Modelling canine rabies elimination in India through mass dog vaccination

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Declaration of originality

I hereby declare that this thesis is my own work. Plans of fieldwork and analyses were formulated with support from my supervisors – Professor Christl Donnelly, Dr. Pierre Nouvellet and Dr. Abi Tamim Vanak. Dr. Reeta Mani, Additional Professor at the National Institute of Mental Health and Neurosciences, Bengaluru, India helped to analyse canine serum samples for rabies virus neutralizing antibodies and provided valuable insights on rabies immunological dynamics. I analysed the data and discussed the results with my supervisors.

Copyright declaration

Abstract

The 'Zero by 30' campaign aims to globally eliminate dog-mediated human rabies deaths by 2030. Theoretical and empirical studies have shown that annual mass rabies vaccination (MRV) campaigns that vaccinate at least 70% of the dog population in an area can effectively control canine rabies outbreaks and eventually eliminate it. Achieving such coverages in free-ranging dog (FRD) populations, the main source of human infections in rabies-endemic regions, can be a major challenge where most FRDs are unowned and so not easily accessible for vaccination. Despite bearing the largest burden of human rabies deaths globally, few studies have explored the population characteristics of FRDs in India in the context of rabies elimination, particularly accessibility for vaccination. Similarly, there are limited studies of dog ownership practices (DOP) relevant to rabies control in India.

We conducted a longitudinal field study over 16 months in a cohort of unowned dogs (UDs), semi-owned dogs (SODs) and ODs at an urban (human population of 240991 individuals) and a semi-urban (25861 individuals) site each in Kerala, south India. The study gathered data on dog population characteristics, DOP and pre- and post-vaccination rabies virus neutralizing antibody (RVNA) dynamics. In round 1 (R1) pre-vaccination blood samples were collected from all dogs, after which they were vaccinated against rabies, collared and microchipped where necessary and released. Data on demographic characteristics (sex, age, body condition etc.) and DOP were also collected. As many dogs as possible from this cohort were recaptured at approximately ~30 days (R2), ~150 – 180 days (R3) and ~365 days (R4) after first capture to collect post-vaccination blood samples. All serum samples were tested to assess post-vaccination RVNA titre dynamics and rates of decline. These data were used to parameterise an age-structured deterministic compartmental Susceptible-Exposed-Infectious-Vaccinated (SEIV) model incorporating assumptions about accessibility for vaccination. The model was used to assess the impacts of varying various demographic, immunological and MRV campaign parameters on prospects of rabies elimination within 20 years of campaign implementation.

In R1, 577 dogs across all ownership categories were captured. Only 12% of FRDs were owned, with about 60% of ODs in R1 being free-ranging. Only 29% of ODs were vaccinated against rabies.

Approximately 26% (95% CIs: 22 – 31%) of all dogs sampled in R1 had RVNA titres ≥ 0.23 IU/ml. Mixed-effects logistic regression models found higher recapture probabilities for sterilised dogs and lower probabilities for UDs, dogs from the semi-urban site and those with pre-vaccination RVNA titres ≤ 0.5 IU/ml or no detectable titres. Over 80% of dogs recaptured in R2 had titres > 0.5 IU/ml, irrespective of age or vaccination history. Mixed-effects linear regression models identified significant associations between post-vaccination RVNA titres and age at vaccination, sterilisation status and RVNA titre levels in R1. Titres were estimated to drop below 0.5 IU/ml approximately 200 days (95% CI: 167 – 256 days) after achieving post-vaccination peak levels. However, titres declined at a faster rate for ODs and completely/partially confined dogs compared to dogs without owners and completely FRDs. We also found evidence suggesting the occurrence of non-lethal rabies infections in FRDs. The SEIV model indicated that as accessibility for vaccination increased, rabies elimination was possible in a wider range of scenarios within shorter timeframes, generally within 10 years of implementation of vaccination campaigns, and required lower vaccination coverages. Where ≤ 20% of dogs were accessible, campaigns needed to consistently vaccinate > 95% of dogs for > 20 years to eliminate rabies. Rabies elimination was possible in most scenarios, typically with annual campaigns, even with < 70% effective vaccination coverages in the total dog population. The model also highlighted the complex interplay of demographic factors and disease transmission, with high birth rates resulting in higher rabies cases, irrespective of juvenile mortality or adult lifespan.

Mass rabies vaccination continues to be the most effective rabies control method; however, the implementation and frequency of MRV campaigns must account for varying accessibility of FRD populations and consider variations in demography and immunological dynamics. Rabies control in India will require a multi-pronged approach incorporating more responsible dog ownership, access to veterinary care, effective MRV and dog population and waste management, while ensuring the use of properly stored, high-quality vaccines and where necessary, the use of alternative vaccination methods such as oral vaccines to access as many dogs as possible.

For TMS & SH,

the loves of my life

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As one approaches the end of a long journey and begins to take stock of all that has happened, it is quite a task to acknowledge all the people who have been encountered along the way. Inevitably, one forgets the contributions of some people. As I say my thanks here, I too will have forgotten some individuals. If you are one of them and happen to read this section, please accept my apologies for the omission. When I read this section at some point in the future (which I'm sure I will), I want to be able to remember as many as possible of those people who accompanied me on this PhD journey, and as many details. Hence the length of this section.

This has been a long and difficult journey. While all PhDs are necessarily so, the COVID-19 pandemic compounded the challenges of the PhD experience by making it nearly impossible to discuss ideas, pick brains and more importantly, live a 'normal' life. It's at times like these that one realises just how much one takes so many things for granted.

It is also at times like these that one begins to appreciate the value of support networks and of supportive supervisors. All PhDs are the product of several people coming together to support the generation of new knowledge. But supervisors have perhaps the most important role in helping a wannabe researcher kick-start a career in research and academia, and maybe even decide to stay on. I have often heard other PhD candidates talk of how they wished their supervisors were more available and supported their research. I can safely say I didn't have that problem. I couldn't have asked for more supportive and encouraging individuals to hold my hand over the course of these past four years. It has been an honour and a privilege to work with Professor Christl Donnelly, Dr. Pierre Nouvellet and Dr. Abi Tamim Vanak. Thank you Christl for being who you are. The cliché 'Never meet your heroes' certainly never applied to you. Pierre's sarcastic humour is something to experience (although it takes something special to trigger it!) and he is always great company. Abi and his great field team in Baramati and Bengaluru, including the two Abhijits at either location, have taught me so much, not least how to work hard and play hard. I look forward to working closely with all of you in the future.

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I've had a whale of a time as a student at DIDE / MRC GIDA, a unit that has probably become quite famous around the world for its work during the ongoing pandemic. I've learnt so much from engaging with the research here, talking to people working on a range of topics and delving into complicated statistical methods that I would probably have scoffed at the prospect of ever engaging with while training as a vet oh so long ago. It's been quite something experiencing, almost from the frontlines, the scientific response to a global pandemic and witnessing the impact it can have on peoples' lives.

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Finally, I would like to dedicate this thesis to all the working parents, people with caring responsibilities and those who have struggled to make ends meet over the course of the pandemic. I hope your struggles are worth it in the end.

Relevant publications

1. Radhakrishnan S, Vanak AT, Nouvellet P, Donnelly CA. Rabies as a Public Health Concern in India—A Historical Perspective. Trop Med Infect Dis. 2020;5: 162. doi:10.3390/tropicalmed5040162

Presentations

1. Poster presentation: 'Low levels of ownership and human interaction of free-roaming dogs and poor dog ownership practices pose significant challenges for effective canine rabies control in India. The 6th World One Health Congress 2020, Edinburgh, October 30 - November 3, 2020. (Virtual).
2. Oral presentation: Low levels of confinement, sterilisation, rabies vaccination and rabies virus neutralizing antibodies among owned dogs in Kerala, south India. 11th Kerala Veterinary Science Congress, Kerala, India, November 2019.
3. Poster presentation: A question of accessibility: modelling the effects of demography and accessibility for vaccination on elimination of rabies in free-ranging dog populations in India. 16th conference on Ecology and Evolution of Infectious Diseases, Glasgow, May 29 - 31, 2018.

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List of abbreviations and acronyms

ABC – Animal Birth Control

ABC-ARV – Animal Birth Control – Anti-Rabies Vaccination

AHD – Animal Husbandry Department

AIC – Akaike Information Criteria

ALP – Alappuzha municipality

BCE – Before the Common Era

BCS – Body condition score

CE – Common Era

CI – Confidence Interval

COVID-19 – Coronavirus disease

CVD – Civil Veterinary Department

CVR – Catch-vaccinate-release

CVS – Challenge Virus Standard

DOP – Dog ownership practices

DPM – Dog Population Management

DWO – Dogs without owners

ELISA – Enzyme Linked Immunosorbant Assay

FAO – Food and Agricultural Organization

FAVN – Fluorescent Antibody Virus Neutralization

FRD – Free-ranging dog

GLMM – Generalized Linear Mixed Models

GMT – Geometric Mean Titre

GoI – British colonial Government of India

GPS – Global positioning system

HA-HI – Haemagglutination – Haemagglutination Inhibition test

HIS – Human interaction score

HRS – High recruitment scenario

IDRV – Intradermal rabies vaccination

IU – International Units

KAP – Knowledge, Attitudes and Practices

LMM – Linear Mixed Models

LRS – Low recruitment scenario

LSGI – Local self-government institution

ml – millilitre

MLE – Maximum likelihood estimate

MNT – Mouse Neutralization Test

MRV – Mass rabies vaccination

MUH – Muhamma panchayat

NAPRE – National Action Plan for dog-mediated rabies elimination

NIMHANS – National Institute of Mental Health and Neurosciences

NTD – Neglected Tropical Disease

OD – Owned dog

OIE – World Organization for Animal Health

OR – Odds Ratio

PEP – Post-Exposure Prophylaxis

PI – Pasteur Institute / Principal Investigator

R1 – Round 1 etc.

RFFIT – Rapid Fluorescent Focus Inhibition Test

RIGs – Rabies immunoglobulins

RVNA – Rabies Virus Neutralizing Antibodies

SD – Standard Deviation

SEIV – Susceptible – Exposed – Infectious – Vaccinated

SOD – Semi-owned dog

UD – Unowned dog

UK – United Kingdom

USA – United States of America

USD – United States Dollars

WHO – World Health Organization

Chapter 1 : Introduction

A section of this chapter was included in a manuscript published in *Tropical Medicine and Infectious Diseases* [1]

1.1 Background

1.1.1 Rabies

The World Health Organization (WHO) identifies neglected tropical diseases (NTDs) as a group of communicable diseases affecting over a billion people globally, primarily those living in poverty in low- and middle-income countries in the tropics and sub-tropics [2], imposing a significant economic burden on these countries. This list includes rabies, a viral infection caused primarily by the bite of infected hosts belonging to the mammalian order Carnivora (although all mammals, and in exceptional circumstances birds [3], can be infected), and less frequently the deposition of saliva on wounds or mucous membranes. In addition, it has the highest mortality rate of all known infectious agents, with nearly all individuals who develop clinical symptoms eventually dying [4]. Approximately 59000 annual human deaths are estimated to occur globally due to rabies, mainly through dog bites [5]. While bat-transmitted rabies continues to be a major concern particularly in countries in Latin America [5], terrestrial rabies has been eliminated (or historically been absent) in Western Europe and several island nations such as Japan, Australia and New Zealand (except for imported cases). In North America, domestic animal rabies in dogs and cats occurs infrequently, mainly through exposure to infected wildlife reservoir hosts [5]. However in most rabies-endemic countries in Africa and Asia including India, domestic dogs, particularly free-ranging dogs (FRDs) are the main reservoir and source of human exposure, with children and people living in rural areas comprising a large proportion of those affected [5,6].

1.1.2 Control of rabies

Since FRDs are the primary rabies reservoir in endemic regions, campaigns to reduce the burden of human rabies must ultimately target control efforts at FRD populations [7]. Historically, rabies control in animal reservoirs has been attempted by reducing reservoir host populations, primarily by indiscriminate culling, such as of foxes in Europe [8,9] and dog populations globally [10–12]. More recently, these have been through attempts to reduce breeding of dogs by sterilisation or animal birth control (ABC) [13,14]. This strategy has been based on an assumption that rabies transmission in reservoir species is density-dependent, and that density reduction would thus result in fewer transmission events between infected and susceptible animals [8,15]. However, density reduction has frequently failed to reduce or eliminate rabies in endemic regions, and an analysis of previous efforts at density reduction has suggested that rabies transmission in host populations is only weakly density-dependent, if at all [15]. Density reduction through culling has even been shown to be counter-productive to control efforts, as indiscriminate culling in areas of high demand for dogs can result in uncontrolled human movement of dogs between regions [16]. This can result in the unintentional reintroduction of rabies into areas where culling has taken place, undermining control efforts [16]. Nevertheless, mass culling and/or ABC are still used as part of control efforts in many parts of the world [17,18].

On the other hand, mass rabies vaccination (MRV) of reservoir species has proven successful in control and elimination of rabies [5]. This is exemplified by the success of Western Europe in eliminating rabies in fox populations through extensive oral vaccination campaigns [19] and the elimination of dog rabies in Japan [20] and Latin America [21]. The WHO now recommends MRV of dogs as the primary means of control in rabies-endemic countries, with ABC use only to supplement the use of MRV [5]. To this end, the WHO in collaboration with the Food and Agricultural Organization (FAO) and the World Organization for Animal Health (OIE) established the ‘Zero by 30’ campaign with a goal of eliminating dog-mediated human rabies deaths by 2030 [22]. In Latin America, this goal was achieved through

systematic implementation of coordinated MRV campaigns across the region over three decades [21], although this progress has been jeopardized by disruptions caused by the coronavirus disease (COVID-19) pandemic [23,24].

Empirical and theoretical studies have shown that vaccinating at least 70% of the dog population in a region, with follow-up annual vaccination campaigns, can lead to local elimination of rabies [25,26] and reductions in the number of human rabies deaths [27]. However, patchy vaccination coverage where even small areas achieve sub-optimal coverage, is predicted to compromise efforts to eliminate rabies over a wider region [17]. Mass vaccination alone has also been shown to be more cost-effective than in combination with host density reduction through ABC campaigns in controlling dog rabies and when compared to human post-exposure prophylaxis (PEP) for reducing human rabies deaths [28].

Several factors influence the choice of method to be used in MRV campaigns, including the size of the dog population, demographic characteristics and the proportion of dogs that are owned [29]. Studies of local FRD ecology and demography are thus critical in informing the design and implementation of regional vaccination campaigns [5]. Reliable estimates of the dog abundance and density are necessary for determining whether target vaccination coverages have been achieved. The method used to estimate population size can depend on time and resources available and the required accuracy of estimates, and these have been summarised by Belo et al. (2015) [30]. Human:dog ratios have been used when no other reliable information is available [31], but such estimates may be affected by the quality of human census data and can vary widely. Census methods such as direct counts can be expensive and time-consuming, particularly when carried out over large areas, and do not account for heterogeneity in detection probabilities [30]. A number of capture-recapture techniques, used widely in wildlife population estimation, are able to account for such heterogeneities [32] and have been used to obtain dog population estimates in India [33]. Photographic mark-resight surveys, which use physical markers captured through photographs to identify individual dogs [34], have been used in India [35] and Bhutan [36]. Tiwari et al. (2018) identified the use of the Application

"SuperDuplicates" online tool as the method requiring the least inputs to reliably estimate dog populations in a village in central India, requiring data collected from just two surveys over consecutive days [37].

Demographic studies of FRD populations have found that most dogs have short lifespans of about 3 years [38,39]. Young pups under one-year of age comprise nearly 30% of the population [38], with a majority failing to survive to adulthood [31]. Such high turnover rates have direct effects on the maintenance of herd immunity after MRV campaigns, necessitating more frequent campaigns [5]. However, rabies vaccination has also been shown to reduce mortality from all other causes in dogs [40]. Thus, rabies vaccination may serve to improve lifespans in dog populations and aid in the maintenance of herd immunity, facilitating control efforts.

One of the key concerns in targeting FRD populations for MRV has been whether enough dogs are accessible for vaccination to achieve target vaccination coverages [7]. Studies of dog demography and ecology in countries in Africa [39], Latin America [38] and Asia [41] have found that a majority of dogs in these regions are owned or have a human caretaker, thus making them accessible for vaccination. Indeed, when accounting for political and organisational factors, the high levels of dog ownership may partly have facilitated the success of MRV campaigns in Latin America. A systematic review of the literature on dog demography and rabies control in Africa also reported that most dogs in Africa were owned [39]. A survey of the dog population in a region of Sri Lanka found that only ~20% were ownerless [42].

Based on an understanding of the dog population in an area being targeted for vaccination, strategies for maximising coverage can include one or a combination of central point vaccination, household-based campaigns, mobile units or capture of FRDs using nets or cages [29]. Which strategy is used can influence the costs of vaccination campaigns, owner participation and thus success in achieving target coverages. For instance, a review of studies analysing vaccination coverages after MRV campaigns reported that providing vaccination free of charge resulted in coverages closer to recommended levels

than when dog owners were charged a fee for vaccination [39]. Owner access to vaccination points [43] and the presence of incentives [44] can also influence participation in campaigns. Regions with a larger proportion of unowned FRDs may require the deployment of mobile units to capture dogs by net or cages, increasing the time, resources, effort and thereby costs required to achieve target coverages in the shortest time possible [29].

A variety of methods have been used to mark vaccinated dogs to enable calculation of post-campaign vaccination coverages. These include the use of paint marks [45,46], collars [44,46] and cable ties [47]. A number of studies have reported achieving 70% vaccination coverages in these dog populations [39,48–50], using different methods to estimate coverage. Sambo et al. (2018) reported that although post-vaccination transect surveys were able to provide cost-effective and timely estimates of coverage than school-based or household surveys, they tended to overestimate vaccination coverage by up to 10% [51]. In another study, vaccinated FRDs in India marked with paint were identified through post-vaccination transects on a motorbike to estimate vaccination coverage [45]. Such a method is highly likely to overestimate coverage since it fails to account for heterogeneities in detection probabilities and is thus based on unreliable dog population estimates.

A further source of uncertainty when implementing MRV campaigns has been whether vaccinated dogs will develop adequate antibody titres, particularly if malnourished or they have concurrent illnesses. The WHO recommends that dogs of all ages are vaccinated during MRV campaigns [5]. A study of dog populations from Indonesia and South Africa found that most vaccinated dogs developed adequate post-vaccination rabies virus neutralizing antibody (RVNA) titres [48] (defined as at least 0.5 International Units (IU)/ml of serum [52]). Adequate immunity was also developed by vaccinated pups under three months of age, contrary to concerns that maternally derived antibodies may interfere with development of active immunity [53]. On the contrary, a single dose of rabies vaccine was reported to be inadequate in generating protective immunity lasting up to a year in a high proportion of unowned dogs and in previously unvaccinated owned pups and juveniles below one year of age in

Sri Lanka [54]. Development and maintenance of adequate RVNA titres can be influenced by a range of intrinsic (e.g. age, breed, sterilisation status, individual variation etc.) and extrinsic factors (e.g. vaccine quality, time of post-vaccination blood sampling, appropriate vaccine storage etc.). These factors are discussed in greater detail in chapter 4.

1.1.3 Rabies in India

India has the dubious distinction of bearing the largest burden of at least 11 of the NTDs identified by the WHO [55], including rabies. Of the 59000 annual human deaths estimated to occur globally due to dog-mediated rabies, about 35% occur in India [5]. Over three-quarters of cases in India occur in rural communities with poor access to diagnostic facilities and PEP which are key to preventing development of disease [6]. More than 95% of cases are caused by dog bites, largely because of the approximately 60 million stray/FRDs in the country [31], and many cases of human rabies go undetected or, are mis-diagnosed [56]. A significant proportion of cases are children and despite the availability of safe and effective vaccines, awareness of and access to PEP, including rabies immunoglobulins (RIGs), continue to be poor [57].

Despite the high burden of human rabies in India, the disease is not notifiable and a structured surveillance system is yet to be put in place [56]. Rabies is also not included in the list of diseases for which surveillance is routinely carried out by states and reported under the Integrated Disease Surveillance Programme of the Indian Ministry of Health and Family Welfare [58]. Instead, dog bites and snake bites are to be reported separately (Diseases under surveillance: Presumptive (P form)) [58]. The absence of an organized national or regional system for rabies surveillance compounds the problem of poor availability of human and animal rabies incidence data [59]. Current estimates of the burden of rabies in India (21000 human deaths annually (95% confidence interval (CI): 17000 - 24000)) are based on an epidemiological study conducted in 2003 [6], and even this may be an underestimate of the true disease burden. Another study estimated that 12700 human deaths from symptomatically identifiable furious rabies occurred in India in 2005 [60]. Most recently, a multicentric survey

conducted in 2017 across seven Indian states estimated an annual incidence of animal exposures (bite, scratch or lick from an animal irrespective of its rabies status) of 1.26%, which was reportedly lower than previous estimates from India [61]. However, the authors acknowledged that, owing to the limited scale of their study, results could not be used to generate a country-level burden of potential rabies exposures.

A review conducted in 2013 of research articles on rabies from Indian institutions published during the period 2001 – 2011 identified a dearth of studies that were relevant for policy formulation, with most publications based on laboratory-based and biomedical research on topics such as virology and vaccine development [62]. Such a disconnect between priorities of policy makers and researchers has implications for informing disease control efforts in India [63]. A 2012 systematic review on dog ecology and post-campaign vaccination coverages did not include any reports from India [38]. Lack of knowledge on dog demography and ecology and impacts of interventions on rabies were identified as some of the key knowledge gaps affecting formulation of control policies [62].

Since the publication of these reviews, a number of studies exploring topics such as FRD demography, ecology and reproductive behaviour [37,64–66], public awareness about rabies [67,68], mathematical models of rabies control [28] and dog population management (DPM) through ABC [69], evaluations of various strategies for rabies control [45,70–72] and the history of rabies as a public health concern [1] in the Indian context have been published. These and previous studies [33,73–78] provide valuable information on the factors regulating FRD populations and have highlighted some of the challenges in implementing MRV as a cost-effective tool for rabies control in India [47,69].

Based on information gathered from disparate publications on dog demography, Gompper (2014) estimated the dog population in India to be approximately 60 million [31]. Reviews of global dog ownership trends have identified the lowest levels of dog owning households [31,38] and the highest proportion of unowned dogs [74] from India. The dog population distribution is likely to be highly heterogenous and influenced by socio-economic factors and human population densities [5]. A

number of studies estimated human:dog ratios of 18:1 in rural Karnataka [75] and 36:1 in rural Maharashtra [33], the latter being one of the highest reported for rural regions globally [31]. The study from Maharashtra used a photographic capture-mark-recapture methodology to estimate dog populations, finding that adults comprised between 67 and 72% of the dog population in five villages [33]. This study also identified the problematic issue of 'ownership' of dogs in Indian villages, using the term 'reference persons' instead for individuals who provided for FRDs but would deny any sort of ownership. Indeed, in contrast to FRDs in African countries which are presented at vaccination camps by 'owners', such reference persons may not necessarily be able to handle these dogs or present them for vaccination [47]. Populations of feral dogs, which have very little to no direct contact with humans, are also known to occur in Sikkim [79], Maharashtra (Abi Vanak, personal communication) and Kerala (Sreejith Radhakrishnan, personal observation). Nearly half of all female dogs captured during ABC campaigns in north India over eleven years were pregnant, with most pregnancies found during the latter half of the year. Expected lifespans in this population was approximately 3.6 years at one year of age [78].

Rabies control efforts have been implemented in a number of states in India in recent years, mostly involving collaborations between international charities and state or local governments [45,79–81]. These have reported success in vaccinating large segments of the dog population, with subsequent reductions or elimination of human rabies deaths, mainly in two of the smallest Indian states – Sikkim (human population of 610,000 , approximately 41000 dogs) [79] and Goa (human population of 2.4 million, approximately 150,000 dogs) [5]. Across the rest of the country, rabies vaccination of the FRDs occurs mainly during ABC campaigns [82], which have been implemented to varying levels across the country [18]. It was only on September 28th, 2021 on the occasion of World Rabies day, that the Government of India launched its first National Action Plan for dog-mediated rabies elimination (NAPRE) by 2030 [83].

Studies within the regional context of the complex interactions between dog demography and disease ecology, physiological responses to vaccination and socio-economic factors that drive human behaviour towards dogs in rabies endemic regions are critical to facilitate precision public health interventions for disease control and elimination [5,84]. Notwithstanding the lack of prioritisation and resourcing of rabies surveillance, it is precisely the lack of an understanding of these complex interactions that hinder the formulation of appropriate rabies control strategies in India [62].

1.1.4 Mathematical models of rabies

Mathematical models are powerful tools to explore hypotheses about the epidemiology of infectious diseases, to evaluate the impact of interventions for disease control and to identify gaps in knowledge about disease transmission [85]. They are particularly useful when demographic and/or disease incidence data are not available to explain disease transmission processes and the impacts of control strategies. In this regard, mathematical models can play an important role in informing public health policy, especially in the case of emerging infections or NTDs for which surveillance data may be sparse or absent.

The first rabies transmission models were developed to explore the feasibility of strategies to control fox rabies in Europe [8]. Subsequent modelling studies helped to elucidate the range of complex factors influencing wildlife rabies transmission such as seasonal births [86] and geographical features of host habitats [87]. The insights from these studies informed the development of canine rabies transmission models.

A range of mathematical models have been used to explore rabies transmission dynamics in a variety of settings. These include compartmental models, individual / agent-based models, metapopulation models, network models as well as branching process models [88]. Of these, compartmental models have been the most commonly used type of model. More recent phylodynamic studies have combined epidemiological data with phylogenetic sequence data from circulating rabies virus strains to provide greater insights into mechanisms influencing infection persistence and spread [89]. Symptomatic

rabies infection is generally considered to be inevitably fatal. Due to this reason, canine rabies transmission models are comprised of three disease classes – Susceptible, Exposed (also referred to as Latent) and Infectious. Using this SEI framework, Coleman and Dye (1996) provided support for of the need for a minimum of 70% vaccination coverages to prevent rabies outbreaks [25]. The incorporation of rabies vaccination gives rise to a fourth class (Vaccinated), resulting in the general SEIV framework, which has been used extensively to explore the use of canine vaccination as a rabies control measure.

Mathematical models of rabies transmission have also been used to highlight fundamental aspects of the disease transmission process in dog populations. One of the key insights provided by models was the low basic reproduction number (R_0) for rabies [17], generally taking values between 1 and 2 [26]. Models also highlighted substantial heterogeneity in transmission of infection, with a small number of dogs being responsible for a majority of onward transmission events [26]. In addition to transmission through bites from infected hosts, human-mediated movement of dogs was also shown to contribute to rabies spread [90,91]. Demographic factors, particularly birth rates, have also been shown to influence rabies transmission dynamics [92,93]. Frequent introductions of rabies infection have been demonstrated to be important for disease maintenance, particularly in urban areas [89,90]. More recently, given the increasing body of evidence suggesting the occurrence of non-fatal rabies exposures that may result in naturally acquired immunity against rabies, mathematical models have also been used to explore the potential influence of this phenomenon on outbreak dynamics in dog populations [94].

Modelling studies have been especially critical in building the evidence base for MRV as the most effective rabies control measure [17,26,95]. High turnover in FRD populations has been shown to necessitate vaccination coverages of 70% or more through annual MRV campaigns, contrary to the low coverages that would be expected given the low R_0 values for rabies outbreaks [26]. Additionally, studies have also highlighted that spatially heterogeneous vaccination coverages can reduce the

probability of and increase the time required for rabies elimination, even in the presence of high mean coverages [17,96]. Dog population structure was also shown to influence elimination prospects, with high proportions of FRDs necessitating very high vaccination coverages for elimination [97]. In rabies-free areas, such as Australia, modelling studies suggested that in the event of entry of rabies-infected dogs, vaccination coverages of ≥ 90% of the dog population with high surveillance were required to eliminate the disease [98,99]. Models incorporating cost-effectiveness analyses showed that MRV campaigns were the most cost-effective means of reducing human rabies deaths in India [28].

A key concern that is highlighted in mathematical models of rabies transmission is the incorporation of density-dependent transmission, where disease transmission rates scale with host density [100]. This assumption in inconsistent with empirical evidence suggesting that rabies transmission is only weakly density-dependent, if at all [15]. Accounting for transmission heterogeneity, as well as incorporating spatial structure into models have been suggested as appropriate strategies to capture the dynamics of rabies observed in FRD populations [100].

1.2 Research motivations

There is a dearth of information in the Indian context on FRD populations and aspects of rabies control such as accessibility and response to rabies vaccination. While there is overwhelming evidence for the effectiveness of MRV to reduce the burden of canine rabies and eventually eliminate it, much of the evidence base demonstrating the feasibility of this strategy relies on most FRDs being owned and therefore directly accessible for vaccination. There is limited information in the scientific literature on FRD ownership or the complex factors that can influence rabies control in the Indian context.

This research study was formulated with the aim of collecting data on these aspects, within a One Health framework. These included the demography of FRD populations, dog ownership practices (DOP), factors influencing FRD capture, seroprevalence of RVNA and post-vaccination antibody dynamics, public awareness about risks of rabies exposure and treatment and attitudes towards FRDs.

These data would inform the development of rabies transmission models that would be used to explore the challenges of implementing MRV as a rabies control strategy for FRDs in India.

This necessitated an ambitious research plan of fieldwork to gather multiple streams of data. Fieldwork was conducted over 16 months between October 2018 and January 2020 at one urban and semi-urban study site each in the south Indian state of Kerala, India. The following data were collected:

1. Data on pre- and post-vaccination RVNA titres in FRDs and owned dogs (ODs) – A total of 577 dogs were captured in the field in the first round of the study (R1) and blood samples were collected, after which they were microchipped to aid in individual identification, vaccinated against rabies and immediately released. Blood samples were collected from as many dogs as possible from this cohort in three subsequent study rounds up to one year post-vaccination to explore RVNA dynamics.
2. Data on dog population characteristics – In the course of the above work, data on individual dog characteristics such as age, sex, body condition, sterilisation status, reproductive activity, vaccination history etc. were collected from dogs captured in all study rounds. These data also helped to evaluate the various factors influencing recapture probability of dogs of different ownership categories.
3. Dog demography – Photographic mark-resight surveys were conducted at both sites to gather data on FRD population structure. A total of 33 line transects of one kilometre length, situated at least one kilometre apart, were surveyed over five consecutive days and individual dog characteristics (age, sex, sterilisation status, reproductive status, coat colour) as well as photographs were recorded for as many sighted dogs as possible.
4. Dog ownership practices, public attitudes towards FRDs and knowledge, attitudes and practices (KAP) in the context of rabies treatment and control – Household surveys were conducted in over 300 households across both sites to gather information on these aspects.

These data would inform the design of mathematical models of rabies transmission and control that would be used to explore the challenges of implementing MRV as a strategy for canine rabies control in India.

Owing to time constraints and COVID-19-related disruptions, data from the photographic mark-resight surveys and dog ownership surveys in households could not yet be analysed and are not included in this thesis. Preliminary results of the KAP survey are presented in Appendix A4.

1.3 Structure of this thesis

This thesis is structured as follows:

1. Chapter 2 addresses the historical treatment of rabies as a public health concern in pre-independence (before 1947) India, as well as in the period immediately after independence.
2. Chapter 3 describes population characteristics of FRDs and ODs in the context of rabies control in India, including factors influencing recapture probability in future study rounds
3. Chapter 4 summarises the pre- and post-vaccination RVNA dynamics in FRDs and ODs, presents estimates of the rates of decline in post-vaccination titres and discusses the factors that affect the development and maintenance of titres.
4. Chapter 5 describes the insights gained from an age-structured SEIV deterministic compartmental model parameterised using demographic and immunological data for FRD populations in India, collected from the published literature and using estimates from chapters 3 and 4.
5. Finally, chapter 6 is a discussion chapter that summarises the key findings of this thesis, the limitations of the study and future research.

Chapter 2: Rabies as a public health concern in India – a historical perspective

Work in this chapter formed the basis of a manuscript published in *Tropical Medicine and Infectious Diseases* [1]

ABSTRACT:

India bears the highest burden of global dog-mediated human rabies deaths. Despite this, rabies is not notifiable in India, and continues to be underprioritized in public health discussions. This review examines the historical treatment of rabies in British India, a disease which has received relatively less attention in the literature on Indian medical history. Human and animal rabies was widespread in British India and treatment of bite victims imposed a major financial burden on the colonial Government of India. It subsequently became a driver of Pasteurism in India and globally and a key component of British colonial scientific enterprise. Efforts to combat rabies led to the establishment of a wide network of research institutes in India and important breakthroughs in development of rabies vaccines. As a result of these efforts, rabies no longer posed a significant threat to the British and it declined in administrative and public health priorities in India towards the end of colonial rule; a decline that has yet to be reversed in modern-day India. The review also highlights features of the administrative, scientific and societal approaches to dealing with this disease in British India which persist to this day.

2.1 Introduction

“A bite from a mad dog is more dreaded than any thing I know; which arises from the horribleness of the disease, the uncertainty of the animal’s being mad, or of the infection being received: The not knowing at what period to expect the effects, or to feel confident of having escaped it, keeps the person in a state of cruel suspence (sic) for months, or even years.” - Daniel Johnson, Sketches of Field Sports as followed by The Natives of India with observations on the animals (1822).

A perusal of the Five-Year Plans for national development in independent India (post-1947) covering the period 1951 (when the first five-year plan was unveiled) to 2002 reveals that rabies was never prioritised for control. During this period, the very term 'rabies' appears only twice – once in the fourth plan (1969 – 1974) while listing the diseases for which research was conducted at the Central Research Institute at Kasauli and once in the sixth plan (1980 – 1985) in a brief description of mortality rates of environmentally linked diseases ("Diseases like TB, Gastro-intestinal infections, malaria, filaria, infectious hepatitis, rabbies (sic) and hook worm ……") [101]. The tenth plan (2002 – 2007) mentions the development of a new animal rabies vaccine "being tested for technology transfer", as well as research projects on a number of infectious diseases including rabies, although no further details are provided.

It is only in the eleventh plan (2007 – 2012) that rabies control efforts are first mentioned in the form of pilot projects for the control of human rabies, for which 8.65 crore rupees (~2.1 million US dollars at the time) were allocated. For the first time in a Five-Year Plan, rabies control in animals, ABC and vaccination of stray dogs are also mentioned in this plan, as components of animal welfare to be handled by the Animal Welfare Board of India [101].

In 2014 the Ministry of Health and Family Welfare of the Government of India announced funding for a National Rabies Control Programme as part of the twelfth five-year plan (2012-2017) [102]. This programme is coordinated by the National Centre for Disease Control, New Delhi and the Animal Welfare Board of India, with the aim of halving human rabies deaths by the end of 2017. However, little information is available on the achievements of the programme, which finds no mention on the website of the national Ministry of Health and Family Welfare (https://mohfw.gov.in/) or the NITI Aayog (https://niti.gov.in/) which replaced the Planning Commission of India in 2015. A search for the term 'rabies' on the Open Government Data Platform India (https://data.gov.in) returns no results [103]. The annual budget for 2018 presented by the Finance Minister of India allocated 40 crore rupees (approximately 6.13 million US dollars) for a few pilot schemes under the National Rural Health

Mission, which includes control of human rabies [104]. This amount has been reduced to 25 crore rupees (approximately 3.51 million US dollars) in 2019 [105] and 2020 [106].

Given this background, it is natural to assume that rabies has always been accorded low priority in India. However, a quick glance through the literature on rabies in pre-independence India (before 1947) suggests otherwise. Rabies was one of several 'tropical' afflictions including cholera, plague, typhoid, tuberculosis, polio and snakebites that were viewed as serious medical and public health problems, particularly for British residents in India. Consequently, it was subjected to much research and control efforts by the British colonial Government of India (hereafter referred to as GoI), frequently using native Indians as subjects for experimentation to develop and refine vaccines [107]. The effort to combat rabies and other infectious diseases was instrumental in the establishment of a wide network of research institutes in India and some important breakthroughs in development of rabies vaccines. However, the disease appears to have gradually lost priority in scientific circles and the colonial GoI, which may be the basis for its continued neglect in modern India. In this historical context, underlying reasons for the present-day under-prioritisation of rabies in post-independence India need to be explored, as these may provide insights into what needs to change in order that rabies control in India receives the priority and resources it deserves.

We used the search terms 'rabies', 'hydrophobia' and 'India' to review a range of historical archives, online and physical documents about rabies in pre-independence India (covering the period from the early 1800s to 1947, when India gained independence from British rule, and the few years immediately after). These included articles published in scientific journals (via PubMed and Google Scholar) and popular magazines, historical documents held at the India Office Records and Private Papers of the British Library and the Wellcome Library at the Wellcome Collection, online archives of the Medical History of British India maintained by the National Library of Scotland (https://digital.nls.uk/indiapapers/), British Parliamentary Papers available via ProQuest UK Parliamentary Papers, historical newspapers available via ProQuest Historical Newspapers and

documents available online at the Hathi Trust (www.hathitrust.org) and libraries of the universities of Oxford and Cambridge.

2.2 Rabies documentation in pre-independence India

As one of the oldest diseases known to man, rabies was widely documented by the earliest human civilizations [108]. A disease akin to rabies was recognised in ancient Indian treatises on health and medicine. The Susruta Samhita (Susruta's Compendium) is an ancient Indian text of Ayurveda (written between 1000 BCE and the first or second century CE), the Indian system of traditional medicine still practised in most parts of the country. This text details various medical conditions and surgical procedures and discusses in detail the symptoms of rabies in humans bitten by rabid dogs or wildlife, recognising that once symptoms develop in human bite victims, the disease is inevitably fatal [109]. The Mughal emperor Jahangir (1569 - 1627) is recorded to have noted the symptoms of rabies in an elephant that he owned [110]. It is also highly likely that rabies was documented extensively in the numerous vernacular languages on the Indian subcontinent.

Accounts of British medical and military personnel who worked in India during the 1800s highlight the fact that rabies, also referred to as hydrophobia, was widespread throughout India, responsible for the deaths of numerous Indian, British and European citizens [10,111,112]. The disease also caused extensive mortality in livestock and pet animals such as purebreed dogs owned by British officials [111,113]. These accounts identified the occurrence of large populations of free-ranging ('stray') dogs and to a lesser extent wildlife, predominantly jackals, as the main source of infection [10,111,114]. A collection of observations on life in India by a former surgeon of the East India Company (1822) includes a chapter titled 'Observations on hydrophobia and rabid animals' that describes symptoms in humans in graphic detail [111]. The same chapter and other reports provide detailed descriptions of the progress of rabies in infected pet dogs and wildlife, recounting behavioural changes as symptoms began to manifest [111,113,114]. These symptoms included changes in temperament with increased displays of affection or mis-directed aggression, changes in vocalisation which were often

noticed by Indian caretakers and changes in appetite, varying from voracious consumption of food to eventual rejection. In one instance, a rabid pup that was bitten by a (presumably rabid) hyena interrupted a dance party, resulting in the party having to be broken up and the pup being killed immediately [111].

These accounts also detail the experiences of British military doctors who often had to treat patients with symptoms of rabies and their agony at having to witness progression of the disease and inevitable death [111]. Much effort was put into discovering ways to treat infected individuals and potential modes of treatment, including traditional Indian cures, were keenly discussed in medical circles [112,115,116]. Even at this time, it was well recognised that treating bite wounds as soon as possible after bites occurred was key to preventing disease progression [111,112]. A letter to the editor of The Lancet in 1829 discusses the symptoms of rabies, disputing whether it should also be referred to as 'hydrophobia', and possible ways of treatment including bleeding of patients in India [116]. A booklet on Ayurvedic treatments for various illnesses published in 1876 from Cochin, in present-day Kerala, includes symptoms of rabies and traditional treatment methods for exposure to 'Peppatti visham' (poison from a rabid/mad dog) such as chants, and pills and powders made from plant parts [117]. Various other treatments including Buisson baths [118] and cauterizing wounds with caustic agents (e.g. nitric acid) [112,113] have also been documented. Rabies was also a significant health concern for British military personnel stationed in India, and pensions were given to the family of military personnel who died of rabies contracted in the line of duty [119].

Various aspects of rabies also found their way into Indian and British newspapers and magazines, ranging from individual theories about how the disease occurs ("a disease engendered by the practice in England of docking the tails of so many of our sporting and household dogs.") (1861) [120]; reports of incidents of rabid dog bites [121] and outbreaks in wildlife [122]; descriptions of encounters with rabid dogs, symptoms observed and suggested control measures ('lunar caustic …. apply it well to every wound..') (1859) [123] and an account of a former army officer who claimed to have successfully

recovered from rabies after being exposed in India with a detailed description of his symptoms (1836) [124]. Indian newspapers also reprinted articles about rabies that were initially published in British newspapers [125].

Such news reports and readers' letters to editors make evident that stray dogs, dog bites and rabies were an important public concern, particularly in major cities like Bombay (present-day Mumbai), Poona (present-day Pune), Lahore and Calcutta (present-day Kolkata) where many British and European citizens lived [126–131] and where significant numbers of cases were often reported [132,133]. Public awareness about rabies among British residents would also have been high when rabies was a major threat in Britain during the Victorian era and for a long time after its elimination in 1902 [134]. Complaints about 'mad dogs' in India can be found in letters published as early as 1861 [120]. In addition to humans, purebred pet dogs were frequently infected [126,130] and British residents constantly demanded action from authorities to control rabies and stray dog populations [126,135,136], even proposing that private contributions be used to fund control measures [131]. Such concerns about rabies control also need to be located within discourses of sanitation, hygiene and urban improvement that were emerging in British India since the late 1800s [137]. These discourses were a product of the burden imposed by infectious diseases on British army personnel in India [137], and in rapidly expanding cities like Bombay and Calcutta, where epidemics of plague, cholera, measles and smallpox were frequently reported, particularly among the city's poor [138,139].

2.3 Pasteur Institutes and rabies vaccination in British India

The discovery of a rabies vaccine by Louis Pasteur, Emile Roux and other colleagues in 1885 [108,140] was a ground-breaking medical milestone, resulting in the establishment of Pasteur Institutes (PIs) in various parts of the world for production of rabies vaccines [141]. Initially, individuals exposed to rabies in India had to undertake a long journey to the PI in Paris for treatment, thereby affecting their chances of survival [141]; such journeys were often reported in newspapers [114,121,142–144]. These journeys were a major financial burden for the GoI, by one estimate costing £100 per person treated

(approximately £12,000 per person in 2019 terms) [145]. Recognising the need to bring rabies treatment to India ("if only for the protection of Europeans, and especially of the troops."), AV Lingard, Imperial bacteriologist at the Imperial Bacteriological Laboratory at Poona proposed in 1891 that "anti-rabic treatment and cure" could be started in the Laboratory [141]. There was a public movement in the 1890s in India to gather support for the establishment of such institutes, described in detail by Chakrabarti (2012) [107]. The first PI in India started functioning at the hill station of Kasauli in 1900 under David Semple, a medical officer of the colonial Indian Medical Service [146]. It has been argued that this shift in the choice of locations from hot and humid Pune to the colder environment of Kasauli was driven primarily by a desire to maintain a distance from the native Indian population and to avoid the hot tropical climate of the Indian plains, rather than by scientific considerations [107].

Within a short period, the PI at Kasauli served as the main destination for treating an increasing numbers of individuals, both civilians and soldiers, exposed to rabies using vaccine produced at the institute [147,148] providing significant financial savings to the GoI by avoiding the costs of travel to Paris for treatment [145]. As a result of political pressure to decentralize rabies vaccination [107], PIs (or Pasteur sections within other institutions) were established throughout British India including at Coonoor in South India (1907) [149], Rangoon (in present-day Myanmar) (1915) [150], Shillong (East India) (1917), Bombay (1922) [151], Calcutta (1924) [107] and Patna (1928) [152]. Patients who were exposed through bites would often seek medical advice by sending a telegram to the PI, before deciding on travelling to the institute for treatment [153]. These PIs served thousands of individuals exposed to rabies from all parts of British India and Ceylon (present-day Sri Lanka) [154], even after India gained independence [155,156] and many continue to serve the same function to the present day [157].

Detailed statistics were collected on bite victims presenting for treatment to record information about which species bit them (dog, jackal etc.), location, number, category and severity of bites (bites on head or face, bites through clothing etc.), whether they completed the full course of vaccinations and

the number of deaths post-vaccination – information which greatly improved scientific understanding about rabies [158]. From 1912 statistics on the number of individuals bitten by rabid animals and not seeking treatment were also compiled at Kasauli [148,159]. Hundreds of animals were also examined every year at PIs, veterinary colleges and other institutions like the Haffkine Institute in Bombay [148,149,160] to confirm a diagnosis of rabies. Thousands of copies of a pamphlet titled 'Rabies and antirabic treatment in India' were printed and sent to local governments, with suggestions to translate these into local languages [150]. Updated editions of this pamphlet were published in subsequent years [161,162]. At one point, the Kasauli institute treated more patients every year than any other PI around the world [148,163].

Kasauli also became the site for extensive research into safer and more effective rabies vaccines, since the vaccines in use at the time often resulted in serious neurological complications [164]. One of the key events in the history of rabies vaccines was the development of a phenol-inactivated nerve tissue vaccine by David Semple based on Pasteur's original work and developed through experiments and trials on patients at Kasauli [165]. Used for decades in large parts of the world, production of the Semple vaccine has now been discontinued, although it is still produced for human or animal use in a few countries in Africa [5]. The development and evolution of these and other modern rabies vaccines have been covered in detail elsewhere [107,166,167].

Eventually post-exposure treatment was also decentralized by opening 'outcentres' throughout India, though not without opposition from John Cunningham, the Director of the PI at Kasauli in the 1920s who wanted to expand research on rabies vaccines at the institute [146,168]. A 1923 news report identifies such centres 'at Karachi, Allahabad, Ahmednagar, Poona, Belgaum and Karwar' as well as Parel in Bombay [129]. These outcentres made it possible to greatly reduce delays in post-bite treatment, and the mortality rate among treated individuals in 1938 was reported to be 0.52%. By 1938, the Kasauli institute had over 140 outcentres in the northern provinces and other Indian states, while the Coonoor institute had 223 outcentres in Madras Presidency and southern states [169]. While

public funds and government grants initially supported the establishment and functioning of PIs at Kasauli and Coonoor, the effectiveness of and demand for rabies vaccines developed at these centres meant that by the 1920s, these institutions started to function fully as private entities, with most of their income coming from the sale of rabies vaccines to government, municipal and local bodies and state hospitals [169].

2.4 Rabies control in animals

One of the earliest documented pieces of legislation for dealing with stray and rabid dogs in British India is regulation II of 1813, which permitted the destruction of ownerless dogs in Bombay city during specific periods of the year. The strict (and often over-enthusiastic) enforcement of this regulation sometimes led to the destruction of ODs as well, and is closely associated with what has been described as the 'Bombay dog riots' of 1832. These riots, which also had communal overtones, have been described in detail elsewhere [170]. Other legislation included section 68 of the Cantonment Code of 1912, and provisions in Municipal Acts, which authorized cantonments or municipalities to detain or destroy confirmed or suspected rabid dogs as well as stray dogs. In municipalities, officers of the Civil Veterinary Department (CVD) were authorized to carry out these functions. Some Local Self-Government Acts also permitted issuing rewards for destruction of 'noxious animals' [171].

A newspaper report from 1912 describes the system in Madras where dog capture was outsourced to "low caste dog-catchers", and dogs were "painlessly destroyed in a lethal chamber" [172]. It was proposed that a similar system be implemented in Bombay. A news report from 1923 describes the efforts of the Health department of Bombay municipality in "diminishing the number of dangerous, diseased and stray dogs in the city". This was carried out by a team of "3 sub-inspectors, 2 dog carts, 8 cart drivers, 18 dog catchers and a lethal chamber ….. in which dogs are destroyed by means of carbonic acid gas". The municipality reportedly spent about 10,000 rupees a year for this purpose, destroying 6579 and 6848 'ownerless dogs' and returning 22 and 6 dogs to their owners in 1921 and 1922 respectively [129]. Similar efforts were also reported from Calcutta [127] and Poona [128]. Lethal

chambers and ‘electrocutors’ for destruction of dogs were installed in local bodies – a report of the CVD of Madras Presidency (1929-30) describes the inspection of lethal chambers in Tiruppur, Coonoor and Pollachi, construction of additional chambers at Erode and Udamalpet and an ‘electrocutor’ at Ootacamund (present-day Ooty) [160]. However, one letter from a reader describing empty dog carts and the number of dogs on the road [126] suggests that such measures may have been no more effective in controlling dog populations and rabies than they were in more contemporary times. These measures and the methods used to kill dogs (carbonic acid gas, strychnine poisoning, clubbing to death, electrocution) [107,173] were opposed by many Indians due to religious reasons [128,170], by Indian vernacular newspapers and many British residents [107]. In addition to dogs, wildlife [161], predominantly jackals [174] were also often destroyed.

A host of other measures targeting ODs were largely modelled on measures implemented in Britain in the 1800s which had proven successful in making the country rabies-free by 1902 [134]. Officials recognised that ODs in India were often unconfined and thus could be infected with or spread rabies – one report proposed that owned female dogs which were allowed to roam freely when in oestrus resulted in increased dog fights and thus the spread of rabies [175]. Purebreed dogs were often allowed to roam freely [176] or used by European soldiers to hunt pariah (unowned mongrels) dogs [107]. Such dogs risked reintroducing rabies into Britain or introducing it into other British colonies when their owners moved around the world [134,177]. Consequently, control measures included enforcing registration of ODs (purebred or pariah), levying a ‘dog tax’ [107,173] and issuing badges or discs to be fitted to the collars of ODs [176]. Local authorities would thus be able to round up unowned dogs for destruction after 72 hours, while straying ODs could be returned to their owners. Such a ‘tax and badge’ policy was implemented in Shimla and Mussoorie and reported to function satisfactorily [178], while some local bodies were reportedly not keen on implementing these measures [179]. In military cantonments, kennels were set aside to isolate suspected rabid dogs for observation and destruction [180]. Some letters to newspapers proposed a ban on importation of dogs into India [136]

while others argued that the quarantine of dogs imported into India would be pointless without first controlling rabies in the country [181].

As early as 1899, an annual administration report of the CVD in India highlighted the rise in number of rabies cases presented at Bombay and Lahore veterinary colleges, and recommended that the GoI issue orders to prevent its spread in India [182]. However, whether rabies could ever be effectively controlled or eliminated in India appears to have been a contentious topic [183]. At the first meeting of veterinary officers in India, held at Lahore in 1919, veterinarians discussed the challenges of controlling stray dog populations and argued for mandatory licensing of all dogs and systematic destruction of strays [184]. The following resolution was adopted at this meeting – "That it is considered that any suitable measure that can be adopted for reducing and destroying the surplus population of dogs is desirable, but that it does not appear to be possible under the conditions prevailing in India to deal more effectively with the disease. Power should be given to veterinary practitioners to order the detention and destruction of dogs suffering from rabies." Based on this resolution, the GoI appears to have advised local bodies to give veterinary officers relevant powers to perform these functions [171]. At the same time, it voiced doubts about the feasibility of implementing such control measures in rural areas and appears to have left it to municipalities and local self-governments to deal with the problem. This approach appears to have persisted throughout the period of British rule in India and there seem to have been no policies for nationwide rabies control in animals. It was also believed that rabies could not be eliminated in India as it was also maintained in wildlife [178].

After the world's first rabies vaccine for dogs was developed in Japan in 1915, Umeno and Doi developed a single dose canine rabies vaccine suitable for mass production in 1920 [185,186]. This vaccine was used for mass vaccination of dogs in Japan from 1924-25 [167,186]. In this context, experimental studies to develop a method of veterinary PEP ("anti-rabic inoculation of dogs bitten by rabid dogs"), presumably for valuable ODs, had begun at the Punjab Veterinary College in Lahore from

1915, also including horses in later years. These studies were inspired by work being conducted at the PI in Kasauli, fully recognising that the control of rabies in animals would benefit people as well [187]. A report of the Principal of this college in 1922-23 determined that rabies PEP of dogs could be considered an established mode of treatment (while also including caveats about conclusively establishing whether a dog was infected or not) [188]. Similar studies to develop preventive rabies vaccination in dogs were also started at the Madras Veterinary College from 1922 using rabies vaccine from the PI, Coonoor. Initial experiments were reported to be inconclusive because following vaccination, dogs from both experimental and control arms remained healthy after being infected with rabies virus [189].

Even prior to these studies, there are frequent reports of treatment of valuable animals exposed to rabies. For instance, two elephants owned by the Raja of Nilambur that were bitten by a rabid dog were given 'anti-rabic treatment' in 1919-20, of which one elephant was confirmed by microscopic examination to have subsequently died of rabies. However the first record of the use of rabies vaccines for veterinary PEP, beyond those reported from the Punjab and Madras Veterinary Colleges, is found in a Madras CVD report from 1923-24, when ten animals were 'treated with anti-rabic vaccine' at the veterinary hospital, Calicut (present-day Kozhikode in Kerala) [189].

From 1923, vaccines for veterinary use were issued from the PI, Coonoor to veterinarians in Madras Presidency and Indian states [169]. The use of PEP to treat valuable animals (ODs, livestock at government livestock farms, equines and even a monkey) eventually started throughout most Indian provinces [189,190]. Vaccines were sourced from regional PIs, the Haffkine Institute in Bombay and the HK Mulford drug company in the USA [191]. By the 1930s and 1940s veterinary PEP was being commonly administered at veterinary colleges and regional veterinary centres [133,160,191,192]. In response to rabies cases in Darjeeling municipality in 1933, legislation was enacted which required dog owners to vaccinate their dogs and to keep them muzzled or on a leash when in public [193]. A letter to a newspaper in 1935 complained that vaccination had failed to prevent the onset of rabies in

some ODs [130]. Statistics of the number of patients treated at the PIs also reported figures for the number of animals vaccinated [155,194]. Between 1923 and 1948, 14,212 animals had been "prophylactically treated" at the PI in Coonoor [195] – these are all likely to have been owned.

However, the use of veterinary PEP seems to have been restricted to treating valuable owned animals and mass vaccination of dogs for rabies control does not appear to have been seriously considered in British India. The studies conducted in Japan and the USA on preventive rabies vaccination of dogs as well as vaccination studies conducted at the Madras Veterinary College were discussed at the second meeting of veterinary officers in India, held at Calcutta in 1923. At this meeting, the opinion that rabies could never be eradicated in India persisted and it was opined that vaccines would be useful only to reduce case numbers [171]. A newspaper report covering the conference stated that "The control of rabies in India constitutes one of the most difficult problems confronting both medical and veterinary authorities. The Conference resolved that the results of investigations upon the prophylactic vaccination of dogs against rabies should be referred to the Central Standing Advisory Committee on Epizootic Diseases and Research with a view to advising Government upon the desirability of enforcing measures of widespread inoculation of dogs against the disease." [196]. We found no records to suggest that mass vaccination of dogs was ever considered by local authorities or the GoI. Mass culling of stray and rabid dogs and registration and, in later years, vaccination of ODs appear to have been the most widely implemented rabies control measures in colonial-era India.

2.5 Historic animal rabies incidence in India

Annual administration reports of the CVD provide a detailed picture of the prevalence of animal diseases in British provinces in India. Provinces and presidencies were comprised of districts and municipalities with veterinary dispensaries and diagnostic laboratories, as well as veterinary colleges in some provinces. These institutions reported the number of cases of animal diseases treated or diagnosed. Infectious disease statistics from the earliest CVD reports (1887 onwards) focused solely on those affecting productive livestock (cattle, buffalo, sheep and goats) or equines such as rinderpest,

foot-and-mouth disease, haemorrhagic septicaemia, anthrax, surra, strangles etc. Rabies was mentioned only when it affected these species, and the disease was often included in the category 'Other' diseases. It is only from 1903-04 onwards that rabies cases in livestock and dogs, most likely owned, were explicitly recorded in tables of disease summaries. Subsequently the number of cases recorded increased significantly (Fig 2.1), which may have been partly driven by a number of provincial administrations framing rules for rabies prevention (e.g. Madras in 1923-24) [197].

Figure 2.1 presents the total number of animal rabies cases reported in all species (domestic and wildlife) between 1887-88 and 1950-51 across all provinces in British India. Case numbers reported from lower administrative levels (districts and municipalities), from regional veterinary colleges and/or diagnostic laboratories have been combined to present a breakdown by province/state in Figure 2.2. These reports indicate that animal rabies was endemic and widespread throughout all provinces in British India, affecting all species of domestic animals, most commonly dogs, and wildlife. Officials frequently highlighted their concerns about alarming increases in rabies cases and recommended implementation of control measures [183,187].

Outbreaks were occasionally reported, necessitating PEP treatment of several animals – for instance, the spike in cases in Bengal province in 1935-36 when 950 animal rabies cases were reported from all districts [132] (Fig 2.2). CVD staff were often exposed to rabies and had to undergo PEP at PIs [198].

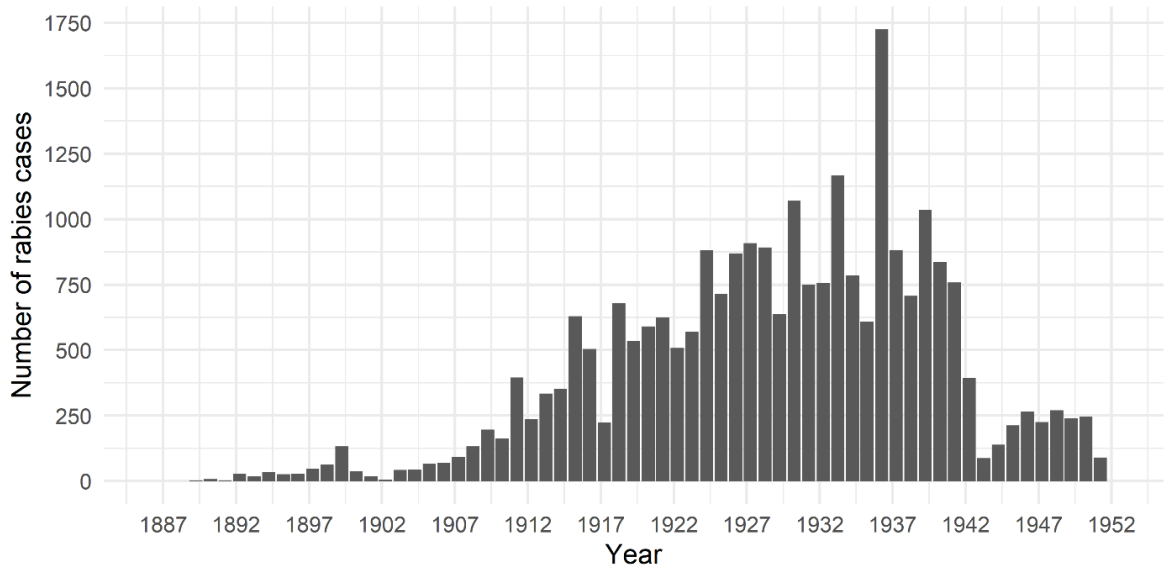

Figure 2.1 Annual rabies incidence in British India, 1887-1951. Total number of rabies cases reported each year in all animal species between 1887-1888 and 1950-51 (denoted 1888, 1951 etc. on the x-axis). Statistics were compiled from annual reports of the Civil Veterinary Department of the colonial British Government of India, available at https://digital.nls.uk/indiapapers/.

CVD reports also highlight the wide variation in numbers of rabies cases reported from various provinces (Figure 2.2). In line with the lack of a consistent policy for rabies control in animals, there was little consistency in reporting of animal rabies cases [199]. Statistics compiled by Chakarabarti (2012) show that between 1880 and 1935 rabies caused an average of 160-170 human deaths per year in Punjab province (Fig. 4.1 in [107]). However, the few animal rabies cases that are reported from Punjab province appear during the late 1800s and early 1900s, following which cases are reported only sporadically (Fig 2.2). It was often acknowledged that reported statistics of animal rabies incidence were likely to be underestimates of the true disease burden [199,200], which was sometimes attributed to a lack of public interest in reporting cases to veterinary officials [179]. There is a marked drop in the number of recorded animal rabies cases after 1941 (Fig. 2.1), possibly because many provinces stopped reporting cases after this period (Fig. 2.2).

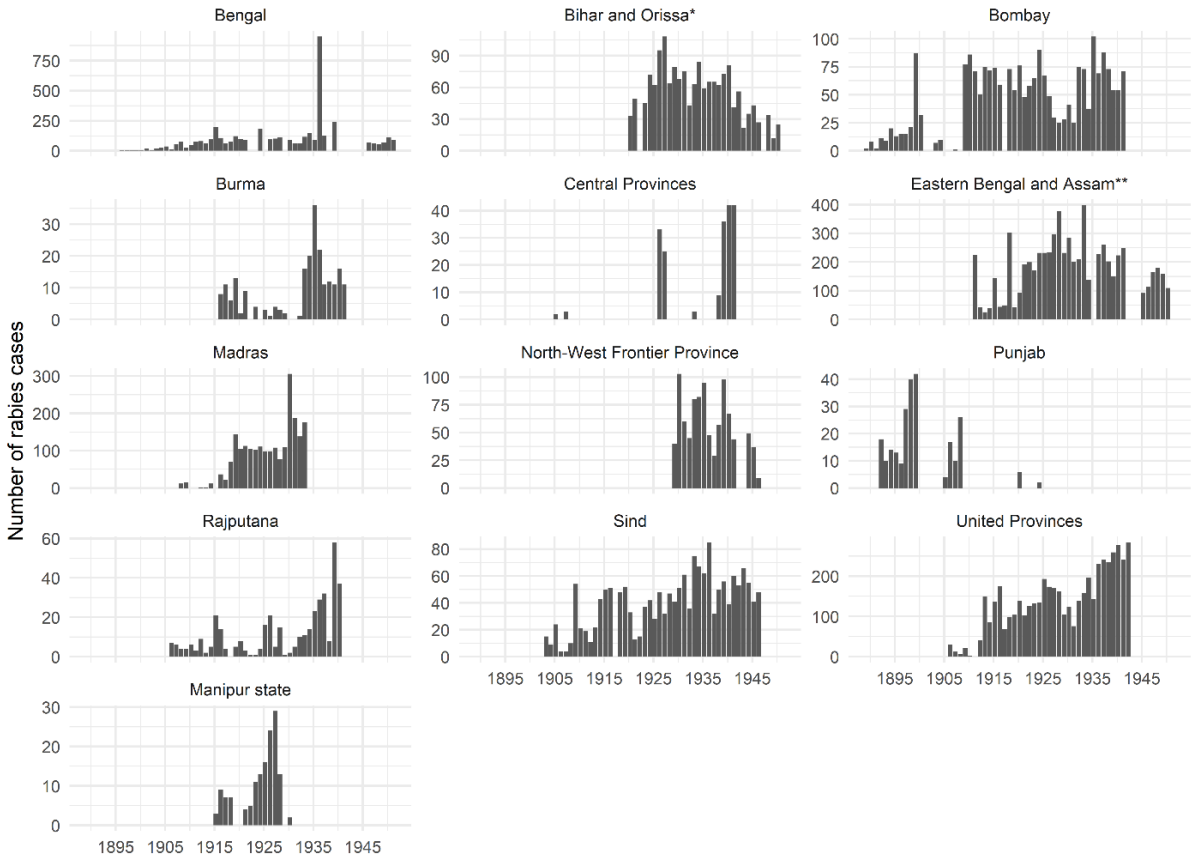

Figure 2.2 Annual rabies incidence by province in British India, 1887 – 1951. Annual rabies incidence in all animal species between 1887-1888 and 1950-51 (denoted 1888, 1951 etc. on the x-axis) in every British province in India (except Baluchistan for which no data was available) and the princely state of Manipur. Statistics were compiled from annual reports of the Civil Veterinary Department of the colonial British Government of India, available at https://digital.nls.uk/indiapapers/. Note the different scale of the y-axis for each region. *1911-12 to 1935-36 – Bihar and Orissa provinces, 1936-37 to 1949-50 – Bihar only; **1907-08 to 1910-11 – Eastern Bengal and Assam provinces, 1912-13 to 1949-50 – Assam only.

Veterinary institutions charged a fee for admission and treatment of cases [201]. Some CVD reports indicate that while there was high demand for rabies PEP, the cost of treatment was unaffordable for poor animal owners and officials were unable to provide it free of cost [133,160]. A 1928-29 CVD report from Madras Presidency describes how poor dog and livestock owners could not afford the cost of PEP for their animals and were advised to administer 'indigo blue' instead [202]. In later years,

PIs charged for testing brain samples (ten rupees per brain sample in 1933-34), which poor farmers could not afford to pay [198]. Such barriers to treatment and diagnosis are likely to have influenced estimates of the true rabies burden and efforts to limit its spread.

2.6 The origins of its neglect?

Chakrabarti (2012) discusses in detail the various ethical, moral and political debates around scientific research and treatment for rabies in colonial India [107]. It is debatable whether the motives behind the research and development of vaccines and control efforts targeting rabies and other diseases in India were purely altruistic or driven by imperial ambitions and a scientific fascination for tropical illnesses [164,203]. However, it is evident that colonial British governments in India invested time and resources to control rabies (among other diseases) in the country and so the disease could hardly be considered 'neglected' from today's perspective.

Given what has been described thus far, what might explain the neglect of rabies by public health practitioners and policymakers in modern-day India? Could it be driven by a perception that rabies had declined sufficiently to justify focusing on other more important human diseases? Dog bites and rabies clearly continued to be major public health concerns in India long after PIs started to save lives from the early 1900s. This is evident from newspaper reports and letters to newspapers from the public [122,126,127,129,180,204,205] as well as official reports and documents of the PIs and the GoI [150,164,206]. Annual reports from the PIs reveal a rapid rise in the number of patients vaccinated against rabies annually. The number of patients treated at the PI in Coonoor and its outcentres increased from under 200 in 1907 to 13000 in 1935 [164], highlighting the high burden of animal bites in regions served by the institute. This increase was frequently attributed to wider awareness of the availability and benefits of "Pasteurian treatment" rather than any actual increase in rabies [148,150,207].

At the same time, it is not clear that human deaths from rabies reduced substantially in British India. As mentioned above, Chakarabarti (2012) found that around 160 to 170 people died of rabies every

year in Punjab province between 1880 and 1935 [107]. This is despite the presence of the PI at Kasauli and the Lahore veterinary college in this province. In 1913, 243 rabies-related deaths were reported from the Central Provinces and Berar [150]. Similarly, 220 deaths were reported in India in 1922, with the report acknowledging that this figure was an under-estimate of the true incidence [206]. A letter to the Times of India in 1911 speculated that the true number of dog bites and rabies deaths in India was likely to be much higher than those stated in reports from the PI [136]. It was recognised that not everyone completed the full course of post-exposure vaccinations and it was not possible to follow up on outcomes for all patients [206]. As human deaths from rabies in the general population outside those attending the PIs was not systematically recorded, the true death toll may have been higher.

An examination of human disease statistics and discourses around public health in British India provides some hints about administrative and health priorities vis-à-vis rabies. Notwithstanding concerns raised by the public or officials of the CVD, provincial administrations did not consider rabies to be a concern compared to deaths from other contagious diseases or snakebites [107]. In the late 1800s, mortality statistics from British India included rabies deaths under the broad heading of 'Injuries', which covered a wide variety of conditions (suicide, wounds or accidents, snake-bite, injuries caused by wild beasts etc.) [208,209]. Before the establishment of the PI in India, rabies occasionally killed several soldiers stationed in India (69 deaths in 1879-80, 146 in 1885-86), though considerably fewer than the thousands of deaths every year due to diseases like cholera or smallpox [208,209].

With the advent of PIs and the race within scientific circles around the world to develop safer and more effective rabies vaccines, detailed records began to be maintained in India of the number of people vaccinated from broad ethnic (European and Indian) or religious (Muslim, Hindu, others) groups, those developing complications or dying post-vaccination, the number of patients that completed the full course of vaccination and mortality rates between European and Indian patients [158,210,211]. Such statistics were published in annual reports of PIs [158], scientific journals [178] and, during the early 1900s, regularly included in annual reports presented to the UK Houses of

Parliament [148–150,207,211–214]. These efforts served to establish the safety and efficacy of different vaccines being developed at the PIs and improve scientific understanding about rabies. For instance, in a letter written in 1911, WF Harvey, the then Director of the PI of Kasauli, recommended the collection of statistics by local bodies on the number of people bitten by rabid animals and who subsequently die without being vaccinated. His aim in suggesting this was to prove that the true mortality rate for rabies was much lower than that reported in statistics from Europe. He stated that this would involve 'two or three years' work only', within which period he expected to prove his hypothesis [159]. In several significant respects, India was at the forefront of global research on rabies and the PI at Kasauli was central to this enterprise [107].

Statistical abstracts and reports of burden of illnesses in British India were split into sections – the first dealt with morbidity and mortality in the European Army in India, followed by the Native army (later referred to as the 'Indian' army), the general population and jails [148–150,154,210,211,214]. Individuals treated for rabies at PIs were categorized as Europeans (including Eurasians/Anglo-Indians) and natives / Indians and further into soldiers and civilians. The number of Europeans vaccinated against rabies did not increase substantially over the years and few ever died of rabies. The number of Indians vaccinated increased annually and concurrently, the number of recorded deaths (Table 2.1).

At the same time, overall rabies-related mortality continued to be much lower than mortality from other contagious diseases. Of 22,579 patients vaccinated between 1912 and 1916, only 135 (including 4 Europeans) died. This is in marked contrast with mortality from diseases like cholera (1,259,012 deaths between 1914 and 1917) and plague (1,599,088 deaths in the same period) [215]. At the second meeting of veterinary officers in 1923, it was even remarked that the money spent on rabies control in India would prove more beneficial if diverted for the control of cholera [216]. Indeed, diseases like cholera, plague, smallpox and malaria frequently caused extensive epidemics in India (e.g. the First Cholera Pandemic (1817-1821) [217], the plague epidemic in Bombay (1896) [137]) and high human mortality requiring active interventions by the state [137]. This focus on epidemic diseases

would have been in marked contrast with rabies which was, and continues to be to this day, characterized by fewer cases and only occasional outbreaks in animals [122,132]. Such outbreaks were handled by mass culling of dogs or jackals [174,176], and following the development of vaccines, by PEP administration to human and animal bite victims.

As mentioned previously, there was also an emerging discourse around sanitation and urban improvement in colonial India from the late 1800s [137–139]. A range of sanitary reforms were implemented from this period, particularly aimed at improving the health of European army personnel who initially suffered significantly higher morbidity and mortality from epidemics in India, compared to Indian soldiers. Sanitary measures such as the provision of piped and filtered water, relocating barracks from swampy areas and improvements in drainage and preventive vaccination against smallpox and plague caused a remarkable and consistent decline in morbidity and mortality among British troops in India [137]. Such sanitary measures would have had little impact on rabies, which would not have been seen to be as amenable to human modification of environmental conditions. Preventive vaccination of humans against rabies, as practised for smallpox, would hardly have been considered necessary, given the sporadic nature of the disease. These epidemiological characteristics of rabies are likely to have greatly influenced colonial perceptions of what diseases could be reasonably controlled through public health interventions.

Table 2.1 Number of people given rabies post-exposure prophylaxis at various Pasteur Institutes in India. Category totals may not always match as the breakdown of the number of patients treated and the number of deaths was not always explicitly reported.

Year	Numbers treated (number of deaths)			Reference
	European	Native/Indian	Total	
1900-1901[1]	146 (1)	175 (9)	321 (10)	[214]
1901-1902[1]	215 (2)	328 (11)	543 (13)	[214]
1902-1903[1]	269 (1)	315 (12)	584 (12)	[214]
1903-1904[1]	248 (0)	364 (10)	612 (10)	[214]
1904-1905[1]	307 (0)	570 (12)	877 (12)	[214]
1905-1906[1]	342 (2)	803 (19)	1145 (21)	[214]
1906-1907[1]	452 (2)	846 (17)	1308 (19)	[214]
Interim, 09/08 – 31/12, 1907[1]	146 (1)	373 (4)	519 (5)	[214]
1908[2]	342 (2)	1047 (24)	1729 (26)	[212,214]
1909[2]	675 (3)	1920 (25)	2595 (28)	[210,214]
1910[2]	575 (0)	2325 (43)	2900 (43)	[211,214]
1911[2]	297(1)	2911 (50)	3208 (51)	[214]
1912[2]	400 (0)	4388 (59)	4788 (59)	[148]
1913[2]	2 (2)	5271 (66)	5273 (68)	[213]
1914[2]	NA (1)	NA (60)	5795 (61)	[150]
1915[2]	468 (1)	6409 (41)	6877 (42)	[207]
1933[1]	1356 (0)	14582 (83)	15938 (83)	[218]
1936[1]	1357 (0)	NA (97)	NA (97)	[219]
1938[3]	NA (NA)	NA (NA)	12396 (21)	[155]

[1] Figures for Kasauli institute only; [2]Figures combined for all Pasteur institutes in India, where available; [3]Coonoor institute and its subsidiary centres only; NA – Not available.

A similar situation existed with rabies in animals in India. CVD officials were more concerned with the treatment, control and prevention of diseases affecting equines and livestock, which were largely unaffected by rabies. For instance, in 1935-36, when Bengal province experienced rabies outbreaks in multiple districts and recorded 950 cases (Figure 2), the number of livestock deaths from rinderpest and haemorrhagic septicaemia was 35281 and 3989, respectively [132]. This is unlike the situation reported in Trinidad, for instance, where between 1925 and 1958 repeated outbreaks of rabies transmitted by bats threatened the livestock industry, prompting widespread vaccination campaigns for cattle and efforts to destroy bat populations. As a result, rabies was accorded high government priority for control and elimination in Trinidad with WHO assistance and much research was conducted on this topic [220]. Dog rabies had also been eliminated in Britain in 1902, and barring occasional outbreaks seeded by dogs brought into the country, rabies ceased to be the significant domestic public health concern it once had been for British politicians and policymakers [134]. This may also have contributed to the gradual loss of interest in investing in rabies control and prevention in British India.

The success of Semple's vaccine in reducing human rabies deaths in India was soon recognized by the global scientific community and it began to be widely used around the world [168]. By the 1930s, statistics from the PIs continued to be published in scientific journals [195,218,219] but was no longer included in reports to the UK Parliament. During this period one also finds a return to the practice of including rabies deaths under the head of 'Injuries' [221,222]. While research to make the Semple vaccine safer did continue, the key personnel driving this research left the PI or were transferred and research on other diseases began to take precedence. The PI at Kasauli was shut down in 1939 with work being shifted to the Central Research Institute next door [107]. Research on rabies vaccines and diagnosis continued to be conducted at the Coonoor PI after Indian independence in 1947, spearheaded by the institute's Director N Veeraraghavan [223], but rabies no longer appears to have been accorded the same priority it once was in British India during the early 20th century.

Thus, despite the importance given to rabies in India with the advent of Pasteurism, a combination of factors is likely to have contributed to its eventual decline in administrative and public health priorities. In particular, the success of Semple's vaccines in preventing human rabies deaths will have influenced administrative officials to prioritise scarce resources towards competing and more pressing public health interventions (e.g. improving sanitation and addressing epidemics). A point to this effect is made in an anecdote in a CVD report about rabies control becoming a priority only 'when the deaths amongst humans numbered some scores annually, and a genuine feeling of alarm for personal safety was felt' [224]. In this respect, rabies in British India may have become a victim of its own success, something which is recognised today in Latin America as canine rabies control becomes more effective and human mortality has reduced dramatically [21].

2.7 Impacts on present-day debates in India

Much literature exists on the medical history of a range of infectious diseases that caused major epidemics in British India, including malaria, cholera, plague and smallpox [137,138,225]. This review has examined the historical treatment in British India of rabies as a public health concern, a topic which has received relatively less attention. From being a widespread and untreatable illness, rabies rose to become a driver of Pasteurism in India and globally and a key component of British colonial scientific enterprise. The disease, however, eventually declined in administrative and public health priorities in India towards the end of colonial rule; a decline that has yet to be reversed in modern-day India. In charting this history of rabies, the review highlights features of the colonial administrative, scientific and societal approach to dealing with this disease in India which remarkably persist in the country nearly a century later.

Key among these are the interrelated issues of an absence of a rabies control policy at the national level and of systems for rabies surveillance in humans and animals [56]. Notwithstanding the existence of a National Rabies Control Program [102], it was only in September 2021 that the government released its national action plan to eliminate rabies (NAPRE) [83]. Until then, the country lacked a well-

considered roadmap with realistic milestones to chart progress towards effective national rabies control, let alone elimination by 2030 [5]. Policy formulation and implementation continue to be the responsibility of states and local bodies and consequently these are inconsistent. For instance, only two states (Tamil Nadu and Sikkim) have made human rabies notifiable [226]. As was the case in colonial India, animal rabies is not seen as an economically relevant disease affecting animal production systems and hence not prioritised for control by agriculture or animal husbandry ministries [227].

Rabies in animals was widespread in space and time across British India (Figure 2). The mean number of animal rabies cases recorded between 1903-04 and 1950-51 was 522. This is likely to be an underestimate given that reporting was unsystematic and not mandatory and reported numbers do not include cases from most princely states and territories not under direct British control. The human population of India has risen from 361 million in 1951 to over 1.2 billion in 2011 [228] and the population of dogs have increased correspondingly. In the absence of any comprehensive rabies control measures, it therefore stands to reason that the number of animal rabies cases will also have increased significantly. Although animal rabies is notifiable in India today, disease reporting is acknowledged to be unreliable even by rabies experts in India [226] and rabies statistics such as those reported by the CVD are difficult to access. This makes the task of estimating the true prevalence in animals extremely difficult. Such gaps in knowledge of the human and animal disease burden and patchy awareness of rabies as a public health threat [67], even among medical health professionals [229], significantly hinder the development of political, scientific and societal urgency to address this burden, particularly in rural areas which bear the biggest brunt [6].

One positive change from the colonial-era approach to rabies control in India is with respect to dog population management (DPM). Although culling of dogs continued to be the mainstay of DPM and rabies control efforts for decades after independence, the Animal Birth Control (Dogs) Rules, 2001 outlawed this inhumane measure [82]. It was replaced by a policy of ABC and anti-rabies vaccination

(ABC-ARV), carried out once during a dog's lifetime, after which it was returned to its original location. However in the absence of scientifically robust methods to obtain reliable estimates of dog population sizes, ABC-ARV is implemented in a haphazard and uncoordinated manner across local bodies and states, and involving various public [230,231] and private entities [232]. The policy also does not account for the need to revaccinate sterilised dogs to maintain anti-rabies immunity in the dog population and consequently, the possibility that these dogs may continue to bite and transmit rabies to other dogs and people [233]. Rule 10 of the ABC Rules prohibits euthanasia of dogs suspected to be rabid, instead requiring such dogs to be isolated until they die naturally of rabies [82], followed by laboratory confirmation of disease [234]. This is clearly a welfare issue for infected dogs.

There is also a flawed perception of ABC-ARV as more than just a DPM tool. Consequently, this measure is increasingly viewed as the primary rabies control measure in India, perceived to be unsuccessful in reducing disease only because of ineffective and/or inadequate implementation [235]. This perception is despite the fact that the WHO itself recommends sterilization of dogs only as a supportive measure to maintain levels of rabies vaccination coverage achieved through MRV, accepted as the most scientific method for rabies control [5]. A recent study was unable to evaluate the role of surgical sterilisation in controlling dog rabies due to poor data collection or reporting, and recommended that mass vaccination should continue to be the control method of choice [236]. The ABC-ARV policy also finds support through discourses that argue for the continued existence of street dogs as 'integral inhabitants of the multispecies city' [235].

Wang (2019) describes the conflict that existed in New York City from the early 20th century, between the American Society for Prevention of Cruelty to Animals and the Department of Health, over population control and muzzling of the city's FRDs [237]. No such conflict appears to have existed over dog culling in colonial India. On the contrary there is in India today an impasse, along the lines of that which existed in New York, between two conflicting perspectives of the place of dogs on the streets, with direct impacts on rabies control efforts. On the one hand is the view that there should be a holistic

approach to control stray dog populations on public health, wildlife conservation [238] and animal welfare grounds. This approach would require enforcing responsible dog ownership, civic waste and humane DPM and a national MRV program [5,233], eventually leading to the elimination of FRDs. On the other hand is the view, held primarily by animal welfare campaigners, that dogs have the right to exist on the streets and to be fed by people [239]. This latter view consequently favours ABC-ARV as the most appropriate DPM and rabies control measure, notwithstanding its drawbacks.

In this respect, the ABC-ARV policy has made it difficult to adopt comprehensive measures to deal with the persistent threat of rabies posed by the large populations of unowned FRDs in India [227], particularly implementation of mass dog vaccination. Despite evidence from the 1920s that mass vaccination of dogs successfully reduces rabies incidence and can eliminate it [185,186], there were no attempts to implement such a measure in colonial India. Instead, it was widely considered that rabies could never be effectively eliminated in India. This perception continues to hold sway at the highest levels of government to this day with the view that logistical constraints make mass vaccination of dogs unfeasible in India [240]. It is left to state administrations to implement mass vaccination policies, commonly in partnership with non-governmental organisations [79,241].

In another unfortunate parallel with the colonial era, there is little emphasis on promoting responsible DOP such as confinement and vaccination of ODs in India. It is unclear how successful attempts were by colonial administrations to enforce registration and identification of ODs. While several local bodies have now made such measures mandatory in India, they are poorly enforced [242]. Rabies vaccination, while largely confined to ODs, primarily valuable pure breeds, is also not mandatory. This often results in poor compliance with vaccination regimens [242], especially in the case of owned non-descript dogs (i.e. those that do not belong to any specific breed), from poorer households with limited access to veterinary services. A high proportion of this latter category of dogs are also poorly confined, resulting in the birth of unwanted pups and increased risk of contracting rabies from interactions with free-ranging unowned dogs (discussed further in Chapter 3).

2.8 Conclusions

Notwithstanding poor availability of disease data, the case may be made that rabies does not impose the kind of human health burden in India that diseases such as tuberculosis, malaria and HIV do. Consequently, rabies control may not be seen as a cost-effective public health investment, a view that was certainly shared by public health practitioners in British India [216]. Such a perspective, however, fails to consider the impact of rabies on individuals from rural backgrounds, particularly children [57] and the near certainty of death in the absence of access to treatment before symptoms appear. As an entirely vaccine-preventable disease disproportionately affecting the poor in low and middle income countries, preventing unnecessary human rabies deaths and suffering by addressing barriers to access to human PEP is an important means of achieving social justice [243]. At the same time, the cost of human PEP provision can be substantial (30 million US dollars over an unspecified timeframe in India, by one estimate) [226]. Rabies control through mass dog vaccination has been consistently shown to be more cost-effective in preventing human rabies deaths [28,244]. With the science and tools for rabies control already existing, rabies elimination is low hanging fruit and a textbook example of the One Health approach in action. This is well recognised even in India, with zoonotic disease prioritisation exercises frequently identifying rabies as one of the main diseases for targeting control efforts [245,246]. Political will has been key to implementing effective control measures in many countries around the world [5], and is the primary factor currently hindering progress on this front in India today.

Rabies in British India was clearly not a 'neglected' public health concern. Early rabies vaccines were highly effective in saving human lives, although there remained a poor understanding of the true disease burden in Indian society. These factors, combined with changing priorities of colonial British governments, in all likelihood contributed to a progressive loss of priority of rabies control in the face of the vast array of competing infectious disease and public health challenges in British India. It may be possible to argue that the current neglect of rabies in India is a legacy, albeit unintended, of British colonial rule. But this clearly is no justification for carrying on in the same vein. Current public health

professionals and policy makers should look to the extensive historic and current scientific literature on evidence-based rabies control measures to formulate a strategy to achieve the lofty goal of elimination of dog-mediated human rabies deaths by 2030.

Chapter 3 : Free-ranging and owned dog population characteristics in Kerala, south India in the context of rabies control

ABSTRACT

While most human rabies cases in India are caused by bites from FRDs, few studies have assessed ownership and population characteristics of Indian dog populations. This longitudinal study focussed on a population of owned (OD), semi-owned (SOD) and unowned (UD) dogs captured at an urban (human population of 240991 individuals) and a semi-urban (25861 individuals) site each in Kerala, south India. Dogs were included in the study either when captured in the field, presented for anti-rabies vaccination at vaccination camps or households, and when captured for local ABC campaigns. Dogs were microchipped and/or collared, blood sample collected, and individual characteristics were recorded in round one. Further blood samples were collected upon recapture approximately 30 days, 150-180 days and one year post-vaccination.

In total, 577 dogs across all ownership categories were captured in the first round. Although only 12% of FRDs were owned, over 90% were in good body condition or overweight. About half of UDs had direct human interactions and less than half were ever recaptured in subsequent rounds. About 60% of ODs were free-ranging and only 29% were vaccinated against rabies, of which only a third had detectable RVNA. About 7% of the dog population were SODs. Mixed-effects logistic regression models found higher recapture probability for sterilised dogs (OR 2.06, 95% CI: 1.23 – 3.47) and lower for UDs (OR 0.18, 95% CI: 0.07 – 0.46), particularly UD pups (OR 0.20, 95% CI: 0.06 – 0.73), dogs from the semi-urban site (OR 0.30, 95% CI: 0.17 – 0.53) and those with pre-vaccination RVNA titres ≤ 0.5 IU/ml (OR 0.27, 95% CI: 0.09 – 0.82) or no detectable titres (OR 0.25, 95% CI: 0.08 – 0.73). This study thus conclusively establishes that most FRDs in India are unowned and highlights important factors influencing recapture probability, particularly the difficulties in accessing FRDs outside urban locations. Rabies control in India will require a multi-pronged approach incorporating more responsible dog ownership, access to veterinary care, effective MRV and dog population and waste management.

3.1 INTRODUCTION

3.1.1 Background

Studies of local FRD ecology and demography are critical in informing the design and implementation of MRV campaigns [5]. Rapid population turnover due to high mortality rates during the first year of life has been reported in owned FRD populations in Africa [26,247] and unowned FRDs in India [73,248]. Human movement of dogs can also introduce rabies-infected individuals into a region [16,249]. Additionally, factors such as individual dog health can have a bearing on maintenance of immunity post-vaccination. For instance, Wera et al. (2021) reported that owned FRDs with poor body condition scores (BCS) were twice as likely to lose adequate levels of rabies binding antibodies, compared to those with good BCS [250]. Such factors can result in rapid declines in herd immunity to rabies, necessitating higher initial vaccination coverages and repeated campaigns [249]. Despite these challenges, the demonstrated feasibility of achieving 70% vaccination coverages in FRD populations has been predicated on observations that most of these populations are in fact owned and therefore readily accessible for vaccination. Most of this literature has focused on countries in Africa [39], Latin America [38] and parts of south-east Asia such as Indonesia and the Philippines [16,38,41].

Although India accounts for the highest burden of human rabies deaths globally, no coherent control policy had been formulated until the launch of the NAPRE in September 2021 [83]. Although pilot MRV campaigns were conducted in some cities [251], there have been no attempts to implement it as a national control strategy. Policymakers have even opined that MRV is not feasible in India due to a perceived lack of infrastructure and logistics to implement it in a systematic manner [240]. Recently, MRV campaigns conducted over five years by an international charity, Mission Rabies, in association with the state animal husbandry department were highlighted as key to substantially reducing the burden of dog rabies in Goa [241]. However, it was not clear if the associated decline and eventual elimination of human rabies deaths in the state could be attributed solely to the MRV campaigns and how significant a role improved access to human PEP played.

Relatively few studies have explored the nature of dog ownership and DOP such as vaccination and confinement in the Indian context [33,45,242]. Studies describing dog populations in India have suggested that the vast majority of FRD are unowned or semi-owned [33,38,45,76,252] and therefore not readily accessible for vaccination. In a study of six villages in rural Maharashtra, Belsare and Gompper (2013) [33] identified a large proportion of 'quasi-owned' FRDs which could be linked to 'reference persons'. These reference persons often provided resources for and had a bond with these dogs [31], but could not necessarily handle them, thereby limiting accessibility for vaccination. A study in the city of Ranchi identified 92% of dogs in the city as being free-ranging, most with no known owner [45], without specifying how this was determined. Most FRDs in Jodhpur were also reported to be unowned, based on the absence of collars [76]. A study of DOP in an urban colony in New Delhi found low levels of responsible pet ownership among dog-owning households [242]. A community survey in Bangalore city found that there were two 'stray' dogs for every pet dog, without specifying if these strays were truly unowned [74]. Bhalla et al. (2021) reported very high FRD densities in Bangalore, a major Indian city, with a large proportion of these dogs being supported by only 10 to 18% of households in the study area [252]. Vaccinating such large populations of FRDs without owners can be a substantial challenge and impact efforts to achieve recommended vaccination coverages, compounded further by poor DOP compromising vaccination coverages in ODs.

There is also a need to understand what factors influence capture/recapture probability in FRD populations as these can have a direct bearing on their accessibility for interventions, be they mass vaccination or ABC / sterilisation. Apart from age [26,73,247,248], FRD survival has also been shown to vary by sex [247,248], extent of urbanisation [248] and the influence of ongoing ABC campaign on dog health [64,253]. Dog population density is known to increase with human population size and human:dog ratios in rural India are some of the highest in the world [31,33]. In a study combining central point and door-to-door vaccination of dogs in six rural Indian villages, Belsare and Gompper (2013) reported vaccination coverages substantially lower than the recommended 70% target coverage [33]. This study highlighted the difficulty of accessing even ODs for vaccination in rural

regions. In a study at two sites in north India, fewer active dogs were reported at the rural site [64], suggesting the presence of fewer FRDs in this location. The extent to which FRDs interact with people in their vicinity can also have a bearing on how readily accessible they are.

The state of Kerala in south India outperforms other Indian states in national health and human development surveys [254]. The state is comprised of 14 districts, each further constituted by local self-government institutions (LSGIs) known as 'panchayats' or village councils, which serve as decentralized local governance institutions. Each panchayat (or less commonly, corporation or municipality) is served by at least one government veterinary institution, commonly a veterinary dispensary, which function under the aegis of the Kerala state Animal Husbandry department (AHD) [255]. There are thus a total of 1200 LSGIs in Kerala [256] served by 1166 veterinary institutions [257]. These veterinary institutions provide clinical and preventive veterinary services within each LSGI, including veterinary preventive and post-exposure rabies vaccination [255]. The veterinary rabies vaccines commonly administered include those procured by the AHD through centralized tender processes as well as commercial cell-culture vaccines [258] which may be provided through the veterinary institution or privately purchased by animal owners.

Based on the most recently available data (2011-2014) [259], the per-capita death rate from rabies in the state is estimated to be approximately 0.041 (95% CI: 0.036 – 0.046) per 100,000 persons, one of the lowest rates globally for rabies endemic regions [56,260], even if accounting for under-reporting of deaths. Dog ownership is widespread and there are high levels of awareness of the need to vaccinate dogs against rabies [261], the risks of rabies exposure and high levels of adherence reported to PEP schedules [262]. A study of canine management practices from central Kerala reported high levels of responsible DOP, such as rabies vaccination and confinement, although most dogs were sexually intact [261]. Issues of FRDs and dog bites have gained much public attention in the past few years, forcing the state government to implement ABC campaign to sterilise FRDs [231]. These campaigns are also commonly conducted at the level of each LSGI, with a fixed number of dogs

captured and sterilised depending on budgetary allocations of each local body [230]. As in other parts of India, it would be reasonable to expect that most FRDs are unowned.

3.1.2 Aims

We conducted a longitudinal field study in four rounds at two sites in Kerala to:

a. Establish conclusively the ownership status of FRDs at these sites;
b. Identify dog population characteristics that influenced FRD recapture probability in subsequent rounds;
c. Investigate the extent of responsible DOP relevant to rabies prevention and control in ODs, and
d. Identify differences in these characteristics between the urban and semi-urban sites, if any.

We discuss the implications of these findings for DPM and rabies control strategies in Kerala and India.

3.2 METHODS

3.2.1 Location of the study

The study was carried out at two locations in Kerala – Alappuzha (ALP), an urban municipality (9°29' N, 76°20' E) and Muhamma (MUH) (9°36' N, 76°21' E), a semi-urban panchayat (Figs 3.1 and 3.3). ALP is a major Indian tourist destination with a human population of 240991 individuals (Census of India, 2011) [263]. The official estimate of the dog population in ALP is 4991 dogs, including 3448 ODs and 1543 'stray' dogs (Livestock Census, 2012 of Kerala state AHD) (Serene Xavier, personal communication). This results in a human:dog ratio of approximately 48:1 or 156:1 when excluding ODs. ALP is bordered by two large water bodies – the Arabian sea along its western border and the Vembanad lake in the north-east. Much of the eastern part of the municipality consists of an extensive wetland system including agricultural land and backwaters. ALP is divided into 52 administrative wards. MUH lies about 13km north of ALP (Fig. 3.1b), with a human population of 25861 individuals [263] and a total dog population of 1059 dogs (887 ODs, 172 'stray' dogs), resulting in a human:dog ratio of approximately 24:1 or 150:1 when excluding ODs. The entire eastern side of the panchayat is

bordered by the Vembanad lake. The panchayat also includes the uninhabited Pathiramanal island, which was not covered in this study. MUH is divided into 14 administrative wards.

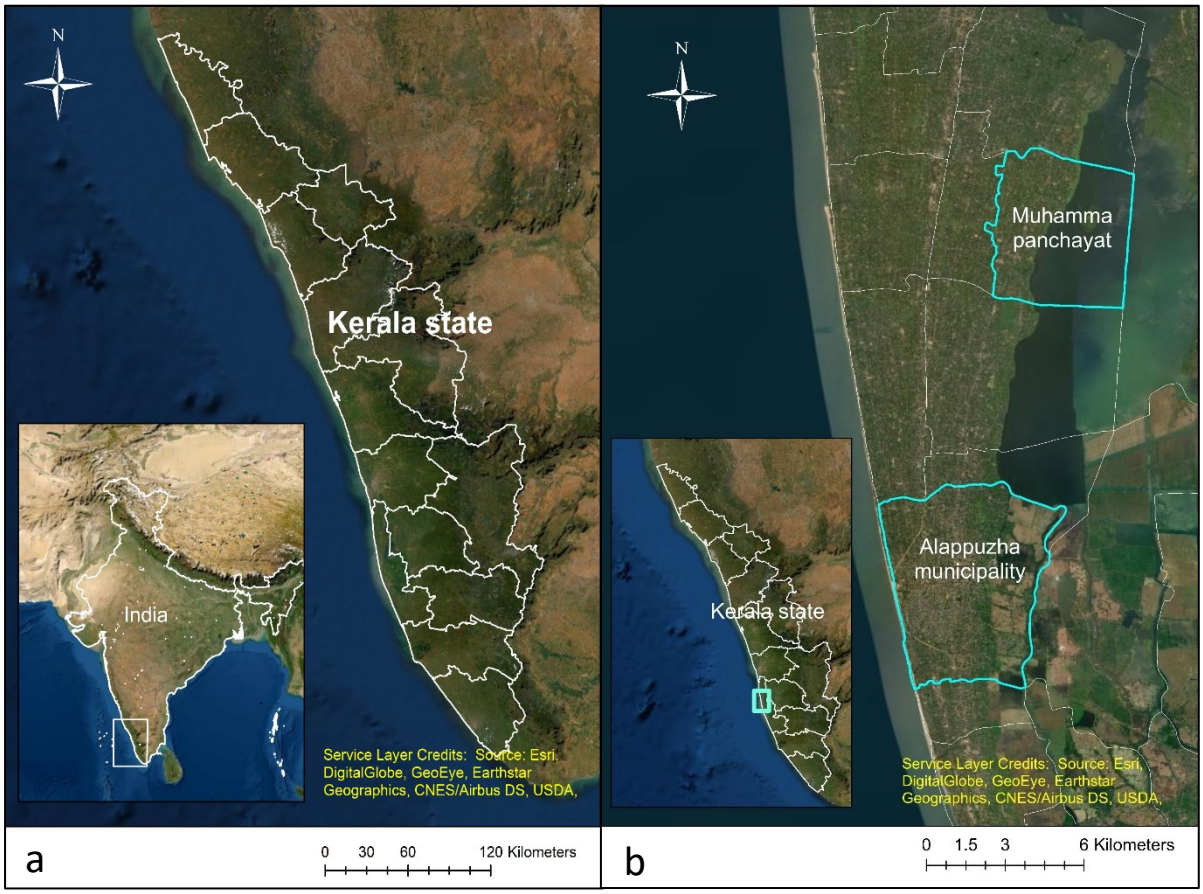

Figure 3.1 The study location. a. Map indicating the location of the state of Kerala in India (inset), b. Map indicating boundaries of Alappuzha municipality and Muhamma panchayat (in cyan) and their locations in Kerala (inset).

3.2.2 Data collection

The study was conducted in four rounds between October 2018 and January 2020 (Table 3.1). The fieldwork team comprised of the principal investigator (PI) Sreejith Radhakrishnan, dog catchers and a field assistant. Two professional dog catchers with over three years' experience in capture, handling and restraint of dogs assisted the PI in capture and sampling of dogs in round 1 (R1). A third dog catcher was engaged in subsequent rounds to enable more effective recapture of vaccinated dogs. The field assistant helped in recording data in an electronic data collection form and with fieldwork logistics. All dog catchers and the PI were vaccinated against rabies before dog capture and handling

started, and staff were trained in responsible dog handling, restraint techniques and welfare. The team made every effort to ensure that dogs were not subjected to undue stress or harm at any stage while carrying out procedures.

The team travelled along main roads at both study sites, choosing routes randomly and attempted to capture any FRDs sighted along these routes. Routes were repeated on the same or subsequent days until it was not possible to capture any new FRDs or there were none left to include in R1 from that route. In the context of this study, the term FRDs applies to all dogs that were entirely free-ranging, including dogs with no known owner and those with/without a reference person (hereafter referred to as unowned dogs, UDs); owned dogs, ODs; and semi-owned dogs (SODs) (defined further in the section on Data Coding). Dogs captured in R1 were encountered in the following ways:

A. In the field – during the capture of UDs/SODs/free-ranging ODs using butterfly nets or when ODs were presented by owners for vaccination
B. During household visits to vaccinate ODs
C. During rabies vaccination camps to vaccinate ODs.
D. When captured as part of official ABC campaign (UDs/SODs/free-ranging ODs).

During R1, a blood sample (day zero) was collected to determine pre-vaccination RVNA titres from dogs encountered for the first time, after which they were vaccinated against rabies. Post-vaccination blood samples to assess levels and persistence of RVNA were collected from as many dogs as possible of this cohort approximately one month (~30 days, round 2 (R2)), five to six months (~150 – 180 days, round 3 (R3)) and 12 months (~365 days, round 4 (R4)) after being first vaccinated for the study. The terms ‘capture’ and ‘recapture’ are used hereafter to refer to instances where dogs were first included in the study (with or without sampling, unless stated explicitly) or resampled in subsequent rounds, respectively, irrespective of their ownership status. In the absence of unique identifying features, each dog was subcutaneously implanted with a Radio-Frequency Identification microchip (Chiphandel, Germany) to enable individual identification in future rounds. Each microchip was encoded with a

unique 15-digit number that could be detected using a microchip reader (Chiphandel, Germany). Blood samples were not obtained from very young pups because of their small body size, general health or inability to collect enough blood. Coloured collars (orange and black collars for male and female dogs, respectively) were also fitted to identify dogs included in the study visually and to avoid unintentional recaptures during the ongoing round of capture (Fig 3.2). However, young pups and juveniles were not collared to prevent tightening of the collar as they matured. Collars were removed if they were found to have tightened when resampling dogs in subsequent rounds or when members of the public informed the fieldwork team of this happening. Except in these instances, and where collars had not fallen off naturally, they were removed only when dogs were recaptured in R4. Photographs of every captured dog were taken in each round.

Figure 3.2 Identification collars applied to free-ranging and some owned dogs. Orange (left) and black (right) collars were used to identify male and female dogs, respectively. All collars had a thin reflective strip in the middle to make them readily visible at night.

Some UDs/SODs could be individually identified based on one or a combination of features such as coat pattern, body size, physical deformities, behaviour or association with local people or specific locations; presence and location of ear-notches (right or left ear) and/or absence of testicles (in males) in dogs sterilised during past or ongoing ABC campaigns. Such dogs were not collared and/or

microchipped. In subsequent rounds, we encountered UDs/SODs which a) were collared but had no detectable microchip, or b) had no collar or detectable microchip but were known to have been included in the study. We attempted to identify such dogs by comparing their characteristics (age, sex, global positioning system (GPS) location of capture and other features mentioned above) with those of dogs previously captured in that locality or as part of the ABC campaign in R1, using photographs and GPS locations recorded during previous rounds. All other FRDs were identified based on their collar and microchip number before blood sampling.

Throughout fieldwork, the fieldwork team interacted with the local public for information about the dogs that were captured, including to assess whether any were known to be owned or vaccinated in the past. In a previous study from India, the presence of collars on FRDs was used as a marker of ownership [76]. Similarly, we considered all FRDs with pre-existing collars on them as owned, even if could not conclusively identify their owners in the course of the study. For FRDs which were later established to be owned, informed consent was obtained from owners to include them in the study, failing which they were removed from the study. All other ODs were included in the study if vaccination and collection of blood samples were undertaken after obtaining informed consent from an owner / reference person over 18 years of age, and if procedures could be carried out without potential harm to the dog, the owner or members of the fieldwork team. No fee was charged for any vaccination. Many dog owners presented ODs for vaccination in the field while we were engaged in capturing FRDs. ODs were also presented at vaccination camps conducted at a few locations in ALP or were vaccinated during household visits. All procedures on ODs were carried out in the presence of their respective owners and preferably with the owners handling the dogs themselves. ODs presented by their owners or free-ranging ODs which were captured and handled in the presence of their owners/reference persons were fitted with coloured collars and/or microchipped only if deemed necessary and with the owner's approval. The vaccination history of each dog was also collected and confirmed by examining vaccination certificates where presented. The owner was contacted by phone before household visits in later rounds for post-vaccination blood sampling. Dogs captured during

ongoing ABC campaigns in ALP were also included in the study. In this case, collaring/microchipping, vaccination, blood sampling and data collection were carried out at the ABC centres.

All dogs were subject to a quick but thorough physical examination, including an assessment of body condition by physical examination, skin / coat condition, reproductive status in females (pregnancy or lactation) and presence of wounds or injuries that needed treatment. They were then vaccinated irrespective of vaccination history, age, health or reproductive status, as recommended by the WHO [5]. However, newborn UD/SOD pups (those with sealed eyes or two to three weeks of age) were included in the study once they were older and if encountered again on returning to the same location after a few weeks. This was done to enable blood collection and to minimise potential loss to follow up and wastage of microchips, since high mortality rates (> 50%) have been reported in young free-ranging pups within the first three to four months of life [248,264]. Once procedures were completed, dogs were immediately released back to their territory (UDs/SODs), returned to owners (ODs) or moved into operation theatres for sterilisation (all dogs at ABC centres).

FRDs have varying levels of interactions with human beings, which may be subjectively described as being friendly or fearful, direct or indirect. However, this descriptive approach makes it difficult to quantify what proportion of dogs are accessible for capture, handling or healthcare interventions such as vaccination or treatment. To address this, we developed human interaction scores (HISs) on a scale of 1 (does not appear in public when people present) to 6 (completely tolerant of human handling or restraint, even by strangers) for UDs and SODs only, representing increasing levels of human interaction (Item 30, Table S1). These scores were assigned based on our observations of the extent to which each dog interacted with people at our study sites and discussions with these people. Throughout the study, the PI was the only person to assign a HIS value to each dog. We decided to assign scores only to UDs and SODs recaptured at least once. This was done to uniquely identify each dog by its microchip number or physical characteristics and have at least one further opportunity to observe its interaction with humans. Therefore, these data are not available for all dogs included in R1. We re-evaluated the HIS assigned to each dog based on such future observations. For analyses,

the HIS assigned in the most recent recapture was used as the dog's HIS throughout the study. Dogs were also grouped into two broad categories – those with (HIS of 4, 5 or 6) or without (HIS of 1, 2 or 3) direct human interactions.

We tried to record the activity of each dog at the time of capture in R1 (Table S1). For ODs, this variable was recorded as 'Not applicable – owned dog', unless the OD was ranging free at the time of capture. For UDs and SODs, these were further broadly grouped as trying to escape from dog catchers, exhibiting normal behaviour (e.g. sleeping, investigating its environment, interacting with other dogs etc.) and interacting with people (local members of the public or staff involved in this study) (Table S2). Further details of dog capture, sampling and data collected are provided in Appendix A1 and Table S1 within. Further details of vaccination, blood sampling and testing for RVNA titres across all study rounds are presented in Chapter 4.

The data collection tool EpiCollect [265] was used to design an electronic data collection form for real-time data collection using Android mobile phones and online data storage and manipulation. Data collected included ownership and confinement status, sex, age, breed, BCS, skin/coat condition, sterilisation status, vaccination history and GPS coordinates of the capture location. The full list of data collected is provided in Table S1 (Appendix A1).

3.2.3 Data coding

The classification of dogs described by Taylor et al. (2017) [266] was used to broadly categorise dogs as ODs (by an individual, family or household) (OD) and UDs. FRDs with pre-existing collars around their necks were also conservatively categorised as ODs, even if we could not conclusively identify their owners. Taylor et al. (2017) defined 'community-owned' dogs as those over which more than one family or household claims ownership. In this study, we use the term 'semi-owned' (SOD) to include such 'community-owned' dogs as well as a broader category of dogs that are closely associated with one or more households as well as public spaces such as places of worship, restaurants, business establishments or marketplaces, fed by family members or members of the public and share close

bonds with these individuals. Such dogs were cared for by one or more people, were often referred to by name (e.g. Suleiman, Susie, Mary etc.), could often be confidently handled by locals (and rarely, even strangers) and had 'reference persons' (13) who spoke for these dogs but did not claim any ownership rights. Such dogs had high acceptance in their respective spaces and if it was suspected that they would come to any harm at the hands of authorities or dog catchers, local members of the public would actively intervene or enquire why the dogs were being handled or caught. Because of their high acceptance in public or private spaces and high level of comfort with human interaction, SODs were also highly accessible for capture, vaccination and blood sampling. However none had claims of ownership over them.

In addition to classifying dogs as UDs, SODs and ODs, we specified a broader category – DWOs, that included both UDs and SODs.

Pups were zero to four months of age, juveniles between five and 12 months, and adults over 12 months of age. A separate category of 'aged' dogs was defined as those over five years of age but these were included as adults for most analyses. Purebreed dogs were identified by their specific breed name (e.g. Labrador, Dachshund), and also analysed together under a broad 'purebreed' heading. Dogs that displayed a mixed phenotype with features characteristic of specific breeds such as bushy or short tails, hairy coats or short statures were identified as a crossbreed of that breed (e.g. Dachshund cross) or, where breed characteristics could not be distinguished, more broadly as a 'crossbreed'. All other dogs were classified as of non-descript breed. Confinement status was recorded as completely confined and partially or completely free-ranging. All DWOs were recorded as completely free-ranging, while ODs could fall under any of these three categories. Skin/coat condition was recorded broadly as poor, fair, good or very good, along with details of signs of illness or parasites, where applicable. The reproductive status of female dogs was recorded as being pregnant, having whelped recently or lactating, with details of when they whelped and the number of pups born, if known. Body conditions scores was assigned on a scale from 1 (no discernible body fat, emaciated) to

9 (massive fat deposits, obese). Dogs were also broadly categorized to have an under ideal (BCS of 1 – 3), ideal (4 – 5) or over ideal (6 – 9) body condition [267].

During ABC campaigns, dogs that are surgically sterilised (by orchiectomy / ovariohysterectomy) are also vaccinated against rabies and a notch placed on one ear pinna to indicate that they are sterilised. Therefore, the presence of an ear notch in dogs encountered during R1 was used as a sign that the dog had been vaccinated against rabies at least once before. If any unsterilised dog included in the study in R1 was later found to have an ear notch, it was recorded as having been sterilised and revaccinated after R1 and the date of revaccination (if known) was also noted. If unknown, an approximate date of revaccination was assigned for analysis purposes. The vaccination history, if known, was recorded as having never been vaccinated, having been vaccinated in the past, regularly vaccinated or of 'unknown' vaccination status. Further vaccination details (dates, frequency) were also recorded where available. Vaccination status was automatically recorded as 'unknown' for all UDs and most SODs.

3.2.4 Data analysis

Data were downloaded from the EpiCollect website (https://five.epicollect.net) and analysed in R [268]. Detailed descriptions of data coding are provided in Table S1 (Appendix A1). Briefly, individual characteristics of dogs (sex, breed, BCS etc.) were coded as categorical variables, grouping multiple responses together where necessary (Table S2), and summarised as proportions. Pearson's chi-squared and Fisher's exact tests were used to test for differences in proportions between groups. A two-tailed exact binomial test was used to test if the male: female sex ratio was significantly different from 1:1. An unpaired two sample t-test was used to test for differences in capture effort between study sites. A p-value of less than or equal to 0.05 was considered to be significant.

3.2.5 Logistic regression

Many studies have identified the influence of factors such as age, sex, sterilisation status, body condition and extent of urbanisation on FRD survival (and in extension, their capture/recapture probability) [31,33,64,247,248,253]. However, no studies in the current literature have explicitly

explored factors influencing FRD capture probability and thereby their accessibility for interventions. In order to explore the potential influence of as many variables as possible, we used univariable and multivariable logistic regression to explore the possible influence of several characteristics (in addition to those previously mentioned) (predictor variables) of each dog recorded when first captured in R1, on the probability of being recaptured in each subsequent round (R2, R3, or R4). The full list of predictors considered in these analyses is detailed in Table S61 (Appendix A1). Univariable models with statistically significant p values based on the chi-squared statistic were then used to purposefully select predictors for inclusion in the multivariable model. After excluding three predictors with a large number of missing values – reproductive status (applicable only for female dogs, 527 missing values), HIS (311 missing values, due to it not being recorded for ODs or DWOs that were never recaptured) and dog's activity (125 missing values) – stepwise selection of variables (using the R function stepAIC from the MASS package) was used to select the model with the lowest Akaike Information Criterion (AIC) statistic as the most parsimonious model. Odds ratios (ORs) and p-values for predictors from this final model are presented in the Results section. These analyses were conducted using data combined for all dogs included in the study, as well as separately for ODs, SODs, UDs and DWOs. We also assessed whether these characteristics significantly influenced individual capture probability across all recapture rounds (R2, R3 and R4 combined), calculated as the number of times a dog was captured (X successes, ranging from 0 to 3 times) out of the total possible number of times it could have been captured (N attempts, ranging from 1 to 3 times). N was calculated based on whether the dog was known to have been present at the study sites during R2 - R4. If the dog was known to have died or to have been lost to the study in the interval between two capture rounds, N was correspondingly reduced. If the fate of a DWO was unknown by the end of R4, it was assumed to be alive but not recaptured (N=3). Dogs that could not have been recaptured because they died or were lost to the study less than 30 days after vaccination (i.e. N = 0) were excluded from all logistic regression analyses, as they provided no information on recapture probability. Mixed-effects logistic regression models (generalised linear mixed models, GLMM) with unique dog identity as a random effect were also used

to model recapture probabilities across R2, R3 and R4. In these models, the fixed effects used were dog characteristics recorded in R1 as well as the study round, but ages in R2, R3 and R4 were inferred based on age at capture in R1. For example, pups from R1 progressed to being juveniles by R3 and adults in R4.

3.2.6 Ethical approval and permits

Local ethical approval to conduct the study was obtained from the Kerala state AHD. All animal procedures were approved by the Animal Welfare and Ethical Review Board of Imperial College London (Reference number 20180705A). All activities at both field sites were carried out in coordination with local elected representatives, the Municipal Chairman in ALP municipality and the panchayat President in MUH panchayat.

3.3 RESULTS

3.3.1 Sample population characteristics

In R1, 577 unique dogs (515 in ALP, 62 in MUH) were included in the study (Table 3.1, Fig 3.3) and blood samples collected from 554 of these. This included 85 UDs and SODs that were captured as part of an ABC campaign in ALP. Two dogs escaped from the butterfly nets before they could be sampled, and two UDs were subsequently established to have been mistakenly captured, vaccinated and microchipped twice during the early stages of fieldwork. One UD died in the field soon after net capture. Two ODs were abandoned by their owners in the course of the study, one of which was recorded as unowned when resampled in R3 and R4.

A breakdown of dogs captured in R1 by site, ownership category, age and sex is provided in Table 3.2, and further in Appendix A1 (Table S3). In the absence of more recent estimates of the dog population at these study sites and assuming that the population size had not changed substantially since the 2012 livestock census, we estimate that we were able to capture 25.3% (390/1543) of DWOs in ALP and 29.7% (51/172) of DWOs in MUH, while substantially lower proportions of ODs were captured in R1 – 3.6% (125/3448) in ALP and 1.2% (11/887) in MUH.

The sample population was comprised primarily of adults (484/577, 84%) at both study sites, and across all ownership categories, in part because we actively excluded young pups among UDs and SODs in R1. However, a higher proportion of ODs were comprised of pups (24/136, 18%) because many were presented for vaccination in the field or at vaccination camps. The overall male:female sex ratio was not significantly different from 1:1 ($p = 0.31$), across sites or ownership categories.

All members of the fieldwork team were from Kerala and the study was conducted over 16 months at the same locations, so the team could familiarise themselves with and build trust among local communities who were, therefore, more willing to disclose the true ownership status of dogs. The ownership status of most FRDs was established during R1 itself while one and two dogs were confirmed to be ODs in R3 and R4, respectively. Only 12% (60/501) of completely FRDs were owned (16% when including ODs that were partially free-ranging), with similar proportions at both study sites (9/60, 15% in MUH; 51/441, 12% in ALP). A total of 262 DWOs (223 UDs and 39 SODs) were recaptured at least once and thus assigned an HIS. There was only a low positive correlation between the final HIS assigned to a dog and the number of times it was captured throughout the study ($r = 0.19$, 95% CI: –0.07 – 0.31, $p = 0.002$). Overall, only 13% (77/577) of all dogs captured in R1 were sterilised.

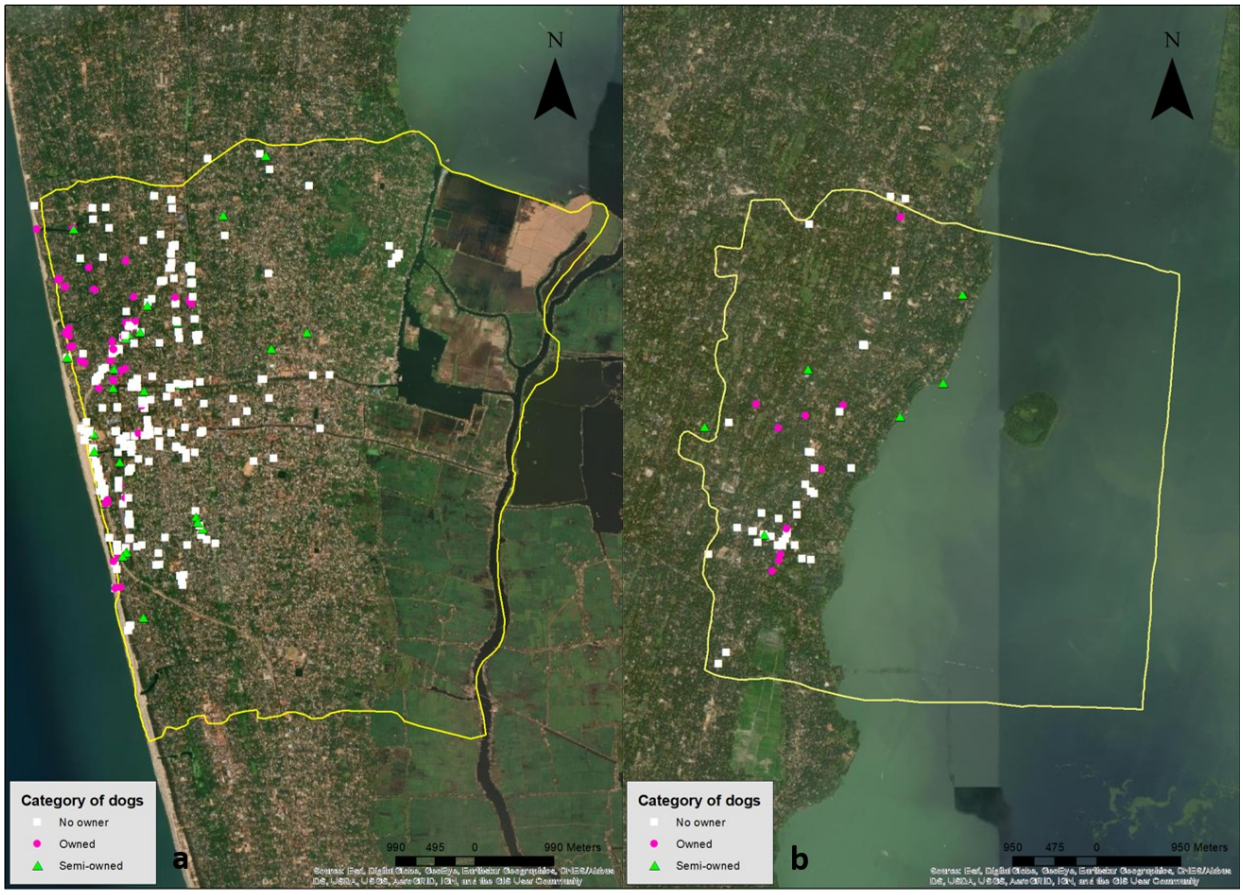

Figure 3.3 Map of study sites with dog capture GPS locations. Map of the study sites highlighting the urban and semi-urban nature of Alappuzha municipality (left) and Muhamma panchayat (right), respectively, and GPS locations of dogs captured in R1 by ownership category.

Table 3.1 The number of dogs captured during each round from both study sites. Values in brackets indicate the proportion of dogs of each category – owned (OD), semi-owned (SOD) or unowned (UD) dogs – captured in round 1, that was recaptured in subsequent rounds. For example, of 356 UDs captured in round 1 in Alappuzha, 166 (47%) were recaptured in round 2. Figures for UDs include dogs whose identities could not be confirmed because microchip numbers were not detected.

Number of dogs	Site	Round 1 (DAY 0) (October 2018 – January 2019)				Round 2 (~DAY 30) (November 2018 – January 2019)				Round 3 (~ DAY 150 – 180) (April – May 2019)				Round 4 (~ DAY 365) (October 2019 – January 2020)			
		OD	SOD	UD	Total	OD	SOD	UD	Total	OD	SOD	UD	Total	OD	SOD	UD	Total
Captured / recaptured	Alappuzha	125	34	356	515	108 (0.86)	32 (0.94)	166 (0.47)	306 (0.59)	82 (0.66)	25 (0.74)	91 (0.26)	198 (0.38)	90 (0.72)	17 (0.50)	104 (0.29)	211 (0.41)
	Muhamma	11	7	44	62	6 (0.55)	4 (0.57)	12 (0.27)	22 (0.35)	8 (0.72)	4 (0.57)	8 (0.18)	20 (0.32)	3 (0.27)	3 (0.43)	3 (0.07)	9 (0.15)
	Total	136	41	400	577	114 (0.84)	36 (0.88)	178 (0.45)	328 (0.57)	90 (0.66)	29 (0.71)	99 (0.25)	218 (0.38)	93 (0.68)	20 (0.49)	107 (0.27)	220 (0.38)

Table 3.2 Age and sex composition of dogs included the study, by ownership status. Age and sex composition of the total number of dogs (n = 577) included in the study in round 1 at both study sites, belonging to various ownership categories (P – Pups (0-4 months), J – Juveniles (5 – 12 months), A – Adults (>12 months, including Aged dogs – those >5 years))

Site	Sex	Unowned dogs (UD)				Semi-owned dogs (SOD)				Owned dogs (OD)				Category totals
		P	J	A	Total	P	J	A	Total	P	J	A	Total	
Alappuzha	Male	7	10	156	173	1	1	10	12	14	2	49	65	250 males
	Female	11	17	155	183	1	1	20	22	9	8	43	60	265 females
	Total	18	27	311	**356**	2	2	30	**34**	23	10	92	**125**	**515 dogs**
Muhamma	Male	2	2	14	18	0	2	2	4	0	1	3	4	26 males
	Female	1	2	23	26	0	0	3	3	1	0	6	7	36 females
	Total	3	4	37	**44**	0	2	5	**7**	1	1	9	**11**	**62 dogs**

3.3.1.1 Unowned (UD) and semi-owned (SOD) dogs

Among 441 UDs and SODs captured in R1, the most common phenotype was that of the non-descript mongrel (n = 431, 98%) (Fig 3.2). Nine UDs, all in ALP, were classified as crossbreed dogs with physical features of purebreeds such as long bushy tails, short snouts or short tails. Some had long-haired coats, or short statures similar to Dachshunds, and one UD had clear features of the Labrador breed.

SODs comprised a small percentage (7%) of the FRD population. None had claims of ownership over them, although two were cared for by more than one household and all had direct interactions with members of the public (Table S5). Many had identifiable reference persons caring for them, even providing food and shelter to female dogs with pups. Only a quarter of SODs captured in R1 tried to escape from dog catchers and this proportion persisted at just over half of all SODs throughout recapture rounds, in contrast to UDs where the proportion trying to escape from dog catchers increased consistently throughout the study (Fig S8). One in five SODs could be vaccinated and blood sampled without net capture and with minimal restraint. Two were known to have been vaccinated in the past.

Only 16% of DWOs captured in R1 (69/441, all of which were adults) were sterilised including only four in MUH (8%). A significantly higher proportion of males (43/207, 21%) were sterilised compared to females (26/234, 11%, p = 0.008). An ABC campaign was conducted in ALP from December 2018 to February 2019 and in MUH during April 2019, when many study dogs were sterilised. Consequently, the proportion of DWOs recaptured in later rounds that were sterilised increased in each round for both sexes (Fig S1), comprising over half of all dogs recaptured one year later (Table S4).

Of 223 UDs which were assigned HISs, only about half (105, 47%) was assessed to interact directly with humans to varying levels (HIS of 4, 5 and 6), being significantly higher in MUH (78%) than in ALP (44%, p = 0.013). However, all 39 SODs that were assigned HISs had direct human interactions (Table S5). The proportion of sterilised dogs did not differ by their HIS (Table S6). Fewer than one in five UDs or SODs were judged to have poor skin and coat condition throughout the study (Fig S2).

Irrespective of age, sex or HIS, most UDs and SODs were in ideal body condition throughout the study (Table S7, Fig S3, Appendix A1). A significantly higher proportion of sterilised UDs were overweight or obese (31% with an above ideal BCS of 6-9), compared to non-sterilised dogs (8.3%, $p < 0.001$), this difference holding true in all rounds except R2. However, an increasing proportion of UDs recaptured in R3 and R4 were overweight or obese, irrespective of sterilisation status (Table S7, Fig S4).

Of 174 non-sterilised juvenile and adult female UDs, 31 adults (18%) were visibly pregnant or suckling between two and 10 pups during R1. Of these females that were recaptured in later rounds and were not sterilised, corresponding percentages are 12%, 27% and 50% for R2, R3 and R4, respectively (Table S8, Fig S5). One UD was pregnant or lactating in all rounds. These percentages were significantly higher in SODs with 10 of 21 non-sterilised juvenile and adult females (48%, $p < 0.001$) visibly pregnant or suckling between two and six pups during R1. Corresponding percentages for R2, R3 and R4 were 27%, 36% and 56% respectively. One SOD was pregnant or lactating in all four capture rounds.

3.3.1.2 Owned dogs (OD) and dog ownership practices (DOP)

Of the 136 ODs included in the study in R1 (125 in ALP, 11 in MUH), 60% were non-descript dogs while purebreeds comprised about one-third of all ODs, the most common breeds being the Spitz breed (45%), Dachshunds (19%), Pomeranians (19%) and Yellow Labradors (10%). Most had an ideal BCS (Table S11, Fig S3) and good or very good coat condition (>60%) throughout the study. A third were judged to be overweight or obese, rising to 47% in R4. Most ODs (60%, including all ODs in MUH) were completely or partially free-ranging, only nine of which had pre-existing collars on at the time of capture in R1. Only eight (6%) were sterilised, all of which were completely or partially free-ranging and so had been mistakenly captured during a past regional ABC campaigns. The proportion of ODs that was sterilised increased to 11% in R4, as some were included in an ongoing ABC campaign in ALP (Table S12, Fig S1). Of 55 non-sterilised juvenile and adult females in R1, nine adults (16%), all partially or completely free-ranging non-descript dogs, were visibly pregnant or suckling between one and seven pups during R1. The proportion reproductively active rose to a third of all non-sterilised female ODs recaptured in R4 (Table S8, Fig S6), 70% of which were non-descript dogs with all but one being

completely or partially free-ranging. One free-ranging OD was lactating or had recently whelped when captured in three of the four capture rounds.

Less than a third of all ODs (including only one OD in MUH) were known to have been vaccinated against rabies by their owners, including only four (3% of all ODs) which were regularly vaccinated every year. This proportion did not change substantially even after excluding pups, which are generally considered too young for vaccinations. The vaccination history of 17 ODs was recorded as 'unknown' because owners were unsure of their dog's vaccination history or because we could not identify the owner despite the dog having a collar around its neck. Of those that had been vaccinated, just over a third (39%, 15/39 ODs with a history of vaccination and whose titres were assessed) had detectable RVNA titres, including only one with titres > 0.5 IU/ml (Table S13). RVNAs were also detected in 20 ODs with no history of vaccination, including one dog with titres > 0.5 IU/ml, and seven ODs with unknown vaccination histories (Table S13). These results are presented further in Chapter 4.

Overall, only a quarter of juvenile and adult ODs (28/112, 25%) were completely confined and had a history of being vaccinated against rabies at least once. There were also clear differences in the care given to owned pure/crossbreed dogs and non-descript dogs (Table 3.3), and these differences largely persisted in later rounds of the study (Fig S6).

Table 3.3 Breed differences in rearing of owned dogs. Differences between care provided to pure/crossbreed and non-descript owned dogs captured in round 1 of the study, using five measures – body condition score (BCS), coat condition, confinement, reproductive activity and vaccination against rabies at least once. Statistically significant differences are highlighted in bold.

Measure	Number of pure/crossbreed owned dogs (Proportion)	Number of non-descript dogs (Proportion)	Significance level (p)
Completely confined	40 (0.74)	14 (0.17)	**< 0.001, Chi-squared test**
Vaccinated against rabies at least once	30 (0.58)	10 (0.17)	**< 0.001, Chi-squared test**
Poor coat condition	0 (0)	12 (0.15)	**0.003, Fisher's exact test**
Reproductively active*	1 (0.03)	8 (0.32)	**0.007, Fisher's exact test**
Under ideal BCS	3 (0.06)	11 (0.13)	0.16, Fisher's exact test

*number lactating, pregnant or recently whelped among all non-sterilised juvenile and adult females

3.3.2 Capture effort

When calculating capture effort, we excluded data for six days from ALP and one day from MUH when fewer than 10 dogs were captured, as these were generally scheduled visits to households to resample ODs. The effort required to capture dogs was especially pronounced in semi-urban MUH with significantly fewer dogs captured per hour of fieldwork (mean 3.55, sd 0.70 dogs per hour over 8 days evaluated) than in urban ALP (mean 5.14, sd 0.96 dogs per hour over 28 capture days evaluated, $p < 0.001$) (Fig S9). These differences were significant even when excluding ODs from analyses. We could only include 44 UDs from MUH (compared to 356 from ALP). The mean interval between capturing two consecutive dogs was significantly higher in MUH (mean 17.49, sd 3.59 minutes) than in ALP (mean 12.09, sd 2.42 minutes, $p < 0.001$). These rates were evaluated over R1 and R2 (30 days post-vaccination) together, as fieldwork for these rounds overlapped for over a month.

3.3.3 Recapture proportions

Of the 577 dogs captured in R1, the numbers and proportions of dogs recaptured in subsequent rounds are – R2 – 328 (0.57), R3 – 218 (0.38) and R4 – 220 (0.38) (Table 3.1, Figs 3.4 and 3.5). Fieldwork could not be carried out as extensively in R3 as in other rounds due to high daytime temperatures during April-May, the limited availability of dog catchers during this period and restrictions in the duration of fieldwork. Thirty dogs across all categories (8 UDs, 16 ODs, 6 SODs) whose identities were confirmed died between 7 and 365 days after first capture in R1. Some UDs in MUH were known to have died, but their identities could not be confirmed. Eight ODs were lost to follow-up between 18 and 282 days after first capture in R1, while 4 UD pups were lost to follow-up within 14 days. Seven of 47 pups (15%) died and eight (17%) were lost to follow-up within 60 days of first capture, the latter because they were moved by their owners, given away or due to unknown reasons. A further five pups died within one year of first capture.

Over 60% of ODs were recaptured in each subsequent round. A similar percentage of SODs was recaptured in R2 and R3, dropping to 49% in R4 (Table 3.1, Fig 3.5). However, less than half of all UDs included in R1 were ever recaptured, being significantly lower than recapture proportions for ODs/SODs in ALP ($p < 0.001$). Recapture proportions were significantly lower in MUH, particularly for UDs where only a quarter of all dogs (0.27) were recaptured in R2, dropping to less than 10% one year later in R4 (Table 3.1). Most dogs from MUH (Table S14) and most unowned pups (0.76) and juveniles (0.58) were never recaptured.

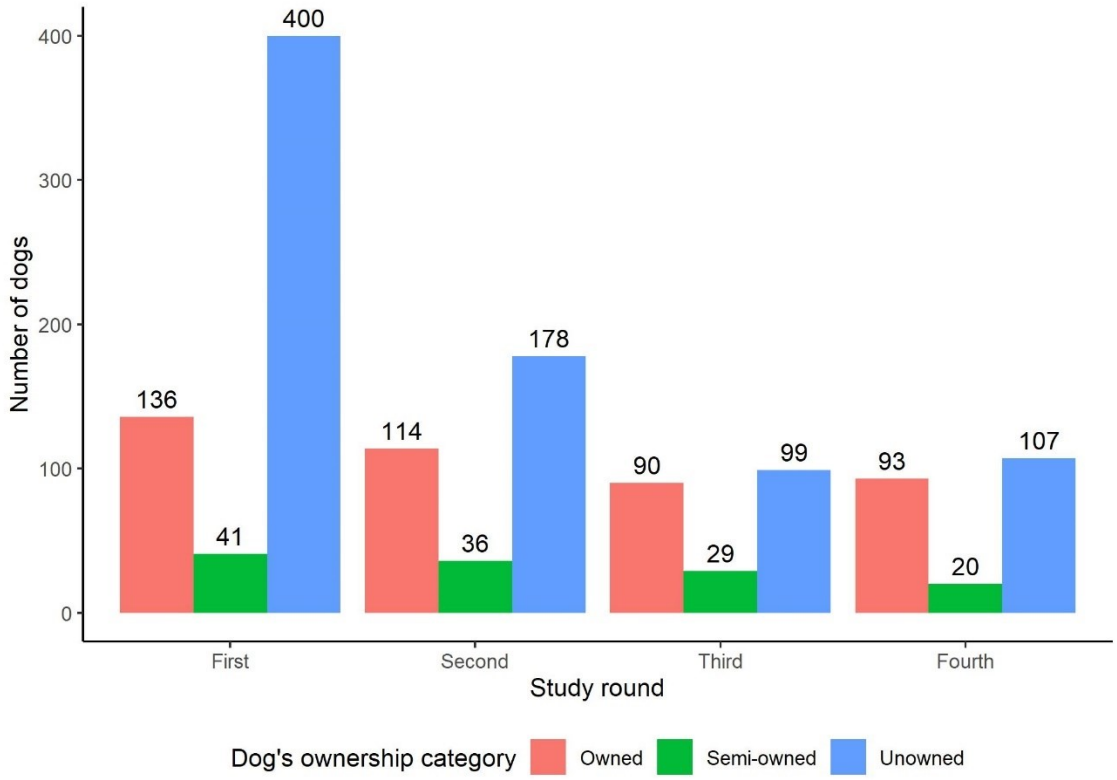

Figure 3.4 Dog captures and recaptures. The number of dogs by ownership category at both study sites that were captured in round 1 and recaptured in subsequent rounds. Numbers above bars indicate sample sizes for each category.

Of all the completely or partially FRDs that were recaptured at least once (including free-ranging ODs) (n = 340), three-quarters were captured within 100 metres of their previous capture locations (Fig S7). Nearly half or more of all dogs recorded to be sterilised in R1 were recaptured in each round. Compared to UDs with no direct human interaction, a higher proportion of UDs that directly interacted with humans (HIS of 4, 5 and 6) was recaptured in R2 and R4 ($p = 0.014$) (Table S30). Finally, most DWOs captured as part of the ABC campaign in ALP were never recaptured (Table S36).

3.3.4 Failure of microchip detection

Of 491 unique dogs across all ownership categories that were microchipped in R1, microchips could not be detected in a total of 50 dog captures across all subsequent rounds (35 unique dogs) (Table S38), representing microchip failure in 7% (35/491) of all microchipped dogs. Most of these dogs were

UDs (26/35, 74%). Among these, one UD recaptured in R2, one SOD from R3 and six UDs recaptured in R4 could not be conclusively identified based on features as described in section 3.2.2.

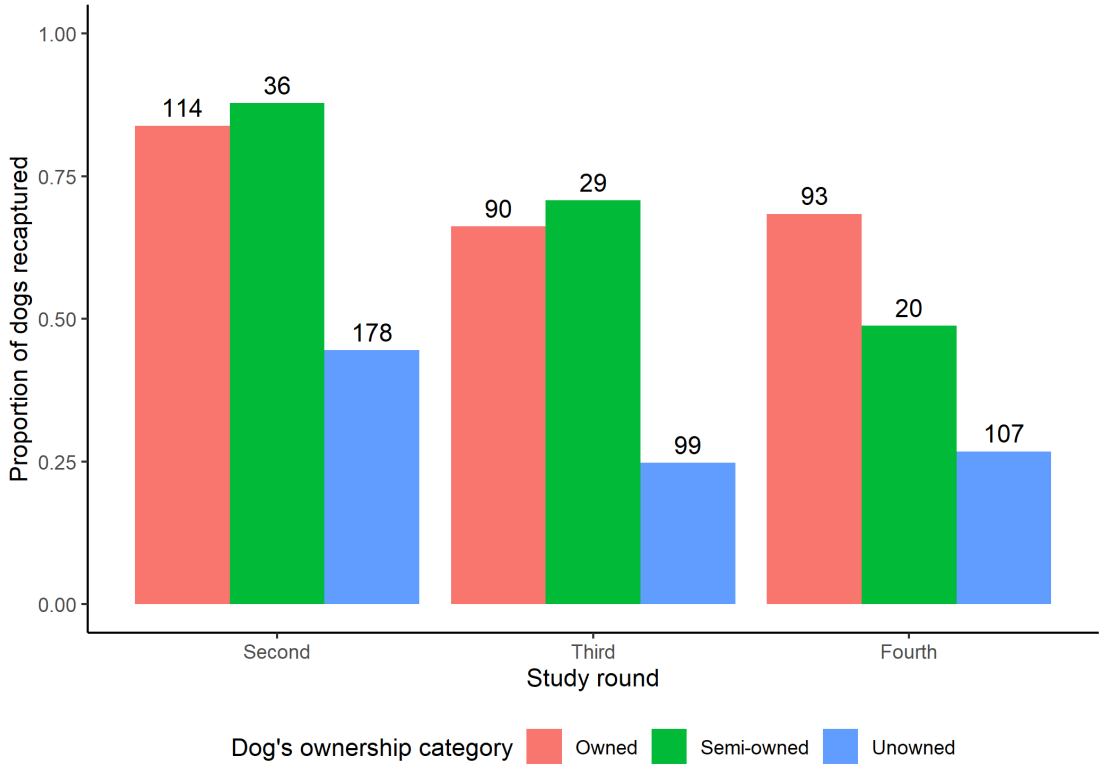

Figure 3.5 The proportion of recaptures in subsequent rounds. The overall proportions of dogs of various ownership categories captured in round 1 that were recaptured in subsequent rounds at both sites. Numbers above bars indicate sample sizes for each category.

3.3.5 Logistic regression

In the final multivariable GLMM for all dogs with unique dog identity as a random effect, recapture probability was significantly associated with ownership category, study site, vaccination history, sterilisation status in R1, pre-vaccination RVNA titre levels in R1, whether the dog was collared as part of our study in R1, whether it was captured as part of ongoing ABC campaigns in R1 as well as the study round during which dogs were recaptured (included as a fixed effect only in the GLMMs) (Tables 3.4, S57). The probability of recapture was lower in R3 (OR 0.30, 95% CI: 0.22 – 0.42) and R4 (OR 0.32, 95% CI: 0.23 – 0.45) compared to R2, holding true in the GLMMs for UDs, SODs and ODs (R3 only) (Table 3.4). UDs in particular were significantly less likely to be recaptured in later rounds compared

to ODs (OR 0.18, 95% CI: 0.07 – 0.46) or SODs (OR 0.10, 95% CI: 0.05 – 0.20, evaluated in the GLMM for UDs and SODs together, Table S61). Recapture probability was significantly lower in semi-urban MUH (OR 0.31, 95% CI: 0.16 – 0.60) compared to urban ALP for all categories of dogs and particularly for UDs and SODs in R2 and R4. Recapture probabilities were significantly higher for dogs that were recorded as sterilised when captured in R1 (OR 2.06, 95% CI: 1.23 – 3.47) and female dogs fitted with a black study collar in R1 (OR 2.08, 95% CI: 1.23 – 3.52). Compared to dogs with RVNA titres > 0.5 IU/ml prior to vaccination in R1, recapture probabilities were significantly lower for dogs with pre-vaccination RVNA titres ≤ 0.5 IU/ml (OR 0.27, 95% CI: 0.09 – 0.82) or without detectable titres (OR 0.25, 95% CI: 0.08 – 0.73). Sterilisation status and pre-vaccination RVNA titres influenced recapture probability only for UDs and SODs. Dogs whose vaccination history was unknown were less likely to be recaptured (OR 0.18, 95% CI: 0.07 – 0.49) compared to those that had never been vaccinated. Dogs included in the study when captured as part of local ABC campaigns during R1 were significantly less likely to be recaptured (OR 0.33, 95% CI: 0.19 – 0.56) compared to dogs encountered in the field.

In addition to the above predictors, the multivariable GLMM for UDs highlighted age at first capture as a significant predictor, pups being less likely to be recaptured (OR 0.20, 95% CI: 0.06 – 0.73) compared to adults (Table S58). Additional details for the various fixed and mixed effects logistic regression models, are presented in Appendix A1.

Table 3.4 Summary of statistically significant predictors and odds ratios from logistic regression analyses. Summary of dog characteristics recorded in round 1 that were predicted to significantly increase (in green) or decrease (in orange) recapture probability in future rounds (AR – All rounds; AR-ME – All rounds, Mixed effects model with unique dog identifier as a random effect). Results are presented for analyses using data for all dogs, unowned (UD), owned (OD), semi-owned (SOD) dogs and dogs without owners (DWO). Black cells indicate predictors not considered for a particular model. See Table S61 for levels used for variables. R2 – round 2 etc. ABC – If caught for sterilisation in R1 as part of animal birth control campaign; HIS – Human interaction score; MUH – Muhamma; No DHI – No direct human interaction; ≤ 0.5 – Less than/equal to 0.5 IU/ml, None – No antibodies detected.

Category of dogs	Predictor (Level(s) for which odds ratio indicated)*	R2	R3	R4	AR	AR-ME
All dogs	Sterilised (Yes)		2.10	2.13	1.77	2.06
	Ownership (UD)		0.10	0.37 SOD 0.13 UD	0.25	0.18
	Vaccination history (Unknown)	0.20			0.30	0.18
	Site (MUH)	0.27		0.26	0.40	0.30
	Rabies immunity (≤ 0.5, None)			0.21, 0.22	0.31, 0.29	0.27, 0.25
	ABC (Yes)	0.33	0.39	0.49	0.45	0.33
	Collared (Yes - black collar)		2.35		1.70	2.08
	Study round (R3, R4)					0.30, 0.32
UD	Age (Pup)				0.37	0.20
	Sterilised (Yes)		1.83	2.57	1.72	2.12
	Site (MUH)	0.32		0.23	0.40	0.26
	Rabies immunity (≤ 0.5, None)			0.08, 0.09	0.26, 0.26	
	ABC (Yes)	0.31	0.36		0.40	0.27
	Study round (R3, R4)					0.27, 0.28
OD	Age (Pup)			0.19		
	Vaccination history (Unknown)	0.17			0.28	0.20
	Site (MUH)			0.14		
	Study round (R3)					0.37
SOD	Study round (R3, R4)					0.18, 0.06
DWO (UD and SOD)	Sterilised (Yes)		2.02	2.17	1.85	2.56
	Coat (Good)	5.19				
	Ownership (UD)		0.31	0.37	0.51	0.10
	Site (MUH)	0.21		0.30	0.51	0.31
	Rabies immunity (≤ 0.5, None)			0.23, 0.23		0.20, 0.20
	ABC (Yes)	0.36			0.65	0.32
	HIS (No DHI)	0.45				
	Study round (R3, R4)					0.30, 0.27

*Reference levels: ABC – No; Age – Adult (incl. Aged); Coat – Poor; Collared – Not collared; HIS – Direct human interaction; Ownership – OD or SOD (for models of UD and SOD together); Rabies immunity – > 0.5 IU/ml; Site – ALP; Sterilised – No; Study round – Second (R2); Vaccination history – Never vaccinated

3.4 DISCUSSION

In this chapter we highlight a number of characteristics of a mixed dog population in India that have important implications for long-term efforts to control dog rabies and improve the effectiveness of DPM measures such as ABC.

We present conclusive evidence to show that only a small proportion of FRDs (16% if including partially free-ranging ODs) are owned. These results agree with similar reports from other parts of India [45,76]. We assessed ownership status directly by consulting members of the public and are therefore reasonably confident of having conclusively established the ownership status of most FRDs. Additionally, as most free-ranging ODs at these sites did not have a pre-existing collar on them, this latter characteristic cannot be considered a reliable indicator of ownership among FRDs in India [76]. The proportion of owned FRDs will vary across India depending on factors like community tolerance for FRDs, dog ownership levels, availability of food and shelter and human population density. Nevertheless, it is unlikely to be as high as levels reported for FRDs from parts of Africa, Latin America and Asia [16,269]. Our findings echo previous observations [33] that central point and door-to-door vaccination, commonly adopted in Africa and parts of Asia, are not feasible strategies in India for achieving the high vaccination coverages required for canine rabies control and elimination.

We identified SODs as comprising a small but highly accessible fraction of the FRD population that can, and should, be readily targeted by MRV and DPM campaigns. Rabies infected dogs can shed virus in their saliva up to three days before the onset of clinical signs [270]. Dogs displaying the paralytic form of rabies may easily be overlooked as being affected by other illnesses [271,272] and handled by people. Given the extent of their direct interactions with people, SODs can thus potentially pose greater risks of zoonotic rabies transmission than UDs. A comparatively higher proportion of SODs was observed to be reproductively active and many female SODs and their pups were actively cared for by their reference persons. SODs may therefore disproportionately contribute to maintaining the FRD population as their pups are likely to have increased survival, compared to pups of UDs.

Logistic regression models indicated that UDs, and UD pups in particular, have significantly lower recapture probabilities compared to ODs or SODs. Previous studies have reported high levels of early life mortality among owned [26,247] and unowned [248] free-ranging pups. Even after intentionally including only 21 UD pups in R1, we could recapture only four pups in R2 and one each in R3 and R4. Nine UD pups either died or were lost to the study due to unknown reasons within 60 days of first capture. The large ALP dog population is frequently targeted by dog catchers seeking to achieve sterilisation targets for ABC campaigns when they struggle to capture dogs in other areas. The constant presence of dog catchers in ALP thus means that FRDs recognise nets (and likely, the dog catchers and/or their vehicles), often advertise these threats to their neighbours by barking or howling and learn to evade capture more effectively. As the study progressed, we observed that UDs were warier of dog catchers and increasingly difficult to recapture. The proportion of UDs trying to escape from dog catchers increased consistently in each subsequent round, reaching 80% of captured UDs in R4 (Fig S8).

We also highlight the additional effort required to capture dogs outside of urban locations. MUH is a semi-urban panchayat with a lower human population and thus likely a smaller baseline dog population than ALP, possibly accounting for why fewer dogs were captured in MUH in R1. The low recapture proportions at this site may partly be explained by higher mortality rates reported in dog populations from semi-urban locations [248]. The few UDs that were recaptured in MUH were determined to interact directly with humans, which may have made them easier to recapture. Alternately, such UDs may have increased survival through better access to resources. Other reasons for the low recapture proportions may include migration (e.g. juvenile dispersal) and failure to identify study dogs due to loss of study collars (or absence of collars in the case of pups, juveniles and some adults which were not collared). However, an additional challenge in MUH was the availability of wide-open spaces offering more opportunities for dogs to escape from dog catchers, including ODs, all of which were free-ranging. Only four of the 62 dogs captured in R1 from MUH were recaptured in all

future rounds (Table S14), similar to figures reported from rural villages in Maharashtra [47]. These challenges may also account for the very low percentage of sterilised dogs (8%) observed at this site.

We found that less than half of all UDs interacted directly with humans and so were readily accessible for vaccination. Logistic regression models did not identify this characteristic as a predictor of recapture probability for UDs, possibly because most UDs were never recaptured and hence not assigned a HIS. We found that DWOs with no direct human interaction had significantly lower probability of recapture only in R2. Net capture and handling by strangers can be stressful for FRDs, so it may be expected that FRDs with direct human interactions would be more easily recaptured soon after being first captured. However, the extent of individual dogs' interactions with humans may not have been accurately assessed, particularly for those recaptured only once after R1. Nevertheless, the HIS proved a useful population-level measure to determine accessibility of DWOs for interventions.

Over 90% of all FRDs were in ideal body condition, overweight or obese, irrespective of their HIS and across all study rounds, suggesting that dogs have ready access to food throughout the year. Similar findings have been reported from North India [64] and Bhutan [36]. ALP is a popular Indian tourist destination with numerous restaurants, particularly along the popular beachfront where a large proportion of dogs were captured. Food waste management is a persistent problem throughout Kerala and most of India [273] and FRDs regularly source food from garbage [64]. Food waste generated from restaurants, slaughterhouses, and fish stalls in ALP and MUH all provide a ready food source to FRDs. We found a relatively stable FRD population around such food sources at both study sites. Contrary to reports from other parts of India [252,274], almost none of the respondents of a household survey conducted at these sites reported directly feeding FRDs, identifying the aforementioned food sources instead (Sreejith Radhakrishnan, unpublished data). However, there were two individuals in ALP who were avowed dog-lovers, owned one or two dogs themselves and regularly fed several FRDs, sometimes in the face of local opposition. They had close bonds with and could be considered 'reference persons' for many FRDs, often facilitating their capture. Such

individuals can be invaluable partners in MRV campaigns and identifying and building trust with them should be an important consideration while planning campaigns. At the same time, such individuals should be made to understand how they exacerbate the problem of FRDs by subsidising these dogs. They should also be incentivised to take responsibility for ensuring that these dogs are vaccinated against rabies and/or sterilised.

Unowned dogs and SODs that were recorded as being sterilised in R1 (based on the presence of an existing ear notch) had significantly higher probability of being recaptured. Owing to the ABC campaign conducted during R1, the proportion of recaptured DWOs that was sterilised also rose consistently in each future round (Fig S1). These high recapture probabilities may be due to increased survival and/or reduced roaming among sterilised dogs. Sterilised dogs, particularly male UDs, were also more likely to be overweight or obese and we occasionally had to remove collars from dogs that had gained weight post-sterilisation. Substantial evidence exists for increased lifespan, weight gain and reduced roaming particularly in sterilised companion dogs [253,275], but a study of sterilised FRDs did not find any reduction in roaming behaviour [276]. Certain UDs may also be easier to capture, thus being among the first dogs to be sterilised during regional ABC campaign and frequently recaptured later on. However, UDs from ALP that were sterilised as part of an ongoing ABC campaign in R1 had significantly lower probabilities of recapture in all later rounds. The stress of capture and sterilisation may make dogs more skittish and even lead to increased post-release dispersal, making them more difficult to recapture. It is also possible that sterilised dogs without collars and non-detectable microchips may have been excluded from our data. While there was no evidence for poor handling or care of UDs/SODs captured for ABC campaigns during this study, post-release mortality was not assessed in this cohort. In regions where high post-sterilisation mortality of FRDs is suspected, studies to identify its causes may be warranted as this is an important animal welfare issue.

Multivariable logistic regression models also identified pre-vaccination RVNA titre levels as a significant and consistent predictor of recapture probability among UDs and SODs. Dogs with non-

detectable RVNA or titres ≤ 0.5 IU/ml had significantly lower recapture probability compared to dogs with titres > 0.5 IU/ml (Table 3.4), even though all dogs in this study were vaccinated against rabies in R1. Additionally, in univariable logistic regression models, dogs with no detectable RVNA titres were less likely to be recaptured compared to those with detectable titres. We also detected RVNA in 20 ODs with no history of vaccination, 17 of which were partially or completely free-ranging. This included one free-ranging juvenile with titres > 0.5 IU/ml, suggesting non-fatal exposure to rabies. These findings are discussed in detail in Chapter 4. There are frequent reports of rabid/suspected rabid dogs biting multiple people in ALP [277,278]. The detection of high RVNA titres in UDs and SODs suggests ongoing rabies virus circulation in the dog population in ALP. Further, dogs that survive rabies infection and develop adequate titres may live longer [40], particularly if subsequent vaccination induces effective anamnestic responses as identified recently [279]. Given the increasing evidence for non-lethal rabies infection in various wild and domestic species [280], the potential implications of non-fatal rabies exposures for survival and rabies control in FRDs requires further research.

We found concerning evidence of poor DOP at both study sites with a direct bearing on rabies control and DPM efforts. We only vaccinated ODs and collected data on DOP when requested by owners at vaccination camps and in the field or when dogs were captured in the field and subsequently confirmed to be ODs. We were also not able to conduct vaccination camps, and therefore excluded several ODs, in MUH. Therefore the 136 ODs included in R1 are not representative of the overall OD population at either site. Nevertheless, our data show that a substantial number of ODs, and consequently their owners and the people around them, are at risk of rabies exposure. Failure to detect RVNA or detection of only low titres in vaccinated ODs may occur due to owners' failure to follow recommended vaccination schedules and/or the use of poor quality or improperly stored vaccine [281]. Poor DOPs were more common for non-descript ODs whereas higher proportions of pure or crossbreed ODs received better levels of care and had vaccination certificates. These differences highlight the relatively higher value placed on purebreed dogs, with many being reared for commercial breeding and sale of pups. The level of care offered may also have influenced the

likelihood that an OD would be resampled in later rounds, as ODs with fair or good coat condition had significantly higher probability of recapture one year later in R4, while those whose vaccination history was unknown were significantly less likely to be recaptured. Our findings are similar to those of another study from North India [242] which reported that only 40% of dog-owning households demonstrated responsible pet ownership. Such poor vaccination coverage places even completely confined dogs at risk, as rabid dogs are known to enter homes and attack owned animals, as was reported during household surveys in MUH (Sreejith Radhakrishnan, unpublished data). Pups born to these free-ranging ODs are likely to have higher survival than those of UDs or SODs [282] and will eventually become part of the FRD population, exacerbating this problem.

Dog abandonment, even of expensive purebreeds, occurs commonly in Kerala [283]. Local residents of ALP often recounted such incidents, particularly along the beachfront [284], and some previously unknown FRDs were observed at some locations in ALP during R3 and R4. Some dog owners even privately admitted to having tried to abandon or successfully abandoning ODs, including two from our study. Reasons for abandonment included financial difficulties, unresolved illnesses in their dogs or multiple unwanted pregnancies. This latter problem may explain the substantial public support observed for ABC campaigns and even some free-ranging ODs at both sites were sterilised during regional ABC campaigns on their owners' requests.

However, these field observations contrasted with the higher levels of responsible DOP reported by dog owners in household surveys (Sreejith Radhakrishnan, unpublished data). This incongruence may be due to a lack of representativeness of the sample of ODs from the field study. Insufficient awareness of the importance of responsible DOP and/or poor enforcement of existing legislation may also be contributing factors. In informal conversations even elected representatives at our study sites admitted that they had not registered ODs with the local authority, despite this being mandatory [242,285]. Additionally, dog owners may be unable or unwilling to fulfil their responsibilities but not admit to this in surveys for fear of penalties. Access to veterinary care can be an important limiting

factor in ensuring timely rabies vaccination coverage in ODs. Nearly all local bodies in Kerala have only one government veterinary clinic providing free or subsidized services (including rabies vaccines) for the entire region and transporting animals to the clinic can be difficult and expensive. As in most parts of Kerala, animal owners depend on private house visits by veterinarians, which are rarely provided free of charge. Nearly all ODs included from ALP in this study were from households located several kilometres from the closest veterinary clinic (data not presented) and most were non-descript dogs. Most households with makeshift fences or walls did not confine their ODs. Owners confirmed having difficulty in accessing veterinary care and readily permitted their dogs to be vaccinated in R1 or revaccinated in R4. There is therefore an urgent need to substantially widen access to veterinary services in India, particularly rabies vaccination and perhaps even sterilisation for ODs.

The findings of our study have important implications for program managers and policymakers who must consider various factors affecting the feasibility, sustainability and effectiveness of DPM and rabies control efforts. These considerations are especially important when campaigns target large populations of FRDs that are not owned (estimated at 60 million in India) [31]. ABC campaigns are conducted throughout India by multiple public and private entities, in the absence of any MRV policy. Most campaigns fail to sterilise any substantial fraction of the FRD population, which is regularly replenished through births, migration and abandonment of ODs and often has ready access to a variety of food sources [252]. Even if effectively implemented, most ABC campaigns will require at least a few years before impacts become evident [69,76]. All will involve the use of stressful capture methods such as net capture with butterfly nets. Campaigns must therefore be implemented in an efficient and targeted manner focusing on capturing as many dogs as possible in the shortest time frame and ensuring that dogs are handled appropriately (e.g. avoiding net capture for dogs amenable to hand restraint). Failing this, extended campaigns that capture only a small fraction of the extant dog population will almost inevitably alter FRD behaviour such that they become increasingly difficult to capture. Such behavioural alterations can make it nearly impossible to achieve and/or maintain high vaccination/sterilisation levels, even with substantial investment in training adequate numbers

of dog catchers. In this respect, the widespread ABC campaigns currently being implemented across India could prove to be counterproductive for efforts at implementing MRV [69].

These challenges may be greater in semi-urban or rural areas with relatively smaller FRD populations and/or more open spaces, where physical capture of FRDs becomes more difficult. Current ABC campaigns are haphazardly implemented and largely focus on the large dog populations in urban areas [241] and few, if any, sterilise FRDs in rural regions, which can act as a source of reproductively active dogs replenishing urban dog populations. Patchy vaccination coverage even within small fractions of a large geographic area has also been shown to jeopardize the effectiveness of otherwise comprehensive rabies control programmes [286]. In such areas, alternative modes of vaccination such as oral rabies vaccines [71,287] and the use of safe, effective and long-acting oral contraceptives (currently not available for use in FRDs) or other non-surgical means [266,275] may need to be considered.

On the other hand, campaign managers also need to be aware that certain dogs are more easily catchable than others, as highlighted in this study by the higher recapture probabilities for sterilised dogs and SODs. As pointed out elsewhere [21], such 'easy-to-reach' dogs may be repeatedly vaccinated during campaigns and inflate vaccination coverage estimates while contributing no additional population-level immunity. In the absence of reliable dog population estimates, these risks may also apply when ABC coverage is calculated for newly covered regions.

Our findings provide empirical evidence for the extensive challenges facing effective rabies control in India. The large population of unowned FRDs in India and the difficulties in capturing them pose significant financial and logistical challenges for achieving the 70% vaccination coverage required for rabies elimination. In June 2021 the government of Goa, the smallest Indian state with one of the lowest human populations [288], announced that no human rabies deaths had been recorded in the state since September 2017, declaring itself a 'rabies-controlled' state [289,290]. MRV campaigns in Goa are conducted by an international charity (Mission Rabies), with financial and logistic support

from the state animal husbandry department [241]. While it is unclear to what extent such MRV campaigns have contributed to the elimination of human rabies deaths in Goa, replicating this model in larger Indian states, many with populations larger than several countries, will be a challenging undertaking. Even if campaigns are successfully implemented, maintaining high vaccination coverages will also be a challenge if large FRD populations persist. Rabies control in India will therefore require a multi-pronged approach incorporating enhancing public awareness about and enforcement of responsible DOP, widening access to affordable veterinary services, implementation of regional and national MRV campaigns, effective dog population and civic waste management. Local / state governments will also need to reconsider approaches to canine rabies control and DPM, tailoring efforts to regional ground realities. In the absence of systematic efforts to reduce the FRD population in India, future MRV campaigns will be unsustainable in the long term. Furthermore, disruptions to the conduct of these campaigns may quickly reverse any gains made in controlling rabies [23,24]. For example, Peru has reported a rise in canine rabies cases after MRV campaigns and rabies surveillance activities were disrupted due to the COVID-19 pandemic [23].

From a wider one health perspective, research is also warranted to identify barriers to access to veterinary services and means of providing these, free-of-cost or at subsidized rates, particularly for underserved communities. Such subsidized veterinary services have been shown to enhance uptake of canine rabies vaccination and improve animal and human welfare [291]. Understanding what drives 'animal lovers' and the general public to feed FRDs [252] may enable policymakers to incentivise more responsible attitudes towards them.

Finally, the results of this study provide useful information on demographic characteristics of dog populations such as accessibility for vaccination, which are used in chapter 5 to parameterise compartmental models of rabies transmission.

Further studies across Kerala and India will be needed to identify features of dog populations and DOP that demand targeted interventions. The nature of the dog populations varies widely across India, in

keeping with the diversity of cultural and religious beliefs, socio-economic conditions and demographic factors. Studies within regional contexts of the complex interactions between dog demography and disease ecology and human-dog interactions in rabies endemic regions are critical to facilitate precision public health interventions for disease control and elimination [5,84]. Notwithstanding the lack of prioritisation and resourcing of rabies surveillance, a greater understanding of these aspects is key to implementing effective rabies control strategies in India [62].

Chapter 4 : Pre- and post- vaccination rabies virus neutralizing antibody dynamics in free-ranging and owned dogs in Kerala, south India

ABSTRACT

Mass rabies vaccination (MRV) of dogs is a key component of the 'Zero by 30' campaign to eliminate dog-mediated human rabies deaths by 2030. However, only two studies have investigated post-vaccination rabies virus neutralizing antibody (RVNA) responses in dogs without owners (DWOs), which comprise the vast majority of the free-ranging dog (FRD) population in India. We conducted a longitudinal serosurvey in a mixed population of DWOs and owned dogs (ODs) over 16 months at two sites in India. Pre-vaccination blood samples were collected and dogs were vaccinated against rabies in round 1 (R1) and data on individual dog characteristics and DOP were collected. Dogs were further resampled at approximately 30 (R2), 150 to 180 (R3) and 365 days (R4) post-vaccination to assess RVNA dynamics and factors affecting these.

Most dogs had no detectable RVNA titres in R1, but approximately 26% (95% CIs: 22 – 31%) were seropositive with titres ≥ 0.23 IU/ml. Over 80% of vaccinated dogs recaptured in R2 developed titres > 0.5 IU/ml, irrespective of age or prior vaccination history. Titres declined in R3, although at a faster rate for ODs and completely/partially confined dogs compared to DWOs and completely FRDs. Mixed-effects linear regression models identified significant associations between post-vaccination RVNA titres and age at vaccination, sterilisation status and RVNA titre levels in R1 and whether dogs had been captured for ABC campaigns in R1. The mean maximum likelihood estimate of the per capita rate of decline in titres was 0.005, translating to titres dropping below 0.5 IU/ml approximately 200 days (95% CI: 167 – 256 days) after achieving peak titres. We also report evidence suggesting the occurrence of non-lethal rabies infections in FRDs in India. MRV continues to be the most effective rabies control method; however, the implementation and frequency of MRV campaigns must be tailored to differential immune responses to vaccination among regional dog populations. Equally crucial will be ensuring the use of high-quality rabies vaccines that have been stored properly.

4.1 INTRODUCTION

4.1.1 Background

The global campaign 'Zero by 30' was jointly formulated by the WHO, the FAO and the OIE with the ambitious goal of eliminating dog-mediated human rabies deaths by 2030 [292]. One of the key pillars of the campaign is the use of MRV of dogs with the aim of reducing risks of human rabies exposure [22]. Empirical and theoretical studies have shown that achieving and maintaining vaccination coverages of 70% or more in the dog population can eventually lead to local rabies elimination [25,26] and reductions in human rabies deaths [27]. The systematic implementation of coordinated MRV campaigns over three decades across the Americas was central to eliminating human rabies in the region [21].

As pointed out in chapter 3, achieving such high vaccination coverages can be a challenge in countries such as India where most FRDs have no owners and therefore are not readily accessible for vaccination. Another important consideration is establishing that such FRDs respond effectively to vaccination by developing RVNA titres above 0.5 IU/ml, the baseline established to indicate adequate seroconversion [5], and maintain those levels until the next phase of an MRV campaign.

While several studies have evaluated post-vaccination RVNA responses in dogs (Table 1), most have focused on ODs, particularly those that are confined. Only a few studies have focused on free-ranging ODs or community dogs [48,250,293–295]. Two studies, one from Peru [293] and one from Indonesia and South Africa [48], found that over 90% of free-ranging ODs developed post-vaccination RVNA titres > 0.5 IU/ml. In the former study 97% of dogs tested one year after vaccination maintained titres above this level, with the vast majority of these being previously unvaccinated dogs. In contrast, in the latter study only 60-80% of ODs sampled one year post-vaccination had titres at this level. A separate study from Indonesia reported that previously unvaccinated free-ranging ODs administered only one dose of vaccine were significantly more likely to lose adequate binding antibodies [250]. A study from Sri Lanka reported similar findings, although the confinement status of the ODs was not specified [54].

Table 4.1 Studies reporting post-vaccination RVNA titres in dog populations. Studies that reported post-vaccination rabies virus neutralizing antibody titres in dog populations, based on study location, ownership category of dogs (OD – owned dogs, DWO – dogs without owners), type of vaccine used (CCV – cell culture vaccine), method used for titre estimation (ELISA – Enzyme linked Immunosorbant Assay; FAVN – Fluorescent Antibody Virus Neutralization test; HA-HI – Haemagglutination – Inhibition test; MNT – Mouse Neutralization test; RFFIT – Rapid Fluorescent Focus Inhibition Test) and time period in which the study was conducted

Sl. No.	Location	Category of dogs	Type of vaccine used	Method used	Time period of study	Reference
1.	Bolivia	OD	Suckling mouse brain vaccine (government manufactured)	ELISA	2007	[296,297]
2.	India	OD	Unspecified	RFFIT	2012	[281]
3.	India	OD, DWO	Unspecified	ELISA	2018	[298]
4.	Indonesia	Free-ranging OD	Rabisin – commercial CCV	ELISA	2018-2019	[250]
5.	Indonesia, South Africa	Free-ranging OD	Rabisin – commercial CCV	FAVN	2010	[48]
6.	Italy	OD	Unspecified	FAVN	2006-2012	[299]
7.	Japan	OD	Unspecified CCV	RFFIT	1999-2000	[300]
8.	Kenya	Free-ranging OD	Rabisin – commercial CCV	FAVN, ELISA	2019	[279]
9.	Mali	OD	Rabisin, DogVac-R, Hexadog – commercial CCVs	FAVN	2010-2011	[301]
10.	Nepal	OD	Defensor, Biocan R – commercial CCVs; NeJa Rab – government manufactured CCV	ELISA	2017	[302]
11.	Peru	Free-ranging OD	Rabisin – commercial CCV	RFFIT	1985	[293,303]
12.	South Africa	OD	Defensor 3 – commercial CCV	FAVN	2016-2018	[304]
13.	Spain	OD	Unspecified commercial CCV	ELISA	1993-1994	[305]
14.	Sri Lanka	OD, DWO	Nobivac Rabies – commercial CCV	RFFIT	2014-2015	[54]
15.	Sweden	OD	Nobivac Rabies, Rabisin – commercial CCVs	FAVN	2005	[306]
16.	Tanzania	Free-ranging OD	Rabisin – commercial CCV	RFFIT, ELISA	1993-1996	[294]
17.	Thailand	OD	Rabdomun – commercial CCV	RFFIT	1989-1990	[307]
18.	Tunisia	OD	Rabisin – commercial CCV, Rabirabta – government manufactured suckling lamb brain vaccine	RFFIT	1987-1994	[308]
19.	Tunisia	OD, Dogs in research facility	Rabisin – commercial CCV	RFFIT, ELISA	Not specified	[309]
20.	UK	OD	Unspecified commercial vaccines	FAVN	1999-2002	[310]
21.	UK	OD	Unspecified commercial vaccines	FAVN	2002	[311]
22.	USA	OD	Unspecified	FAVN	2006-2010	[312]
23.	USA	Dogs in research facility	Continuum Rabies, Imrab-TF – commercial CCVs; PureVax – live canarypox vector vaccine	RFFIT	Unspecified	[313]
24.	USA	OD	Rabdomun – commercial CCV	RFFIT	1991-1992	[314]
25.	Zambia	OD	Rabisin, Rabies Vet, Rabigen-mono – commercial CCVs	FAVN	2015	[315]

More importantly, only two studies have reported post-vaccination RVNA titres in 'stray' or unowned FRDs [54,298] (Table 4.1). Pimburage et al. (2017) reported that most dogs without owners (DWOs) in Sri Lanka failed to maintain RVNA titres > 0.5 IU/ml one year after receiving a single dose of rabies vaccine [54]. Nale et al. (2021) reported that only a third of vaccinated 'stray' dogs in Mumbai, India had titres > 0.5 IU/ml six months after vaccination [298]. This represents a major gap in the literature, as DWOs are very likely to have limited access to resources and care compared to ODs, as well as increased exposure to stressors such as infectious and non-infectious disease, physical injury, adverse environmental conditions, and inter-specific competition for resources. Such stressors can cause immune dysfunction that impacts on response to vaccination [316] resulting in failure to seroconvert, lower antibody titres or shorter duration of immunity. All these factors can cause rapid declines in herd immunity, necessitating high initial vaccination coverages and/or more frequent MRV campaigns [249].

In addition to a history of previous vaccination, response to vaccination and immune dynamics have been shown to be influenced by a wide range of intrinsic and extrinsic factors. Important intrinsic factors include age [53,304,306,310,311], sterilisation status [281,310,311], general health and body condition [48,250] and breed [281,306,311]. There can also be substantial individual variation in responses to vaccination [48,317]. Morters et al. (2017) reported development of RVNA titres > 0.5 IU/ml in all pups below three months of age born to free-ranging ODs and administered a single dose of vaccine [53], showing that maternal antibodies did not interfere with development of post-vaccination titres in pups. At the same time, several studies have consistently shown that young dogs below one year of age develop lower titres than adults after one dose of vaccination [306,310]. Pimburage et al. (2017) reported that nearly 80% of pups and 90% of juveniles had titres > 0.5 IU/ml when tested six months later, with these proportions dropping to less than 50% when titres were assessed one year after vaccination [54]. Sterilised ODs had significantly higher titres at levels > 0.5 IU/ml compared to non-sterilised dogs [281,310,311]. Large dog breeds were more likely to have titres < 0.5 IU/ml in a study from the UK [311]. A study from Sweden found that small dogs and mixed breed

dogs were more likely to have titres > 0.5 IU/ml compared to larger and purebreed dogs [306]. In contrast, a report from India found that non-descript ODs had lower titres compared to purebreeds [281]. Owned FRDs with poor BCS were twice as likely to lose adequate levels of rabies binding antibodies when assessed using ELISA kits, compared to those with good BCS [250]. Lactating females and dogs with clinical signs of illnesses such as generalized dermatitis have also been found to have significantly lower RVNA titres [48].

Extrinsic factors that can have an impact on estimation of RVNA titres include the interval between vaccination and post-vaccination blood sampling [310–312], vaccine quality [306,310,311,317,318], proper storage of vaccine under cold-chain conditions [281,317,319], the route of vaccine administration [317,320] and country of origin of animals [299,310]. Antibody titres rise rapidly after primary vaccination before peaking and then declining at a slower rate [317], suggesting that blood sampling too early or too late after vaccination could lead to detection of low titres and spuriously concluding that dogs are failing to seroconvert. Wallace et al. (2017) reported that peak RVNA titres after primary rabies vaccination were expected to occur around 12-18 days post-vaccination, dropping below 0.5 IU/ml at 160 days after vaccination [312]. A number of studies have shown that sampling dogs approximately four weeks after vaccination ensured the lowest rates of failure to achieve recommended titre levels [310,311]. These latter studies and others also identified significant differences in immunity induced by different vaccines [320]. Poor canine rabies vaccine quality was determined to be an important factor contributing to the high dog rabies incidence in China [318]. Maintenance of vaccine under cold-chain conditions is a constant challenge in low- and middle-income countries with direct impacts on vaccine quality and induction of immunity [281,317], necessitating the use of more thermostable vaccines [319] and low-cost technologies for cold-chain maintenance [321]. Administration of rabies vaccine by the subcutaneous route has been shown to induce lower titres compared to intramuscular administration [317,320], although the use of high potency vaccines has since reduced these differences [317]. Dogs imported into the UK from Australia and New Zealand were reported to be more likely to fail antibody titre tests compared to dogs from the UK [310].

Similarly, higher failure rates were reported for dogs brought into Italy compared to those vaccinated within the country [299].

An additional factor that can impinge on both dog survival and response to vaccination is the stress experienced during ABC campaigns. A number of studies have pointed out the benefits of sterilisation as part of ABC for DPM and protection from rabies through concurrent vaccination [232,253,322,323]. This intervention is now the primary means of rabies control in India, especially in the absence of MRV campaigns [1]. However, it is unclear whether there are any differences in antibody responses among dogs captured as part of ABC campaigns, compared to those vaccinated and immediately released back into the field.

At the same time, it has been argued that there is no absolute protective level of antirabies immunity [317,320]. Animals with high RVNA levels have been known to succumb to challenge with rabies virus, while previously vaccinated dogs with no detectable titres at the time of challenge survived [317]. Vaccine-induced immunity has also been shown to persist for several years in the absence of detectable titres. Under experimental conditions, dogs with RVNA titres > 0.1 IU/ml had a 100% survival rate on challenge with rabies virus [317]. Dodds et al. (2020) reported that of five dogs administered two doses of rabies vaccine nearly seven years ago, four survived experimental virus challenge, while all unvaccinated dogs in the control arm died [313]. Thus absence of detectable RVNA titres in previously vaccinated dogs, or even titres below 0.5 IU/ml, does not necessarily indicate that such dogs are not protected against rabies infection. However, as pointed out by Aubert (1992), dogs with detectable RVNA prior to challenge, or those that seroconverted post-vaccination, have the highest probability of surviving challenge with rabies virus [317].

There is also increasing evidence for the occurrence of non-fatal rabies infections in a wide range of species, including dogs [280]. This phenomenon has primarily been identified by RVNA detection in animals with no known vaccination history, presumably due to a sub-lethal exposure to rabies virus. It is unclear whether such sub-lethal exposure results in the development of clinical signs of rabies,

from which animals subsequently recover after mounting an immune response, or if they mount immune responses without ever developing clinical signs. While difficult to study under natural conditions, insights from experimental rabies virus challenge studies in unvaccinated dogs suggest the occurrence of both these phenomena [317]. Manickam et al. (2008) reported that four unvaccinated dogs in the control arm of a vaccination study survived challenge with rabies virus for up to 90 days, by which time point all had developed RVNA titres > 0.5 IU/ml [324]. While six other dogs in the control arm displayed clear signs of the furious or paralytic forms of rabies and subsequently died, the four that survived showed only transient neurological signs such as incoordination or restlessness which disappeared after about a week. All four tested negative for virus in brain smears [324]. In another study, Fekadu et al. (1992) reported that unvaccinated dogs survived challenge with street rabies virus without showing any clinical signs of rabies or developing RVNA titres. When these dogs were challenged again two years later, they developed high anamnestic RVNA titres and did not develop disease [325]. A recent study of unvaccinated ODs in Kenya found evidence for anamnestic immune responses four to five days after primary rabies vaccination, suggesting the presence of pre-existing immunity, possibly from non-fatal rabies infections [279].

Virus neutralization tests are recommended as gold standard tests for assessment of RVNA titres [5]. The two approved tests for RVNA titre estimation in canine serum are the Fluorescent Antibody Virus Neutralization (FAVN) test [326] and RFFIT [5]. Both these tests measure antibody levels in serum by assessing the extent to which rabies virus in cell culture is neutralized by RVNA in test serum. The results of these tests may be affected by non-specific inhibitors in serum which can cause false positive results, particularly at low serum dilutions [327]. Similarly cross-reactive antibodies, other immune-modulating proteins or factors in serum that causes cell death can also lead to inaccurate or false positive test results [328]. Few studies have reported serum quality characteristics or explored their association with test results [48]. Additionally, these tests are expensive to perform, requiring several days before results can be made available and involve handling live rabies virus by well-trained staff

[329]. As a result, assessment of post-vaccination RVNA titres to establish seroconversion after a MRV campaign is not recommended as a routine procedure by the WHO [5].

However, given the limited evidence base for immune responses to rabies vaccination in DWOs, such studies are critical in identifying the factors that can maximise the impacts of MRV campaigns. They can aid public health authorities in rabies endemic countries develop the confidence to implement MRV campaigns. We report the findings of a longitudinal serosurvey conducted in a mixed population of ODs and DWO at two locations in India. These findings include factors influencing pre-vaccination RVNA titre levels, post-vaccination responses and estimates of the duration for which titres persisted above 0.5 IU/ml, estimated from serum samples collected up to 180 days post-vaccination.

4.1.2 Aims

This component of the longitudinal field study in Kerala was conducted to:

a. Evaluate the prevalence of RVNA in FRDs and ODs and relate them to known history of vaccination;
b. Evaluate response to vaccination and identify factors influencing the development and persistence of titres in these populations;
c. Explore influence of serum quality factors (haemolysis and turbidity) on measured titres, and
d. Estimates rates of decline in titres, and identify factors influencing these rates.

We discuss the implications of our findings for the design and implementation of MRV campaigns in India.

4.2 METHODS

4.2.1 Data collection

Full details of the study sites and fieldwork methodology are provided in Chapter 3.

Briefly, this longitudinal study was conducted in four rounds (Table 3.1) between October 2018 and January 2020 at two locations in Kerala state, south India - an urban municipality (ALP) and a semi-

urban panchayat (MUH) (Figs 3.1 and 3.3, chapter 3). In R1, dogs included in the study were captured in the field (UDs, SODs and ODs), encountered at vaccination camps or during household visits (ODs) or those captured for local ABC campaigns in ALP (UDs and SODs). A pre-vaccination (day zero) blood sample was collected from each dog to assess RVNA titres, after which they were vaccinated against rabies and microchipped/collared (Fig. 3.2, chapter 3) where necessary. Individual dog details (age, sex, sterilisation status, BCS, HIS etc., photographs and GPS locations) and in the case of ODs, information on DOP relevant to rabies control (confinement and vaccination history), were also collected (Appendix A1, Table S1), after which dogs were released back to their environment or returned to their owners. Sterilisation status was used as a marker of prior rabies vaccination, as ABC campaigns vaccinate dogs during the sterilisation procedure.

Further blood samples, as well as data, were collected from as many dogs as possible of this cohort to assess levels and persistence of RVNA approximately 30 days (R2), 150 – 180 days (R3) and 365 days (R4) after being first vaccinated for the study. All procedures on ODs were conducted after obtaining informed consent from owners.

4.2.2 Vaccination and estimation of RVNA titres

Raksharab® (Indian Immunologicals Ltd., Hyderabad, India), a commercially available inactivated cell culture rabies vaccine containing ≥ 2.5 IU/ml of rabies virus antigen (Challenge Virus Standard (CVS) strain), was used to vaccinate all dogs in R1 by subcutaneous administration of 1ml of the vaccine. The vaccine was transported under cold chain from the manufacturer's distribution unit and stored at 4°C until use in the field when it was held within an insulated icebox at all times. All dogs recaptured in R4 were revaccinated using the same vaccine. Blood samples were collected from all dogs (except some very young pups and difficult-to-handle adults in R1) via the cephalic or saphenous veins into red-topped Vacutainer® serum separator tubes (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) and stored at 4°C until serum separation within 24 hours. Serum samples were held at -20°C until overnight transport on dry ice to the WHO Collaborating Centre for Reference and Research in Rabies, National Institute of Mental Health and Neurosciences (NIMHANS) in Bengaluru, India. RVNA titre

estimation was done by the WHO- [5] and OIE- recommended [330] RFFIT using the CVS-11 strain of rabies virus by a protocol described elsewhere [331]. The lower and upper limits of reported RVNA titres were 0.23 IU/ml and 15 IU/ml, respectively. The results of 18 samples from R2 were reported as ≤ 0.11 IU/ml.

After being vaccinated as part of the study in R1, several dogs were revaccinated on different occasions – when they were recaptured as part of regional ABC campaigns or when ODs were given boosters by the PI or by another veterinary professional. Three UDs were also mistakenly revaccinated in the initial stages of R1 because their microchips were not detected at the time of recapture and so were assumed to be dogs not encountered previously. Revaccination dates were recorded wherever available. Where these were not known, we assigned approximate dates for data analysis purposes.

The data collection tool EpiCollect [265] was used to design an electronic data collection form for real-time data collection using Android mobile phones and online data storage and manipulation. Data collected included ownership and confinement status, sex, age, breed, BCS, skin/coat condition, sterilisation status, vaccination history and GPS coordinates of the capture location. The full list of data collected is provided in Table S1 (Appendix A1).

4.2.3 Data coding

Dogs were grouped into three categories based on their RVNA titre levels – those with no rabies antibodies detected, those having titres ≤ 0.5 IU/ml and those with titres > 0.5 IU/ml. The variable 'Ever vaccinated' was created to account for whether a dog may have ever been vaccinated in the past before capture in R1, either because their owner got them vaccinated (also captured by the variable 'vaccination history') or because they had been sterilised as part of a past ABC campaign (also captured by the variable 'Sterilisation status'). The variable 'Ever revaccinated' captured data on whether any dog was known to have been revaccinated after we vaccinated them in R1, as described in section 4.2.2.

Serum quality was visually assessed at the time of sample processing and the degree of haemolysis and turbidity was recorded subjectively as being absent, or present on a scale between mildest to severe. For statistical analysis, these observations were converted to numeric scores on a scale of 1 (absent) to 10 ('Completely haemolysed, blackish red' in the case of haemolysis, 'Milky' for turbidity) (Tables S7-S14, Appendix A2) and summarised. These data were analysed to detect correlations between the degree of haemolysis and turbidity, associations with reported RVNA titres as well as any differences based on factors such as age, sex, ownership category and so on.

Additional details of data coding are presented in section 3.2.2.

4.2.4 Data analysis

Data were downloaded from the EpiCollect website (https://five.epicollect.net) and analysed in R [268]. Detailed descriptions of data coding are provided in Table S1 (Appendix A1). Briefly, individual characteristics of dogs (sex, breed, BCS etc.) were coded as categorical variables, grouping multiple responses together where necessary, and summarised as proportions. Pearson's chi-square and Fisher's exact tests were used to test for differences in proportions between groups. We used a Wilcoxon rank sum test for independent samples to test for significant group differences in median scores of serum sample characteristics (haemolysis and turbidity).

To estimate geometric mean titres (GMTs), titres reported as < 0.23 IU/ml or < 0.11 IU/ml were transformed by two methods. In method one, these titres were transformed by dividing the numeric values by two – hence titres reported as < 0.23 IU/ml became 0.115 IU/ml and < 0.11 IU/ml became 0.055 IU/ml. In method two, all such titres were coded as 0.10 IU/ml. We used t-tests on log10-transformed titre values to test for significant differences in GMTs between groups [332] (Table 4.2).

4.2.5 Linear regression

We used univariable and multivariable linear regression to explore the influence of the following characteristics recorded in R1 on pre-vaccination (R1) titres – age, sex, breed, BCS, HIS and general extent of (direct or no direct) human interaction, coat condition, ownership status, sterilisation and

reproductive status, study site, vaccination history, confinement, whether the dog had ever been vaccinated ('Ever vaccinated' as defined in section 4.2.3) and serum quality characteristics, namely haemolysis and turbidity scores. Similar analyses were also conducted for titres from each post-vaccination round (R2, R3 and R4). For these models, in addition to the above predictors, each dog's R1 titre, the interval between vaccination in R1 and blood sampling in each corresponding round, whether the dog had been sterilised during the ABC campaign in R1 and information on whether the dog was ever revaccinated after R1 ('Ever revaccinated' as defined in section 4.2.3) were also considered as predictors. All titres were log10 transformed before fitting these models. The full list of predictors considered in these analyses is detailed in Table S49. Univariable models with statistically significant p-values based on the chi-squared statistic were then included in the multivariable model. Stepwise selection of variables (done manually or using the R function stepAIC from the MASS package) was used to select the model with the lowest Akaike Information Criterion (AIC) statistic as the most parsimonious model. Model estimates and p-values for predictors from this final model are presented in the Results section. These analyses were conducted using data combined for all dogs included in the study, as well as separately for ODs, SODs, UDs as well as DWOs. Mixed-effects linear regression models (linear mixed models, LMMs) with unique dog identity as a random effect were also used fitted to the longitudinal titre data after excluding R1 titre data from the dataset (thus R2 titres were the reference level in LMMs). In these models, the fixed effects used included the predictors detailed above as well as the study round, but ages in R2, R3 and R4 were also inferred based on age at capture in R1. For example, pups from R1 progressed to being juveniles by R3 and adults in R4.

4.2.6 Estimating the probability of protection and rate of decline in titres from serum data

We used RVNA titre data from R2, R3 and R4 to obtain maximum likelihood estimates (MLEs) of two parameters – the probability of protection/proportion protected (π) and an exponential rate of decline in titres below 0.5 IU/ml (β). This rate was calculated from the time when peak RVNA titres were assumed to be achieved. We assumed peak titres would be achieved 18 days post-vaccination [312]. We compared these results with those obtained for π and β when assuming that peak titres would be

achieved 28 days post-vaccination [311]. Each titre (not controlling for multiple observations being obtained from some dogs) was assigned a binomial probability of 0 (titres below 0.5 IU/ml) or 1 (greater than or equal to 0.5 IU/ml).

Hence

$$P(1|\,\pi,\beta) = \pi e^{-\beta t} \text{ and}$$

$$P(0|\,\pi,\beta) = 1 - \pi e^{-\beta t}$$

where t is the number of days post-peak titres (assumed to be achieved 18/28 days post-vaccination). Thus the log-likelihood function to be maximised was

$$l = \sum_{i=1}^{n} x_i(\log \pi - \beta t_i) + (1 - x_i)\log\left(1 - \pi e^{-\beta t_i}\right)$$

x_i being the immune status of each dog (0 or 1) and t_i the time duration in days after peak titres were assumed to have been reached.

When including RVNA titre data from R4 (up to 420 days after achieving peak titres) in the estimation process, near-zero rates of decline (β) were estimated because titres of nearly all dogs recaptured in R4 were higher than in R3 (Fig 4.2). We observed these trends even after excluding dogs known to have been revaccinated after R1 (and therefore would be expected to have higher RVNA titres due to an anamnestic immune response). To control for this inconsistency in titres, we used titre data only from R2 and R3 in the MLE analyses by excluding all titres obtained more than 180 days after achieving peak titres. An overall estimate of π and β was obtained, in addition to separate estimates when controlling for the ownership status (OD vs DWO), vaccination history (dogs with and without a known history of vaccination prior to the study or revaccination after R1) and breed in the case of OD. A chi-squared test of twice the difference in log likelihoods between two nested models was used to test whether the model with a higher number of parameters was significantly better than the model with fewer parameters (the degrees of freedom being the difference in the number of parameters

estimated in each model). MLEs were obtained using the *optim* and *optimx* functions in R. We compared parameter estimates obtained using two methods – L-BFGS-B and bobyqa – which are optimisation algorithms used to minimize a log-likelihood function for parameter estimation in machine learning [333].

4.2.7 Ethical approval and permits

Local ethical approval to conduct the study was obtained from the Kerala state Animal Husbandry Department. All animal procedures were approved by the Animal Welfare and Ethical Review Board of Imperial College London (Reference number 20180705A). All activities at both field sites were carried out in coordination with local elected representatives, the Municipal Chairman in ALP municipality and the panchayat President in MUH panchayat.

4.3 RESULTS

The proportion of dogs of different ownership categories recaptured in subsequent rounds is presented in Table 3.1. RVNA titres were obtained for all four time points (day 0, ~ 30 days, ~150 – 180 days and ~365 days) from 118 dogs – 65 OD, 16 SOD and 37 UD. Only 20% of all dogs (117/577), mostly UDs and SODs (60%), were known to have been vaccinated prior to R1. Only two dogs, both adult ODs in R1, that had been vaccinated in the past were revaccinated after R1. Altogether, the number of all dogs known to have received one, two or three vaccine doses by R4 was 416, 159 and two, respectively. Unless stated otherwise, all group comparisons of geometric mean titres are reported for dogs that were known to have received only one dose of rabies vaccine.

Thirty eight serum samples from R3 and R4 were tested more than once for comparing the consistency in titres reported by RFFIT. Of these, one sample was tested as four replicates, 10 samples as three replicates and 27 samples as two replicates. The variation in titres for 27 samples (71%) did not exceed normal inter-assay variation of two-fold or less [48,334] (Table S15, Appendix A2). However, there were important differences within rounds with 7/13 (54%) and 4/25 (16%) samples from R3 and R4, respectively, exceeding this level of inter-assay variation.

Geometric mean titres calculated using both transformations were significantly different only in R1 ($p = 0.006$) as it contained nearly all of the titres reported as < 0.23 IU/ml (Table 4.2). However, when titre data transformed using either method were used for further comparisons between groups and linear regression modelling, results obtained were consistent irrespective of which transformation was used. All results reported below have used titres transformed using method one. A p-value of less than or equal to 0.05 was considered to be significant.

Table 4.2 Geometric mean titres by method of transformation of titres. Summary of geometric mean titres (and 95% confidence intervals) by study round and mean interval (in days) after vaccination, calculated using the two methods of transforming titre values described in methods

Study round	Mean interval (in days) after vaccination (range)	Method 1	Method 2
First	0 (day of vaccination)	0.16 (0.15,0.17)	0.14 (0.14,0.15)
Second	35 (18 – 89)	1.98 (1.74,2.27)	2.02 (1.77,2.31)
Third	157 (112 – 191)	0.60 (0.52,0.68)	0.59 (0.51,0.67)
Fourth	372 (309 – 431)	1.59 (1.42,1.78)	1.59 (1.42,1.78)

4.3.1 Serum characteristics

Serum characteristics were recorded for a total of 1299 samples across all four rounds. Characteristics were not recorded for 23 samples collected on the first day of fieldwork in R1. Most samples (> 70%) from each round had haemolysis scores ranging from 1 (absent) to 3 (mild haemolysis) (median scores of 1 in R1 – R3, 2 in R4, Table S7, Appendix A2). Similarly, over 75% of samples from each round had low turbidity scores (median score of 1 in all rounds, Table S8, Appendix A2). There was a small but significant positive correlation between haemolysis and turbidity scores across all rounds ($r = 0.16$ (95% CI: 0.11 – 0.21), $p < 0.001$) as well as within each round except R2 (Table S6). There was no correlation between haemolysis scores and the log10 of its RVNA titre within each round, but a small and significant correlation when compared across all rounds ($r = 0.12$, (95% CI: 0.06 – 0.17), $p < 0.001$) (Table S4). Similarly, there was a small but significant correlation between turbidity scores and the log10 of RVNA titres when compared across all rounds ($r = 0.14$ (95% CI: 0.09 – 0.19), $p < 0.001$) as well as in R2 ($r = 0.13$ (95% CI: 0.02 – 0.24), $p = 0.02$) (Table S5).

Linear regression models highlighted sterilisation status, confinement status, body condition, coat condition and breed as statistically significant predictors of serum haemolysis across the study with their influence within each study round varying (Table 4.3, Figs S2-S6, Appendix A2). Nearly all these predictors were associated with ODs having higher median haemolysis scores. While no predictors of serum turbidity appeared consistently within each study round, when we considered data for all

rounds together, sterilisation status was found to be significantly associated with turbidity scores, with sterilised dogs having higher median turbidity scores compared to non-sterilised dogs (p = 0.03).

Table 4.3 Predictors significantly influencing haemolysis scores. Summary of predictors highlighted by linear regression models as significantly influencing haemolysis scores when using data for all rounds together

Predictor	Levels	Median score	p-value
Sterilisation status	Sterilised	3	< 0.001
	Not sterilised	1	
Confinement	Completely/partially confined	2	< 0.001
	Free-ranging	1	
Body condition	Over ideal body condition	2.5	< 0.001
	Under ideal / Ideal body condition	1	
Coat condition	Good or Very good	2	< 0.001
	Poor or Fair	1	
Breed	Pure/Crossbreed	2	< 0.001
	Non-descript	1	

4.3.2 Pre-vaccination (day zero, R1) titres

In R1, the GMT was 0.16 IU/ml (95% CI: 0.15 – 0.17 IU/ml), with similar titres across all ownership categories (Table 4.4), irrespective of past vaccination history, confinement status or study site. Among 359 UDs and SODs across both sites with no known vaccination history, 10 had RVNA titres > 0.5 IU/ml in R1 (Fig. 4.1), giving a seroprevalence of 2.8% (95% CIs: 1.3 – 5%). At a lower test cut-off of 0.2 IU/ml, 26% (94/359, 95% CIs: 22 – 31%) were seropositive. Of 68 ODs known not to have been vaccinated (as reported by their owners) or not sterilised, 16 (24%, 95% CIs: 14 – 35%) were seropositive, 13 of which were completely or partially free-ranging. These included one completely free-ranging OD (1.5%, 95% CIs: 0.04 – 7.9%) with titres > 0.5 IU/ml. Irrespective of vaccination history or ownership status, none of the dogs in MUH had R1 titres > 0.5 IU/ml.

A significantly higher proportion of sterilised UDs (26/58, 45%), which would have been vaccinated at least once at the time of sterilization, had detectable RVNA compared to non-sterilised UDs (86/328, 26%, p = 0.006) (Table S2, Appendix A2). This difference was driven by the significantly higher proportion of sterilised male UDs with detectable RVNA (19/36, 53%) compared to 34/150 (23%) non-sterilised males (p< 0.001), while there were no differences between sterilised and non-sterilised females (p = 0.99). Only two SODs were known to have been previously vaccinated, but neither were seropositive.

Table 4.4 Geometric mean titres by ownership category in all rounds. Geometric mean titres (and 95% confidence intervals) (in International Units per ml) for rabies virus neutralizing antibodies detected in each study round for dogs of various ownership categories, not accounting for past vaccination history or future revaccination after round 1

Ownership category	Round 1 (DAY 0) (October 2018 – January 2019)	Round 2 (~DAY 30) (November 2018 – January 2019)	Round 3 (~ DAY 150 – 180) (April – May 2019)	Round 4 (~ DAY 365) (October 2019 – January 2020)
Owned	0.16 (0.15 – 0.18)	2.12 (1.72 – 2.61)	0.44 (0.36 – 0.54)	1.48 (1.25 – 1.76)
Semi-owned	0.15 (0.13 – 0.19)	1.79 (1.06 – 3.01)	0.88 (0.59 – 1.34)	1.41 (0.97 – 2.06)
Unowned	0.16 (0.15 – 0.17)	1.94 (1.62 – 2.33)	0.70 (0.58 – 0.83)	1.73 (1.46 – 2.05)

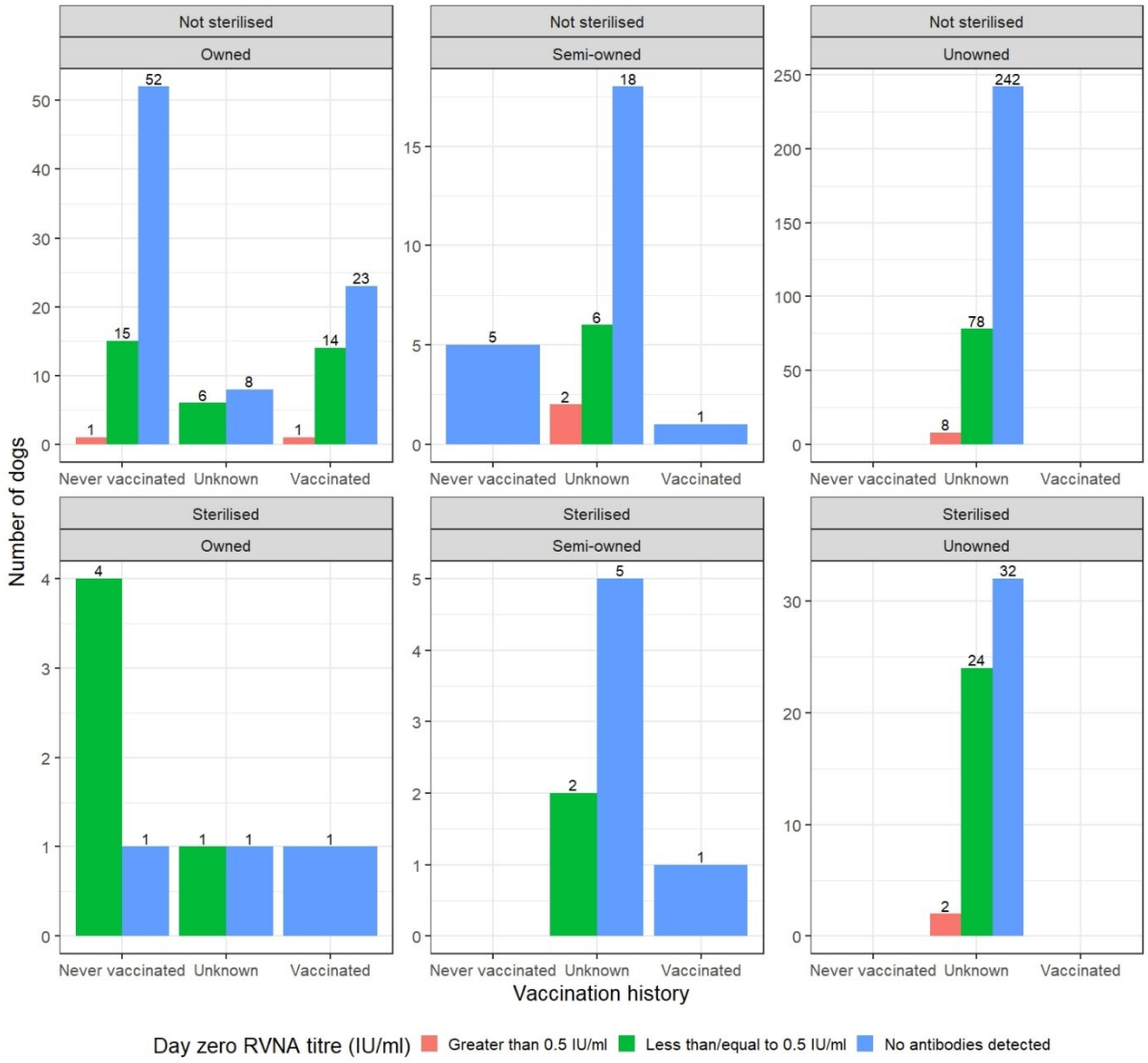

Figure 4.1 Day zero (round 1) rabies virus neutralizing antibody (RVNA) titres. Titres (broadly categorized as Greater than 0.5 IU/ml, Less than/equal to 0.5 IU/ml and No antibodies detected) by ownership category, sterilisation status and vaccination history of dogs whose titres were assessed. Numbers above bars indicate sample sizes for each category.

Less than a third of all ODs (including only one OD in MUH) were known to have been vaccinated by their owners, including only four (3% of all ODs) which were regularly vaccinated every year. This proportion did not change substantially even after excluding 19 pups, which are generally considered too young for vaccinations. Of those that had been vaccinated by their owners, just over a third (15/38, 39%) were seropositive including only one with titres > 0.5 IU/ml.

4.3.3 Post-vaccination titres

The mean interval between vaccination in R1 and blood sampling in R2, R3 and R4 was 34.5 days (range: 18 – 89 days), 156.6 days (112 – 191) and 372.2 days (309 – 431). Most dogs of all ownership categories had titres > 0.5 IU/ml in R2 – 91% (104/114, 95% CI: 84.5 - 95.7%) of OD, 87.6% (155/177, 95% CI: 81.8 – 92%) of UD and 78% (28/36, 95% CI: 61 - 90%) of SOD. The percentages of dogs with R2 titres ≥ 0.23 IU/ml and ≥ 0.11 IU/ml were 94% (308/328) and 96% (316/328), respectively. However there was variation in individual titres and within study sites (Fig. 4.2). The GMT in R2 was 1.98 IU/ml (95% CI: 1.74 – 2.27 IU/ml), with no significant differences between ownership categories (Table 4.4).

Geometric mean titres declined in R3 (GMT 0.6 IU/ml, 95% CIs: 0.52 – 0.68 IU/ml) for all ownership categories. Only 43% (33/76) of DWOs and 22% (12/54) of ODs that received a single dose of rabies vaccine had titres > 0.5 IU/ml in this round. However titres increased in R4 (GMT 1.59 IU/ml, 95% CIs: 1.42 – 1.78 IU/ml) (Fig. 4.2, Table 4.4) without attaining R2 levels. Mean titres increased for 94% (129/137) of dogs whose R3 and R4 titres could be compared. This proportion was similar, 95% of dogs (118/124), after excluding those that had been revaccinated after R1, suggesting a systematic over-estimation of titres at the diagnostic laboratory where samples were tested.

Mean titre levels in R2 were significantly higher for dogs with a history of vaccination before R1, across all ownership categories (Tables 4.5 and 4.7). All but one of 85 dogs with a prior vaccination history had titres > 0.5 IU/ml in R2, while 83.8% of those with no known vaccination history attained these levels ($p < 0.001$). These differences were significant in R3 as well ($p < 0.001$), while similar proportions had titres > 0.5 IU/ml in R4 (Table S16, Appendix A2). Among ODs in R2, dogs whose vaccination history was unknown had mean titres significantly higher than that of previously unvaccinated dogs, and similar to GMTs of previously vaccinated ODs (Table 4.5). These results suggest that at least some ODs with unknown vaccination history may in fact have been vaccinated in the past. Non-descript ODs had higher mean post-vaccination titres than pure/crossbreed ODs in all rounds, being significantly higher in R4 ($p = 0.03$) (Table 4.6).

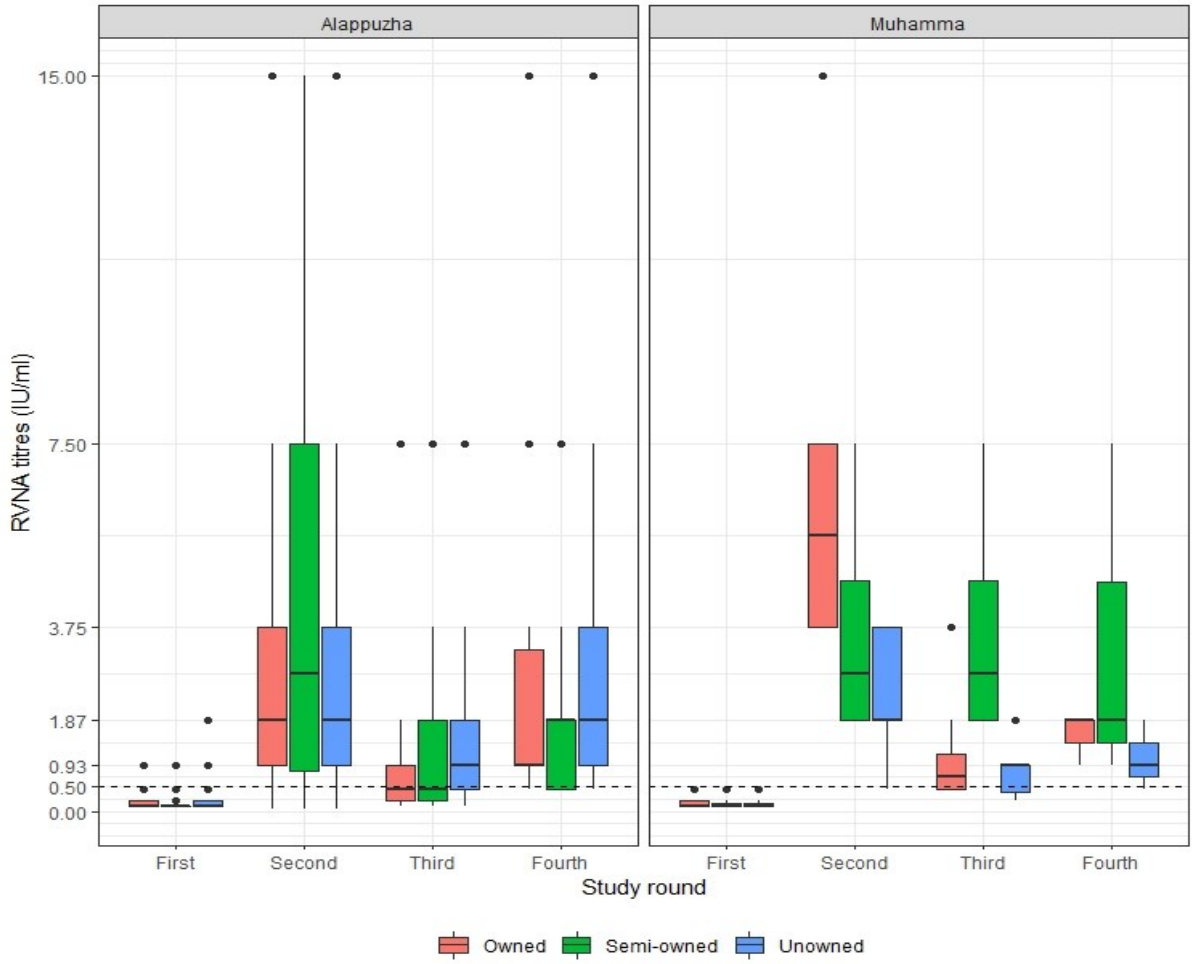

Figure 4.2 Distribution of RVNA titres by study round, ownership category and site. Distribution of rabies virus neutralizing antibody (RVNA) titres (in IU/ml) by study round and ownership category at both sites, highlighting variation in post-vaccination titres in post-vaccination rounds. The horizontal dotted line indicates a titre level of 0.5 IU/ml.

Table 4.5 Geometric mean titres by ownership category and vaccination history in R2. Summary of geometric mean titres (and 95% confidence intervals) in round 2 (~30 days post-vaccination) by vaccination history and ownership category, after excluding dogs known to have been revaccinated after R1

Ownership	If previously vaccinated before being included in the study		
	No	Unknown	Yes
Owned	1.51 (1.07,2.13)	4.05 (2.51,6.53)	3.06 (2.43,3.84)
Semi-owned	1.86 (0.18,19.79)	1.31 (0.67,2.56)	5.04 (2.46,10.34)
Unowned	-	1.64 (1.31,2.07)	3.46 (2.53,4.73)

Table 4.6 Geometric mean titres in all rounds for dogs vaccinated only once. Summary of geometric mean titres (and 95% confidence intervals) attained after one dose of vaccine in adult owned dogs in R1, categorized by breed and study round and after excluding previously vaccinated dogs and those known to have been revaccinated after R1

Breed	Study round			
	Round 1	Round 2	Round 3	Round 4
Non-descript	0.16 (0.14, 0.190	3.03 (2.04, 4.51)	0.45 (0.31, 0.68)	1.73 (1.2, 2.49)
Pure/ crossbreed	0.15 (0.12, 0.19)	1.47 (0.63, 3.45)	0.27 (0.17, 0.42)	0.93 (0.64, 1.34)

4.3.4 Linear regression

The best-fitting linear regression models identified only prior vaccination history as predictive of pre-vaccination (R1) RVNA titres (Table 4.7, Table S27, Appendix A2). This included a history of vaccination by their owners in ODs or during sterilisation as part of regional ABC campaigns in the case of DWOs, both of which were captured by the variable 'Ever vaccinated'. Previously vaccinated dogs had significantly higher R1 titres (GMT 0.18 IU/ml, 95% CIs: 0.16 – 0.20 IU/ml) than those with no vaccination histories (GMT 0.15 IU/ml, 95% CIs: 0.13 – 0.16 IU/ml) or unknown vaccination histories

(GMT 0.15, 95% CIs: 0.15 – 0.16 IU/ml). However, none of the characteristics considered specifically for ODs predicted their R1 titres.

In the best fitting multivariable LMM for all dogs with unique dog identity as a random effect, statistically significant associations were observed between RVNA titres and age, pre-vaccination (R1) sterilisation status, pre-vaccination RVNA titres, study round (included as a fixed effect in the LMMs only) and whether dogs had been captured for ABC campaigns in R1. Similar effects were seen for UDs or DWOs that were sterilised at the time of capture in R1 (Tables 4.7 and 4.8).

Table 4.7 Summary of statistically significant predictors of RVNA titres from linear regression analyses. Summary of dog characteristics recorded in round 1 (R1) that were found to significantly ($p < 0.05$) increase (in green) or decrease (in orange) rabies virus neutralizing antibody titres in future rounds and across all recapture rounds for the most parsimonious models i.e with lowest AIC scores (AR – All rounds, fixed effects model; AR-ME – All rounds, Mixed-effects linear regression model with unique dog identifier as a random effect). Results are presented using data for all dogs, unowned (UD), owned (OD), semi-owned (SOD) dogs and dogs without owners (DWO). Black cells indicate predictors not considered for a particular model. See Table S49 in Appendix A2 for levels used for variables. R1 – round 1 etc.; ABC – If caught for sterilisation in R1 as part of animal birth control campaign; MUH – Muhamma; VIP – vaccinated in the past; C/PC – completely/partially confined.

	Predictor*	R1	R2	R3	R4	AR-ME
All dogs	Sterilised (Yes)		0.16	0.25	0.14	0.19
	Confinement (C/PC)			-0.23		
	Vaccination history (VIP)			0.26		
	Site (MUH)			0.22		
	Ever vaccinated (Yes)	0.10				
	ABC (Yes)		-0.59	-0.30		-0.33
	Age (Juvenile; Pup)		-0.31; -0.61			-0.29; -0.58
	R1 titre (log10)				0.19	0.18
	Study round (R3; R4)					-0.57; -0.17
UD	Sterilised (Yes)	0.09		0.22		
	ABC (Yes)		-0.56	-0.32		-0.38
	Age (Juvenile; Pup)		-0.40; -0.48			-0.43; -0.50
	R1 titre (log10)		0.27			0.19
	Study round (R3)					-0.48
OD	Site (MUH)		0.42			
	Age (Juvenile; Pup)		-0.44 Pup			-0.24; -0.51
	Study round (R3; R4)					-0.73; -0.24
SOD	Sterilised (Yes)					0.42
	Site (MUH)			0.28		0.53
	Age (Pup)		-1.08			-1.05
	R1 titre (log10)			0.82		0.62
	Study round (R3)					-0.46
DWO (UD and SOD)	Sterilised (Yes)	0.07		0.25		0.17
	ABC (Yes)		-0.55	-0.33		-0.34
	Age (Juvenile; Pup)		-0.42; -0.68			-0.35; -0.86
	R1 titre (log10)		0.28		0.22	0.26
	Turbidity scale		0.04			
	Study round (R3; R4)					-0.47; -0.13

*Reference levels: ABC – No; Age – Adult (incl. Aged); Confinement – Completely free-ranging; Ever vaccinated – No; Site – Alappuzha; Sterilised – No; Study round – Second (R2); Vaccination history – Never vaccinated; Turbidity scale - 1

Table 4.8 Regression estimates for predictors of RVNA titres from multivariable mixed-effects linear regression model. Regression estimates and p-values for predictors of rabies virus neutralizing antibody titres for all dogs in the best-fitting multivariable mixed-effects linear regression model. Statistically significant p-values ($p < 0.05$) are highlighted in bold.

Predictor*	Estimate	Standard error	95% confidence intervals	p-value
(Intercept)	**0.50**	**0.06**	**0.37, 0.62**	**< 0.001**
Age: Juvenile	**-0.29**	**0.07**	**-0.42, -0.16**	**< 0.001**
Age: Pup	**-0.58**	**0.11**	**-0.79, -0.37**	**< 0.001**
Sterilisation status in round 1: Sterilised	**0.19**	**0.05**	**0.09, 0.29**	**< 0.001**
If captured during on-going animal birth control in round 1: Yes	**-0.33**	**0.07**	**-0.47, -0.19**	**< 0.001**
Study round: Third	**-0.57**	**0.04**	**-0.64, -0.50**	**< 0.001**
Study round: Fourth	**-0.17**	**0.04**	**-0.24, -0.09**	**< 0.001**
Round 1 (pre-vaccination) RVNA titre (log10)	**0.18**	**0.07**	**0.04, 0.32**	**0.014**

*Reference levels: Age – Adult (incl. Aged); Sterilisation status – Not sterilised; If captured during on-going animal birth control in round 1– No; Study round – Second

Among dogs with no known vaccination history that received only one vaccine dose in R1 and were not known to have been subsequently revaccinated, pups and juveniles had lower GMTs compared to adults in all subsequent post-vaccination study rounds (Table 4.9). Mean titres were significantly lower in R2 for these age categories (Pups – GMT 0.57 IU/ml, 95% CI: 0.32 – 1.01 IU/ml, $p < 0.001$; Juveniles – GMT 0.89 IU/ml, 95% CI: 0.53 – 1.51 IU/ml, $p = 0.001$). Only 56% (14/25) of pups and 68% (17/25) of juveniles across all ownership categories had titres > 0.5 IU/ml in R2. These differences were pronounced in DWOs where only one in three pups and half of all juveniles had adequate titres. At the same time, only 63% of OD pups (12/19) had R2 titres > 0.5 IU/ml. These proportions dropped to one in three DWOs and 21% (4/19) of ODs recaptured in R3, before rising to nearly all dogs in R4 (Table 4.9).

Table 4.9 Geometric mean titres in all rounds for dogs vaccinated only once, by age in R1. Summary of geometric mean titres (and 95% confidence intervals) among dogs across all ownership categories that received their first rabies vaccine dose in R1 and were not subsequently revaccinated, by study round and age at first capture in R1

Age at first capture in round 1	Study round			
	Round 1	Round 2	Round	Round 4
Adult	0.16 (0.15,0.17)	1.98 (1.64,2.4)	0.5 (0.42,0.6)	1.55 (1.31,1.84)
Juvenile	0.14 (0.12,0.16)	0.89 (0.53,1.51)	0.4 (0.23,0.68)	1.2 (0.78,1.83)
Pup	0.13 (0.12,0.15)	0.57 (0.32,1.01)	0.34 (0.21,0.53)	1.41 (0.85,2.34)

Sterilisation status in R1 was a significant predictor of RVNA titres in all post-vaccination study rounds, particularly in DWOs. As mentioned above and discussed in section 4.3.2, dogs sterilised during ABC campaigns are also vaccinated against rabies, which would explain the significant influence on post-vaccination titres in DWOs. Dogs that were recorded as sterilised in R1 had higher mean titres in all post-vaccination rounds, being statistically significant in R2 and R3 ($p < 0.001$) (Table 4.10). These differences were also present in previously unvaccinated ODs, although not statistically significant.

Table 4.10 Geometric mean titres in all rounds for dogs vaccinated only once, by sterilisation status in R1. Summary of geometric mean titres (and 95% confidence intervals) by study round and sterilisation status in R1, after excluding dogs known to have been revaccinated after R1

Sterilisation status	Study round			
	Round 1	Round 2	Round 3	Round 4
Not sterilised	0.15 (0.15,0.16)	1.63 (1.36,1.95)	0.47 (0.4,0.55)	1.5 (1.29,1.75)
Sterilised	0.18 (0.16,0.21)	3.58 (2.74,4.68)	1.13 (0.82,1.56)	2.01 (1.49,2.71)

Dogs that were captured as part of a regional ABC campaign in R1 were found to have lower GMTs in future rounds, being significantly lower in R2 ($p = 0.002$) and R3 ($p = 0.01$) (Table 4.11). However, there were no differences observed in R4. Compared to R2 titres, mean titres were significantly lower in R3 and R4, although the magnitude of the reduction in titres was much lower in R4 than R3 (Table 4.2).

Pre-vaccination RVNA levels predicted future titres, with dogs having higher pre-vaccination titres in R1 having correspondingly higher titres in each post-vaccination round.

Table 4.11 Geometric mean titres in post-vaccination rounds for dogs vaccinated only once, by whether captured for ABC in R1. Summary of geometric mean titres (and 95% confidence intervals) of dogs recaptured in post-vaccination study rounds, based on whether they were captured as part of regional animal birth control (ABC) campaigns in R1. Dogs known to be revaccinated after R1 were excluded in this analysis.

If captured for ABC in round 1	Study round		
	Round 2	Round 3	Round 4
No	1.96 (1.63, 2.35)	0.61 (0.49, 0.76)	1.53 (1.22, 1.91)
Yes	0.6 (0.31, 1.13)	0.33 (0.22, 0.48)	1.78 (1.12, 2.82)

Additional significant predictors in individual study rounds included confinement practice in R3, study site in R2 and R3, and extent of serum turbidity in R2. In R3, completely or partially confined dogs were found to have significantly lower mean titres than FRDs. When accounting for breed differences and comparing only non-descript ODs, completely FRDs had higher GMTs than completely confined dogs (Table 4.12) in R3 and R4, being statistically significant in R3 ($p = 0.01$).

Previously unvaccinated OD in MUH had higher RVNA titres compared to OD in ALP in all post-vaccination rounds, being significantly higher in R2 ($p < 0.001$, Table 4.13) and marginally significant in R3 ($p = 0.053$). Similarly, GMTs for all dogs were significantly higher in MUH in R2 ($p = 0.005$). Serum sample turbidity was found to significantly influence RVNA titre levels in R2, with a unit increase in the turbidity score increasing log10 titres by 0.04 units. This suggested that true titres in samples with high turbidity scores were lower than those reported in laboratory results (Table S52).

Table 4.12 Geometric mean titres in all rounds for non-descript owned dogs vaccinated only once, by confinement status. Summary of geometric mean titres (and 95% confidence intervals) by study round and confinement among non-descript owned dogs, after excluding dogs known to have been vaccinated in the past and/or revaccinated after R1.

Confinement status	Study round			
	Round 1	Round 2	Round 3	Round 4
Completely / partially confined	0.16 (0.11, 0.23)	2.97 (1.67, 5.28)	0.23 (0.14, 0.38)	1.23 (0.48, 3.11)
Free-ranging	0.16 (0.15, 0.17)	2.07 (1.71, 2.5)	0.56 (0.46, 0.68)	1.7 (1.41, 2.06)

Table 4.13 Geometric mean titres in post-vaccination rounds for owned dogs vaccinated only once, by study site. Summary of geometric mean titres (and 95% confidence intervals) by study round and site among owned dogs, after excluding dogs known to have been vaccinated in the past and/or revaccinated after R1.

Site	Study round		
	Round 2	Round 3	Round 4
Alappuzha	1.57 (1.15, 2.14)	0.36 (0.28, 0.48)	1.41 (1.11, 1.79)
Muhamma	4.95 (3.55, 6.9)	0.65 (0.44, 0.97)	1.87 (NA, NA)*

*Single observation

4.3.5 Estimates of the probability of protection and rate of decline in titres

Similar estimates of π and β were obtained when assuming peak titres being achieved 18 and 28 days-post-vaccination (Table S50, Appendix A2) and using the two optimisation methods (Table S51). All further analyses were conducted assuming peak titres achieved at 18 days-post-vaccination and using the L-BFGS-B optimisation method.

Using titre data for only R2 and R3 resulted in an MLE of 0.93 (95% CI 0.89 – 0.98) for π (the proportion achieving RVNA titres ≥ 0.5 IU/ml post-vaccination) and 0.005 per day (95% CI: 0.0038 – 0.006) for β (the exponential rate of post-vaccination decline in titres to < 0.5 IU/ml) (Model 2, Table S51). This latter rate translated to titres dropping below 0.5 IU/ml approximately 200 days ((95% CI: 167 – 256 days) post-peak titre (assumed to be achieved 18 days post-vaccination).

However, these rates differed significantly by vaccination history and ownership status of dogs. Dogs with a) a known history of rabies vaccination prior to the study (by their owners, reference persons or as part of local ABC campaigns) or b) revaccination after R1 (by their owners or reference persons, by the PI during the course of the study or as part of on-going local ABC campaigns) had a higher π (0.99, 95% CI 0.95 – 1) (Table 4.14, Model 6a) compared to those without a history of vaccination/revaccination (0.90, 95% CI 0.84 – 0.97, $p < 0.001$). Similarly, π was higher for ODs (OD) (0.99, CI 0.92 – 1) than dogs without owners (DWO) (0.90, 95% CI 0.84 – 0.95, $p = 0.04$) (Table 4.14, Model 4). However, RVNA titres declined at a faster rate for unvaccinated / un-revaccinated dogs (0.0066, 95% CI 0.0049 – 0.0084) compared to those with a history of vaccination/revaccination (0.0031, 95% CI – 0.002 – 0.0042, $p < 0.001$) (Table 4.14, Model 6a). This translated to titres dropping below 0.5 IU/ml by about 323 days post-peak titre for the latter category, while in dogs known to have received only one dose of the vaccine, titres dropped below 0.5 IU/ml within 151 days or 5 months. These differences in β persisted even after excluding pups and juveniles (which would be expected to have lower peak titres to start with after just one dose of vaccine) (Table 4.14, model 6b) from the analyses. We also found significant differences in the persistence of titres above 0.5 IU/ml by ownership status.

Notably, titres declined significantly faster in ODs (0.0086, 95% CI 0.0062 – 0.011) compared to DWOs (0.0031, 95% CI 0.0019 – 0.0042, $p < 0.001$) (Model 4). These differences between ODs and DWOs were consistent even when accounting for vaccination history (Model 8), with titres declining faster in vaccinated/revaccinated ODs compared to vaccinated/revaccinated DWOs, respectively. The mean duration of adequate immunity in previously unvaccinated dogs receiving a single dose of vaccine was 105 days (beta of 0.0095, 95% CI: 0.0063 - 0.0127) and 204 days (beta of 0.0049, 95% CI: 0.0031 - 0.0068) for ODs and DWOs, respectively. However, in revaccinated dogs, titres were predicted to remain above 0.5 IU/ml for 196 days (0.0051, 95% CI: 0.0028 - 0.0075) and 500 days (0.0020, 95% CI: 0.0013 - 0.0028) in ODs and DWOs, respectively. The above differences in rates of declines in titre between OD and DWO persisted even after excluding purebreed dogs from the analyses (results not shown). These differences in the rates of decline are shown in Fig. 4.3, indicating a good fit of the MLE estimates with titre data.

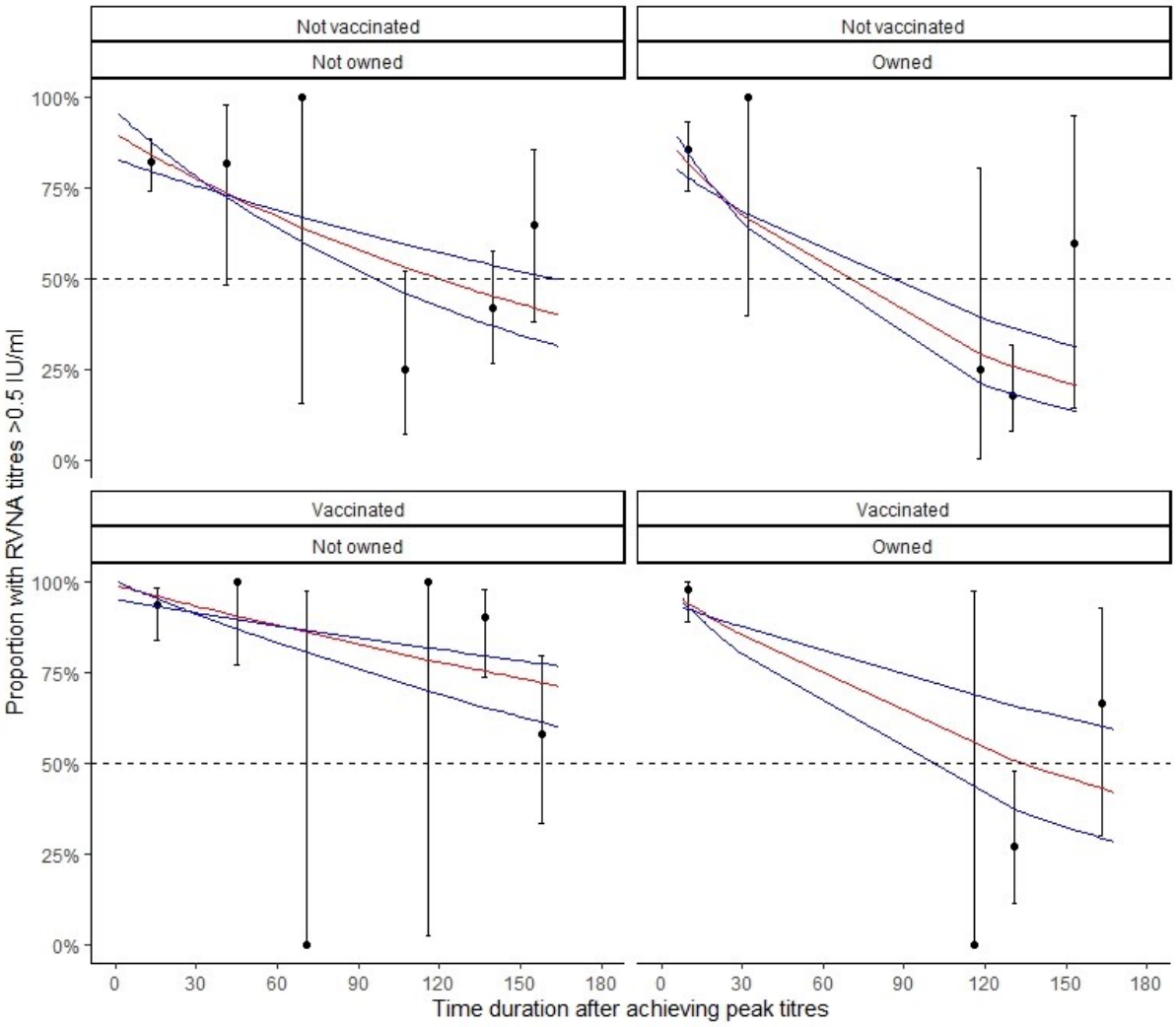

Figure 4.3 Proportions of dogs with RVNA titres > 0.5 IU/ml and rates of decline. Proportions (with exact binomial confidence intervals) of dogs with rabies virus neutralizing antibody (RVNA) titres > 0.5 IU/ml at thirty-day intervals after peak post-vaccination titres are achieved (assumed to be at 18 days post-vaccination). Proportions have been plotted at points on the x-axis corresponding to the median duration for each thirty-day interval. These are further categorized by the dog's vaccination history (Vaccinated – dogs with a history of vaccination prior to round 1 and/or revaccination after round 1; Not vaccinated – dogs vaccinated only once during round 1) and ownership status. The mean estimate (in red) and 95% confidence intervals (in blue) for the maximum likelihood estimate of the rate of decline in immunity are displayed. Horizontal dotted lines represent 50% of dogs with RVNA titres > 0.5 IU/ml.

Table 4.14 Maximum likelihood estimates for π and β. Maximum likelihood estimates obtained for the parameters π and β using the L-BFGS-B optimisation method, based on the ownership and/or vaccination status of dogs, when assuming that peak rabies virus neutralizing antibody titres are achieved 18 days-post-vaccination.

Model number	Number of parameters	Parameters	Description of population	π (95% confidence intervals)		β (95% confidence intervals)		Log likelihood	Model comparisons (degrees of freedom)	p-value
1	2	1 π, 1 β	All dogs	0.93 (0.89 - 0.98)		0.0050 (0.0039 - 0.0060)		-275.8137	-	-
2	3	2 π, 1 β	Owned dogs (OD) vs Dogs without owners (DWO)	OD: 0.92 (0.84 - 1)	DWO: 0.94 (0.89 - 0.99)	0.0049 (0.0038 - 0.0060)		-275.7075	1 and 2 (1)	0.64
3	3	1 π, 2 β		0.93 (0.89 - 0.97)		OD: 0.0077 (0.0056 - 0.0098)	DWO: 0.0034 (0.0023 - 0.0046)	-267.6536	1 and 3 (1)	< 0.001
4	4	2 π, 2 β		OD: 0.99 (0.92 - 1)	DWO: 0.90 (0.84 - 0.95)	OD: 0.0086 (0.0062 - 0.011)	DWO: 0.0031 (0.0019 - 0.0042)	-265.6055	1 and 4 (2); 2 and 4 (1); 3 and 4 (1)	< 0.001 < 0.001 0.04
5	3	2 π, 1 β	Dogs with (V) and without (NV) a history of vaccination before/ revaccination after round 1	V: 0.99 (0.94 - 1)	NV: 0.85 (0.79 - 0.92)	0.0044 (0.0034 - 0.0055)		-265.2156	1 and 5 (1)	< 0.001
6	3	1 π, 2 β		0.96 (0.91 - 1)		V: 0.0026 (0.0017 - 0.0036)	NV: 0.0075 (0.0058 - 0.0093)	-262.4626	1 and 6 (1)	< 0.001
6a	4	2 π, 2 β		V: 0.99 (0.95 - 1)	NV: 0.90 (0.84 - 0.97)	V: 0.0031 (0.0020 - 0.0042)	NV: 0.0066 (0.0049 - 0.0084)	-259.2068	1 and 6a (2); 5 and 6a (1); 6 and 6a (1)	< 0.001 < 0.001 0.011
6b	4	2 π, 2 β (excl. young)		V: 0.99 (0.95 - 1)	NV: 0.99 (0.94 - 1)	V: 0.0028 (0.0018 - 0.0039)	NV: 0.0069 (0.0051 - 0.0086)	-200.1371	-	-
7	5	4 π, 1 β	OD and DWO with (V) and without (NV) a history of vaccination before / revaccination after round 1	OD-V: 0.99 (0.76 - 1); OD-NV: 0.85 (0.74 - 0.95); DWO-V: 0.99 (0.94 - 1); DWO-NV: 0.86 (0.78 - 0.93)		0.0044 (0.0033 - 0.0056)		-265.106	5 and 7 (2)	0.896
8	6	2 π, 4 β		V: 0.99 (0.95 - 1)	NV: 0.90 (0.83 - 0.96)	OD-V: 0.0051 (0.0028 - 0.0075); OD-NV: 0.0095 (0.0063 - 0.0127); DWO-V: 0.0020 (0.0013 - 0.0028); DWO-NV: 0.0049 (0.0031 - 0.0068)		-251.7877	6a and 8 (2)	< 0.001

4.4 DISCUSSION

In this chapter we highlight features of the response to rabies vaccination and immunological dynamics of RVNA in a mixed dog population in India. These results have important implications for the implementation and impact of MRV campaigns for rabies control in regions with substantial populations of DWOs.

This study reinforces findings from multiple other studies about the effectiveness of rabies vaccination in inducing antirabies immunity. Over 90% of dogs in our study seroconverted after vaccination in R1 irrespective of prior vaccination history, with over 80% developing titres > 0.5 IU/ml. However, a single vaccine dose was insufficient to maintain titres above this level up to one year post-vaccination in previously unvaccinated dogs, irrespective of ownership status or breed. Less than half of previously unvaccinated DWOs maintained RVNA titres > 0.5 IU/ml when sampled approximately six months post-vaccination and titres were predicted to drop below these levels approximately seven months post-vaccination. These findings agree with previous studies in 'stray' dogs from Sri Lanka [54] and free-ranging ODs in Indonesia [250]. Previously vaccinated dogs were predicted to maintain titres for more than a year. Dogs < 1 year of age had lower initial titres which declined faster than in adults. Young ODs may thus benefit from a booster dose of rabies vaccine one month after primary vaccination [250]. Currently, different vaccine manufacturers in India provide inconsistent advice on pre-exposure rabies vaccination, recommending a single primary vaccination after three months of age followed by annual revaccination [335]. Where a booster is recommended one month after primary vaccination, the advice is not consistent across media (e.g. vaccine leaflet vs website) [336]. Vaccination recommendations must be made consistent to account for failure of young dogs to maintain titres > 0.5 IU/ml in response to a single vaccine dose. Veterinarians must make it a practice of administering two doses of rabies vaccine to young animals, as is done with other multivalent vaccines such as for canine distemper and parvovirus. Additionally, MRV campaigns targeting DWOs and populations with a high proportion of young dogs may need to be conducted twice a year in areas where no campaigns have been previously conducted. Doing so may enable a sufficiently large

proportion of the dog population to maintain adequate levels of immunity long enough for annual vaccination campaigns to be subsequently implemented. A corollary of this observation is that conducting campaigns less frequently than once a year is very unlikely to maintain high levels of herd immunity in FRDs in India.

We found concerning evidence that even previously vaccinated ODs maintained titres > 0.5 IU/ml for much shorter periods than the one to three years claimed by vaccine manufacturers (Fig. 4.3). Pure- and crossbreed dogs in particular had lower titres by day 180 post-vaccination, despite all vaccinated dogs developing robust RVNA titres approximately 30 days post-vaccination. This is possibly due to failure of owners to vaccinate dogs as per schedule, combined with the use of poor-quality rabies vaccines. Most ODs in our study had never been vaccinated previously by their owners or had been vaccinated over a year ago. The majority of ODs with a prior history of vaccination had no detectable titres in R1. Cold-chain maintenance of vaccines is also a constant problem in India (Sreejith Radhakrishnan, personal observation), which can adversely affect vaccine quality. In addition, most ODs are likely to be vaccinated at public sector veterinary clinics which provide government procured rabies vaccines at a subsidized rate. Although anecdotal, there have been long-held concerns among public sector veterinarians over the quality of these vaccines provided at public sector clinics, with even reports of rabies deaths in previously vaccinated ODs (Biju S, personal communication). Such poor-quality vaccines may have reduced antigenic potency that fails to induce adequate immunity in vaccinated ODs. In contrast, DWOs that had been previously vaccinated with commercially available vaccines as part of past regional ABC campaigns developed higher RVNA titres after vaccination in R1 and these titres were predicted to stay above 0.5 IU/ml for more than a year. There is thus an urgent need to ensure that only high-quality rabies vaccines stored under appropriate cold-chain conditions are used across all veterinary facilities in India. Failure to do so will defeat the whole purpose of vaccinating dogs and do little to reduce the burden of rabies in India.

The use of titre data from R4 would have enabled us to obtain more precise and accurate estimates of the rate of decline in immunity. However, there was an unexpected rise in titres consistently across all ownership categories in R4, compared to R3, even after excluding dogs known to have been revaccinated prior to R4. There are a number of possible reasons for this observation. While we tried to record any instance of revaccination after R1, it is likely that we have not accounted for revaccination of some dogs in our study cohort. Some dog owners may have failed to mention that their dogs received a booster dose after R1, although we consider this highly unlikely. In September 2019, a month before R4 was started, a rabid dog bit 32 people in a part of ALP close to where much of our fieldwork was conducted [277]. Thus, potential exposure to circulating rabies virus through bites or contact with saliva may have stimulated an anamnestic immune response in FRDs in the locality, including some from our study cohort. In response to the bite incidents, local veterinary authorities attempted to vaccinate as many FRDs as possible in the locality, although reportedly only 30 dogs were eventually vaccinated (Dr. Vysakh Mohan, personal communication). A proportion of these 30 dogs may have included those from our study cohort. Nevertheless, none of these scenarios explain the consistent rise in titres in nearly all of the 220 dogs recaptured in R4. Over 50% of DWOs recaptured in R4 comprised sterilised dogs (Fig. S1, Appendix A1) which would not have been revaccinated by ABC teams. Even completely confined purebreed ODs had increased titre levels despite being very unlikely to encounter FRDs or to have been revaccinated before R4. At the same time, various immunological trends observed in previous rounds, such as the higher mean titres among sterilised and non-descript breed dogs, and the impact of pre-vaccination titre levels, were still captured in the R4 titre data.

It is unclear what explains this uniform rise in titres – a systematic over-estimation of RVNA titres, while possible, is reportedly unlikely as the laboratory is a WHO reference centre for rabies which regularly handles large numbers of serum samples from clinical trials. Any systematic errors in the RFFIT test would have been picked up quickly and rectified (Reeta Mani, personal communication). It is recommended that longitudinal serum samples from individual animals must be tested in the same

assay simultaneously to account for inter-assay variation that can occur when even the same sample is tested at different times [334]. In this study we chose to submit samples for testing at regular intervals, where possible within a few weeks to months after collection, as we were concerned about potential disruptions to storage facilities where samples were being stored. Indeed, at one point between R3 and R4, the -20°C freezer where we stored serum samples before transport to NIMHANS experienced power disruptions resulting in temperature fluctuations. All samples collected until then had to be stored in a separate -4°C freezer until the deep freezer was repaired. These temperature fluctuations could potentially have affected sample quality and compromised RFFIT titre results. Postponing testing of samples until completion of all study rounds would have meant that nearly 1400 serum samples collected over 16 months needed to be tested starting late January to early February 2020. With the benefit of hindsight, our decision not to postpone testing proved fortuitous as it is almost certain these serum samples would not have been tested until mid- to late-2021, as lockdowns necessitated by the COVID-19 pandemic took hold in India from March 2020. In addition, the testing laboratory at NIMHANS was charged with COVID-19 surveillance in India and did not conduct routine laboratory activities for several months in 2020, and again in 2021 during the catastrophic second COVID-19 wave in India (Reeta Mani, personal communication).

Non-descript breed dogs had consistently higher post-vaccination titres compared to purebreed dogs throughout the study. Using data from R2 and R3, we also showed that immunity declined at a significantly higher rate for ODs than for DWO irrespective of their vaccination history, despite the better care received and overall health of the former group of dogs. This is in contrast with a previous study from India which reported that purebreed dogs had higher RVNA titres compared to non-descript ODs [281], the latter group comprising nearly all of the DWOs in our study population. Similarly completely FRDs had higher mean titres than completely or partially confined dogs. Mean titres for ODs in MUH, all of which were completely free-ranging, were higher than for ODs in ALP in all rounds of the study. In chapter 3, multivariable logistic regression models showed that dogs in R1 with pre-vaccination RVNA titres ≤ 0.5 IU/ml or non-detectable titres had significantly lower recapture

probability compared to dogs with titres > 0.5 IU/ml (Table 3.4), even though all dogs in this study were vaccinated against rabies in R1. Additionally, in univariable logistic regression models, dogs with no detectable RVNA titres were less likely to be recaptured compared to those with detectable titres. We also detected RVNA in 20 ODs with no history of vaccination, 17 of which were partially or completely free-ranging. This included one free-ranging juvenile with titres > 0.5 IU/ml. Additionally, as highlighted above, there are frequent reports of rabid/suspected rabid dogs biting multiple people in ALP [277,278]. These observations suggest ongoing rabies virus circulation in the dog population in ALP. Further, dogs that survive rabies infection and develop adequate titres may live longer [40], particularly if subsequent vaccination induces effective anamnestic responses as identified recently [279]. Dogs have even been observed to lick saliva drooling from the mouth of suspect/confirmed rabid dogs (Abi Tamim Vanak, personal observation). It is unclear what role, if any, oral exposure to rabies virus may have in priming or boosting the immune system of healthy dogs against rabies infection. All these findings bolster evidence for the occurrence of non-lethal rabies infection in FRDs [280], which may also serve to induce anamnestic immune responses in vaccinated dogs or vice versa, leading to longer duration of antirabies immunity. However we could not establish whether any dogs survived such rabies exposures after developing clinical signs of rabies as we did not encounter suspected or confirmed rabid dogs in the course of fieldwork. The potential implications of the occurrence of non-lethal rabies infections for vaccination efforts and rabies control in FRDs requires further research.

Nevertheless, these observations must not detract from the continued importance of ensuring all dogs are regularly vaccinated against rabies, and the need for MRV campaigns to maintain adequate herd immunity. Experimental studies have shown that dogs vaccinated with high-quality rabies vaccines are able to respond effectively to rabies virus challenge by developing robust anamnestic responses even several years post-vaccination. All dogs with RVNA titres > 0.1 IU/ml were shown to survive challenge with rabies virus under experimental conditions [317]. While booster vaccinations are recommended when RVNA titres drop below 0.5 IU/ml [5], detection of titres below this level does

not necessarily mean that dogs are no longer protected against rabies. Additionally, rabies vaccination has been demonstrated to reduce mortality due to other causes among FRDs [40]. Thus, even a single dose of rabies vaccine may prove to be beneficial in priming immune responses and protecting against future rabies infections in FRDs.

The finding that dogs captured for ABC campaigns in R1 (as opposed to dogs that were already sterilised at capture in R1, and hence previously vaccinated) had significantly lower RVNA titres is not unexpected. FRDs captured as part of these efforts experience multiple concurrent stressors – net capture in the field, transport in vehicles and pre-operative confinement in close contact with similarly stressed and often aggressive dogs that they are unfamiliar with, handling by strangers, anaesthesia, surgery and vaccination; and further post-surgical confinement for up to three days prior to release. The chronic stress induced by this sequence of events is certain to cause immunosuppression [316], with inevitable consequences for development of post-vaccination immunity. An additional factor that may potentially contribute to lowered titres is appropriate storage under cold-chain conditions of the vaccine administered during ABC campaigns. The same vaccine used in the field study was administered as part of the regional campaign in R1, however it was procured separately by local authorities and we did not consistently observe its storage and handling. At the same time, we also reported in chapter 3 that dogs captured for ABC in R1 had significantly lower odds of recapture in later rounds, suggesting lower survival among this cohort. Our findings thus highlight a major pitfall in relying on ABC as a rabies prevention strategy, even as this intervention continues to be promoted as a suitable alternative in the absence of MRV campaigns [1] despite not being recommended by the WHO [5]. Government bodies and animal welfare organisation must ensure that dogs captured for ABC are treated with care throughout a campaign and establish that they are healthy prior to release. Failure to do so can result in reduced post-release survival, lowered antirabies immunity and subsequent lowered herd immunity levels.

Haemolyzed serum samples and those with high levels of lipemia, which increases serum turbidity, can interfere with serum neutralization tests, and their effects may vary based on the assay being used [327]. We found that ODs had higher median haemolysis scores compared to DWOs. We attribute this finding to the fact that most ODs in our study were restrained by their owners during sampling, and therefore would have struggled much more than DWOs which were effectively restrained by dog catchers using nets. Such struggling often made it difficult to quickly and effectively collect blood samples from ODs, possibly leading to shear forces rupturing red blood cells during sampling. While we found no consistent associations between serum quality and reported RVNA titres, increased serum turbidity in R2 was found to significantly increase the value of final reported titres. This highlights the challenges of blood sampling dogs in the field. Further, although we advised dogs owners not to feed dogs before blood sampling, this advice was not always adhered to or we could visit households only later in the day by when they might have been fed, and we had not control over the feeding status of DWOs. Many studies reporting RVNA titre levels do not explicitly report serum sample quality parameters, making it difficult to assess their effects on final results.

4.4.1 Conclusions

Our findings highlight the challenges and opportunities for achieving and maintaining herd immunity in FRDs and ODs in India. Rabies vaccination is highly effective in inducing antirabies immunity in dogs. However, public health agencies will need to consider differential immune responses based on individual dog characteristics such as age, breed and past vaccination history to tailor the implementation and frequency of MRV campaigns to regional dog populations. Doing so will be crucial to ensure that adequate herd immunity is induced and maintained in dog populations. An equally important aspect is the use of high-quality rabies vaccines that have been properly stored to prevent loss of antigenic potency. Any compromises in this regard will render rabies control efforts fruitless and merely lead to wastage of scarce public health funds.

Chapter 5 : **A question of accessibility: modelling the influence of accessibility for vaccination and demographic factors on elimination of rabies in free-ranging dog populations in India.**

ABSTRACT

Typically, mathematical models of rabies transmission in dogs assume uniform accessibility for vaccination. However, the substantial populations of free-ranging dogs (FRDs) that are unowned in regions like India mean that this assumption does not reflect real-world situations. An assumption of uniform mortality rates in models also goes against evidence suggesting high rates of mortality in the first year of life in FRD populations. We parameterised a deterministic compartmental age-structured Susceptible-Exposed-Infectious-Vaccinated (SEIV) model, incorporating demographic and immunological parameters reported for Indian FRD populations, as well as gathered during fieldwork in India. Model results highlighted the importance of incorporating assumptions about accessibility for vaccination when modelling rabies elimination strategies. As accessibility for vaccination increased, rabies elimination was possible in a wider range of scenarios within shorter timeframes, with less frequent campaigns achieving lower vaccination coverages in the accessible population. Where elimination was possible, this occurred generally within 10 years of implementation of vaccination campaigns. However, in scenarios where ≤ 20% of dogs were accessible, more frequent campaigns (typically annual) were needed to maintain high vaccination coverages (> 95% of the accessible population) consistently for > 20 years to eliminate rabies. Rabies elimination was possible in most demographic scenarios and transmission settings, typically with annual campaigns, even with < 70% effective vaccination coverages in the total dog population. The model also highlighted the complex interplay of demographic factors and disease transmission, with high birth rates resulting in higher rabies cases, irrespective of juvenile mortality or adult lifespan. Finally, human interventions

such as animal birth control that fail to substantially reduce birth rates, while improving juvenile survival at the cost of reduced adult lifespan, may be counterproductive to rabies control efforts.

5.1 INTRODUCTION

5.1.1 Background

Mathematical models of rabies transmission were first used to inform fox rabies control efforts in Europe [8] and subsequent models served to identify several important demographic and ecological features of wildlife rabies transmission in Europe and North America [337]. Similar approaches were then used to develop models of canine rabies transmission and control, which have regularly highlighted the effectiveness of MRV as a cost-effective strategy for rabies elimination. Coleman and Dye (1996) used a simple Susceptible – Latent (Exposed) – Infective (SEI) compartmental transmission model to support empirical evidence for the need to vaccinate at least 70% of the dog population in order to eliminate rabies [25]. Since then, a variety of mathematical models of rabies have been used to elucidate various aspects of rabies spread and control [88], including disease dynamics [338], to investigate introductions of the pathogen into rabies-free regions [339] and the impact of interventions on future incidence [17,340], to inform control efforts [8,17,97] and for economic analyses [28]. For instance, modelling studies highlighted that patchy vaccination coverage in small areas making up otherwise comprehensively covered regions can compromise rabies control efforts by acting as pockets of infection [17]. Bourhy et al. (2018) combined epidemiological and phylogenetic sequence data to show that rabies did not persist in urban areas and outbreaks were seeded by frequent introductions from outside the urban areas.

While mathematical models have reinforced the feasibility of canine rabies elimination through MRV, many models assume complete accessibility of dogs for vaccination, possibly owing to the observation that most FRDs in Africa and Asia are owned. Thus, the key factor influencing the probability of rabies elimination has been shown to be vaccination coverages achieved, with high rates of dog population turnover necessitating higher vaccination coverages than would otherwise be predicted solely based

on the low R_0 values typically associated with rabies outbreaks [26]. However, such assumptions about accessibility for vaccination are unlikely to apply in regions like India where a large proportion of FRDs are truly unowned and therefore not directly accessible for vaccination. Typically, conventional rabies transmission models have also assumed uniform mortality rates within dog populations. This assumption goes against research that has consistently highlighted very high levels of mortality in young FRDs below one year of age in India [73,248], with potential implications for vaccination coverages required for rabies control and elimination. Belsare and Vanak (2020) demonstrated that juvenile mortality and litter size were important factors regulating FRD population sizes and thus DPM efforts such as ABC [69]. As highlighted in chapter 4, there may also be differences between disparate dog populations (e.g. ODs vs DWOs) in development and persistence of RVNA titres. Incorporating such heterogeneity in immunological responses into transmission models could generate different infection dynamics and influence probability of elimination.

Despite having the largest burden of dog-mediated human rabies deaths globally, no modelling studies have evaluated the feasibility of canine rabies elimination in India through MRV campaigns. While this is likely to be in large part due to the long-standing neglect of rabies as a public health concern in India [1] (as discussed in chapter 2), a lack of data on the disease burden in humans and dogs as well as dog ecology and demography can also hinder efforts to model control strategies [100]. Only two studies have used mathematical models to evaluate strategies for rabies control in India. Fitzpatrick et al. (2016) fit a compartmental transmission model to data on human rabies from the south Indian state of Tamil Nadu and showed that MRV campaigns targeting dogs were highly cost-effective in reducing human rabies deaths [28]. Their models suggested that vaccinating as low as 13% of the FRD population reduced human rabies cases by nearly 90%. Belsare and Vanak (2020) explored the use of rabies vaccination in the context of ABC campaigns in India and showed that even in best-case scenarios, only 35% of the FRD population could be vaccinated [69]. Crucially, their model was possibly the first to account for varying accessibility of FRD populations for interventions, specifically

for ABC. This was done by incorporating a parameter – catchability – to reflect heterogeneity in capture effort required. Using this approach, these models enabled estimation of the financial resources, time and effort required to effectively reduce FRD populations in India.

Failing to incorporate assumptions about accessibility when designing and implementing MRV campaigns may result in unrealistic expectations for elimination timelines and misdirection of scarce financial and human resources, rendering the goal of canine rabies elimination infeasible in the long term. It can also have direct implications on resource requirements for rabies control efforts. For instance, Gibson et al. (2020) detailed examples of hypothetical resource requirements for MRV campaigns in cities in India [72]. However, the framework used to calculate these requirements did not formally incorporate any estimates of accessibility because, as they point out, such estimates are not available, particularly for UDs. In the absence of such data, mathematical models that incorporate varying levels of accessibility for vaccination can provide valuable insights into the challenges in eliminating rabies in hard-to-reach FRD populations.

5.1.2 Aims

We developed a deterministic compartmental age-structured SEIV model of rabies transmission in FRD populations in the Indian context. This model incorporated demographic parameters reported for Indian FRD populations, as well as demographic and immunological data gathered during fieldwork in Kerala and presented in chapters 3 and 4.

The aims of developing this model were to:

a. Explore the influence of assumptions about accessibility of FRDs for vaccination on the probability of rabies elimination in India and
b. Explore the feasibility of rabies elimination in FRD populations in India

5.2 METHODS

5.2.1 Description of the model

In the model, each time step represented one day, and all rates were expressed as daily rates. We incorporated age-structure into our model to account for the substantially higher mortality rates recorded for free-ranging pups and juveniles below one year of age, where only 19% of dogs in this age category were reported to survive to sexual maturity in India [248]. Thus the model comprised of young (< nine months of age, with superscript y) & adult dogs (≥ nine months, with superscript A), both of which comprised susceptible, exposed, infected and vaccinated dogs (Fig 5.1, Table 5.2). In addition, the model was parameterised using a range of demographic and MRV campaign parameters reported in the literature for FRD populations in India or gathered through data from fieldwork conducted in Kerala, as detailed in chapters 3 and 4 (Table 5.3).

5.2.1.1 Demography

We assumed a carrying capacity (*K*) of 600,000 dogs, the most recent official estimate of Kerala's 'stray' dog population [230] (Table 5.2). The starting population was comprised of a proportion of adults p^A and the proportion of young p^y was $1 - p^A$. Susceptible dogs comprised those that were accessible (S_v) or not accessible (S_{nv}) for vaccination, based on the parameter 'acc', representing assumed proportions of dogs accessible for vaccination.

The per capita birth rate was calculated using a range of published estimates of the proportion of adults in the population p^A, the proportion of female dogs in the population p^{female}, the proportion of adult females that reproduce annually (assumed to be 0.5) [78], and the number of pups per litter (l_{size}) (Table 5.1).

Therefore, per capita birth rate b was calculated as

$$b = p^A * p^{female} * 0.5 * l_{size} \quad (1)$$

Using these parameters, a list of values was generated for per capita birth rate (Table 5.3), to explore its influence on rabies dynamics and control efforts.

Table 5.1 Parameters used to calculate per capita birth rate per day. List of demographic parameters used to calculate values of the per capita birth rate per day, b, as per equation 1 above, for use in the age-structured SEIV compartmental rabies transmission model for free-ranging dog populations.

Sl. No	Parameter	Symbol	Value from literature	Location	Reference	List of values used
Demographic parameters						
1.	Proportion of adults,	p^A	i. 0.76 (summer), 0.82 (autumn) ii. 0.67 – 0.86 iii. 0.82 iv. 0.87 (maximum)	West Bengal Maharashtra Maharashtra Kerala	[341] [33] [64] Field data	0.67, 0.72, 0.77, 0.82, 0.87
2.	Adult sex ratio (Male:Female), used to calculate proportion of females	p^{female}	i. 0.5 (1:1) – 0.29 (2.5:1) ii. 0.42 (1.37: 1) iii. 0.5 (1:1) iv. 0.43 (1.34: 1) – 0.29 (2.5:1) v. 0.5 (1:1)	Maharashtra West Bengal West Bengal Maharashtra Kerala	[33] [73] [341] [64] Field data	0.34, 0.42, 0.5, 0.56
3.	Proportion of females conceiving	-	0.475 (95% CI: 0.44 – 0.51)	Rajasthan	[78]	0.5
4.	Litter size	l_{size}	i. 6 (IQR 4 – 7) ii. 4 (IQR 3 – 5) iii. 5 (range 2 – 9) iv. 5.7 (range 3 – 9) v. 5.6 vi. 4.6 (4 – 5.3) vii. 4 (range 2 – 10)	Goa and Tamil Nadu West Bengal West Bengal West Bengal Rajasthan Rajasthan Kerala	[66] [248] [73] [342] [343] [77] Field data	5, 6, 7

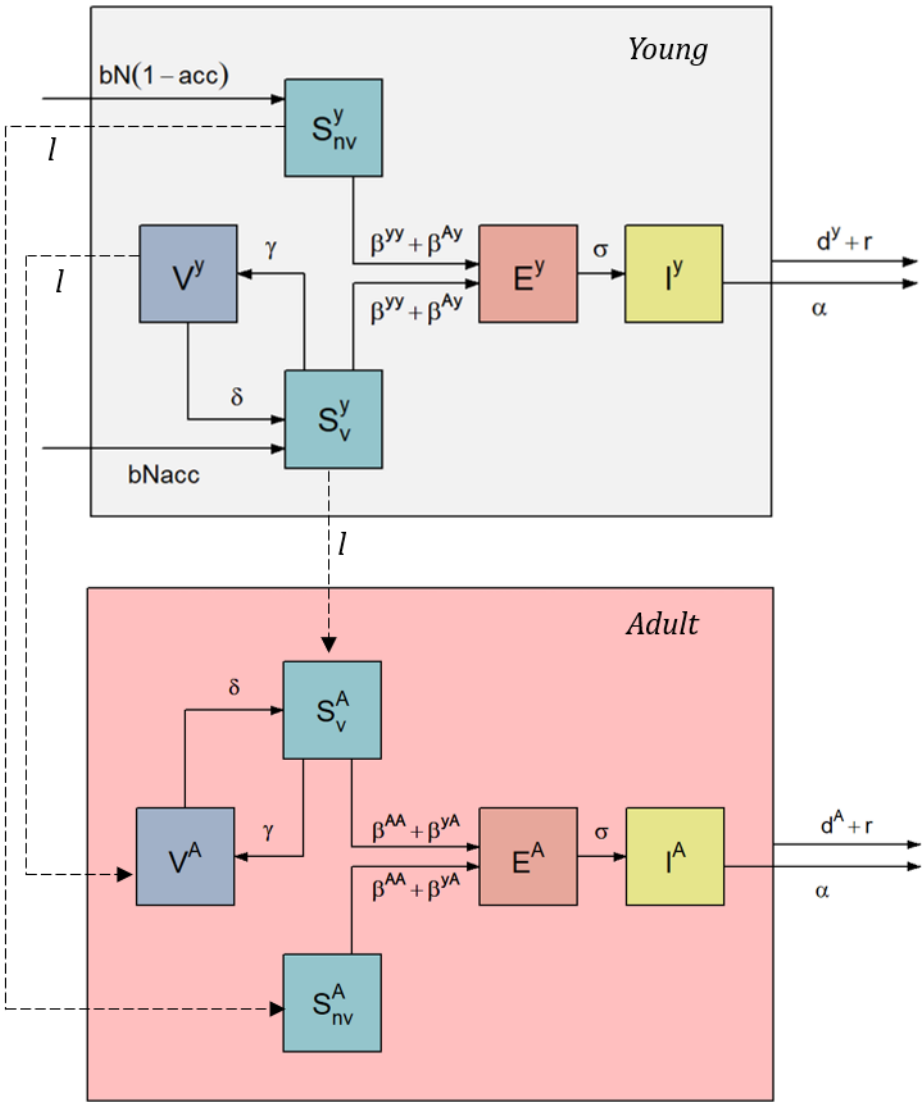

Figure 5.1 Schematic of age-structured SEIV model. Schematic of the age-structured SEIV model comprising young (with superscript y) and adult dogs (with superscript A), with the susceptible compartment further divided into dogs that are accessible (with subscript v) or not accessible (with subscript nv) for vaccination. Dashed arrows represent transition of young dogs to adults at a rate l. S – susceptible, V – vaccinated, E – Exposed, I – infectious. Parameters are detailed in Table 5.3.

All births entered the susceptible young compartment. For simplicity, all dogs contributed to births, with a constant proportion of dogs being born accessible ($bNacc$) or non-accessible ($bN(1-acc)$). Individuals left respective compartments because of death due to natural causes (d) and density-

dependent constraints. We assumed that birth rate was constant and density regulation acted on mortality ($d^x + rN/K$, with x='*y*' or '*A*' for young and adult dogs, respectively) (see Table 5.2). As such, with a low population size (i.e. N~0) the population grew exponentially at rate *r*; while when the approach carrying capacity (i.e.N~K), birth remain constant but increase mortality in young and adult led to a null per capita birth rate (given *r* = *b* - *d*). In the absence of rabies, a population *N* is assumed to be governed by logistic growth and regulated to a carrying capacity *K*, as given by the equation,

$$\frac{dN}{dt} = rN\left(1 - \frac{N}{K}\right) \tag{2}$$

The mortality rate for young dogs (d^y) was calculated using the formula

$$p = 1 - e^{-d^y t} \tag{3}$$

where p is the proportion of young dogs reported to die before sexual maturity and t the period by which sexual maturity is reached, assumed to be seven months (210 days). Thus, juvenile mortality rate d^y was calculated as

$$d^y = \frac{\log\left(\frac{1}{1-p}\right)}{t} \tag{4}$$

Adult mortality (d^A), in the absence of density-dependence, was calculated using lifespan estimates reported in the literature. Consequently, overall mortality rate *d* was calculated to be

$$d = d^y p^y + d^A p^A \tag{5}$$

After considering the two-month canine gestation period, young dogs (male or female) from only susceptible and vaccinated compartments transitioned to become adults at nine months of age at a per capita rate $'l'$ per day.

5.2.1.2 Disease transmission

Rabies transmission occurs when susceptible hosts are bitten by rabid/infectious individuals. In the model, susceptible individuals (*S*) become exposed (*E*) at a rate governed by the transmission rate β and is proportional to the product of the number of infectious individuals and the number of susceptibles at any time point. Individuals become infectious (*I*) at a rate equal to σ, the inverse of the mean incubation period (assumed to be $1/\sigma$ = 25.5 days [340]). The average duration of illness was assumed to be 5.7 days ($1/\alpha$) [340], after which infectious dogs die. The transmission rate β was calculated using next-generation matrices [344] derived from the system of equations detailed in Table 5.2 and based on assumed R_0 values. We assumed that transmission occurred at the same rate within and between age compartments (i.e. young to young β^{yy}, young to adult β^{yA}, adult to young β^{Ay}, adult to adult β^{AA}). Therefore, when

$$\beta^{yy} = \beta^{yA} = \beta^{Ay} = \beta^{AA} = \beta,$$

the equation to calculate β from R_0 is

$$\beta = \frac{R_0}{\sigma K Z} \tag{6}$$

where

$$Z = \frac{p^y}{(\sigma+b+p^A(d^y-d^A))(\alpha+b+p^A(d^y-d^A))} + \frac{p^A}{(\sigma+b+p^y(d^A-d^y))(\alpha+b+p^y(d^A-d^y))}.$$

When $p^y = p^A = 0.5$, and $d^y = d^A$, equation 6 reduced to

$$\beta = \frac{R_0(\sigma+b)(\alpha+b)}{\sigma K}$$

as derived by Anderson et al. (1981) [8].

The model was initiated with one infected dog I_0 present at time t_0, and allowed to run for 250 years until the number of infectious dogs had achieved an endemic equilibrium.

5.2.1.3 Vaccination

Vaccination was introduced on the first day of the 251st year (t = 91251 days ignoring leap years) in the form of pulsed MRV campaigns implemented for a fixed number of days per campaign (campaign duration, $camp.durn$, 30 days in all models) and covering a proportion of the dog population ($coverage$, with assumed values between 0.1 and 0.95). Thus, the per capita vaccination rate per day, γ, was calculated as

$$\gamma = \frac{\log\left(\frac{1}{1-coverage}\right)}{camp.durn}. \quad (7)$$

The effective vaccination coverage required in the entire dog population was calculated as the product of the proportion of dogs accessible for vaccination and the target vaccination coverage in the accessible proportion. Thus,

$$\text{Effective vaccination coverage} = coverage * acc.$$

Campaigns were implemented at intervals specified by the parameter '$camp.int$', as opposed to reactive vaccination implemented in response to outbreaks [340]. Dogs moved into the vaccinated compartment at the time of vaccination. Vaccinated dogs, regardless of age (i.e V^y or V^A) lost immunity at an exponential rate δ (values between one per 220 days, one year (365 days) and three years (1095 days)) which relates to duration of immunity induced by most inactivated rabies vaccines [48], and returned to being susceptible. Vaccination did not stop at any point in the model.

Once the proportion of adults p^A in the starting dog population was incorporated into the per capita birth rate b, the former no longer influenced the dog population or rabies transmission dynamics in the models. Therefore, p^A was set to be 0.77 in all models. The model was run for 20 years after introduction of vaccination, and the effects of varying various parameters (Table 5.3) on elimination of new rabies cases during this period were explored

All models were implemented and numerically solved in R (version 4.1.1) [268] using the *deSolve* package.

Table 5.2 System of ordinary differential equations describing rabies transmission dynamics in the age-structured SEIV compartmental model for free-ranging dogs.

Category		Demography*	Enter compartment	Leave compartment	Death due to rabies	Vaccination
Young dogs						
Susceptible, accessible for vaccination	$\frac{dS_v^y}{dt} =$	$bNacc - (d^y + rN/K)S_v^y$	$+ \delta V^y$	$-(\beta^{yy}I^y + \beta^{Ay}I^A)S_v^y - lS_v^y$		$-\gamma S_v^y$
Susceptible, not accessible for vaccination	$\frac{dS_{nv}^y}{dt} =$	$bN(1-acc) - (d^y + rN/K)S_{nv}^y$		$-(\beta^{yy}I^y + \beta^{Ay}I^A)S_{nv}^y - lS_{nv}^y$		
Exposed	$\frac{dE^y}{\mathrm{dt}} =$	$-(d^y + rN/K)E^y$	$+(\beta^{yy}I^y + \beta^{Ay}I^A)(S_v^y + S_{nv}^y)$	$-\sigma E^y$		
Infectious	$\frac{dI^y}{dt} =$	$-(d^y + rN/K)I^y$	$+\sigma E^y$		$-\alpha I^y$	
Vaccinated	$\frac{dV^y}{\mathrm{dt}} =$	$-(d^y + rN/K)V^y$	$+\gamma S_v^y$	$-\delta V^y - lV^y$		
Adult dogs						
Susceptible, accessible for vaccination	$\frac{dS_v^A}{\mathrm{dt}} =$	$-(d^A + rN/K)S_v^A$	$+lS_v^y + \delta V^A$	$-(\beta^{AA}I^A + \beta^{yA}I^y)S_v^A$		$-\gamma S_v^A$
Susceptible, not accessible for vaccination	$\frac{dS_{nv}^A}{\mathrm{dt}} =$	$-(d^A + rN/K)S_{nv}^A$	$+lS_{nv}^y$	$-(\beta^{AA}I^A + \beta^{yA}I^y)S_{nv}^A$		
Exposed	$\frac{dE^A}{dt} =$	$-(d^A + rN/K)E^A$	$+(\beta^{AA}I^A + \beta^{yA}I^y)(S_v^A + S_{nv}^A)$	$-\sigma E^A$		
Infectious	$\frac{dI^A}{\mathrm{dt}} =$	$-(d^A + rN/K)I^A$	$+\sigma E^A$		$-\alpha I^A$	
Vaccinated	$\frac{dV^A}{dt} =$	$-(d^A + rN/K)V^A$	$+lV^y + \gamma S_v^A$	$-\delta V^A$		
$N = (S_v^y + S_{nv}^y + E^y + I^y + V^y) + (S_v^A + S_{nv}^A + E^A + I^A + V^A)$						

*Including births and deaths

Table 5.3 Parameters used in the age-structured SEIV compartmental model of rabies transmission in free-ranging dogs. Parameters used in the age-structured SEIV compartmental rabies transmission model for free-ranging dog populations. Parameters whose values were varied in the model to evaluate their influence on rabies dynamics and elimination within 20 years of initiating vaccination campaigns are highlighted in bold

Sl. No	Parameter	Symbol	Value from literature	Location	Reference	List of values explored
Demographic parameters						
1.	**Birth rate, (per capita per day)**	$\boldsymbol{b}$	**Calculated using values of parameters detailed in Table 5.1**	-	**-**	**0.00210, 0.00313, 0.00417, 0.00467**
2.	Sex ratio at birth (Male:Female)	-	i. 1.26:1 ii. 1.41:1	West Bengal West Bengal	[73] [342]	1:1
3.	Transition of young to adulthood	$1/l$	7-13 months (age at which females achieved first copulatory 'lock') + 60 days (canine gestation period)	West Bengal	[345]	9 months (270 days)
4.	**Adult life span, to calculate per capita adult mortality rate**	$\boldsymbol{1/d^A}$	**i. 2.6 years** **ii. 3.8 years (sterilised females)**	**West Bengal** **Rajasthan**	[73] [78]	**2.6, 3, 3.8 and 4.5 years**
5.	**Proportion of young dying before sexual maturity (to calculate per capita juvenile mortality rate, d^y)**	**-**	**i. 0.81 by 7 months** **ii. 0.82 by one year** **iii. 0.75 by one year**	**West Bengal** **West Bengal** **Rajasthan**	[248] [73] [78]	**0.69, 0.75, 0.81**
6.	Carrying capacity	K	Official estimate of Kerala 'stray' dog population	Kerala	[230]	600,000
7.	Population growth rate	r	Calculated using assumed values of parameters birth rate, juvenile and adult mortality rates as $b - (d^y p^y + d^A p^A)$	-	-	-
Disease transmission parameters						
8.	Incubation period	$1/\sigma$	25.5 days	Tanzania	[340]	25.5 days
9.	Duration of illness	$1/\alpha$	5.7 days	Tanzania	[340]	5.7 days
10.	**Reproduction number, (used to calculate transmission rate β)**	$\boldsymbol{R_0}$	**Various**	**Various locations**	[26]	**Transmission settings: Low – 1.12, Medium – 1.48, High – 1.65**

Vaccination parameters						
11.	**Interval between campaigns**	$\boldsymbol{camp.int}$	**Assumed**	**-**	**-**	**1 year, 2 years, 3 years)**
12.	Campaign duration (in days),	$camp.durn$	Assumed	-	-	30 days
13.	**Assumed vaccination coverage, used to calculate per capita vaccination rate per day, γ**	**-**	**i. 8% - 35%** **ii. Assumed**	**Using ABC in India** **-**	[69]	**10%, 25%, 40%, 60%, 80%, 95%**
14.	**Accessible proportion**	$\boldsymbol{acc}$	**i. 40% easily captured** **ii. 50%**	**India** **Kerala**	[69] **Field data**	**0.2, 0.4, 0.6, 0.8, 1**
15.	**Duration of immunity induced by one vaccine dose**	$\boldsymbol{1/\delta}$	**i. 1 – 3 years (Nobivac Rabies)** **ii. 1 – 3 years (Raksharab)** **iii. 220 days**	**MSD animal health India** **Indian Immunologicals Ltd.** **Kerala**	[335] [336] **Field data**	**220 days, 365 days (1 year), 1095 days (3 years)**

5.2.2 Transmission settings and demographic scenarios

Three different transmission settings were modelled based on assumed values of R_0 – low (R_0 =1.12), medium (R_0 =1.48) and high (R_0 =1.65). Within each transmission setting, a total of 12960 unique combinations of the parameters highlighted in bold in Table 5.3 were explored (hereafter, each unique combination is referred to as a 'scenario'). In addition, three broad demographic scenarios were defined – a baseline scenario incorporating mean values of demographic parameters reported in the literature for FRD populations in India and thus presumed to reflect real-world dynamics; a high recruitment scenario (HRS) where a large number of pups would be born, a high proportion survived to sexual maturity and adult dogs (in the absence of density regulation) lived for the longest period assumed in these models; and a low recruitment scenario (LRS) where very few pups would be born, a low proportion survived to sexual maturity and adult lifespan (in the absence of density regulation) was the shortest assumed in the models (Table 5.4). In addition to these scenarios, rabies elimination endpoints (detailed below in section 5.2.4) were also estimated across all scenarios implemented (referred to as 'All scenarios').

The lowest per capita birth rate implemented in the model was 0.00210 per day. When this value was used to define the LRS scenario, dog populations were not self-sustaining in the presence of rabies transmission. Therefore, the next highest value of the per capita birth rate (0.00313 births per day) was used.

Table 5.4 Demographic scenarios and their parameters. Summary of the three demographic scenarios explored and parameters used to define them.

Demographic scenario	Parameters				Implications
	Birth rate (per capita per day), b	Proportion of young dying before sexual maturity	Adult life span (in years), $1/d^A$ (assuming no density dependence)	Mean adult life span (in years), $1/(d^A + r)$ (at carrying capacity)	
Baseline scenario (Low birth rate, high juvenile mortality, moderate adult mortality)	0.00313	0.81	3	1.80	Moderate population growth, slower replenishment of susceptibles
High recruitment scenario (HRS) (high birth rate, low juvenile and adult mortality)	0.00467	0.69	4.5	0.78	Susceptibles rapidly replenished, high adult mortality as population nears carrying capacity
Low recruitment scenario (LRS) (low birth rate, high juvenile and adult mortality)	0.00313	0.81	2.6	1.76	Low population growth and slower replenishment of susceptibles

The lowest per capita birth rate implemented in the model was 0.00210 per day. When this value was used to define the LRS scenario, dog populations were not self-sustaining in the presence of rabies transmission. Therefore, the next highest value of the per capita birth rate (0.00313 births per day) was used.

5.2.3 Key assumptions

The models assumed density-dependent rabies transmission in a closed population with no immigration or emigration, and no introduction of rabies cases into the population. Dogs that were inaccessible for vaccination (S_{nv}) remained inaccessible throughout the model. Although FRDs

reproduce throughout the year, seasonal reproduction has been demonstrated in Indian FRD populations [66]. We did not incorporate any seasonality and births occured at a constant rate throughout each year. While we accounted for accessibility, we did not model capture effort explicitly as done by Belsare and Vanak (2020) [69].

A completely effective vaccine was assumed and thus all dogs were assumed to develop post-vaccination RVNA titres > 0.5 IU/ml. This may not necessarily hold in reality, and poor immune responses have been reported even in dogs that were routinely vaccinated [346]. We also implemented uniform vaccination rates for young and adult dogs, when in fact these may differ considerably in practice, particularly for very young FRD pups. The rate of loss of vaccine-induced immunity (and thus returning to the Susceptible class) was calculated based on reported estimates of the time for RVNA titres to drop below 0.5 IU/ml, although dogs with titres below this level may still be protected [317].

Rabies transmission was assumed to occur at the same rate within and between age groups. We also assumed that all canine rabies cases will be detected. In reality a substantial proportion of cases may go undetected due to poor surveillance, lack of diagnostic laboratory facilities, lack of awareness about rabies symptoms in dogs or confusing rabies symptoms with other canine neurological diseases such as canine distemper, especially in dogs that may present with the paralytic/'dumb' form of rabies. Although DPM interventions such as ABC are routinely implemented for FRDs in India, the impacts of these interventions on rabies dynamics were not explored in the model.

5.2.4 Analysis of model results

Prior to analyses of model output, scenarios in which parameter combinations resulted in either a) dog populations failing to sustain themselves, or b) rabies cases declining to near zero (i.e. $I^{y} + I^{A} <$ 0.5) at the time of initiation of MRV campaigns (t = 91251) were excluded. In a limited number of scenarios, the number of new rabies cases dropped below 0.5 per month after implementation of vaccination but subsequently rose again above this level. In such scenarios, the number of new monthly rabies cases were converted to zero once it was < 0.5, before further analyses.

Once MRV campaigns were implemented, we defined t = 0 as the time of first implementation of MRV. To calculate time to elimination (in years), rabies elimination was defined to occur at the first time point in each unique scenario when new monthly (every 30 days) rabies cases (Rab) dropped below 0.5 ($Rab < 0.5$). Alternatively, for the period ten years after MRV implementation (between simulated years 11 – 20), we calculated the mean monthly Rab across a range of scenarios sharing the same combination of the parameters interval between campaigns, vaccination coverage, duration of immunity and accessibility i.e. Rab_{11-20}^{mean}.

Once MRV campaigns were implemented, we evaluated the impacts of various parameters on rabies elimination by:

a. Calculating the proportion of scenarios where rabies was eliminated i.e. $Rab_{11-20}^{mean} < 0.5$ – To do this, the mean number of new rabies cases generated every 30 days between years 11 – 20 after MRV implementation was calculated for each scenario and then rounded to integer values. We then calculated the proportion of scenarios with each unique combination of the parameters interval between campaigns, vaccination coverage, duration of immunity and accessibility (e.g. interval between campaigns = 1, coverage = 10%, duration of immunity = 220 days, accessibility = 0.2 and so on) where $Rab_{11-20}^{mean} < 0.5$. The interaction between accessibility, coverage and interval between campaigns (at a fixed value of duration of immunity) was summarised as heatmaps representing the lowest vaccination coverage for each combination of accessibility and interval between campaigns, where all of the scenarios with each combination of these parameters had $Rab_{11-20}^{mean} < 0.5$ (Fig. 5.2). This process was repeated for the three different demographic scenarios defined above (Fig. 5.3).
b. Calculating the mean time to elimination (in years), i.e. $t_{Rab<0.5}^{mean}$,– For this calculation, monthly Rab from t = 91251 days was calculated for each unique scenario and then rounded to integer values. The first time point where Rab < 0.5 was then used to calculate the time to elimination for that particular scenario. Then, $t_{Rab<0.5}^{mean}$ for various combinations of the parameters interval between campaigns, vaccination coverage, duration of immunity and accessibility across all

scenarios was calculated. We also calculated the proportion of scenarios with every combination of these parameters where Rab < 0.5 (Figs 5.5, S1, S3). The interaction between accessibility, coverage and interval between campaigns (at a fixed value of duration of immunity) was summarised as heatmaps representing $t^{mean}_{Rab<0.5}$ for the lowest vaccination coverage for each combination of accessibility and interval between campaigns where all of the scenarios with each combination of these parameters achieved Rab < 0.5. This process was repeated for the three different demographic scenarios defined above (Figs. S5, S6).

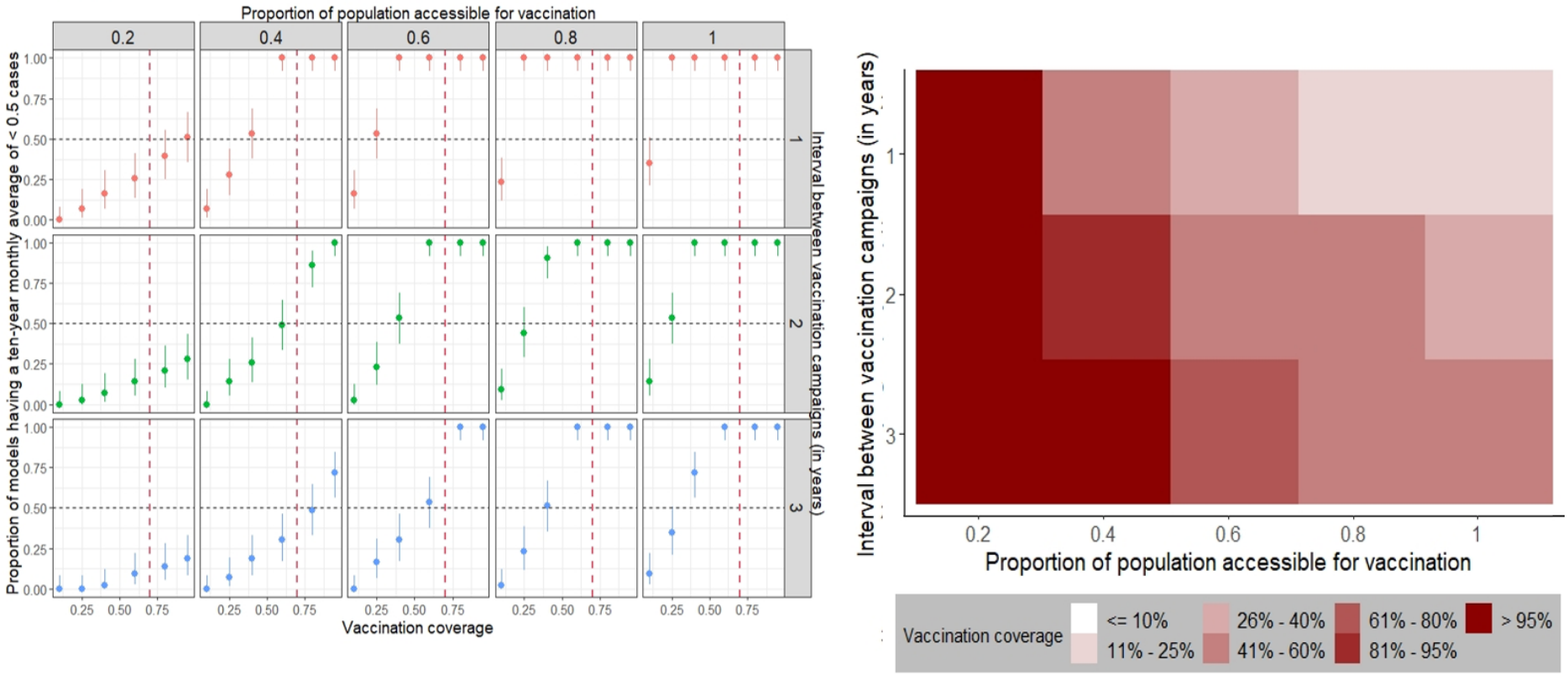

Figure 5.2 Proportion of models with monthly average < 0.5 new rabies cases and generation of heatmap. (Left) The proportion of models achieving elimination (defined as having a ten-year monthly average of < 0.5 rabies cases 10 years after implementation of MRV) in a low-transmission setting (R_0 =1.12) shown as a function of vaccination coverage in the accessible proportion of the population, the proportion of the population accessible for vaccination (acc) and interval between vaccination campaigns (in years) ($camp.int$). Vaccine-induced immunity is assumed to last for one year. (Right) Heatmap representing the lowest vaccination coverage at which rabies was elimination in all models for each combination of interval between campaigns and accessibility ($camp.int$ and acc).

5.3 RESULTS

Of 12960 unique scenarios generated within each transmission setting using values of the parameters highlighted in bold in Table 5.3, dog populations failed to sustain themselves or rabies cases declined to < 0.5 at the time of initiation of MRV campaigns in 1350 scenarios. All these scenarios had the lowest per capita birth rate explored of 0.0021 per day, and were excluded from further analyses.

As expected, the proportion of scenarios which achieved $Rab_{11-20}^{mean} < 0.5$ increased consistently with an increase in vaccination coverage and/or accessibility, and a decrease in interval between campaigns (Fig. 5.2). Conversely, as accessibility decreased or the interval between campaigns increased, higher vaccination coverages were required for rabies elimination. Similarly, as coverage increased, mean time to elimination decreased consistently for all values of accessibility, with only a small difference (2 – 3 years) in the mean time to elimination across different values of accessibility at the highest vaccination coverage (95%), particularly with annual MRV campaigns (Figs 5.4, 5, S1-S4). Where rabies elimination was possible within 20 years of MRV implementation, mean time to elimination was generally less than 10 years, irrespective of vaccination coverage, accessibility or interval between campaigns (Fig. 5.5). When only 20% of dogs were accessible, vaccination of > 95% of the accessible population (Fig. 5.3) over > 20 years (Figs. 5.5, S1, S3) was required to ensure rabies elimination across all transmission settings and demographic scenarios, except the baseline and LRS scenarios of the low-transmission setting, as detailed below. Similarly, when all dogs were accessible, the mean time to elimination was generally ≤ 6 years across all demographic scenarios and transmission settings (Figs 5.4, 5.5), with the lowest vaccination coverage required increasing with greater intervals between campaigns (Fig 5.3). Except in low-transmission settings, implementing MRV campaigns less frequently than annually required at least 60% of the dog population to be accessible for vaccination and vaccination coverages greater than 95% for more than 20 years. The LRS and HRS scenarios required the lowest and highest vaccination coverages respectively, across all transmission settings (Fig. 5.3).

The duration of vaccine-induced immunity did not substantially influence rabies transmission dynamics or probability of elimination in the scenarios explored. As duration of immunity increased, time to rabies elimination decreased slightly while elimination was predicted to be possible even if only a lower proportion of the population was accessible for vaccination (Figs. S12, S15-S17) or lower vaccination coverages were achieved (Figs S14-S17).

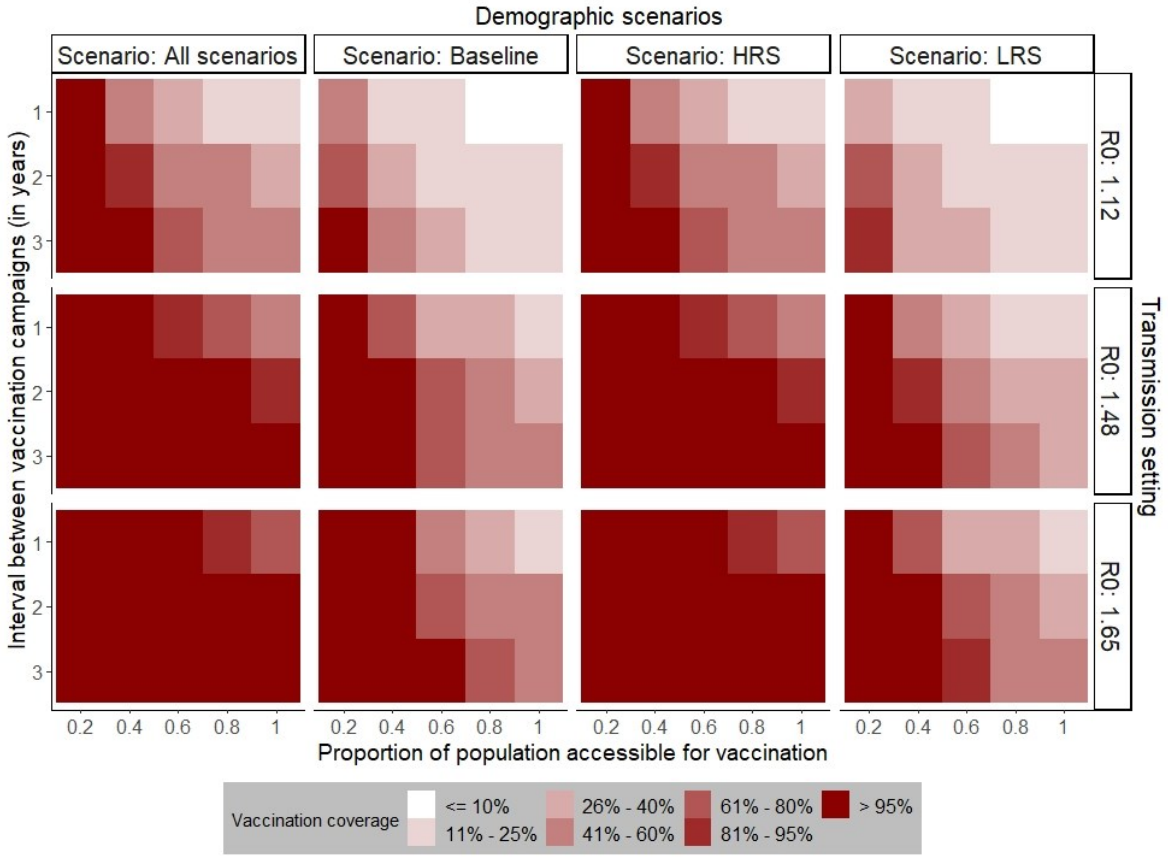

Figure 5.3 Heatmap of lowest vaccination coverages at which rabies is eliminated, by interval between campaigns, demographic scenarios and transmission setting. Heatmap showing the lowest vaccination coverages at which rabies was eliminated in all models (defined as having a ten-year monthly average of < 0.5 rabies cases, ten years after implementation of mass rabies vaccination). The vaccination coverages required for certain elimination are presented as a function of the interval between vaccination campaigns, proportion of dogs accessible for vaccination, demographic scenario and transmission setting. Parameters used to define the demographic scenarios (Baseline, HRS, LRS) are summarised in Table 5.4. Vaccine-induced immunity is assumed to last for one year. R0 – Reproduction number.

There was some incongruence in the lowest vaccination coverages required for rabies elimination, when assessed based on either the proportion of scenarios where rabies was eliminated ($Rab_{11-20}^{mean} < 0.5$) or the mean time to elimination ($t_{Rab<0.5}^{mean}$) (as defined in section 5.2.4) (Figs 5.3, S6). This incongruence arose due to the slightly different methods used to assess rabies elimination according to either criterion. We calculated the mean time to elimination for each unique scenario using a single time point at which the number of new rabies cases dropped to < 0.5, and then averaging over all scenarios sharing values of the parameters interval between campaigns, vaccination coverage, duration of immunity and accessibility. For example, in the high-transmission setting there were 43 scenarios where MRV campaigns conducted every two years achieved 95% vaccination coverage in a population in which all dogs were accessible and their immunity lasted a year. Rabies elimination occurred in all of these models within 20 years of MRV implementation. Thus, the proportion of models achieving elimination was 1 (Fig. S3) and so Figure S6 indicates that rabies elimination is possible using this combination of parameters, with the mean time to elimination being 1.6 years. However, in four of these 43 models, rabies elimination occurred in the 19th year of MRV implementation. These four models had values of Rab_{11-20}^{mean} of between 1 and 7 cases per month and so the proportion of models where rabies was eliminated was 0.91. As we chose the lowest vaccination coverages for this endpoint only when rabies was eliminated in all scenarios, Figure 3 shows elimination for the above combination of parameters requiring a higher vaccination coverage (i.e. > 95%). This incongruence is repeated in a few other contexts as well. It could partly be overcome by, for instance, modelling vaccination for a longer duration (e.g. 30 years) and calculating the proportion of models achieving elimination over the last ten years, i.e. $Rab_{21-30}^{mean} < 0.5$.

5.3.1 Low-transmission setting ($\boldsymbol{R_0}$ =1.12)

Vaccination coverages required to eliminate rabies were lowest in the low-transmission setting across all demographic scenarios (Fig. 5.3) and in some cases elimination was possible even with MRV campaigns conducted every three years. Even when only 20% of dogs were accessible for vaccination, annual campaigns that achieved vaccination coverages of 41 – 60% and 26 – 40% could eliminate

rabies within 8.3 years (baseline) and 13.4 years (LRS) respectively (Figs 5.3, S6). When all dogs were accessible, annual MRV campaigns achieving coverages of ≤ 10% (baseline, LRS) and 11 – 25% (HRS) could eliminate rabies within approximately six years. In the LRS scenario, it was also possible to eliminate rabies within 9.2 years (Fig S6) with campaigns held every three years when only 20% of dogs were accessible, provided campaigns consistently vaccinated 81 – 95% of the accessible population (Fig. 5.3).

5.3.2 Medium-transmission setting ($\boldsymbol{R_0}$ =1.48)

In this transmission setting, across all three demographic scenarios, vaccination coverages above 95% (Fig. 5.3) were required for more than 20 years (Fig. 5.5) for rabies elimination when only 20% of dogs were accessible for vaccination. When all dogs were accessible, annual MRV campaigns achieving coverages of 11 – 25% (baseline, LRS) and 41 – 60% (HRS) could eliminate rabies within approximately three years. However, in the HRS scenario, rabies elimination was possible only when ≥ 60% of the population was accessible with annual campaigns achieving vaccination coverages of > 40% (Fig 5.4).

5.3.3 High-transmission setting ($\boldsymbol{R_0}$ =1.65)

As in the medium-transmission setting, across all demographic scenarios, vaccination coverages of > 95% (Fig. 5.3) over > 20 years (Fig. S6) were required for rabies elimination when only 20% of the population was accessible. Generally, at least 60% of dog population needed to be accessible for vaccination to eliminate rabies through annual campaigns in the baseline and LRS scenarios, with vaccination coverages of 41 – 60% or 26 – 40%, respectively. Rabies elimination was possible in the HRS scenario only if very high proportions of dogs were accessible for vaccination (≥ 0.8) and with campaigns achieving coverages of > 60%.

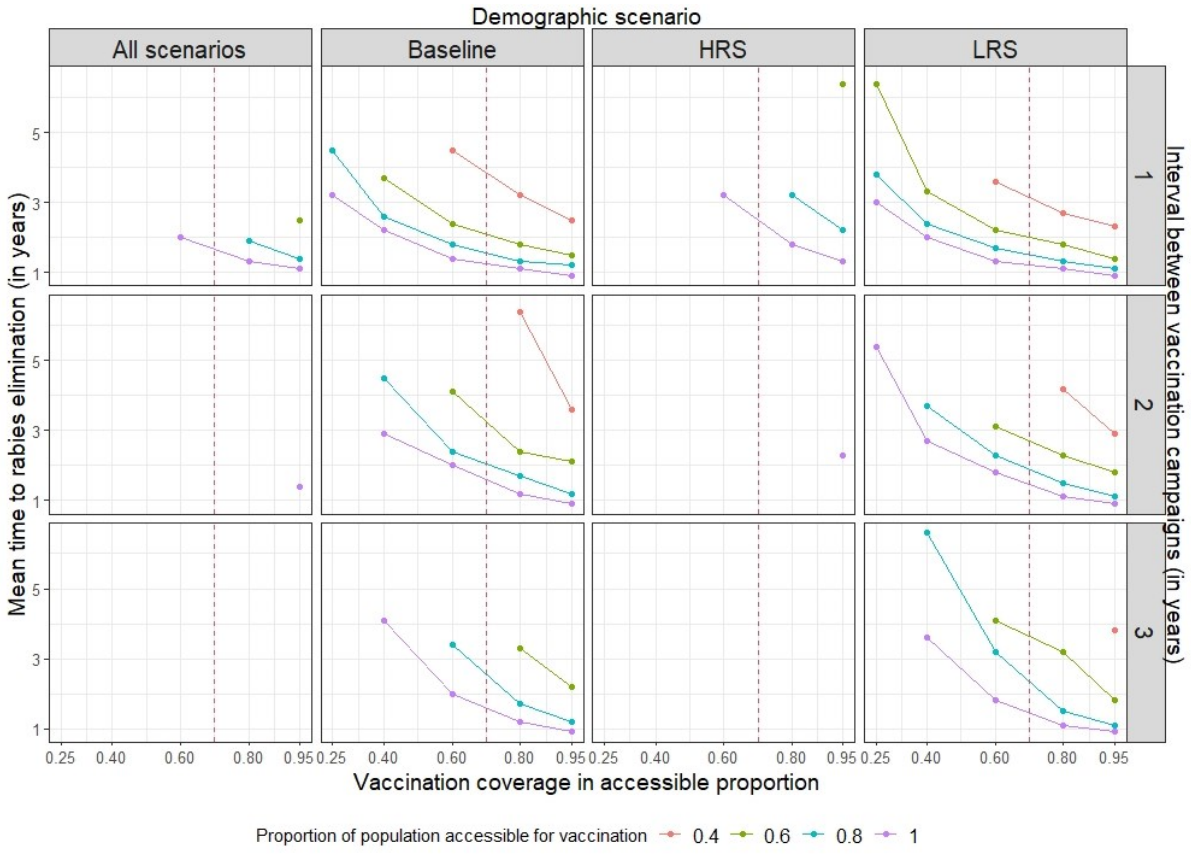

Figure 5.4 Mean time to rabies elimination by vaccination coverage, demographic scenarios and interval between campaigns. The mean time to rabies elimination (in years) as a function of the vaccination coverage across various demographic scenarios in a medium-transmission setting (R_0 =1.48), with pulse mass rabies vaccination campaigns of 30 days each, conducted every one, two or three years and vaccine-induced immunity lasting for one year. The figure shows only the lowest vaccination coverages at which rabies was eliminated in 100% of scenarios sharing values of campaign frequency and accessibility of dog populations. Vaccine-induced immunity is assumed to last for one year. Black dashed vertical line – 70% vaccination coverage.

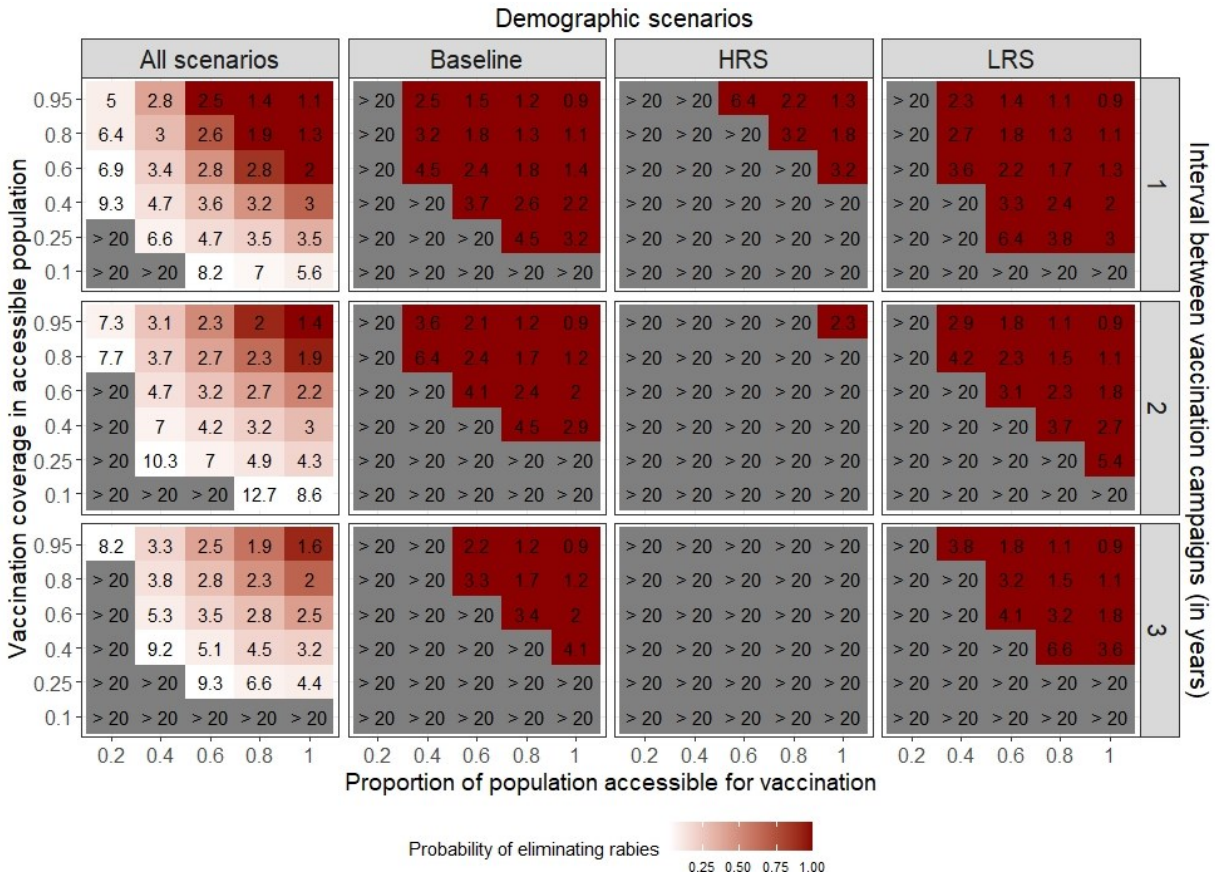

Figure 5.5 Heatmap of mean time to elimination by proportion accessible, vaccination coverage, demographic scenarios and interval between campaigns. Heatmap showing the mean time in years (figures within cells) for elimination (i.e. new rabies cases below 0.5 after implementation of MRV) in a medium-transmission setting (R_0 = 1.48), shown as a function of the proportion of population accessible for vaccination, the vaccination coverage in the accessible population, demographic scenarios and interval between vaccination campaigns (in years). The colour of each cell represents the probability of elimination. Cells in grey indicate that rabies elimination was not possible within 20 years after implementation of campaigns. Vaccine-induced immunity is assumed to last for one year.

5.3.4 Effective vaccination coverages

In low-transmission settings (R_0 = 1.12), < 70% effective vaccination coverage was required in the total dog population (including inaccessible dogs) across all demographic scenarios to eliminate rabies within 20 years, irrespective of accessibility for vaccination or interval between campaigns (Fig. 5.6). This was also the case in the baseline and LRS scenarios in medium- (R_0 = 1.48) and high-transmission (R_0 = 1.65) settings. However, in the HRS scenario and across all unique scenarios (All scenarios) in medium-transmission settings, only annual campaigns were able to eliminate rabies at coverages < 70%. In high-transmission settings, only annual campaigns vaccinating more than 70% of the total dog population could eliminate rabies across all unique scenarios.

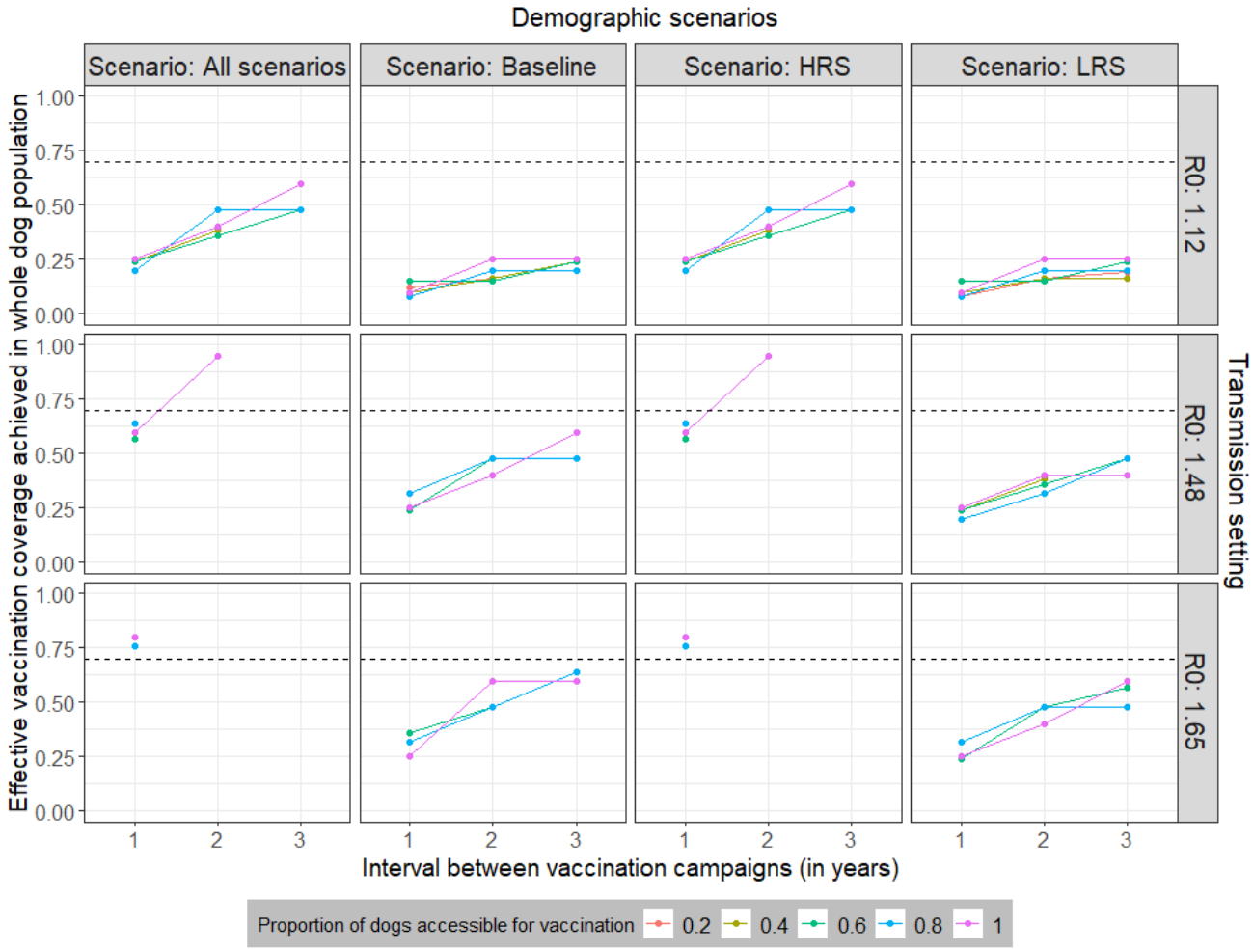

Figure 5.6 Lowest effective vaccination coverages required for rabies elimination in whole dog population, by interval between campaigns, demographic scenarios and transmission setting. The lowest effective vaccination coverage required for rabies elimination in the whole free-ranging dog populations (including inaccessible dogs) where elimination was shown to be possible within 20 years of implementation of pulse mass rabies vaccination campaigns of 30 days each. Effective coverages are shown as a function of the proportion of the population accessible for vaccination and interval between campaigns, across various demographic scenarios and transmission settings. Vaccine-induced immunity is assumed to last for one year. Black dashed horizontal line – 70% effective vaccination coverage.

5.3.5 Influence of demographic parameters on average rabies cases

Mean rabies incidence after MRV implementation was most substantially influenced by the per capita birth rate. In dog populations with higher birth rates, more frequent vaccination campaigns targeting higher coverages were required to eliminate rabies (Fig. 5.7). Rabies elimination was feasible in populations with the highest per capita birth rate (0.00467 per day) only in low-transmission settings, with annual campaigns vaccinating at least 40% of the accessible population. Conversely, annual campaigns achieving the latter coverage could eliminate rabies in dog populations with the lowest birth rate (0.0021) even in high-transmission settings. As birth rates increased, the mean time to elimination also increased correspondingly (Fig S20).

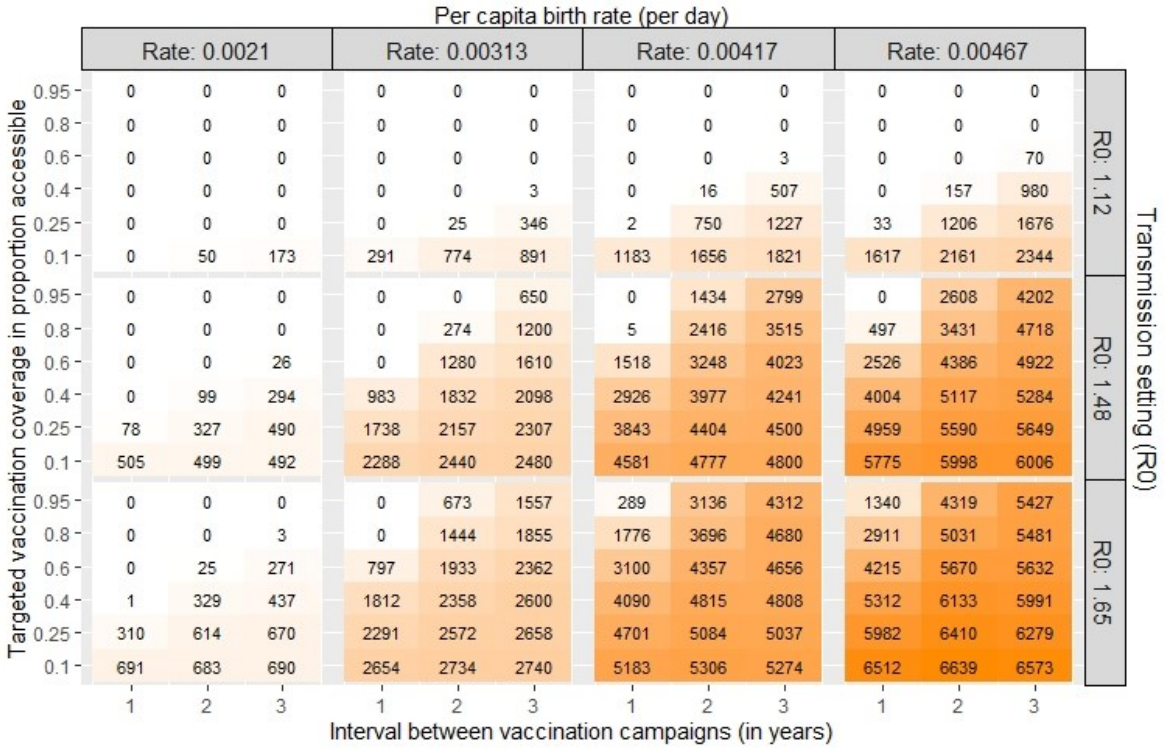

Figure 5.7 Monthly average number of rabies cases by interval between campaigns, vaccination coverage, per capita birth rate and transmission setting. The ten-year monthly average number of rabies cases ten years after implementation of mass rabies vaccination campaigns is shown as a function of the interval between vaccination campaigns, vaccination coverage in the proportion of the population accessible for vaccination, per capita birth rate (per day) and disease transmission setting. Vaccine-induced immunity is assumed to last for one year, with 60% of the population accessible for vaccination. R0 – Reproduction number.

Variation in juvenile mortality had a less substantial but noticeable impact on rabies elimination, with Rab_{11-20}^{mean} decreasing with an increase in the proportion of young dogs dying before sexual maturity (Fig. 5.8). At the same time, the time to elimination decreased with increasing juvenile morality (Fig S19). Rabies elimination was not influenced by adult lifespan (in the absence of density-dependence), although the mean rabies incidence increased slightly as adult dogs lived longer (Fig. 5.9) while the mean time to elimination decreased slightly (Fig S21).

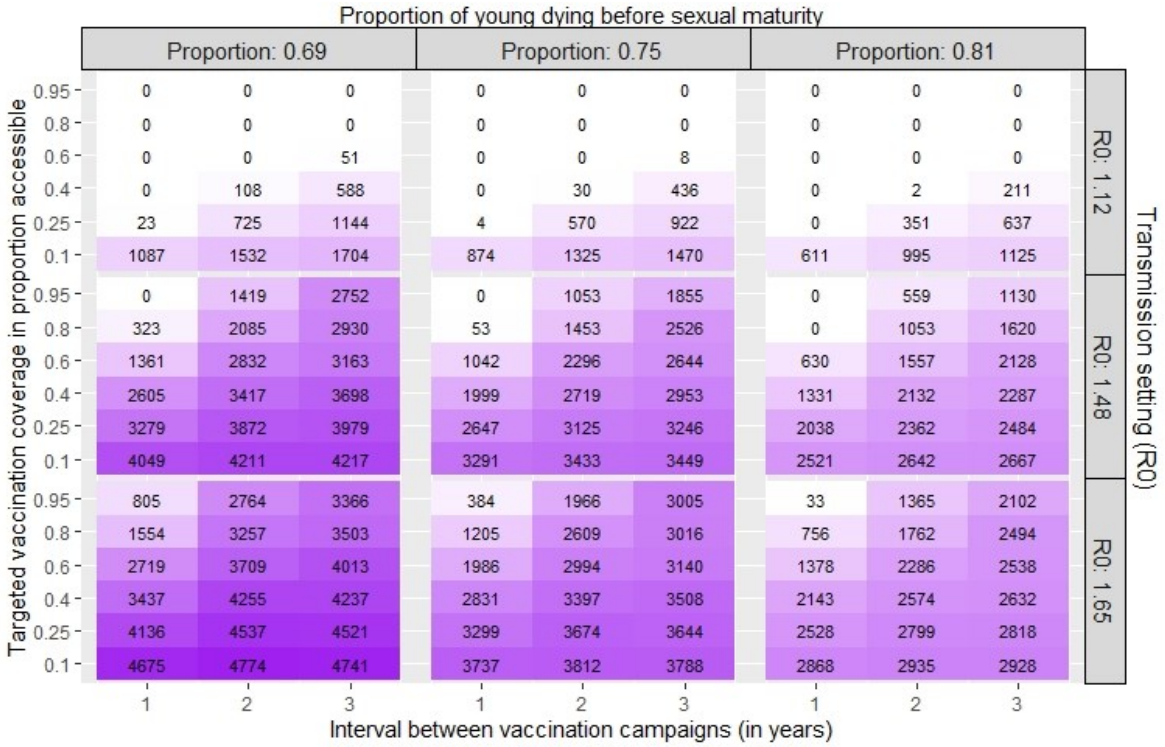

Figure 5.8 Monthly average number of rabies cases by interval between campaigns, vaccination coverage, proportion of young dying before sexual maturity and transmission setting. The ten-year monthly average number of rabies cases ten years after implementation of mass rabies vaccination campaigns is shown as a function of the interval between vaccination campaigns, vaccination coverage in the proportion of the population accessible for vaccination, the proportion of young dogs dying before sexual maturity and disease transmission setting. Vaccine-induced immunity is assumed to last for one year, with 60% of the population accessible for vaccination. R0 – Reproduction number.

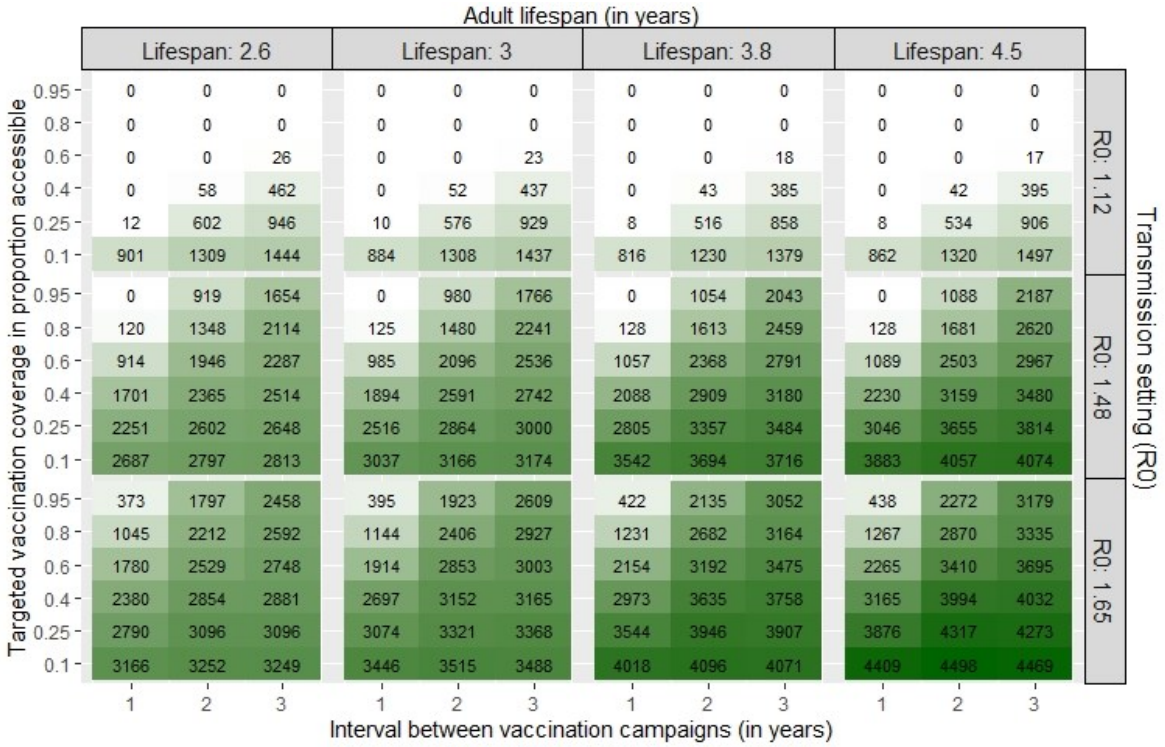

Figure 5.9 Monthly average number of rabies cases by interval between campaigns, vaccination coverage, adult lifespan and transmission setting. The ten-year monthly average number of rabies cases ten years after implementation of mass rabies vaccination campaigns is shown as a function of the interval between vaccination campaigns, vaccination coverage in the proportion of the population accessible for vaccination, adult dog lifespan and disease transmission setting. Vaccine-induced immunity is assumed to last for one year, with 60% of the population accessible for vaccination. R0 – Reproduction number.

5.4 DISCUSSION

While the 'Zero by 30' campaign targets the elimination of human rabies deaths caused by dog-bites, we have specifically modelled canine rabies elimination in the Indian context. The burden of human rabies deaths can be substantially reduced by ensuring timely access to effective human PEP [5]. However, human PEP alone cannot ensure human rabies elimination since the persistence of rabies in FRD populations poses a constant infection risk to people. This chapter highlights the importance of considering some key aspects of FRD populations, viz. accessibility for vaccination and age-specific survival, when formulating strategies to eliminate canine rabies through mass vaccination and when

modelling these strategies. These aspects will be especially relevant in regions like India which has one of the largest FRD populations globally [31], most of which are unowned (chapter 3) and so not easily vaccinated.

The model confirmed many previously known and intuitive results – e.g. that higher vaccination coverages and more frequent MRV campaigns result in higher probabilities of rabies elimination within shorter timelines. As expected, an increase in accessibility for vaccination correspondingly increased the feasibility of elimination in a wider range of scenarios, with lower vaccination coverages required in the accessible population (Fig. 5.3). However, incorporating age-structure into the models enabled us to explore various demographic scenarios and to disentangle the relative contributions of demographic parameters on rabies elimination prospects. A key insight provided by these demographic scenarios is that if less than 20% of the dog population is accessible for vaccination, rabies elimination can be achieved only with more frequent MRV campaigns (typically annual) that consistently achieve very high vaccination coverages (> 95% of the accessible population) for more than 20 years. The only exception appears to be in dog populations with low recruitment rates (baseline and LRS scenarios) in low transmission settings.

The model also provided unexpected insights into the complex dynamics between demographic factors and disease transmission, and how human interventions may potentially alter these dynamics, with implications for disease control and elimination. In the relative absence of interventions that support dog survival, juvenile survival is expected to be density-dependent such that when the total population sizes reach carrying capacity, juvenile mortality increases [69], as does overall population mortality rates. Similarly, as population birth rates increase, rabies incidence increases correspondingly (Fig. 5.7). Both these factors (increased mortality and high birth rates) result in high population turnover, necessitating more frequent vaccination with high coverages to ensure that adequate herd immunity levels are maintained between vaccination cycles [26]. Thus reduction of overall population mortality is seen to be beneficial for rabies control efforts, as it enables lower

frequency of vaccination and/or lower coverages to maintain adequate herd immunity levels and thus increases probability of elimination.

However, our model predicted an increase in rabies incidence with reductions in juvenile mortality (Fig. 5.8) or an increase in adult lifespan (in the absence of density dependence), the latter in medium- and high-transmission settings (Fig. 5.7). This counterintuitive observation may be explained by considering the influence of birth rates and juvenile mortality on mean adult lifespan (at carrying capacity). At low birth rates, effective mean adult lifespan decreases with a reduction in juvenile mortality (Fig. S22, Appendix A3). However, at high birth rates, effective mean adult lifespan is substantially lower (< 1 year) than assumed lifespans due to density-dependent regulation, irrespective of juvenile mortality (Table S1, Fig. S22, Appendix A3). Thus, the increased rabies incidence observed as juvenile mortality decreases is possibly due to adults living for shorter periods and the resultant loss of vaccinated individuals from the population. In such situations, unvaccinated young dogs < 1 year of age comprise an increasingly higher proportion of the dog population. Conversely, as adult lifespan appears to be substantially influenced by both birth rates and juvenile mortality, the impacts of assuming higher adult lifespan (in the absence of density dependence) are likely being modified by these two factors, meaning that an increase in adult lifespan does not necessarily translate into a reduction in rabies incidence. The comparatively similar mean adult lifespans may also explain why we did not observe any substantial influence of duration of immunity on rabies elimination prospects, despite marked differences in assumed adult lifespans in the absence of density -dependence (Fig. S21, Appendix A3).

The HRS scenario assumes high birth rates and low juvenile mortality. This is unlikely to be commonly observed, particularly as high juvenile mortality in the first year of life (81%) has been consistently reported in Indian FRD populations [73,248]. Nevertheless, the scenario provided an opportunity to explore the challenges of rabies control in situations where human interventions may have knock-on effects of improving health or longevity of young dogs, while potentially increasing birth rates by

enabling more females to reproduce successfully. Such scenarios are likely where human subsidisation of FRD populations occurs through supplementary feeding and better veterinary care, as reported in Indian cities [252]. However, such supplementary feeding is more likely to occur near residential areas [252] and so not all FRDs may be uniformly subsidized. Sterilisation of dogs has also been demonstrated to improve general health of the larger dog population [76]. In chapter 3, we highlighted that DWOs with direct human interactions, and sterilised dogs in particular, had higher recapture probabilities in future study rounds. Such dogs may live longer on average and thus be more likely to be repeatedly vaccinated across multiple MRV campaigns. Thus, human interventions that are implemented haphazardly may result in a combination of unequal subsidization of dogs, pockets of highly accessible dogs and high turnover with an increasing proportion of unvaccinated young dogs as the population reaches carrying capacity. Implementing MRV campaigns using conventional catch-vaccinate-release (CVR) methods in such scenarios may result in patchy vaccination coverages while a substantial fraction of dogs remain unvaccinated, hindering rabies control efforts [17], particularly in urban regions.

Belsare and Vanak (2020) highlighted the extensive costs and time scales involved in relying on ABC alone to reduce dog population sizes [69]. While we did not model the impacts of simultaneously implementing ABC and MRV on probability of rabies elimination, our results suggest that interventions that do little to control birth rates while potentially improving juvenile survival, at the cost of reducing adult lifespans, may in fact be counterproductive to rabies control efforts. In this respect, the beneficial effects of ABC may be evident only if they are able to substantially reduce birth rates and thus the replenishment of susceptibles, rather than increasing dog survival. However, these benefits may be evident only when ABC campaigns are implemented effectively and alongside MRV campaigns.

The baseline demographic scenario is based on published estimates of demographic parameters for FRD populations in India (Table 5.2). As such, this scenario could therefore be assumed to reflect general population dynamics for Indian FRD populations. In this scenario, elimination was possible

even with vaccination coverages as low as 11 – 25% of the accessible population, even if only 40% of dogs were accessible in low-transmission settings, provided MRV campaigns were conducted annually. However, the required coverages increased substantially in medium- and high-transmission settings, with elimination in the latter setting requiring coverages > 95% if accessibility was less than 60% (Fig 5.3).

The LRS demographic scenario is likely to resemble dog population dynamics in rural areas in India, where smaller dog populations, lower proportions of females and higher proportions of dogs with poor body condition have been reported [64]. Interventions such as ABC programs tend to target urban cities in India [241], thus depriving rural dogs of the health benefits of sterilisation. As pointed out in chapter 3, accessing dogs for vaccination in rural areas may be a major challenge, with even ODs more likely to be free-ranging [33]. However, our models indicate that rabies elimination is feasible in such scenarios even in high-transmission settings and where only 40% of the population is accessible for vaccination (Fig. 5.3).

There are limited data on canine rabies incidence in India, making it difficult to estimate R_0 values. Nevertheless, the range of R_0 values explored in this chapter captures a potential range of transmission settings that may occur in a country as large and diverse as India. High-transmission settings are likely to occur in densely populated large Indian cities with substantial FRD populations. For example, a single animal welfare charity (ResQ) in the city of Pune in western India, which has approximately three million people, reported an annual average of 120 – 140 confirmed canine rabies cases between 2018 and 2020, with multiple co-circulating rabies virus strains (Abi Tamim Vanak, personal communication). While there are no official estimates of the FRD population in Pune, it is often commonplace to see large packs of dogs, possibly comprising up to 100 individuals, within small residential areas where local residents often feed these dogs (Sreejith Radhakrishnan, personal observation). Achieving high vaccination coverages in such large FRD populations, where accessing sufficiently large proportions of dogs is difficult, will require thorough planning of MRV campaigns and

likely involve the implementation of a combination of vaccination methods such as CVR and oral vaccines.

Considerations about accessibility can thus be important when planning MRV campaigns, allocation of resources and man-power and developing realistic and achievable rabies elimination targets. Failure to vaccinate hard-to-reach dogs may result in patchy coverage and pockets of unvaccinated dog populations that can threaten rabies control efforts in the wider region [286]. More dogs may be accessed for vaccination by increasing resource allocation to training and deploying more dog catchers. Incorporating assumptions about accessibility can enable local bodies to more effectively estimate the effort required and associated costs for increasing vaccination coverage. We did not consider the effort required to achieve vaccination coverages within different conditions of accessibility. Models that incorporate capture effort [69] can provide insights into the cost-effectiveness of different vaccination methods (parenteral vs oral vaccines) and the feasibility of rabies elimination using these methods under various assumptions of accessibility.

However, it is far from straightforward to directly estimate accessibility for vaccination, particularly as it will be influenced by the vaccination method [72]. The use of parenteral rabies vaccines requires that dogs are physically handled, necessitating the use of capture methods such as net capture to vaccinate FRDs and sometimes even ODs. Such methods are highly stressful and make dogs harder to access as campaigns progress, even with the involvement of a large workforce of dog catchers. On the other hand, Gibson et al. (2019) showed that by manually handing out an oral bait construct, a significantly higher proportion of inaccessible dogs could be reached than by using CVR methods in India [71]. Animal charities and institutions involved in DPM efforts such as ABC are probably best suited to assess what proportions of dogs are accessible for vaccination. We showed in chapter 3 that despite involving an additional dog catcher, less than half of all UDs included in our study in R1 were recaptured in R2, merely 30 days after first being vaccinated. Such low recapture probabilities are unlikely to be due to demographic processes such as migration or mortality and highlight the

challenges in accessing FRDs for parenteral vaccination. These challenges can be especially evident in Indian cities where interventions like ABC are likely to have been present for several years and so will have familiarised dogs to avoid capture.

A lack of readily available canine rabies incidence data from India precluded the possibility of relating our model results to real-world scenarios by fitting to such data. Despite this drawback, these models reinforce the feasibility of eliminating rabies in FRD populations in India, even with effective vaccination coverages less than the WHO-recommended coverage of 70%, particularly if campaigns are conducted annually. Calculations of effective vaccination coverage may not be of practical relevance where a certain proportion of dogs are truly inaccessible for vaccination and MRV campaigns should aim to vaccinate as many dogs as possible in the shortest duration. Nevertheless, our results suggest that campaigns that are unable to vaccinate 70% of the dog population may still be able to substantially reduce rabies cases to the point of elimination, which will contribute substantially towards the goal of eliminating dog-mediated human rabies deaths in India by 2030. At the same time, the models also reinforce the need for annual MRV campaigns, with rabies elimination being increasingly infeasible with larger intervals between campaigns. The increasing levels of vaccination coverage required in less-accessible populations are also likely to increase costs of campaigns targeting rabies elimination, thus pointing to the need for reliable data on dog demography and ecology in regional contexts to inform the design and implementation of campaigns.

The results of these models, particularly the impacts of demographic parameters such as birth rates, are heavily reliant on the assumption of density-dependent rabies transmission, the validity of which has been questioned [100]. Additionally, our models assume that rabies transmission occurs only between dogs within the population and does not incorporate potential introduction of rabies from infected dogs entering the population from elsewhere or (less commonly) transmission from other (e.g. wildlife) species. Such simplified scenarios are unlikely to be applicable in the real world, except in the case of islands such as Indonesia [17]. Phylogenomic [89] and metapopulation modelling studies

[90] indicate that frequent introduction of rabies from outside cities was necessary for sustained outbreaks in urban dog populations. Incorporating transmission heterogeneity, where most rabies cases fail to seed further infections, human movement of dogs and the influence of spatial and metapopulation dynamics can help to overcome these drawbacks [90,100].

We have also assumed a perfect vaccine and development of adequate RVNA titres in all vaccinated dogs indefinitely. As pointed out in chapter 4, the use of poor quality or improperly stored vaccines may substantially affect duration of immunity and coverage levels achieved. While approximately 90% of dogs were shown to develop post-vaccination titres > 0.5 IU/ml (chapter 4), there may be variation in seroconversion rates in dog populations that have poor access to resources and greater exposure to stressors such as infectious diseases or adverse environmental conditions. From a modelling perspective, these phenomena may be characterised as reductions in the effective per capita vaccination rate during a campaign. Such factors may substantially influence herd immunity levels and thus increase the vaccination coverages and time required for rabies elimination, and points to ensuring the use of high-quality thermostable vaccines [319] stored under appropriate cold-chain conditions [321].

Our model results are also contingent on assumed values of the basic reproduction number R_0. Li (2018) questioned the validity of the low R_0 estimates (< 2) with narrow confidence intervals estimated for previous rabies outbreaks, and suggested that these may be larger and more uncertain than previously thought [347]. This has implications for model results and prospects for elimination.

Despite these drawbacks, this chapter details the first mathematical model of canine rabies control through mass vaccination that has been parameterised using local dog demographic data in the Indian context. The results of our model provide valuable insights into the challenges in implementing MRV campaigns for rabies control in unowned FRD populations in India and elsewhere. They also highlight the complex interplay of factors influencing rabies transmission dynamics and their impacts on elimination prospects.

5.4.1 Conclusions

MRV campaigns have been repeatedly shown to be the most cost-effective means of controlling canine rabies and every effort should be made to vaccinate as many dogs as possible. However, campaign managers and governments must consider accessibility of dog populations when designing strategies that target canine rabies elimination, particularly in regions with large populations of DWOs. Doing so can highlight potential pitfalls in the campaign strategy, and potential benefits of alternative modes of vaccination such as oral vaccines to access hard-to-reach dog populations. At the same time, the potential impact of human interventions on differential survival rates in juvenile and adult dogs can complicate control efforts, and local realities need to be considered carefully when implementing campaigns.

Chapter 6 : Discussion

The underlying motivation of the research in this thesis was to explore the feasibility of eliminating canine rabies through mass vaccination in India. Fieldwork conducted in India over 16 months provided data on dog population characteristics, including the ownership status of FRDs, recapture probabilities, pre- and post-rabies vaccination RVNA titres and immunological dynamics in the field for ODs and DWOs. These data were used in combination with previous reports in the literature of FRD demographic characteristics to parameterise an age-structured deterministic compartmental model of rabies transmission. This model was used to explore the impacts of varying levels of accessibility, assumed vaccination coverages and demographic characteristics of FRD populations on the probability of rabies elimination following the implementation of MRV campaigns in India.

6.1 Summary of findings

In chapter 2, historical records from pre-independence India (prior to 1947) and the immediate post-independence era were reviewed to understand trends in rabies incidence, efforts to control the disease and historical perceptions of the disease as a public health concern in India. It was shown that rabies was widespread and endemic and, far from being neglected, a key driver of Pasteurism in India. As an important component of British colonial scientific enterprise, rabies was integral to the establishment of some of the most important scientific and research institutions in India. Research conducted in India on rabies led to development of the Semple vaccine that was used across the world for several decades and saved countless lives. The success of these vaccines may have contributed to a gradual decline in prioritisation of rabies for control in India, in turn potentially establishing a foundation for its continued neglect to the present day in Indian public health discourses [1].

In chapter 3, data on population characteristics of DWOs and ODs from two sites in India were used to establish that 88% of FRDs had no owners and were therefore not readily accessible for vaccination. Over 90% of FRDs were in good body condition or overweight. There was evidence of poor DOP, with

approximately 60% of ODs being free-ranging and less than a third having been vaccinated against rabies. Of the latter, only a third had detectable RVNA titres. Less than half (47%) of all UDs included in the study in R1 were ever recaptured in later study rounds, and recapture probability was shown to be significantly lower at the semi-urban study site and for pups. Interestingly, dogs with pre-vaccination titres > 0.5 IU/ml had significantly higher probability of recapture in future rounds.

In chapter 4, pre- and post-vaccination RVNA titres collected at four time points across 16 months were used to estimate rates of decline in titres among ODs and DWOs. It was found that while most unvaccinated dogs had no detectable pre-vaccination RVNA titres, about 26% were seropositive with detectable titres ≥ 0.23 IU/ml. Most (> 80%) dogs that were vaccinated in R1 developed titres > 0.5 IU/ml 30 days later, irrespective of age or prior vaccination history. However, titres were estimated to drop below this level approximately 200 days (95% CI: 167 – 256 days) after attaining peak levels (assumed to occur 18 days after vaccination), with more rapid declines among ODs and completely or partially confined dogs, compared to DWOs and dogs that were completely free-ranging. These results provided further supporting evidence for the occurrence of non-lethal rabies in FRDs.

Finally, in chapter 5, an age-structured SEIV model incorporating assumptions about the accessibility of FRDs for vaccination indicated that accessibility is an important parameter that should be incorporated into transmission models exploring the feasibility of rabies elimination through MRV campaigns. As larger proportions became inaccessible for vaccination, higher vaccination coverages were required in the accessible fraction to eliminate rabies, with coverages of > 95% of accessible dogs for more than 20 years required when only 20 – 40% of dogs were accessible. These coverages were also influenced by the assumed transmission setting and intervals between MRV campaigns. The model also highlighted the complex interactions between accessibility, demographic characteristics (particularly per capita birth rate) and human interventions that subsidize FRD populations and how these interactions could potentially confound rabies control efforts, particularly in urban FRD

populations. The model also reinforced the feasibility of rabies elimination in FRDs in India, even if campaigns failed to achieve the WHO-recommended 70% vaccination coverage.

6.2 Limitations and future work

There are several limitations to this study, many that were identified in the course of research. Key among these are the challenges of capturing and identifying FRDs, with implications for the quality of data collected during fieldwork. There is a substantial body of literature on humane DPM [282,348–350], covering the principles of population management [282], guidance on dog handling [349] and operational requirements [282,350]. However, these documents are necessarily focused on the practical aspects of implementing effective and humane DPM interventions where any particular dog would be captured only once during its lifetime. In this respect, these documents are less suited to explaining the challenges of capturing FRDs as part of research studies, or even MRV campaigns, where the same dogs may be repeatedly recaptured for longitudinal data and sample collection or revaccination. These gaps in knowledge meant that we could not anticipate events such as failure of microchips or removal of collars from presumed DWOs by members of the public, leading to some DWOs being unintentionally vaccinated more than once in the course of the study. Ultimately, there is no substitute for practical field experience. However, we have summarised some of the insights gained during fieldwork in Appendix A5 in the hope that this will provide practical pointers on the challenges of FRD capture, thereby informing the design and implementation of future research studies. A future area of research in this regard would be to identify safe but long-lasting coloured compounds (lasting for one to two weeks) that can be used to physically mark animals (such as by spraying) that have been subjected to an intervention. Doing so would prevent subjecting them to the undue stress of unintentional recapture in the course of a study.

Regarding testing challenges, there is only one laboratory in south India that conducts regular testing of serum samples for RVNA by using RFFIT – the WHO reference lab for rabies based at NIMHANS, Bengaluru. However, this laboratory no longer accepts animal samples as they deal with large volumes

of human samples, particularly from clinical trials of rabies vaccines (Reeta Mani, personal communication). This dearth of reliable test facilities means that conducting similar studies of RVNA dynamics in India is cumbersome and expensive and precludes the possibility of conducting blind testing of samples at alternate facilities to validate test results. A new OIE reference laboratory for rabies was recently established at the Karnataka Veterinary, Animal and Fisheries Sciences University in Bengaluru. It is hoped that this facility will enable repeat testing of serum samples, thus providing further confidence in our results.

Another related concern is the lack of reliable long-term storage facilities for serum samples and the potential for power disruptions to affect sample quality. Canine serum samples collected during fieldwork are currently stored in a -20°C deep freezer of the District Veterinary Centre at the field site in ALP. In mid-2019, this freezer experienced power disruptions that caused temperature fluctuations, necessitating transfer of samples to a different -4°C freezer until the deep freezer was repaired. These temperature fluctuations may have affected sample quality and can complicate interpretation of future test results. If of sufficient quality, these serum samples could also provide a valuable resource to assess the seroprevalence of other canine diseases such as canine distemper, parvo- and adeno-virus infections in FRD populations in India. Due to this reason, there must be greater efforts to develop reliable cold-storage facilities to safeguard such biological samples.

An important limitation on the interpretation of the serum results remains an inability to account for the unexpectedly high RVNA titres in serum samples collected in R4. These high values meant that data from R4 could not be included in the MLE analyses to estimate rates of post-vaccination decline in RVNAs. These results highlight the challenges in the use and interpretation of results of virus neutralization tests, even when gold-standard tests are conducted at internationally recognized reference laboratories. Testing these samples using alternative tests such as the WHO-approved competitive ELISA test kits [5] may help to reconcile these differences in expected and observed titres.

In the course of fieldwork, substantial data were collected through photographic mark-resight surveys on FRD demography at both study sites. We also conducted household surveys in over 300 households to assess DOP, public attitudes towards FRDs and knowledge, attitudes and practices (KAP) surrounding the risks of rabies infection, treatment and rabies control. These data could not be analysed and included in this thesis due to time constraints. Conducting the mark-resight surveys involved extensive planning and the recruitment and training of several undergraduate and postgraduate students. The comparatively small number of dogs in MUH, a semi-urban site, meant that compiling the mark-resight data for downstream analyses was relatively straightforward. However, there was a markedly larger FRD population in urban ALP and surveyors frequently struggled to photograph and record data on all the dogs sighted at various transects. It has also proven a challenge to distinguish individual dogs using the photographs taken in ALP, and similar challenges have been encountered during surveys conducted in Bengaluru, one of the most densely populated urban cities in India (Abi Tamim Vanak, personal communication). The recent development of a mobile app that partially automates the identification of FRDs through photographs, although not without challenges, is a step in the right direction (Abi Tamim Vanak, personal communication). Such technology advances the possibility of adopting a citizen-science approach to estimation of dog population sizes across India.

Data from the household surveys can provide valuable insights into social attitudes towards dog ownership and FRD control in India. Preliminary results of the KAP surveys indicate very high levels of public awareness in Kerala about medical aspects of rabies and the need for prompt post-exposure PEP (Appendix A4). However, the latter was counterbalanced by comparatively lower awareness about rabies control in dogs and surveillance to assess the burden of canine rabies. These gaps in public awareness highlight the major role of the Kerala state AHD in promoting responsible DOP, and in increasing investment in surveillance and epidemiological analyses of rabies incidence to inform evidence-based implementation of control strategies such as MRV campaigns. The high awareness levels of medical treatment for rabies exposure possibly account for the low number of human rabies

deaths reported from Kerala. However, the high demand for human rabies PEP has its financial implications for the state (see section 2.3.1 below). Future research should explore the drivers of public health-seeking behaviour vis-à-vis perceived rabies exposures and the potential to use protocols like integrated bite case management to streamline the administration of human PEP [351], thereby minimising the unnecessary use of a valuable resource.

As pointed out in chapter 5, we were unable to relate the results of the compartmental model to observed rabies incidence data in Kerala as such data were not readily available. Data on symptomatic and laboratory confirmed rabies cases are compiled separately by the veterinary universities and the state AHD in Kerala. This means that laboratories have to be individually contacted to compile state-level prevalence data. While rabies incidence data from 2019 onwards was made available to us from the statistics wing of the Kerala state AHD, these were not used as we had not obtained prior departmental approval to do so. Future work focussing on fitting our model to these data will help to validate model assumptions and could provide invaluable insights into rabies transmission dynamics within the state of Kerala.

6.3 Implications of research and challenges

As discussed in chapter 3, the Goa state government reported in June 2021 that it had recorded zero human rabies deaths since September 2017 [289,290]. This achievement was reported to have been the result of implementation in the state of MRV campaigns since 2014, in collaboration with the international charity Mission Rabies [241]. The launch of India's NAPRE in September 2021 was a landmark moment that set the stage for the country with the largest burden of dog-mediated human rabies deaths to implement systematic efforts to eliminate this scourge. The action plan emphasized the need to make rabies a notifiable disease in India, improving access to human PEP and the importance of intersectoral collaboration within a One Health framework [352]. The animal health component of the plan focused on achieving 70% vaccination coverages 'in a defined geographical area annually for 3 consecutive years.'

In this context, developments over the past few years in the south Indian state of Kerala (Fig. 6.1) could make it a model among large Indian states for successful state-led rabies control efforts, provided these efforts are evidence-based and well-coordinated.

6.3.1 A 'Kerala model' for rabies control in India?

Kerala has a human population of 33 million (2011 census) with a mean density of 860 individuals /km^2 [353]. The development trajectory of Kerala has been well discussed in the development studies literature, and the 'Kerala model of development' is frequently referred to in discussions about alternative models of development [354]. The state's achievements in literacy and health have long been recognised [355], with many health and demographic indicators on par with high-income countries [254], and the state has consistently outperformed all other Indian states in national health and human development surveys [254]. An epidemiological transition from high mortality (from infectious diseases) to high morbidity (due to chronic illnesses), as found in most high-income countries, was evident in Kerala as early as 1993 [356].

As mentioned in chapter 3, data from the state's Integrated Disease Surveillance Programme indicated that the number of human rabies deaths reported in Kerala was 10 (2011), 13 (2012), 11 (2013) and 10 (2014, at the time of publication) [259]. When accounting for a further 20% of paralytic rabies cases that may potentially have gone mis-diagnosed [6], this translates to a per capita death rate of approximately 0.041 (95% confidence intervals: 0.036 – 0.046) per 100,000 persons (all-India estimate – 3 per 100,000 persons), one of the lowest rates globally for rabies-endemic regions [56,260]. Dogs are the main source of infection, and the majority of diagnosed animal rabies cases are in dogs [346].

As in most parts of India, canine rabies control in Kerala is currently dealt with under the broader aim of controlling FRD populations through ABC. The state witnessed a spurt in the annual incidence of dog bites since 2012, with over 100,000 dog bites reported between 2015 and 2016 [357]. FRD bites have become an emotive topic in the public consciousness in Kerala, with instances of deaths from

attacks by packs of dogs [358] resulting in widespread reactive killing of FRD [359], despite the culling of stray dogs being illegal in India [82].

Figure 6.1 Location of rabies diagnostic laboratories in Kerala. Map indicating location of the state of Kerala in India (inset), depicting sites of existing (green points) and newly established (red point) veterinary rabies diagnostic laboratories.

The Kerala state AHD conducts a livestock census every five years, most recently in 2017, and dog populations are also estimated under the broad headings of 'domestic' or ODs, and 'stray' or FRD. FRD populations are likely to be underestimated, as these numbers are gathered solely through direct

counts (Sreejith Radhakrishnan, personal observation). As per the most recently available data from the national livestock census of 2012, the dog population in Kerala was approximately 930,000 'domestic' dogs and 270,000 'stray' dogs, which translated to 'stray' dogs comprising about a quarter of the total dog population [360]. The most recent estimate of the 'stray' dog population in the state is about 600,000 [230], although no indication is given as to how this was estimated.

As part of the global framework for the elimination of dog-mediated human rabies, five pillars of rabies elimination were identified as key to achieving the 'Zero by 30' goal [22]. These are summarised by the acronym STOP-R, indicating socio-cultural, technical, organizational, political and resource factors. These are discussed in relation to the preparedness of Kerala to achieve this target:

1. Socio-cultural factors – Dog ownership is widespread in Kerala, with a variety of pure-bred dogs raised in households and exorbitant sums often spent on procuring exotic breeds from within and outside India (pers. obs.). Non-descript dogs or mongrels are usually raised by households with low incomes (chapter 3). There is widespread public awareness of the need for vaccinating dogs, particularly against rabies (Sreejith Radhakrishnan, unpublished data; [261]), of the risks of rabies exposure and the importance of seeking prompt treatment (Appendix A4). This awareness has also been highlighted by reports of people seeking rabies PEP even after cat and rodent bites [262]. Additionally, press reports of dog bites and deaths from rabies have driven public discussions about the threats posed by FRDs, on civic issues such as responsible dog ownership and waste management as potential means to tackle this issue [231,361]. Professional bodies such as the Indian Veterinary Association conduct rabies awareness events at the community level, for instance, by celebrating World Rabies Day (September 28th).

2. Technical factors – Ensuring capacity for timely and accurate diagnosis by setting up well-equipped laboratories with trained personnel is one of the key components in combatting rabies in endemic regions [5]. Kerala has at least four public sector laboratories providing diagnostic services for animal rabies by the Fluorescent Antibody Technique [5], with an additional facility recently

established in north Kerala (Bhagyalakshmi P.S., pers. comm.) (Fig. 6.1). The presence of a WHO collaborating centre on rabies research at NIMHANS in the adjoining state of Karnataka is also an advantage.

Kerala was the first state in India to shift to exclusive use of safer cell culture vaccines after the use of nerve tissue vaccines for rabies prophylaxis was halted in 1993 [362]. The use of intradermal rabies vaccination (IDRV) as a cost- and dose-sparing alternative to intramuscular administration for pre-exposure prophylaxis (the vaccination of individuals before they are exposed to rabies) and PEP was started in 2009. This is now provided free of cost at all public sector hospitals to individuals potentially exposed to rabies [363], with good compliance reported to specified vaccination schedules [262]. Equine or human rabies immunoglobulins (RIGs) use for treating most severe category III bite wounds is also widespread at rabies referral centres [364]. A national assessment conducted in 2017 of PEP facilities and services in antirabies clinics in India found that both urban and rural clinics in Kerala had adequate facilities to ensure wound washing, vaccine storage facilities and did not report any shortages of RIGs [365]. Such widespread access to PEP, combined with high levels of public awareness of the risks of rabies exposure, may account for the low incidence of human rabies deaths in Kerala.

3. Organizational factors – Kerala was the first state in India to develop a state-level antimicrobial resistance strategic action plan in 2018 [366] and was lauded for its handling of the Nipah outbreak in 2018 [367] and the ongoing COVID-19 pandemic during its early stages in 2020 [368,369]. The need for intersectoral collaboration between medical and veterinary institutions was recognised at a WHO evaluation of Kerala's implementation of IDRV [262]. Sukumaran and Pradeepkumar (2015) also emphasized the need for collaborative efforts between the AHD, the state veterinary university, Directorate of Health Services and the Animal Welfare Board of India and the need to adopt a One Health approach for tacking rabies in Kerala [259].

The AHD has an Assistant Director (Rabies Eradication) with the responsibility for coordinating rabies control programmes in the state (https://ahd.kerala.gov.in/index.php/administration). This office also collates data on rabies cases diagnosed at laboratories of the AHD. The next step will be to ensure timely collation and analysis of diagnostic data from all the laboratories in the state, which will strengthen surveillance. Statistics on animal bites, PEP doses administered and rabies deaths are compiled by the Directorate of Health Services [259]. Access to veterinary care in Kerala is also good, with at least one veterinary institution functioning within each grama panchayat (the lowest administrative unit of decentralised local government in India), where animal rabies vaccines are provided at highly subsidised rates under a Rabies Free Kerala campaign launched in 2016 (112).

4. Political factors – Political will was identified as one of the key factors in enabling the widespread distribution and use of IDRV throughout hospitals in Kerala [370]. There is currently widespread political interest in addressing the social and public health challenges posed by FRD. The state government has implemented ABC-ARV campaigns across the state [18], where FRD are captured, sterilised at ABC centres, vaccinated and released at sites where they were originally captured, with ear notches used to mark sterilised animals [231]. The state government also announced plans to establish a production facility for cell culture rabies vaccines for veterinary use which, once established, would make Kerala the first state in India to do so [357].

5. Resources – In addition to substantial funds assigned for the free provision of PEP across the state, there has been increased allocation of funds by the state government and local panchayats for ABC campaigns. However, the state's annual budget for PEP provision has reportedly been insufficient to meet actual demand [259]. The establishment of facilities to manufacture veterinary rabies vaccines is expected to cost about 150 crore rupees (1500 million rupees, about 22 million USD) [357]. Efforts are also being made to raise awareness among officials of local self-government institutions about the need for prioritising and funding control activities and the potential for external funding.

Considering these factors, Kerala may be able to achieve local elimination of dog-mediated human rabies by 2030. However, poor understanding of reasons for the continued existence of a large FRD population, including its ecology and demography, is likely to hinder these efforts. Experience from ABC campaigns in the municipal corporation of Kochi, where a systematic campaign has been ongoing since 2014, suggests that capturing more than 40% of the FRD population at this location is challenging (Kishorekumar KJ, personal communication). As we pointed out in chapter 3, many FRD are also fed by the public, who however may deny ownership of these dogs.

In this context, the results presented in this thesis have important implications for building on rabies elimination strategies outlined in the NAPRE and highlight the extensive challenges that India faces in controlling canine rabies. The NAPRE focused on achieving 70% vaccination coverages in dog populations through parenteral vaccines alone, through standard methods such as door-to-door and central point vaccination and the use of dog catchers to vaccinate FRDs. There was no mention at all about the need to consider alternative methods, particularly oral rabies vaccines. While these strategies are in line with current global recommendations and consensus on how to eliminate human rabies deaths, the action plan highlights a major gap in understanding of FRD populations in India.

We established that the vast majority of FRDs at our study sites are unowned and therefore not readily accessible for vaccination. Using parenteral vaccines to achieve target vaccination coverages in such populations will require large investments in training personnel, equipment and implementation. Such investments may be beyond the capacities and resources available to local or state governments, particularly in the face of arguably more pressing health concerns such as the ongoing tuberculosis and HIV epidemics and the recent COVID-19 pandemic in India. Our research highlights the need to develop a greater understanding of accessibility of dog populations for vaccination within regional contexts and adapting control efforts accordingly. In regions where human subsidization of dogs leads to unequal accessibility for vaccination, or in less densely populated regions where net capture of FRDs

may be extremely difficult, MRV campaigns may need to be conducted using a combination of both parenteral and oral vaccines.

While the immediate goal of eliminating dog-mediated human rabies deaths is much needed and may be feasible by enhancing availability of and access to affordable human PEP, the only way to ensure the sustainability of this goal is through eliminating dog rabies. The experience of countries in Latin America highlights the enormous financial and resource commitments required and the time scales involved (in decades) to eliminate dog rabies [21]. While Mexico has achieved this goal, it remains to be seen how effectively they are able to sustain this progress in the presence of large FRD populations. These challenges will certainly be magnified in India, considering its substantially larger FRD population [31].

As the NAPRE is rolled out across India, governments need to ensure that the benefits of elimination efforts are distributed equally across society. We highlighted in chapter 3 how many dog-owning households reported difficulty in accessing veterinary care for ODs, indicating the need to widen access to affordable veterinary services in India. Kerala's experience in rabies control may also provide important lessons in this regard. Despite the state's achievements in reducing the burden of human rabies deaths, human cases continue to be reported by the press in Kerala [371,372]. In September and October 2021 alone, five people in different parts of the state were reported to have died of rabies [373–376]. These included three children under 18 years of age and one 'migrant labourer', an Indian citizen from one of various north Indian states who work in Kerala (Jereesh Jerry, personal communication). One of the children died despite having received three doses of PEP, prompting the government to institute an enquiry into the causes of potential vaccine failures [377]. Three of these deaths were reported from two separate tribal colonies in south [375] and north Kerala [376]. These reports suggest that rabies continues to primarily affect vulnerable, underserved and/or disadvantaged communities – in these instances, children and tribal and migrant labourer communities. Tribal communities are one of the most disadvantaged communities in Kerala and India,

with poor access to and utilization of healthcare facilities [378,379] and high prevalence of illnesses [380,381]. Nearly all ODs in these communities are completely free-ranging and difficult to handle, making it challenging to vaccinate them against rabies (Sreejith Radhakrishnan, personal observation). Recent reports of rabies cases in tribal hamlets in north Kerala have forced veterinary services to adopt novel means of vaccinating these dogs, such as administering vaccine in darts delivered through blow-pipes (Dilip Falgunan, personal communication). Similarly, in a survey of migrant labourers in Kerala, over half were assessed to have major illnesses such as diabetes, high blood pressure and cancer [382], highlighting poorer living conditions and/or unequal access to healthcare.

Our research has also highlighted the complex cultural contexts within which DPM and rabies control efforts need to function in India. Further research within the One Health framework is required to understand how to effectively ensure responsible DOP among dog owners and what drives people to support FRD populations in India, often in the face of public opposition. Such research can help to develop ways of incentivising responsible behaviours, such as ensuring that all dogs are vaccinated and sterilised. There is a need to conduct field-based research on fundamental aspects such as population sizes, ownership and accessibility for vaccination, to inform decision-making and economic evaluations for dog-mediated rabies control and elimination across India.

6.4 Conclusions

Even as India has set forth a national action plan to eliminate dog-mediated human rabies deaths by 2030, the control of canine rabies will require concerted efforts to vaccinate sufficiently large proportions of FRD populations and to maintain these high coverages for several years. These can be overwhelming challenges, given that the vast majority of FRDs in India are unowned and therefore not readily accessible for vaccination, as we have shown in chapter 3. This problem is compounded by the lack of responsible DOP and the widespread subsidization of FRD populations by the general public and the ready availability of food waste. While elimination of human rabies deaths may be achieved through canine rabies control and provision of affordable human PEP, ensuring that any success

achieved is not short-lived will require a long-term plan to effectively reduce FRD populations in India. Thus, the rabies control landscape in India is highly complex, requiring the effective and sustained implementation of MRV campaigns that may necessarily involve the use of both parenteral and oral vaccines; enforcement of responsible DOP; effective waste management and humane DPM efforts. At the same time, several operational hurdles also need to be considered, such as ensuring storage facilities to hold rabies vaccines under cold-storage and building a well-trained workforce capable of implementing MRV campaigns. Failing these efforts, any attempts to control canine rabies in India may be rendered fruitless.

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Appendix A1: Supplementary information for chapter 3

All information presented here relates to chapter 3: Free-ranging and owned dog population characteristics in Kerala, south India in the context of rabies control

METHODS

Dog capture/inclusion and sampling in Alappuzha (ALP) municipality and Muhamma (MUH) panchayat

A. In the field - during the capture of UDs/SODs/free-ranging ODs using butterfly nets or when ODs were presented by owners for vaccination

FRDs were humanely captured by dog catchers using butterfly nets. Resting or stationary dogs were captured by quietly approaching them and quickly dropping a net over them before they became aware of the presence of the dog catcher. Where possible, FRDs were enticed with an edible reward to approach the PI or dog catcher, to facilitate quick net capture or to even avoid it if the dog was amenable to hand restraint. FRDs were also net-captured with the help of local people who were friendly with or trusted by the dog. Where neither of these methods were possible, one of the dog catchers would try to direct the dog in the direction of the other catchers or chase the dog for a short distance before they could evade the net. As soon as a dog was captured in the net, the net was tightened to restrict the dog's movement and prevent prolonged struggling.

B. During household visits for ODs

Dog-owning households were visited either when vaccination of ODs was requested by dog owners during ongoing capture of FRD in the field in round 1 (R1) or as part of pre-arranged visits to several households in a local area. Where possible, ODs were always handled by their owners. Net capture and restraint or manual handling by dog catchers were done only if owners could not safely handle dogs by themselves. These households were visited for the collection of post-vaccination blood samples during subsequent rounds.

C. During rabies vaccination camps for ODs

Central point vaccination camps for ODs were organised in parts of ALP in consultation with elected representatives and dog owners. No camps could be conducted in MUH. Dog owners presented dogs for vaccination and handled them themselves or were assisted by dog catchers. Dogs that could not be safely handled by owners were restrained by net or were vaccinated but not included in the study. Dog owners were contacted by phone to organise household visits for blood sample collection in subsequent rounds.

D. When captured as part of official ABC campaigns

FRDs were captured from the study locations for surgical sterilisation and rabies vaccination as part of regional ABC campaigns. Dog catchers avoided capturing visibly pregnant or lactating females, or females with young pups. Dogs anaesthetised before surgery were vaccinated and scanned with a microchip reader to detect any existing microchip implanted as part of the study. If no microchip was detected in a dog without a study collar, a new microchip was implanted, a coloured collar applied, and the dog was recorded as being included in the study. If a microchip was detected or no microchip was detected but the dog had a study collar (orange or black), blood samples were collected, and the dog was recorded as having been resampled as part of the second round of the study. For dogs that had previously been resampled in the field and were subsequently captured for sterilisation, their details were recorded, including the date of revaccination, and additional blood samples were collected as appropriate. An ear notch was also applied on the left ear to identify each dog as having been sterilised. All dogs captured as part of ABC campaigns were released back to where they were originally captured, as per the Animal Birth Control (Dogs) Rules, 2001 in India [82]. These dogs were later recaptured in the field for collection of further post-vaccination blood samples.

RESULTS

Animal Birth Control and reproductive activity in female dogs

Around one in five (18%) non-sterilised juvenile and adult female UDs and ODs, and nearly half of SODs, were pregnant or lactating during R1. These proportions may be higher, particularly in UDs, as only females with visible signs of pregnancy were recorded as such and less than half of all juvenile and adult female UDs captured in R1 were ever recaptured. Many pregnant and lactating female UDs will also not have been included in the ABC campaign and thus in R1. Sterilisation of pregnant dogs is currently prohibited in India by the Animal Birth Control (Dogs) Rules, 2001 [82]. Dog catchers preferentially capture male dogs as these can be quickly sterilised at lower cost (Sajeev Kumar, personal communication). While contrary to current international recommendations to sterilise more female dogs [282], this practice enhances daily sterilisation rates at lower overall cost and regional sterilisation targets are achieved more quickly. Such practices also account for the significantly higher proportion of male dogs found to be sterilised in ALP. When we assessed only those UDs captured in the field, the proportion of reproductively active non-sterilised females rose slightly to 19.5%. A study from Jaipur in north India estimated that nearly half of all females recruited into a population became pregnant every year [78]. The number and proportion of reproductively active female dogs were highest during October to January (R1 and R2 combined, R4) and dropped during April – May (R3), holding true for all ownership categories. This observed seasonality in reproductive activity agrees with previous reports from south [66] and west India [248].

We also observed a consistent rise across ownership categories in the overall proportion of reproductively active non-sterilised adult females in R4 (51%, 95% CI: 34% - 68% among UDs and SODs) (Fig S5), even as the number and proportion of sterilised dogs recaptured increased (Fig. S1). This trend highlights how if high proportions of female dogs are not sterilised during ABC campaigns, more non-sterilised female dogs will successfully reproduce, compensating for any loss of females of reproductive age. Another threat to the successful implementation of regional ABC campaigns lies in

how sterilisation targets are set. Presently dog sterilisation targets in Kerala are determined by how much funding each local body allocates for ABC, which is rarely informed by reliable baseline dog population estimates. Once this target is achieved, dog catchers (either government-appointed or coordinated by charities like Humane Society International) move on to the next local body and may return to a previously completed location months or even years later. Such a strategy possibly accounts for the low proportion of sterilised FRDs captured across both sites (15%) in our study. Combined with the practice of targeting male dogs for sterilisation, this strategy is highly unlikely to sterilise any significant fraction of the local dog population, defeating the very purpose of DPM activities and resulting only in substantial expenditure for local bodies [241]. Indeed, Belsare and Vanak (2020) showed that a 'high-intensity ABC' strategy that substantially reduced the adult dog population in an area would require significant financial expenditure and sterilising large numbers of dog every year [69]. As discused in the main chapter, it may also be more challenging to implement ABC campaigns effectively in regions with smaller dog populations (e.g. rural areas) or where dogs are more able to evade capture.

Table S1 - Details of data collected for every dog included in the study in the EpiCollect data collection form

Sl. No.	Data collected	Possible responses (where applicable)	Comments
1.	Date of capture / sampling	-	
2.	GPS location where sighted / captured	Latitude, longitude and accuracy values	This also included locations of sampling (in the case of ODs presented in the field), locations of households in the case of ODs sampled at houses, locations of vaccination camps and animal birth control centres. Where possible, this was also recorded for failed capture attempts, when previously marked dogs were seen or when capture was not attempted
3.	Ownership status	a. Free-ranging dogs captured in the field b. Free-ranging dogs sampled from ABC campaigns, c. OD sampled at vaccination camps d. OD sampled during household visits e. OD sampled in the field f. Semi-owned dogs	Option e. was chosen if 1. Captured FRDs (as defined above) were subsequently established to be free-ranging OD, or 2. ODs were presented for vaccination by owners during on-going FRD capture in the field. Unowned dogs (UDs) – Categories a and b; Owned dogs (ODs) – categories c, d and e.
4.	Time of sighting	-	Time when a dog was first seen in the field, and before capture was attempted, or time when procedures were first initiated, where appropriate
5.	Whether the dog was captured	a. Capture not attempted b. Yes c. No d. Previously caught / study collar present	Capture was not attempted if a dog was too far away or if they moved away before dog catchers could try to capture it. Failed capture attempts were recorded as 'No'.
6.	Time of capture	-	In the case of dogs that were successfully captured / brought under the control of the primary investigator (PI).
7.	Dog's activity at the time of capture	a. Sleeping, b. Resting, c. Trying to escape from dog catcher d. Feeding, e. Interacting with local people f. Interacting with field staff	

		g. Interacting with other dogs h. Other normal behaviour (e.g. exploring territory, fighting etc.) i. Not applicable in the case of OD	
8.	Unique identification code	A1, M2 etc.	A – ALP, M – MUH; Numbered in sequential order. This code was also used to mark serum sample tubes / cryovials
9.	Sex	Male / Female	
10.	Age	a. Pup – 0 – 4 months b. Juvenile – 5 to 12 months c. Adult – more than 12 months d. Aged – more than 5 years	Age was determined based on body size, size and development of genitalia, reproductive status (e.g. lactating females) or dentition (young pups). For most analyses, the 'Aged' category was combined with 'Adult' category.
11.	Breed	Free text	Recorded as being of a recognised purebreed, a crossbreed or non-descript
12.	Body condition score (BCS)	On a scale of 1 to 9 (1-3 – under ideal; 4 & 5 – ideal; 6-9 – over ideal)	Based on World Small Animal Veterinary Association (WSAVA) guidelines [267]
13.	Confinement status	a. Unconfined / completely free-ranging b. Mostly tethered or restricted within owner's property, but occasionally free-ranging c. Always confined, but allowed to roam within owner's private property only d. Completely confined or restricted in owner's house / cage or pen	All UDs and SODs (as defined above) (captured in field / ABC) were recorded as being unconfined / completely free-ranging. Broad categories – Unconfined – category a, Partially confined – category b, Completely confined – category c and d.
14.	Coat condition	Free text	Visually assessed as very good, good, fair or poor. Presence of skin diseases (e.g. dermatitis, mange), parasites etc. also recorded
15.	Whether a collar was present	No / Owner-applied collar / Collar applied as part of this study	
16.	Whether ear-notched	Yes / No	To indicate that the dog was sterilised during past (or on-going) ABC campaigns, and hence also vaccinated against rabies at least once in the past.

17.	Vaccination history	a. Unknown b. Never vaccinated (except as part of ABC or this study, where applicable) c. Regular vaccination d. Vaccinated in the past e. Other (Specify)	FRDs with no known owners were recorded as having an 'Unknown' vaccination history. Very young pups (< 3 months old) born to free-ranging dogs, all UDs and most SODs were recorded as 'Never vaccinated'. Vaccination history was collected from owners / reference persons for all other dogs.
18.	Vaccination details	Free text	Based on history presented by owner / caretaker or based on vaccination certificate if presented
19.	Whether captured for regional ABC campaigns	Yes / No	Whether the dog was captured for regional ABC campaigns, where they were sterilised and vaccinated / revaccinated against rabies
20.	Microchip number	-	A unique 15-digit code. Dogs not implanted with microchips were given unique names (e.g. Bakery dog). If no microchip was detected, this was also recorded (e.g. 'No microchip detected').
21.	Capture / Sampling occasion	a. First (pre-vaccination (prv)) b. Second (~30 days post-vaccination (pov)) c. Third (~160 – 180 days pov) d. Fourth (~365 days pov) e. Not applicable	'Not applicable' in instances where dogs were unintentionally recaptured / recaptured again for ABC campaigns and additional samples taken
22.	If blood sample collected	Yes / No	Recorded as 'No' when venipuncture was not possible (e.g. young pups) / insufficient blood was obtained / if the dog escaped before sampling / repeat recapture of dogs for ABC campaigns
23.	If study collar was applied	Yes – orange collar (males) / Yes - black collar (females) / No	Study collars were not applied in the case of pups and young juveniles / if an owner's collar was present / if the dog did not permit collaring / the dog escaped before collaring
24.	Photos (1 and 2)	-	Where possible, two different photos of the dog were taken to aid in identification
25.	Time released	-	Time of release from PI's control / return to the owner
26.	Comments	Free text	Any additional relevant information

27.	Human interaction score (Level of interaction with humans)	1. Does not appear in public when people present 2. Appears in public spaces only occasionally, especially when people present 3. Constant public presence, but avoids direct human interaction 4. Constant public presence, interacts with certain individuals (with little to no direct physical contact) 5. Constant public presence, permits physical contact with humans 6. Completely tolerant of human handling / restraint, even by strangers	

Table S2 – Grouping of data for variables from Table S1 for further analyses

Sl. No.	Grouping variable	Grouped responses
1	Ownership2	a. Owned = {"C. Owned - from vaccination camp", "D. Owned - from household visit", "E. Owned - in field"}; b. Unowned = {"A. Free-ranging - in field", "B. Free-ranging - from ABC program"}; c. Semi-owned = {F. Semi-owned}
2	Confinement	a. Free-ranging = {A. Unconfined / completely free-ranging} b. Partially free-ranging = {B. Mostly tethered or restricted within owner's property, but occasionally free-ranging} c. Confined = {C. Always confined, but allowed to roam within owner's private property only, D. Completely confined or restricted in owner's house / cage or pen
3	Titre level	a. No antibodies detected = {Less than 0.11, Less than 0.23} b. Antibodies detected = All other RFFIT titres
4	Rabies immunity	a. No antibodies detected = {Less than 0.11, Less than 0.23} b. Less than/equal to 0.5 IU/ml = {0.11, 0.23, 0.46} c. Greater than 0.5 IU/ml = {0.93, 1.87, 3.75, 7.5, 15}
5	Breed2	a. Non-descript = {Non-descript} b. Crossbreed = Where breed recorded as either non-specific 'crossbreed' or a cross of a specific breed c. Purebreed = Where a specific breed is recorded (e.g. Pomeranian, Spitz, Dachshund etc.)
6	HIS2	a. No direct human interaction = when Human interaction score is 1,2 or 3 b. Direct human interaction = when Human interaction score is 4, 5 or 6
7	BCS2	a. Under ideal BCS = when BCS is 1, 2 or 3 b. Ideal BCS = when BCS is 4 or 5 c. Over ideal BCS = when BCS is 6, 7, 8 or 9
8	Confinement2	a. Free-ranging = when Confinement is 'Free-ranging' b. Completely/ partially confined = when Confinement is {b. Partially free-ranging, c. Confined}
9	Dogs activity2	a. Normal behaviour = {"A. Sleeping", "B. Resting", "D. Feeding", "H. Other normal behaviour (e.g. exploring territory, walking, fighting etc.)"} b. Interacting with people = {"E. Interacting with local people", "F. Interacting with project staff"} c. Trying to escape = {C. Trying to escape from dog catcher} d. Owned dog = {I. Not applicable – Owned dog}
10	Vaccination history2	a. Unknown = when Vaccination history is {a. Unknown} b. Never vaccinated = when Vaccination history is {b. Never vaccinated (except as part of ABC or this study)} c. Vaccinated = when Vaccination history is {c. Regular vaccination, d. Vaccinated in the past}
11	Reproductive status	Dogs which were recorded as being 'pregnant' or 'lactating', or to have given birth / 'whelped' recently
12	Reproductive status 2	a. Active – when reproductive status is 'pregnant', 'lactating' or 'whelped' b. Not active – all other dogs

Table S3 - Breakdown of dogs included in the study in Round 1 at both study sites (ALP and MUH) (n = 577)

Sl. No.	Ownership	Breed	Sex	Earnotched	Vaccination history	Age*	n
1.	Unowned	Crossbreed	Male	No	Unknown	Adult	2
2.						Pup	1
3.				Yes		Adult	2
4.			Female	No		Adult	4
5.		Non-descript	Female	No		Adult	152
6.						Juvenile	16
7.						Pup	14
8.				Yes		Adult	23
9.			Male	No		Adult	130
10.						Aged	1
11.						Juvenile	9
12.						Pup	10
13.				Yes		Adult	35
14.						Aged	1
Total							**400**
15.	Owned	Crossbreed	Female	No	Never vaccinated	Adult	1
16.					Vaccinated in the past		4
17.					Unknown		2
18.				Yes			1
19.			Male	No	Never vaccinated		1
20.						Juvenile	1
21.					Vaccinated in the past	Adult	1
22.				Yes			1
23.		Non-descript	Female	No	Never vaccinated		12
24.						Aged	3
25.						Juvenile	2
26.						Pup	9
27.					Unknown	Adult	3
28.					Vaccinated in the past		5
29.				Yes	Never vaccinated		1
30.			Male	No	Never vaccinated	Adult	16
31.						Aged	1
32.						Juvenile	1
33.						Pup	13
34.					Regular vaccination	Aged	1
35.					Unknown	Adult	6
36.					Vaccinated in the past		3
37.						Aged	1
38.				Yes	Never vaccinated	Adult	4
39.					Unknown		1
40.		Purebreed	Female	No	Never vaccinated	Adult	2
41.						Juvenile	5
42.						Pup	1

43.					Regular vaccination	Adult	1
44.					Unknown		2
45.					Vaccinated in the past		11
46.						Aged	1
47.						Juvenile	1
48.			Male		Never vaccinated	Adult	3
49.						Aged	1
50.						Juvenile	1
51.						Pup	1
52.					Regular vaccination	Adult	2
53.					Unknown	Adult	2
54.					Vaccinated in the past	Adult	4
55.						Aged	4
Total							**136**
56.	Semi-owned	Non-descript	Female	No	Never vaccinated	Adult	2
57.						Pup	1
58.					Unknown	Adult	17
59.						Juvenile	1
60.				Yes		Adult	3
61.			Male	No	Never vaccinated	Juvenile	1
62.						Pup	1
63.					Unknown	Adult	6
64.						Juvenile	2
65.					Vaccinated in the past	Adult	1
66.				Yes	Unknown		4
67.					Vaccinated in the past		1
68.		Crossbreed	Female	No	Unknown		1
Total							**41**
Overall number of dogs included in the study							**577**

*Adult (more than 12 months), Pup (0-4 months), Juvenile (5-12 months), Aged (more than 5 years)

Table S4 – Number and percentage (in brackets) of all dogs comprised of sterilised and non-sterilised dogs in all study rounds (UD – Unowned dogs, OD – Owned dogs, SOD – Semi-owned dogs)

Sterilisation status	Round 1				Round 2				Round 3				Round 4			
	UD	OD	SOD	Total	UD	OD	SOD	Total	UD	OD	SOD	Total	UD	OD	SOD	Total
Not sterilised	339 (85)	128 (94)	33 (81)	500 (87)	121 (68)	107 (94)	24 (67)	252 (77)	52 (53)	81 (90)	14 (48)	147 (67)	47 (44)	83 (89)	12 (60)	142 (65)
Sterilised	61 (15)	8 (6)	8 (19)	77 (13)	57 (32)	7 (6)	12 (33)	76 (23)	47 (47)	9 (10)	15 (52)	71 (33)	60 (56)	10 (11)	8 (40)	78 (35)
Total	400	136	41	577	178	114	36	328	99	90	29	218	107	93	20	220

Table S5 – Distribution of human interaction scores (HIS) for unowned (UD) and semi-owned (SOD) dogs captured in round 1 in Alappuzha (ALP) and Muhamma (MUH).

Ownership category	Site	HIS = 2	HIS = 3	HIS = 4	HIS = 5	HIS = 6	Total
UD	ALP	1 (0.05)	113 (0.55)	62 (0.30)	22 (0.11)	7 (0.03)	205
	MUH	2 (0.11)	2 (0.11)	11 (0.61)	2 (0.11)	1 (0.06)	18
Total		3 (0.01)	115 (0.52)	73 (0.33)	24 (0.11)	8 (0.04)	223
SOD	ALP	0	0	11 (0.33)	15 (0.45)	7 (0.21)	33
	MUH	0	0	1 (0.17)	4 (0.67)	1 (0.17)	6
Total		0	0	12 (0.31)	19 (0.49)	8 (0.21)	39

Table S6 – Number and proportion (in brackets) of non-sterilised (NS) and sterilised (S) unowned dogs based on whether they directly interact or not with humans

Extent of human interaction	Round 1			Round 2			Round 3			Round 4		
	NS	S	Total	NS	S	Total	NS	S	Total	NS	S	Total
Direct human interaction	86 (0.82)	19 (0.18)	105	58 (0.66)	30 (0.34)	88	19 (0.41)	27 (0.59)	46	24 (0.40)	36 (0.60)	60
No direct human interaction	94 (0.80)	24 (0.20)	118	63 (0.72)	25 (0.28)	88	33 (0.62)	20 (0.38)	53	23 (0.49)	24 (0.51)	47
Total	180	43	223	121	55	176	52	47	99	47	60	107

Table S7 – Number and proportion (in brackets) of unowned and semi-owned dogs captured in each study round across both study sites having an under ideal, ideal or over ideal body condition score (BCS) based on whether they are sterilised (S) or not sterilised (NS)

Overall body condition score	Round 1			Round 2			Round 3			Round 4		
	NS	S	Total	NS	S	Total	NS	S	Total	NS	S	Total
Under ideal BCS	31 (0.08)	1 (0.01)	32 (0.07)	5 (0.03)	1 (0.01)	6 (0.03)	3 (0.05)	0 (0)	3 (0.02)	4 (0.07)	0 (0)	4 (0.03)
Ideal BCS	307 (0.83)	47 (0.68)	354 (0.80)	133 (0.92)	59 (0.86)	192 (0.90)	54 (0.82)	32 (0.52)	86 (0.67)	39 (0.66)	35 (0.52)	74 (0.58)
Over ideal BCS	34 (0.09)	21 (0.30)	55 (0.13)	7 (0.05)	9 (0.13)	16 (0.08)	9 (0.14)	30 (0.48)	39 (0.31)	16 (0.27)	33 (0.49)	49 (0.39)
Total	372	69	441	145	69	214	66	62	128	59	68	127

Table S8. Number and percentage (in brackets) of non-sterilised juvenile and adult female dogs of all ownership categories captured in each study at both study sites, which were reproductively active (lactating, pregnant or whelped) at the time of capture

Reproductive status	Round 1				Round 2				Round 3				Round 4			
	OD	SOD	UD	Total	OD	SOD	UD	Total	OD	SOD	UD	Total	OD	SOD	UD	Total
Lactating	7 (13)	8 (38)	22 (13)	37 (15)	0 (0)	4 (27)	7 (10)	11 (8)	6 (15)	3 (27)	9 (27)	18 (21)	4 (10)	3 (33)	9 (32)	16 (21)
Pregnant	1 (2)	1 (5)	9 (5)	11 (4)	1 (2)	0 (0)	2 (3)	3 (2)	1 (2)	1 (9)	0 (0)	2 (2)	5 (12)	2 (22)	3 (11)	10 (13)
Whelped / given birth	1 (2)	1 (5)	0 (0)	2 (1)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2)	0 (0)	0 (0)	1 (1)	5 (12)	0 (0)	2 (7)	7 (9)
Not reproductively active	46 (84)	11 (52)	143 (82)	200 (80)	45 (98)	11 (73)	64 (88)	120 (90)	32 (80)	7 (64)	24 (73)	63 (75)	27 (66)	4 (44)	14 (50)	45 (58)
Total	55	21	174	250	46	15	73	134	40	11	33	84	41	9	28	78

Table S9. Detection of pre-vaccination rabies virus neutralising antibodies (RVNA) in unowned dogs captured in round 1 at both study sites. Figures in brackets represent proportion of dogs with detectable/non-detectable RVNA titres.

Site	Antibodies detected	No antibodies detected	Total
Alappuzha	101 (0.29)	242 (0.71)	343 (1)
Muhamma	11 (0.26)	32 (0.74)	43 (1)
Total	112 (0.29)	274 (0.71)	386 (1)

Table S10. Detection of pre-vaccination rabies virus neutralising antibodies (RVNA) in unowned dogs captured in round 1 at both study sites, based on their sterilisation status. Figures in brackets represent proportion of dogs with detectable/non-detectable RVNA titres.

Earnotched	Antibodies detected	No antibodies detected	Total
Not sterilised	86 (0.26)	242 (0.74)	328 (1)
Sterilised	26 (0.45)	32 (0.55)	58 (1)
Total	112 (0.29)	274 (0.71)	386 (1)

Table S11. Number and proportion (in brackets) of owned dogs captured in each study round, having under ideal, ideal or over ideal body condition scores (BCS)

Overall body condition score (BCS)	Study round				
	First	Second	Third	Fourth	Total
Under ideal BCS	14 (0.10)	5 (0.04)	3 (0.03)	2 (0.02)	24 (0.06)
Ideal BCS	75 (0.55)	85 (0.75)	56 (0.62)	47 (0.51)	263 (0.61)
Over ideal BCS	47 (0.35)	24 (0.21)	31 (0.34)	44 (0.47)	146 (0.34)
Total	136 (1)	114 (1)	90 (1)	93 (1)	433 (1)

Table S12. Number and proportion (in brackets) of owned dogs captured in each study round, based on their sterilisation status

Sterilisation status	Study round				
	First	Second	Third	Fourth	Total
Not sterilised	128 (0.94)	107 (0.94)	81 (0.90)	83 (0.89)	399 (0.92)
Sterilised	8 (0.06)	7 (0.06)	9 (0.10)	10 (0.11)	34 (0.08)
Total	136 (1)	114 (1)	90 (1)	93 (1)	433 (1)

Table S13. Number and proportion (in brackets) of owned dogs captured in each study round, based on their vaccination history and day-zero (pre-vaccination) rabies virus neutralising antibody (RVNA) titres (in international units (IU)/ml)

Vaccination history	< 0.23	0.23	0.46	0.93	Total
Never vaccinated	53 (0.73)	12 (0.16)	7 (0.10)	1 (0.01)	73 (1)
Unknown	9 (0.56)	7 (0.44)	0 (0)	0 (0)	16 (1)
Vaccinated	24 (0.62)	5 (0.13)	9 (0.23)	1 (0.03)	39 (1)
Total	86 (0.67)	24 (0.19)	16 (0.12)	2 (0.02)	128 (1)

Table S14. Number and proportion of all dogs captured a certain number of times at both study sites, and whether differences in proportions between sites are statistically significant. Statistically significant differences are highlighted in bold.

Site	Caught once	Caught twice	Caught thrice	Caught four times
Alappuzha (n = 515)	157 (0.30)	127 (0.25)	113 (0.22)	118 (0.23)
Muhamma (n = 62)	30 (0.48)	17 (0.27)	11 (0.18)	4 (0.07)
p-value	**0.0068**	0.75	0.53	**0.001 (Fisher's exact test)**

Table S15 – Number of female and male dogs of all ownership categories captured in R1, with number (and proportions in brackets) recaptured in later rounds

Sex	Round 1	Round 2	Round 3	Round 4
Female	301	180 (0.60)	120 (0.40)	112 (0.37)
Male	276	148 (0.54)	98 (0.36)	108 (0.39)
Total	577	328	218	220

Table S16. Number of female and male unowned dogs captured in R1, with number (and proportions in brackets) recaptured in later rounds

Sex	First	Second	Third	Fourth	Total
Female	209 (0.52)	102 (0.57)	57 (0.58)	52 (0.49)	420 (0.54)
Male	191 (0.48)	76 (0.43)	42 (0.42)	55 (0.51)	364 (0.46)
Total	400	178	99	107	784

Table S17. Recapture proportions by age at first encounter in R1

Age	Round 1	Round 2	Round 3	Round 4
Pup	47	25 (0.53)	17 (0.36)	11 (0.23)
Juvenile	46	26 (0.57)	12 (0.26)	15 (0.33)
Adult (incl. Aged)	484	277 (0.57)	189 (0.39)	194 (0.40)
Total	577	328 (0.57)	218 (0.38)	220 (0.38)

Table S18. Number and proportion of pups captured in R1, which were recaptured in subsequent rounds, by ownership category

Ownership status	Round 1	Round 2	Round 3	Round 4
No owner	21	4 (0.19)	1 (0.05)	1 (0.05)
Owned	24	19 (0.80)	15 (0.63)	9 (0.38)
Semi-owned	2	2 (1.00)	1 (0.05)	1 (0.05)
Total	47	25 (0.53)	17 (0.36)	11 (0.23)

Table S19. Number of pups captured in the first round and numbers of same pups recaptured in subsequent rounds, based on confinement status

Sampling occasion	First	Second	Third	Fourth
Confined	10	7 (0.70)	6 (0.60)	4 (0.40)
Free-ranging	33	14 (0.42)	7 (0.21)	6 (0.18)
Partially free-ranging	4	4 (1)	4 (1)	1 (0.25)
Total	47	25 (0.53)	17 (0.36)	11 (0.23)

Table S20. Number and proportion of juveniles captured in R1, which were recaptured in subsequent rounds, by ownership category

Ownership status	Round 1	Round 2	Round 3	Round 4
No owner	31	13 (0.42)	3 (0.10)	6 (0.19)
Owned	11	10 (0.91)	8 (0.73)	8 (0.73)
Semi-owned	4	3 (0.75)	1 (0.25)	1 (0.25)
Total	46	26 (0.57)	12 (0.26)	15 (0.33)

Table S21. Number of juveniles captured in R1, and numbers and proportions recaptured in subsequent rounds, based on confinement status

Ownership status	First	Second	Third	Fourth
Confined	5	4 (0.80)	3 (0.60)	4 (0.80)
Free-ranging	38	19 (0.50)	6 (0.16)	8 (0.21)
Partially free-ranging	3	3 (1.0)	3 (1.0)	3 (1.0)
Total	46	26 (0.57)	12 (0.26)	15 (0.33)

Table S22. Number of pups of different ownership categories captured once, twice, thrice or four times in the course of the study

Ownership status	Number of pups captured only once in Round 1	Number of pups captured twice	Number of pups captured thrice	Number of pups captured four times
No owner (n = 21)	16 (0.76)	4 (0.19)	1 (0.05)	0 (0)
Owned (n = 24)	4 (0.17)	4 (0.17)	9 (0.38)	7 (0.29)
Semi-owned (n = 2)	0 (0)	1 (0.5)	0 (0)	1 (0.5)
Total (n = 47)	20 (0.43)	9 (0.19)	10 (0.21)	8 (0.17)

Table S23. Number of juveniles of different ownership categories captured once, twice, thrice or four times in the course of the study

Ownership status	Number captured only once in Round 1	Number captured twice	Number captured thrice	Number captured four times
No owner (n = 31)	18 (0.58)	7 (0.23)	3 (0.10)	3 (0.10)
Owned (n = 11)	0 (0)	3 (0.27)	1 (0.09)	7 (0.64)
Semi-owned (n = 4)	1 (0.25)	2 (0.50)	0 (0)	1 (0.25)
Total (n = 46)	19 (0.41)	12 (0.26)	4 (0.09)	11 (0.24)

Table S24 – Number and proportion (in brackets) of sterilised and non-sterilised dogs of all ownership categories captured in R1 which were recaptured in subsequent rounds, and whether these differences were statistically significant (in bold).

Sterilisation status	Total captured in Round 1	Number recaptured in Round 2	Number recaptured in Round 3	Number recaptured in Round 4
Sterilised in Round 1	77	49 (0.64)	38 (0.49)	39 (0.51)
Not sterilised in Round 1	500	279 (0.56)	180 (0.36)	181 (0.36)
p-value (differences in proportions recaptured between sterilised and non-sterilised dogs)	-	0.24	**0.03**	**0.02**
Total	577	328 (0.57)	218 (0.37)	220 (0.38)

Table S25 – Number and proportion (in brackets) of sterilised and non-sterilised dogs of all ownership categories from Round 1 that were captured one, two, three or four times. Statistically significant differences are highlighted in bold.

Sterilisation status	Number of dogs captured only once in Round 1	Number of dogs captured twice	Number of dogs captured thrice	Number of dogs captured four times
Sterilised in Round 1 (n = 77)	19 (0.25)	15 (0.19)	18 (0.23)	25 (0.32)
Not sterilised in Round 1 (n = 500)	168 (0.34)	129 (0.26)	106 (0.21)	97 (0.19)
p-value (differences between sterilised and non-sterilised dogs in proportions captured a certain number of times)	0.15	0.29	0.78	**0.014**
Total (n = 577)	187 (0.32)	144 (0.25)	124 (0.22)	122 (0.21)

Table S26 – Number and proportion (in brackets) of owned dogs of different breeds captured in round 1, which were recaptured in subsequent rounds.

Breed	Round 1	Round 2	Round 3	Round 4
Crossbreed	14	13 (0.93)	6 (0.43)	10 (0.71)
Non-descript	82	66 (0.80)	57 (0.70)	50 (0.61)
Purebreed	40	35 (0.88)	27 (0.68)	33 (0.83)
Total	136	114 (0.84)	90 (0.66)	93 (0.68)

Table S27. Number and proportion (in brackets) of unowned dogs assigned various human interaction scores captured in round 1, which were recaptured in subsequent rounds.

Human interaction score	First	Second	Third	Fourth
2	3	1 (0.33)	2 (0.67)	0 (0)
3	115	87 (0.76)	51 (0.44)	47 (0.41)
4	73	64 (0.88)	29 (0.40)	37 (0.51)
5	24	18 (0.75)	(0.46)	17 (0.71)
6	8	6 (0.75)	6 (0.75)	6 (0.75)
Total	223	176	99	107

Table S28 – Number and proportion (in brackets) of semi-owned dogs assigned various human interaction scores captured in round 1, which were recaptured in subsequent rounds.

Human interaction score	First	Second	Third	Fourth
4	12	11 (0.92)	8 (0.67)	6 (0.50)
5	19	18 (0.95)	14 (0.74)	8 (0.42)
6	8	7 (0.88)	7 (0.88)	6 (0.75)
Total	39	36	29	20

Table S29. Number and proportion (in brackets) of unowned and semi-owned dogs assigned various human interaction scores captured in round 1, which were recaptured in subsequent rounds.

Human interaction score	First	Second	Third	Fourth
2	3	1 (0.33)	2 (0.67)	0 (0)
3	115	87 (0.76)	51 (0.44)	47 (0.41)
4	85	75 (0.88)	37 (0.44)	43 (0.51)
5	43	36 (0.84)	25 (0.58)	25 (0.58)
6	16	13 (0.81)	13 (0.81)	12 (0.75)
Total	262	212	128	127

Table S30. Number and proportion (in brackets) of unowned dogs captured in round 1 which were assessed to have direct or no direct human interaction, which were recaptured in subsequent rounds.

HIS2	First	Second	Third	Fourth
Direct human interaction	105	88 (0.84)	46 (0.44)	60 (0.57)
No direct human interaction	118	88 (0.75)	53 (0.45)	47 (0.40)
Total	223	176	99	107

Table S31. Number of unowned and semi-owned dogs captured in round 1 whose activity at the time of capture was recorded, and the number and proportion (in brackets) of these dogs recaptured in subsequent rounds.

Dog's activity in Round 1	First	Second	Third	Fourth
Trying to escape from dog catcher	214	110 (0.51)	64 (0.30)	61 (0.29)
Normal behaviour	90	43 (0.48)	27 (0.30)	23 (0.26)
Interacting with people (local public, study members)	14	12 (0.86)	9 (0.64)	9 (0.64)
Total	318	165	100	93

Table S32. Number of unowned and semi-owned dogs captured in round 1 whose day zero (pre-vaccination) rabies virus neutralising antibody (RVNA) titres (in IU/ml) were recorded, and the number and proportion (in brackets) of these dogs recaptured in subsequent rounds.

RVNA titres	Total dogs caught in R1	Dogs recaptured in R2	Dogs recaptured in R3	Dogs recaptured in R4
< 0.23	304	144 (0.47)	81 (0.27)	79 (0.26)
0.23	63	31 (0.49)	22 (0.35)	18 (0.29)
0.46	47	23 (0.49)	13 (0.28)	12 (0.26)
0.93	10	8 (0.80)	6 (0.60)	6 (0.60)
1.87	2	2 (1)	1 (50)	2 (1)

Table S33. Number of unowned and semi-owned dogs captured in round 1 based on whether they had detectable levels of day zero (pre-vaccination) rabies virus neutralising antibodies (RVNA) and the number and proportion (in brackets) of these dogs recaptured in subsequent rounds.

Whether RVNA detected or not	Total dogs caught in R1	Dogs recaptured in R2	Dogs recaptured in R3	Dogs recaptured in R4
Antibodies detected	122	64 (0.52)	42 (0.34)	38 (0.31)
No antibodies detected	304	144 (0.47)	81 (0.27)	79 (0.26)

Table S34. Number of unowned and semi-owned dogs captured in round 1 based on whether day zero (pre-vaccination) rabies virus neutralising antibodies (RVNA) titres were greater than or less than/equal to 0.5 IU per ml (IU/ml), or not detected, and the number and proportion (in brackets) of these dogs recaptured in subsequent rounds. Chi-square and Fisher's exact tests were used to check of significant differences between groups in proportions recaptured. Statistically significant p-values are highlighted in bold

RVNA levels	Total dogs caught in R1	Dogs recaptured in R2	Dogs recaptured in R3	Dogs recaptured in R4
a. > 0.5 IU/ml	12	10 (0.83)	7 (0.58)	8 (0.67)
b. ≤ 0.5 IU/ml	110	54 (0.49)	35 (0.32)	30 (0.27)
c. No antibodies detected	304	144 (0.47)	81 (0.27)	79 (0.26)
Level of significance (p-value) between a. & b.		0.051	0.11 (Fisher's exact test)	**0.009 (Fisher's exact test)**
Level of significance (p-value) between a. & c.		**0.031**	**0.04 (Fisher's exact test)**	**0.005 (Fisher's exact test)**

Table S35. Number of unowned dogs captured in round 1 based on whether day zero (pre-vaccination) rabies virus neutralising antibodies (RVNA) titres were greater than 0.5 IU per ml (IU/ml), less than/equal to 0.5 IU/ml or not detected, and the number and proportion (in brackets) of these dogs recaptured in subsequent rounds. Chi-square and Fisher's exact tests were used to check of significant differences between groups in proportions recaptured. Statistically significant p-values are highlighted in bold

RVNA levels	Total dogs caught in R1	Dogs recaptured in R2	Dogs recaptured in R3	Dogs recaptured in R4
a. > 0.5 IU/ml	10	8 (0.80)	5 (0.50)	8 (0.80)
b. ≤ 0.5 IU/ml	102	47 (0.46)	29 (0.28)	25 (0.25)
c. No antibodies detected	274	118 (0.43)	62 (0.23)	65 (0.24)
Level of significance (p-value) between a. & b.		0.051	0.17 (Fisher's exact test)	**< 0.001 (Fisher's exact test)**
Level of significance (p-value) between a. & c.		**0.025 (Fisher's exact test)**	0.059 (Fisher's exact test)	**< 0.001 (Fisher's exact test)**

Table S36. Number of unowned and semi—owned dogs included in the study in round 1 based on whether they were first included in the study when captured as part of local animal birth control campaigns, and the numbers and proportions (in brackets) recaptured in subsequent rounds. Statistically significant differences between recapture proportions (Chi-squared test) are highlighted in bold.

Whether captured for ABC in R1	First	Second	Third	Fourth
Yes	85	26 (0.31)	12 (0.14)	15 (0.18)
No	356	188 (0.53)	116 (0.33)	112 (0.31)
Total	441	214 (0.49)	128 (0.29)	127 (0.29)
p-value		**< 0.001**	**0.0012**	**0.017** (not significant if including 4 earnotched dogs wo microchips

Table S37. Number of unowned and semi—owned dogs included in the study based on whether they were captured and sterilised as part of local ABC campaigns at any stage of the study, and the numbers and proportions (in brackets) recaptured in subsequent rounds. Statistically significant differences between recapture proportions (Chi-squared test) are highlighted in bold.

Whether captured for ABC in any study round	First	Second	Third	Fourth
Yes	116	57 (0.49)	22 (0.19)	30 (0.26)
No	325	157 (0.48)	106 (0.33)	97 (0.30)
Total	441	214 (0.49)	128 (0.29)	127 (0.29)
p-value		0.96	**0.007**	0.49

Table S38. Number of dogs microchipped in round 1, which were recaptured in subsequent rounds and in which no microchip could be detected, from all rounds of the study. Figures in brackets indicate the number of dogs whose identities could not be confirmed

Study round	No owner	Owned	Semi-owned	Total
Second	16 (1)	2 (0)	3 (0)	21 (1)
Third	6 (0)	3 (0)	3 (1)	12 (1)
Fourth	11 (6)	4 (0)	2 (0)	17 (6)
Total	33 (7)	9 (0)	8 (1)	50 (8)

Table S39: Summary of the various fixed and mixed effects logistic regression models, including predictors included and AIC values for the preliminary and final multivariable models

Model Number	Study round	Dog category (n)	Important variables identified from univariate analyses*	AIC for model with all these variables (excluded variables due to missing values)	Variables in reduced model*	AIC for reduced model	Table Number for ORs
1.	Round 2	All dogs (566)	BCS, BCS2, Breed2, Coat, Ownership2, Confinement2, HIS2, Dogs_activity, Dogs_activity2, Vaccination history2, ABC, Site, Study collar applied, Study collar applied2, Rabies immunity	633.47 (HIS2, Dogs_activity, Dogs_activity2)	Coat, Ownership2, Confinement, Vaccination history2, ABC, Site, Rabies immunity	623.03	S40
2.		UD (393)	Earnotched, Coat, ABC, Site	515.32	Coat, ABC, Site	514.79	S43
3.		OD (132)	Vacc_history2, Site	101.79	Vaccination history2	101.27	S46
4.		SOD (41)	NA	NA	NA	NA	NA
5.		UD+SOD (434)	Sex, Earnotched, Coat, Ownership2, HIS2, Dogs_activity, Dogs_activity2, ABC, Site, Rabies immunity	241.46 (Dogs_activity, Dogs_activity2)	Coat, Ownership2, HIS2, ABC, Site, Rabies immunity	237.61	S49
6.	Round 3	All dogs (566)	BCS, BCS2, Earnotched, Breed2, Coat, Ownership2, Confinement2, Human_interaction, Dogs_activity, Dogs_activity2, Vaccination history2, ABC, Study collar applied, Study collar applied2	650.92 (Human_interaction, Dogs_activity, Dogs_activity2)	Earnotched, Ownership2, ABC, Study collar applied	641.73	S41
7.		UD (393)	Age2, Earnotched, Repr_status, ABC, Study collar applied, Study collar applied2	427.68 (Repr_status)	Age2, Earnotched, ABC	426.17	S44
8.		OD (132)	Coat	158.21	Coat	158.21	S47

9.		SOD (41)	NA	NA	NA	NA	NA
10.		UD+SOD (434)	Age2, Earnotched, Ownership2, Human_interaction, Dogs_activity, Dogs_activity2, Vaccination history2, ABC, Study collar applied, Study collar applied2	356.29 (Dogs_activity, Dogs_activity2)	Age2, Earnotched, Ownership2, Human_interaction, ABC	350.13	S50
11.	Round 4	All dogs (566)	BCS, BCS2, Earnotched, Breed2, Coat, Ownership2, Confinement2, Dogs_activity, Dogs_activity2, Vaccination history2, ABC, Site, Study collar applied, Study collar applied2, Rabies immunity	626.1 (Dogs_activity, Dogs_activity2)	Earnotched, Ownership2, Confinement2, ABC, Site, Study collar applied2, Rabies immunity	611.47	S42
12.		UD (393)	Earnotched, Site, RFFIT_titre, Rabies immunity	415.45	Earnotched, Site, Rabies immunity	412.39	S45
13.		OD (132)	Age2, Site, Confinement2	147.34	Age2, Site, Confinement2	147.34	S48
14.		SOD (41)	NA	NA	NA	NA	NA
15.		UD+SOD (434)	Earnotched, Ownership2, Dogs_activity, Dogs_activity2, ABC, Site, RFFIT_titre, Rabies immunity	476.69 (Dogs_activity, Dogs_activity2)	Earnotched, Ownership2, ABC, Site, Rabies immunity	474.59	S51
16.	All rounds (AR)	All dogs (566)	BCS, BCS2, Earnotched, Breed2, Coat, Ownership2, Confinement2, Human association, HIS2, Dogs_activity, Dogs_activity2, Vaccination history2, ABC, Site, Study collar applied, Study collar applied2, RFFIT_titre, Titre_level, Rabies immunity	1282.7 (Dogs_activity, Dogs_activity2, Human association, HIS2)	Earnotched, Coat, Ownership2, Vaccination history2, ABC, Site, Study collar applied, Rabies immunity	1275.84	S52
17.		UD (393)	Age2, Earnotched , Dogs_activity, ABC, Site, Study collar applied,	966.77 (Dogs_activity)	Age2, Earnotched , ABC, Site, Rabies immunity	963.2	S53

			Study collar applied2, RFFIT_titre, Titre_level, Rabies immunity				
18.		OD (132)	Vaccination history2, Site	248.51	Vaccination history2, Site	248.51	S54
19.		SOD (41)	NA	NA	NA	NA	NA
20.		UD+SOD (434)	Age2, BCS, Earnotched, Coat, Ownership2, Human association, HIS2, Dogs_activity, Dogs_activity2, Vaccination history2, ABC, Site, Study collar applied, Study collar applied2, RFFIT_titre, Rabies immunity	589.87 (Dogs_activity, Dogs_activity2, Vaccination history2)	Earnotched, Ownership2, HIS2, ABC, Site, Rabies immunity (intentionally excluded Vaccination history2 here, due to very large ORs in final model)	577.17	S55
21.	All rounds – mixed effects (AR-ME)	All dogs (566)	Dogs_activity_R1, Dogs_activity2_R1, Breed2_R1, BCS_R1, BCS2_R1, Confinement2_R1, Coat_R1, Earnotched_R1, Vaccination history2_R1, ABC_R1, Sampling_occasion, Study collar applied_R1, Stduy_collar_applied2_R1, RFFIT_titre_R1, Rabies immunity_R1, Human_interaction_R1, HIS2_R1, Site_R1, Ownership2_R1, **Repr_status_R1**	1711.86 (Dogs_activity_R1, Dogs_activity2_R1, Human_interaction_R1, HIS2_R1, Repr_status_R1)	Coat_R1, Earnotched_R1, Vaccination history2_R1, ABC_R1, Sampling_occasion, Study collar applied_R1, Rabies immunity_R1, Site_R1, Ownership2_R1	1702.42	S56
22.		UD (393)	Dogs_activity_R1, Dogs_activity2_R1, Age2_R1, Earnotched_R1, ABC_R1, Sampling_occasion, Study collar applied2_R1, Site_R1, **Repr_status_R1**	1311.58 (Dogs_activity_R1, Dogs_activity2_R1, Repr_status_R1)	Age_R1, Earnotched_R1, ABC_R1, Sampling_occasion, Site_R1	1309.42	S57

23.		OD (132)	Vaccination history2_R1, Sampling_occasion, Site_R1	338.95	Vaccination history2_R1, Sampling_occasion, Site_R1	338.95	S58
24.		SOD (41)	Sampling_occasion, Human_interaction_R1	119.1	Sampling_occasion, Human_interaction_R1	119.1	S59
25.		UD+SOD (434)	Dogs_activity_R1, Dogs_activity2_R1, Age2_R1, Earnotched_R1, Vaccination history2_R1, ABC_R1, Sampling_occasion, Study collar applied_R1, Rabies immunity_R1, Site_R1, Ownership2_R1**, Repr_status_R1**	1391.15 (Dogs_activity_R1, Dogs_activity2_R1, Repr_status_R1, Vaccination history2_R1)	Age2_R1, Earnotched_R1, Ownership2_R1, ABC_R1, Site_R1, Rabies immunity_R1, Sampling_occasion, Study collar applied_R1 (intentionally excluded Vaccination history2 here, due to very large ORs in final model)	1391.15	S60

Table S40. Odds ratios and p-values for variables included in the final multivariable logistic regression model for probability of being captured in round 2 for all dogs. Statistically significant p-values ($p < 0.05$) are highlighted in bold.

Variable	Odds_Ratio	Standard_error	95% confidence intervals	p_value
(Intercept)	**11.21**	**1.02**	**1.73-104.92**	**0.018**
Coat: Fair	1.10	0.32	0.59-2.07	0.760
Coat: Good	1.62	0.33	0.86-3.08	0.139
Coat: Very good	1.07	0.70	0.28-4.64	0.926
Ownership2: Semi-owned	3.97	0.72	0.99-17.48	0.057
Ownership2: Unowned	0.47	0.56	0.15-1.37	0.173
Confinement2: Completely/partially free-ranging	3.24	0.65	0.89-12	0.072
Vaccination history2: Unknown	**0.20**	**0.67**	**0.05-0.74**	**0.017**
Vaccination history2: Vaccinated	2.23	0.77	0.53-11.68	0.296
ABC: Yes	**0.33**	**0.29**	**0.19-0.58**	**< 0.001**
Site: Muhamma	**0.27**	**0.33**	**0.14-0.51**	**< 0.001**
Rabies immunity: ≤ 0.5IU/ml	0.32	0.81	0.05-1.33	0.159
Rabies immunity: No antibodies detected	0.25	0.80	0.04-1.01	0.082

Table S41. Odds ratios and p-values for variables included in the final multivariable logistic regression model for probability of being captured in round 3 for all dogs. Statistically significant p-values (p < 0.05) are highlighted in bold.

Variable	Odds_Ratio	Standard_error	95% confidence intervals	p_value
(Intercept)	**1.89**	**0.20**	**1.3-2.81**	**0.001**
Earnotched: Sterilised	**2.10**	**0.29**	**1.2-3.69**	**0.009**
Ownership2: Semi-owned	0.68	0.44	0.29-1.63	0.384
Ownership2: Unowned	**0.10**	**0.31**	**0.05-0.18**	**< 0.001**
ABC: Yes	**0.39**	**0.35**	**0.19-0.76**	**0.008**
Study collar applied: Yes - black	**2.35**	**0.31**	**1.31-4.37**	**0.005**
Study collar applied: Yes - orange	1.49	0.32	0.81-2.82	0.210

Table S42. Odds ratios and p-values for variables included in the final multivariable logistic regression model for probability of being captured in round 4 for all dogs. Statistically significant p-values (p < 0.05) are highlighted in bold.

Variable	Odds_Ratio	Standard_error	95% confidence intervals	p_value
(Intercept)	**23.30**	**0.74**	**5.86-109.15**	**< 0.001**
Earnotched: Sterilised	**2.13**	**0.29**	**1.21-3.76**	**0.008**
Ownership2: Semi-owned	**0.37**	**0.45**	**0.15-0.87**	**0.025**
Ownership2: Unowned	**0.13**	**0.33**	**0.06-0.24**	**< 0.001**
Confinement2: Completely/ partially free-ranging	**0.36**	**0.47**	**0.14-0.89**	**0.032**
ABC: Yes	0.55	0.32	0.28-1.02	0.066
Site: Muhamma	**0.26**	**0.41**	**0.11-0.56**	**0.001**
Study collar applied2: Collared	1.62	0.30	0.92-2.96	0.104
Rabies immunity: ≤ 0.5IU/ml	**0.21**	**0.65**	**0.05-0.69**	**0.015**
Rabies immunity: No antibodies detected	**0.22**	**0.63**	**0.06-0.72**	**0.016**

Table S43. Odds ratios and p-values for variables included in the final multivariable logistic regression model for probability of being captured in round 2 for unowned dogs. Statistically significant p-values (p < 0.05) are highlighted in bold.

Variable	Odds_Ratio	Standard_error	95% confidence intervals	p_value
(Intercept)	0.83	0.30	0.46-1.48	0.534
Coat: Fair	1.23	0.33	0.64-2.39	0.543
Coat: Good	1.92	0.35	0.98-3.82	0.061
Coat: Very good	0.80	0.96	0.1-5.29	0.819
ABC: Yes	**0.31**	**0.30**	**0.17-0.55**	**< 0.001**
Site: Muhamma	**0.32**	**0.36**	**0.15-0.64**	**0.002**

Table S44. Odds ratios and p-values for variables included in the final multivariable logistic regression model for probability of being captured in round 3 for unowned dogs. Statistically significant p-values (p < 0.05) are highlighted in bold.

Variable	Odds_Ratio	Standard_error	95% confidence intervals	p_value
(Intercept)	**0.39**	**0.15**	**0.29-0.52**	**< 0.001**
Age: Juvenile (5-12 months)	0.33	0.63	0.08-0.99	0.080
Age: Pup (0-4 months)	0.17	1.04	0.01-0.87	0.090
Earnotched: Sterilised	**1.83**	**0.30**	**1.01-3.3**	**0.046**
ABC: Yes	**0.36**	**0.38**	**0.16-0.72**	**0.007**

Table S45. Odds ratios and p-values for variables included in the final multivariable logistic regression model for probability of being captured in round 4 for unowned dogs. Statistically significant p-values ($p < 0.05$) are highlighted in bold.

Variable	Odds_Ratio	Standard_error	95% confidence intervals	p_value
(Intercept)	3.45	0.80	0.85-23.06	0.120
Earnotched: Sterilised	**2.57**	**0.31**	**1.39-4.72**	**0.003**
Site: Muhamma	**0.23**	**0.61**	**0.05-0.66**	**0.017**
Rabies immunity: ≤ 0.5IU/ml	**0.08**	**0.83**	**0.01-0.36**	**0.003**
Rabies immunity: No antibodies detected	**0.09**	**0.81**	**0.01-0.38**	**0.003**

Table S46. Odds ratios and p-values for variables included in the final multivariable logistic regression model for probability of being captured in round 2 for owned dogs. Statistically significant p-values ($p < 0.05$) are highlighted in bold.

Variable	Odds_Ratio	Standard_error	95% confidence intervals	p_value
(Intercept)	**8.37**	**0.37**	**4.28-18.91**	**< 0.001**
Vaccination history2: Unknown	**0.17**	**0.62**	**0.05-0.58**	**0.004**
Vaccination history2: Vaccinated	1.47	0.71	0.4-7.03	0.584

Table S47. Odds ratios and p-values for variables included in the final multivariable logistic regression model for probability of being captured in round 3 for owned dogs. Statistically significant p-values ($p < 0.05$) are highlighted in bold.

Variable	Odds_Ratio	Standard_error	95% confidence intervals	p_value
(Intercept)	1.75	0.63	0.53-6.68	0.372
Coat: Fair	0.61	0.73	0.14-2.49	0.501
Coat: Good	2.22	0.68	0.53-8.27	0.244
Coat: Very good	0.29	0.94	0.04-1.72	0.185

Table S48. Odds ratios and p-values for variables included in the final multivariable logistic regression model for probability of being captured in round 4 for owned dogs. Statistically significant p-values (p < 0.05) are highlighted in bold.

Variable	Odds_Ratio	Standard_error	95% confidence intervals	p_value
(Intercept)	**7.11**	**0.42**	**3.3-17.6**	**< 0.001**
Confinement2: Completely/ partially free-ranging	0.44	0.47	0.17-1.07	0.078
Age: Juvenile (5-12 months)	0.77	0.77	0.18-4.14	0.733
Age: Pup (0-4 months)	**0.19**	**0.53**	**0.06-0.53**	**0.002**
Site: Muhamma	**0.14**	**0.75**	**0.03-0.58**	**0.009**

Table S49. Odds ratios and p-values for variables included in the final multivariable logistic regression model for probability of being captured in round 2 for unowned and semi-owned dogs. Statistically significant p-values (p < 0.05) are highlighted in bold.

Variable	Odds_Ratio	Standard_error	95% confidence intervals	p_value
(Intercept)	**17.16**	**1.27**	**2.01-410.57**	**0.025**
Coat: Fair	1.51	0.45	0.61-3.63	0.357
Coat: Good	**5.19**	**0.55**	**1.79-16.04**	**0.003**
Coat: Very good	0.83	1.23	0.09-18.43	0.883
Ownership2: Unowned	0.36	0.69	0.08-1.22	0.134
HIS2: No direct human interaction	**0.45**	**0.38**	**0.2-0.93**	**0.036**
ABC: Yes	**0.36**	**0.47**	**0.14-0.91**	**0.028**
Site: Muhamma	**0.21**	**0.55**	**0.07-0.63**	**0.005**
Rabies immunity: ≤ 0.5 IU/ml	0.75	1.14	0.04-5.06	0.802
Rabies immunity: No antibodies detected	0.65	1.10	0.03-4	0.698

Table S50. Odds ratios and p-values for variables included in the final multivariable logistic regression model for probability of being captured in round 3 for unowned and semi-owned dogs. Statistically significant p-values (p < 0.05) are highlighted in bold.

Variable	Odds_Ratio	Standard_error	95% confidence intervals	p_value
(Intercept)	6.43	1.31	0.53-152.6	0.155
Age: Juvenile (5-12 months)	0.32	0.63	0.08-1	0.066
Age: Pup (0-4 months)	0.25	0.98	0.03-1.43	0.151
Earnotched: Sterilised	**2.02**	**0.35**	**1.03-4.05**	**0.044**
Ownership2: Unowned	**0.31**	**0.46**	**0.12-0.75**	**0.011**
Human association: 3	0.40	1.24	0.02-4.36	0.465
Human association: 4	0.32	1.25	0.01-3.57	0.369
Human association: 5	0.46	1.28	0.02-5.31	0.541
Human association: 6	1.92	1.42	0.07-30.17	0.645
ABC: Yes	0.48	0.41	0.21-1.05	0.073

Table S51. Odds ratios and p-values for variables included in the final multivariable logistic regression model for probability of being captured in round 4 for unowned and semi-owned dogs. Statistically significant p-values (p < 0.05) are highlighted in bold.

Variable	Odds_Ratio	Standard_error	95% confidence intervals	p_value
(Intercept)	**4.18**	**0.70**	**1.11-18.18**	**0.041**
Earnotched: Sterilised	**2.17**	**0.30**	**1.21-3.89**	**0.009**
Ownership2: Unowned	**0.37**	**0.36**	**0.18-0.74**	**0.005**
ABC: Yes	0.56	0.33	0.29-1.04	0.075
Site: Muhamma	**0.30**	**0.47**	**0.11-0.69**	**0.009**
Rabies immunity: ≤ 0.5IU/ml	**0.23**	**0.67**	**0.06-0.81**	**0.027**
Rabies immunity: No antibodies detected	**0.23**	**0.64**	**0.06-0.78**	**0.022**

Table S52. Odds ratios and p-values for variables included in the final multivariable logistic regression model for probability of being captured across all rounds for all dogs. Statistically significant p-values ($p < 0.05$) are highlighted in bold.

Variable	Odds_Ratio	Standard_error	95% confidence intervals	p_value
(Intercept)	**18.06**	**0.46**	**7.47-45.89**	**< 0.001**
Earnotched: Sterilised	**1.77**	**0.17**	**1.26-2.48**	**0.001**
Coat: Fair	0.85	0.19	0.59-1.24	0.403
Coat: Good	1.01	0.19	0.7-1.47	0.948
Coat: Very good	0.91	0.40	0.42-2.02	0.809
Ownership2: Semi-owned	1.18	0.35	0.6-2.39	0.631
Ownership2: Unowned	**0.25**	**0.31**	**0.13-0.45**	**< 0.001**
Vaccination history2: Unknown	**0.30**	**0.34**	**0.16-0.58**	**< 0.001**
Vaccination history2: Vaccinated	1.00	0.33	0.53-1.91	0.988
ABC: Yes	**0.45**	**0.19**	**0.31-0.64**	**< 0.001**
Site: Muhamma	**0.40**	**0.20**	**0.27-0.59**	**< 0.001**
Study collar applied: Yes - black	**1.70**	**0.18**	**1.19-2.44**	**0.004**
Study collar applied: Yes - orange	1.32	0.19	0.92-1.91	0.135
Rabies immunity: ≤ 0.5 IU/ml	**0.31**	**0.39**	**0.14-0.65**	**0.003**
Rabies immunity: No antibodies detected	**0.29**	**0.38**	**0.13-0.59**	**0.001**

Table S53. Odds ratios and p-values for variables included in the final multivariable logistic regression model for probability of being captured across all rounds for unowned dogs. Statistically significant p-values (p < 0.05) are highlighted in bold.

Variable	Odds_Ratio	Standard_error	95% confidence intervals	p_value
(Intercept)	2.20	0.40	1.03-5.11	0.050
Age: Juvenile (5-12 months)	0.69	0.26	0.41-1.14	0.162
Age: Pup (0-4 months)	**0.37**	**0.46**	**0.14-0.86**	**0.031**
Earnotched: Sterilised	**1.72**	**0.18**	**1.21-2.44**	**0.003**
ABC: Yes	**0.40**	**0.19**	**0.27-0.57**	**< 0.001**
Site: Muhamma	**0.40**	**0.25**	**0.24-0.64**	**< 0.001**
Rabies immunity: ≤ 0.5 IU/ml	**0.26**	**0.42**	**0.11-0.57**	**0.001**
Rabies immunity: No antibodies detected	**0.26**	**0.41**	**0.11-0.56**	**0.001**

Table S54. Odds ratios and p-values for variables included in the final multivariable logistic regression model for probability of being captured across all rounds for owned dogs. Statistically significant p-values (p < 0.05) are highlighted in bold.

Variable	Odds_Ratio	Standard_error	95% confidence intervals	p_value
(Intercept)	**6.08**	**0.20**	**4.18-9.16**	**< 0.001**
Vaccination history2: Unknown	**0.28**	**0.36**	**0.14-0.57**	**< 0.001**
Vaccination history2: Vaccinated	0.87	0.32	0.47-1.67	0.674
Site: Muhamma	0.45	0.44	0.19-1.11	0.074

Table S55. Odds ratios and p-values for variables included in the final multivariable logistic regression model for probability of being captured across all rounds for unowned and semi-owned dogs. Statistically significant p-values (p < 0.05) are highlighted in bold.

Variable	Odds_Ratio	Standard_error	95% confidence intervals	p_value
(Intercept)	**6.26**	**0.48**	**2.57-17.15**	**< 0.001**
Earnotched: Sterilised	**1.85**	**0.21**	**1.23-2.81**	**0.003**
Ownership2: Unowned	**0.51**	**0.25**	**0.31-0.81**	**0.006**
HIS2: No direct human interaction	0.77	0.17	0.56-1.07	0.123
ABC: Yes	**0.65**	**0.22**	**0.42-1**	**0.049**
Site: Muhamma	**0.51**	**0.26**	**0.3-0.86**	**0.012**
Rabies immunity: ≤ 0.5 IU/ml	0.48	0.46	0.18-1.13	0.109
Rabies immunity: No antibodies detected	0.44	0.44	0.17-0.99	0.062

Table S56. Odds ratios and p-values for variables included in the final multivariable mixed effects logistic regression model (with each unique dog identifier as the random effect) for probability of being captured across all rounds for all dogs. Statistically significant p-values ($p < 0.05$) are highlighted in bold.

Variable	Odds Ratio	Standard error	95% confidence intervals	p value
(Intercept)	**68.73**	**47.38**	**17.79-265.42**	**<0.001**
Coat: Fair	0.89	0.25	0.51-1.55	0.681
Coat: Good	1.11	0.32	0.64-1.95	0.702
Coat: Very good	0.80	0.46	0.25-2.5	0.698
Earnotched: Sterilised	**2.06**	**0.55**	**1.23-3.47**	**0.006**
Vaccination history2: Unknown	**0.18**	**0.09**	**0.07-0.49**	**0.001**
Vaccination history2: Vaccinated	1.04	0.46	0.43-2.48	0.937
ABC: Yes	**0.33**	**0.09**	**0.19-0.56**	**<0.001**
Sampling occasion: Third	**0.30**	**0.05**	**0.22-0.42**	**<0.001**
Sampling occasion: Fourth	**0.32**	**0.05**	**0.23-0.45**	**<0.001**
Study collar applied: Yes - black	**2.08**	**0.56**	**1.23-3.52**	**0.006**
Study collar applied: Yes - orange	1.52	0.41	0.89-2.59	0.124
Rabies immunity: ≤ 0.5IU/ml	**0.27**	**0.15**	**0.09-0.82**	**0.021**
Rabies immunity: No antibodies detected	**0.25**	**0.14**	**0.08-0.73**	**0.011**
Site: Muhamma	**0.30**	**0.09**	**0.17-0.53**	**<0.001**
Ownership2: Semi-owned	1.60	0.85	0.56-4.53	0.380
Ownership2: Unowned	**0.18**	**0.09**	**0.07-0.46**	**<0.001**

Table S57. Odds ratios and p-values for variables included in the final multivariable mixed effects logistic regression model (with each unique dog identifier as the random effect) for probability of being captured across all rounds for unowned dogs. Statistically significant p-values ($p < 0.05$) are highlighted in bold.

Variable	Odds Ratio	Standard error	95% confidence intervals	p value
(Intercept)	1.11	0.20	0.79-1.57	0.549
Age2: Juvenile (5-12 months)	0.59	0.24	0.26-1.33	0.202
Age2: Pup (0-4 months)	**0.20**	**0.13**	**0.06-0.73**	**0.015**
Earnotched: Sterilised	**2.12**	**0.62**	**1.2-3.77**	**0.010**
ABC: Yes	**0.27**	**0.08**	**0.15-0.48**	**<0.001**
Sampling occasion: Third	**0.27**	**0.05**	**0.19-0.4**	**<0.001**
Sampling occasion: Fourth	**0.28**	**0.06**	**0.19-0.42**	**<0.001**
Site: Muhamma	**0.26**	**0.10**	**0.12-0.54**	**<0.001**

Table S58. Odds ratios and p-values for variables included in the final multivariable mixed effects logistic regression model (with each unique dog identifier as the random effect) for probability of being captured across all rounds for owned dogs. Statistically significant p-values ($p < 0.05$) are highlighted in bold.

Variable	Odds Ratio	Standard error	95% confidence intervals	p value
(Intercept)	**15.16**	**6.65**	**6.41-35.81**	**<0.001**
Vaccination history2: Unknown	**0.20**	**0.11**	**0.07-0.59**	**0.003**
Vaccination history2: Vaccinated	0.81	0.35	0.35-1.87	0.619
Sampling occasion: Third	**0.37**	**0.14**	**0.18-0.78**	**0.009**
Sampling occasion: Fourth	0.71	0.28	0.33-1.54	0.388
Site: Muhamma	0.38	0.25	0.11-1.36	0.138

Table S69. Odds ratios and p-values for variables included in the final multivariable mixed effects logistic regression model (with each unique dog identifier as the random effect) for probability of being captured across all rounds for semi-owned dogs. Statistically significant p-values ($p < 0.05$) are highlighted in bold.

Variable	Odds Ratio	Standard error	95% confidence intervals	p value
(Intercept)	**17.13**	**15.70**	**2.84-103.21**	**0.002**
Sampling occasion: Third	**0.18**	**0.14**	**0.04-0.85**	**0.031**
Sampling occasion: Fourth	**0.06**	**0.05**	**0.01-0.33**	**0.001**
Human association: 5	0.90	0.65	0.22-3.69	0.881
Human association: 6	6.61	7.81	0.65-67.02	0.110

Table S60. Odds ratios and p-values for variables included in the final multivariable mixed effects logistic regression model (with each unique dog identifier as the random effect) for probability of being captured across all rounds for unowned and semi-owned dogs (all free-ranging dogs without owners). Statistically significant p-values ($p < 0.05$) are highlighted in bold.

Variable	Odds Ratio	Standard error	95% confidence intervals	p value
(Intercept)	**48.29**	**38.64**	**10.06-231.71**	**<0.001**
Age2: Juvenile (5-12 months)	0.60	0.29	0.23-1.55	0.292
Age2: Pup (0-4 months)	0.41	0.29	0.1-1.64	0.209
Earnotched: Sterilised	**2.56**	**0.74**	**1.45-4.51**	**0.001**
ABC: Yes	**0.32**	**0.09**	**0.19-0.56**	**<0.001**
Sampling occasion: Third	**0.30**	**0.06**	**0.21-0.43**	**<0.001**
Sampling occasion: Fourth	**0.27**	**0.05**	**0.19-0.4**	**<0.001**
Study collar applied: Yes - black	1.32	0.57	0.57-3.06	0.516
Study collar applied: Yes - orange	0.87	0.38	0.37-2.04	0.754
Rabies immunity: ≤ 0.5IU/ml	**0.20**	**0.13**	**0.06-0.69**	**0.011**
Rabies immunity: No antibodies detected	**0.20**	**0.12**	**0.06-0.66**	**0.008**
Site: Muhamma	**0.31**	**0.10**	**0.16-0.6**	**<0.001**
Ownership2: Unowned	**0.10**	**0.04**	**0.05-0.2**	**<0.001**

Table S61. Details of variables used as predictors in logistic / mixed effects logistic regression models. In brackets, the reference level and remaining levels.

Variable	Variable description
Sex	Sex (Female; Male)
Age2	Age2: (Adult (incl. Aged); Juvenile, pup)
BCS	Body condition score (BCS = 2; 3 - 8)
BCS2	BCS2 (Under ideal (2-3); Ideal (4-5), Over ideal (6-8))
Earnotched	Sterilisation status (Not sterilised; Sterilised)
Breed2	Breed (Non-descript; Crossbreed, Purebreed)
Coat	Coat condition (Poor; Fair, Good, Very good)
Ownership2	Ownership status (Owned; semi-owned, Unowned)*
Confinement2	Confinement status (Completely confined; Completely/partially free-ranging)
Human interaction	Human interaction score (HIS = 2; 3 - 6)
HIS2	HIS2 (Direct human interaction (4-6); No direct human interaction (1-3))
Dogs activity	Dogs activity at the time of capture (Trying to escape from dog catcher; sleeping, resting, feeding, interacting with local people, interacting with project staff, other normal behaviour, not applicable – owned dog
Dogs activity2	Dogs activity2 (Trying to escape; Normal behaviour, Interacting with people, Owned dog)
Repr status	Reproductive status (Pregnant; Lactating, whelp)
Repr status2	Reproductive status (Active; Not active)
Vaccination history	Vaccination history (Never vaccinated; Regular vaccination, unknown, vaccinated in the past)
Vaccination history2	Vaccination history2 (Never vaccinated; Unknown, Vaccinated)
ABC	If captured as part of on-going ABC (Yes; No)
Site	Study Site: (Alappuzha; Muhamma)
Study collar applied	If study collar applied or not (Not collared; Yes (black - female), Yes (orange - male))
Study collar applied2	If study collar applied or not (Collared; Not collared)
RFFIT titre	RFFIT titre when first captured (Less than 0.11; 0.11, Less than 0.23, 0.23, 0.46, 0.93, 1.87, Not applicable)
Titre level	Titre level (if antibodies detected or not) (Antibodies detected; No antibodies detected)
Rabies immunity	Rabies immunity (if titre level above or below 0.5) (Greater than 0.5 IU/ml; No antibodies detected, Less than/equal to 0.5 IU/ml)
Sampling occasion	Round of study during which recaptured (used in mixed effects models only) (Second; Third, Fourth)

*When running analyses with data for unowned and semi-owned dogs combined, the ownership category ‘semi-owned dogs’ was used as the reference level.

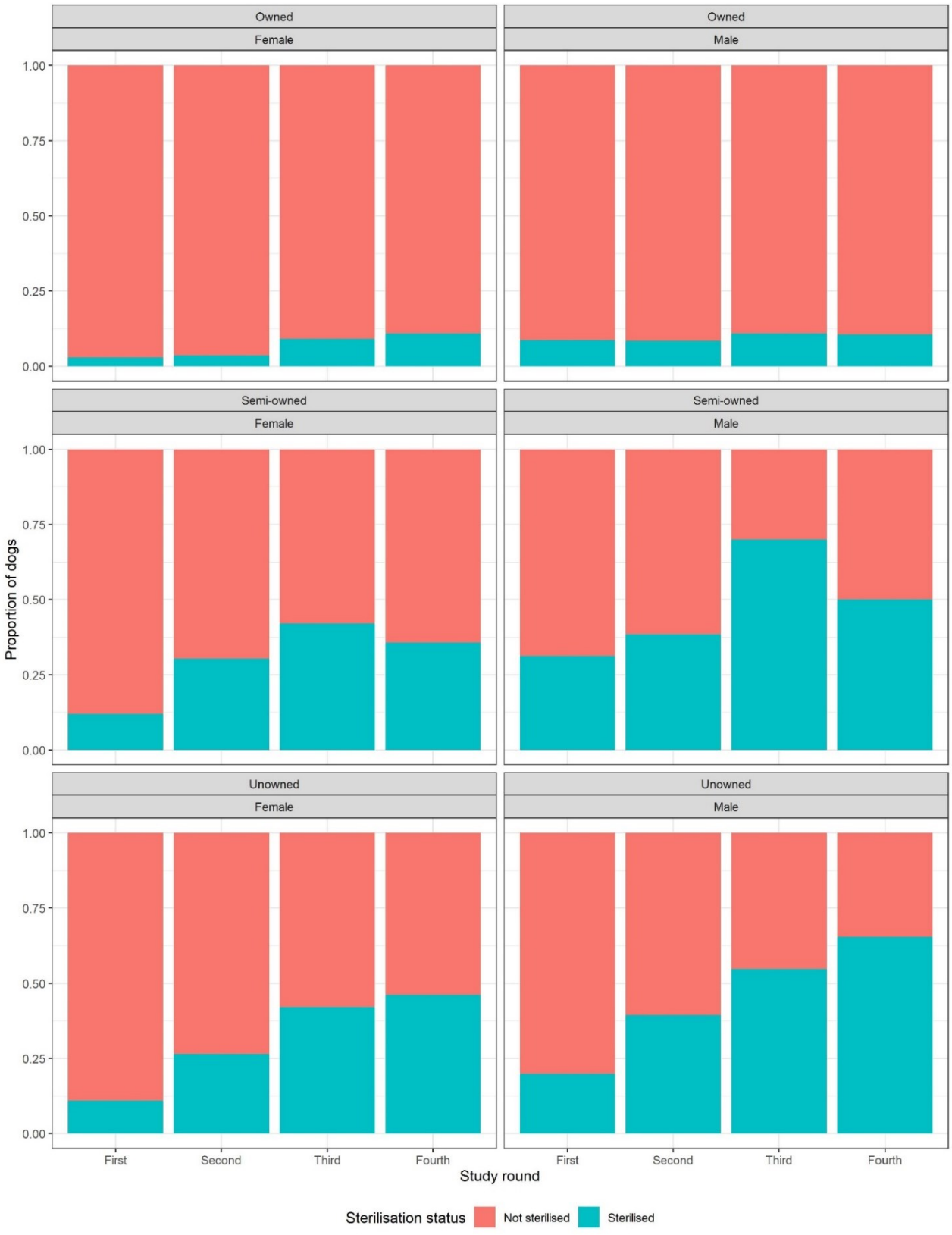


Fig S1. Proportion of female and male dogs of each ownership category captured in each study round at both sites based on their sterilisation status. The proportion of sterilised unowned dogs captured increased in each round, for both sexes (bottom panel).

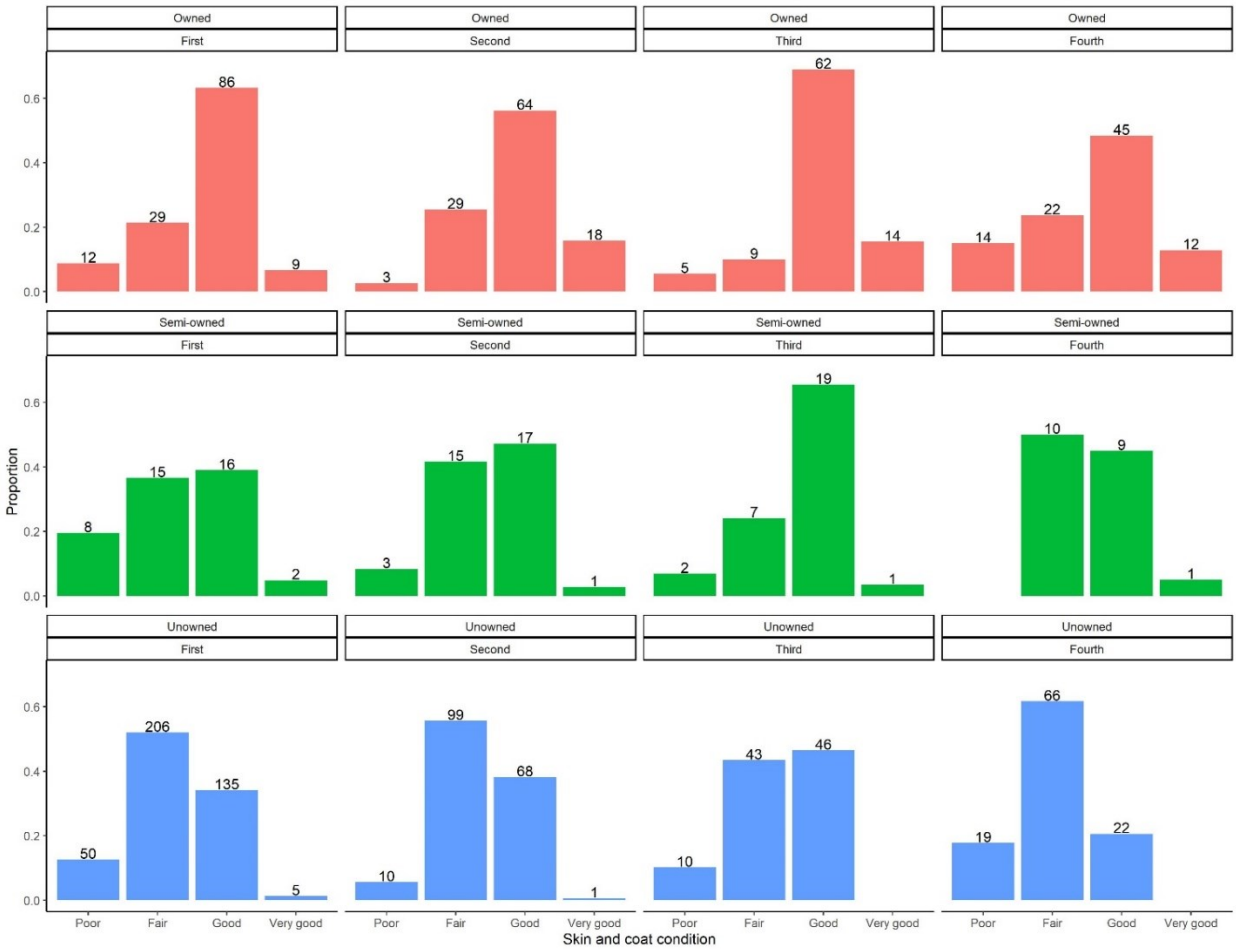


Fig S2. Distribution of skin and coat condition of unowned dogs captured in all study rounds at both study sites

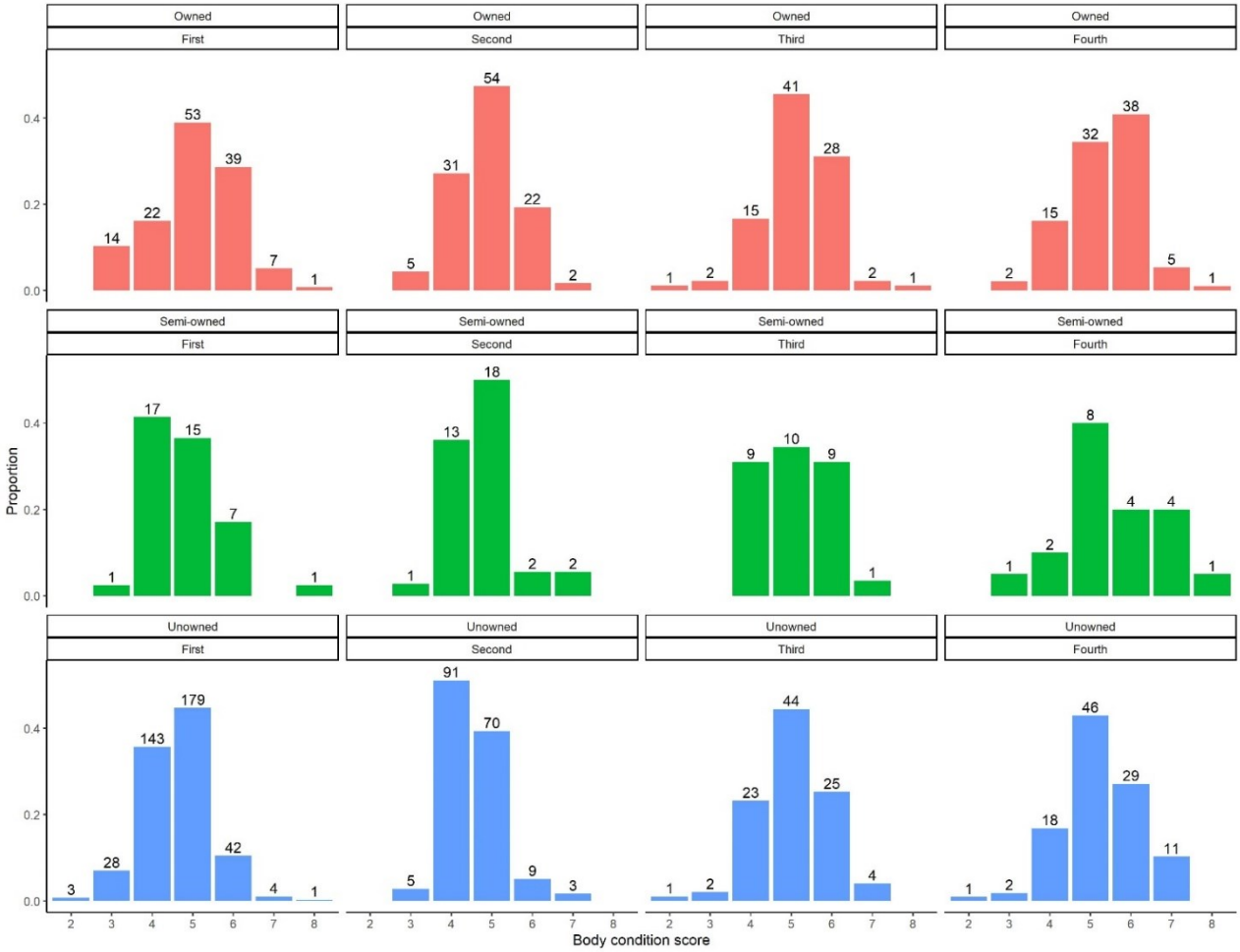


Fig S3. Distribution of body condition scores of unowned dogs captured in all study rounds at both study sites

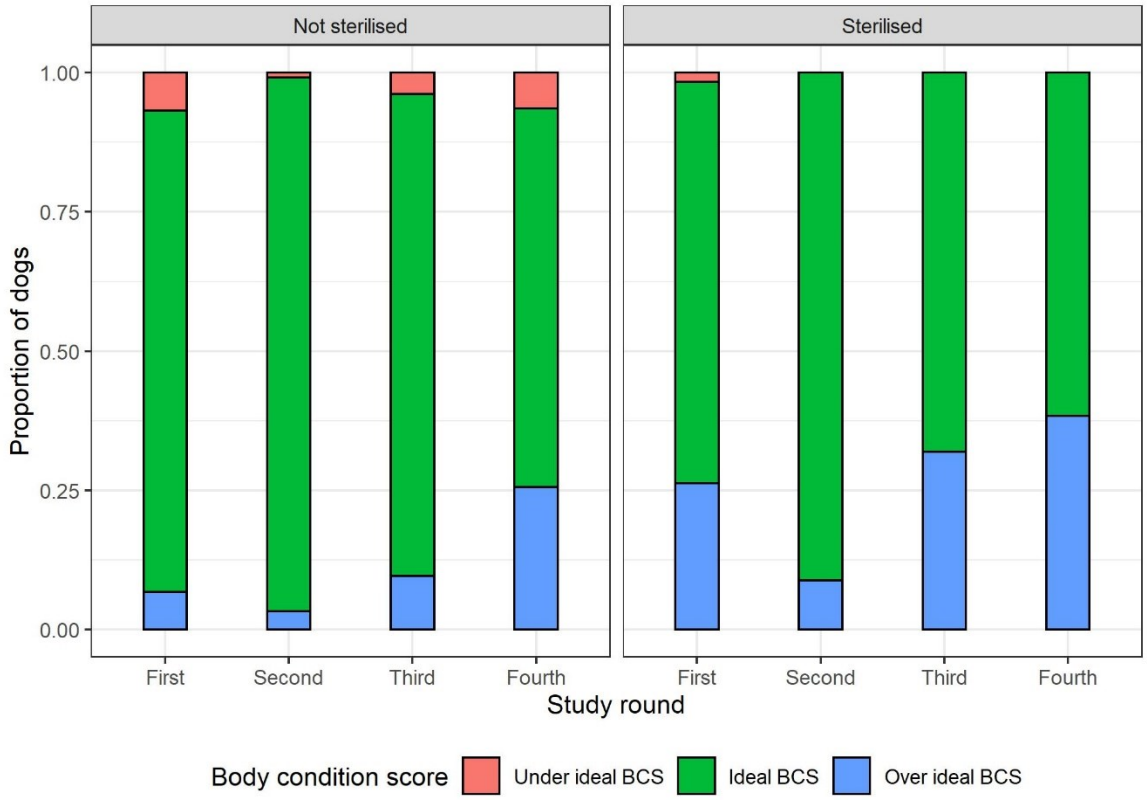


Fig S4. Proportion of unowned dogs captured in each study round across both study sites, with a body condition score (BCS) that was under ideal (BCS of 1-3), ideal (4-5) or over ideal (6-9), based on whether they were sterilised or not. The proportion of dogs with an over ideal BCS increased in the third and fourth rounds for both non-sterilised and sterilised dogs.

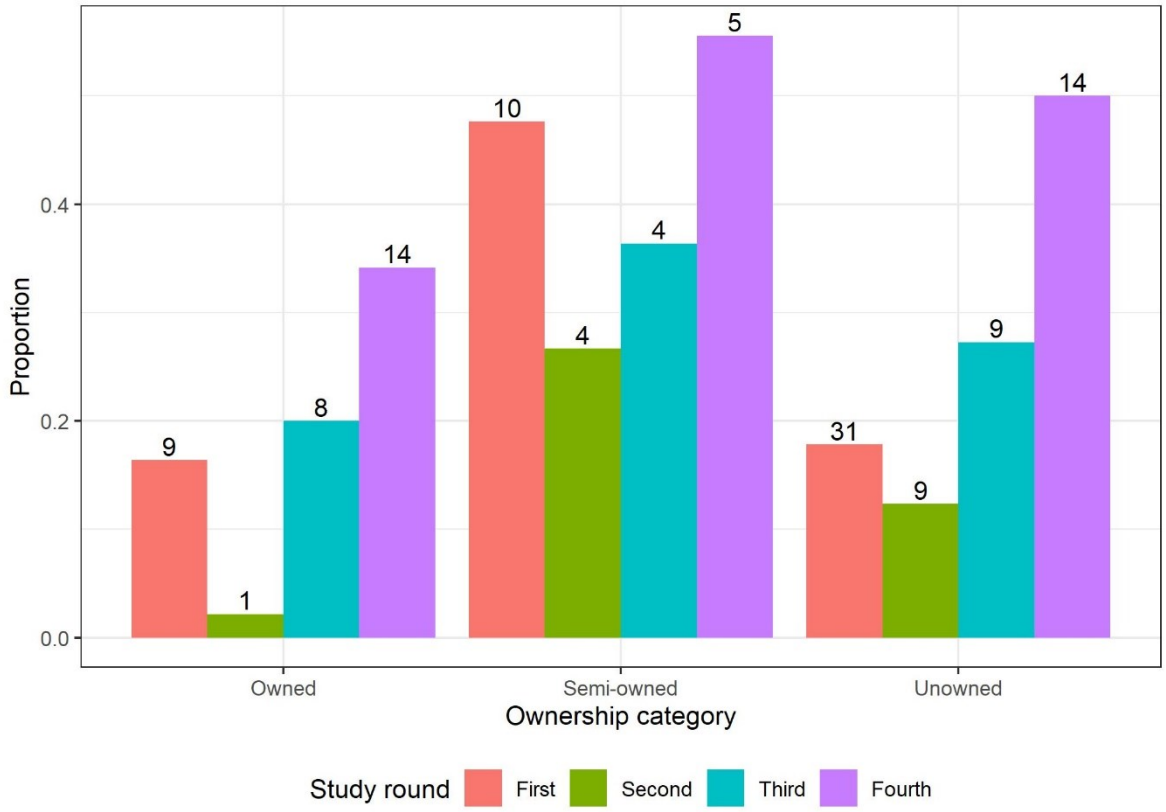


Fig S5. Proportion (and actual number of dogs represented above each bar) of owned, semi-owned and unowned dogs captured in all study rounds across both sites, which were reproductively active (lactating, pregnant or recently given birth).

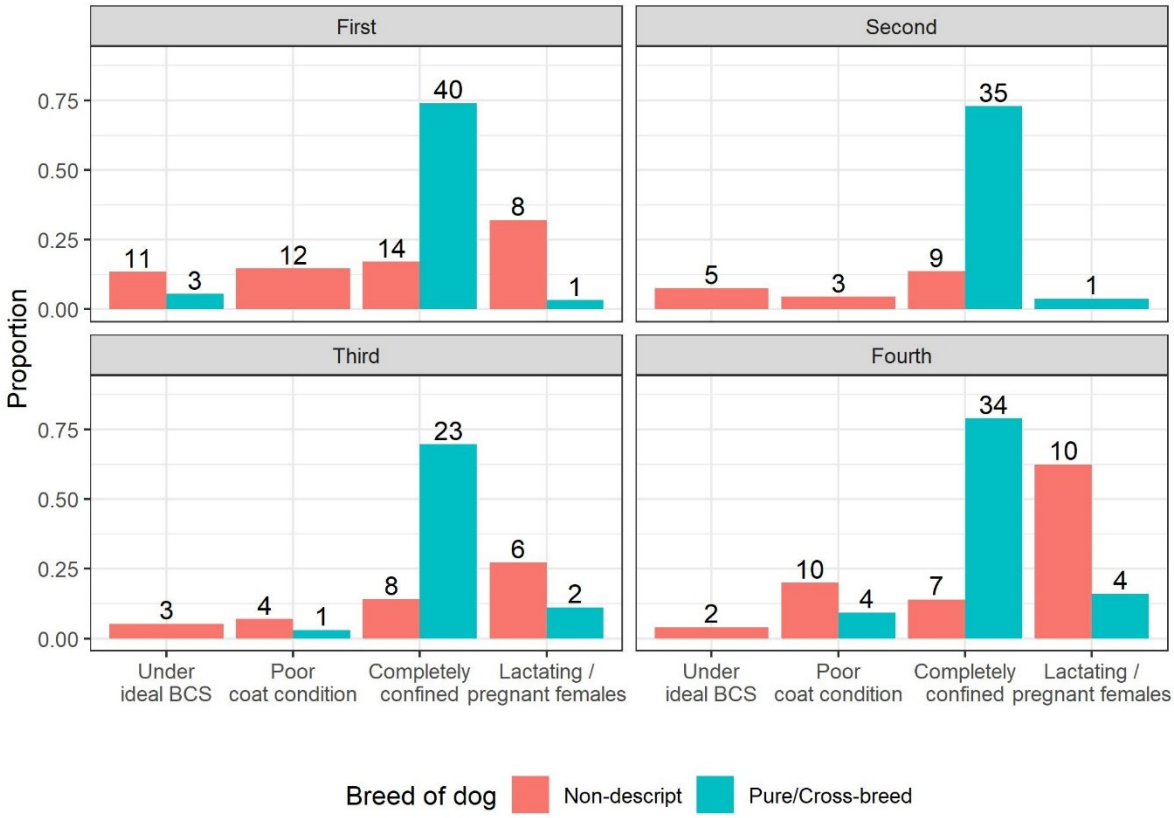


Fig S6. Breed differences in rearing of owned dogs. Differences between non-descript and pure/cross-breed owned dogs in all study rounds, in the proportions that had under ideal body condition scores (BCS), poor coat condition, were completely confined and the proportions of non-sterilised juvenile and adult females that were pregnant or lactating.

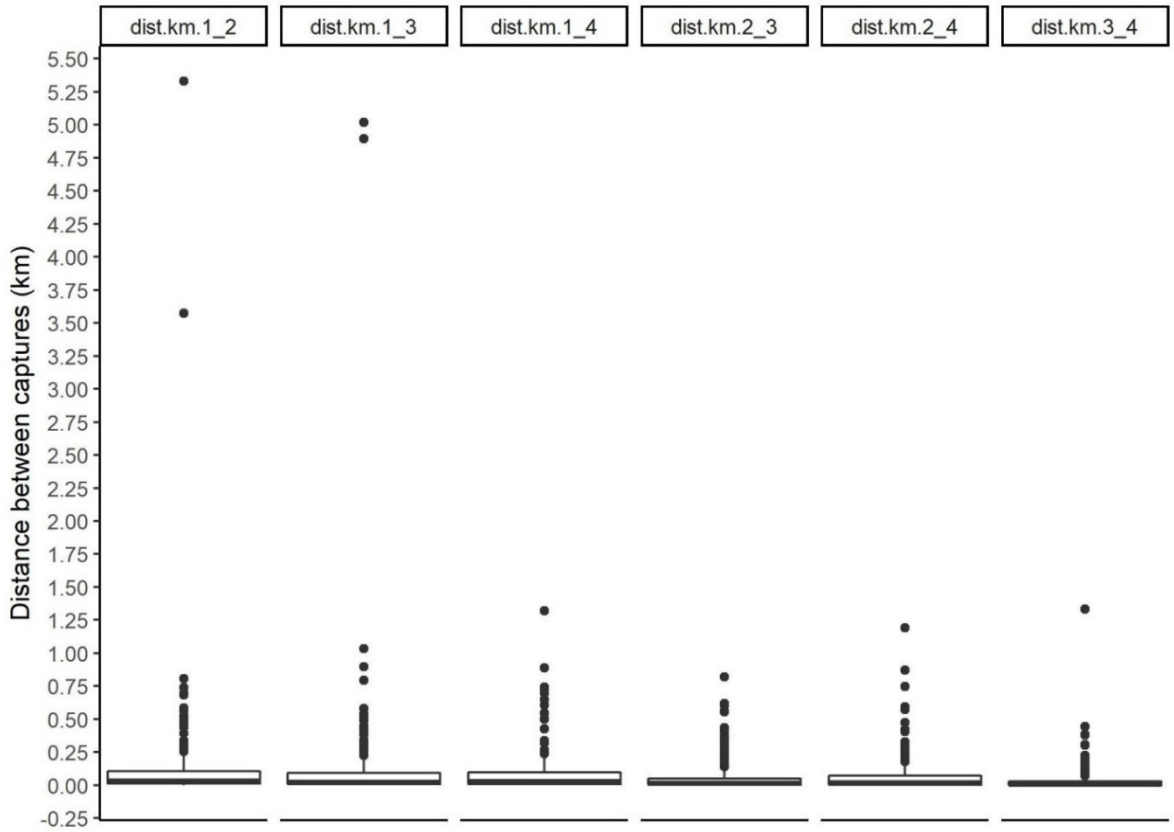


Fig S7. Boxplot of pairwise distances between recaptures for partially or completely free-ranging dogs, including owned dogs, at both study sites. For example, dist.km.1_2 refers to distance (in kilometres) between capture locations in rounds 1 and 2.

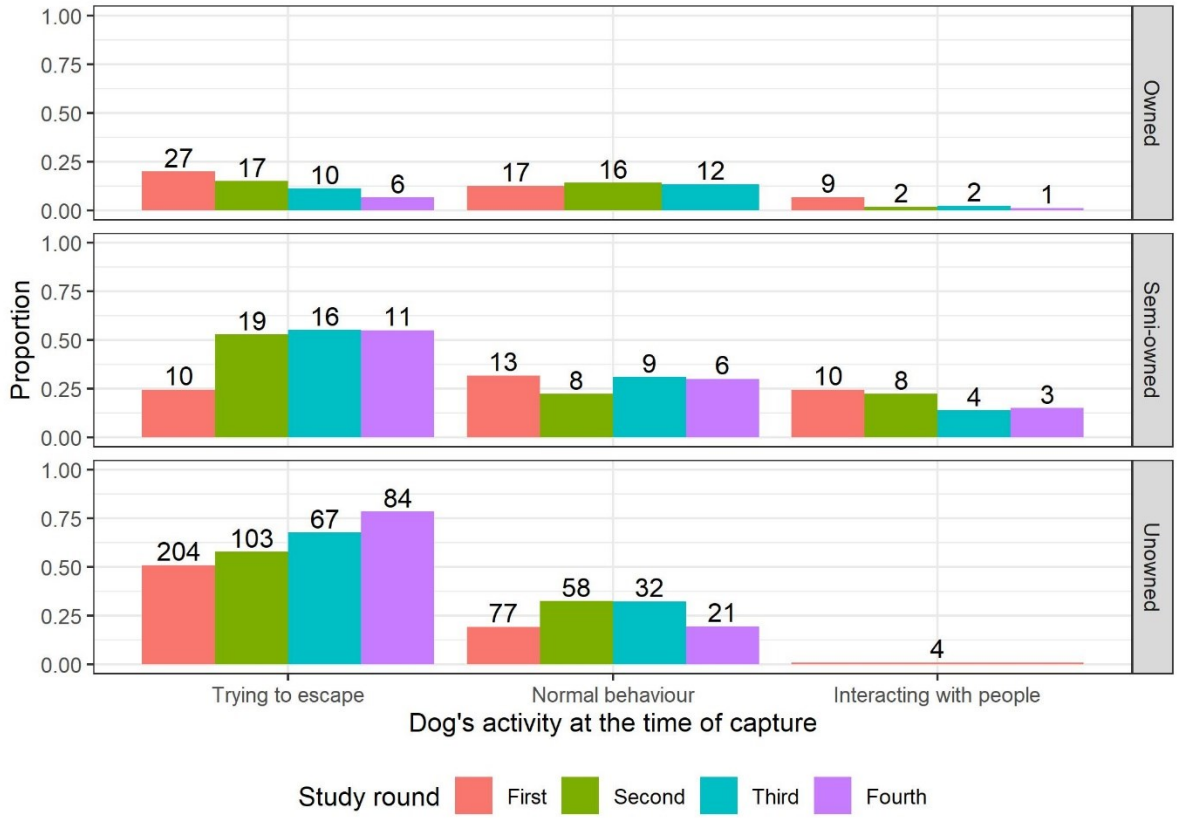


Fig S8. Proportion (and number of dogs represented above each bar) of owned, semi-owned and unowned dogs captured in all study rounds across both sites, based on their activity at the time of capture

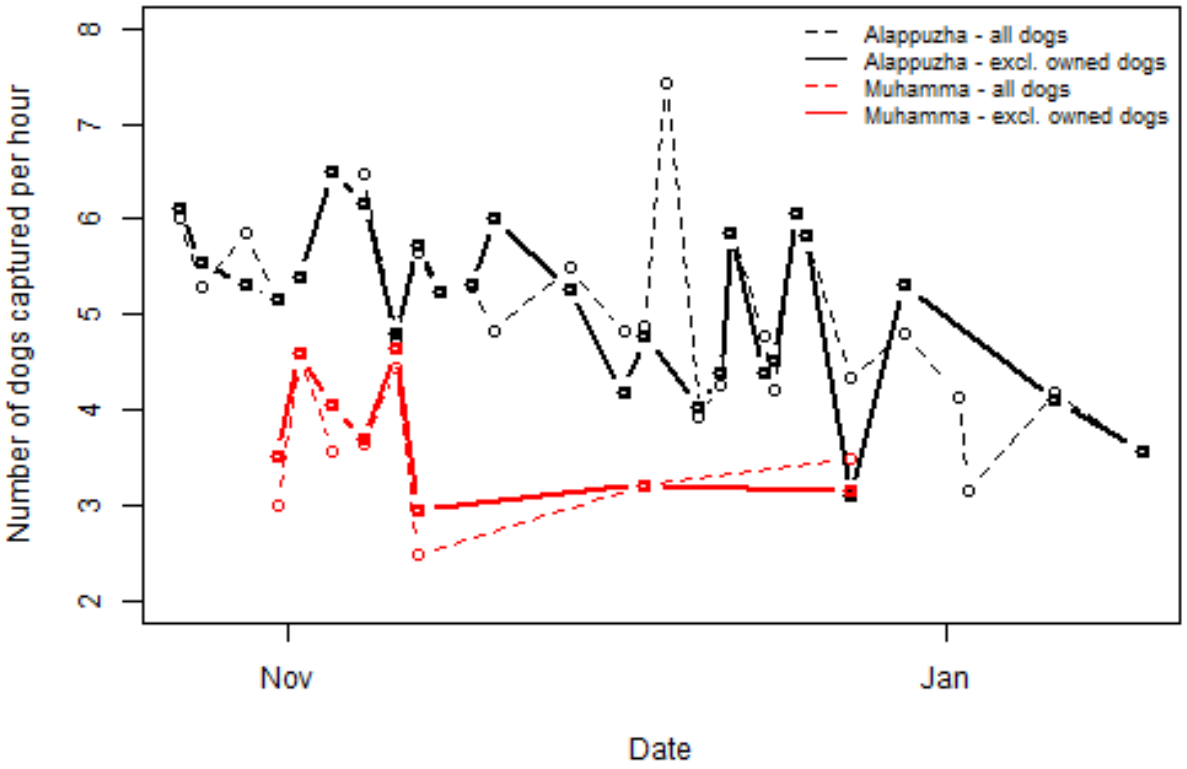


Fig. S9. Number of dogs captured per hour during rounds 1 and 2 in Alappuzha and Muhamma

Appendix A2: Supplementary information for chapter 4

All information presented here relates to chapter 4: Pre- and post- vaccination rabies virus neutralizing antibody dynamics in free-ranging and owned dogs in Kerala, south India

Table S1. Detection of pre-vaccination rabies virus neutralizing antibodies (RVNA) in unowned dogs captured in round 1 at both study sites. Figures in brackets represent percentages of dogs with detectable/non-detectable RVNA titres.

Site	Antibodies detected	No antibodies detected	Total
Alappuzha	101 (29)	242 (71)	343 (100)
Muhamma	11 (26)	32 (74)	43 (100)
Total	112 (29)	274 (71)	386 (100)

Table S2. Detection of pre-vaccination rabies virus neutralizing antibodies (RVNA) in unowned dogs captured in round 1 at both study sites, based on their sterilisation status. Figures in brackets represent percentages of dogs with detectable/non-detectable RVNA titres.

Earnotched	Antibodies detected	No antibodies detected	Total
Not sterilised	86 (26)	242 (74)	328 (100)
Sterilised	26 (45)	32 (55)	58 (100)
Total	112 (29)	274 (71)	386 (100)

Table S3. Number and percentages (in brackets) of owned dogs captured in each study round, based on their vaccination history and day-zero (pre-vaccination) rabies virus neutralizing antibody (RVNA) titres

Vaccination history	< 0.23	0.23	0.46	0.93	Total
Never vaccinated	53 (73)	12 (16)	7 (10)	1 (1)	73 (100)
Unknown	9 (56)	7 (44)	0 (0)	0 (0)	16 (100)
Vaccinated	24 (62)	5 (13)	9 (23)	1 (3)	39 (100)
Total	86 (67)	24 (19)	16 (12)	2 (2)	128 (100)

Table S4. Correlation between extent of hemolysis and log10 of RVNA titres serum samples collected in all rounds

Study round	Correlation coefficient	95% confidence intervals	p-value
Round 1	-0.04	-0.13, 0.4	0.31
Round 2	0.06	-0.05, 0.16	0.31
Round 3	-0.01	-0.14, 0.12	0.88
Round 4	-0.05	-0.19, 0.08	0.42
All rounds	0.12	0.06, 0.17	< 0.001

Table S5. Correlation between extent of turbidity and log10 of RVNA titres in serum samples collected in all rounds

Study round	Correlation coefficient	95% confidence intervals	p-value
Round 1	-0.04	-0.13, 0.04	0.31
Round 2	0.13	0.02, 0.24	0.02
Round 3	-0.03	-0.16, 0.11	0.69
Round 4	-0.01	-0.14, 0.12	0.89
All rounds	0.14	0.09, 0.19	< 0.001

Table S6. Correlation between extent of hemolysis and turbidity in serum samples collected in all rounds

Study round	Correlation coefficient	95% confidence intervals	p-value
Round 1	0.11	0.02,0.19	0.01
Round 2	0.065	-0.04, 0.17	0.24
Round 3	0.24	0.11, 0.36	< 0.001
Round 4	0.19	0.06, 0.32	0.003
All rounds	0.16	0.11, 0.21	< 0.001

Table S7. Summary of the number (and percentage) of serum samples collected in each round by the extent of hemolysis present

Hemolysis present	Hemolysis score	First	Second	Third	Fourth	Total
No	1	358 (67)	170 (52)	116 (53)	98 (45)	742 (57)
Mildest	2	19 (4)	34 (10)	17 (8)	21 (10)	91 (7)
Mild	3	81 (15)	51 (16)	30 (14)	43 (20)	205 (16)
Mild - Moderate	4	14 (3)	14 (4)	10 (5)	12 (5)	50 (4)
Moderate	5	45 (8)	29 (9)	27 (12)	28 (13)	129 (10)
Moderate - Marked	6	2 (0)	4 (1)	10 (5)	11 (5)	27 (2)
Marked	7	8 (1)	17 (5)	5 (2)	4 (2)	34 (3)
Marked - Severe	8	5 (1)	2 (1)	0 (0)	0 (0)	7 (1)
Severe	9	2 (0)	5 (2)	2 (1)	1 (0)	10 (1)
Completely hemolysed, blackish red	10	0 (0)	2 (1)	0 (0)	2 (1)	4 (0)
Total		534 (100)	328 (100)	217 (100)	220 (100)	1299 (100)

Table S8. Summary of the number (and percentage) of serum samples collected in each round by the extent of turbidity present

Turbidity present	Turbidity score	First	Second	Third	Fourth	Total
No	1	427 (80)	208 (63)	130 (60)	120 (55)	885 (68)
Mildest	2	16 (3)	31 (9)	24 (11)	14 (6)	85 (7)
Mild	3	48 (9)	44 (13)	25 (12)	35 (16)	152 (12)
Mild - Moderate	4	9 (2)	3 (1)	3 (1)	5 (2)	20 (2)
Moderate	5	19 (4)	25 (8)	16 (7)	23 (10)	83 (6)
Moderate - Marked	6	0 (0)	2 (1)	3 (1)	7 (3)	12 (1)
Marked	7	8 (1)	8 (2)	9 (4)	11 (5)	36 (3)
Marked - Severe	8	1 (0)	0 (0)	0 (0)	0 (0)	1 (0)
Severe	9	5 (1)	4 (1)	6 (3)	3 (1)	18 (1)
Milky	10	1 (0)	3 (1)	1 (0)	2 (1)	7 (1)
Total		534 (100)	328 (100)	217 (100)	220 (100)	1299 (100)

Table S9. Summary of the number (and percentage) of serum samples collected from dogs of different ownership categories by the extent of hemolysis present

Hemolysis present	Hemolysis score	Owned	Semi-owned	Unowned	Total
No	1	223 (53)	71 (58)	448 (59)	742 (57)
Mildest	2	33 (8)	17 (14)	41 (5)	91 (7)
Mild	3	61 (15)	18 (15)	126 (17)	205 (16)
Mild - Moderate	4	21 (5)	4 (3)	25 (3)	50 (4)
Moderate	5	52 (12)	6 (5)	70 (9)	128 (10)
Moderate - Marked	6	9 (2)	5 (4)	13 (2)	27 (2)
Marked	7	14 (3)	2 (2)	18 (2)	34 (3)
Marked - Severe	8	1 (0)	0 (0)	6 (1)	7 (1)
Severe	9	6 (1)	0 (0)	4 (1)	10 (1)
Completely hemolysed, blackish red	10	0 (0)	0 (0)	4 (1)	4 (0)
Total		420 (100)	123 (100)	755 (100)	1298 (100)

Table S10. Summary of the number (and percentage) of serum samples collected from dogs of different ownership categories by the extent of turbidity present

Turbidity present	Turbidity score	Owned	Semi-owned	Unowned	Total
No	1	280 (67)	73 (59)	532 (70)	885 (68)
Mildest	2	33 (8)	8 (7)	44 (6)	85 (7)
Mild	3	63 (15)	20 (16)	68 (9)	151 (12)
Mild - Moderate	4	7 (2)	1 (1)	12 (2)	20 (2)
Moderate	5	21 (5)	15 (12)	47 (6)	83 (6)
Moderate - Marked	6	2 (0)	2 (2)	8 (1)	12 (1)
Marked	7	4 (1)	4 (3)	28 (4)	36 (3)
Marked - Severe	8	0 (0)	0 (0)	1 (0)	1 (0)
Severe	9	8 (2)	0 (0)	10 (1)	18 (1)
Milky	10	2 (0)	0 (0)	5 (1)	7 (1)
Total		420 (100)	123 (100)	755 (100)	1298 (100)

Table S11. Summary of the number (and percentage) of serum samples collected from dogs of different confinement categories by the extent of turbidity present

Turbidity present	Turbidity score	Completely/partially confined	Free-ranging	Total
No	1	147 (62)	738 (70)	885 (68)
Mildest	2	19 (8)	66 (6)	85 (7)
Mild	3	41 (17)	110 (10)	151 (12)
Mild - Moderate	4	5 (2)	15 (1)	20 (2)
Moderate	5	14 (6)	69 (7)	83 (6)
Moderate - Marked	6	1 (0)	11 (1)	12 (1)
Marked	7	4 (2)	32 (3)	36 (3)
Marked - Severe	8	0 (0)	1 (0)	1 (0)
Severe	9	5 (2)	13 (1)	18 (1)
Milky	10	2 (1)	5 (0)	7 (1)
Total		238 (100)	1060 (100)	1298 (100)

Table S12. Summary of the number (and percentage) of serum samples collected from dogs of different confinement categories by the extent of haemolysis present

Hemolysis present	Hemolysis score	Completely/partially confined	Free-ranging	Total
No	1	100 (42)	642 (61)	742 (57)
Mildest	2	25 (11)	66 (6)	91 (7)
Mild	3	34 (14)	171 (16)	205 (16)
Mild - Moderate	4	13 (5)	37 (3)	50 (4)
Moderate	5	41 (17)	87 (8)	128 (10)
Moderate - Marked	6	7 (3)	20 (2)	27 (2)
Marked	7	11 (5)	23 (2)	34 (3)
Marked - Severe	8	1 (0)	6 (1)	7 (1)
Severe	9	6 (3)	4 (0)	10 (1)
Completely hemolysed, blackish red	10	0 (0)	4 (0)	4 (0)
Total		238 (100)	1060 (100)	1298 (100)

Table S13. Summary of the number (and percentage) of serum samples collected from dogs of different broad breed categories by the extent of haemolysis present

Hemolysis present	Hemolysis score	Crossbreed	Non-descript	Purebreed	Total
No	1	34 (53)	652 (59)	56 (42)	742 (57)
Mildest	2	6 (9)	68 (6)	17 (13)	91 (7)
Mild	3	8 (12)	181 (16)	16 (12)	205 (16)
Mild - Moderate	4	4 (6)	40 (4)	6 (5)	50 (4)
Moderate	5	9 (14)	100 (9)	19 (14)	128 (10)
Moderate - Marked	6	0 (0)	23 (2)	4 (3)	27 (2)
Marked	7	2 (3)	23 (2)	9 (7)	34 (3)
Marked - Severe	8	0 (0)	6 (1)	1 (1)	7 (1)
Severe	9	1 (2)	4 (0)	5 (4)	10 (1)
Completely hemolysed, blackish red	10	0 (0)	4 (0)	0 (0)	4 (0)
Total		64 (100)	1101 (100)	133 (100)	1298 (100)

Table S14. Summary of the number (and percentage) of serum samples collected from OD across all study rounds by the extent of hemolysis present by breed

Hemolysis present	Hemolysis score	Crossbreed	Non-descript	Purebreed	Total
No	1	18 (43)	150 (61)	55 (42)	223 (53)
Mildest	2	4 (10)	12 (5)	17 (13)	33 (8)
Mild	3	6 (14)	39 (16)	16 (12)	61 (15)
Mild - Moderate	4	4 (10)	11 (4)	6 (5)	21 (5)
Moderate	5	7 (17)	26 (11)	19 (14)	52 (12)
Moderate - Marked	6	0 (0)	5 (2)	4 (3)	9 (2)
Marked	7	2 (5)	3 (1)	9 (7)	14 (3)
Marked - Severe	8	0 (0)	0 (0)	1 (1)	1 (0)
Severe	9	1 (2)	0 (0)	5 (4)	6 (1)
Total		42 (100)	246 (100)	132 (100)	420 (100)

Table S15. Details of serum samples that were tested as blind replicates and titre results, showing the variation in titres for each sample

Sample number	RVNA titre - replicate 1	RVNA titre - replicate 2	RVNA titre - replicate 3	RVNA titre - replicate 4
1.	0.230	0.46	0.93	NA
2.	7.500	0.46	1.87	NA
3.	0.930	0.46	NA	NA
4.	0.930	0.46	1.87	NA
5.	1.870	0.46	1.87	NA
6.	1.870	0.93	1.87	NA
7.	0.460	0.23	NA	NA
8.	0.930	0.46	0.93	NA
9.	0.115	0.46	1.87	NA
10.	0.115	0.46	0.93	NA
11.	0.230	0.46	0.46	NA
12.	0.115	0.46	0.46	NA
13.	0.460	0.46	0.46	NA
14.	0.460	1.87	NA	NA
15.	1.870	0.93	NA	NA
16.	7.500	3.75	NA	NA
17.	0.930	1.87	NA	NA
18.	1.870	0.93	NA	NA
19.	0.930	1.87	NA	NA
20.	0.460	1.87	NA	NA
21.	0.930	1.87	NA	NA
22.	0.930	1.87	NA	NA
23.	1.870	1.87	NA	NA
24.	0.460	1.87	NA	NA
25.	0.930	1.87	NA	NA

Sample number	RVNA titre - replicate 1	RVNA titre - replicate 2	RVNA titre - replicate 3	RVNA titre - replicate 4
26.	0.930	0.93	NA	NA
27.	0.930	1.87	NA	NA
28.	3.750	1.87	NA	NA
29.	1.870	1.87	NA	NA
30.	0.930	0.93	NA	NA
31.	0.460	0.93	NA	NA
32.	0.930	0.46	NA	NA
33.	1.870	0.93	NA	NA
34.	3.750	1.87	NA	NA
35.	0.930	1.87	NA	NA
36.	1.870	0.93	NA	NA
37.	0.460	1.87	NA	NA
38.	1.870	3.75	NA	NA

Table S16. Number (and percentages) of dogs that had RVNA titres > 0.5 IU/ml in each study round, based on whether they were known to have been vaccinated before R1, after excluding dogs revaccinated after R1.

Study round	Vaccinated prior to R1	No known history of vaccination before R1
R2	85/86 (99%)	202/241 (84%)
R3	38/61 (62%)	45/130 (35%)
R4	59/68 (87%)	103/123 (84%)

Table S17. Summary of geometric mean titres (and 95% confidence intervals) of OD by study round and history of ever being vaccinated, after excluding dogs known to have been revaccinated after R1

Study round	No	Unknown	Yes
First	0.15 (0.13,0.17)	0.16 (0.13,0.2)	0.18 (0.15,0.22)
Second	1.51 (1.07,2.13)	4.05 (2.51,6.53)	3.06 (2.43,3.84)
Third	0.38 (0.29,0.49)	0.4 (0.15,1.1)	0.61 (0.41,0.93)
Fourth	1.43 (1.11,1.84)	1.32 (0.61,2.84)	1.52 (1.15,2.01)

Table S18. Summary of geometric mean titres (and 95% confidence intervals) of UD by study round and history of ever being vaccinated, after excluding dogs known to have been revaccinated after R1

Study round	Unknown	Yes
First	0.15 (0.15,0.16)	0.19 (0.16,0.22)
Second	1.64 (1.31,2.07)	3.46 (2.53,4.73)
Third	0.56 (0.44,0.7)	1.03 (0.73,1.47)
Fourth	1.6 (1.28,1.99)	2.17 (1.53,3.06)

Table S19. Summary of geometric mean titres (and 95% confidence intervals) of SOD by study round and history of ever being vaccinated, after excluding dogs known to have been revaccinated after R1

Study round	No	Unknown	Yes
First	0.11 (0.11,0.11)	0.15 (0.12,0.19)	0.14 (0.11,0.2)
Second	1.86 (0.18,19.79)	1.31 (0.67,2.56)	5.04 (2.46,10.34)
Third	0.74 (0.22,2.46)	0.49 (0.3,0.8)	1.57 (0.7,3.51)
Fourth	1.87 (1.87,1.87)	1.41 (0.79,2.53)	1.31 (0.56,3.07)

Table S20. Summary of geometric mean titres (and 95% confidence intervals) of UD and SOD by study round and history of ever being vaccinated, after excluding dogs known to have been revaccinated after R1

Study round	No	Unknown	Yes
First	0.11 (0.11,0.11)	0.15 (0.15,0.16)	0.18 (0.16,0.21)
Second	1.86 (0.18,19.79)	1.58 (1.27,1.97)	3.68 (2.76,4.9)
Third	0.74 (0.22,2.46)	0.54 (0.44,0.67)	1.14 (0.82,1.59)
Fourth	1.87 (1.87,1.87)	1.57 (1.28,1.92)	1.98 (1.44,2.74)

Table S21. Summary of geometric mean titres (and 95% confidence intervals) by study round and ownership category, after excluding dogs known to have been vaccinated in the past and/or revaccinated after R1, i.e. those that could have been vaccinated only in R1 as part of this study

Study round	Dogs without owners	Owned dogs
First	0.15 (0.15,0.16)	0.15 (0.14,0.17)
Second	1.59 (1.28,1.98)	1.73 (1.26,2.37)
Third	0.55 (0.45,0.67)	0.38 (0.3,0.49)
Fourth	1.58 (1.29,1.92)	1.41 (1.12,1.79)

Table S22. Summary of geometric mean titres (and 95% confidence intervals) by study round and ownership category, after excluding pups and juvenile as well as those known to have been vaccinated in the past and/or revaccinated after R1 i.e. including only those that could have been vaccinated in R1 as part of this study

Study round	Dogs without owners	Owned dogs
First	0.16 (0.15,0.17)	0.16 (0.14,0.18)
Second	1.84 (1.48,2.29)	2.47 (1.69,3.61)
Third	0.56 (0.46,0.69)	0.39 (0.28,0.55)
Fourth	1.62 (1.31,2)	1.44 (1.08,1.93)

Table S23. Summary of geometric mean titres (and 95% confidence intervals) of pups and juveniles captured in R1 by study round and ownership category, after excluding those known to have been vaccinated in the past and/or revaccinated after R1, i.e. including only those that could have been vaccinated in R1 as part of this study

Study round	Dogs without owners	Owned dogs
First	0.13 (0.12,0.15)	0.14 (0.12,0.18)
Second	0.48 (0.25,0.91)	0.92 (0.58,1.48)
Third	0.37 (0.15,0.91)	0.36 (0.24,0.52)
Fourth	1.17 (0.66,2.09)	1.35 (0.91,2.01)

Table S24. Summary of geometric mean titres (and 95% confidence intervals) by study round and ownership category, including only those known to have been vaccinated in the past and revaccinated after R1

Study round	Owned dogs
First	0.16 (0.08,0.32)
Second	2.64 (0.34,20.43)
Third	0.46 (NA,NA)
Fourth	1.87 (0.48,7.32)

Table S25. Summary of geometric mean titres (and 95% confidence intervals) by study round and ownership category, excluding those known to have been vaccinated in the past but including those revaccinated after R1

Study round	Dogs without owners	Owned dogs
First	0.15 (0.12,0.19)	0.11 (0.11,0.11)
Second	1.99 (1.32,2.99)	1.41 (0.7,2.84)
Third	1.14 (0.78,1.68)	0.38 (0.17,0.87)
Fourth	1.62 (1.04,2.54)	1.87 (1.07,3.26)

Table S26. Summary of geometric mean titres (and 95% confidence intervals) by study round and ownership category and all ages including only those dogs known to have been vaccinated before R1 but excluding those revaccinated after R1

Study round	Dogs without owners	Owned dogs
First	0.18 (0.16,0.21)	0.18 (0.15,0.22)
Second	3.68 (2.76,4.9)	3.06 (2.43,3.84)
Third	1.14 (0.82,1.59)	0.61 (0.41,0.93)
Fourth	1.98 (1.44,2.74)	1.52 (1.15,2.01)

Table S27: Summary of the various linear regression models, including predictors included and AIC values for the preliminary and final multivariable models

Model Number	Study round	Dog category (n)	Important variables identified from univariate analyses*	AIC for model with all these variables	Variables in reduced model*	AIC for reduced model
26.	Round 1 titres	All dogs	RFFIT_num1_log10 & RFFIT_num2_log10 – Age2, Earnotched_R1, Ever_vaccinated	Num1 – 5.83; Num2 – 113.09	Ever_vaccinated	Num1 – 3.35; Num2 – 110.88
27.		UD	Both – Earnotched_R1, Ever_vaccinated	Num1 – 11.16; Num2 – 84.76	Earnotched_R1	Num1 – 11.16; Num2 – 84.76
28.		OD	None	NA	NA	NA
29.		SOD	Both – Repr_status (Perfect fit, summary may be unreliable)	Num1 - -1415; Num2 - -1437	Repr_status	Num1 - -1415; Num2 - -1437
30.		UD+SOD	Both – Earnotched_R1, Ever_vaccinated	Num1 – 17.9; Num2 – 98.6	Earnotched_R1	Num1 – 15.5; Num2 – 96.2
31.	Round 2 titres	All dogs	Both - Age2, BCS, BCS2, Earnotched_R1, Vaccination_history.x, Vaccination_history2, ABC, Ever_vaccinated, Turbidity_scale, RFFIT_num1_log10 (or num2)	Num1 –444.39; Num2 – 420.13	Age2, Earnotched_R1, ABC, RFFIT_num1_log10, Turbidity_scale	Num1 – 433.6; Num2 –409.41
32.		UD	Both - Age2, Earnotched_R1, ABC, Ever_vaccinated, RFFIT_num1_log10, Turbidity_scale (or num2)	230.44; 217.76	Age2, Earnotched_R1, ABC, RFFIT_num1_log10, Turbidity_scale	230.44; 217.76
33.		OD	Both - Age2, BCS2, Vaccination_history.x, Vaccination_history2, Site, Ever_vaccinated, Int1_2	151.74;143.82	Age2, Site, Ever_vaccinated	143.16;135.40
34.		SOD	Age2, Earnotched_R1	70.52; 66.83	Age2, Earnotched_R1	70.52; 66.83
35.		UD+SOD	Both - Age2, Earnotched_R1, ABC, Ever_vaccinated, RFFIT_num1_log10 (or num2)	291.96; 275.41	Age2, Earnotched_R1, ABC, RFFIT_num1_log10, Turbidity_scale	288.08; 271.54
36.	Round 3 titres	All dogs	Both - Age2, Earnotched_R1, Breed2, Ownership2, Confinement2, Vaccination_history.x, Vaccination_history2, ABC, Site, Ever_vaccinated, Ever_revaccinated, Int1_3	238.19; 248.27	Earnotched_R1, Confinement2, Vaccination_history.x, ABC, Site	223.26; 233.05
37.		UD	Earnotched_R1, ABC, Ever_vaccinated	87.7; 91.57	Earnotched_R1, ABC	87.7; 91.57
38.		OD	Confinement2, Int1_3, Site (num2 only)	104.83; 110.57	Confinement2, Int1_3	104.83; 110.07

39.		SOD	Site, Int1_3, RFFIT_num1_log10 (or num2)	33.03; 33.55	Site, RFFIT_num1_log10 (num2)	32.52; 33.1
40.		UD+SOD	Earnotched_R1, ABC, Ever_vaccinated, Int1_3	131.73; 135.91	Earnotched_R1, ABC	125.9; 130.1
41.	Round 4 titres	All dogs	Earnotched_R1, RFFIT_num1_log10	183.14; 182.89	Earnotched_R1, RFFIT_num1_log10	183.14; 182.89
42.		UD	Dogs_activity2	75.94	Dogs_activity2	75.94
43.		OD	None	-	NA	-
44.		SOD	Repr_status, RFFIT_num1_log10	-1.22	Repr_status	-1.22
45.		UD+SOD	RFFIT_num2_log10 only	108.24	RFFIT_num2_log10 (num2 only)	108.24
46.	Mixed effects model	All dogs	Age_infer ,Age_R1 ,Age2_infer ,Age2_R1 ,BCS , Confinement_R1, Confinement2_R1 ,Coat2, Earnotched_R1, Vaccination_history2_R1 ,ABC_R1, Sampling_occasion, RFFIT_num1_log10_R1 , Ever_vaccinated	943.38	Age2_infer, Earnotched_R1, ABC_R1, Sampling_occasion, RFFIT_num1_log10_R1	878.74
47.		UD	Age_infer, Age_R1, Age2_infer, Age2_R1, Earnotched_R1, ABC_R1, Sampling_occasion, RFFIT_num1_log10_R1, Ever_vaccinated	442.5064	Age2_R1, ABC_R1, Sampling_occasion, RFFIT_num1_log10_R1	435.16
48.		OD	Age_infer, Age_R1, Age2_infer, Age2_R1, Vaccination_history2_R1, Sampling_occasion, Ever_vaccinated	372.65	Age2_infer, Sampling_occasion	348 .52
49.		SOD	Age_infer, Age2_infer, Earnotched_R1, Sampling_occasion, RFFIT_num1_log10_R1, Site_R1	131.401	Age2_infer, Earnotched_R1, Sampling_occasion, RFFIT_num1_log10_R1, Site_R1	131.401
50.		UD+SOD	Age_infer, Age_R1, Age2_infer, Age2_R1, Earnotched_R1, ABC_R1, Sampling_occasion, RFFIT_num1_log10_R1, Ever_vaccinated	559.96	Age2_infer, Earnotched_R1, ABC_R1, Sampling_occasion, RFFIT_num1_log10_R1	550.13

Table S27. Linear regression estimates and p-values for R1 titres for all dogs for variables included in the final multivariable linear regression model. Statistically significant p-values (p < 0.05) are highlighted in bold.

Variable	Estimate	Standard_error	95% confidence intervals	p_value
(Intercept)	**-0.84**	**0.03**	**-0.89--0.78**	**< 0.001**
Ever_vaccinatedUnknown	0.03	0.03	-0.04-0.09	0.412
Ever_vaccinatedYes	**0.10**	**0.04**	**0.03-0.17**	**0.008**

Table S28. Linear regression estimates and p-values for R1 titres for UD for variables included in the final multivariable linear regression model. Statistically significant p-values (p < 0.05) are highlighted in bold.

Variable	Estimate	Standard_error	95% confidence intervals	p_value
(Intercept)	**-0.81**	**0.01**	**-0.84--0.79**	**< 0.001**
EarnotchedSterilised	**0.09**	**0.03**	**0.02-0.16**	**0.013**

Table S29. Linear regression estimates and p-values for R1 titres for SOD for variables included in the final multivariable linear regression model. Statistically significant p-values (p < 0.05) are highlighted in bold. (exclude table?)

Variable	Estimate	Standard_error	95% confidence intervals	p_value
(Intercept)	**-0.64**	**0**	**-0.64--0.64**	**< 0.001**
Repr_statusLactating	**-0.30**	**0**	**-0.3--0.3**	**< 0.001**
Repr_statusWhelp	**0.00**	**0**	**0-0**	**< 0.001**

Table S30. Linear regression estimates and p-values for R1 titres for UDSOD for variables included in the final multivariable linear regression model. Statistically significant p-values (p < 0.05) are highlighted in bold.

Variable	Estimate	Standard_error	95% confidence intervals	p_value
(Intercept)	**-0.81**	**0.01**	**-0.84--0.79**	**< 0.001**
EarnotchedSterilised	**0.07**	**0.03**	**0.01-0.14**	**0.024**

Table S31. Linear regression estimates and p-values for R2 titres for all dogs for variables included in the final multivariable linear regression model. Statistically significant p-values (p < 0.05) are highlighted in bold.

Variable	Estimate	Standard_error	95% confidence intervals	p_value
(Intercept)	**0.45**	**0.09**	**0.28-0.63**	**< 0.001**
Age2Juvenile	**-0.31**	**0.10**	**-0.5--0.11**	**0.002**
Age2Pup	**-0.61**	**0.11**	**-0.82--0.4**	**< 0.001**
EarnotchedSterilised	**0.16**	**0.08**	**0.01-0.31**	**0.042**
SedatedYes	**-0.59**	**0.10**	**-0.78--0.39**	**< 0.001**
RFFIT_num1_log10	0.15	0.10	-0.05-0.36	0.134
Turbidity_scale	0.03	0.01	0-0.06	0.067

Table S32. Linear regression estimates and p-values for R2 titres for UD for variables included in the final multivariable linear regression model. Statistically significant p-values (p < 0.05) are highlighted in bold.

Variable	Estimate	Standard_error	95% confidence intervals	p_value
(Intercept)	**0.51**	**0.11**	**0.29-0.73**	**< 0.001**
Age2Juvenile	**-0.40**	**0.14**	**-0.67--0.13**	**0.004**
Age2Pup	**-0.48**	**0.24**	**-0.94--0.01**	**0.046**
EarnotchedSterilised	0.12	0.09	-0.06-0.3	0.177
SedatedYes	**-0.56**	**0.11**	**-0.78--0.33**	**< 0.001**
RFFIT_num1_log10	**0.27**	**0.13**	**0.01-0.52**	**0.042**
Turbidity_scale	0.03	0.02	0-0.07	0.064

Table S33. Linear regression estimates and p-values for R2 titres for SOD for variables included in the final multivariable linear regression model. Statistically significant p-values (p < 0.05) are highlighted in bold.

Variable	Estimate	Standard_error	95% confidence intervals	p_value
(Intercept)	**0.29**	**0.12**	**0.05-0.54**	**0.019**
Age2Juvenile	-0.74	0.36	-1.48-0.01	0.052
Age2Pup	**-1.08**	**0.44**	**-1.97--0.19**	**0.019**
EarnotchedSterilised	0.48	0.27	-0.07-1.03	0.085

Table S34. Linear regression estimates and p-values for R2 titres for OD for variables included in the final multivariable linear regression model. Statistically significant p-values (p < 0.05) are highlighted in bold.

Variable	Estimate	Standard_error	95% confidence intervals	p_value
(Intercept)	**0.29**	**0.08**	**0.14-0.44**	**< 0.001**
Age2Juvenile	-0.05	0.15	-0.36-0.26	0.75
Age2Pup	**-0.44**	**0.13**	**-0.69--0.19**	**0.001**
SiteMuhamma	**0.42**	**0.19**	**0.04-0.79**	**0.028**
Ever_vaccinatedUnknown	0.27	0.16	-0.06-0.6	0.104
Ever_vaccinatedYes	0.18	0.10	-0.02-0.38	0.07

Table S35. Linear regression estimates and p-values for R2 titres for UDSOD for variables included in the final multivariable linear regression model. Statistically significant p-values ($p < 0.05$) are highlighted in bold.

Variable	Estimate	Standard_error	95% confidence intervals	p_value
(Intercept)	**0.51**	**0.11**	**0.3-0.72**	**< 0.001**
Age2Juvenile	**-0.42**	**0.13**	**-0.67--0.17**	**0.001**
Age2Pup	**-0.68**	**0.20**	**-1.07--0.28**	**0.001**
EarnotchedSterilised	0.16	0.09	0-0.33	0.057
SedatedYes	**-0.55**	**0.10**	**-0.75--0.34**	**< 0.001**
RFFIT_num1_log10	**0.28**	**0.12**	**0.04-0.52**	**0.023**
Turbidity_scale	**0.04**	**0.02**	**0.01-0.07**	**0.024**

Table S36. Linear regression estimates and p-values for R3 titres for all dogs for variables included in the final multivariable linear regression model. Statistically significant p-values ($p < 0.05$) are highlighted in bold.

Variable	Estimate	Standard_error	95% confidence intervals	p_value
(Intercept)	**-0.34**	**0.07**	**-0.47--0.2**	**< 0.001**
EarnotchedSterilised	**0.25**	**0.08**	**0.11-0.4**	**0.001**
Confinement2Completely/partially confined	**-0.23**	**0.08**	**-0.4--0.07**	**0.006**
Vaccination_history.xRegular vaccination	-0.30	0.40	-1.1-0.49	0.452
Vaccination_history.xUnknown	0.15	0.07	0-0.29	0.052
Vaccination_history.xVaccinated in the past	**0.26**	**0.10**	**0.07-0.45**	**0.008**
SedatedYes	**-0.30**	**0.12**	**-0.54--0.06**	**0.016**
SiteMuhamma	**0.22**	**0.09**	**0.03-0.4**	**0.023**

Table S37. Linear regression estimates and p-values for R3 titres for UD for variables included in the final multivariable linear regression model. Statistically significant p-values (p < 0.05) are highlighted in bold.

Variable	Estimate	Standard_error	95% confidence intervals	p_value
(Intercept)	**-0.18**	**0.05**	**-0.27--0.09**	**< 0.001**
EarnotchedSterilised	**0.22**	**0.09**	**0.05-0.39**	**0.013**
SedatedYes	**-0.32**	**0.13**	**-0.59--0.06**	**0.015**

Table S38. Linear regression estimates and p-values for R3 titres for SOD for variables included in the final multivariable linear regression model. Statistically significant p-values (p < 0.05) are highlighted in bold.

Variable	Estimate	Standard_error	95% confidence intervals	p_value
(Intercept)	**0.51**	**0.23**	**0.02-0.99**	**0.042**
SiteMuhamma	**0.64**	**0.22**	**0.19-1.09**	**0.007**
RFFIT_num1_log10	**0.82**	**0.28**	**0.24-1.4**	**0.007**

Table S39. Linear regression estimates and p-values for R3 titres for OD for variables included in the final multivariable linear regression model. Statistically significant p-values (p < 0.05) are highlighted in bold.

Variable	Estimate	Standard_error	95% confidence intervals	p_value
(Intercept)	**-1.29**	**0.53**	**-2.34--0.24**	**0.017**
Confinement2Completely/partially confined	-0.17	0.09	-0.35-0.01	0.062
Int1_3	0.01	0.00	0-0.01	0.05

Table S40. Linear regression estimates and p-values for R3 titres for UDSOD for variables included in the final multivariable linear regression model. Statistically significant p-values ($p < 0.05$) are highlighted in bold.

Variable	Estimate	Standard_error	95% confidence intervals	p_value
(Intercept)	**-0.16**	**0.04**	**-0.25--0.08**	**< 0.001**
EarnotchedSterilised	**0.25**	**0.08**	**0.09-0.41**	**0.002**
SedatedYes	**-0.33**	**0.12**	**-0.56--0.09**	**0.008**

Table S41. Linear regression estimates and p-values for R4 titres for all dogs for variables included in the final multivariable linear regression model. Statistically significant p-values ($p < 0.05$) are highlighted in bold.

Variable	Estimate	Standard_error	95% confidence intervals	p_value
(Intercept)	**0.33**	**0.08**	**0.17-0.48**	**< 0.001**
EarnotchedSterilised	**0.14**	**0.07**	**0.01-0.28**	**0.036**
RFFIT_num1_log10	**0.19**	**0.09**	**0.01-0.38**	**0.041**

Table S42. Linear regression estimates and p-values for R4 titres for UD for variables included in the final multivariable linear regression model. Statistically significant p-values ($p < 0.05$) are highlighted in bold.

Variable	Estimate	Standard_error	95% confidence intervals	p_value
(Intercept)	**0.32**	**0.05**	**0.22-0.42**	**< 0.001**
Dogs_activity2Normal behaviour	**-0.30**	**0.11**	**-0.51 - -0.08**	**0.007**
Dogs_activity2Interacting with people	-0.50	0.28	-1.06-0.05	0.076

Table S43. Linear regression estimates and p-values for R4 titres for UDSOD for variables included in the final multivariable linear regression model. Statistically significant p-values ($p < 0.05$) are highlighted in bold.

Variable	Estimate	Standard_error	95% confidence intervals	p_value
(Intercept)	**0.41**	**0.10**	**0.22-0.6**	**< 0.001**
RFFIT_num2_log10	**0.22**	**0.11**	**0-0.44**	**0.046**

Table S44. Regression estimates and p-values for RVNA titres for UD for variables included in the final multivariable mixed effects linear regression model. Statistically significant p-values ($p < 0.05$) are highlighted in bold.

Variable	Estimate	Standard_error	95% confidence intervals	p_value
(Intercept)	**0.53**	**0.08**	**0.37-0.68**	**< 0.001**
Age2_R1Juvenile	**-0.43**	**0.11**	**-0.64--0.21**	**< 0.001**
Age2_R1Pup	**-0.50**	**0.19**	**-0.86--0.13**	**0.008**
Sedated_R1Yes	**-0.38**	**0.08**	**-0.53--0.23**	**< 0.001**
Sampling_occasionThird	**-0.48**	**0.05**	**-0.57--0.38**	**< 0.001**
Sampling_occasionFourth	-0.08	0.05	-0.17-0.02	0.106
RFFIT_num1_log10_R1	**0.19**	**0.09**	**0.01-0.38**	**0.039**

Table S45. Regression estimates and p-values for RVNA titres for OD for variables included in the final multivariable mixed effects linear regression model. Statistically significant p-values ($p < 0.05$) are highlighted in bold.

Variable	Estimate	Standard_error	95% confidence intervals	p_value
(Intercept)	**0.42**	**0.04**	**0.33-0.51**	**< 0.001**
Age2_inferJuvenile	**-0.24**	**0.08**	**-0.41--0.08**	**0.005**
Age2_inferPup	**-0.51**	**0.12**	**-0.74--0.28**	**< 0.001**
Sampling_occasionThird	**-0.73**	**0.05**	**-0.84--0.62**	**< 0.001**
Sampling_occasionFourth	**-0.24**	**0.05**	**-0.35--0.13**	**< 0.001**

Table S46. Regression estimates and p-values for RVNA titres for OD for variables included in the multivariate mixed effects linear regression model that was not the most parsimonious. Statistically significant p-values ($p < 0.05$) are highlighted in bold.

Variable	Estimate	Standard_error	95% confidence intervals	p_value
(Intercept)	**0.42**	**0.06**	**0.3-0.54**	**< 0.001**
Age2_inferJuvenile	-0.16	0.09	-0.33-0.02	0.077
Age2_inferPup	**-0.43**	**0.12**	**-0.67--0.2**	**< 0.001**
Sampling_occasionThird	**-0.72**	**0.05**	**-0.83--0.61**	**< 0.001**
Sampling_occasionFourth	**-0.22**	**0.06**	**-0.33--0.11**	**< 0.001**
Confinement2_R1Completely/partially confined	**-0.16**	**0.06**	**-0.28--0.04**	**0.007**
Ever_vaccinatedUnknown	0.17	0.11	-0.04-0.38	0.119
Ever_vaccinatedYes	**0.17**	**0.07**	**0.04-0.3**	**0.012**

Table S47. Regression estimates and p-values for RVNA titres for SOD for variables included in the final multivariable mixed effects linear regression model. Statistically significant p-values ($p < 0.05$) are highlighted in bold.

Variable	Estimate	Standard_error	95% confidence intervals	p_value
(Intercept)	**0.71**	**0.22**	**0.26-1.15**	**0.003**
Age2_inferJuvenile	-0.47	0.24	-0.96-0.03	0.063
Age2_inferPup	**-1.05**	**0.34**	**-1.74--0.37**	**0.004**
Site_R1Muhamma	**0.53**	**0.18**	**0.16-0.9**	**0.006**
Earnotched_R1Sterilised	**0.42**	**0.16**	**0.1-0.74**	**0.013**
Sampling_occasionThird	**-0.46**	**0.11**	**-0.68--0.24**	**< 0.001**
Sampling_occasionFourth	-0.25	0.12	-0.49-0	0.05
RFFIT_num1_log10_R1	**0.62**	**0.25**	**0.11-1.13**	**0.019**

Table S48. Regression estimates and p-values for RVNA titres for UDSOD for variables included in the final multivariable mixed effects linear regression model. Statistically significant p-values ($p < 0.05$) are highlighted in bold.

Variable	Estimate	Standard_error	95% confidence intervals	p_value
(Intercept)	**0.55**	**0.08**	**0.39-0.7**	**< 0.001**
Age2_inferJuvenile	**-0.35**	**0.10**	**-0.55--0.16**	**< 0.001**
Age2_inferPup	**-0.86**	**0.20**	**-1.25--0.47**	**< 0.001**
Earnotched_R1Sterilised	**0.17**	**0.06**	**0.05-0.29**	**0.006**
Sedated_R1Yes	**-0.34**	**0.07**	**-0.49--0.2**	**< 0.001**
Sampling_occasionThird	**-0.47**	**0.04**	**-0.56--0.38**	**< 0.001**
Sampling_occasionFourth	**-0.13**	**0.05**	**-0.23--0.04**	**0.004**
RFFIT_num1_log10_R1	**0.26**	**0.09**	**0.08-0.43**	**0.004**

Table S49. Details of variables used as predictors in linear/ mixed effects linear regression models. In brackets, the reference level and remaining levels.

Variable	Variable description
Sex	Sex (Female; Male)
Age	Age (Adult; Aged, Juvenile, pup)
Age2	Age2: (Adult (incl. Aged); Juvenile, pup)
BCS	Body condition score (BCS = 2; 3 - 8)
BCS2	BCS2 (Under ideal (2-3); Ideal (4-5), Over ideal (6-8))
Earnotched:	Sterilisation status (Not sterilised; Sterilised)
Breed2	Breed (Non-descript; Crossbreed, Purebreed)
Coat2	Coat condition (Poor; Fair, Good, Very good)
Ownership2	Ownership status (Owned; semi-owned, Unowned)*
Confinement	Confinement status (Confined; free-ranging, partially free-ranging)
Confinement2	Confinement status (Completely/partially confined; Free-ranging)
Human interaction	Human interaction score (HIS = 2; 3 - 6)
HIS2	HIS2 (Direct human interaction (4-6); No direct human interaction (1-3))
Dogs activity	Dogs activity at the time of capture (Trying to escape from dog catcher; sleeping, resting, feeding, interacting with local people, interacting with project staff, other normal behaviour, not applicable – owned dog
Dogs activity2	Dogs activity2 (Trying to escape; Normal behaviour, Interacting with people, Owned dog)
Repr status	Reproductive status (Pregnant; Lactating, whelp)
Repr status2	Reproductive status (Active; Not active)
Vaccination history	Vaccination history (Never vaccinated; Regular vaccination, unknown, vaccinated in the past)
Vaccination history2	Vaccination history2 (Never vaccinated; Unknown, Vaccinated)
Sedated (ABC)	If Sedated as part of on-going ABC (Yes; No)
Site	Study Site: (Alappuzha; Muhamma)
Study collar applied	If study collar applied or not (Not collared; Yes (black - female), Yes (orange - male))
Study collar applied2	If study collar applied or not (Collared; Not collared)
RFFIT titre	RFFIT titre when first captured (Less than 0.11; 0.11, Less than 0.23, 0.23, 0.46, 0.93, 1.87, Not applicable)
Titre level	Titre level (if antibodies detected or not) (Antibodies detected; No antibodies detected)
Rabies immunity	Rabies immunity (if titre level above or below 0.5) (Greater than 0.5 IU; No antibodies detected, Less than 0.5 IU)
Sampling occasion	Round of study during which recaptured (used in mixed effects models only) (Second; Third, Fourth)

*When running analyses with data for unowned and semi-owned dogs combined, the ownership category 'semi-owned dogs' was used as the reference level.

Table S50: Comparison of maximum likelihood estimates obtained for the parameters π and β using the L-BFGS-B optimisation method, when assuming that peak rabies virus neutralizing antibody titres are achieved 18 or 28 days-post-vaccination (dpv).

Model number	Time period when peak titres achieved (in days post-vaccination, dpv) (assumed)	Number of parameters	Parameters	Description of population	π (95% confidence intervals)		β (95% confidence intervals)		Log likelihood	Model comparisons (degrees of freedom)	p-value (Chi-square test)
1	18 dpv	2	1 π, 1 β	Overall population	0.95 (0.887 - 0.998)		0.0051 (0.0039 - 0.0063)		-238.2195	-	-
2	28 dpv		1 π, 1 β		0.901 (0.849 - 0.943)		0.0050 (0.0039 - 0.0062)		-238.214	-	-
3	18 dpv	3	2 π, 1 β	Dogs with (V) and without (NV) a history of vaccination before/ revaccination after round 1	V - 0.999 (0.92 - 1)	NV - 0.86 (0.78 - 0.94)	0.0044 (0.0033 - 0.0056)		-231.4923	1 and 3 (1)	< 0.001
4	28 dpv		2 π, 1 β		V - 0.99 (0.96 - 1)	NV - 0.83 (0.76 - 0.90)	0.0048 (0.0037 - 0.0060)		-230.3505	2 and 4 (1)	< 0.001
5	18 dpv	3	1 π, 2 β		0.97 (0.92 - 1)		V - 0.0028 (0.0018 - 0.0039)	NV - 0.0075 (0.0057 - 0.0093)	-225.8475	1 and 5 (1)	< 0.001
6	28 dpv		1 π, 2 β		0.91 (0.86 - 0.96)		V - 0.0025 (0.0014 - 0.0037)	NV - 0.0074 (0.0056 - 0.0092)	-227.377	2 and 6 (1)	< 0.001
7	18 dpv	4	2 π, 2 β		V - 0.99 (0.93 - 1)	NV - 0.94 (0.86 - 1)	V - 0.0031 (0.0020 - 0.0043)	NV - 0.0070 (0.0051 - 0.0089)	-224.9254	1 and 7 (2); 3 and 7 (1); 5 and 7 (1)	< 0.001 < 0.001 0.01
8	28 dpv		2 π, 2 β		V - 0.98 (0.93- 1)	NV - 0.87 (0.81 - 0.94)	V - 0.0032 (0.002 - 0.0044)	NV - 0.0070 (0.0051 - 0.0089)	-224.8968	2 and 8 (2); 4 and 8 (1); 6 and 8 (1)	< 0.001 < 0.001 0.174

Table S51: Comparison of maximum likelihood estimates obtained for the parameters π and β using two optimisation methods – bobyqa and L-BFGS-B - when assuming that peak rabies virus neutralizing antibody titres are achieved 18 days-post-vaccination (dpv).

Model number	Optimisation method	Number of parameters	Parameters	Description of population	π (95% confidence intervals)		β (95% confidence intervals)		Log likelihood	Model comparisons (degrees of freedom)	p-value (Chi-square test)
1	bobyqa	2	1 π, 1 β	Overall population	0.93 (0.89 - 0.97)		0.0049 (0.0038 - 0.006)		-275.8021	-	-
2	L-BFGS-B		1 π, 1 β		0.93 (0.89 - 0.98)		0.0050 (0.0038 - 0.006)		-275.8137	-	-
3	bobyqa	3	2 π, 1 β	Dogs with (V) and without (NV) a history of vaccination before/ revaccination after round 1	V 0.99	NV 0.85	0.0043		-265.2023	1 and 3 (1)	< 0.001
4	L-BFGS-B		2 π, 1 β		V 0.99 (0.94 - 1)	NV 0.85 (0.79 - 0.92)	0.0044 (0.0034 - 0.0055)		-265.2156	2 and 4 (1)	< 0.001
5	bobyqa	3	1 π, 2 β		0.95		V - 0.0025	NV - 0.0074	-262.4287	1 and 5 (1)	< 0.001
6	L-BFGS-B		1 π, 2 β		0.96 (0.91 - 0.998)		V - 0.0026 (0.0017 - 0.0036)	NV - 0.0075 (0.0058 - 0.0093)	-262.4626	2 and 6 (1)	< 0.001
7	bobyqa	4	2 π, 2 β		V - 0.99	NV 0.9	V 0.003	NV 0.0066	-259.1821	1 and 7 (2); 3 and 7 (1); 5 and 7 (1)	< 0.001 < 0.001 0.011
8	L-BFGS-B		2 π, 2 β		V - 0.99 (0.95 - 1)	NV 0.9 (0.84 - 0.97)	NV 0.0031 (0.002 - 0.0042)	NV 0.0066 (0.0048 - 0.0084)	-259.2068	2 and 8 (2); 4 and 8 (1); 6 and 8 (1)	< 0.001 < 0.001 0.026

Table S52. Predicted rabies virus neutralizing antibody (RVNA) titres based on the reported titre and the turbidity score of each sample

Reported RVNA titre	Predicted titre at turbidity score = 1	2	3	4	5	6	7	8	9	10
0.23	0.23	0.21	0.19	0.17	0.16	0.15	0.13	0.12	0.11	0.10
0.46	0.46	0.42	0.38	0.35	0.32	0.29	0.26	0.24	0.22	0.20
0.93	0.93	0.85	0.77	0.71	0.64	0.59	0.54	0.49	0.45	0.41
1.87	1.87	1.71	1.56	1.42	1.29	1.18	1.08	0.98	0.90	0.82
3.75	3.75	3.42	3.12	2.84	2.59	2.37	2.16	1.97	1.79	1.64
7.50	7.50	6.84	6.24	5.69	5.19	4.73	4.32	3.94	3.59	3.27
15.00	15.00	13.68	12.48	11.38	10.38	9.46	8.63	7.87	7.18	6.55

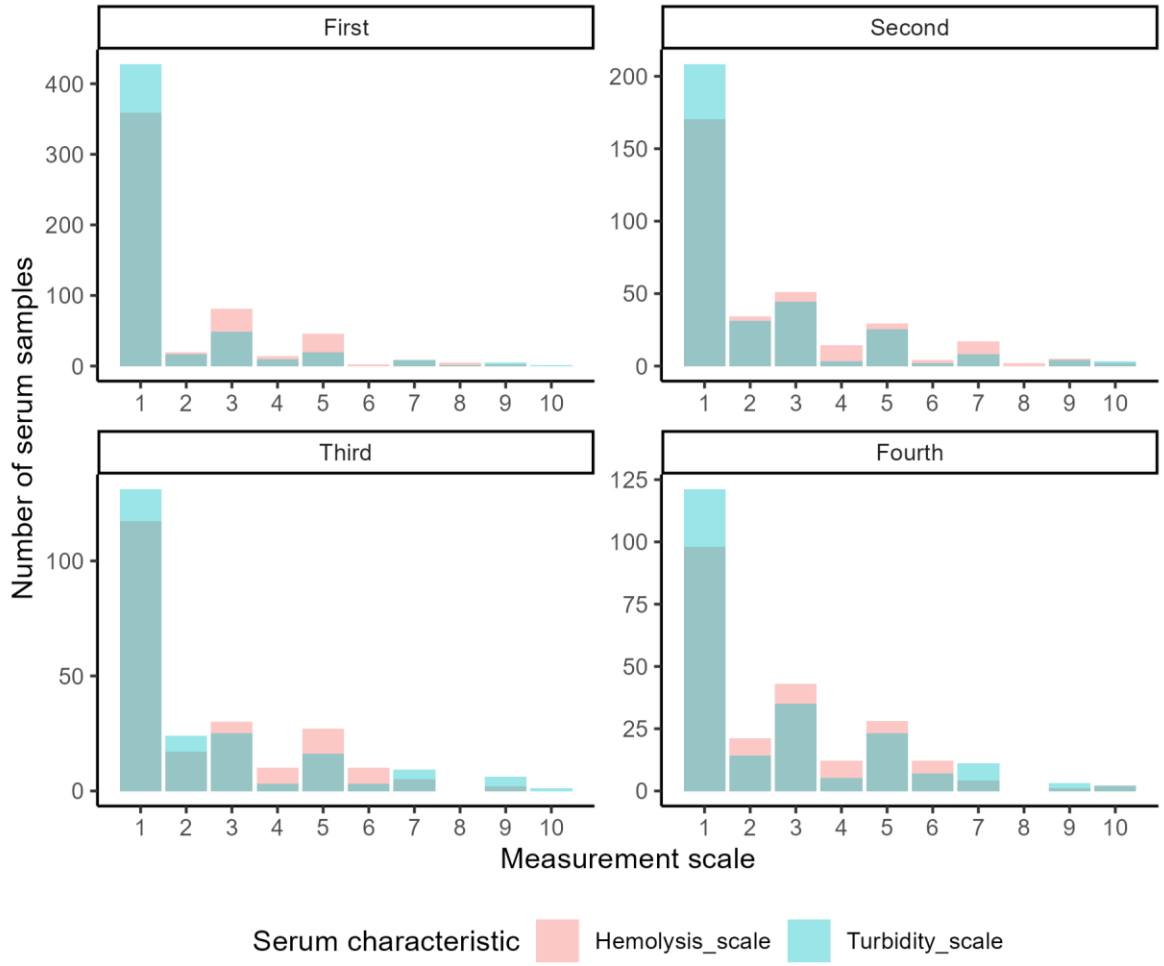


Fig. S1. Distribution of hemolysis and turbidity scores of serum samples collected in each study round

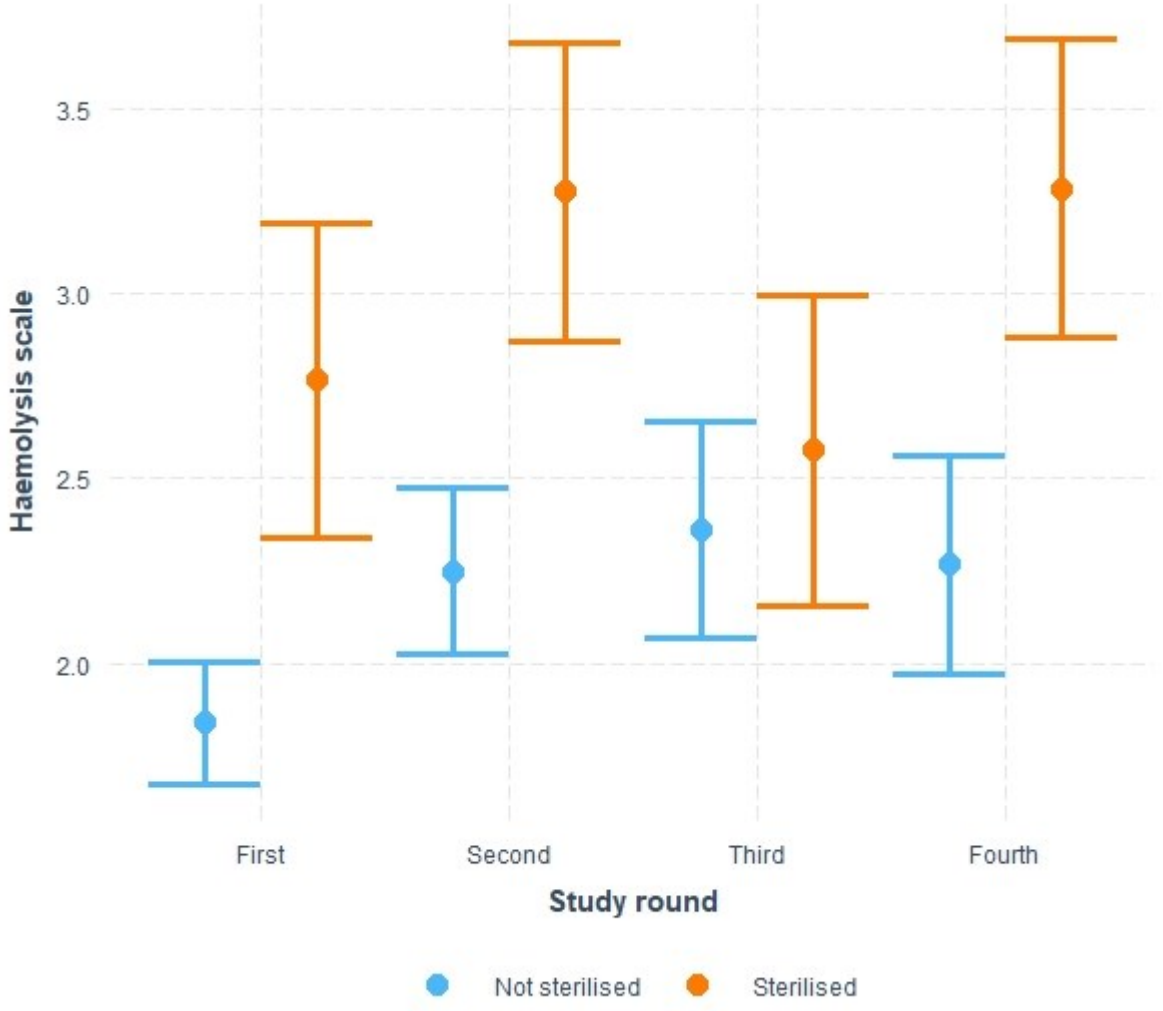


Fig. S2. Differences in hemolysis scores of serum samples by study round, based on the dog's sterilisation status

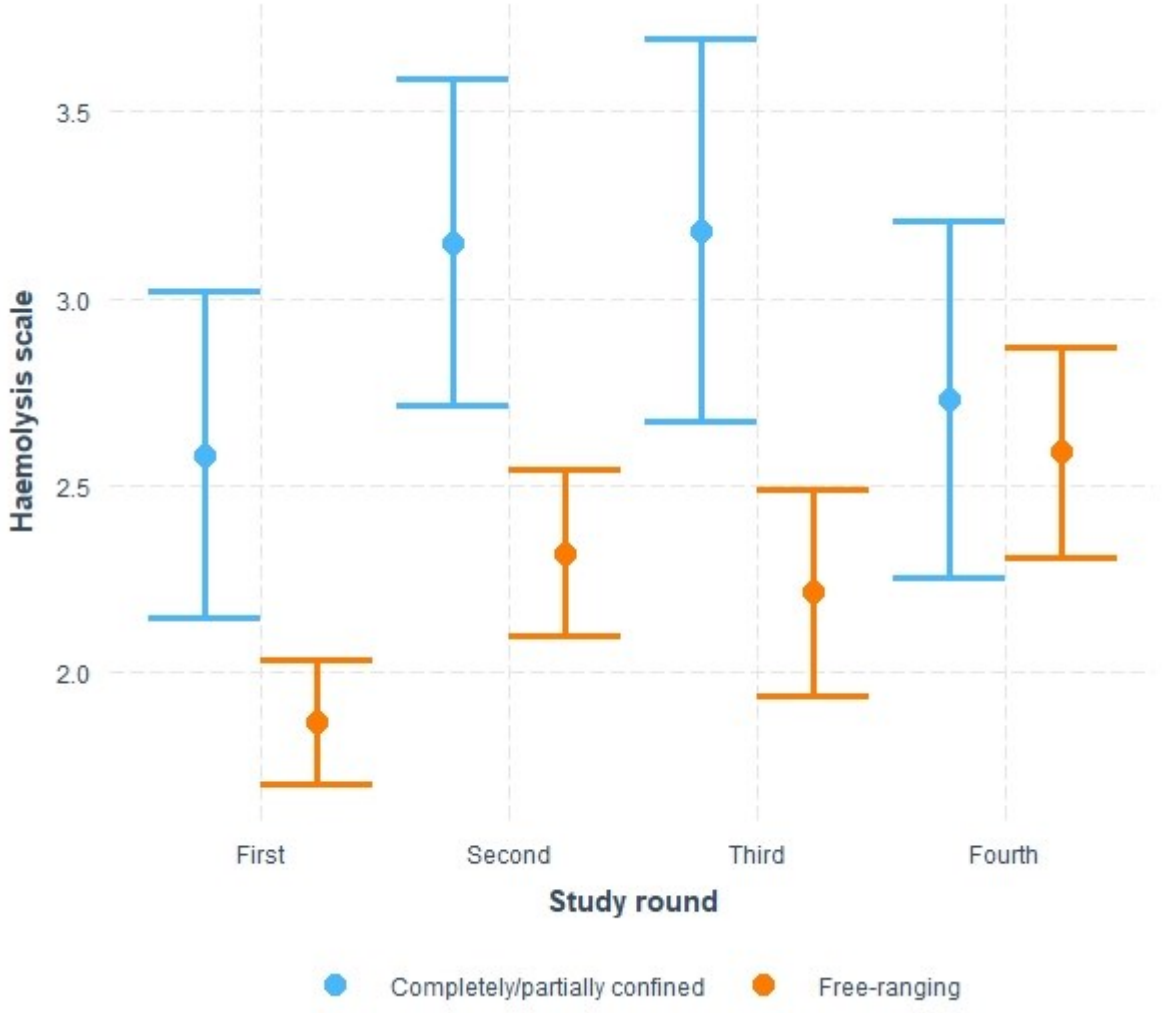


Fig. S3. Differences in hemolysis scores of serum samples by study round, based on the dog's confinement status

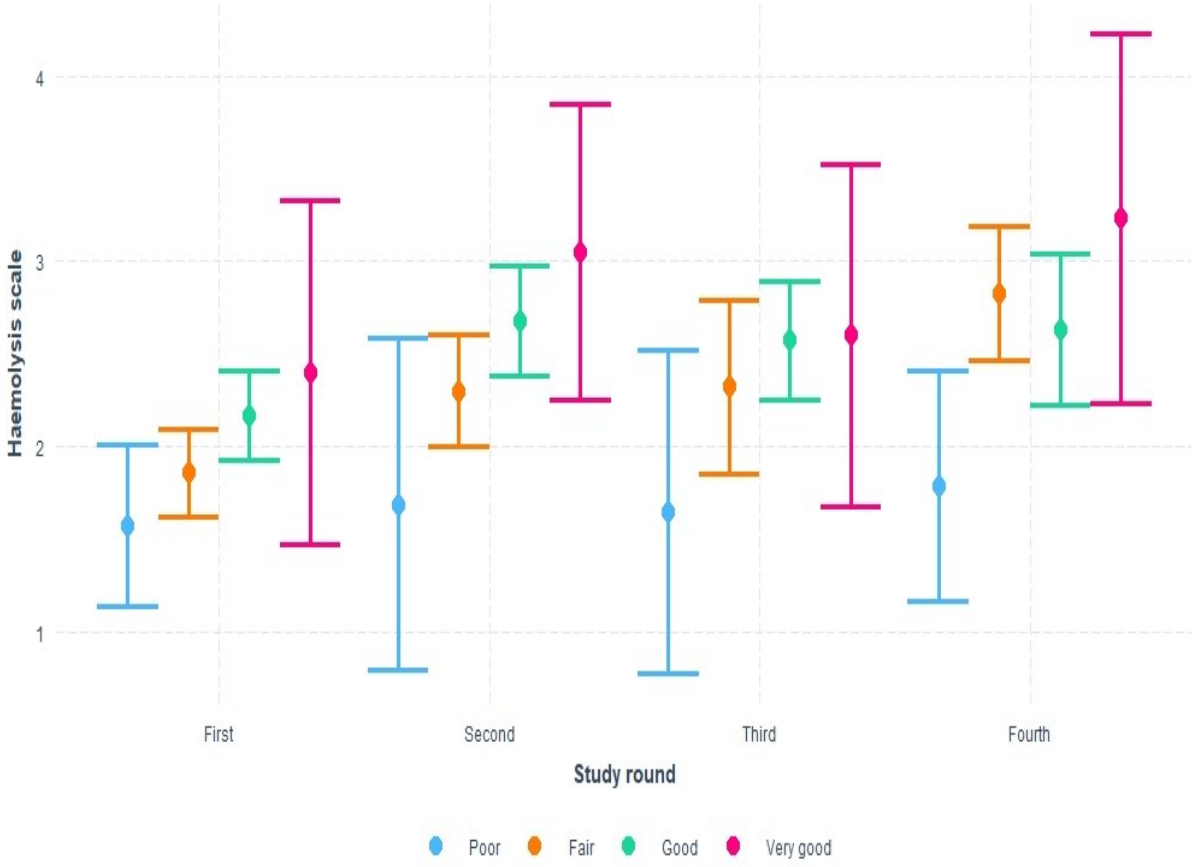


Fig. S4. Differences in hemolysis scores of serum samples by study round, based on the dog's coat condition

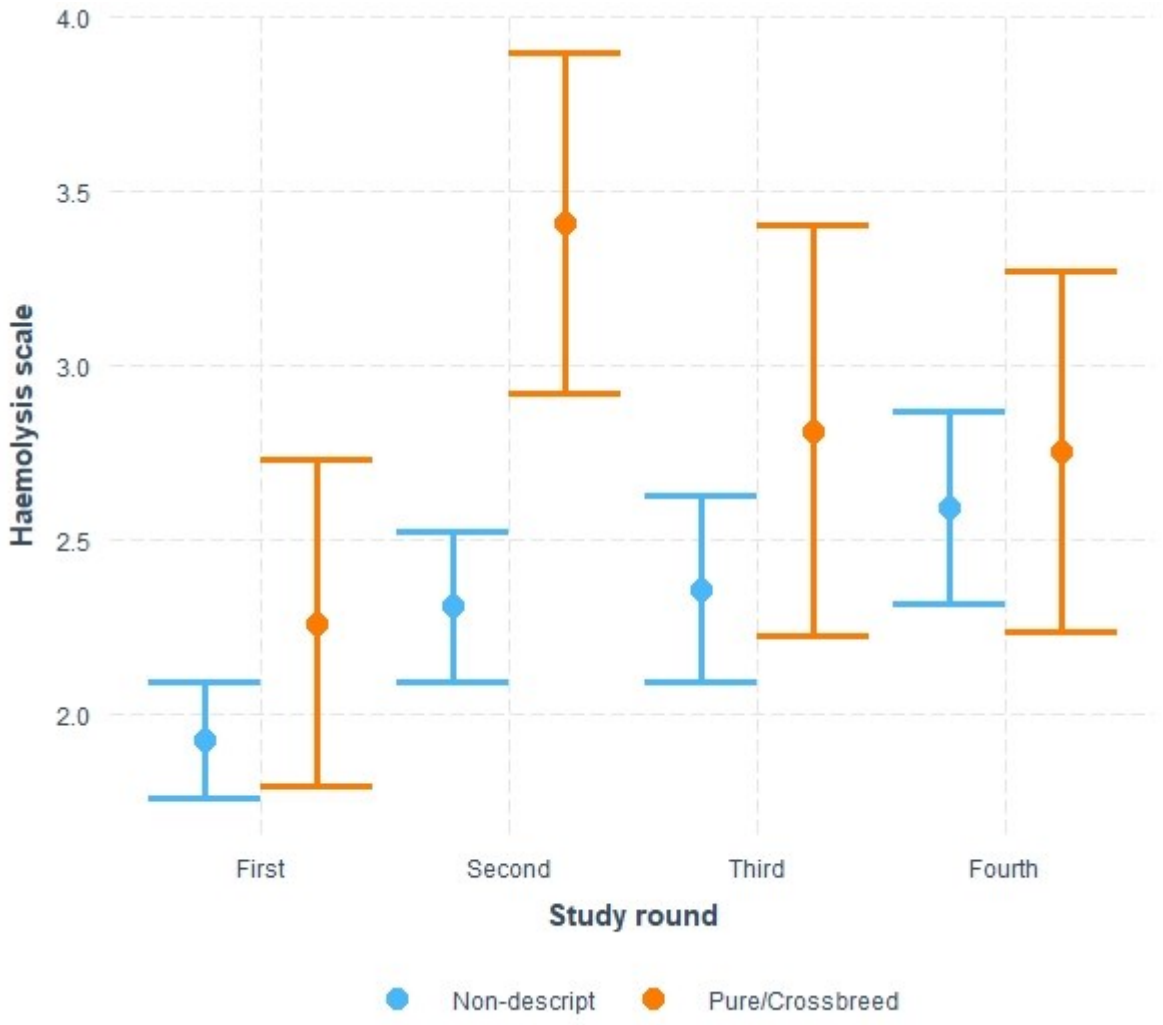


Fig. S5. Differences in hemolysis scores of serum samples by study round, based on the dog's breed

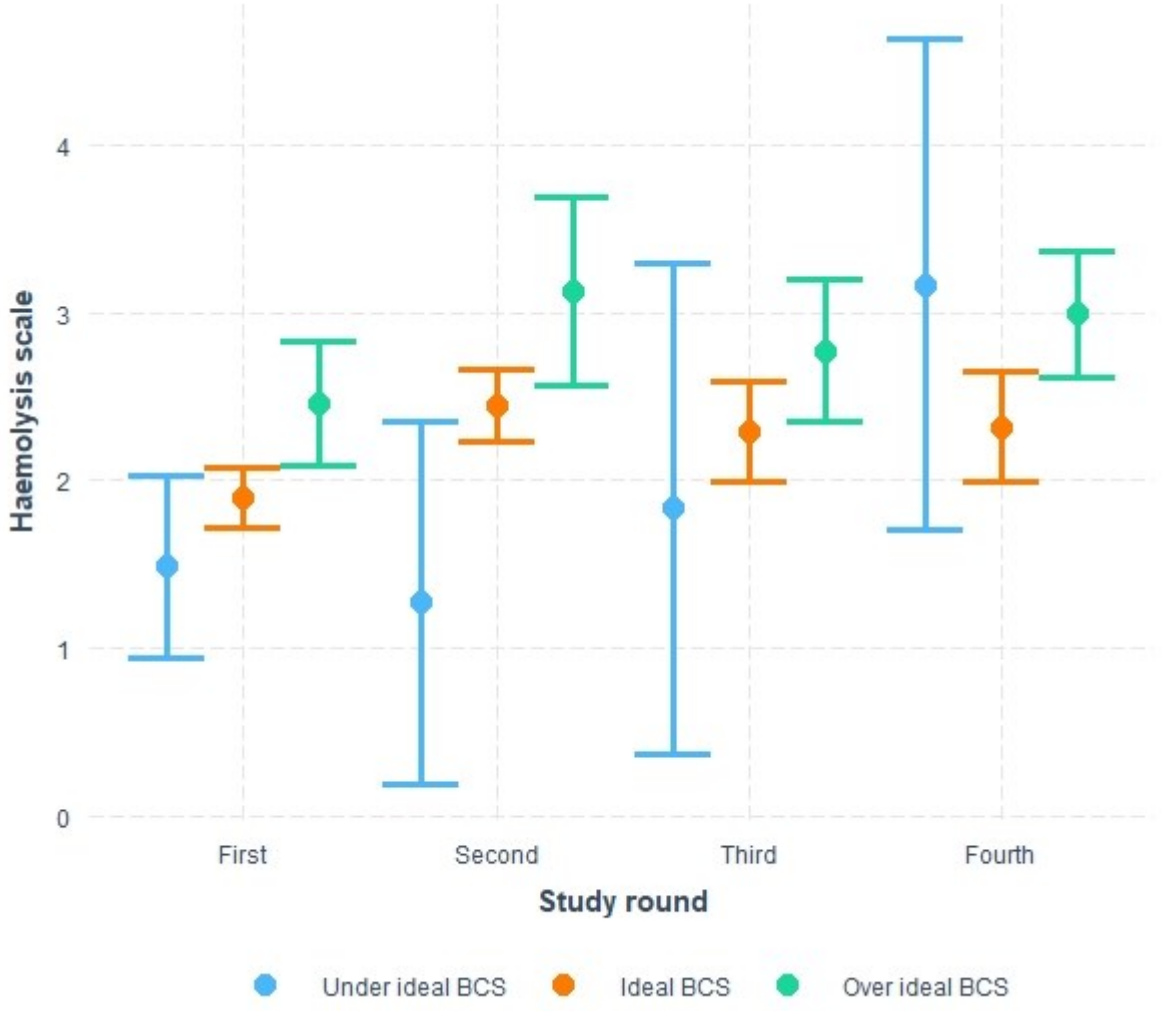


Fig. S6. Differences in hemolysis scores of serum samples by study round, based on the dog's body condition score (BCS)

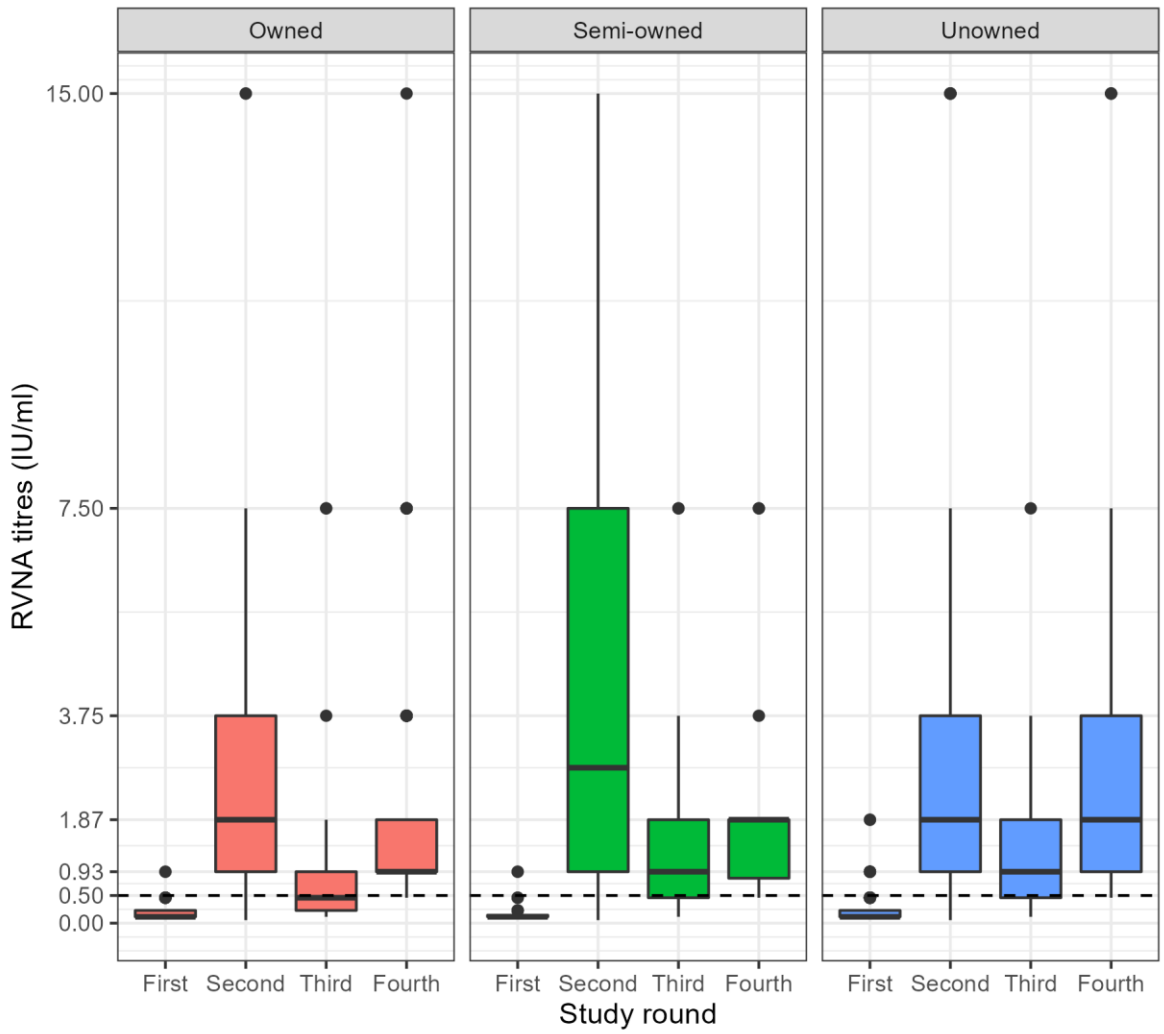


Fig. S7. Distribution of rabies virus neutralizing antibody (RVNA) titres (in IU/ml) in each study round, highlighting variation in titres by ownership status. The horizontal dotted line indicates a titre level of 0.5 IU/ml.

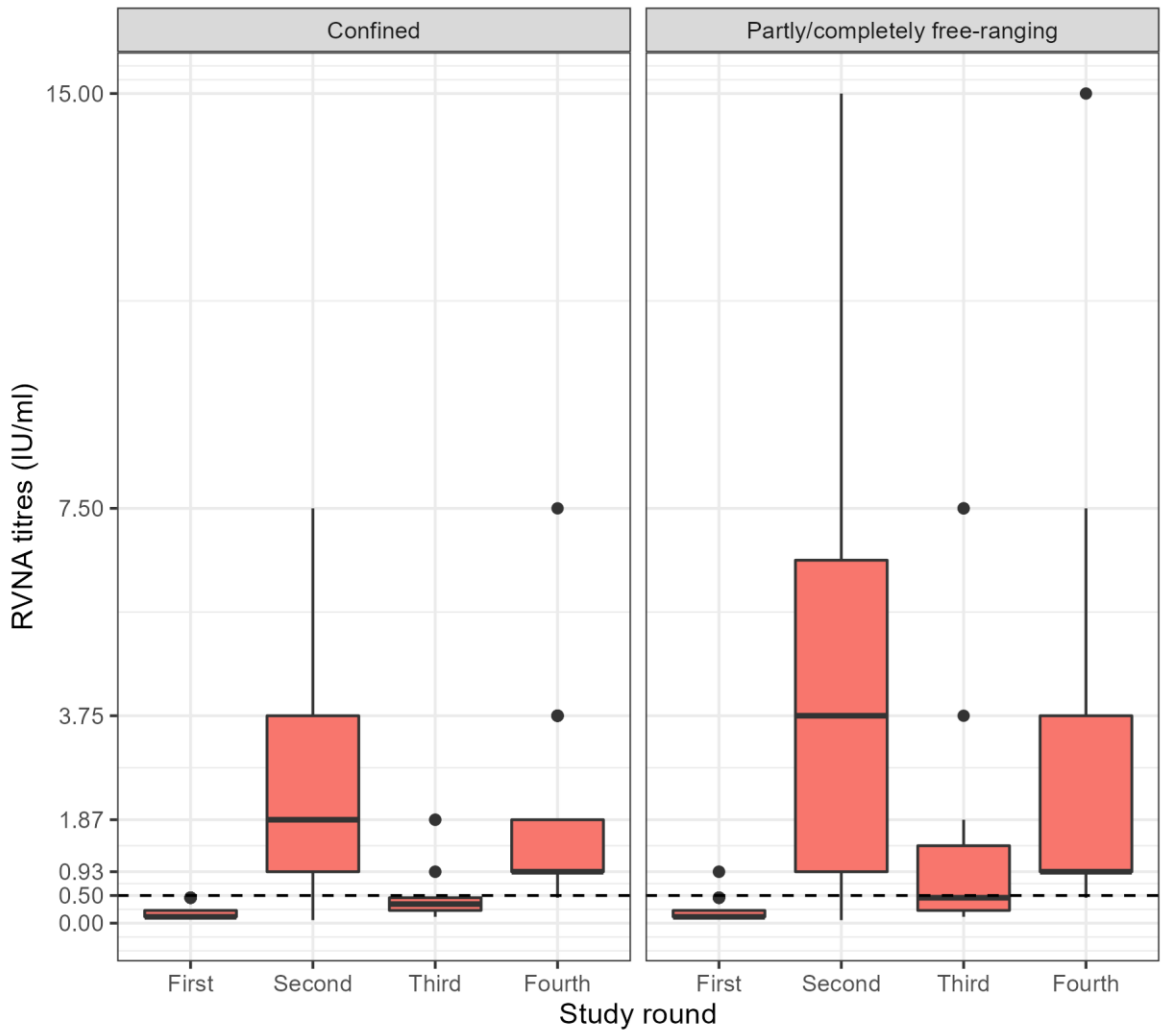


Fig. S8. Distribution of rabies virus neutralizing antibody (RVNA) titres (in IU/ml) in each study round highlighting variation in titres by confinement status. The horizontal dotted line indicates a titre level of 0.5 IU/ml.

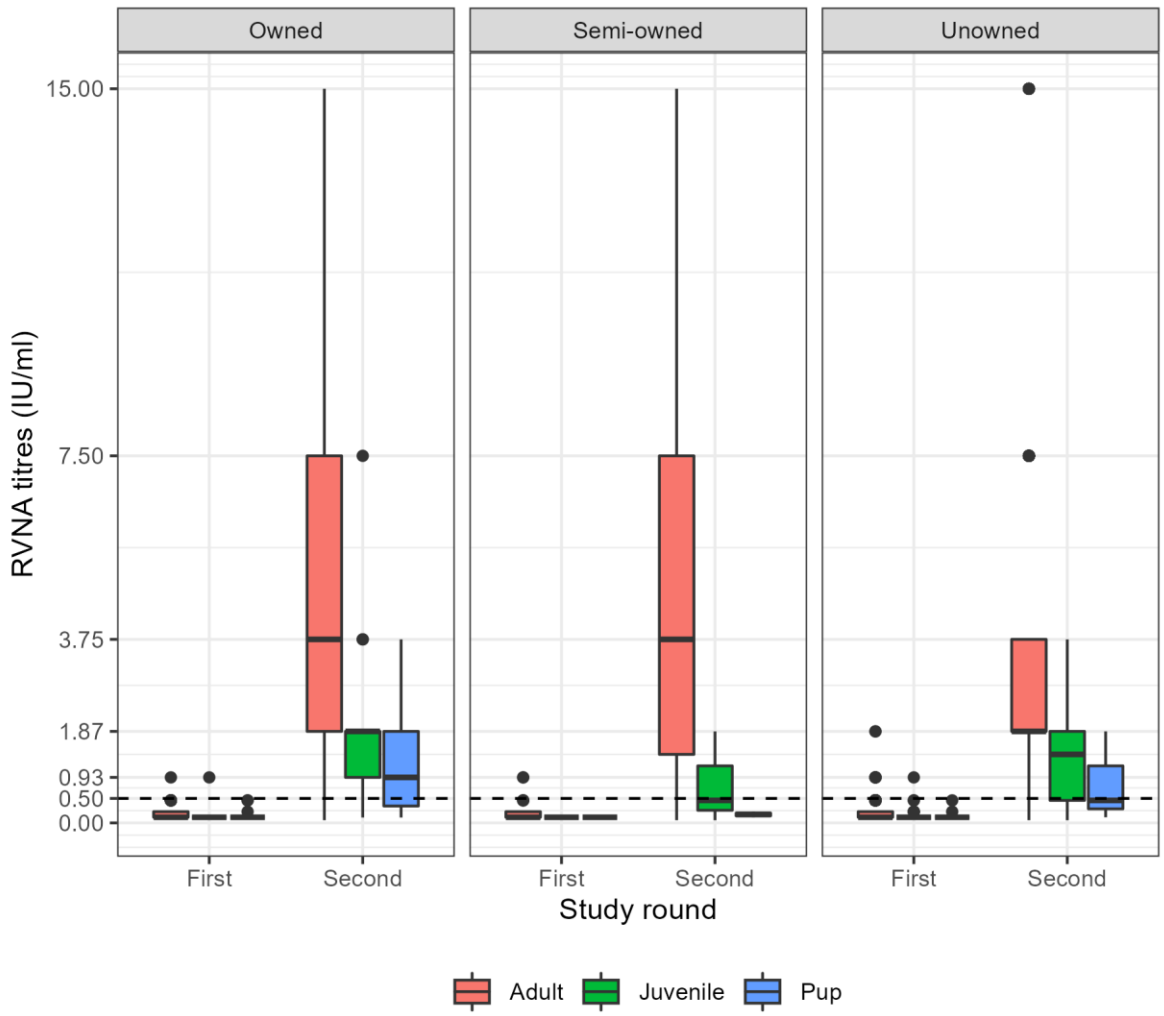


Fig. S9. Distribution of rabies virus neutralizing antibody (RVNA) titres (in IU/ml) in round 1 (first) and 2 (second), highlighting variation in post-vaccination titres by age at first vaccination. The horizontal dotted line indicates a titre level of 0.5 IU/ml.

Appendix A3: Supplementary information for chapter 5

All information presented here relates to chapter 5: A question of accessibility: modelling the influence of accessibility for vaccination on elimination of rabies in free-ranging dog populations in India.

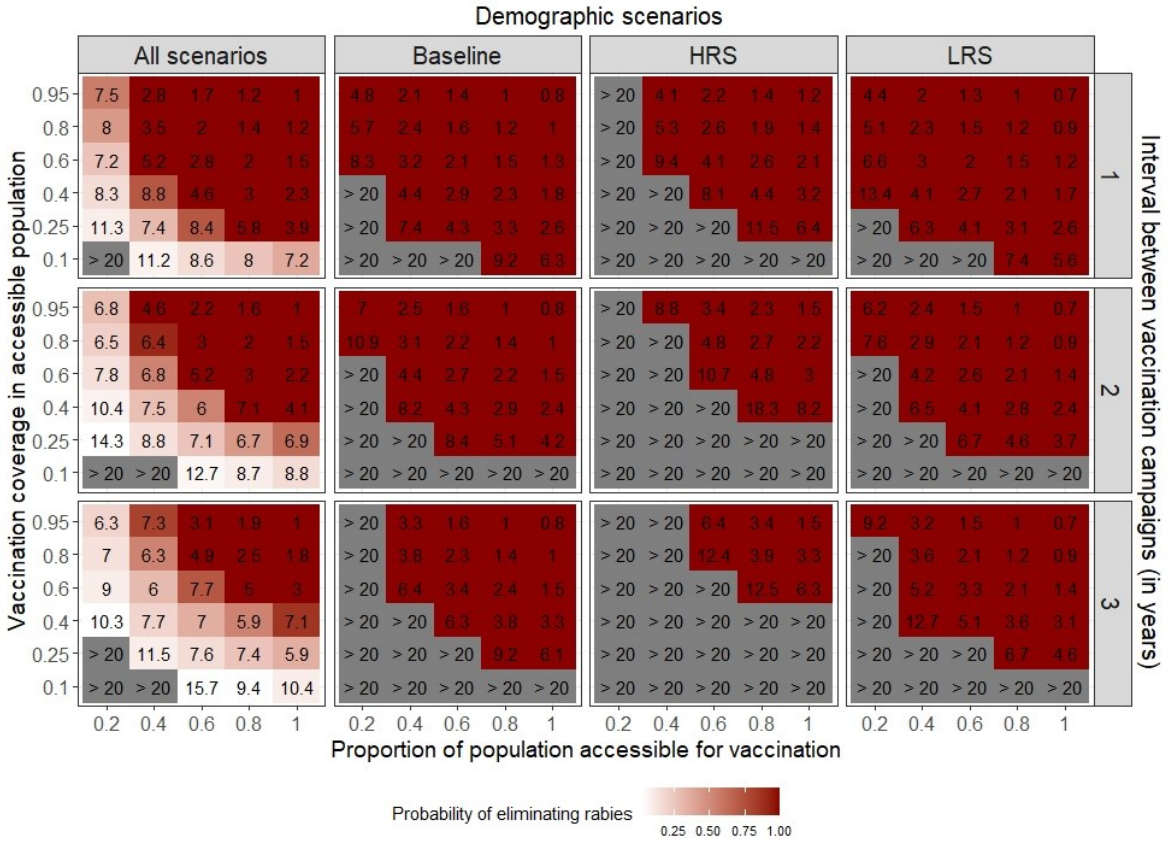


Fig S1. As in Fig. 5.5 (main text) but with R0 = 1.12 (instead of R0 = 1.48). Heatmap showing the mean time in years (figures within cells) for elimination (i.e. new rabies cases below 0.5 after implementation of MRV) in a low transmission setting , shown as a function of the proportion of population accessible for vaccination, the vaccination coverage in the accessible population, demographic scenarios and interval between vaccination campaigns (in years). The colour of each cell represents the probability of elimination. Cells in grey indicate that rabies elimination was possible only after more than 20 years after implementation of campaigns. Vaccine-induced immunity is assumed to last for one year.

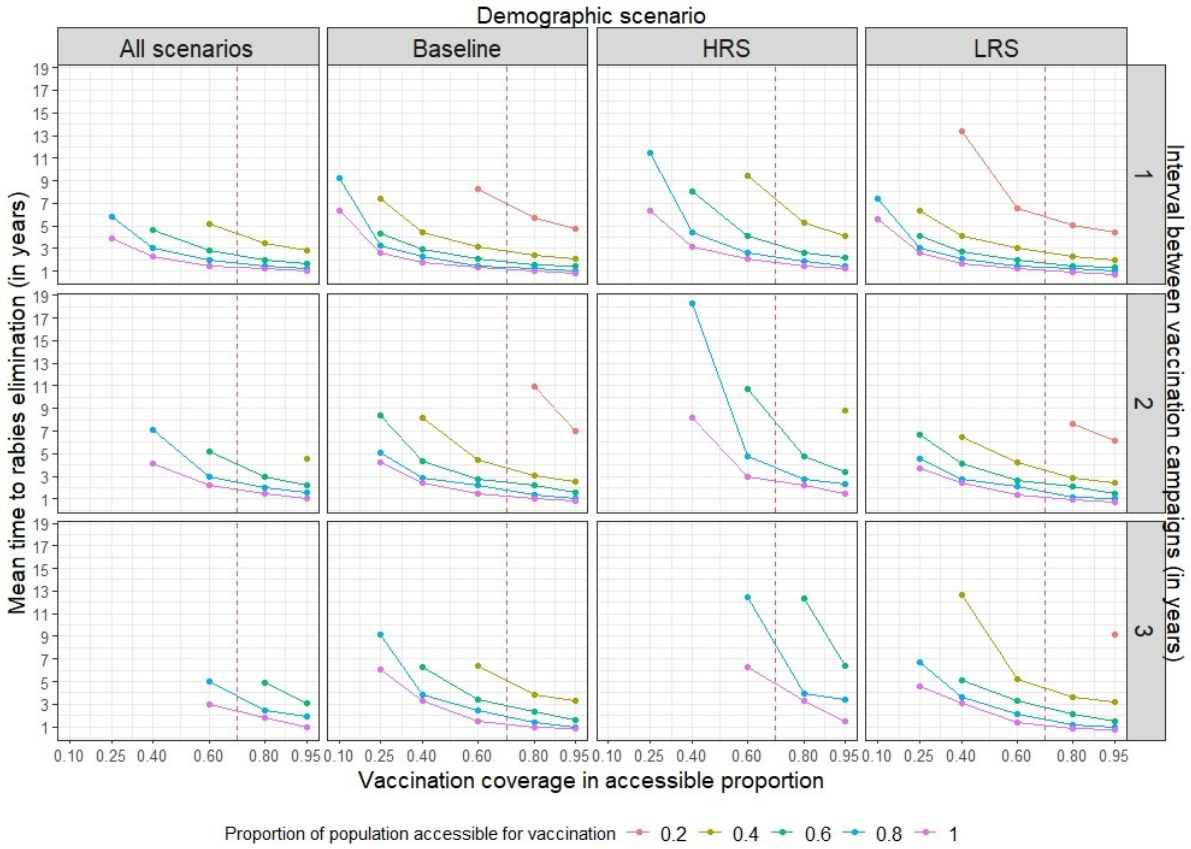


Figure S2. As in Fig. 5.4 (main text) but with R0 = 1.12 (instead of R0 = 1.48). The mean time to rabies elimination (in years) with vaccination coverage across various demographic scenarios in a low-transmission setting, with pulse mass rabies vaccination campaigns of 30 days each, conducted every one, two or three years and vaccine-induced immunity lasting for one year. Only the lowest vaccination coverages with a 100% probability of eliminating rabies are depicted. Black dashed vertical line – 70% vaccination coverage.

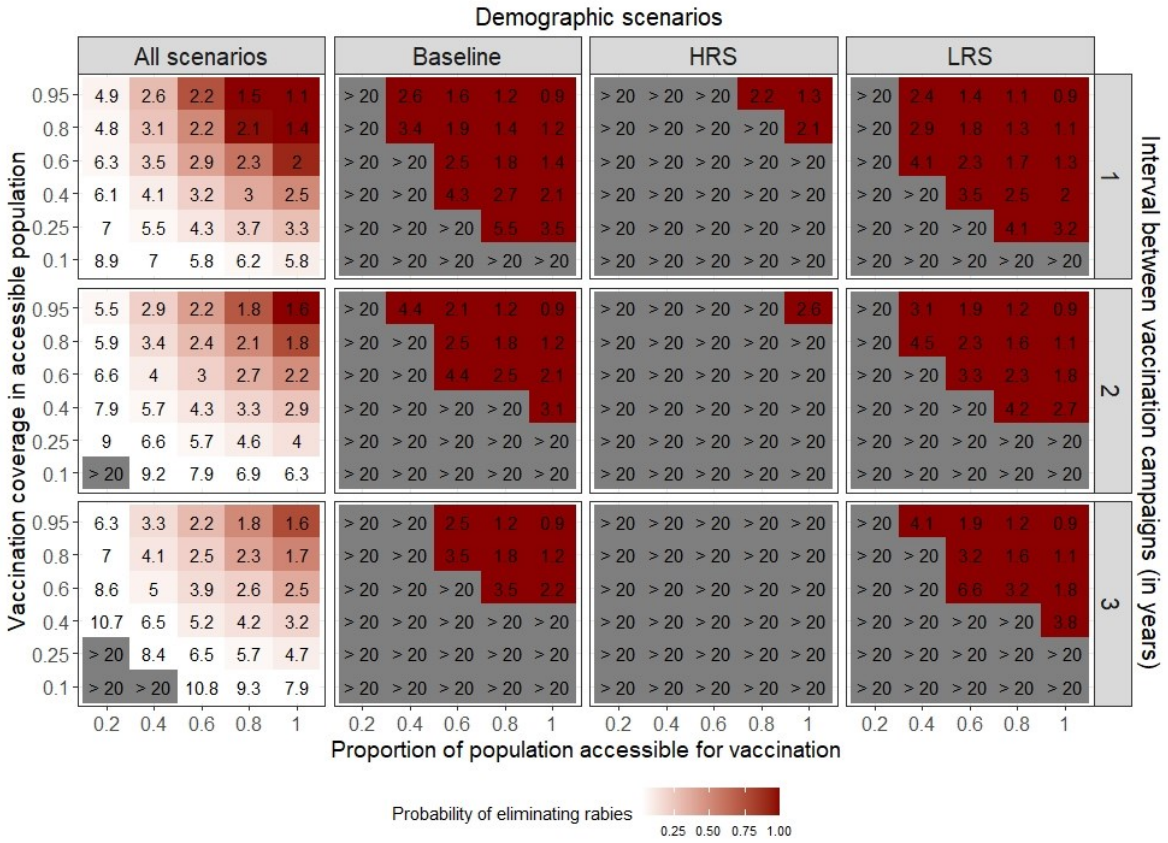


Fig S3. As in Fig. 5.5 (main text) but with R0 = 1.65 (instead of R0 = 1.48). Heatmap depicting the mean time in years (figures within cells) for elimination (i.e. new rabies cases below 0.5 after implementation of MRV) in a high transmission setting, shown as a function of the proportion of population accessible for vaccination, the vaccination coverage in the accessible population, demographic scenarios and interval between vaccination campaigns (in years). The colour of each cell represents the probability of elimination. Cells in grey indicate that rabies elimination was possible only after more than 20 years after implementation of campaigns. Vaccine-induced immunity is assumed to last for one year.

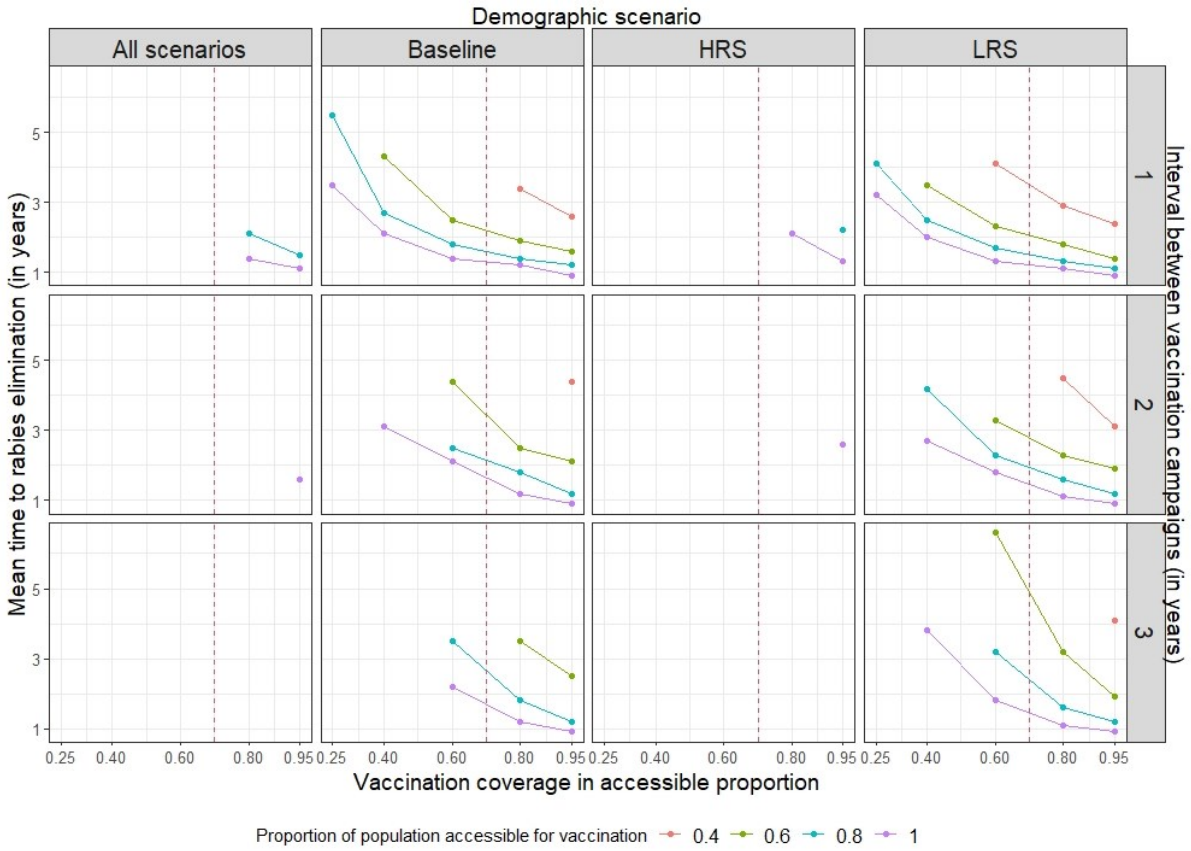


Figure S4: As in Fig. 5.4 (main text) but with R0 = 1.65 (instead of R0 = 1.48). The mean time to rabies elimination (in years) with vaccination coverage across various demographic scenarios in a high-transmission setting, with pulse mass rabies vaccination campaigns of 30 days each, conducted every one, two or three years and vaccine-induced immunity lasting for one year. Only the lowest vaccination coverages with a 100% probability of eliminating rabies are depicted. Black dashed vertical line – 70% vaccination coverage.

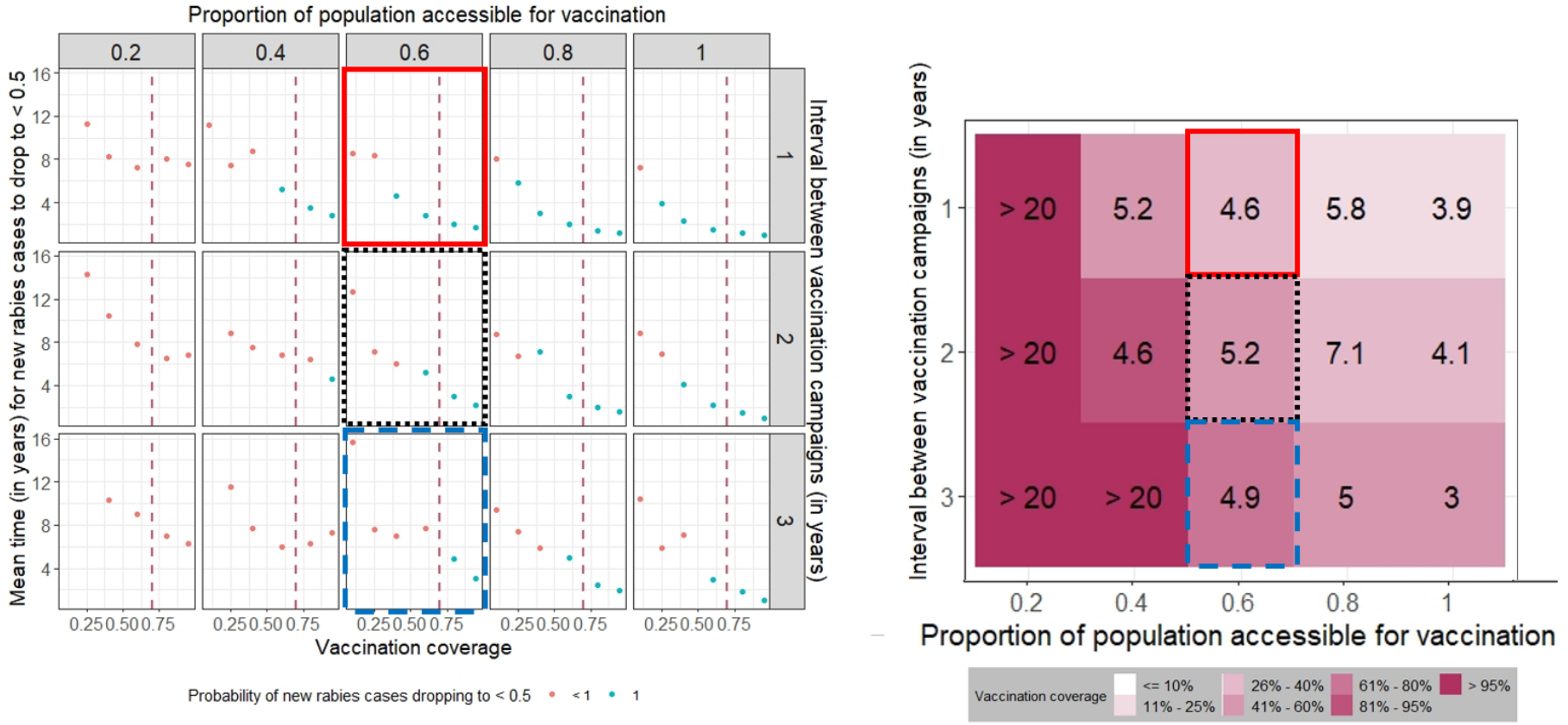


Figure S5: (Left) The mean time (in years) for new rabies cases to drop to < 0.5 cases per month (rabies elimination) after implementation of mass rabies vaccination campaigns in a low-transmission setting (R_0 =1.12) is shown as a function of targeted vaccination coverage in the accessible proportion of the population, the proportion of the population accessible for vaccination (acc) and interval between vaccination campaigns (in years) ($camp.int$). Points in light blue indicate vaccination coverages with a 100% probability of rabies elimination. The red vertical dashed line represents a vaccination coverage of 70%. Vaccine-induced immunity is assumed to last for one year. (Right) Heatmap representing the lowest vaccination coverage with a 100% probability of rabies elimination for each combination of $camp.int$ and acc, with figures within each cell representing the mean time (in years) required for elimination.

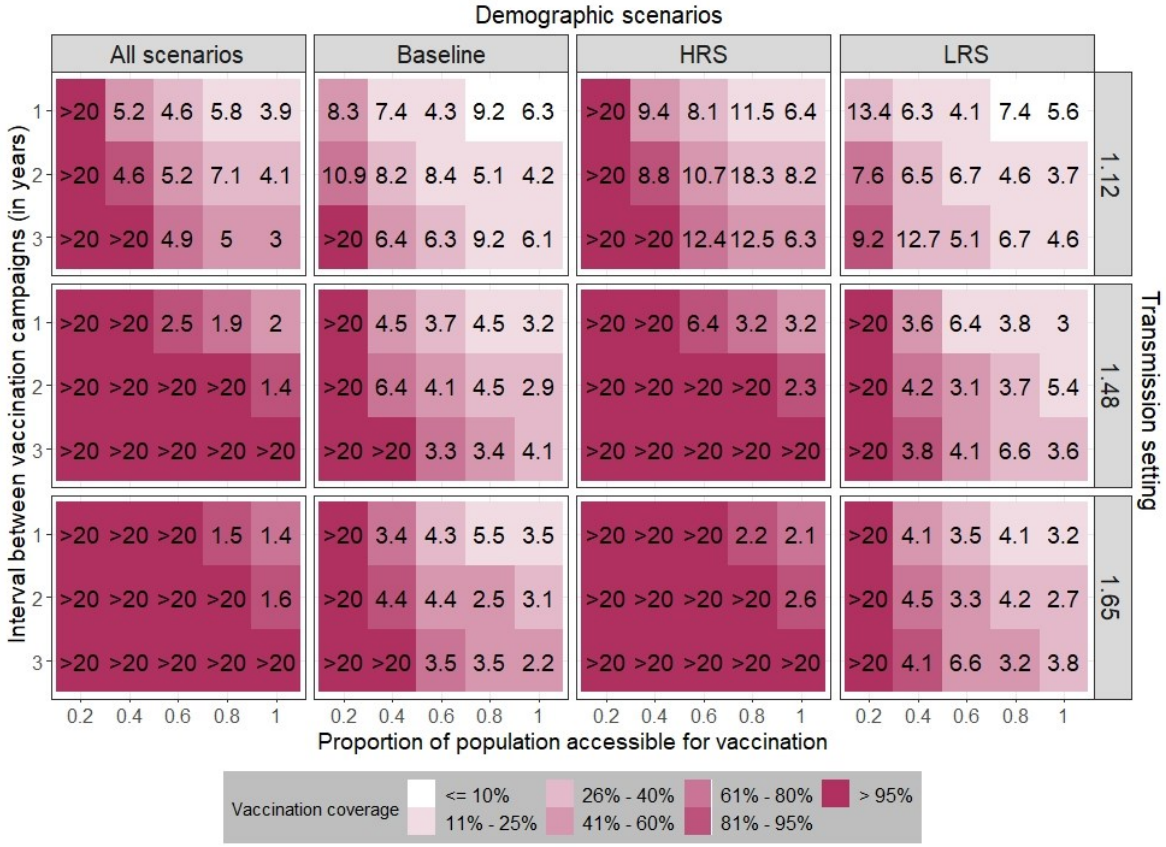


Figure S6: Heatmap of the lowest vaccination coverage levels at which the probability is 100% of eliminating rabies (defined as having a ten-year monthly average of zero rabies cases, ten years after implementation of mass rabies vaccination). Figures within each cell represent the mean time (in years) required for rabies elimination. The effects of campaigns being implemented every one, two or three years, with different proportions of the dog population accessible for vaccination, under different demographic scenarios and transmission settings are explored. Parameters used to define each of the three demographic scenarios (Baseline, HRS, LRS) are summarised in Table 4. Vaccine-induced immunity is assumed to last for one year.

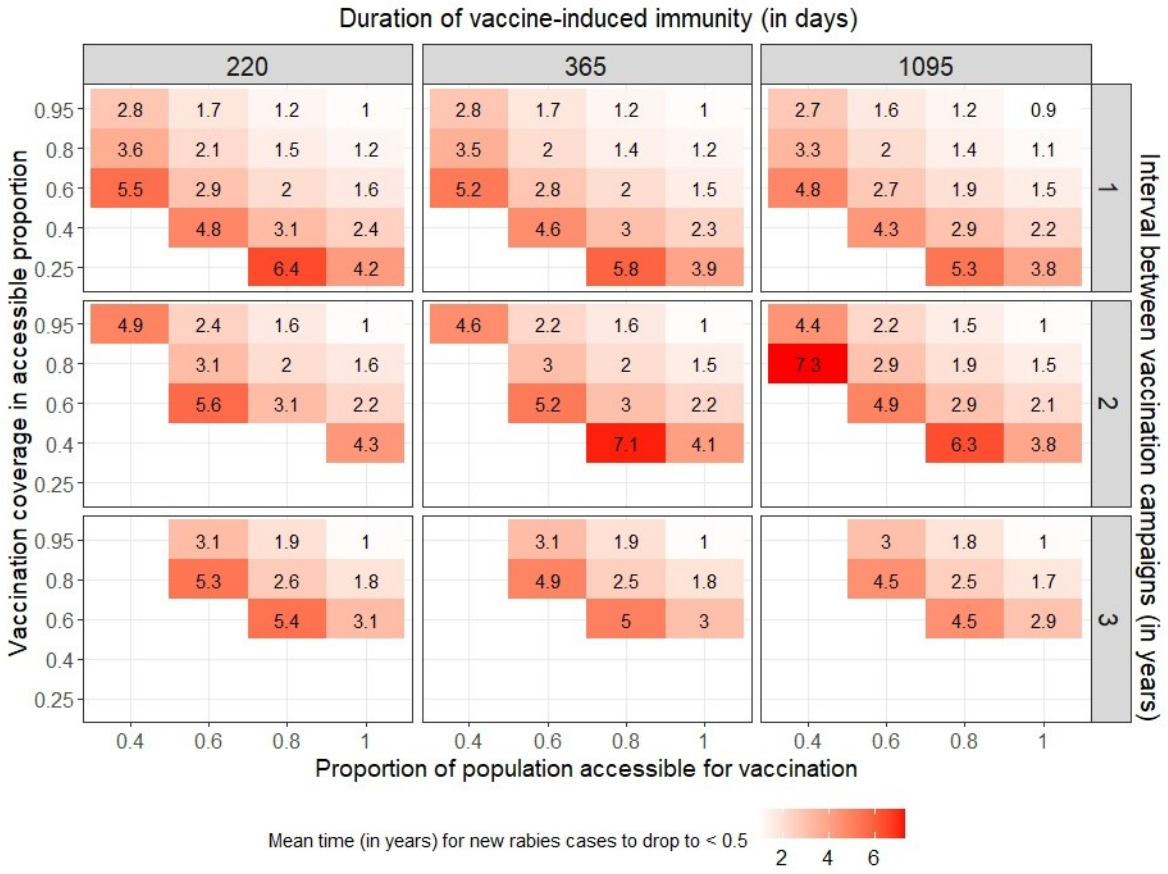


Fig S7. Heatmap depicting the mean time in years (figures within cells) for new rabies cases to drop below 0.5 (time to elimination) after implementation of mass rabies vaccination campaigns for all scenarios in a low transmission setting, shown as a function of the proportion of population accessible for vaccination, the lowest vaccination coverage in the accessible proportion that had a 100% probability of rabies elimination, duration of vaccine-induced immunity (in days) and interval between campaigns (in years). Empty cells without figures represent vaccination coverages and accessibility levels at which time to elimination was > 20 years

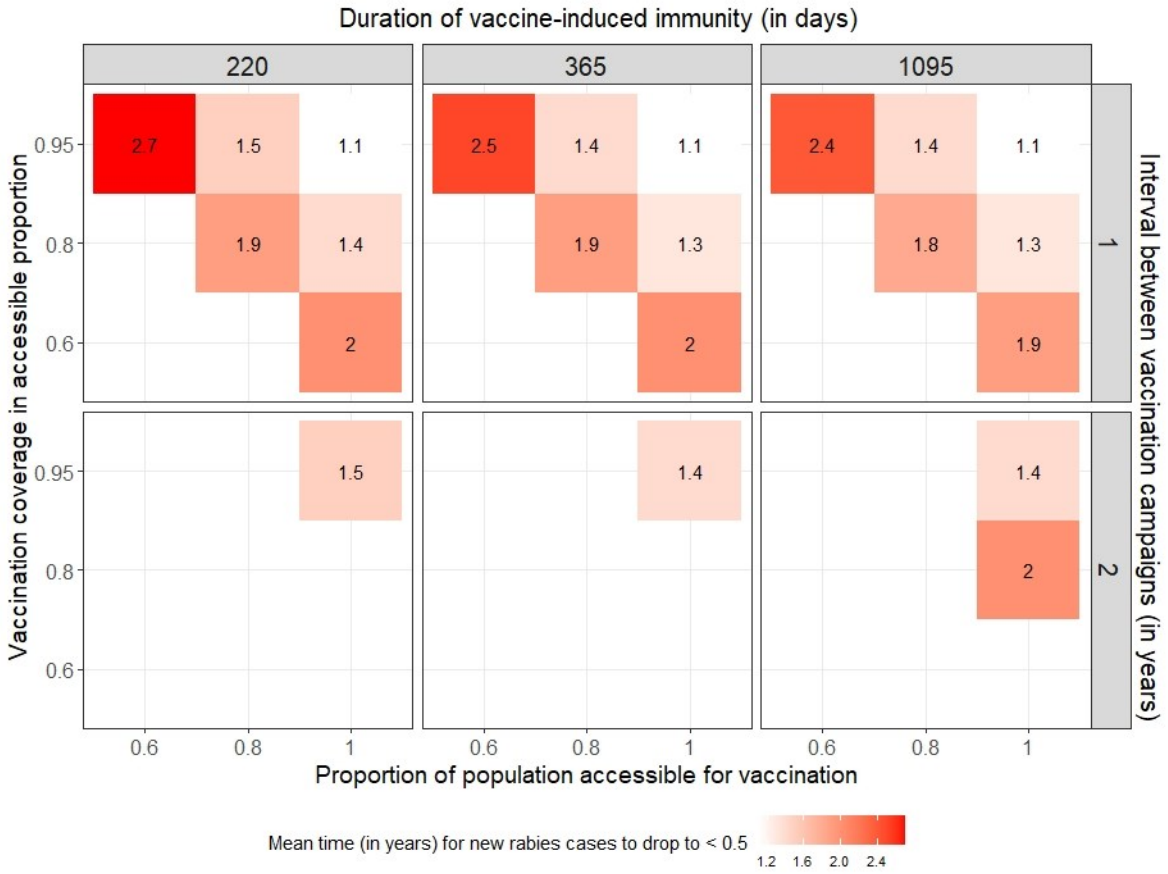


Fig S8. Heatmap depicting the mean time in years (figures within cells) for new rabies cases to drop below 0.5 (time to elimination) after implementation of mass rabies vaccination campaigns for all scenarios in a medium transmission setting, shown as a function of the proportion of population accessible for vaccination, the lowest vaccination coverage in the accessible proportion that had a 100% probability of rabies elimination, duration of vaccine-induced immunity (in days) and interval between campaigns (in years). Empty cells without figures represent vaccination coverages and accessibility levels at which time to elimination was > 20 years

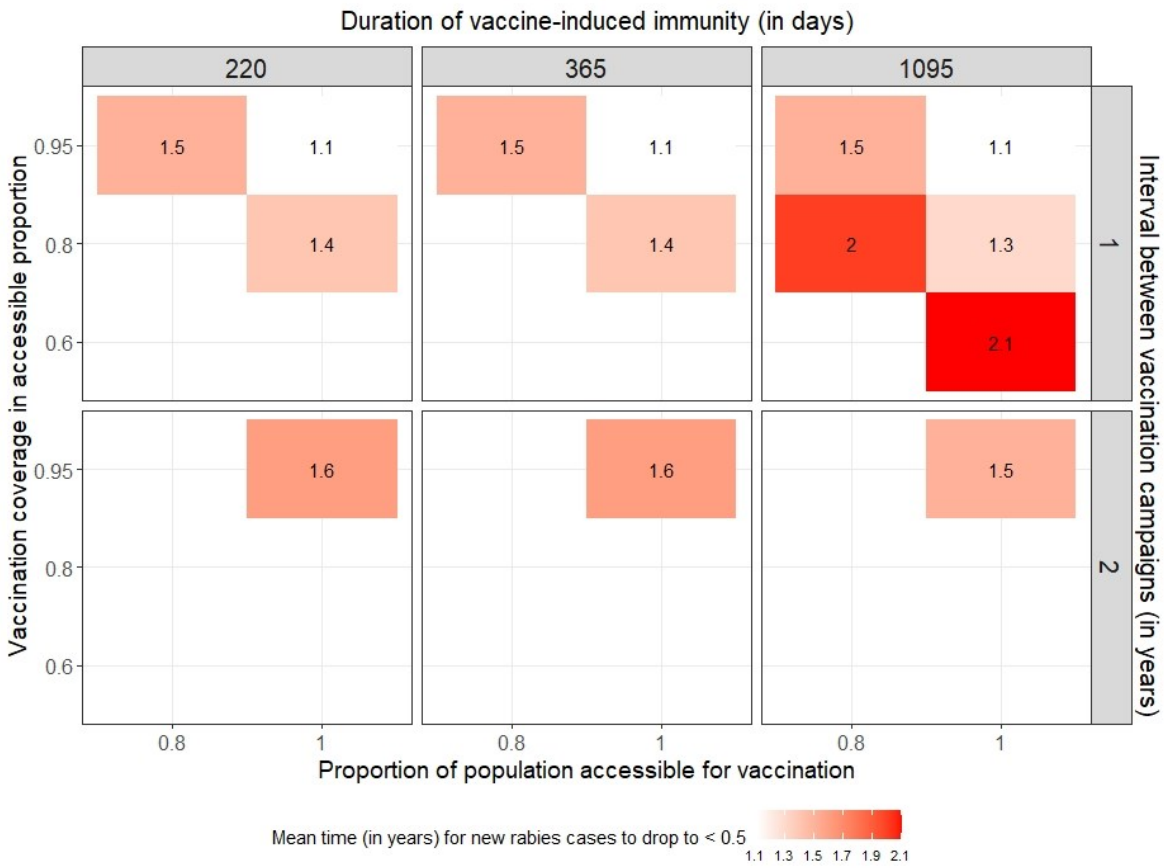


Fig S9. Heatmap depicting the mean time in years (figures within cells) for new rabies cases to drop below 0.5 (time to elimination) after implementation of mass rabies vaccination campaigns for all scenarios in a high transmission setting, shown as a function of the proportion of population accessible for vaccination, the lowest vaccination coverage in the accessible proportion that had a 100% probability of rabies elimination, duration of vaccine-induced immunity (in days) and interval between campaigns (in years). Empty cells without figures represent vaccination coverages and accessibility levels at which time to elimination was > 20 years

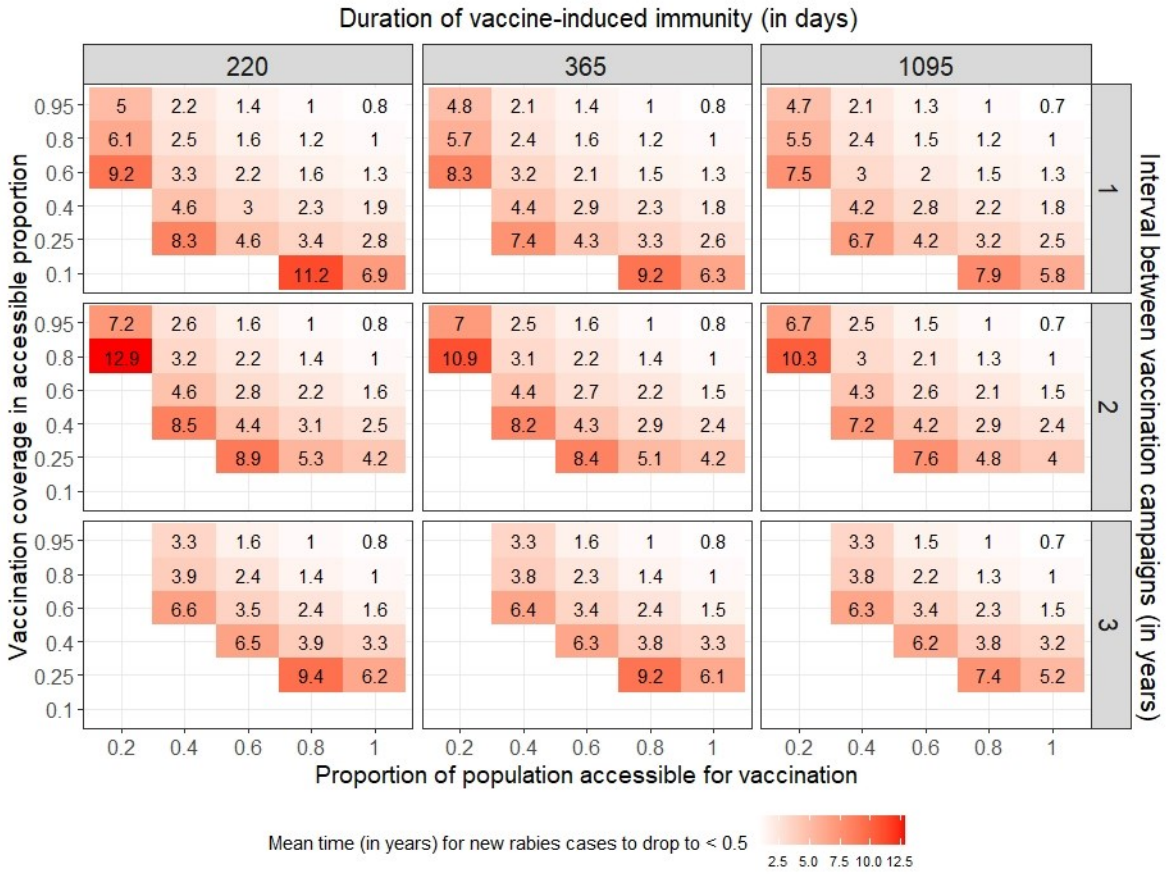


Fig S10. Heatmap depicting the mean time in years (figures within cells) for new rabies cases to drop below 0.5 (time to elimination) after implementation of mass rabies vaccination campaigns for the baseline scenario in a low transmission setting, shown as a function of the proportion of population accessible for vaccination, the lowest vaccination coverage in the accessible proportion that had a 100% probability of rabies elimination, duration of vaccine-induced immunity (in days) and interval between campaigns (in years). Empty cells without figures represent vaccination coverages and accessibility levels at which time to elimination was > 20 years

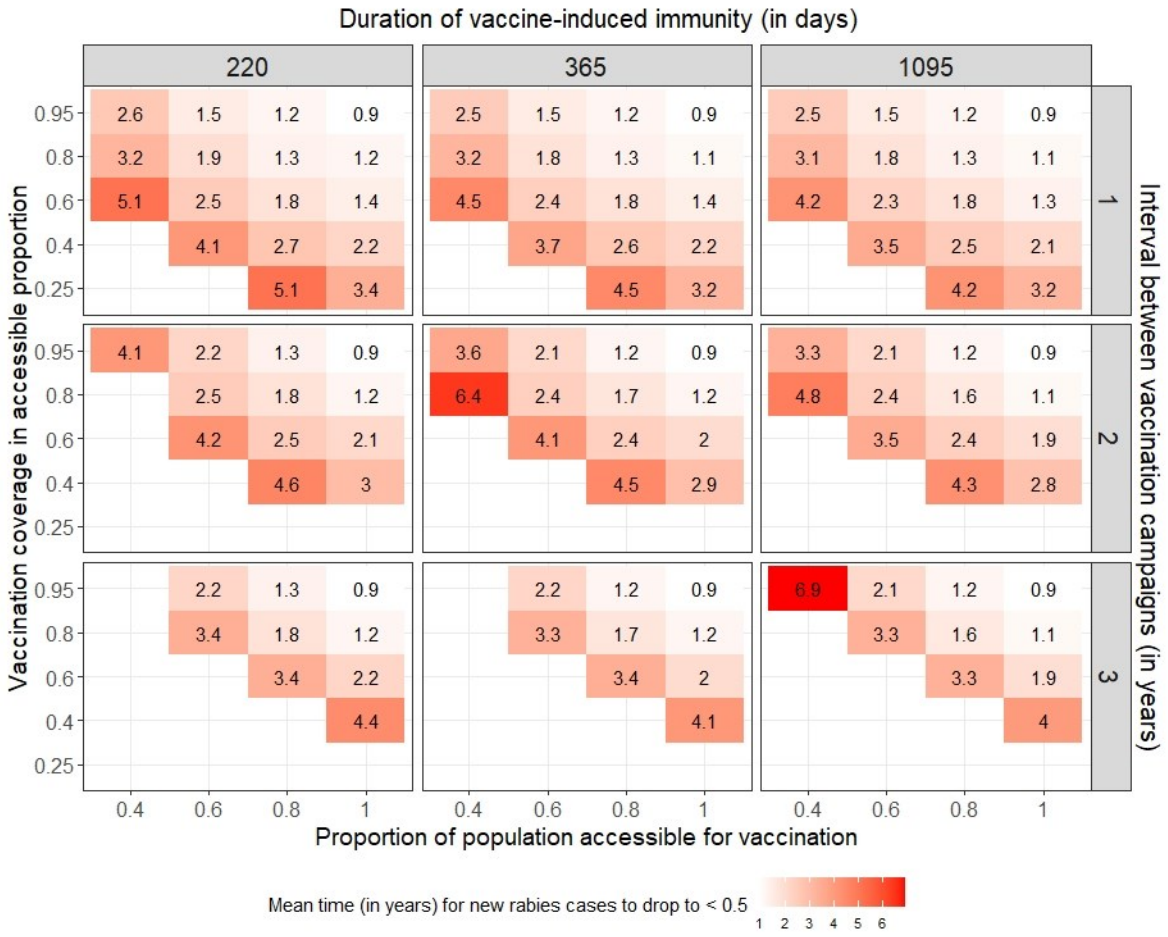


Fig S11. Heatmap depicting the mean time in years (figures within cells) for new rabies cases to drop below 0.5 (time to elimination) after implementation of mass rabies vaccination campaigns for the baseline scenario in a medium transmission setting, shown as a function of the proportion of population accessible for vaccination, the lowest vaccination coverage in the accessible proportion that had a 100% probability of rabies elimination, duration of vaccine-induced immunity (in days) and interval between campaigns (in years). Empty cells without figures represent vaccination coverages and accessibility levels at which time to elimination was > 20 years

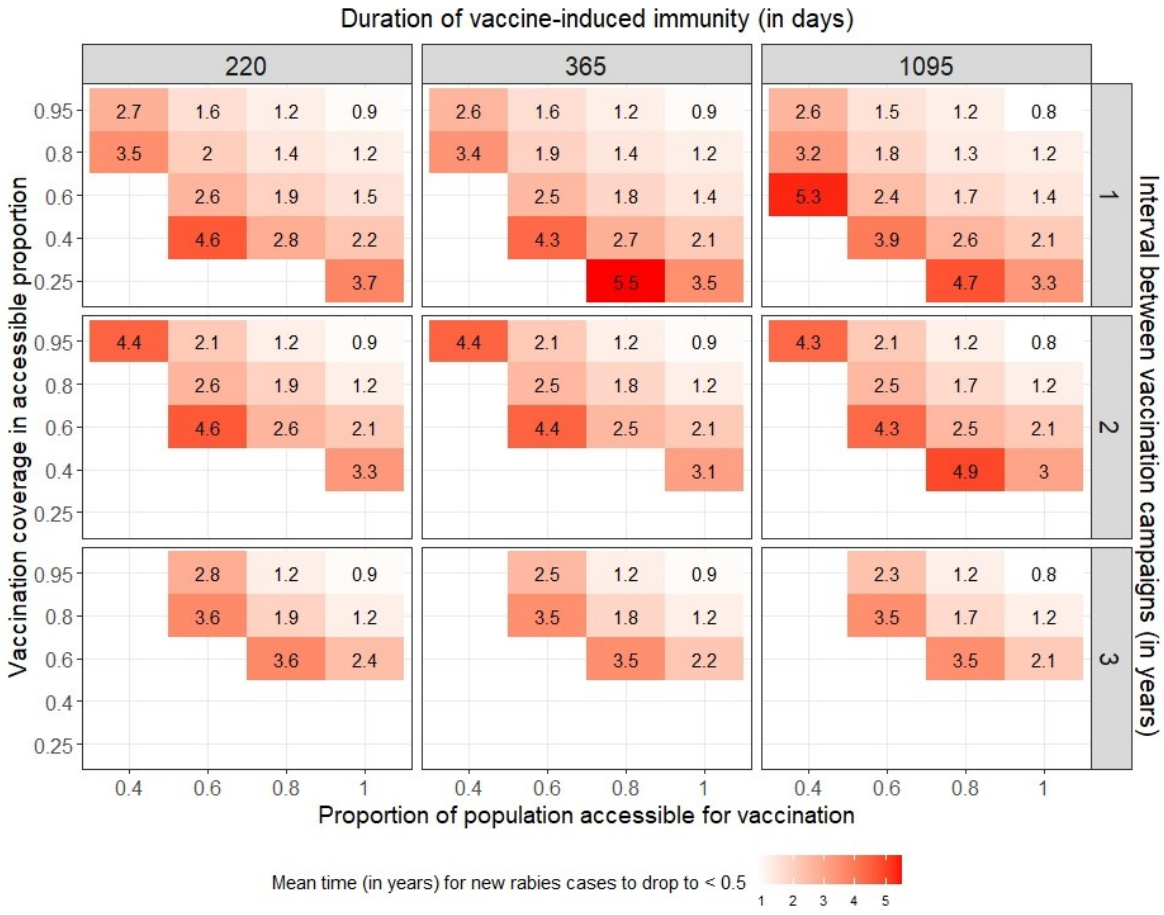


Fig S12. Heatmap depicting the mean time in years (figures within cells) for new rabies cases to drop below 0.5 (time to elimination) after implementation of mass rabies vaccination campaigns for the baseline scenario in a high transmission setting, shown as a function of the proportion of population accessible for vaccination, the lowest vaccination coverage in the accessible proportion that had a 100% probability of rabies elimination, duration of vaccine-induced immunity (in days) and interval between campaigns (in years). Empty cells without figures represent vaccination coverages and accessibility levels at which time to elimination was > 20 years

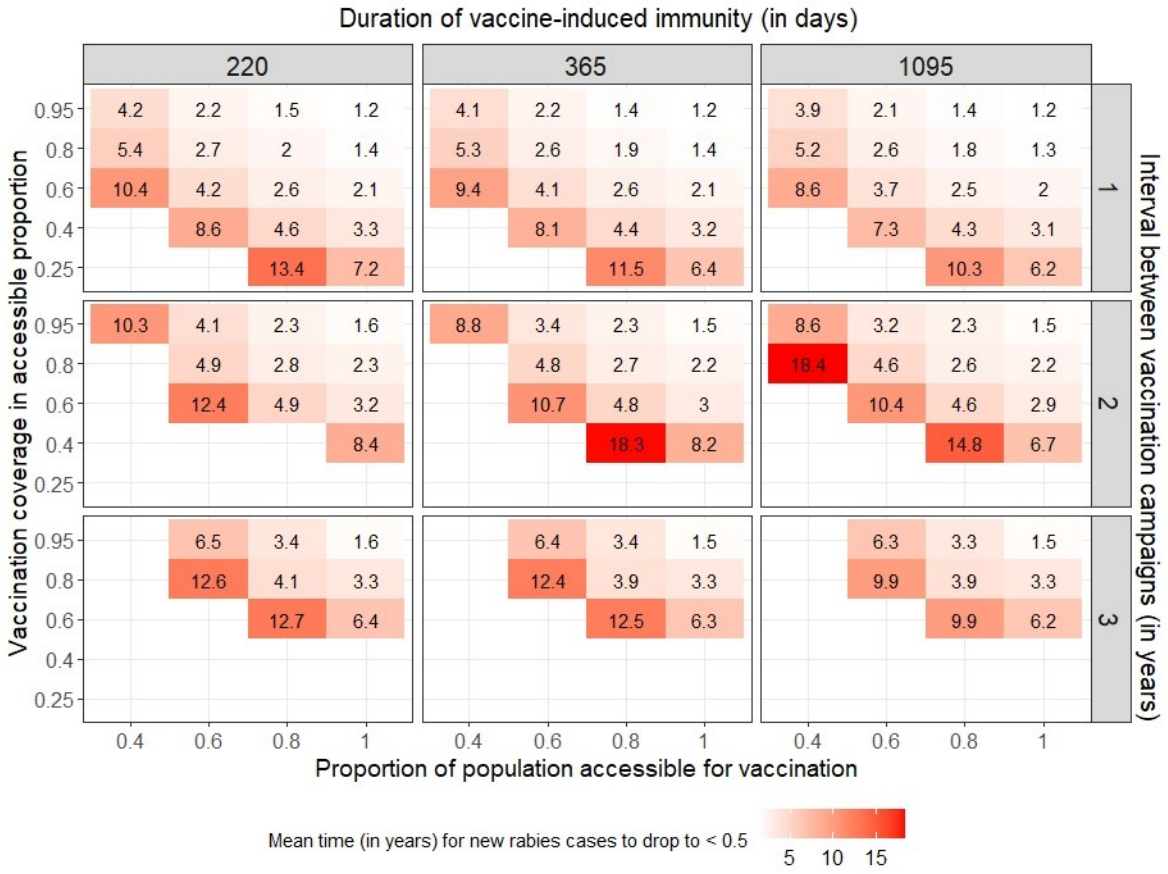


Fig S13 Heatmap depicting the mean time in years (figures within cells) for new rabies cases to drop below 0.5 (time to elimination) after implementation of mass rabies vaccination campaigns for the HRS scenario in a low transmission setting, shown as a function of the proportion of population accessible for vaccination, the lowest vaccination coverage in the accessible proportion that had a 100% probability of rabies elimination, duration of vaccine-induced immunity (in days) and interval between campaigns (in years). Empty cells without figures represent vaccination coverages and accessibility levels at which time to elimination was > 20 years

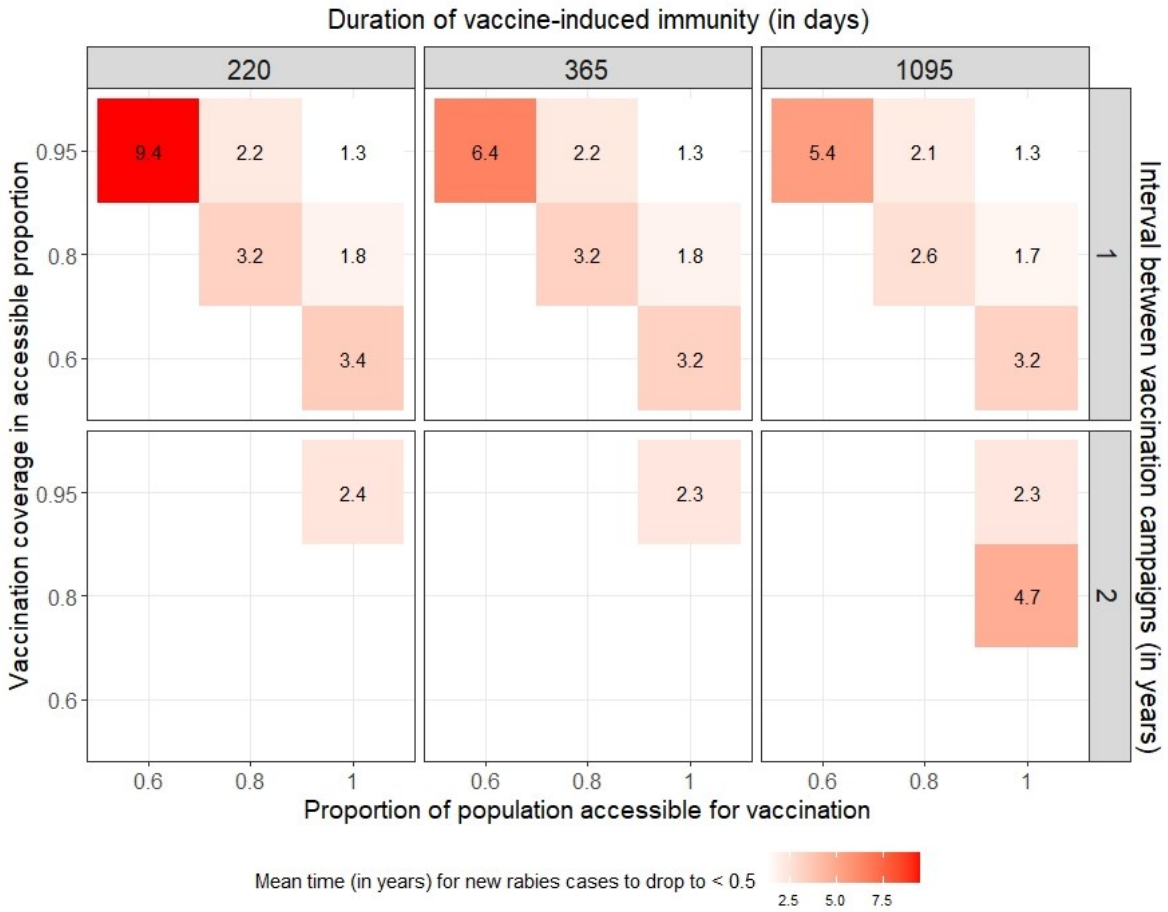


Fig S14. Heatmap depicting the mean time in years (figures within cells) for new rabies cases to drop below 0.5 (time to elimination) after implementation of mass rabies vaccination campaigns for the HRS scenario in a medium transmission setting, shown as a function of the proportion of population accessible for vaccination, the lowest vaccination coverage in the accessible proportion that had a 100% probability of rabies elimination, duration of vaccine-induced immunity (in days) and interval between campaigns (in years). Empty cells without figures represent vaccination coverages and accessibility levels at which time to elimination was > 20 years

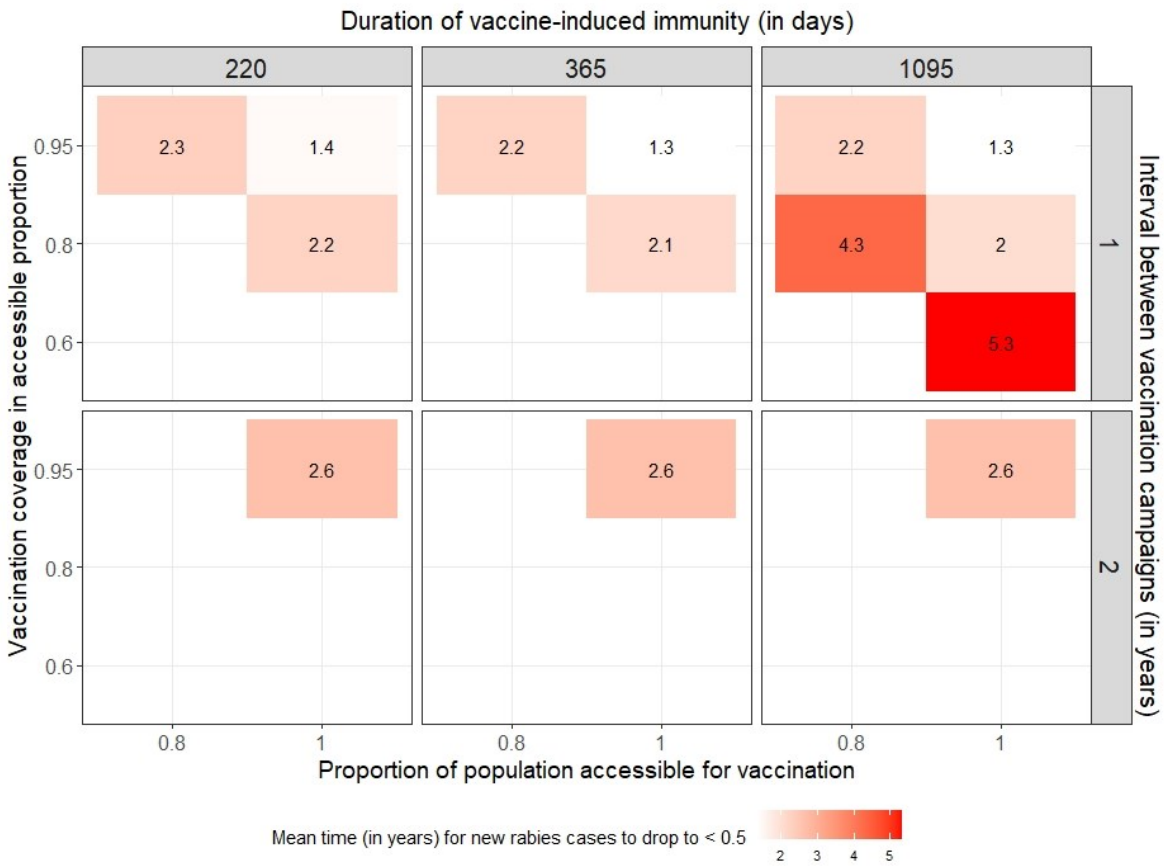


Fig S15. Heatmap depicting the mean time in years (figures within cells) for new rabies cases to drop below 0.5 (time to elimination) after implementation of mass rabies vaccination campaigns for the HRS scenario in a high transmission setting, shown as a function of the proportion of population accessible for vaccination, the lowest vaccination coverage in the accessible proportion that had a 100% probability of rabies elimination, duration of vaccine-induced immunity (in days) and interval between campaigns (in years). Empty cells without figures represent vaccination coverages and accessibility levels at which time to elimination was > 20 years

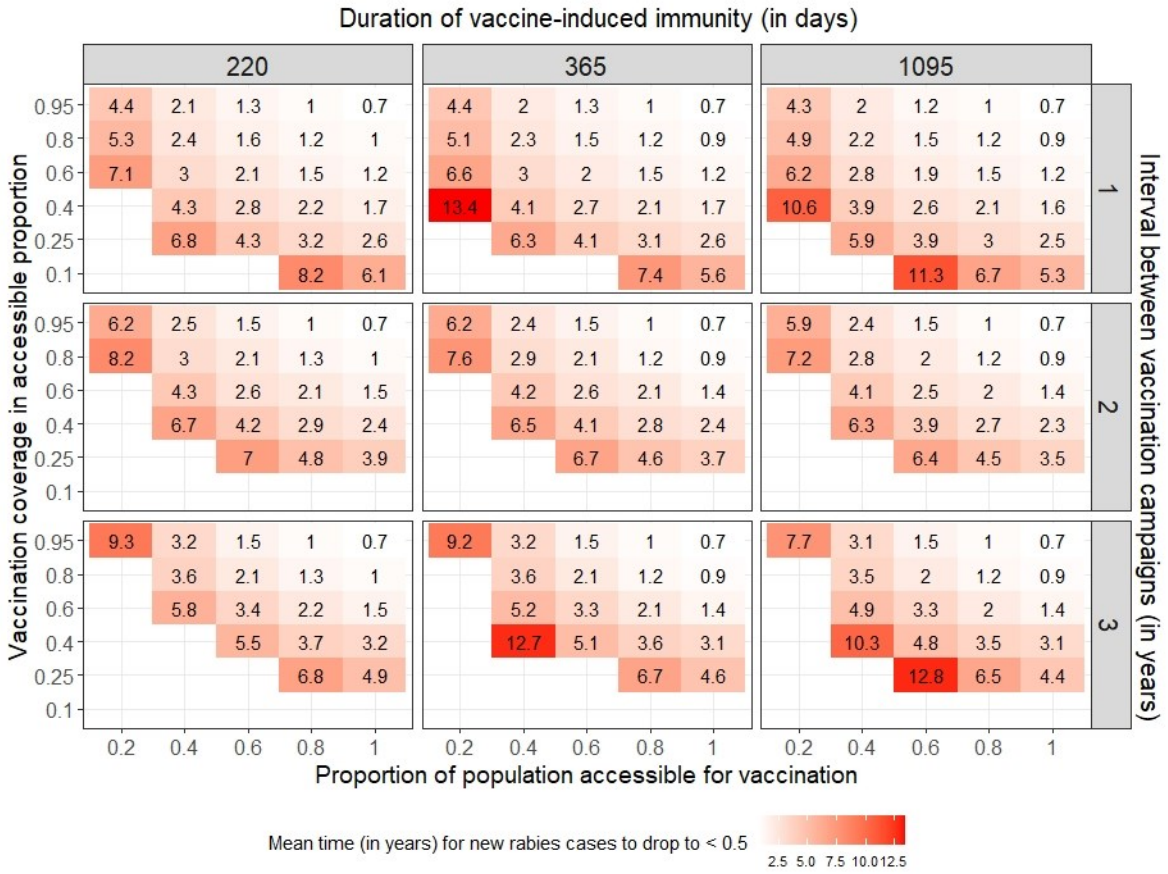


Fig S16. Heatmap depicting the mean time in years (figures within cells) for new rabies cases to drop below 0.5 (time to elimination) after implementation of mass rabies vaccination campaigns for the LRS scenario in a low transmission setting, shown as a function of the proportion of population accessible for vaccination, the lowest vaccination coverage in the accessible proportion that had a 100% probability of rabies elimination, duration of vaccine-induced immunity (in days) and interval between campaigns (in years). Empty cells without figures represent vaccination coverages and accessibility levels at which time to elimination was > 20 years

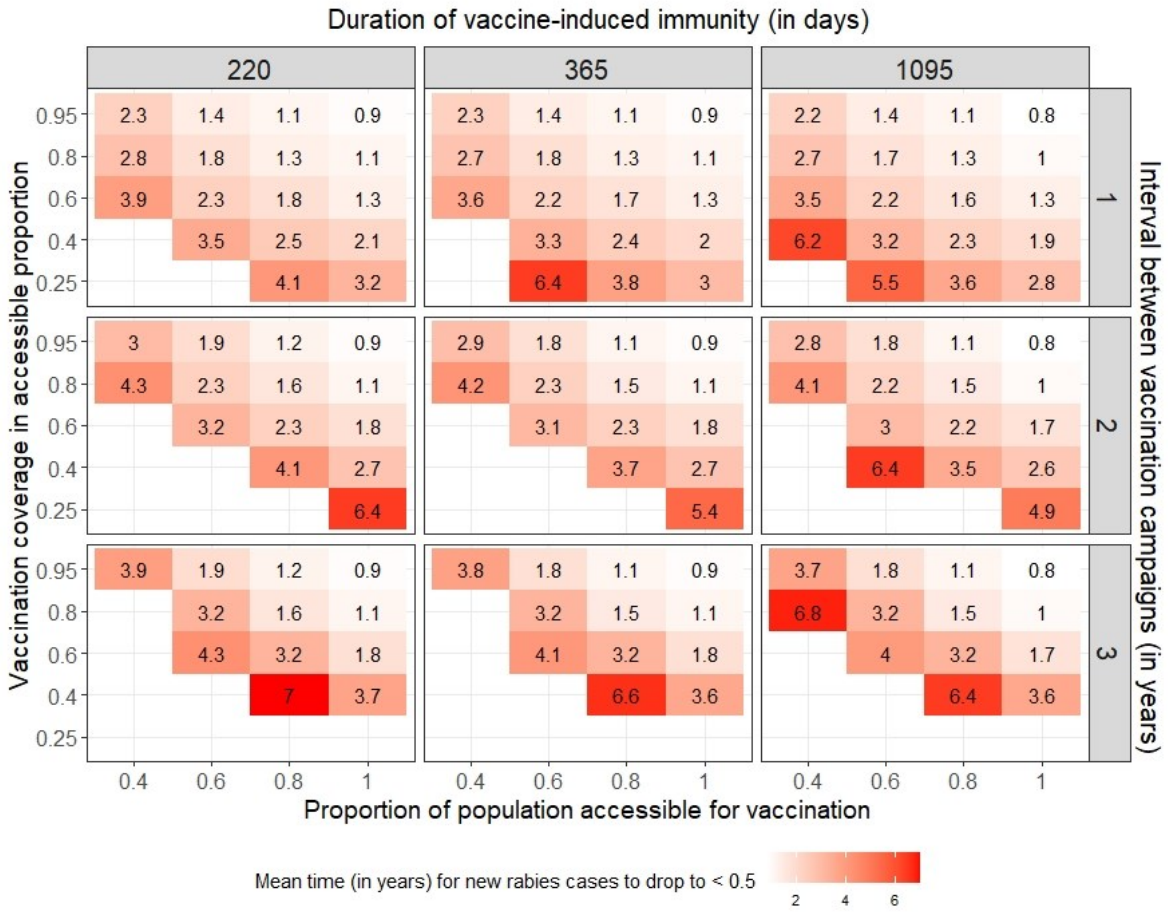


Fig S17. Heatmap depicting the mean time in years (figures within cells) for new rabies cases to drop below 0.5 (time to elimination) after implementation of mass rabies vaccination campaigns for the LRS scenario in a medium transmission setting, shown as a function of the proportion of population accessible for vaccination, the lowest vaccination coverage in the accessible proportion that had a 100% probability of rabies elimination, duration of vaccine-induced immunity (in days) and interval between campaigns (in years). Empty cells without figures represent vaccination coverages and accessibility levels at which time to elimination was > 20 years

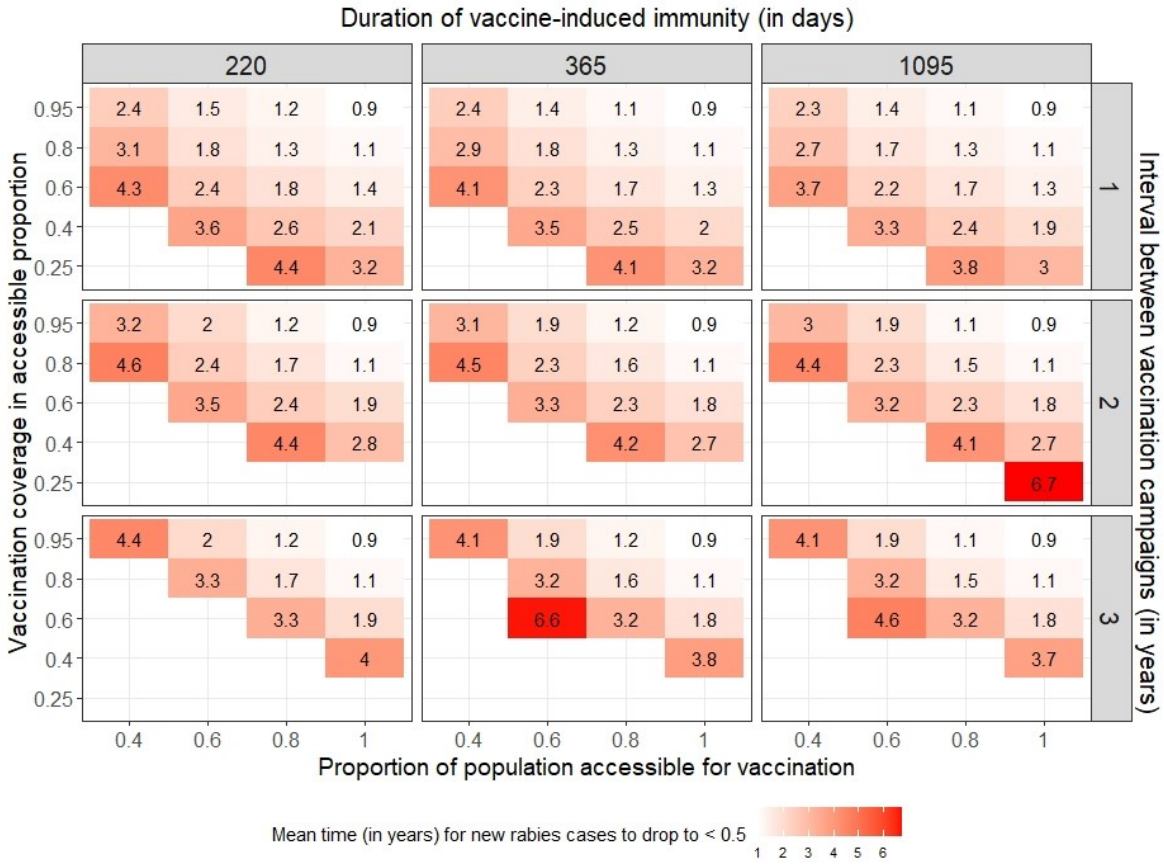


Fig S18. Heatmap depicting the mean time in years (figures within cells) for new rabies cases to drop below 0.5 (time to elimination) after implementation of mass rabies vaccination campaigns for the LRS scenario in a high transmission setting, shown as a function of the proportion of population accessible for vaccination, the lowest vaccination coverage in the accessible proportion that had a 100% probability of rabies elimination, duration of vaccine-induced immunity (in days) and interval between campaigns (in years). Empty cells without figures represent vaccination coverages and accessibility levels at which time to elimination was > 20 years

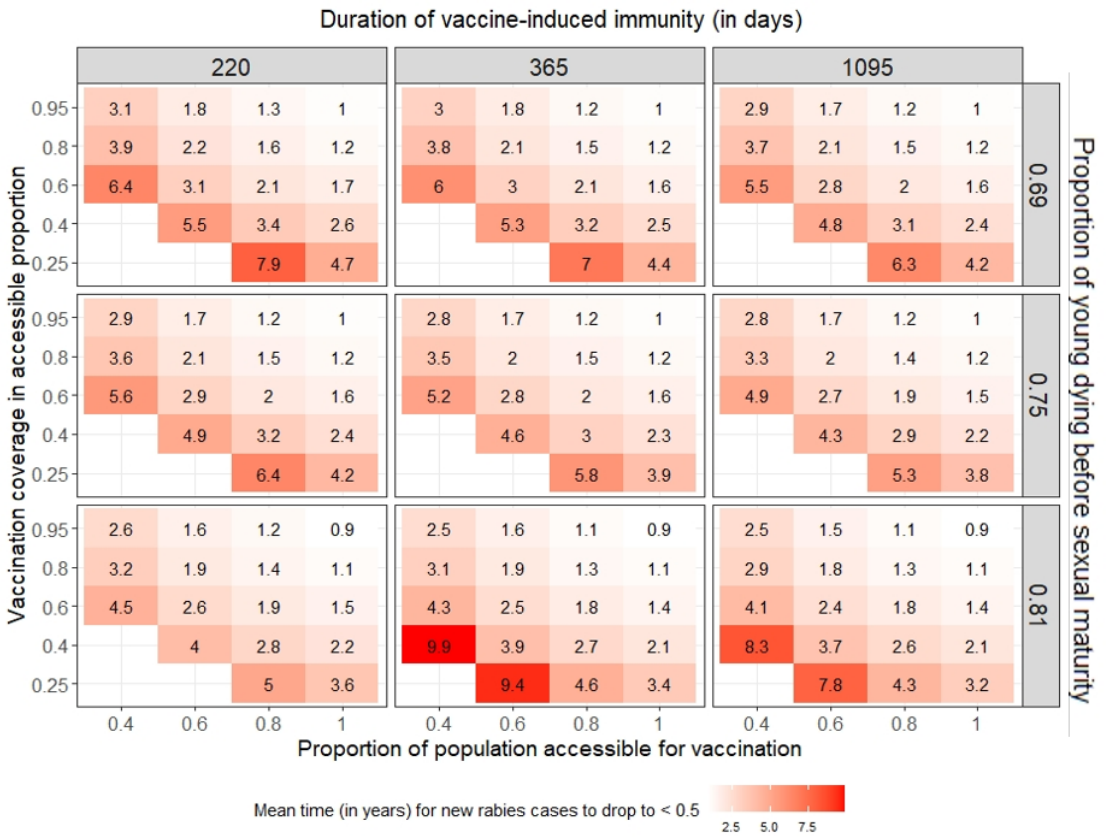


Fig S19. Heatmap depicting the mean time in years (figures within cells) for new rabies cases to drop below 0.5 (time to elimination) after implementation of mass rabies vaccination campaigns in a low transmission setting, shown as a function of the proportion of population accessible for vaccination, the lowest vaccination coverage in the accessible proportion that had a 100% probability of rabies elimination, duration of vaccine-induced immunity (in days) and the proportion of young dogs dying before sexual maturity.

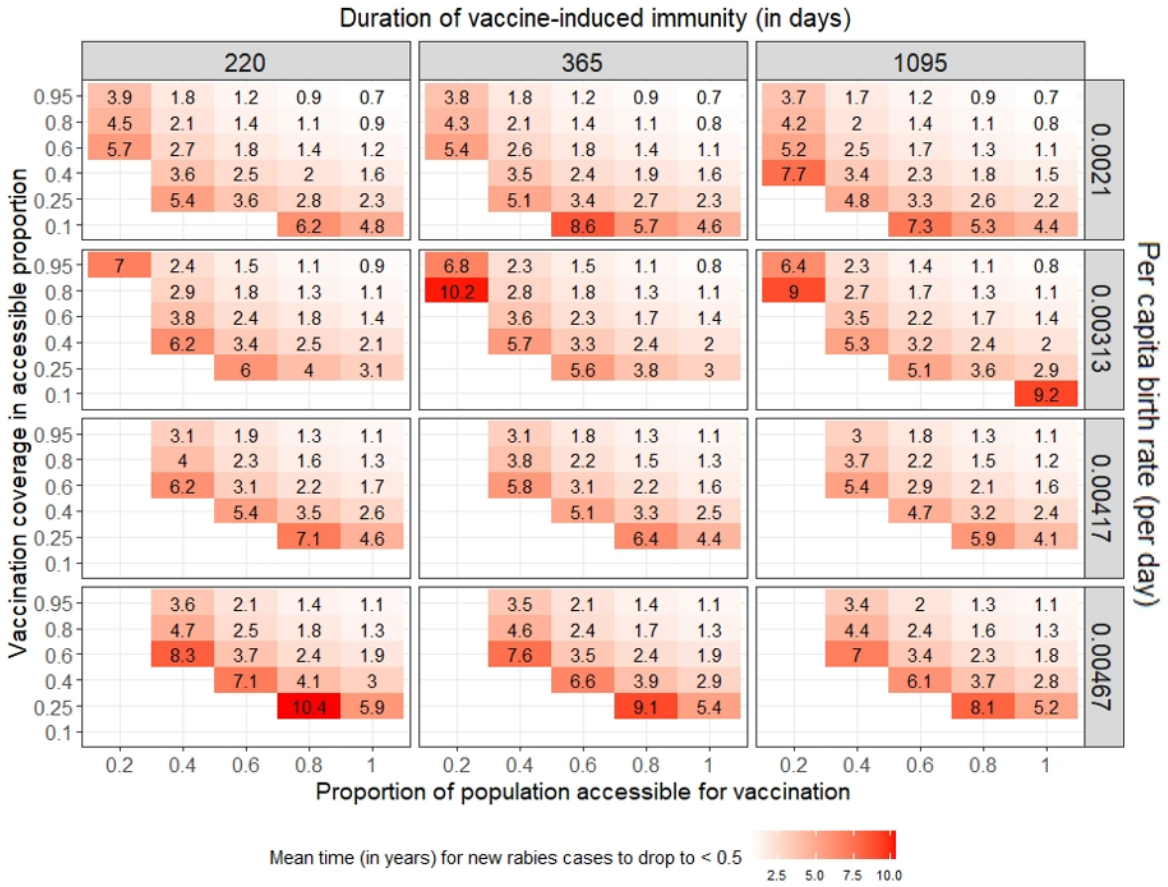


Fig S20. Heatmap depicting the mean time in years (figures within cells) for new rabies cases to drop below 0.5 (time to elimination) after implementation of mass rabies vaccination campaigns in a low transmission setting, shown as a function of the proportion of population accessible for vaccination, the lowest vaccination coverage in the accessible proportion that had a 100% probability of rabies elimination, duration of vaccine-induced immunity (in days) and per capita birth rate (per day).

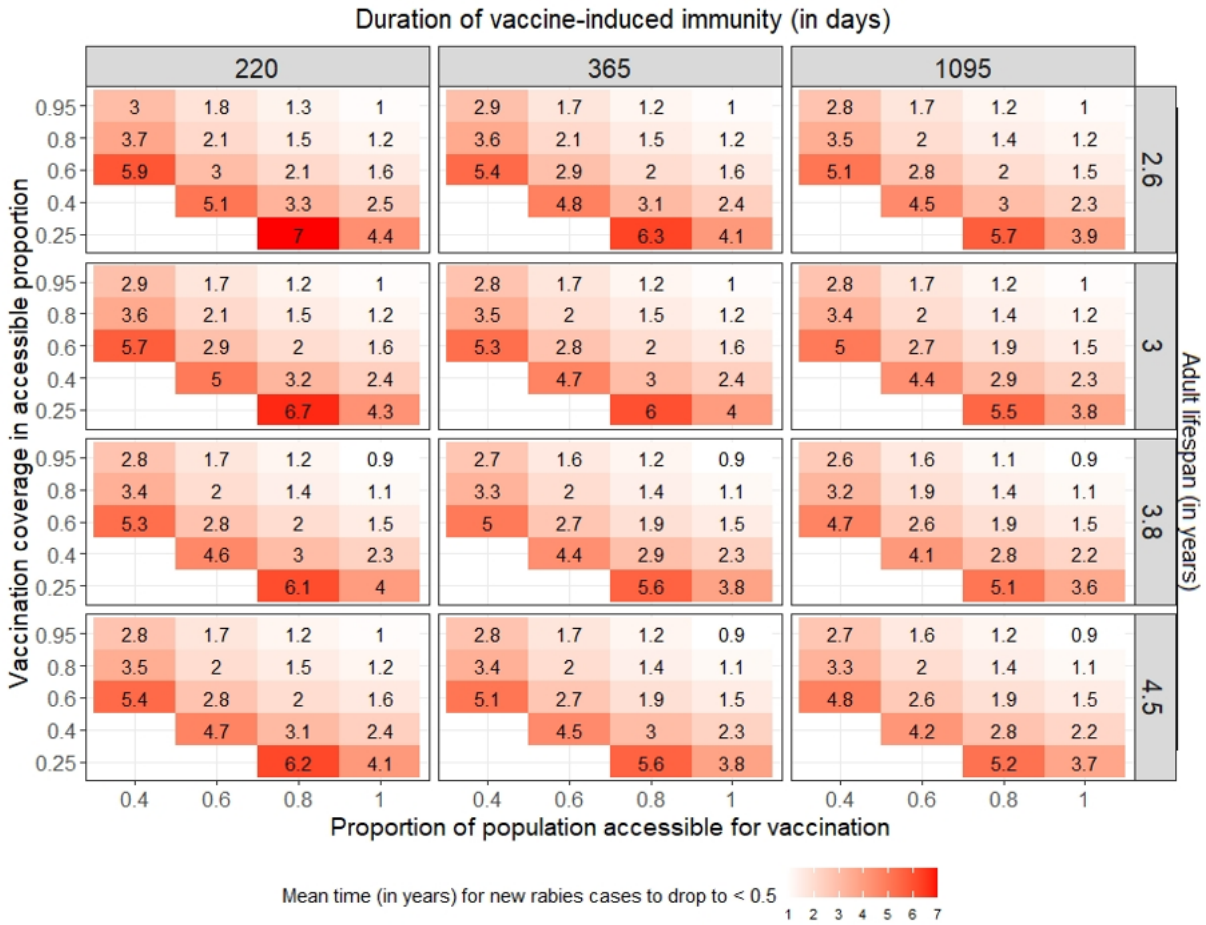


Fig S21. Heatmap depicting the mean time in years (figures within cells) for new rabies cases to drop below 0.5 (time to elimination) after implementation of mass rabies vaccination campaigns in a low transmission setting, shown as a function of the proportion of population accessible for vaccination, the lowest vaccination coverage in the accessible proportion that had a 100% probability of rabies elimination, duration of vaccine-induced immunity (in days) and average adult lifespan (in years).

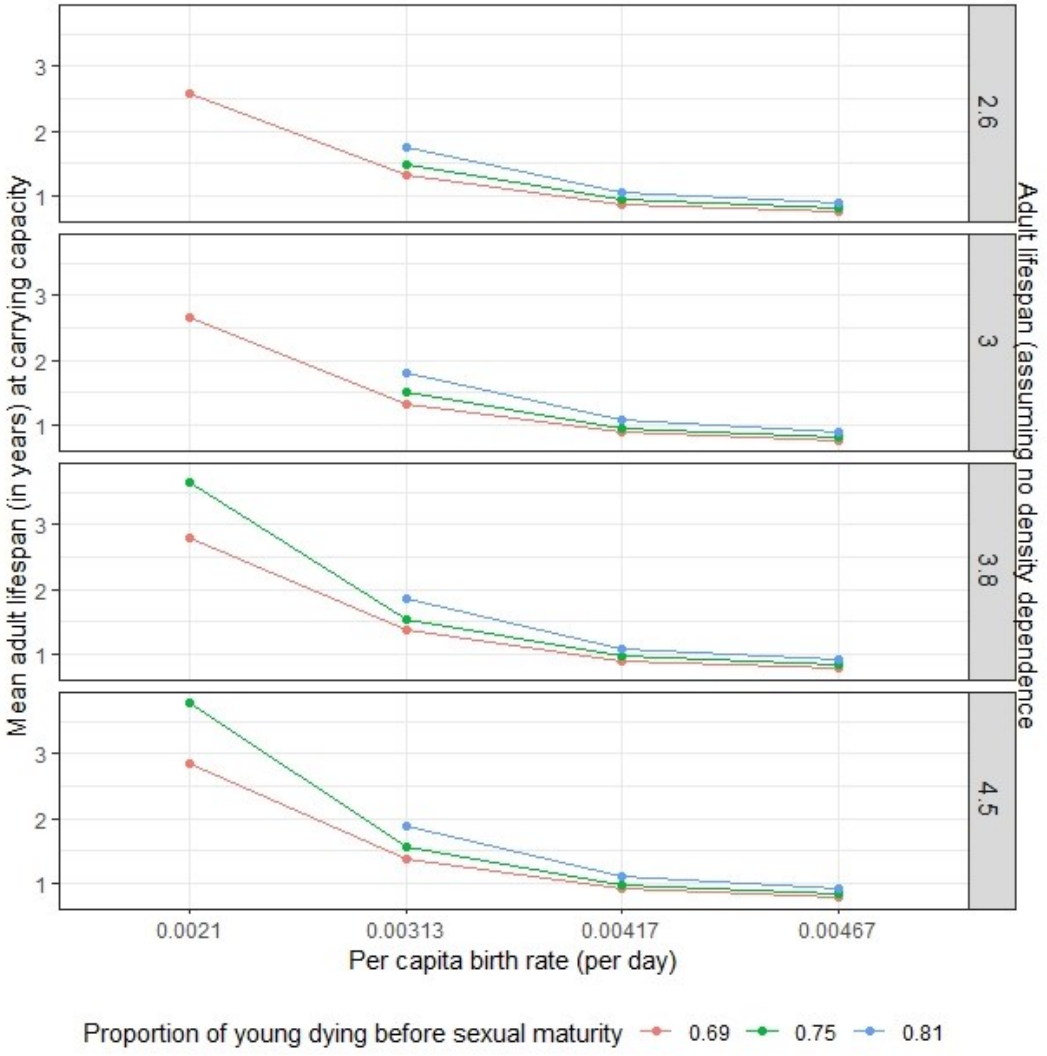


Fig S22. Scaling of mean adult lifespan (in years) at carrying capacity, with per capita birth rate (per day) and the proportion of young dogs dying before sexual maturity

Table S1. Influence of per capita birth rate and proportion of young dying before sexual maturity on mean adult lifespan at carrying capacity (in years). HRS – High recruitment scenario, LRS – Low recruitment scenario

Demographic scenario	Birth rate (per capita per day), b	Proportion of young dying before sexual maturity	Adult life span (in years), $1/d^A$ (assuming no density dependence)	Mean adult life span (in years), $1/(d^A + r)$ (at carrying capacity)
Baseline	0.00313	0.81	3	1.80
HRS	0.00467	0.69	4.5	0.78
HRS-2	0.00467	0.81	2.6	0.89
HRS-3	0.00467	0.81	4.5	0.92
LRS	0.00313	0.81	2.6	1.76
LRS-2	0.00313	0.69	4.5	1.38

Appendix A4: Analyses of rabies Knowledge, Attitudes and Practices (KAP) data

Introduction

The WHO recommends mass vaccination of at least 70% of the dog population in a region for rabies control and elimination. Studies in countries in Africa and Asia have shown that this is feasible, since most free-ranging dogs were owned, making them accessible for vaccination. However, achieving this coverage in India remains a challenge without knowledge about the nature of free-ranging and owned dog populations and ownership practices.

This cross-sectional study conducted surveys at the household level at two study sites in Kerala, India to evaluate public knowledge, attitudes and practices (KAP) in relation to rabies prevention and control using semi-structured open-ended questionnaires.

The survey of ownership and rearing practices can provide insights into key factors influencing rabies transmission risk in dogs, such as population-level rabies vaccination coverage and confinement of owned dogs. In combination with the results of on-going fieldwork conducted at these sites from October 2018, the findings from these surveys will help to determine accessibility for rabies vaccination of free-ranging and owned dogs and identify gaps in public awareness about rabies prevention and control. All the findings will inform the design and interpretation of mathematical models of rabies transmission and control.

Methods

A proportionate, stratified random sample of at least 265 household addresses will be surveyed over both study sites, based on the proportion of households in each ward (details in table below) or ward size.

Study site	Muhamma panchayat	Alappuzha Municipality
Number of wards	16	52
Number of households*	6446	57415
Domestic (owned) dog population**	887	3448
Expected frequency of dog ownership	0.1376	0.06005
Number of households to be surveyed, at a 5% margin of error and 95% confidence level	178	87

*Census of India data (2011)

**Animal census – Kerala state Animal Husbandry department (2012)

Household data held by the local bodies (panchayat / municipality) comprised the sampling frame. Residents of selected addresses were surveyed irrespective of length of stay or their identities. If selected households could not be surveyed (lack of consent/absence of members over 18 years /logistical constraints), the next closest household was chosen. No financial incentives were offered. During visits to selected households, the household head or another responsible adult over the age of 18 (the 'participant') was interviewed. Before the survey, they will be informed about the purpose of the dog ownership questionnaire and KAP surveys, why their household has been chosen and what kinds of questions will be asked. Where phone numbers are available, selected households may be telephoned in advance of the visit to inform them of these details and to seek their consent to visit them. They will be offered the Participant Information Sheet (PIS) and informed consent form (ICF) (drafts of English version attached). Illiterate participants or those unable to read will have the contents of the PIS and ICF explained to them to ensure they are fully understood. Participants will be informed of what personal data will be collected, data anonymisation, access and sharing with other parties. It will be explained that there are no right or wrong answers, that they can refuse to respond to any question and can withdraw at any point during the survey, without giving any reason for doing so or

any effect on their statutory rights or access to government benefits. They will also be given the opportunity to ask questions and clarify doubts.

Where the participant is willing to sign the ICF and participate in one or both of the surveys (dog ownership and KAP), their signature will be collected in two copies of the ICF. One copy will be returned to the them and the second retained by the surveyor. Following this, the surveys will be conducted immediately. However, the household may be visited again at a future date and time, agreed upon in consultation with the head / members, in the following circumstances:

1. where more time is requested to decide whether to participate and/or sign the ICF,
2. the participant is unable to respond to questions on that visit,
3. absence of household head or another responsible adult over 18 years of age.

In these circumstances, the phone number of Sreejith Radhakrishnan will be provided to the respondent (also included in the PIS).

All communication (verbal, and written), will be in the local language Malayalam. Sreejith Radhakrishnan is a native speaker of Malayalam, and additional surveyors selected from the local college will also be native Malayalam speakers. Before the surveys, the questionnaires, PIS and ICF will be translated into Malayalam and reviewed by a local person (e.g. a local councillor) to ensure clarity and ease of understanding.

Personal data that may be collected at each household include religion, educational level and socio-economic status. Thus, participant anonymity and data protection are highly important. Measures to address and mitigate these ethical concerns are detailed in Sections 7 and 14. In short, any personal data will be collected only after obtaining specific written informed consent, and participants will be given the option to refuse to provide this information in the ICF.

Researcher / surveyor safety is not considered to be of concern at these field sites. Fieldwork is being carried out at these sites since October 2018, and many residents and elected representatives are

familiar with the study and the lead researcher Sreejith Radhakrishnan. Measures to mitigate potential risks are described in detail in Section 13.

Undergraduate and postgraduate students from the local college (SD College, Alappuzha) previously assisted during dog population surveys (January 2019) as part of Sreejith's PhD research. Students will be recruited to assist in the household surveys, in consultation with college authorities, and provided training in the survey methodology, interacting with household members and ensuring their own safety. They will also be provided a printed script of the survey questions in Malayalam, to ensure questions/responses are not misinterpreted or misquoted. To ensure their safety, all surveys will be conducted by a pair of surveyors and may be in the company of a local guide, unless determined by Sreejith to be unnecessary.

To improve the quality of the questionnaires and make them more relevant to the Indian situation, previous versions of the dog ownership questionnaire and KAP survey were piloted with members of the public and elected representatives in a Public-Patient Involvement (PPI) format (July 2018, summary of interactions attached), and questions have been framed while incorporating feedback from these interactions. The PIS and ICF were reviewed by members of a Public Involvement panel of the Health Protection Research Unit (HPRU) in Modelling Methodology of the National Institute for Health Research (NIHR) on August 22nd, 2019. Feedback from this meeting were also incorporated into these forms.

The Knowledge, Attitudes and Practices (KAP) survey was adapted from Sambo et al. (2014) [383] to assess

(1) knowledge of rabies, its transmission and outcome, species affected, and means of prevention and control, and

(2) attitudes and practices towards rabies prevention, and suspect rabid animals and carcasses.

Scores will be based on completeness and accuracy of responses (0 – 3), depending on the question.

Question	Answer	Scores	Binary outcome
KNOWLEDGE OF RABIES			
What is rabies?	Rabies described as a disease	2	NA
	Rabies described as change of behaviour of a dog/animal	1	NA
	Unknown/wrong answer	0	NA
How is rabies transmitted / caught?	Through bites	2	NA
	Through scratches	1	NA
	Unknown/wrong answer	0	NA
Which animals can be infected by rabies and transmit to humans?	Three or more animals mentioned (dog, cat, mongoose, wildlife)	2	NA
	One or two animals mentioned	1	NA
	Unknown/wrong answer	0	NA
What are the signs of rabies in animals? Possible responses: a. General signs (poor appetite, dullness) b. Change in behaviour (e.g. restlessness, aggression, unprovoked biting) c. Increased salivation/ drooling / drooped jaw d. Fear of water e. Weakness of back portion / paralysis f. Abnormal vocalisation	Two or more signs known	2	NA
	One sign known	1	NA
	Unknown / wrong answer	0	NA
What treatment should you seek if exposed to rabies?	Human post-exposure prophylaxis (PEP)	2	NA
	Antibiotic and anti-tetanus treatment without mentioning PEP	1	NA
	I do not know/advice from medical practitioner sought	0	NA
What treatment should you seek if your pet or another animal in your household is exposed to rabies?	Post-exposure prophylaxis (PEP) Take it to veterinarian / Veterinary treatment without mentioning PEP Wound treatment and observation of animal Treat wound / do nothing	3 2 1 0	NA NA NA NA
Is rabies fatal?	Fatal nature of the disease known	1	NA
	Fatal nature of the disease unknown	0	NA
What are the methods of rabies control in animals? Possible responses: a. Mass dog vaccination	Three or four methods known	2	NA
	One or two methods known	1	NA
	Unknown/wrong answer	0	NA

b. Confinement / restraint of dogs c. Culling of suspect rabid animals d. Rabies surveillance and testing e. Responsible dog ownership f. Inter-sectoral / cross-border collaborations Note: mass culling of dogs is not an accepted response			
Overall highest score possible		16	NA
Knowledgeable of rabies	Respondents whose score was ≥ 10/16	≥ 10	1
Not knowledgeable of rabies	Respondents whose score was ≤ 10/16	≤ 10	0
KNOWLEDGE OF INTERVENTIONS AGAINST RABIES			
What first aid and medical attention would you seek if exposed to rabies?	Wound cleaning with water, soap, and/or kerosene, as well as subsequently reporting to the hospital	3	1
	Report to the hospital	2	1
	Report to police or public representative	1	0
	Do nothing	0	0
When would you go to the hospital after you were bitten by an animal and you thought you were exposed to rabies?	Report to the hospital immediately after a bite	3	1
	Report to the hospital the following day after a bite	2	1
	Report to the hospital 2 to 14 days after a bite	1	1
	Report to the hospital 14 days after a bite or would do nothing	0	0
What would you do if you suspected that an animal was rabid?	Report to veterinarian / local authorities and kill the animal	2	1
	Kill the animal	1	1
	Do nothing	0	0
What would you do with the carcass of animal that had been suspected to be rabid?	Cut the head and send it / the whole body to veterinary hospital / laboratory for testing	2	1
	Bury/burn the carcass	1	1
	Dispose of / Do nothing	0	0
Overall highest score possible		10	NA
Knowledgeable of interventions	Respondents whose score was ≥ 6/10	≥ 6/10	1

Not knowledgeable of interventions	Respondents whose score was ≤ 6/10	≤ 6/10	0

1. Have you/anyone in your household ever been bitten by a stray dog or another animal which could transmit rabies? Yes / No
2. Do you know of anyone else in your community who has been bitten similarly? Yes/ No
3. Do you know of anyone who contracted and died of rabies? Yes / No

Socio-economic details

4. Number of people living in this household
5. Number of children under 18 years.
6. Family religion – Hindu/Muslim/Christian/Buddhist/other.
7. Do you have a below poverty line (BPL) card? – Yes / No/ Prefer Not to Answer
8. Occupation of household head
9. Highest level of education of household head – Below high-school/high-school/higher-secondary school or pre-degree or vocational higher secondary education/ degree or higher / other/ prefer not to answer
10. Age of household head (within a broad age range).

Results

Knowledge of rabies

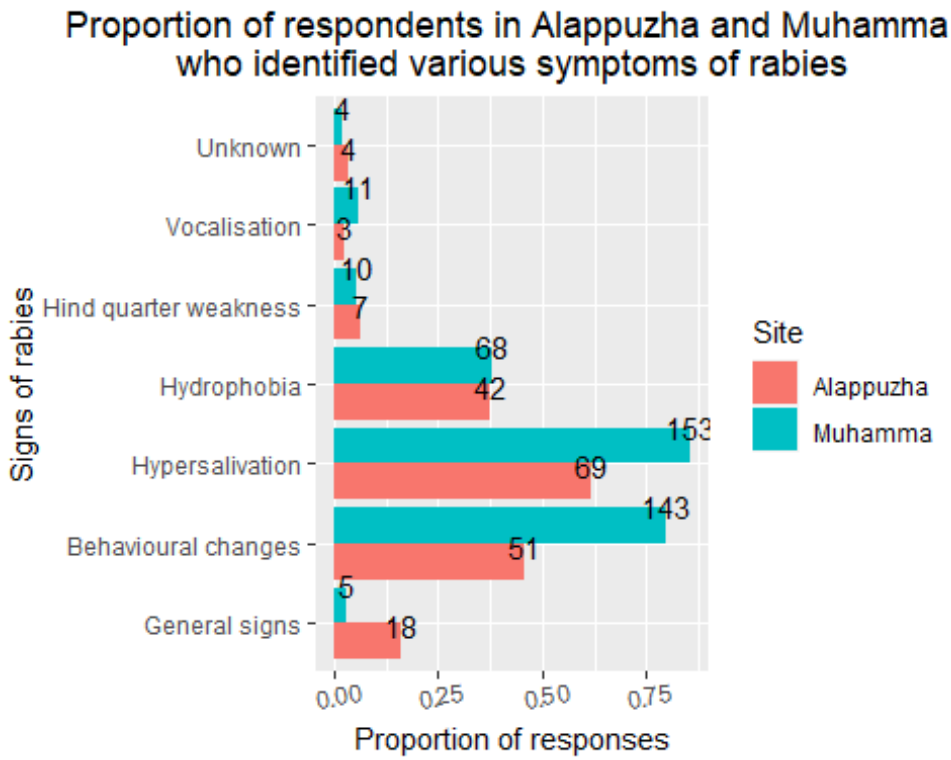
Testing whether significant relationship between Site (Alappuzha, A & Muhamma, M) and different variables related to knowledge of rabies.

Similar proportions of people identified rabies as a disease i.e. no differences in proportions between the two sites. Overall, 68% of respondents identified rabies as a disease, while 23% identified it as a behavioural change. Only 9% of respondents could not define rabies properly, or did not know.

No significant differences between sites in knowledge of how rabies is transmitted. 92% of respondents knew that it was transmitted via bites, while 6% said it was through scratches. Only 1.7% did not know how it was transmitted.

No differences between sites. Overall, 97% of respondents knew at least one animal that transmitted rabies, mainly the dog. 64% knew one or two animals, while 33% knew of three or more species, although this may not be a true reflection as many people stated animals such as rats and squirrels. Many respondents were aware that bats could transmit rabies, although the number of people stating a particular species hasn't been explicitly recorded.

The most commonly identified signs of rabies were hypersalivation and drooped jaws (222/291, 76.3%) and behavioural changes such as roaming, aggression and unprovoked biting (194/291, 66.67%), at both sites. However, significantly higher proportions ($p< 0.001$) of respondents in Muhamma identified these signs (~80%), compared to Alappuzha (61% for hypersalivation, 45% for behavioural changes). Similarly, a significantly higher proportion of Alappuzha respondents (18/112, 16.1%) identified general signs such as poor appetite or dullness as a symptom of rabies, compared to only 5/179 (2.8%) respondents in Muhamma ($p < 0.001$). Could this reflect a more widespread awareness of rabies in Muhamma, compared to Alappuzha. See below for possible reasons for this difference.



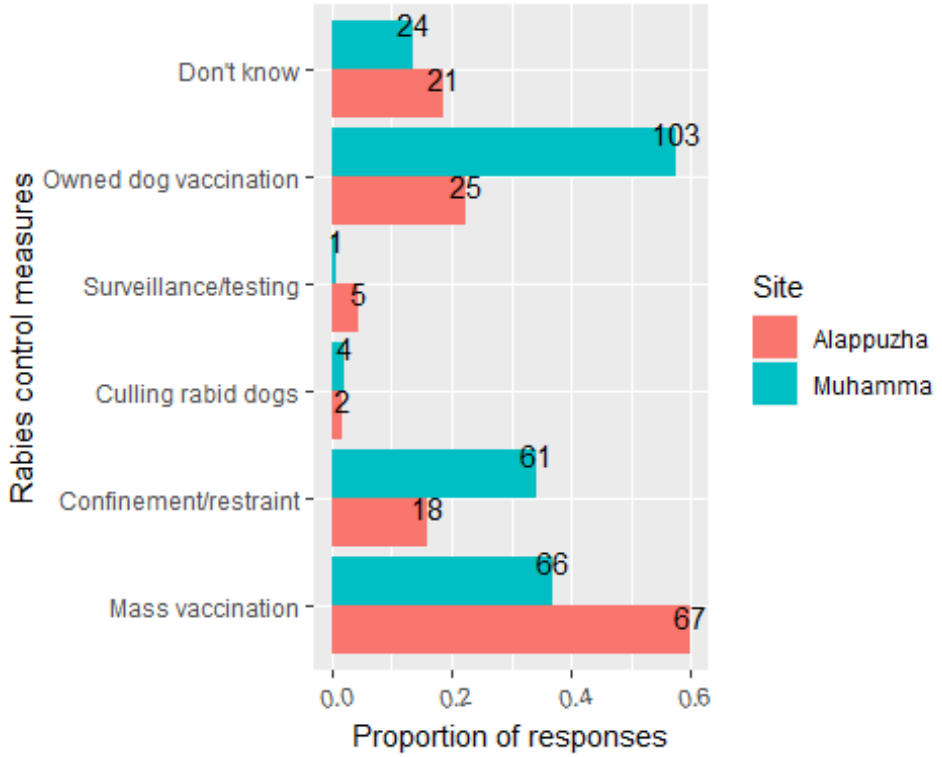
Overall, 69% of respondents knew two or more symptoms of rabies and 97% of respondents knew at least one symptom of rabies. Many respondents stated symptoms seen in humans rather than animals themselves; however this has not been recorded explicitly. An interesting point was that many respondents said that they had never seen a rabid dog or person in real life, only in films or books. The main movies named were Mrugaya and Karumadikuttan. One respondent also mentioned an unnamed Prem Nazir movie. Another important point to note was that respondents would often state only one symptom when asked the question. However, when prodded further, they would also be able to identify additional symptoms. Thus while there appears to be a statistically significant difference between Alappuzha (56/112, 50%) and Muhamma (145/179, 81%, $p < 0.001$) in the proportions of respondents with knowledge of two or more signs, this difference is may have arisen due to how other survey teams asked this question, perhaps without pursuing responses further.

Overall, 85.5% of respondents (249/291) said that they would obtain injections if bitten, with over half (54.6%) aware of human PEP. This was not very different at both sites - Alappuzha (99/112, 88%) and Muhamma (150/179, 83.8%). A significantly higher proportion of respondents in Alappuzha were

aware of human PEP (72/112, 64%) than in Muhamma (87/179, 48.6%) (p = 0.012, Chisq. test). This may reflect a greater awareness of dog bites and necessary treatments in Alappuzha, where the dog population is also much higher, than in Muhamma. Also possibly due to the presence of the general hospital and Alappuzha medical college within their vicinity?

Overall, 88% of the total respondents said they would seek some sort of veterinary treatment if their animal was exposed to rabies. However, only 27% of the total respondents (79/291) were aware of PEP for animals exposed to rabies; 62% (180/291) said they would present the animal for treatment to a veterinarian and follow their advice. This is in contrast to double the proportion of people who knew about human PEP. A significantly higher prop. of respondents from Alappuzha (42/112, 37.5%) knew about veterinary PEP, compared to Muhamma (37/179, 20.7%, p = 0.002). This may be due to the presence of the District Veterinary Clinic in Alappuzha municipality which provides veterinary treatment for longer hours (8am - 8pm), compared to the veterinary dispensary in Muhamma (9am - 3pm). Veterinary services in Muhamma are also likely to be less accessible/available than in Alappuzha

Overall, 83.5% of respondents (243/291) knew that rabies was fatal. There were no differences between sites



The most commonly identified rabies control measure was mass dog vaccination (133/291, 45.7%) followed by owned dog vaccination (128/291, 44%) and confinement and restraint of dogs (79/291, 27.14%). Overall, 230 respondents (79%) identified vaccination of dogs as a rabies control measure. However, there were significant differences in responses between sites. In Alappuzha, the most commonly identified control measure was mass dog vaccination (67/112, 60%), compared to Muhamma (66/179, 36.9%, $p < 0.001$), while it was owned dog vaccination in Muhamma (103/179, 57.5%) compared to Alappuzha (25/112, 22.3%, $p < 0.001$). Similarly, a significantly higher proportion of respondents in Muhamma (61/179, 34.1%) identified confinement and restraint of dogs, compared to Alappuzha (18/112, 16.7%, $p = 0.0012$).

A more concerning finding was the relatively high proportion of respondents who did not know of any rabies control measures - 45/291 (15.5%). This proportion was higher in Alappuzha (21/112, 18.8%) than in Muhamma (24/179, 13.4%), although not significant ($p = 0.28$).

Overall, about 15% of respondents did not know of any rabies control measures, while ~80% knew of one or two methods. There were no differences between sites.

Knowledge of rabies interventions

Testing whether significant relationship between Site (Alappuzha, A & Muhamma, M) and different variables related to knowledge of interventions for rabies.

Overall, ~99% of respondents (288/291) said that they would report to a hospital if bitten, and these proportions were the same at both sites. However, a significantly higher proportion of respondents in Muhamma (126/179, 70.4%) than in Alappuzha (61/112, 54.5%, p = 0.008) knew about wound washing with soap and water before presenting to the hospital. Why is this?

Overall, nearly all respondents (290/291, 99.6%) said they would report to a hospital immediately after or within a day of being bitten/exposed to rabies. There were no differences between sites.

Overall, the majority of respondents reported that they would kill a suspect rabid animal (131/291, 45%), and 37.8% (110/291) said that they would report it to a veterinarian or the local authorities, although some of them would still kill the animal. This latter proportion was higher in Muhamma (73/179, 40.8%) than in Alappuzha (37/112, 33%). Nearly a fifth of the respondents (50/291, 17.2%) were recorded as doing nothing - this included measures to avoid the animal such as chasing it away, warning neighbours and moving into their houses.

The majority of respondents (229/291, 78.7%) reported that they would bury or burn the carcass of an animal suspected to be rabid. Only 18% (53/291) said that they would inform a veterinarian or the local authorities, and only one respondent said that the carcass should be tested to confirm whether it had rabies or not. This latter proportion was significantly higher in Alappuzha (31/112, 27.7%) than in Muhamma (22/179, 12.3%) (p = 0.0016), which may be attributed to the fact that Alappuzha municipality authorities collect animal carcasses for disposal and so many residents would be aware of this. There is no such carcass disposal mechanism known to exist in Muhamma, and residents would have to dispose of any carcasses themselves or get someone else to do it.

Overall, over a fifth of respondents (65/283, 23%) or their household members had been bitten in the past. A significantly higher proportion (more than double) of Muhamma respondents (50/178, 28%) than Alappuzha respondents (15/105, 14.3%, p = 0.011) or their family members had been bitten in the past. This might reflect a higher incidence of dog bites in semi-urban locations like Muhamma than in urban locations such as municipalities. This needs to be analysed further by looking at the demographic characteristics of the respondents (particularly age - hypothetically a higher proportion of older respondents in Muhamma may have resulted in reports of dog bites from several decades ago?)

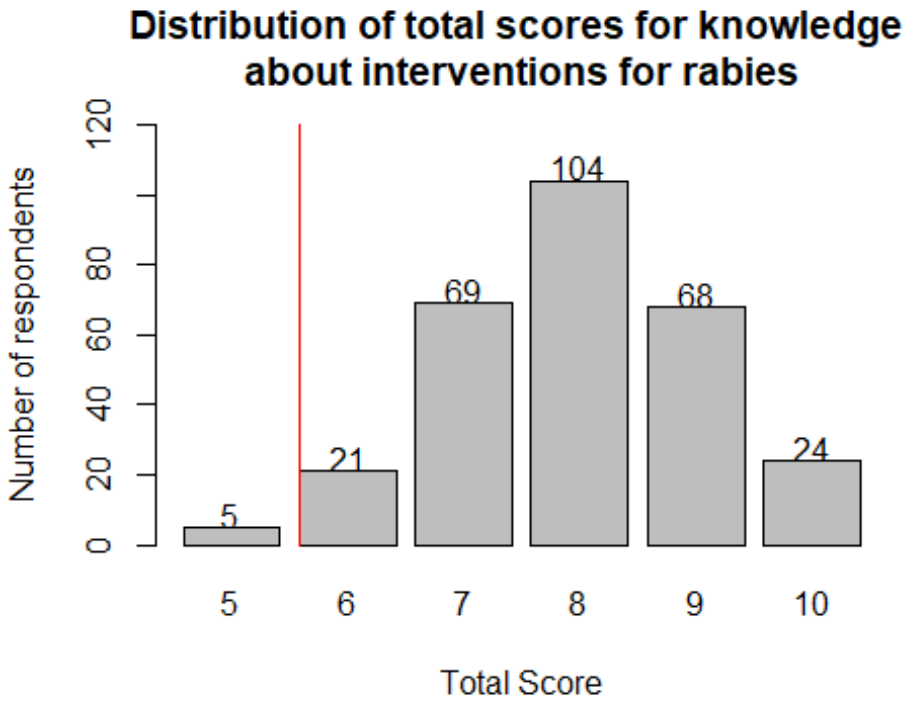
Overall, more than a fifth of respondents (79/280, 28.2%) knew of someone in their community who had been bitten by a dog or another animal that could transmit rabies. Of those who answered this question a significantly higher proportion (more than double) of Muhamma respondents (61/174, 35%) than Alappuzha respondents (18/106, 17%, p = 0.0018) knew of someone in their community who had been bitten. This again might reflect a higher incidence of dog bites in Muhamma than in Alappuzha.

Over a fifth of respondents (62/282, 22%) knew of someone who had died of rabies, this being higher in Muhamma (42/176, 23.9%) than in Alappuzha (20/106, 18.9%), although this difference was not statistically significant. This possibly links back well with the findings that in Muhamma significantly higher proportions of respondents, or their family members had been bitten, and also knew someone in the community who had been bitten

Total Scores

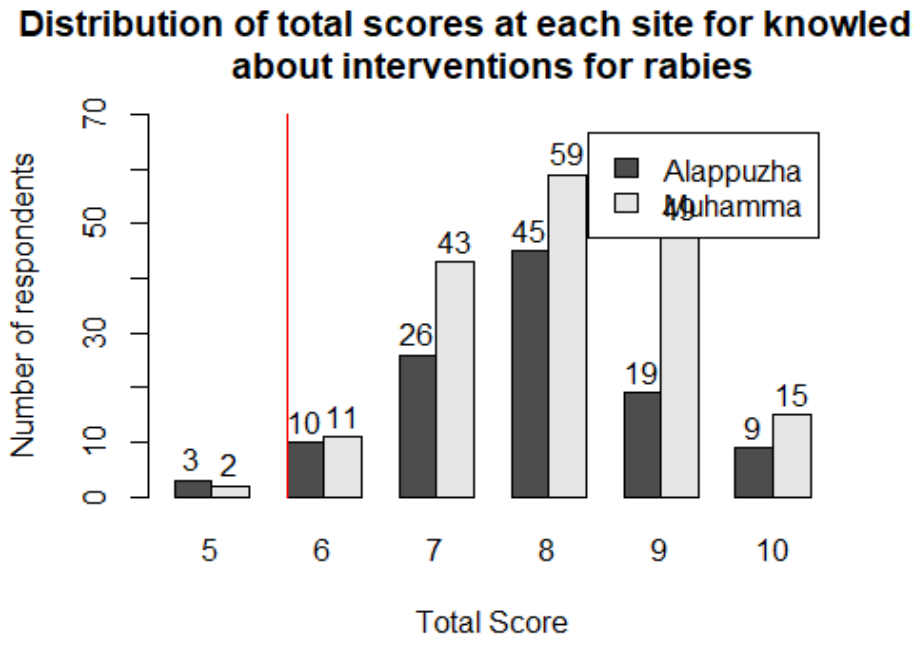
Calculating scores for the two sections on knowledge of rabies and knowledge of interventions for rabies. This is done by adding the scores for responses to each question.

Scores for knowledge about rabies interventions (For both sites (n = 291))



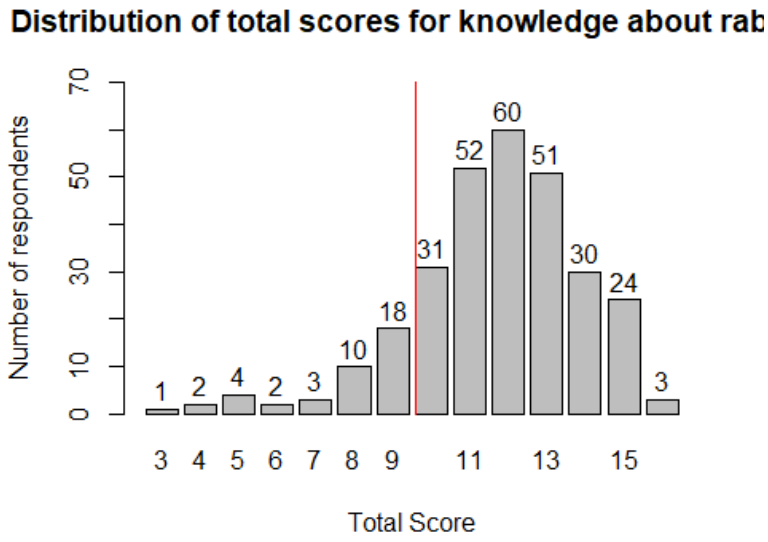
The highest score achievable in this section of the survey is 10. Bars to the right of the vertical red line are the number of respondents who have scored 60% or more (>= 6/10) in this section.

By site



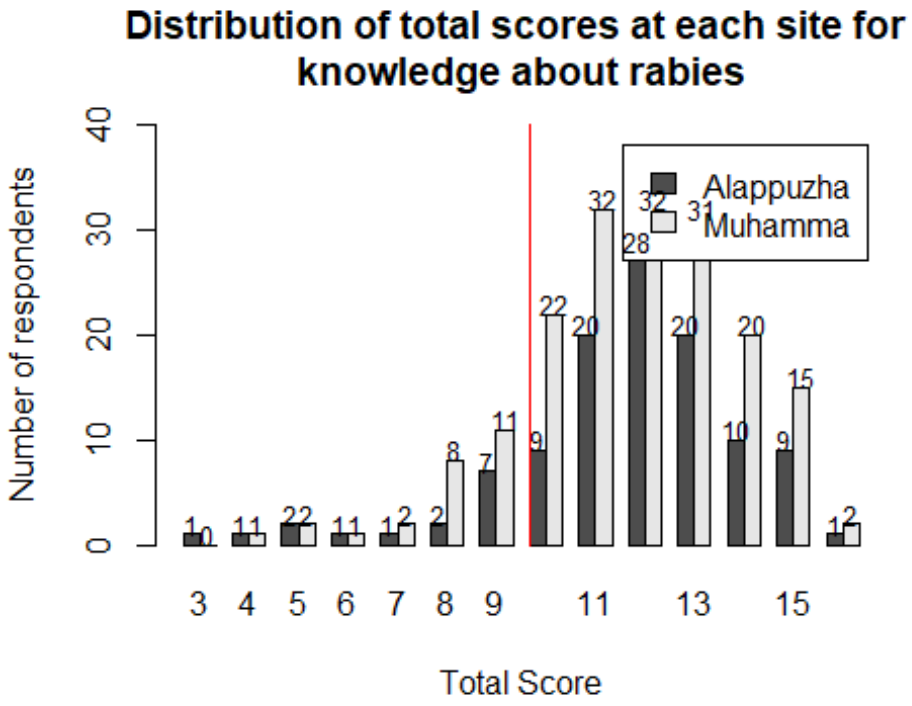
The mean total score for knowledge of rabies interventions was 7.97 (95% CI - 7.9, 8.03) and the median score was 8. Overall, 286/291 respondents (98.2%) scored 60% or more (>=6/10) when questioned about interventions for rabies - 109/112 (97.3%) in Alappuzha (mean: 7.84) and 177/179 (98.9%) in Muhamma (mean: 8.04). The difference in mean scores between sites was not significant.

Scores for knowledge about rabies (For both sites (n = 291))



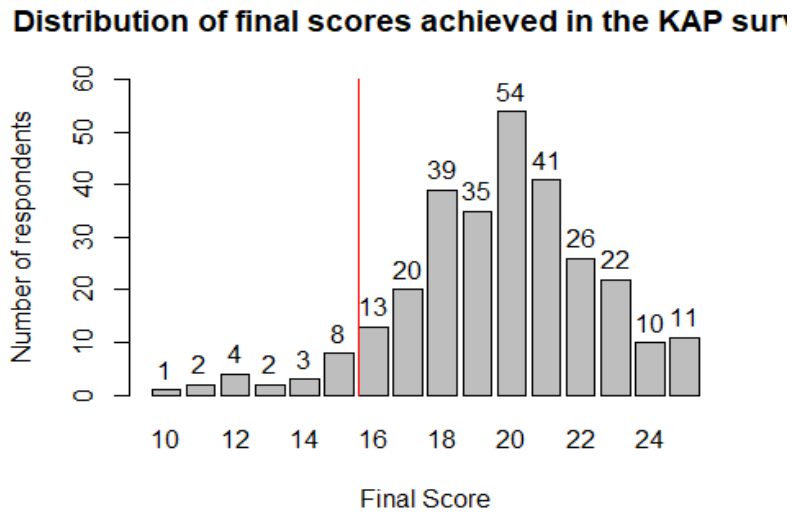
The highest score achievable in this section of the survey is 16. Bars to the right of the vertical red line are the number of respondents who have scored 60% or more (>= 9.6/16) in this section.

By site



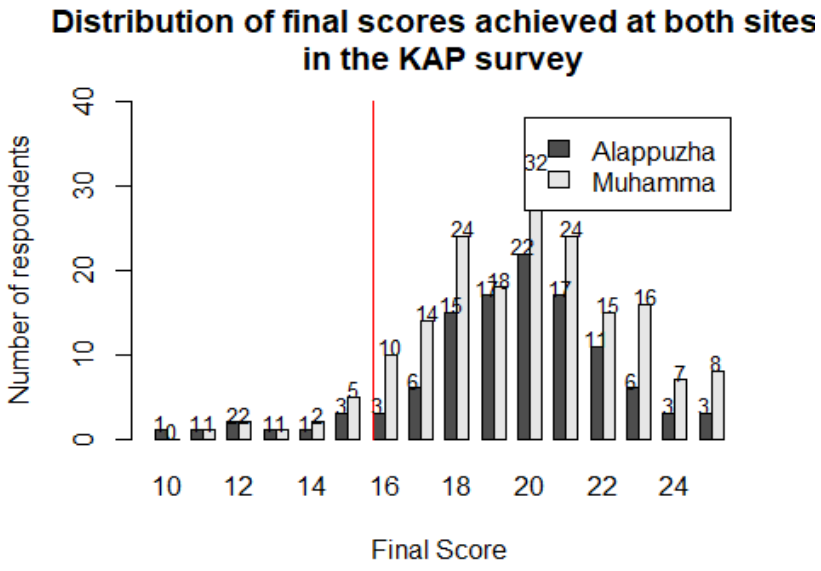
The mean total score for knowledge of rabies was 11.68 (95% CI - 11.54, 11.81) and the median score was 12.Overall, 251/291 respondents (86.2%) scored 60% or more when questioned on their knowledge about rabies - 97/112 (86.6%) in Alappuzha (mean: 11.66) and 154/179 (86%) in Muhamma (mean: 11.69). The difference in scores between sites was not significant by an independent samples t-test.

Final scores achieved in the KAP survey (For both sites (n = 291))



The highest final score achievable in the KAP survey is 26. Bars to the right of the vertical red line are the number of respondents who have scored 60% or more (>= 15.6/26) in the KAP survey.

By site



The mean final score for the KAP survey was 19.65/26 (95% CI - 19.48, 19.81) and the median score was 20. Overall, 271/291 respondents (93%) achieved a final score of 60% or more in the KAP survey - 103/112 (91.9%) in Alappuzha (mean: 19.5) and 168/179 (93.9%) in Muhamma (mean: 19.74). The difference in scores between sites was not significant by an independent samples t-test

Appendix A5: Capturing free-ranging dogs – lessons from the field

The capture, restraint and handling of stray/free-ranging dogs (FRDs) is a routine exercise conducted globally during dog population management (DPM) and mass rabies vaccination (MRV) campaigns [5] or for research [384]. Capture and restraint are necessary particularly when large segments of the FRD population have no distinct owner, such as in India, where most FRDs are unowned [45]. They may be required even for free-ranging owned dogs when owners cannot confidently handle them.

Despite the ubiquity of this procedure, surprisingly little information is available on practical aspects of FRD capture and handling, particularly factors that influence capture rates and dog welfare, such as dog behaviour, dog catcher skills and conditions under which capture is attempted. Some aspects of capturing FRDs are discussed in the 'Revised module for street dog population management, rabies eradication, reducing man-dog conflict' published by the Animal Welfare Board of India [234]. A general lack of information means that authorities and managers may be uninformed about the challenges in capturing and handling FRD and have unrealistic expectations about capture rates and the associated costs and timelines involved in conducting DPM or MRV campaigns. Such misunderstandings can directly impact the design and implementation of campaigns, particularly if there is a shortage of skilled dog catchers.

This communication aims to fill this gap in the literature by detailing insights on the challenges of capturing FRDs. These insights are based on our experience of capturing FRD for research and DPM efforts through ABC in India, home to one of the largest FRD populations in the world [31] and the largest burden of annual human rabies deaths globally [5]. For the research study, FRD (both unowned and owned) were captured using butterfly nets (and at times, catch poles), vaccinated against rabies, fitted with identification collars, microchipped and a blood sample collected before release. Blood samples were collected on three further occasions to monitor post-vaccination antirabies antibody dynamics, necessitating the capture and handling of scores of FRD on multiple occasions, with each

recapture becoming increasingly more difficult. Dogs captured for DPM were net captured and held in captivity until release post-sterilisation. These activities provided extensive opportunities to observe FRD behaviour and the strategies they adopt to evade capture or direct physical contact with strangers more generally. We hope that these field insights will be helpful to researchers, officials of local administrative bodies and trainee dog catchers and can act as a set of guidelines for what to expect when trying to capture FRDs.

It isn't always easy for strangers to handle dogs, particularly FRDs. In the case of dogs without owners (DWO) and even many owned dogs (OD), it's almost impossible to handle them without first catching them using nets, snares or loops and then firmly restraining them, all the while making sure that they don't get a chance to bite you. These are some of the aspects that researchers and animal welfare practitioners should keep in mind as they set out to work with FRDs in the field.

1. FRD can be notoriously difficult to catch, especially if they have been caught in a net before. Many of the dogs in our study had been previously caught in a net for ABC campaigns. As a result, they recognised a net and often even the dog catchers themselves, sometimes from several metres away. In an area where no catching had happened for some time, some dogs did not immediately recognise the nets until they were quite close. Such dogs would immediately become quite wary, staring intently at the dog catcher to monitor their actions, trying to identify if they were approaching said dog. At the slightest hint of danger, they would slowly but surely make their way to the closest escape route – perhaps an open field, the entrance to a house or factory or in the case of less experienced dogs, the underside of a vehicle. When trying to escape from dog catchers, they unhesitatingly ran in the direction of any person who did not have a net.
2. Dogs will defecate when severely stressed. The smell of dog faeces on a net can be detected from metres away – dogs can use it to recognise an approaching dog catcher.

3. You can only catch as many dogs as the number of dog catchers you have – if you're lucky and/or your dog catchers are exceptionally talented
4. The range of dogs you can catch is zero to the number of dog catchers
5. There are no benefits from going to an area with a lot of dogs – all it takes is for one dog to recognise the threat of the dog catcher and vocalise to advertise the threat, and all the dogs will become immediately wary and impossible to catch
6. The ideal stray dog is one that does not cry out during any stage from capture to release – doing so means that other dogs will be warned and, therefore, uncatchable
7. Some dogs may not return to their home range for hours or even days if they realise that dog catchers are around, so make each capture opportunity count. Many may even relocate to new territories.
8. The number of dogs that can be captured will significantly depend on a dog catchers' skill, experience, and knowledge of dog behaviour. A great dog catcher is worth their weight in gold!
9. Butterfly nets are unwieldy pieces of equipment, and only skilled dog catchers know how to make the best use of them.
10. The more dogs you have in an area, the more you can expect to capture over several capture sessions. However, the number of unique dogs you can capture in an area depends on how many there are and how many days you've been catching in that area. Once dog catchers have visited a site, the dogs there will constantly be on guard unless no further catching happens there for the next several days to weeks. You will probably catch the most dogs on the first day, and this number may drop off drastically from day two. By day three, you may not be able to catch any new dogs.
11. As a result, don't have capture sessions spread out over an extended period in any one area. The ideal policy may be to hit the area hard with several dog catchers and move on to a new location after two or three days.

12. Some dogs are very good (or lucky) at evading capture, so you could have three dog catchers trying to catch one dog, only to have it escape through a narrow gap between them!
13. Narrow alleys and streets with walls and fences are your best friends when catching dogs – they help experienced dog catchers to work together to capture a dog – for example, when one catcher drives a dog into the net of their colleague. Wide-open spaces make it very difficult to catch dogs as they have ample space to run and evade dog catchers, and even the best dog catcher cannot outrun a dog
14. Dogs can recognise individuals using visual, auditory and/or olfactory cues [385]. They will recognise dog catchers and researchers sometimes without even having been captured themselves.
15. If a dog can be handled or restrained without using a net, try to do this. Net capture is traumatic for dogs. Some dogs may never need to be net captured. Using a net for such dogs is a sure way of making them difficult to catch in the future.
16. Although hand catching is recommended as the most humane, it is highly dependent on the dog's temperament. Most dogs that associate with people are likely to permit physical contact and handling in reward for food. Still, not all may allow restraint for painful procedures such as vaccination or blood sampling.
17. Dogs can recognise the appearance and sound of various vehicles and may be able to identify the vehicles used by dog catchers or researchers if they use the same vehicle regularly.
18. If a particular local person is friendly with the dog and can handle/restrain the dog, get their help in carrying out procedures on the dog – provided they are confident enough to do so without getting bitten. Similarly, use such person's help to distract dogs with treats or food so that dog catchers can net them. But be warned, doing so may break the dog's trust in that local person, making them difficult to catch or even uncatchable in the future.
19. Use butterfly nets to catch dogs as far as possible. Using snares or catch poles are painful and unethical and may even be fatal if the dog gets choked for a long time.

20. Don’t underestimate the reaction times of dogs – it only takes a millisecond for an improperly restrained head or snout to lash out and cause a severe bite. Unfortunately, some dogs may not let go immediately after biting down on a finger or hand, and this can lead to life-changing injuries and severe blood loss.
21. Stealth is important, much like a hunter hunting their prey.
22. Be careful when trying to catch dogs on busy streets – they might get hit or run over by vehicles while trying to escape the dog catcher.
23. Try to get a brief history of each dog if they are known to members of the local public. This may help you avoid stressing dogs that are sick or old or even help you decide to avoid them in the first place. For example, I had a dog die within a few minutes after net capture and restraint, only to be told later by the locals that it had been sick for the past several weeks!
24. How easy or difficult it is to catch dogs in an area can also greatly depend on the relationship between humans and dogs in that area. In areas where human-dog conflict is common (due to dogs scavenging on garbage, chasing people or vehicles or biting people), dogs are likely to be very wary and flighty, and it may be challenging to catch more than a few dogs, even on the first day of capture.