, although our evaluation was performed retrospectively. As demonstrated previously, multiple vaccinations (boosters) enhance seroconversion and induce long-lasting antibody retention (Pimburage et al., 2017; Sugiyama et al., 1997). In this study, dogs that received multiple vaccinations had a higher GMT and a higher probability of vaccination success than those observed in the single-vaccinated dogs.

This study observed a certain proportion of seronegative dogs among vaccinated dogs despite their valid vaccination certificates. This fact suggests two situations. First, there is a possibility that those seronegative dogs had seroconverted once after the vaccination and, subsequently, the antibody titer decreased below the threshold titer by the date of sampling. The second possible situation is that the seronegative dogs had truly never seroconverted after the vaccination at the time of sampling. Although the reasons for the presence of seronegative dogs, despite a valid vaccination status, remain unclear in this study, it should be considered that a certain proportion of dogs will not seroconvert in a mass vaccination campaign. This is important to note when calculating the desired target vaccination coverage during the planning phase of the mass vaccination campaigns. The reasons for vaccine failure may be various factors, such as a break in the cold chain, inadequate vaccination technique, or host animal factors.

Regarding a break in the cold chain, the Nobivac Rabies vaccine (Merck Animal Health, Madison, NJ, USA), one of the high-quality commercially inactivated canine rabies vaccines, is thermotolerant (Lankester et al., 2016). Power loss occurs in Lusaka District from time to time; however, information on the thermotolerance of Rabisin, a commercially inactivated vaccine used commonly in Lusaka District, is lacking. Furthermore, another earlier study demonstrated that a vast majority of dogs in endemic rabies countries seroconverted successfully (with the threshold of 0.5 IU/mL), regardless of health status. However, there were substantial variations in titers that arose partly from clinical conditions and lactation at vaccination (Morters et al., 2014a). The study, being cross-sectional and retrospective in nature, did not analyze the association between the seroconversion or level of antibody titer and the health status or lactation at the time of vaccination. However, this may be another concern for seroconversion and the introduction of a long-lasting antibody titer.

This chapter presented the findings that help understand the current achievements and situations of rabies control programs in Lusaka. The data presented in this chapter have great potential to guide the planning and implementation of rabies vaccination programs in Lusaka city and contribute positively to achieving the global goal of “Zero by 30.”

5 SUMMARY

Although rabies control programs have progressed in targeting dogs, which are the main vectors of rabies to humans, the disease remains endemic in Zambia. Despite conducting canine mass vaccination campaigns and vaccinating domestic dogs in many veterinary clinics in Lusaka District, the vaccination coverage and actual seropositivity in the dog population in Lusaka District are rarely evaluated. This study estimated the certificate-based vaccination coverage and the seropositivity-based immunization coverage in the owned dog population in Lusaka District using the EPI cluster survey method. The time-series trend of neutralizing antibodies against rabies in vaccinated dogs was also evaluated. Of the 366 dogs in the 200 dog-owning households in Lusaka District, blood samples were collected successfully from 251 dogs. In the sampled dogs, 19.9% (50/251) had valid rabies vaccination certificates. Meanwhile, 42.2% (106/251) had an antibody titer ≥ 0.5 IU/mL, and 52.6% (132/251) had a protective level of antibody titer ≥ 0.2 IU/mL. When the 115 dogs whose blood was not collected were assumed to be unvaccinated or seronegative, the minimum certificate-based vaccination coverage in Lusaka District's owned dog population was estimated at 13.7% (95% CI: 8.7–18.6). In the same manner, the minimum immunization coverage in Lusaka District was estimated at 29.0% (95% CI: 22.4–35.5) with a threshold titer of 0.5 IU/mL and 36.1% (95% CI: 29.1–43.0) with a threshold titer of 0.2 IU/mL. It was also found that a single vaccination with certified vaccines is capable of inducing protective levels of antibodies. In contrast, higher antibody titers were observed in multiple-vaccinated dogs than in single-vaccinated dogs, coupled with the observation of a decline in antibody titer over time. These results suggest the importance of continuous booster immunization to maintain herd immunity and provide useful information to plan mass vaccination against rabies in

Zambia.

Table 17. Selected wards in the EPI cluster survey.

Constituency	No.	Ward	Number of households	Cumulative number of households	Cluster number
Chawama	1	Nkoloma	16,501	16,501	1
	2	Chawama	15,264	31,765	
	3	John Howard	6,093	37,858	
	4	Lilayi	3,050	40,908	
Kabwata	5	Kamwala	10,049	50,957	3
	6	Kabwata	4,835	55,792	
	7	Libala	4,487	60,279	
	8	Chilenje	10,521	70,800	
	9	Kamulanga	5,185	75,985	
Kanyama	10	Kanyama	36,834	112,819	5,6
	11	Harry Mwaanga Nkumbula	35,989	148,808	
	12	Munkolo	6,172	154,980	
Lusaka Central	13	Silwizya	1,595	156,575	9
	14	Independence	3,638	160,213	
	15	Lubwa	7,635	167,848	
	16	Kabulonga	12,704	180,552	
Mandevu	17	Roma	14,120	194,672	10
	18	Mulungushi	2,950	197,622	
	19	Ngwerere	14,164	211,786	
	20	Chaisa	4,566	216,352	
	21	Justine Kabwe	8,560	224,912	
	22	Raphael Chota	18,999	243,911	
	23	Mpulungu	11,490	255,401	
Matero	24	Muchinga	8,202	263,603	15
	25	Kapwepwe	10,952	274,555	
	26	Lima	13,195	287,750	
	27	Mwembeshi	13,016	300,766	
	28	Matero	11,688	312,454	
Munali	29	Chainda	8,485	320,939	18
	30	Mtendere	22,729	343,668	
	31	Kalingalinga	8,714	352,382	
	32	Chakunkula	6,647	359,029	
	33	Munali	9,335	368,364	
Sampling interval [†]				18,418	
Random number				15,120	

[†] Sampling interval = Total population (households) to be surveyed / Number of clusters

Table 18. Age distribution of the dogs involved in the EPI cluster survey.

Male	Age (Months)	Female
15	3–11	23
17	12–23	22
11	24–35	10
18	36–47	12
9	48–59	9
16	60–71	12
5	72–83	4
5	84–95	2
8	Over 96	0
24	Unidentified	29
128	Total	123

Table 19. Validity of the vaccination status and seropositivity.**a. Seropositivity with a threshold of 0.5 IU/mL.**

	Valid	Uncertain	Expired	Never vaccinated before	Total
Seropositive	40	38	24	4	106 (42.2)
Seronegative	10	34	23	78	145 (57.8)
Total	50 (19.9)	72 (28.7)	47 (18.7)	82 (32.7)	251

Values in parentheses are the proportion of the corresponding status (%).

b. Seropositivity with a threshold of 0.2 IU/mL.

	Valid	Uncertain	Expired	Never vaccinated before	Total
Seropositive	43	45	32	12	132 (52.6)
Seronegative	7	27	15	70	119 (47.4)
Total	50 (19.9)	72 (28.7)	47 (18.7)	82 (32.7)	251

Values in parentheses are the proportion of the corresponding status (%).

Table 20. Immunization coverage (proportion of dogs that had actual antibodies against rabies).

	Immunization coverage (<i>n</i> = 251)		Minimum immunization coverage (<i>n</i> = 366) [†]	
	Threshold: 0.5 IU/mL	Threshold: 0.2 IU/mL	Threshold: 0.5 IU/mL	Threshold: 0.2 IU/mL
Coverage (%)	42.2 (33.6–50.9)	52.6 (43.9–61.3)	29.0 (22.4–35.5)	36.1 (29.1–43.0)

[†] Including 115 dogs excluded from blood sampling, assuming that they were seronegative.
Values in parentheses are obtained at 95% confidence intervals.



a. Location of Lusaka District in Zambia

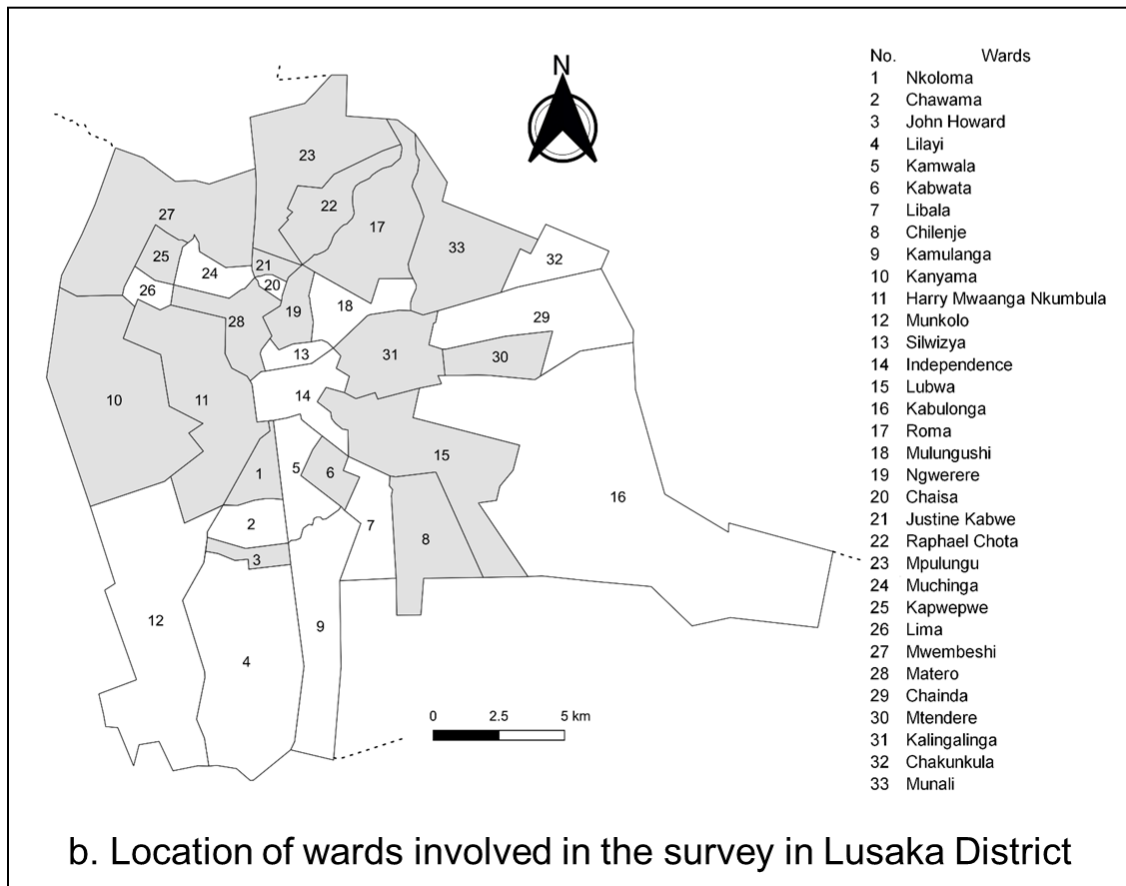


Figure 3. Location of the study area: (a) location of Lusaka District in Zambia; (b) location of the wards involved in the survey in Lusaka District. The selected wards and corresponding numbers are as follows: (1) Nkoloma, (3) John Howard, (6) Kabwata, (8) Chilenje, (10) Kanyama*, (11) Harry Mwaanga Nkumbula*, (15) Lubwa, (17) Roma, (19) Ngwerere, (21) Justine Kabwe, (22) Raphael Chota, (23) Mpulungu, (25) Kapwepwe, (27) Mwembeshi, (28) Matero, (30) Mtendere, (31) Kalingalinga, and (33) Munali. Asterisks (*) denote the wards where double clusters were selected.

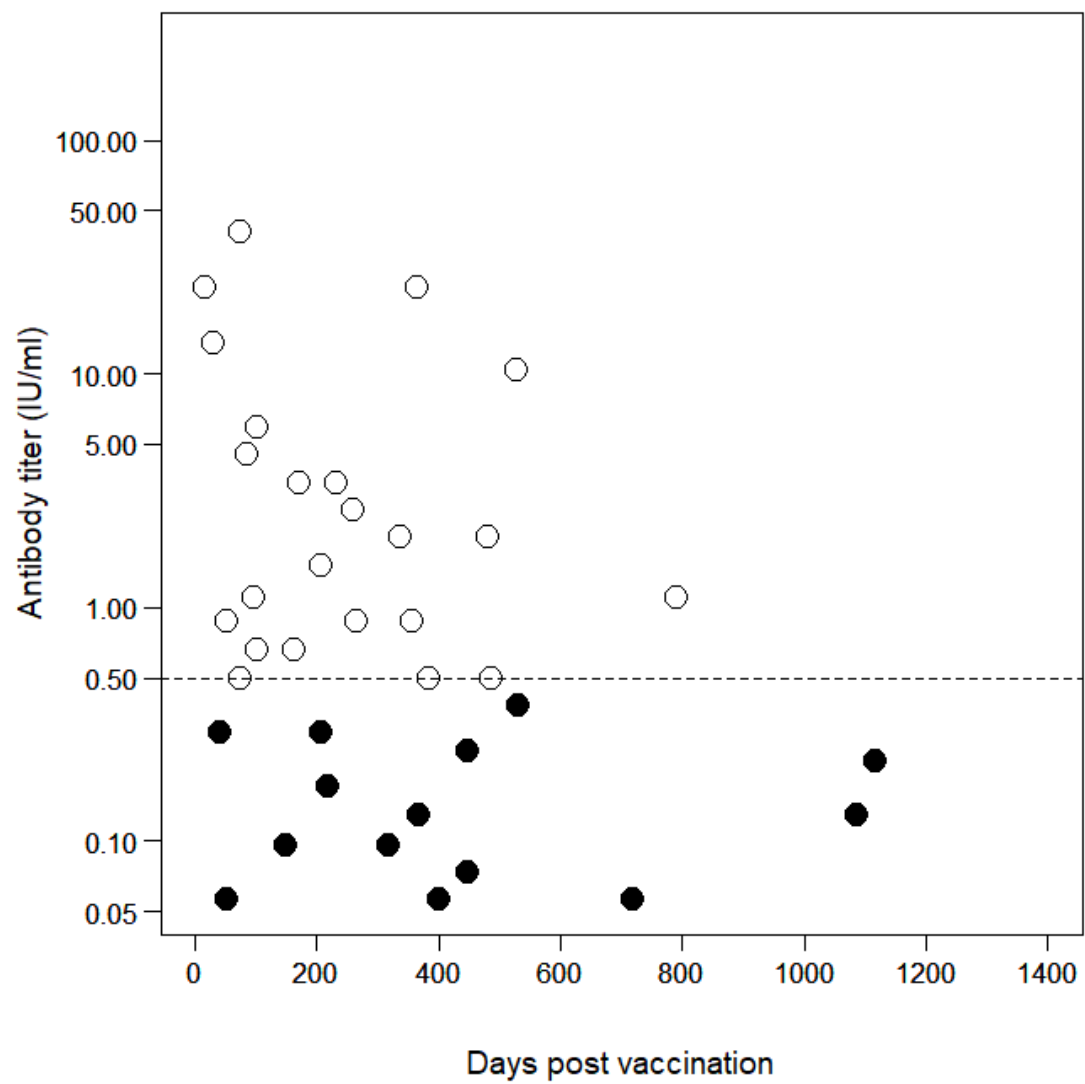


Figure 4. Antibody decline in single-vaccinated dogs ($n = 37$). Open circles represent samples that had antibody titers ≥ 0.5 IU/mL. Filled circles represent samples that had antibody titers < 0.5 IU/mL. The dashed line shows the antibody titer's threshold level (0.5 IU/mL) required for international dog movement.

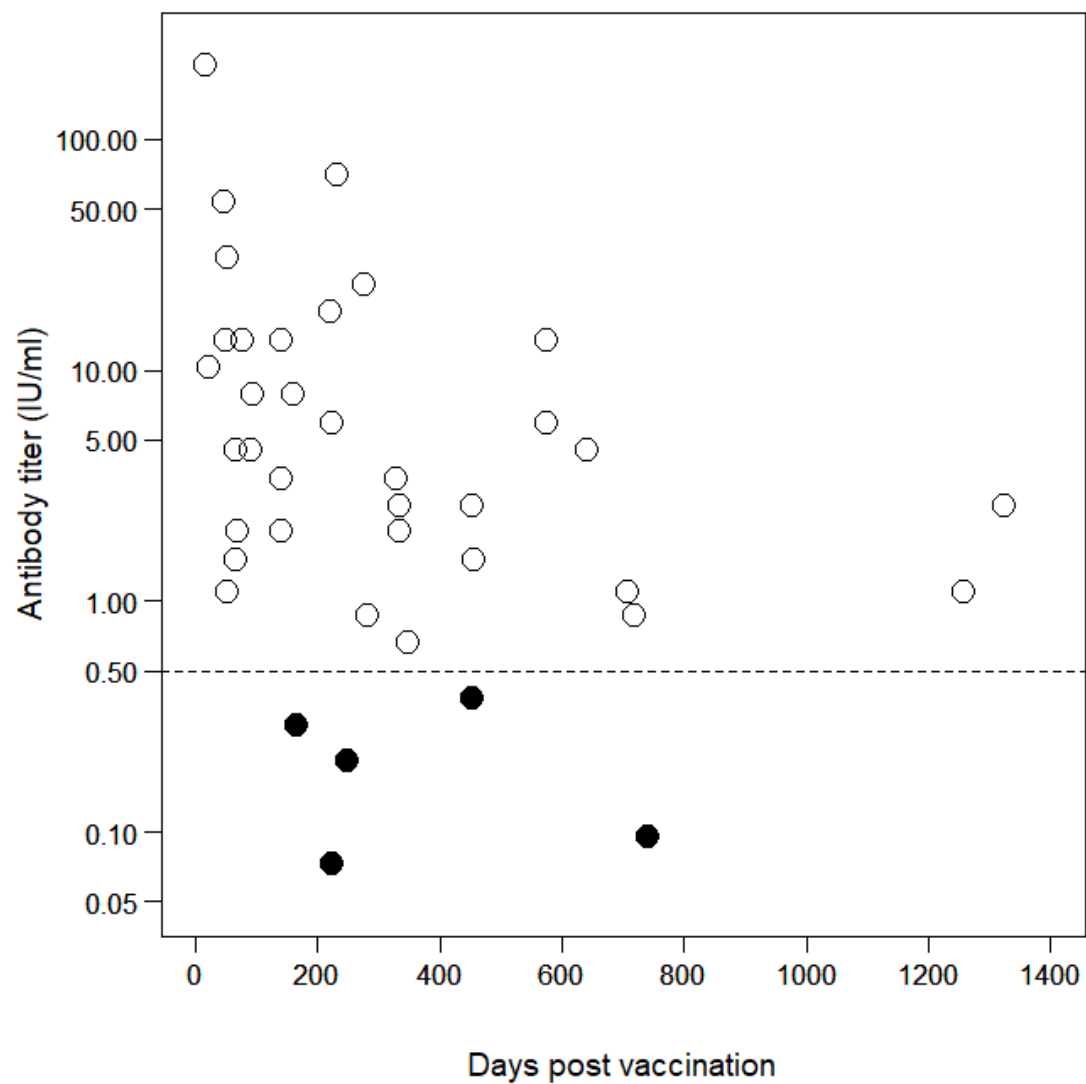


Figure 5. Antibody decline in multiple-vaccinated dogs ($n = 39$). Open circles represent samples that had antibody titers ≥ 0.5 IU/mL. Filled circles represent samples that had antibody titers < 0.5 IU/mL. The dashed line shows the antibody titer's threshold level (0.5 IU/mL) required for international dog movement.

GENERAL CONCLUSION

Controlling rabies in humans and dogs in rabies endemic African countries greatly relies on success in canine vaccination. Therefore, designing and planning effective canine vaccination program based on the information of dog demographics, conducting the program, and assessing achievement and progress of the program are all steps for successful rabies control and prevention.

The study presented in chapter I is the first report on rural dog demographics and canine vaccination coverage attained by conducting a free mass vaccination campaign in Zambia; it also provides an estimate of the ownerless dog population in the rural part of Zambia. This study indicated that the number of ownerless dogs was quite low compared with the number of owned dogs in a rural setting in Zambia. Thus, there is a potential to control rabies through canine mass vaccination campaigns targeted at owned dogs, although the first mass vaccination campaign attained only low vaccination coverage. To achieve the 70% coverage recommended by WHO, we propose including puppies younger than three months old in rabies vaccination programs. Although puppies are currently not included in rabies vaccination in Zambia, the puppy population is not negligible and would be necessary to attain the 70% coverage and obtain the maximum outcome of rabies mass vaccination. This study also suggests that increasing education on rabies and its control, responsible dog ownership, good dog handling, and mass vaccination campaigns are necessary for dog owners to achieve a higher vaccination coverage. Moreover, better advertising to and education of the community (particularly the key community leaders such as local chiefs, teachers, and others) on the importance of rabies and responsible dog ownership cannot be overemphasized to ensure the promotion and sustainability of the rabies mass vaccination campaigns. Furthermore, our

study re-emphasized that regular annual mass vaccinations are necessary to secure owners with vaccination opportunities and to maintain protective herd immunity among dogs. In chapter I, the study highlighted the potential for controlling rabies in rural parts of Zambia and identified key issues that require attention for the success of future rabies campaign/control programs.

In chapter II, the study demonstrated the vaccination coverage and actual immunization coverage in the owned dog population in Lusaka District, Zambia. Although the estimated vaccination coverage based on vaccination certificates' validity was low, the actual immunization coverage was moderately acceptable to confer herd immunity against rabies. This discordance was attributed to owners' improper storage of vaccination certificates for their dogs. Therefore, it is important to continue providing information and education on responsible dog ownership to dog owners to promote effective rabies control in dogs in Lusaka and Zambia. This study further verified that a single vaccination with certified vaccines could induce protective antibodies up to 180 dpv; however, regular boosters are necessary to enhance and maintain protective antibody titers and improve herd immunity. The data presented in chapter II will further strengthen the execution of rabies control programs in Zambia and other rabies-endemic countries and contribute to achieving the goal of the "Zero by 30" global strategic plan for rabies control.

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和 文 要 旨

狂犬病は古くから最も恐れられる人獣共通感染症の一つであり、狂犬病による死亡者は世界で年間約 59,000 人と推定される。発生の大部分はアジアおよびアフリカ地域であり、これらの地域における人の狂犬病の大部分が犬によって媒介されることから、その制圧には犬における狂犬病制御が必須である。犬の狂犬病制御のためには犬の集団ワクチン接種が重要とされ、犬集団における狂犬病ウイルスの伝播を防ぐためには集団の 20–45%が常に免疫されていなければならない(限界ワクチン接種率)。狂犬病が流行するアジア・アフリカ地域で一般的に年一回実施される集団ワクチン接種においては、経験的に 70%のワクチン接種率を達成することが目標とされてきた。これはアジア・アフリカ地域の犬集団では高い死亡率および出生率、移入・移出により集団を構成する個体が短期間で入れ替わり、結果として集団免疫率が急速に低下することに起因する。個体の入れ替わりにより翌年の集団ワクチン接種までに集団免疫率が限界ワクチン接種率を下回ることを防ぐために、集団ワクチン接種時には高い接種率を達成しておく必要がある。しかしながら、国や地域によって犬集団の個体群動態の特徴は様々であり、60–70%の接種率で狂犬病の流行を制御できる集団もあれば、制御できない集団もある。よって、限られた資源を有効に利用し効果的・効率的に狂犬病を制御するためには、地域の犬集団の個体群動態・人口統計に基づいたワクチン接種計画を立案する必要がある。さらに、実施されたワクチン接種によって達成された接種率の評価に加え、集団免疫の形成を左右するフィールドにおけるワクチンの有効性を評価することは、狂犬病流行国における効果的・効率的な狂犬病制御に資する。こうした犬集団の個体群動態・人口統計やワクチン接種率・抗体保有率の評価は、アフリカ諸国ではこれまでにタンザニア、ケニア、チャド、マリ、マラウイ、南アフリカ、ナイジェ

リアなどの国々で報告がある。犬が媒介する人の狂犬病の長期的な制御を成功させるには地域・大陸レベルでの協調した制御対策が必要とされる。そのため、アフリカ諸国における狂犬病制御を成功させるには、各国において対策立案に必要な知見を収集し、国内はもとより汎大陸規模での制御対策を講じなければならない。本研究では、アフリカの狂犬病常在国であるザンビア共和国を対象とし、既存の知見では不足していた、人および犬の狂犬病制御を成功させる鍵となるこれらの項目（犬の個体群動態・人口統計、達成可能なワクチン接種率、ワクチンの有効性）について評価するための調査・研究を行った。

アフリカで発生する狂犬病の約 75%以上が農村地域で発生していると推定される。このため第一章では、ザンビアの農村地域における飼育犬集団の人口統計学的特徴を明らかにし、飼い主のいない野犬の頭数を推定した。また、農村地域で無料集団ワクチン接種を実施することで達成可能なワクチン接種率を調査した。その結果、対象地域の飼育犬集団の 29%が 1 歳以下の若い個体であり、うち 57.5%(全体の 16.7%)がザンビアでは狂犬病ワクチン接種の対象とならない 3 か月齢未満の子犬であった。一方、野犬の頭数は飼育犬の頭数に比較してごくわずかであると推定され(野犬:飼育犬比として 0.01–0.06)、飼育犬を対象としたワクチン接種によって地域の犬集団の集団免疫を維持することが可能であることが示された。しかしながら、対象地域で実施した初回の無料集団ワクチン接種では、飼育犬集団におけるワクチン接種率は 20.9–52.6%(野犬も含めた対象地区の犬集団全体では 19.8–51.6%)と推定された。アンケート調査の結果から、飼い主へ集団ワクチン接種の開催情報が行き届かなかったこと、飼い主が当日不在であったこと、飼い主が飼い犬を取り扱えなかったことが要因として挙げられた。しかしながら、すべての世帯に広告を配布したうえで実施した追加集団ワ

クチン接種によって達成された最終的なワクチン接種率は 57.9–77.8%(犬集団全体では 54.8–76.2%)であり、一部地区では目標とする 70%に到達しなかった。このことから、犬のワクチン接種の重要性を飼い主に広く認識させるために、地域の関係機関を巻き込んだ普及啓発の必要性が示唆された。加えて、現時点ではワクチン接種の対象となっていない 3 か月齢未満の子犬に対するワクチン接種の必要性も示唆された。

一方、大きな一つの連結した高密度の集団を形成する都市部の犬集団では、集団免疫の空間的不均一性が犬集団における狂犬病の流行を拡大・長期化させる。そのため、都市部における狂犬病制御のためには、都市全体を対象とした戦略的なワクチン接種計画の樹立が重要である。第二章では、首都ルサカ(ルサカ郡)の飼育犬集団を対象としてワクチン証明書に基づくワクチン接種率および血中中和抗体価に基づく抗体保有率を推定した。加えて、証明書の情報と測定した血中抗体価をもとに、ワクチン接種犬における抗体価の経時的推移を遡及的に評価し、ザンビアのフィールドにおけるワクチンの有効性について検証した。EPI クラスタ調査法に基づき訪問されたルサカ郡の犬飼育 200 世帯で血液採取に成功した 251 頭の犬のうちワクチン証明書が有効期限内であった犬は 19.9%(50/251)であった。一方、42.2%(106/251)の犬が狂犬病ウイルスに対する 0.5 IU/mL 以上の中和抗体を保有していた。また、52.6%(132/251)の犬が 0.2 IU/mL 以上の中和抗体を保有していた。世帯訪問時に血液採取ができなかった 115 頭についてワクチン未接種かつ抗体陰性と仮定し、ルサカ郡の飼育犬集団全体における最小ワクチン接種率および最小抗体保有率の推定を行うと、最小ワクチン接種率 13.7%(95% CI: 8.7–18.6)、閾値を 0.5 IU/mL とした場合の最小抗体保有率 29.0%(95% CI: 22.4–35.5)、閾値を 0.2 IU/mL とした場合の最小抗体保有率 36.1%(95% CI: 29.1–43.0)であった。また、血中抗体価とワクチン証明書から遡

及的に評価した 0.5 IU/mL を閾値としたワクチン成功確率は市販ワクチン 1 回接種群では 78.6% (95% CI: 49.2–95.3)、複数回接種群で 89.3% (95% CI: 71.8–97.7) であった。閾値を 0.2 IU/mL とした場合のワクチン成功確率は、1 回接種群で 85.7% (95% CI: 57.2–98.2)、複数回接種群で 96.4% (95% CI: 81.7–99.9) であった。上記の結果から、ルサカ郡の飼育犬集団は狂犬病流行を阻止するために必要な集団免疫を概ね維持していると考えられた。市販ワクチン 1 回接種群でも、犬において狂犬病発症を阻止できるとされる 0.2 IU/mL 以上の抗体価を約 86% の個体が保持したことから、ルサカ郡で広く使用される市販ワクチンのフィールドにおける有効性が示された。一方、集団免疫を維持するための追加免疫 (Booster) の重要性もあわせて示唆された。

本研究では、ザンビア共和国の農村部および都市部のそれぞれにおいて効果的・効率的な狂犬病対策を計画・実施する上で重要な知見を提供した。現在、世界保健機関、国際獣疫事務局、国際連合食糧農業機関および the Global Alliance for Rabies Control が協同で推進する「Zero by 30: the global strategic plan」が掲げられ、2030 年までに犬によって媒介される人の狂犬病の死者をゼロにするという取り組みが世界的に進められている。本研究によって得られた知見が、ザンビアおよび世界における“Zero by 30”の達成に寄与することが期待される。