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Use of surveillance data to measure rabies risk and the impact of intervention in Cambodia using novel modelling techniques

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Use of surveillance data to measure rabies risk and the impact of intervention in Cambodia using novel modelling techniques

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ABSTRACT

Rabies is a fatal zoonotic neurotropic viral disease estimated to cause nearly 60,000 deaths worldwide each year. Most of these deaths occur in developing nations in Asia and Africa where the virus is maintained in free-roaming dog populations. Some wild carnivore species such as raccoon-dogs and foxes can also be viral reservoirs. Rabies is most-commonly transmitted through a bite and all mammals are susceptible to it. The virus slowly transits from the bite location to the central nervous system where it replicates. After a long incubation, rabies symptoms are typified by neurological disorders and eventual organ failure and death. Although rabies is fatal once symptoms set in, post-exposure prophylaxis is highly effective at preventing disease from happening if administered prior to symptom start. Furthermore, mass canine vaccination is a proven tool to control rabies at its source and is considered to most cost-effective way to control the disease in dogs and prevent it in humans. Unfortunately, in countries where rabies is endemic, application of these prevention and control tools is often underwhelming due to limited resources and knowledge to apply them in the comprehensive and sustained way that is necessary to achieve full control. This means PEP accessibility is often limited or too costly for bite victims and vaccination campaigns are not sustained enough to fully control disease in the reservoir. Furthermore, due to the lack of accessibility to care for victims, rabies is often underreported and so does not feature as a key priority in many areas. Thus, epidemiological studies are key to provide knowledge that will both provide evidence of rabies burden and help strategize allocation of limited resources for intervention. In recent years, under the guidance of WHO and OIE there has been a push in many countries to achieve canine rabies eradication by 2030.

Cambodia is a nation in Southeast Asia with a human population of 15 million and an estimated dog population of five million. The majority of dogs in Cambodia are owned but allowed to roam freely and are poorly vaccinated. Cambodia is endemic with rabies and has one of the highest mortality rates

from the disease worldwide. The main institution in charge of rabies prevention and surveillance in Cambodia is the Pasteur Institute of Cambodia (IPC). Since it's opening in 1995 it has provided post-exposure prophylaxis to bite victim, collected information on bite attack victims and tested animals, the majority of which were dogs, for rabies, providing passive surveillance on rabies. Until 2018, IPC only had one institution in Phnom Penh, meaning access to PEP and surveillance was limited, with most patients and tested animals coming from Phnom Penh and its surroundings. Furthermore, there is no program for widespread canine vaccination in Cambodia with prevention relying solely on PEP. Nevertheless, to meet eradication targets, Cambodia has been expanding control and prevention in recent years. Two new PEP centers have opened in rural provinces in 2018 and 2019. Pilot vaccination studies have also been conducted to help inform future campaigns. Epidemiological studies could help guide future opening of PEP centers and vaccination campaigns in the years to come. These are the goals of the following three chapters.

The first chapter investigated the impact of physical accessibility to PEP centers on the rate of PEP patients in the population using Bayesian Poisson regression. The model used geographical accessibility data in the form of travel time to a center or a provincial capital and demographic data in the form of urban proportion of the population as predictors of PEP numbers and rates. Regression models were then used to test PEP expansion scenarios where we measured the impact of opening new centers in specific locations. From 2000 to 2016, 294,000 patients presented to IPC for PEP. The majority of these were in Phnom Penh and the neighboring province of Kandal. We observed strong evidence that travel time to a PEP center had a negative association with PEP rate, with an increase in one hour leading to a PEP rate reduction of 70% to 80%. We identified five provincial capitals in which the opening of a new center would maximize PEP access: Banteay Meanchey, Siem Reap, Takeo, Kampot, and Svay Rieng. Adding a center in every provincial capital would increase the number of people living within 60 minutes of a PEP center from 27% to 65%.

The second chapter investigated predictors of rabies positive tests in biting animals. We used patient interview information on the patient, the biting animal and the attack collected by IPC doctors during the first consultation as predictor variables in a Bayesian spatio-temporal logistic regression. From 2000 to 2016, 1.5% (2,500) of PEP patients brought the head of the biting animal for testing. Tested animals, as patients, mostly from Phnom Penh and nearby provinces though not as centrally distributed as PEP patients were. Of the tested animals, 60% tested positive for rabies using direct fluorescent antibody test. A number of variables were predictive of a positive test, notably if a dog was not owned, if the attack was unprovoked and if there was a large number of victims. However, the most predictive variable was a disease suspicion variable assigned by IPC doctors based on behavior and symptoms descriptions from the patient, showing IPC has a strong protocol to identify rabies suspect animals. Finally, we identified three provinces at higher risk of returning positive tests: Kandal, Kampong Cham and Kampong Thom.

The third chapter used data collected in the pilot vaccination campaign to build a spatially explicit rabies transmission agent-based model to help study the impact of demographic turnover on vaccination coverage, and the level of vaccination required to prevent rabies spread. The model was conducted at a small spatial scale in five villages of Kandal province. We characterized the contact probabilities within a 100m infection radius that led to basic reproduction number (R_0) values within the range of 1 and 2, where most estimates from field observational and mathematical modelling studies lie. Within this range, 70% target vaccination coverage annually was sufficient in all cases to reduce R_0 to below one, which theoretically stops disease spread. However, we observed large outbreaks where still possible in up to 8% of simulations with this coverage, in the worst-case infection scenario, and a 90% target vaccination coverage was necessary to reduce these to below 1%. We also observed that one year after vaccination occurred, coverage had reduced by 40% with rapid population turnover, showing the need for sustained annual vaccination to maintain high levels of immunity over time.

Through these three chapters, we provide information that could help guide where rabies risk and needs are the greatest in Cambodia, to inform future PEP center opening. We also provide information on the effectiveness of vaccination and the need for vaccination to be sustained over time to be effect.

INTRODUCTION: RABIES IN CAMBODIA

1. History and general context of rabies

Rabies is one of the oldest viral zoonotic diseases known to humankind, which is caused by the rabies virus, a Lyssavirus. Early on in history, it was understood as a disease transmitted from animals to humans. There is evidence of dogs being associated with death and madness in ancient societies around the world, from the Mediterranean basin to India [1]. The first recorded mentions of the rabies transmission mechanism date back from ancient Greece in the 5^{th} and 4^{th} century BC, during the Greek classical period. The Greek philosopher Aristotle wrote, "That dogs suffer from madness. This causes them to become very irritable and all animals they bite become diseased" and Roman writers, several centuries later, described that the dog's saliva spread a virus ("poison") that caused the infection [2]. The Latin word *rabies* and the Greek word *lyssa* are associated with the meanings of madness, rage, and violence. Written accounts of rabies outbreaks have been numerous worldwide throughout history [2]. The scientific history of rabies is also notable in that it is one of the first viruses to be identified and for which a vaccine was developed. In 1769, Italian anatomist Giovanni Battista Morgagni first observed that the disease seem to spread through the nerves rather than the vascular system [2]. The transmission mechanism was first experimentally confirmed in 1804 by German physician and naturalist Georg Gottfried Zink who used saliva from a rabid dog to infect another, followed by a description of the symptoms he observed [2,3]. Though the virus itself was not yet identified, French Chemist Louis Pasteur more precisely identified, through experimentation, the transmission pathway and the affected organs during the 1880s whilst hypothesizing that the agent was smaller than a bacteria [2]. He also experimented by injecting saliva from infected rabbits intravenously into sheep, which lead to protection instead of infection. Further developments allowed him to create the first attenuated rabies vaccine which was successfully administered to a nine-year old boy who had been bitten by a rabid dog in 1885 [3]. Microscopic evidence of rabies in brain and spinal cord tissue was then first identified by Italian pathologist Aldechi Negri in 1903 allowing for microscopic diagnosis of rabies, though he mistook the pathologic manifestations, now known as the Negri bodies, as protozoan parasites [2–4]. The virus itself was finally identified in 1963 thanks to the development of the electron microscope, revealing its bullet shape [2].

It is estimated that nearly 59,000 people die from rabies every year, with an overall cost of $8.6 billion, including direct and indirect cost from human cases and loss of livestock [5,6]. The societal cost is compounded by the violent nature of the disease, the long period of uncertainty following potential exposure, the young mean age of victims, and the lack of appropriate care to help symptomatic patients. The vast majority of cases occur in developing countries in Africa and Asia, being the result of endemicity in domestic dog reservoirs [5,6]. Although it has a high impact and its existence is widely known to the general public in developed and developing countries alike, rabies is often considered a neglected disease. This is despite the fact that very effective technical tools exist to help control and prevent disease in both humans and animals. Low prioritization is partly due to the lack of information regarding its burden caused by severe under-reporting of cases, showing the importance of epidemiological studies that estimate the burden of rabies from limited surveillance data as well as canine ecology studies and disease transmission models that allow to estimate the most cost-effective control strategies [7–9]. Data collection for rabies often relies of passive surveillance system based on laboratory confirmed human and dog cases. These systems capture a limited number of cases as areas where rabies risk is higher are also areas where access to healthcare and testing infrastructure are low [8,9]. Nevertheless, rabies has become an increasing concern for governments on these continents over the last few decades leading to increasing national and international efforts being put into prevention and control [7,10,11]. In this context, the World Organization for Animal Health (OIE) and World Health Organization (WHO) have established a goal to eradicated dog-mediated rabies by 2030 [12,13].

2. Ecology and cycles

Today, we know that rabies is a viral disease caused by the rabies virus, a neurotropic negative-stranded RNA virus in the Rhabdoviridae family and the genus Lyssavirus [14]. Until 2008, the genus Lyssavirus was divided into 2 phylogroups and 7 genotypes [15,16]. This has since grown to 3 phylogroups and 14 species to date, most of which have fructivore and insectivore bats as their reservoirs [17,18]. Three of these, rabies excluded, can spillover into humans and some domestic species with another three only spillover in humans and one in domestic animals only [17–19]. However, transmission of these viruses to humans remains extremely rare which partly explains why bat rabies was identified much later than canine rabies in the 1930s [2]. Rabies lyssavirus (RABV), a member of phylogroup I and formerly known as Lyssavirus 1, finds its reservoir in multiple terrestrial carnivore species, and is of great concern for humans in terms of both health and economic impact. Most mammals are susceptible, including humans and common domestic species [19].

From an epidemiological standpoint, rabies transmission follows two cycles: the sylvatic and the urban cycle. The sylvatic cycle is the cause of most concern in developed countries where canine rabies is well controlled. It involves the virus being maintained in wild carnivore species, including foxes, raccoons, raccoon dogs, skunks, and mongooses [20,21]. Human encroachment into natural habitats can lead to the exposure to themselves, their pets, or their livestock [19,20]. In developing countries, the urban cycle of rabies is responsible for more than 99% of human cases [5]. This cycle is maintained in free-roaming dog populations, bringing the reservoir much closer to humans. Despite its name, nearly 87% of cases occur in rural but settled areas [6]. The urban cycle is still connected to the sylvatic cycle. This in turn can lead to problems in controlling the urban cycle if intervention is not sustained or ignores the impact of wildlife, as there is always a risk of resurgence in dog populations coming from wildlife species [22].

3. Human dog relationship

Wolf domestication started in the late Mesolithic, around 12,000 to 15,000 years ago, around the same period when humans started developing agriculture and building settlements. This makes the wolf the earliest animal to be domesticated by humans [23]. Early contacts between humans and wolves is thought to have occurred in the form of a symbiotic hunting relationship. Over the long period of domestication, adaptation, and selective breading that followed these initial encounters, dogs became increasingly adapted to human environments, lifestyle, and communication, more so than any other animal species and becoming an integral and beneficial part of most human societies [23,24]. Throughout history, dogs have served in many roles including that of hunter or food source, guard or combatant, or providing mobility and companionship. Today, dogs can be categorized in three broad groupings: constrained owned, free-roaming owned, and free-roaming feral [23,24]. The latter two groups are the ones of primary concern in regards to rabies in developing countries. Beyond rabies, dogs (free-roaming and constrained) can be the source of other public health risks including dog bite-injuries and a number of bacterial and parasitic zoonotic diseases [23,25–32]. They can also be responsible for other nuisances, such as waste, noise, and destruction of wildlife, livestock, or property [25]. Many of these problems can be resolved through appropriate canine population management measures and behavioral training both for dogs and humans [25].

4. Pathogenesis and clinical outcome

The virus is transmitted through the saliva of an infected animal, most commonly through a bite. The virus first replicates in the muscle tissue surrounding the entry site before making its way to the central nervous system via peripheral nerves [14,33]. It will then replicate in motor neurons and spread to the brain, eyes, sensory nerves, and salivary glands [14]. Following exposure, there is an incubation period of varying length depending on the location and severity of the bite injury. In both experimental and field studies, incubation in dogs has been recorded to range between 20 and 30 days, but this can be as short as a few days or as long as years [34–39]. In humans, incubation appears to be longer with observed median incubation around 2 months [40]. In experimental inoculation of dogs it was demonstrated that the virus can start shedding a from 1 to 14 days before the onset of symptoms, during the prodromal phase [35,41]. During this phase, the dog's behavior might show early behavioral changes [33]. Most symptoms are due to the effect of the virus on the central and peripheral nervous system, affecting behavior, perception of the environment, muscle control, and salivation. There are two syndromes known as furious rabies and paralytic rabies. The former typically involves restlessness, aggressiveness, convulsions and spasm, excessive salivation as well as hypersensitivity to water, light, and air movements (hydro, photo, and aerophobia) [14,33,40]. Transmission of the virus is usually through the furious form, with paranoid animals being more likely to bite whilst shedding virus in saliva [14,33,34]. Death can occur through spasm attacks with this form. If an individual survives the furious rabies syndrome, flaccid paralysis of the muscles settles in and individuals eventually die of respiratory or heart failure. Furious rabies is the most commonly known as it is the most obvious and impactful in terms of disease transmission, and appears to be the most common in observational studies [42]. However experimental inoculation of dogs have shown that paralytic rabies is the most common form, in up to 80% of cases [34,43]. The symptomatic period is relatively short in dogs, lasting between 1 and 7 days [34–38,42] and around 14 days in humans [40]. The syndromes are the same in all mammals [33]. Rabies is nearly always fatal, except in anecdotal cases, with the symptomatic period lasting from 7 to 15 days [19,44].

In the event of clinical rabies in human and in the absence of proven curative treatments, only palliative care is available to reduce suffering. This involves a combination of sedative and analgesic medications and rehydration [19]. More aggressive protocols have been attempted including the Milwaukee protocol which gained fame when an American teenager survived bat rabies in 2004 following treatment, but this success could never be replicated despite follow-up attempts [19,45,46].

5. Diagnosis and surveillance

Diagnostic testing for rabies history starts in 1903 with the discovery of a pathological manifestation in brain tissue, known as the Negri bodies, by an Italian pathologist of the same name. Early testing methods relied on the staining and detection of these bodies from the 1920s to the 1950s and 1960s when virus isolation methods where developed as well as fluorescent microscopy techniques to detect antibodies [2,47]. Today there exists numerous methods for diagnostic testing of rabies virus, antigens or antibodies [48,49]. Considerations that guide the selection and use of a specific method depend on the need for an ante-mortem or post-mortem diagnostic, as well as the type and quality of tissue available. The current WHO gold standard for rabies detection and the one that is typically used for animal surveillance is direct fluorescent antibody test (DFAT), which was developed in the 1950s. The principle of this method is to target viral antigens with fluorescent antibodies that are then detected via fluorescent microscopy. This method benefits from very high sensitivity (>99%) as well a quick turnaround with results being available within 1-3hrs. The main drawback however is the need for fresh brain tissue which limits this test to post-mortem use and make it less applicable in areas where access to conditions or infrastructure that allow to properly preserve the brain tissue are limited [47–49]. The second main method in use, which can be conducted both ante- and post-mortem, is direct viral RNA detection using RT-PCR in cerebral spinal fluid (CSF), saliva, nuchal skin or hair follicles. This method is more typically used in human diagnostic testing where the ante-mortem need is higher [47–49].

Surveillance for rabies, as advised by WHO, ideally requires both passive and active elements. Passive surveillance should be used to detect human and animal rabies cases as well as human bite injuries, and help establish spatio-temporal measures of disease burden such as incidence. This requires testing infrastructure, with the advised gold standard tests described above which can be complimented lower sensitivity field tests in the case of logistic limitations. The Surveillance system also requires a data recording and reporting frameworks, which use standardized case-definition. Passive surveillance should be complemented by active surveillance to monitor the sustained application intervention in dog populations such as vaccination. Furthermore, OIE members have to report animal rabies cases through the World Animal Health Information System. WHO also advises data sharing through global and regional reporting systems [19]. Unfortunately, in rabies-endemic developing countries, limited healthcare and testing infrastructure and accessibility often result in deaths occurring at home, poor clinical follow-up of suspect cases, and few laboratory confirmations. Furthermore, lack of training in health personnel and existence of other, sometimes more common, infectious diseases with neurological syndromes can lead to case misidentification. Consequently, surveillance systems often have low sensitivity resulting in underestimated disease incidence. Furthermore, the reporting of this data nationally and internationally is often infrequent [8].

6. Prevention and control

Prevention of human disease relies on two main approaches, prevention in humans and dogs. In humans, prevention relies mainly on offering post-exposure prophylaxis (PEP) to individuals exposed to rabies, and in some case pre-exposure prophylaxis (PrEP) in high-risk individuals such as veterinarians. Beyond the medical tools available to protect humans, it is also recommended to wash the wound with soap for 15 minutes right after the exposure occurs [50]. In dogs two broad tools are used: vaccination and population control [51]. It is necessary to consider these methods together in a one-health approach [52]. Applying these approaches has successfully led to the eradication of canine rabies in many parts of the world throughout the 20th century, including Europe, North America, select countries in Asia and more recently Central and South America [20,53,54]. However, successful implementation of these tools require both effective surveillance and public outreach and education campaigns. Surveillance is necessary to allocate scarce resources where the need is greatest. Education on the other hand is key in improving awareness of the steps to take following exposure, notably rapidly seeking PEP after exposure, but also in increasing social acceptability of interventions in dogs such as vaccination and population management campaigns through sterilization.

6 .1. Vaccination

The first major dog vaccination program occurred in Japan in 1921 [1] . WHO has advised 70% as the target vaccination rate for years and this was estimated to be effective through modelling due to the relatively low basic reproduction number (R0) [19,36]. Canine vaccination campaigns in dogs have been modelled to be more cost-effective than relying on human PEP alone for prevention [53,55–58]. In developing countries, most canine vaccination are conducted through government campaigns as opposed to individual visit to a veterinarian [59] and cost remains a clear barrier responsible for the low vaccination rates observed in the absence of government free vaccination [60]. In practice, when well implemented, vaccination campaigns reaching 60 to 70% target have been highly effective at reducing rabies incidence in dogs and humans as has been seen in Tanzania, the Philippines, Indonesia and many other locations [61–64], but heterogeneous coverage can lead to failure to control disease spread [65]. However, the achievement of long-term control of rabies, requires long term sustained commitment and costly investment which is often beyond the means of local governments in charge in developing countries as well as good record-keeping of previous vaccination efforts [56,59,65–67]. Often free-roaming canine populations are young, with a short life-span and a high turnover rate requiring frequent (annual) and repeated vaccination efforts as new susceptible animals quickly replace immunized ones [59,68]. Furthermore the overall health condition and nutritional status of the animal can impact seroconversion and length of immunity, also requiring re-vaccination efforts in previously vaccinated dogs [68]. In some settings there is also a need to consider wildlife when vaccinating dogs both in term of averting risk of re-introduction from wild reservoirs [22] and to protect non-reservoir wild carnivore species that can be infected by dogs [69] .

Different vaccination strategies can be used such as central-point administration in a village or door to door campaigns, and effectiveness of these can be locally specific in different communities [70]. Vaccination campaigns of free-roaming dogs typically use single dose vaccines due to the necessity to rapidly vaccinate large numbers of individuals. However, as not every dog is easy to capture and restrain when administering injectable vaccines, oral vaccination has been use to supplement or replace injectable vaccination with some success and have been shown is some cases to be both quicker and more cost-effective [71–76].

In Humans, preventive vaccination or PrEP is only recommended in high-risk groups such as individuals with occupational exposure or living in remote endemic areas with little access to health-care [19,50]. WHO recommends a two-dose regimen following a one week two-site ID or one-site IM injection on days 0 and 7. If exposure occurs, a booster dose is recommended [50].

6.2. Post-exposure prophylaxis

As discussed above, the first use of a rabies vaccine by Louis Pasteur in 1885 was actually used in what would be characterized today as post-exposure prophylaxis (PEP) [3] as administration happened after exposure to rabies but prior to the development of symptoms. The next big step in the development of effective PEP was in the addition of serum containing rabies antibodies to the vaccine regimen in 1954. Modern PEP is composed of two main components, a vaccine regimen and serum containing human rabies immune globulin (HRIG). PEP is very effective at preventing disease and death when administered in time [77]. Many regimens are described which can vary based on length, number of vaccine injections, the type and site of injection and the presence of HRIG or not, and these can be modulated based on the severity of exposure. The current WHO standard recommends a one-week PEP regimen involving the administration of HRIG soon after exposure in combination with a two-site intradermal (ID) injection of vaccine. Two subsequent vaccine doses are then administered on days 3 and 7 following the first [50,78,79]. This regimen has been shown to be less costly than other regimens, allowing to treat more patients in low resource settings [80]. Other regimens might use intramuscular injections (IM) with up to five doses over 28 days [50,77,78,81].

Though, as discussed above, canine vaccination is considered more cost-effective than PEP alone, PEP is nevertheless more cost-effective than no intervention at all [82]. Furthermore, even in the presence of canine vaccination campaigns, ethical considerations require that PEP remain available for bite-injury patients as long as rabies remains present in an area or at risk of re-introduction. However access to PEP remains uneven across Africa and Asia due to limited availability of vaccines and mostly HRIG doses, as well as financial costs to patients and sometimes physical inaccessibility to centers providing PEP [83].

6.3. Population management

Canine population management follows three main approaches: containment, sterilization and culling. Culling is considered to be ineffective with no evidence of reducing density nor disease transmission and having a negative ethical and societal impact [84]. Population reduction through culling can lead to population vacuums that increase movements as new animals come in to replace the former ones, and is often unpopular in communities where these dogs live [85]. This strategy can lead to investing resources in ineffective culling programs and failure to invest in more-effective rabies control methods and should therefore be advised against [7].

Sterilization can be achieved by encouraging routine sterilization in individual dog owners or providing public sterilization campaigns in free-roaming populations. This can be done in combination with vaccination campaigns in free-roaming dogs as has been done in Bohol, Philippines and India [62,86]. However, sterilization is costly and is often perceived negatively by local communities that rely on new births to replace their dogs [66]. Containment can take the form of fences and leashes for owned dogs, or public shelters for strays, but it is rarely implemented in developing countries.

7. Rabies context in Southeast Asia

A majority of the 59,000 yearly death of rabies estimated in Hampson et al. 2015 occur in Asia, with the majority of these (21,000) being in India alone [5]. However, in this same study, South-East Asia, also suffered approximately 6,000 yearly deaths making it one of the regions most impacted by rabies. South-East Asia is composed of a number of countries South of China and East of India that are most easily defined by their memberships to the economic and political union known as the Association of Southeast Asian Nations (ASEAN), though other regional definitions exist, notably the WHO one. ASEAN is composed of the nations of Brunei, Cambodia, Indonesia, Laos, Malaysia, Myanmar, the Philippines, Singapore, Thailand and Vietnam with an overall population of 667 million people [87]. However, in the context of rabies we should also consider non-member Bangladesh, with a population of 165 million, in this section due to its geographical location and high level of rabies endemicity [88,89]. ASEAN nations have a wide disparity in wealth and development, whit it's wealthiest nation, Singapore, having a gross domestic product (GDP) per capita of $66,260 and a human development index (HDI) of 0.938 in 2021 and compared to Myanmar's GDP per capita of $1,250 and HDI of 0.583 [90,91]. These countries also vary greatly in their geographical features, notably with some being island or archipelago nations and Singapore being a city-state. Population also varies greatly, between 0.4 million in Brunei to 273.5 million in Indonesia [87]. These economic and geographic disparities can be observed in the differences in rabies status in the region. However all countries in the region have national control plans to reach OIE and WHO's goal of rabies eradication by 2030 [92,93]. This involves regional cooperation through the ASEAN which has targeted rabies as a public health priority [94,95].

In Singapore and Malaysia rabies was initially eradicated through canine vaccination campaigns in 1953 and 1954 respectively, thought it was recently re-introduced in Malaysia in 2015, with the situation remaining largely controlled [88,92,96]. The case of Malaysia however, shows that regional cooperation is necessary to maintain rabies free status, particularly amongst nations that share a land border. Furthermore, Brunei has never reported a rabies case whilst the remaining countries in the region are all endemic with canine rabies [88,92]. In Southeast Asian countries where rabies is endemic, dogs are mostly owned, non-confined and most often serve as guard dogs, as was observed in studies in Vietnam, Thailand, Cambodia and the Philippines [85,97–99]. Dogs are also used as a food source in certain regions, and there are concerns about transmission risks for workers in slaughterhouses and consumers [100,101]. However, in urban areas such as Bangkok, they are also increasingly being kept as pets [102] .

Amongst endemic countries, Thailand has made some notable progress through canine vaccination campaigns and increased vaccination awareness in dog owners, as well as increased access to PEP leading to an important reduction of dog and human cases in the 1990s [88,98,102–105]. Human cases for example dropped from an average of 300 per year in the decades prior to less than 100 by 1995, and then under 30 by 2002 [102,105,106]. Thailand is notable in that large proportion of dogs are vaccinated in situations other than government campaigns [98]. Vietnam has also seen a strong reduction in human cases in the last 20 years mostly thanks to the expansion of PEP centers throughout the country, seeing cases drop from an average of 400 in the early 1990s to 100 or less in the early 2000s [93]. Furthermore, Vietnam has also made efforts to improve dog management and canine vaccination campaigns, though with mixed-successes, seeing disparate vaccination coverage in different provinces, with a national average well below the recommended 70% [93]. As a large archipelago, Indonesia has seen mixed fortunes in recent times with strong interventions helping control rabies in some islands but with rabies expanding in previously free Islands [88]. For many decades rabies was endemic in the Western part of Indonesia, notably on the Island of Java, which holds more than half of the country's population, with the Eastern islands being considered free [107]. However, rabies has spread to previously free islands recently, such as Flores in 1998 and Bali in 2008 [63,108,109]. This in turn has sparked concern in Australia of continued Eastward expansion [110]. Despite sustained vaccination efforts which have achieved some success, rabies remains endemic in these islands [56,111,112]. In the Philippines, the only country not to share a land border with another country, efforts to control rabies through dog vaccination campaigns have been ongoing at the community level since the 1960s with local successes [85,113,114]. Nevertheless rabies remains endemic and a public health concern with an average of 250 human deaths per year recorded nationally in the 1958-68 period, compared to 90 yearly in one hospital in Manilla alone in the 1987-2006 period [113,115]. Current efforts continue through vaccination and education campaigns throughout the Philippines [62,116–118]. Much less has been published about the rabies situation in Laos and Myanmar, with Laos reportedly having lower reported mortality than its neighbors, though this might be due to severe underreporting [119]. Finally, though not an ASEAN nation, Bangladesh is geographically relevant as it is the only Asian nation other than China and India to share a land border with ASEAN nations. More importantly, it has a very high estimated burden of disease with more than 2000 estimated annual deaths though recent in mass dog vaccination appear to be bringing a reduction in reported deaths [89,120].

PEP accessibility and availability in rabies endemic countries in the region also varies greatly with some nations such as Bangladesh and the Philippines having high accessibility to free vaccines whilst other such as Vietnam require a fee and others such as Cambodia have poor accessibility [83,121]. Beyond physical accessibility and cost, knowledge gaps is also a major barrier. Numerous surveys in various Southeast Asian countries have shown that though the existence of rabies is often know by a large majority of respondents, as is the association with dogs and dog bites, the prevention methods pre and post-exposure are often less-well known, though this can improve in the presence of outreach campaigns [122–127].

8. Rabies context in Cambodia

Cambodia is a relatively small constitutional monarchy in Southeast Asia of about 16.7 million people and 3-5 million dogs [87,99,128–130]. A former French protectorate, the Kingdom of Cambodia regained its independence in 1953 but went through a long and severe period of civil war and political instability from 1970 to 1992 that left the country with deep demographic, socio-cultural, and economic scars that are still visible today. The earliest recorded attempt to control rabies in Cambodia, through vaccination and culling campaigns, date back to 1934 under the direction of the Pasteur Institute of Saigon, now known as the Ho-Chi-Minh City Pasteur Institute in Viet-Nam [131]. In Cambodia, a branch of the Pasteur Institute was initially opened in 1953, but was eventually closed and destroyed in 1975 under the Khmer Rouge regime. The current Pasteur Institute of Cambodia (IPC) located in Phnom Penh, gradually re-opened starting 1986 until it's full official opening in 1995 [132].

Like its neighbors, Cambodia is rabies endemic and has one of the highest incidence rates of human rabies with an estimated 6 cases per 100,000 people annually for approximately 800 cases and 600,000 dog bite injuries per year [128,130,133,134]. IPC has made available PEP, with an average of

21,000 regimens being administered each year since 1998 at its center in Phnom Penh, making it the main source of PEP in Cambodia [121,128,130]. The PEP protocol at IPC follows the current one-week intradermal WHO standard mentioned above [77,79,128]. IPC has also recently stepped up its efforts through education campaigns and by opening, in 2018 and 2019, new PEP centers in the Battambang and Kampong Cham provinces as part of a plan to meet the OIE, WHO and by extension ASEAN goal of rabies eradication by 2030 [133–136]. In terms of surveillance, IPC has been recording PEP administration and human rabies cases since 1998, though the latter is very anecdotal. There is also an ongoing passive surveillance program testing samples from suspect dogs responsible of bite injuries using the DFAT method. Through this effort, nearly 200 dogs have been tested each years with more than half returning positive [128,130,137,138].

However, the centralized nature of IPC in Phnom Penh until 2018 meant that access to PEP was very limited and that the burden was likely underestimated, especially in regions away from the capital [83]. With limited travel infrastructure, travelling to the capital can take precious time resulting in prohibitive loss of income and travel costs, which are doubled in the case of children which need to be accompanied and represent a large portion of bite injury victims [128,139]. Additionally, the cost of PEP varies from $30 to $70 when median monthly income in Cambodia is $100 [128,140]. Field surveys, as in other Southeast Asian countries, have also shown a relatively high awareness of the disease and its mode of transmission, but low awareness of the ways to prevent it following exposure, leading to many not seeking proper care or preferring traditional medicine [129,141,142]. Another major limitation of the rabies control effort in Cambodia is the current lack of a government organized canine vaccination program, making PEP the sole tool in preventing human infection [128]. However a pilot vaccination campaign and demographic study has been conducted in several villages of the provinces of Kandal and Battambang to inform future efforts [99].

9. General aims

The goal of this research is to help bridge knowledge gaps in Cambodia to help inform the improvement of current interventions, such as PEP, through the use of surveillance data, and the introduction of new ones, such as canine vaccination through disease modelling. Using data on PEP patients provided by IPC, the first chapter looks at the impact of accessibility to a PEP center using a Bayesian Poisson regression, and assesses locations for new centers that would maximize future access for bite victims. The second chapter focuses on animal surveillance data to help identify predictors of rabies with a Bayesian spatio-temporal logistic regression, in the dual goal of identifying areas where rabies is more prevalent and patients more likely to be exposed in the goal of informing allocation of PEP resources. Finally, the third chapter is the development of a spatially explicit rabies transmission agent-based model at the village level, using detailed local demographic data, to investigate the impact of vaccination efforts.

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CHAPTER 1: Impact of accessibility to rabies centers on human rabies post-exposure prophylaxis rates in Cambodia and identification of optimal future center locations using spatio-temporal Bayesian regression modelling

Short title: accessibility to rabies post-exposure prophylaxis in Cambodia

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Abstract

Rabies is endemic in Cambodia. For exposed humans, Post-Exposure Prophylaxis (PEP) is very effective in preventing this otherwise fatal disease. The Institut Pasteur du Cambodge (IPC) in Phnom Penh was the primary distributor of PEP in Cambodia until 2018. Since then, and to increase distribution of PEP, two new centers have been opened by IPC in the provinces of Battambang and Kampong Cham. Data on bitten patients, who sometimes bring the head of the biting animal for rabies analyses, have been recorded by IPC since 2000. However, human cases are not routinely recorded in Cambodia making it difficult to establish a human burden of disease and generate risk map of dog bites to inform the selection future PEP center locations in high-risk areas. Our aim was to assess the impact of accessibility to rabies centers on the yearly rate of PEP patient in the population and generate a risk map to identify the locations where new centers would be the most beneficial to the Cambodian population. To accomplish this, we used spatio-temporal Bayesian regression models with the number of PEP patients as outcomes. The primary exposure variable considered was travel time to IPC centers. Secondary exposure variables consisted in travel time to a provincial capital and urban proportion of the population. Between 2000 and 2016, a total of 293,955 PEP patient records were identified. Our results showed a significant negative association between travel time to IPC and the rate of PEP patients: an increase in one hour of travel time from the living location to IPC PEP centers leads to a reduction in PEP rate of 70% to 80%. Five provinces were identified as the ideal locations for future centers to maximize PEP accessibility: Banteay Meanchey, Siem Reap, Takeo, Kampot and Svay Rieng. Adding a PEP center in every provincial capital would increase the proportion of Cambodians living within 60 minutes of a PEP center from 26.6% to 64.9%, and living within 120 minutes from 52.8% to 93.3%, which could save hundreds of lives annually.

Author Summary

Rabies is a fatal viral disease that affects the nervous system. It is endemic in many countries in Africa and Asia where free roaming dogs form a reservoir. Transmission to humans occurs most often through a dog bite. However, post-exposure prophylaxis (PEP) if administered before symptoms start is highly effective at preventing the disease. In Cambodia, a few number of centers offer PEP, with the Institut Pasteur du Cambodge in Phnom Penh, being the main one. These limited locations lead to limited accessibility for rural areas distant from Phnom Penh and underestimations of the dog-bite burden and PEP needs. Through statistical modelling, we measured the impact of accessibility on the number of PEP patients and predicted the impact of opening new centers in other locations. We found that travel time was significantly associated with the rate of PEP patients. IPC opened new rabies centers in Battambang and Kampong Cham provinces in 2018 and 2019 and we identified four provinces where future openings would be the most beneficial: Banteay Meanchey, Siem Reap, Takeo, Kampot and Svay Rieng. This study is part of a broader drive to eradicate rabies in Cambodia by 2030 through increased PEP infrastructure and control measures in the dog population.

1. Introduction

Rabies is a neurotropic virus member of the genus Lyssavirus in the Rhabdoviridae family, for which multiple carnivore species are reservoirs [1,2]. Most mammals can be infected, including humans and common domestic species [3]. It is of great concern to humans both in terms of health and economic impacts with nearly 59,000 people dying from rabies every year and an overall cost of $8.6 billion, including direct and indirect cost from human cases and loss of livestock [4,5][5]. The societal cost is compounded by the violent nature of the disease, the young age of victims, with a median age of 26 in Cambodia [6], and the absence of treatment for symptomatic patients. The virus is transmitted through the saliva of an infected animal, most commonly through a bite, leading to an usually long incubation period, typically three to eight weeks, but sometimes months, and an almost 100% fatal outcome once clinical symptoms appear [7,8]. The vast majority of cases occur in developing countries in Africa and Asia with nearly 99% of human cases resulting from dog bites. Eighty seven percent of these cases occur in rural areas [3]. Cambodia is one such country where rabies is endemic and has one of the highest incidence rates of human rabies in the world, with an estimated 6 cases per 100,000 people annually for approximately 800 cases and more than 375,000 dog bite injuries per year [6,9,10].

Rabies vaccines have proven very effective for protecting humans, dogs, and wildlife, but the use of the vaccine is highly variable globally. Moreover, even in the event of human exposure, most cases can be avoided with timely administration of post-exposure prophylaxis (PEP) [11]. Since 2018, the WHO has recommended a one-week PEP regimen involving the administration human rabies immune globulin (HRIG) in combination with a 2-site intradermal (ID) injection of the first dose of rabies vaccine, ideally on the day of exposure. Two subsequent vaccine doses are administered 3 and 7 days after the first one [12,13]. However, other longer regimens in use can be intramuscular (IM) or ID, 1 or 2-site, and add a fourth and sometimes fifth dose between 14 to 28 days after the first [11,12,14]. In comparison, typical rabies pre-exposure prophylaxis (PrEP) involves three vaccine doses on days 0, 7 and 21 [15].

With these tools and effective control and management of the dog population, rabies has been controlled and nearly eradicated in most of Europe and the Americas [16]. In Southeast Asia, without achieving full elimination, the situation improved with canine vaccination and expanded access to PEP, especially in Vietnam and Thailand, seeing a dramatic reduction of human and dog cases [17–20]. However, in areas where rabies remains endemic, successful mitigation of human rabies requires rapid and reliable availability of PEP for bite victims, in addition to effective and sustained dog vaccination strategies and appropriate dog population management.

Institut Pasteur du Cambodge (IPC), located in Cambodia's capital Phnom Penh, has made PEP available since 1998, with an average of 21,000 regimens being administered each year [9]. IPC has used the WHO-recommended one-week PEP regimen since 2018 [21]. PEP administration has been recorded alongside bitten patient interviews about the characteristics of the exposure. In addition, an ongoing passive surveillance program tests head samples from suspected animals responsible for bite injuries using PCR [22]. This program relies on patients voluntarily bringing biting animal heads when coming to IPC to seek PEP. However, with limited travel infrastructure, travelling to the capital can take precious time resulting in prohibitive loss of income and travel costs. Additionally, the cost of PEP varies from $30 to $70 in a country where the median monthly income is $100 [23]. Field surveys have also shown a relatively low awareness of rabies in rural areas, its mode of transmission, and the ways to prevent it following exposure, leading to many people not seeking proper care or preferring traditional medicine [24,25]. Thus, the centralized nature of IPC in Phnom Penh associated with the cost and lack of information means that access to this critical care is unevenly distributed across the country and that the rabies burden and the need for PEP is likely underestimated, especially in rural regions away from the capital. A few other locations such as the Angkor Children Hospital in Siem Reap and the National Institute of Public Health clinic in Phnom Penh also offer PEP, as well as a few private clinics. However, the quality and cost of PEP regimens at these private clinics remains unknown and a large majority of recorded PEP patients come to IPC [26]. In addition to the Phnom Penh PEP center, IPC opened two new locations for PEP treatment, one in Battambang city in July 2018, located in the far northwest of the country, and the second one in Kampong Cham, the third largest city of Cambodia, located 120km from Phnom Penh on the right bank of the Mekong, in March 2019. Unfortunately, there is no national systematic surveillance for human cases and the passive surveillance for dog rabies is biased by the centralized nature of IPC, with most tested animals being from areas close to Phnom Penh. Thus, the direct assessment of a human or animal rabies cannot be established to guide risk-based allocation of future PEP clinics. We can however guide the geographic allocation of resources through indirect means from human PEP data. Using methods that better control for spatial heterogeneity and missing data whilst that adjust for the geographical accessibility (subsequently referred to as accessibility) to PEP centers we can provide a more detailed picture of the rabies burden and the PEP needs in Cambodia.

A number of studies have successfully used Bayesian statistics to create disease risk maps in settings where case data is either unequally distributed, incomplete, or both, as is often the case with veterinary and neglected zoonotic diseases [27–30]. These methods can also fit complex regression model structures that can include spatial and temporal autocorrelation [31–41]. The primary goal of this paper was to assess the impact of accessibility to a PEP center and urbanization level of the province or district of origin on the observed number of PEP patients using Bayesian regression modeling. This will help identify provinces where new PEP centers would be the most beneficial to the Cambodian population by investigating the potential impact of opening new PEP centers on expected numbers of PEP patients. This study is part of a broader effort to control rabies in Cambodia, helping to determine resource allocation, risk-based strategies, and guide policies to meet eradication targets.

2. Methods

We considered three variables potentially influencing the access to the PEP center for bite victims: travel time to the nearest PEP center, travel time to the closest provincial capital, and urban population proportion as a proxy for socio-economic status and accessibility to general healthcare and health information.

2.1 Data collection and management

2.1.1 Rabies surveillance data

Since 1998, IPC has made PEP available to dog-bite victims at its Phnom Penh location, initially for free, and then with a fee starting in 2010 [23]. In parallel, each patient was given a questionnaire upon arrival to collect information related to the characteristics of the attack and the victims such as name, age, and address in order to guide allocation of HRIG and the PEP regimen. These data were then completed over time with follow-up visit information, number of injections, and results from animal testing when the head of the biting animal was brought with patients. The victim's residence was recorded at the province level from 1998 to 2013, then at the district level from 2013 to 2016.

2.1.2 Administrative and demographic data

Cambodia has four main levels of administrative divisions. The largest division is the province, followed by the district, the commune, and the village. Demographic data (population size and urban population proportion) by province and district where obtained from the 1998,2008 and 2019 official population censuses of Cambodia [42–44]. A linear population growth was assumed and projected between the census 1998 and 2008 population values to estimate population values from 2000 to 2007. The same method was used between the census 2008 to 2019 population values to estimate values from 2009 to 2016 (Fig SP1).

Administrative maps were obtained from open-sourced data platforms for the year 2010 and 2016 [45,46]. We performed our modelling analyses at both province and district levels. Certain areas, such as Phnom Penh, have special nomenclature but follow the same overall structure in their divisions. Cambodia has undergone numerous administrative changes within the time frame of our study. The most important were: 1) the split of Kampong Cham province into 2 provinces in 2013, creating the new province of Tboung Khmum; 2) the absorption of a number of densely populated communes from Kandal province into Phnom Penh province causing the administrative transfer of approximatively 250,000 people between the two provinces; 3) the creation of numerous new districts across the country, increasing their number from 183 in 1998 to 204 by 2019. We standardized the population data to arbitrary fixed time points in terms of administrative divisions. For the province level modelling, the 2010 map, which had the same administrative divisions as the 2008 census, i.e. 24 provinces and 192 districts, was used. For the district level modelling, the 2016 map comprising 25 provinces and 197 districts was used. Population totals for all years were adjusted as described below, to take into account the administrative geography of the selected maps. For the province level model, population values of the 2019 census for Tboung Khmum and Kampong Cham provinces were aggregated into the single Kampong Cham province to reflect the 2010 map boundaries. The same was done for IPC data for the years 2013 to 2016. We identified individual communes that were transferred from Kandal Province to Phnom Penh to adjust projections when the transfer was made in 2013. Thus, projections from 2008 to 2012 assumed the administrative division of 2008, and projections from 2013 to 2017 assumed the administrative divisions of 2016. Individual patient records at the district level were manually crosschecked using province and commune names to make sure the information matched the administrative map in use.

The urban proportions of provinces and districts were calculated from the definition of urban communes used in the 1998 and 2008 censuses. This definition was modified in the 2019 census. However, we kept using the older standard for consistency in the projections.

2.1.3 Accessibility data

We used the global friction raster from the Malaria Atlas Project to create a variable measuring accessibility to PEP centers and provincial capitals from patient's residence origin [47]. Using primary data covering transport infrastructure, land coverage, geographical features such as slope, altitude, and water bodies, this dataset estimates the travel speeds across the globe with a resolution of one square kilometer. Using the coordinates of PEP centers or provincial and district capitals, and the friction raster, the R package "gdistance" allows the computation of the shortest travel time from any pixel on the map to the nearest point (PEP center or provincial/district capital) by assessing all possible paths between them [48,49]. This produces a raster of travel time from any given location to the nearest point with the same resolution as the friction raster.

2.1.4 Prediction scenarios for PEP patients

We considered five accessibility scenarios for predicting expected number of PEP patients (Table 1). The first scenario (Scenario 1) represents the situation that was in place from the post-war re-opening of IPC in 1995 with one PEP center in IPC Phnom Penh until 2017 (Fig SP2A). This scenario was used to construct the statistical model as it represents the situation in place when the IPC surveillance data were collected from 2000 to 2016. This scenario was also used to predict patient numbers if no new centers were opened after 2016. The second scenario (Scenario 2) incorporates 3 PEP centers: the IPC Phnom Penh center as well as the two new PEP centers, one at the department of health facility in the city of Battambang, which opened in 2018, and the other at the Kampong Cham hospital which opened in 2019 (Fig SP2B). This scenario was used to predict the numbers of patients based on the PEP access available in Cambodia from 2019 through 2020. The three last scenarios represented a situation with theoretical openings of new PEP centers (Fig SP2C & SP2D). The first one assumed a PEP facility in every provincial capital (Scenario 3) and the other assumed a PEP facility in every district capital (Scenario 4). As Scenario 3 assumed the un-realistic simultaneous addition of centers in every provincial capitals at the same time, a set of multiple simulations (scenario 5) adding a center in a single provincial capital at a time (in addition to the three centers already in existence) were used to observe the specific impact of each location and identify locations where a new center would maximize the number of new patients. Scenario 5 was run with the district level model as earlier models showed that the higher spatial resolution better captured population distribution in regards to accessibility and was considered more accurate for advising the location of future centers. The accessibility measure was aggregated by means of extracting the median travel time to a PEP center or provincial/district capital in each province or district. These provinces and district could then be linked to the patient's province or district of residence.

Table 1: Description of accessibility scenarios used for model fitting and predictions of future PEP patients rates and numbers.

	Description	Source	Model usage	Spatial scale of model
Scenario 1	One PEP center available in Phnom Penh. This represents the situation in place from 1995 to 2017 and includes the data collection period from 2000 to 2016.	Coordinates of IPC were used to create accessibility raster	Model fitting and 2017 predictions	Province and district
Scenario 2	Three PEP centers available in Phnom Penh, Battambang and Kampong Cham. This represents the situation in place currently, following the	Coordinates of all three centers were used to create accessibility raster	2017 predictions	Province and district

	opening of two new IPC centers in Battambang city (2018) and Kampong Cham city (2019).			
Scenario 3	Theoretical scenario: a PEP center present in each provincial capital.	A spatial point data set of the centroid of provincial capitals was included with the 2010 administrative boundaries shapefiles [49]	2017 predictions	Province and district
Scenario 4	Theoretical scenario: a PEP center present in each district capital.	A spatial point data set of the centroid of district capitals was included with the 2010 administrative boundaries shapefiles [49]. Provincial capitals are also the administrative centers of their own district.	2017 predictions	Province and district
Scenario 5	21 scenarios of four PEP centers which include the three current PEP centers (Phnom Penh, Battambang and Kampong Cham) and one of each of the 21 remaining scenarios .	as scenario 1, 2 and 3	2017 predictions	District

2.2 Statistical analysis

2.2.1 Accessibility descriptive analysis

For each of the five accessibility scenarios, we used our 2016 demographic projections at the district level and the aggregated accessibility data to estimate the proportion of the population living in districts that where within 60 minutes and 120 minutes travelling time from the nearest PEP center.

2.2.2 PEP models

To estimate the impact of accessibility on PEP patient rates and predict new patients we used a Bayesian modelling framework using Integrated Nested Laplace Approximations (INLA). Analysis was performed with the R package “R-INLA” [50–53]. We investigated two spatial scales (province and district) and two temporal scales (year and month). The predicted PEP patient numbers were modelled using a Poisson regression with a population offset in which time was used as a non-parametric auto-correlated temporal effect. Three fixed effects were considered: travel time to the closest PEP center, urban population proportion of the district or province of the patient residence, and travel time to the closest provincial capital. The graphical representation did not suggest any seasonal pattern. This was confirmed by a preliminary analysis of the number of patients every month by year using a generalized additive model (gam) with a smooth term over month, using the R-package “mgcv” [54] (Fig SP3). The result of this analysis was not significant (p-value=0.815).Therefore, we constructed all prediction models using a year-level temporal random effect only. Model selection was done using the Deviance Information Criterion (DIC) and the Watanabe-Akaike Information Criterion (WAIC). All models in this section and beyond used minimal non-informative priors as set by default in R-INLA. The default prior distribution for a Poisson model in R-INLA is a Gamma distribution with the following parameters *Gam*(1, 0.00005) [55]. Once the best fitting model was obtained, both at the provincial and district level, models were fitted using a dataset that was expanded to include the prediction year 2017. This involved adding observations with demographic and accessibility data for 2017 but with NA values for the outcome, to be completed when running the model. Due to the different spatial aggregation scale, resulting models were not directly comparable with DIC and WAIC. Therefore, we used intra-class correlation coefficients (ICC) to assess the agreement between observed and fitted values. These ICCs where computed using the R package “irr” [56].

All data management and statistical analysis was conducted in R Version 4.0.3 [25].

3. Results

3.1 Descriptive data

Between 2000 and 2016, 293,955 PEP patient records were identified and associated to a given province, representing a rate of 12.97 patients per 10,000 person-years. For another 85 records (0.03%), the province could not be identified. From 2013 to 2016 we observed 85,780 records with an identifiable district, whereas a further 42 patients (0.05%) could not be located. Yearly data are summarized in Fig 1. We observed two phases in PEP patient numbers with a plateau between 12,000 and 14,000 patients per year from 2000 to 2007, followed by a major increase to a second plateau between 20,000 and 22,000 patients per year from 2008 to 2016. Despite the population increase during this time, the rate of PEP per 10,000 people also showed a major increase from 2007 to 2008.

The distribution of geographic origins of PEP patients was very heterogeneous, with 158,009 patients (53.8% of patients) coming from the capital city of Phnom Penh and another 60,267 (20.5%) coming from the province of Kandal, which surrounds Phnom Penh (Fig 2). In comparison, seven provinces had less than 100 PEP patients in the 17 years of data, and another seven provinces had between 100 and 1,000 patients. Phnom Penh and Kandal also had by far the highest rates of patient recruitment with 65.3 and 29.3 patients per 10,000 person-years, respectively. The patient recruitment of all other provinces was below 10 (suppl table ST1). These patterns were also visible at the district level, with a ring of districts with high patient numbers and rates surrounding Phnom Penh, and very low values further away, with 13 districts without any patients between 2013 and 2016 (Fig 3).

3.2 Population accessibility

Based on our population projections, Cambodia had a population of 14.70 million people in 2016. Of these, 26.6% lived in districts with a median travel time of 60 minutes or less to the IPC PEP center in Phnom Penh, and another 26.2% lived in districts with a median travel time between 60 and

120 minutes of that center, for a total of 52.8% below 120 minutes. In comparison, from 2013 to 2016 74.9% of PEP patients came from districts within 60 minutes of IPC and another 21.5% between 60 and 120 minutes. Adding the two new centers in Battambang and Kampong Cham provinces results in 36.6% of the population living within 60 minutes of a PEP center and 34.8% living between 60 and 120 minutes, bringing the total to 71.5% below 120 minutes from a PEP center. Testing the location of new provincial PEP centers individually in each of the remaining provinces (Scenario 5) increased the proportion of the population living within 60 minutes of a center up to 41.3% (with a center added in Svay Rieng province) and 78.3% within 120 minutes (with a center added in Siem Reap province). The worst-case scenarios, were adding a center to Kandal or Mondul Kiri Provinces, which increased the population living within 60 min of center by 0% and 0.1% respectively and within 120min by 0.3% and 0.1% respectively. The four provinces where adding a center had the biggest accessibility impact were Banteay Meanchey, Siem Reap, Svay Rieng, Takeo, each adding more than 500,000 people living within 60 minutes of a PEP center. Another three provinces, Kampot, Prey Vaeng and Pursat, added between 315,000 and 380,000 people living within 60 minutes of a PEP center with the rest yielding values below 215,000. Adding a PEP center in every provincial capital would increase the proportion of Cambodians living within 60 minutes of a PEP center to 64.9% and within 120 minutes to 93.3%.

3.2 PEP model results

At both the province and district levels, the PEP patient model was fitted with three variables: travelling time to the closest IPC PEP center, urban population proportion, and travel time to the provincial capital. At both province and district level, and in both univariate and multivariate models, travel time to the closest IPC PEP center had a significantly negative association with the rate of PEP patients. These rate ratios varied from 0.20 to 0.30 (Table 2), thus increasing travel time by 1 hour reduced PEP rates by a range of 70% to 80% depending on the model. Time to provincial capital also had a strongly significant negative association with rate of PEP in univariate models at both the province and district levels (RR=0.06 and 0.16, respectively). However, once adjusted for the other two variables, this relationship turned to be strongly positive in both cases (RR=2.33 and 2.93, respectively). Finally, urban population proportion had a positive association with PEP rate in both univariate models (RR=1.33 and 1.17, respectively). In both the province and district level models, adjusting for accessibility to PEP centers and provincial capital reduced the effect size of urban proportion bringing the rate ratios closer to the null (rate ratio of 1) with values of 1.09 at the province level and 1.02 at the district level.

Table 2. Rate ratios for fixed effects (with 95% credibility intervals) in univariate and multivariate Bayesian Poisson regression models

Fixed effect variable	**Province level**		**District level**	
	Univariate	**Multivariate**	**Univariate**	**Multivariate**
Time to vaccination center (1h)	0.275 (0.273 to 0.276)	0.265 (0.261 to 0.279)	0.297 (0.294 to 0.300)	0.196 (0.192 to 0.199)
Time to provincial capital (1h)	0.061 (0.061 to 0.062)	2.334 (2.244 to 2.426)	0.157 (0.154 to 0.160)	2.934 (2.824 to 3.049)
Urban proportion (10%)	1.329 (1.328 to 1.331)	1.092 (1.090 to 1.094)	1.168 (1.166 to 1.169)	1.017 (1.015 to 1.019)

Coefficients for the random effect of year mostly follows the pattern of the observed rate of testing, with a plateau from 2000-2007, before a high increase in 2008 before slowly reducing after 2008. The district level model, which only fitted 2013-16 data, only shows the downwards trend of PEP rates. When comparing the ICC of the two multivariate models, we observe a closer fit of the data at the provincial level with an ICC of 0.97, compared to the district model with an ICC of 0.90 (Table 3).

Table 3. Intraclass correlation coefficient (with 95% confidence intervals) between fitted and observed values for the multivariate Bayesian Poisson regression models.

Model outcome	Province level	District level
PEP patients	0.969 (0.962-0.974)	0.898 (0.883-0.911)

3.3 Predictions for 2017

Predictions for 2017 had broadly similar topline numbers at the district and province levels. In Scenario 1, which corresponds to the initial situation with only one PEP center in Phnom Penh, the province level model predicted 21,885 PEP patients for 2017, compared to 21,611 observed patients in 2016, and the district model predicted 21,643 PEP patients (Table ST2). To be able to compare both province and district level models, results from the district model were aggregated at the province level in the following descriptions.

The opening of the two new centers in Battambang and Kampong Cham provinces (Scenario 2) led to predictions of 28,040 patients for the province model and 29,950 patients for the district model, which corresponds to an increase of the number of predicted patients of 6,155 and 8,307 respectively. In both models, the two provinces with the biggest increases in number of patients were Battambang and Kampong Cham, where the new PEP centers are located. These combined provinces went from 1,067 to 5,290 predicted patients in the province model, which represents 69% of the national predicted increase. In the district model, the two provinces went from 1,321 to 5,457 predicted patients, representing 50% of the national predicted increase. The new province of Tbong Khmum, which is included as part of Kampong Cham in the province model, also experienced a major increase of 1856 patients (from 305 to 2,161) in the district level model (22% of the national increase). Most other changes came from provinces bordering Battambang and Kampong Cham, with Banteay Meanchey experiencing the largest increase in the number of predicted patients in both models, from 19 to 972 at the province level, and from 7 to 1,055 at the district level. The number of predicted patients slightly increases in non-neighboring provinces. The localized impact of these two new centers is very visible when mapped at the district level, with concentric rings of increasing PEP rates in districts closer to the PEP center (Fig 4A & B). Full provincial predictions are detailed in Table ST3.

The theoretical scenarios 3 and 4 led to larger numbers of predicted patients. The province level model predicted 42,992 patients with a PEP center in each province (Scenario 3) and 76,638 with a PEP center in each district (Scenario 4). The district level model predicted 50,944 and 92,601 for these two scenarios, respectively. These new patients are much more widely distributed across the country, but rates seem to remain higher in districts and provinces surrounding Phnom Penh as well as in provinces in the North West (Fig 4C & D).

For each scenario, except Scenario 1, the district level model had higher predicted patient numbers than the province level model. Simulating the opening of a new center in each province individually (Scenario 5) using the district level model led to increases in the number of predicted patients, ranging from 193 (PEP center in Mondul Kiri) to 3,336 (PEP center in Siem Reap). Four locations led to more than 2,300 additional predicted patients: Siem Reap, Svay Rieng, Takeo and Beantay Meanchey. Another three provinces yielded increases between 1,500 and 1,900: Kampot, Kampong Speu and Kampong Thom. Results for Scenario 5 are summarized in table ST4.

4. Discussion

4.1 PEP in Cambodia

The last estimation of rabies burden in Cambodia was published by Ly et al in 2009, with 800 estimated human deaths per year [6]. A recent survey performed in Battambang and Kandal provinces showed that the yearly bite incidence remains among the highest in the worlds, with 2.3% in Kandal and 3.1% in Battambang respectively [10]. Thanks to the commitment of IPC in rabies burden mitigation in Cambodia, this survey provides an updated picture of the needs of Cambodia in terms of PEP. A rough extrapolation to the whole country would imply 375,000 bite injuries per year: even with an over-estimation, a large proportion of bitten people probably remain untreated. The proportion of victims who were infected by rabies remains unknown. From 2000 to 2007, PEP patient numbers were relatively stable with an average 13,020 patients per year, *i.e.* a rate of 10.4 patients per person-year. In 2008, this jumped to a new level of stability, with 21,100 patients a year or 15.0 patients per person-year. This major shift was associated with a shift in the geographical distribution of patients with 65% of cases originating from Phnom Penh in 2000-2007 compared to 45% in 2008-2016, suggesting improved awareness in rural provinces. Despite this, Cambodia has comparatively low PEP rates compared to neighboring countries where PEP is accessible in multiple centers. Vietnam reported 43.0 PEP patients per 10,000 person-year between 2005 and 2015 with nearly 500,000 patients in 2017. Although patients where still clustered in certain areas, access to PEP in Vietnam appeared more homogeneously distributed than in Cambodia [19,20]. However, both countries showed wide ranges of PEP rates between provinces with values ranging from 0.14 patients per person-year in Otdar Meanchey to 65.33 in Phnom Penh for Cambodia compared to a range of 2.21 to 156.27 in Vietnam. In Thailand, another country which has increased its PEP capabilities, the number of patients has increased from 90,000 in 1991 to 400,000 in 2003, which approximately represents an increase in rate from 16 to 60 patients per 10,000 person [57].

4.2 Accessibility and surveillance

As expected, accessibility was significantly associated with the rate at which individuals sought PEP following an animal attack. In both univariate and multivariate associations, and at both provincial and district levels, increasing travel time led to a reduction of the rate of PEP within the population. Between 2013 and 2016, 75% of PEP patients at IPC Phnom Penh, came from districts with a median travel time to IPC below 60min, and 96% of patients came from districts with a median travel time to IPC below 120min. For comparison, 27% of the Cambodian population in 2016 was living in districts located within 60min of IPC and 53% in districts within 120min.

The effect of the other two variables, urban population proportion and travel time to provincial capitals, varied with the model type (univariate or multivariate). This can be explained by the correlation between the three variables. There is a very high correlation between travel time to provincial capital and travel time to IPC, with a Pearson's correlation coefficient value of 0.78 at the province level and 0.74 at the district level. Provincial capitals, including Phnom Penh where IPC center is located, have better access to Phnom Penh than other parts of the country as they are on major roads, thus provinces or districts with higher accessibility to their own provincial capital tend to have higher accessibility to IPC in Phnom Penh. This explains that univariate results show similar results between these two variables: increasing travel time to IPC center or to the provincial capitals both decrease the PEP rates. Once we adjusted for time to IPC center, the multivariate results showed a negative association between time to provincial capital and PEP rate, suggesting that more remote rural areas have higher than expected PEP rates, which could be due to higher bite incidence in rural areas. A survey published in 2016 estimated a biting rate of 4.8 per 100 person-years in the rural province of Siem Reap [25] compared to 1.1 per 100 person years in both the urban province of Phnom Penh and the peri-urban province of Kandal in a 2011 survey [24], supporting this theory. Another more recent study found similar results with bite rates of 3.1 per 100 person-years in the rural province of Battambang compared to 2.3 per 100 in Kandal [10].

Conversely, urban proportion is negatively correlated with travel time to provincial capital and by extension with travel time to IPC, meaning that urbanized areas are closer to provincial centers. However, this correlation is stronger at the district level (Pearson's r = -0.42) compared to the province level (Pearson's r = -0.25). Cambodia is a rural country and has only one urban province where the majority of the population lives in urban areas, Phnom Penh, which is also the smallest in area as it is limited to the city of Phnom Penh. In the 2008 census, 93.6% of Phnom Penh Province's population was living in urban areas. In comparison, the proportion of people living in urban areas varied from 1.7% to 40.4% in other provinces. Therefore, as high urban proportion is strongly correlated to being close to Phnom Penh, we would expect that they would similarly be associated with higher PEP rates. Once adjusted for travel time, the association between PEP rate and the urban population proportion remained positive but with a much smaller odds ratio, still suggesting that more urban areas have higher PEP rates. This could be explained by the fact that urban areas, especially Phnom Penh, have higher economic and development metrics, thus better access to general health-care and information about PEP availability at IPC as well as higher incomes [43]. Evidence of cost being a barrier to PEP can be seen in the drop of PEP rates in 2010 and 2011, following the introduction of a $10 fee for PEP at IPC, compared to 2009 when PEP was free. On the other hand, at the district level we observed 30 highly urban districts, where more than 50% of people lived in urban areas as defined by the census. However, nearly all highly urban districts outside of the Phnom Penh area are where provincial capitals are located, thus urban proportion is highly correlated with travel time to provincial capital, and, by extension, time to IPC. In this case, adjusting for time to IPC brought the association of urban proportion and PEP rates even closer to the null. The impact of urbanization level on PEP seeking behavior should be cautiously interpreted as urbanization can serve as a proxy for socio-economic indicators and PEP awareness, which would increase expected patient numbers in urban areas, but also as a proxy of lower risk of rabid dog attack, which would decrease expected patients in urban areas relative to rural areas. A survey looking at awareness showed high levels of rabies awareness in both the peri-urban province of Kandal and the urban Phnom Penh, but lower awareness of IPC's existence in Kandal (12%) compared to Phnom Penh (32%) [24]. Similarly, individuals in Kandal were less likely to go to a clinic or hospital (47%) compared to Phnom Penh (66%) following a dog bite. A study performed in the province of Siem Reap showed higher education was associated with higher knowledge, and farmers had lower knowledge compared to other professions [58].

Overall, our results suggest an underreporting dog-bite incidence, and that accessibility to the PEP center is a significant barrier for bitten individuals. This is in accordance with a previous study looking at the rate of completion of PEP regimen in Cambodia: patients living further away being less likely to complete their regimen [59].

As a tropical country, Cambodia has a dry and wet season with a monsoon. We suspected that flooding from the Mekong and Tonle Sap during the wet season might lead to lower accessibility and thus lower rates of PEP from June through October. However, preliminary data observation showed no evidence of seasonal pattern in PEP rate nationally or at the provincial level suggesting that the rainy season is not a significant barrier to care (Fig SP3). Tarantola et al. also found no association between the climate seasonality and the rate of PEP completion [59].

4.3 Expanding accessibility and predictions

When using the models to predict the impact of increased accessibility on the expected number of patients, we observed similar large increases in the number of patients at both province and district level models. In 2017, with only one center in Phnom Penh, the observed number of patients was 22,421, which is very close to our prediction of 21,712 in the district level model. In July 2018, a new center was opened in the city of Battambang and another was opened in March 2019 in the city of Kampong Cham. Our district level model predicted some 4,010 patients at the Battambang PEP center and 4,228 at the Kampong Cham PEP center for 29,950 total patients in Cambodia. In 2019, with all three centers operational, 78,691 patients were recorded of which 15,070 were at the Battambang center and 11,132 at the Kampong Cham center. These numbers are much higher than our predictions; however, patients at IPC in Phnom Penh also dramatically increased to 52,498. This is not related to any increase in accessibility but presumably to the impact of the story of a young girl bitten by a cat in December 2018 who died of rabies in February 2019. This event was widely distributed on social media and lead to a dramatic increase of PEP requests in the following months [60,61]. Indeed, the number of patients at the IPC Phnom Penh reliably averaged 1,811 a month from January 2012 to November 2018, with a narrow range between 1,512 and 2,319 patients. In December 2018, patient numbers increased to 2,906 and then continued increasing to a peak of 7,593 in March before coming back down to an average of 3,397 patients a month from April 2019 through December 2020. The number of patients in 2019 represents an increase of 134% in Phnom Penh compared to 2017. If we assume a similar rate of increase nationwide due to this event, our total expected number would be closer to 33,600 patients in 2019, of which 6,400 in Battambang and 4,800 in Kampong Cham. Though we have few data points from the opening of the Battambang center to when these events unfolded, we do observe a similar trend. The center opened in July 2018 and only recorded 84 patients in its first two months of operation, but then averaged 172 patients a month from September through November. In December, the number of patients at the Battambang center jumped to 600 and continued increasing to a peak of 1,501 in April 2019 before coming back down to an average of 1,157 from May 2019 to December 2020. Our projection for Battambang estimated 327 patients a month, which is actually higher than what was initially observed at the Battambang center prior the increases of December 2018, but much lower than the values that followed. As the Kampong Cham center did not open until March 2019, a similar comparison could not be conducted. As a whole, patient numbers in 2020 decreased from the surge of 2019 but remained much higher than expected with some 36,634 patients in Phnom Penh, 12,957 in Battambang and 9,593 in Kampong Cham. However, the drop in numbers in 2020 might also be attributable to movement restrictions caused by the Coronavirus pandemic, with all three centers reaching the lowest number of patients in April 2020.

In prediction scenarios with higher accessibility to a PEP center, the difference between the province level and district level model predictions increased. This is likely due to the district level model assuming a more accurate population distribution than the province level aggregation. Within each province, the population is more likely to be concentrated within districts that are close to travel infrastructure and urban centers. The province level model does not account for this more localized information. Scenarios representing a theoretical universal access to PEP more than doubled the expected number of patients if we assumed a center in each provincial capital, and tripled (province level model) or quadrupled (district level model) if we assumed a PEP center in every district. Nevertheless, even in Scenario 4, which modelled a center in each district, we still observed higher rates of PEP per population in district near Phnom Penh compared to others. Districts of Phnom Penh and neighboring provinces tend to be smaller and have a denser road network, thus they still have higher accessibility to their own administrative center compared to districts in the forested and mountainous regions in the Southwest and Northeast. Similarly, a corridor of high rates can be observed in districts surrounding the Tonle Sap Lake (Fig 4C & D), as these are along the two main North-South highways of Cambodia and thus have better accessibility to nearby administrative centers. Even with a PEP center in each district, around 91% of the population would live within 60min of a center.

Given the cost of opening new centers, it is impossible to implement in practice a center in every Cambodian district. Even a PEP center in every provincial capital is unlikely in the short to medium term. We used the district level model with Scenario 5, with its higher spatial and demographic resolution, to identify which individual provincial capital would have the most beneficial impact. Four provinces stood out as the most effective location to increase access to PEP: Banteay Meanchey, Siem Reap, Svay Rieng and Takeo. Opening a PEP center in one of these four provinces increased the number of yearly patients by a range of 2,300 to 3,300, and increased the population living within 60 minutes of center by a range of 503,000 to 683,000. A fifth location in Kampot would increase the number of patients by 1,800, and the population living within 60 min of a center by 381,000.

However, even when considering accessibility, predictions led to PEP rates that are much lower than bite rates reported from surveys. Our highest estimate projected 0.6 PEP patients per 100 person-years in 2017, against 1.1 bites per 100 person-years in Phnom Penh and Kandal provinces and 4.8 bites per 100 person-years in Siem Reap province [24,25]. These results show that even in the best-case accessibility scenario, there would still be under-reporting and under-coverage of bite injuries. This is despite the very high awareness of rabies in the population, with more than 90% of people knowing of the presence of the disease in dogs and more than 70% knowing it is fatal according to surveys conducted in Phnom Penh, Kandal and Siem Reap Provinces [24,58]. However, the Angkor Hospital for Children in Siem Reap and the National Institute of Public Health clinic in Phnom Penh also provide PEP in Cambodia [23,26]. Thus, IPC data are not reflective of all PEP patients in Cambodia, even if these two centers combined report 3,500 patients per year, which is much lower than the average of 22,000 patients per year at IPC. Furthermore, Ponsich reported that 12% of bite victims in Siem Reap received PEP from private clinics. [25]. Thus, a number of bite victims seek PEP in institutions that are not captured in our data, whether they receive effective PEP or not. Next, a majority (75%) of bitten and interviewed people in Siem Reap reported using traditional treatments and a minority reported any sort of modern medical treatment (36%) [25]. Similarly only 56% of bitten respondents in Kandal and Phnom Penh sought medical treatment and even fewer, 21%, were aware of the existence of IPC [24]. In both cases, these numbers were lower in Kandal than in Phnom Penh. The massive increase in patients in 2019 due to a media event is a clear example of the impact that media outreach can have in increasing awareness of the disease's impact and the ways of preventing it.

4.4 Model and data limitations

As Bayesian statistics using Markov chain Monte Carlo (MCMC) can be computationally intensive and difficult to parametrize, a method using integrated nested Laplace approximation (INLA) to approximate the posterior marginal distribution and developed for R was used in our study [51,53]. Studies comparing INLA to other regression modeling methods have shown that INLA can be simpler to use and quicker in computation whilst yielding similar estimates to other Bayesian or generalized linear approaches [55,62]. We believe this was the most cost-effective approach in this particular study.

However, the data used for this study had several limitations. First, the accessibility data were only available as one data point repeated over time, meaning that we could not capture the impact of the rapid improvement of Cambodian road infrastructure in the last three decades on PEP rates. We initially considered using a spatially auto-correlated random effect in the PEP model. However, because our accessibility variables were themselves spatially auto-correlated (e.g., districts close to IPC are close to other districts close to IPC and its inverse) and the lack of variation in accessibility over time within each geographical unit, the random effect negated the impact of accessibility as a fixed effect. Therefore, we did not use random effects in our PEP models, meaning that we could not adjust for the impact of specific geographic location beyond accessibility to IPC or its level of urbanization. This meant we could not identify areas with higher rates of PEP when adjusted for accessibility and urbanization. Furthermore, the global friction raster used to calculate accessibility relies on broad assumptions regarding mobility on a given type of terrain or infrastructure that might not be applicable to every segment of the population in every country. This might be particularly true for bite injury victims, which could have impaired mobility. Moreover, travel speed depends on accessibility to a vehicle which is limited in a country where only 5% of households owned a car and 44% owned a motorbike in 2008, though this would have likely increased since then [43,63]. However, in the absence a more locally specific mobility study, we assumed this would still be reflective of the broad accessibility issues for the country, and that if the assumptions from the Global Malaria Atlas project introduced a bias, this bias would be mostly similar throughout the country. Nevertheless, this does create unmeasured uncertainty around our estimates.

Secondly, given the infrequency of census data, demographic data were estimated for most years using linear projections, which is a simplistic approach to demographic projections. Finally, the lack of socio-economic indicators meant that the use of urban proportion was difficult to interpret. This indicator can be used as a proxy for many factors, such as wealth, education, how dogs are maintained, or exposure risk to a rabid dog. The 2008 census included such indicators, and likely the full 2019 census will as well. However, the provisional 2019 census did not yet include this information. Similarly, we did not have detailed information about the distribution of the dog population for all provinces and districts of Cambodia, which could also be a meaningful indicator of bite risks.

Finally, the models relied on data from 2000 to 2016 making them unable to take into account the opening of centers in Battambang and Kampong Cham provinces, which would have provided data actually measuring the impact of new center openings. Moreover, as is clearly shown by the discrepancy in numbers between projections and patient numbers actually observed in 2019, recent events have dramatically changed awareness in a way that could have lasting impact, limiting the validity of our prediction, which clearly underestimate the current situation. It can also be assumed that the COVID-19 pandemic might have major medium to long-term effects on mobility and accessibility as well as on IPC and other health infrastructure, further reducing the certainty around forecasted estimates.

4.5 PEP in the broader context of rabies prevention

It is important to note that PEP is not the only tool available to combat rabies. However, it is the main tool being currently implemented in Cambodia on a large scale. Beyond PEP access, IPC has launched large scale education campaigns to increase awareness of the disease and how to prevent it [64]. However, it is widely recognized that the most cost-effective to combat rabies is canine vaccination, which can be supplemented by population management strategies other than culling [16,65–69]. However there are currently no large scale canine vaccination programs in Cambodia, with control plans focused on expanding PEP accessibility, surveillance and population awareness [70]. Nevertheless a pilot vaccination and canine demographic study has been recently been conducted in two provinces of Cambodia [10]. This work is aimed at studying the feasibility and requirements of vaccination campaigns in Cambodia and will inform disease transmission and vaccination models currently under development.

5. Conclusion

Accessibility to a PEP center is one of the main barriers to obtaining PEP, and with only one main center in operation up to 2018, large portions of the country had little access to this life saving treatment. As a consequence, PEP rates in Cambodia varied between 9.9 and 16.2 per 10,000 people from 2000 to 2016. In comparison, neighboring Vietnam, which has broader access to PEP, showed rates varying from 38.9 to 65.5 per 10,000 between 2005 and 2015 [19]. Furthermore, with the centralization of most PEP at IPC and the lack of data collection from private clinics, the data collection with regard to both bite victim estimates and animal testing was highly centralized, making it difficult to establish a detailed picture of the distribution of the rabies risk and burden, and the need for PEP and canine vaccination. In 2018, IPC started expanding its capabilities in terms of PEP distribution and data collection by opening of two new centers, one in Battambang and the other in partnership with a provincial hospital in Kampong Cham. Based on our results, more centers would clearly be necessary to provide a broader access to PEP, as large portions of the population remain in areas that are distant from the existing centers. This study helps identify provincial capitals that should be prioritized as ideal locations for future centers. Two broad areas stand out as ideal future locations, one in the Northwest of the country, where the neighboring provinces of Banteay Meanchey and Siem Reap both showed potential for large increases in patients, despite the fact that both are bordering Battambang where a center is now located. In the South, the two neighboring provinces of Takeo and Kampot represent another area where at least one center would be advised. Finally, the province of Svay Rieng to the South East could be seen as a possible third location.

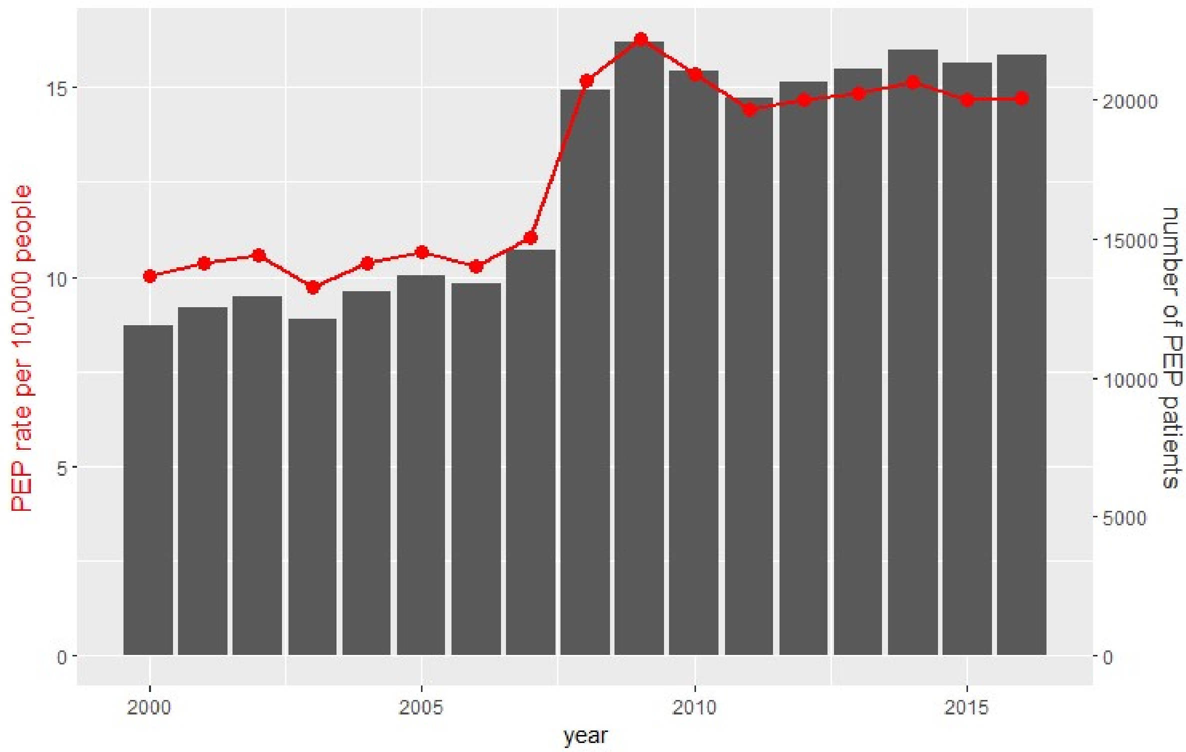


Fig 1. Observed number and rates of PEP patients, in Cambodia from 2000 to 2016.

Red curves represent rates per 10,000 people and histogram bars represent absolute numbers.

Fig 2. Time series of PEP patient numbers and rates by province.

Red curves represent rates per 10,000 people and histogram bars represent absolute numbers.

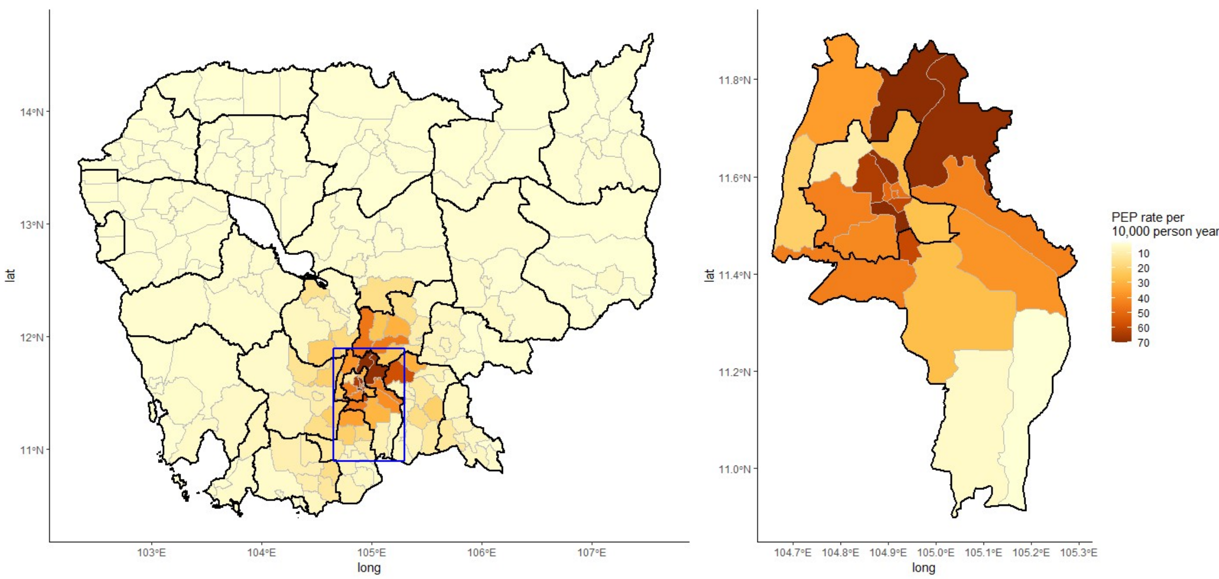
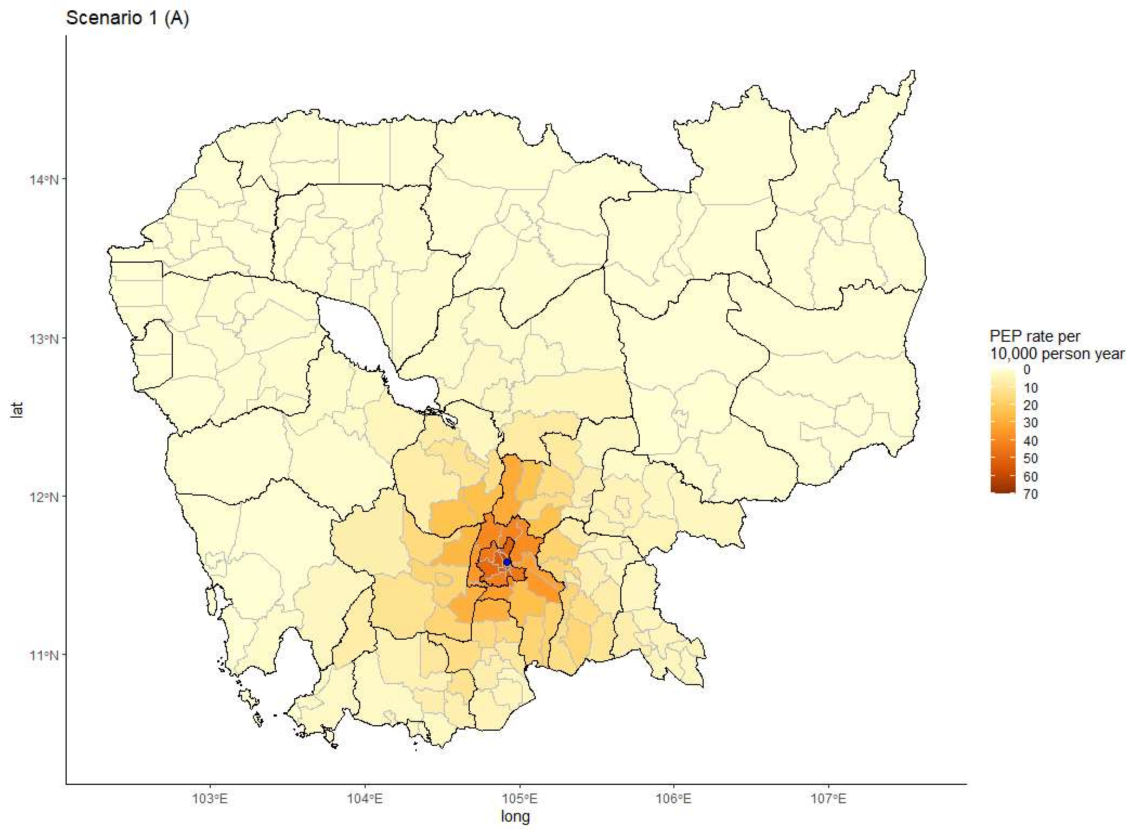
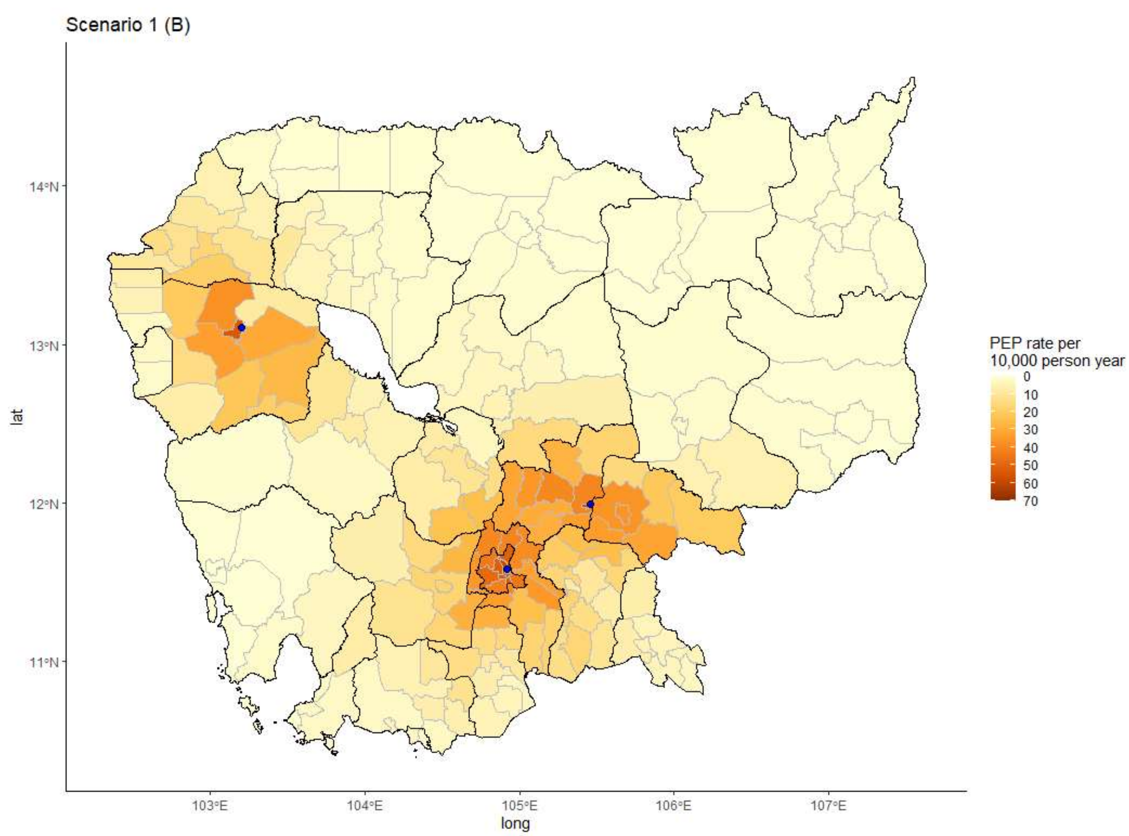


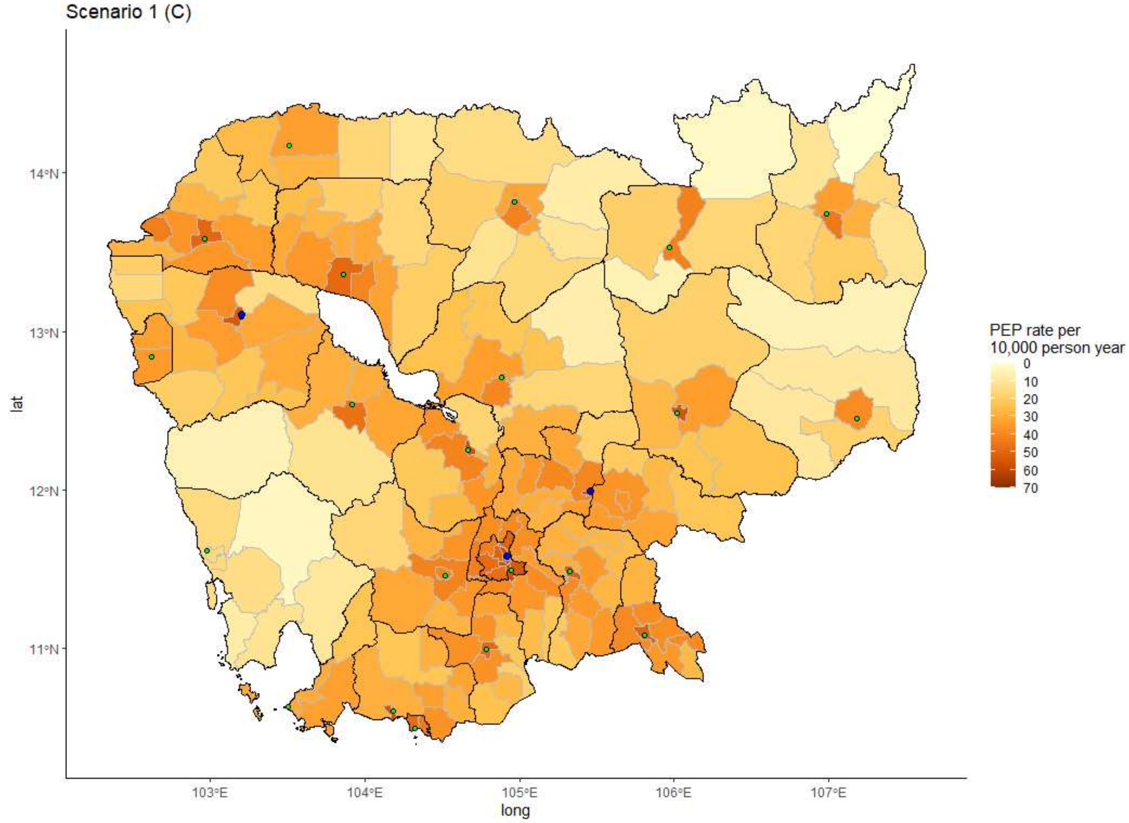
Fig 3. Observed average rates of PEP patients per district for the years 2013 to 2016.

The inlet focuses on the provinces of Kandal and Phnom Penh where the majority of PEP patients at IPC come from. Bold lines represent provincial boundaries.


Scenario 1 (A)
14°N
13°N
12°N
11°N
lat
103°E
104°E
105°E
106°E
107°E
long
PEP rate per
10,000 person year
0
10
20
30
40
50
60
70


Scenario 1 (B)
14°N
13°N
12°N
11°N
lat
103°E
104°E
105°E
106°E
107°E
long
PEP rate per
10,000 person year
0
10
20
30
40
50
60
70

Scenario 1 (C)



Scenario 1 (D)

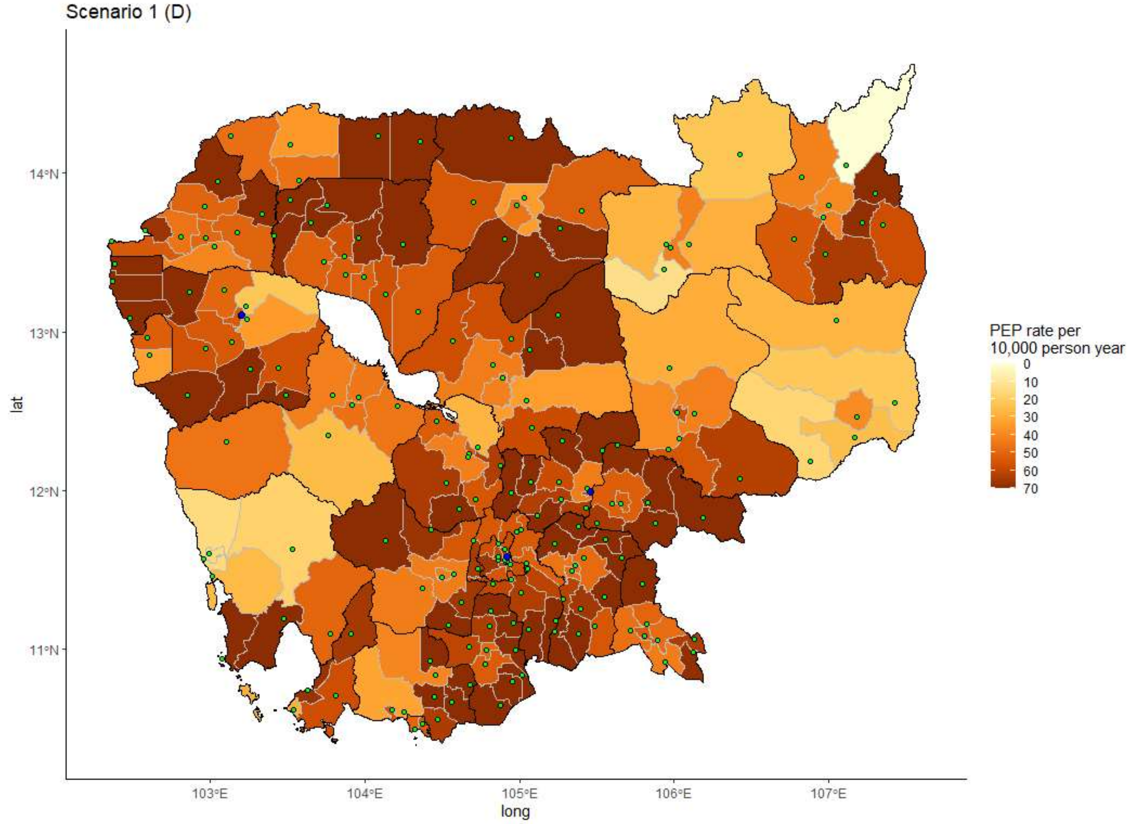


Fig 4A, B, C & D. Predictions of the rate of PEP patients in the population for the year 2017 based on three scenarios.

(A) Scenario 1 represents the situation prior to the opening of new centers in Battambang and Kampong Cham provinces with a single center in Phnom Penh. (B) Scenario 2 represents the current situation, with the opening of two new centers in Battambang and Kampong Cham provinces that actually opened in 2018 and 2019 respectively, bringing the total number of centers to three. (C & D) Scenario 3 and 4 represents the theoretical opening of a center in every provincial capital and district capital respectively. Blue dots represent currently existing centers as of 2020, green dots represent provincial or district capitals where future centers could be opened.

Supporting Information

Supplementary table ST1. Observed cumulative PEP patients by province from 2000 to 2016.

Province	PEP patients	PEP rate per 10,000 person-year
KH01 Banteay Mean Chey	229	0.19
KH02 Battambang	507	0.31
KH03 Kampong Cham	22,809	8.09
KH04 Kampong Chhnang	4,143	5.19
KH05 Kampong Speu	9,870	8.05
KH06 Kampong Thom	3,432	3.22
KH07 Kampot	4,465	4.57
KH08 Kandal	60,267	29.30
KH09 Koh Kong	291	1.45
KH10 Kratie	545	1.01
KH11 Mondul Kiri	46	0.45
KH12 Phnom Penh	158,009	65.33
KH13 Preah Vihear	67	0.23
KH14 Prey Veaeng	12,507	7.60
KH15 Pursat	435	0.65
KH16 Ratanakiri	66	0.26
KH17 Siem Reap	356	0.24
KH18 Preah Sihanouk	482	1.35
KH19 Stueng Treng	40	0.21
KH20 Svay Rieng	1,738	2.09
KH21 Takeo	13,563	9.47
KH22 Otdar Meanchey	42	0.14
KH23 Kep	12	0.20
KH24 Pailin	34	0.33

Supplementary table ST2. Nation-wide predictions of PEP patients and rates according to four prediction scenarios.

Results from both province and district level models are presented. Scenario 1 represents the situation prior to the opening of new centers in Battambang and Kampong Cham provinces with a single center in Phnom Penh. Scenario 2 represents the current situation, with the opening of two new centers in Battambang and Kampong Cham provinces that actually opened in 2018 and 2019 respectively, bringing the total number of centers to three. Scenario 3 represents the theoretical opening of a center in every provincial capital. Scenario 4 represents the theoretical opening of a center in every district.

Measure	Scenario	province level numbers	province level rates	district level numbers	district level rates
PEP patients (rate per 10,000 people)	2016 observed	21,611		21,611	
	2016 fitted values	21,609 (21,290 to 21,932)	14.02 (14.49 to 14.92)	21,623 (21,259 to 22,002)	14.71 (14.46 to 14.97)
	2017 (scenario 1)	21,885 (17,477 to 27,440)	14.73 (11.78 to 18.43)	21,712 (20,471 to 23,048)	14.61 (13.77 to 15.51)
	2017 (scenario 2)	28,040 (22,425 to 35,095)	18.87 (15.09 to 23.62)	29,950 (28,235 to 31,819)	20.15 (19.00 to 21.41)
	2017 (scenario 3)	42,992 (34,365 to 53,825)	28.93 (23.12 to 36.22)	50,944 (48,012 to 54,128)	34.28 (32.31 to 36.42)
	2017 (scenario 4)	76,638 (61,206 to 96,046)	51.57 (41.19 to 64.63)	92,601 (86,899 to 98,789)	62.31 (58.47 to 66.47)

Supplementary table ST3. Predicted number of patients by province based on four prediction scenarios.

Results from both province and district level models are presented. Scenario 1 assumes no opening of new vaccination centers. Scenario 2 assumes the opening of two new centers in Battambang and Kampong Cham provinces that actually opened in 2018 and 2019 respectively. Scenario 3 assumes the opening of a center in every provincial capital. Scenario 4 assumes the location of a vaccination center in every district. This is a theoretical scenario used as a proxy for universal access.

Provinces	Province level model					District level model				
	2016	2017-I	2017-II	2017-III	2017-IV	2016	2017-I	2017-II	2017-III	2017-IV
KH01 Banteay Mean Chey	11	19	972	2248	3951	19	7	1055	2819	4589
KH02 Battambang	26	34	1472	2131	5324	58	31	2515	2948	6104
KH03 Kampong Cham	1751	1033	3818	3914	8404	1677	1290	2942	2963	6122
KH04 Kampong Chhnang	380	546	547	1146	2434	417	722	724	1709	2880
KH05 Kampong Speu	873	1021	1021	1968	3879	903	1638	1638	2931	5046
KH06 Kampong Thom	235	177	263	1075	2789	255	253	433	1658	3735
KH07 Kampot	256	333	333	1449	2694	278	332	332	1832	3524
KH08 Kandal	4213	3170	3170	3520	5312	4391	3681	3681	4311	7569
KH09 Koh Kong	14	4	4	119	460	10	16	16	246	753
KH10 Kratie	31	14	67	703	1133	38	14	109	982	1703
KH11 Mondul Kiri	4	0	1	72	167	2	0	2	114	206
KH12 Phnom Penh	11260	13277	13277	13844	15440	10622	10671	10671	10919	12473
KH13 Preah Vihear	8	2	3	297	1175	7	1	3	411	1812
KH14 Prey Veaeng	1303	1011	1435	2444	5247	1399	1145	1546	3216	7513
KH15 Pursat	33	42	141	483	1656	48	76	269	1150	1696
KH16 Ratanakiri	10	0	0	262	918	8	0	0	462	1159
KH17 Siem Reap	23	49	290	2181	5419	33	31	291	3276	5890
KH18 Preah Sihanouk	31	103	103	662	1298	29	45	45	682	1083
KH19 Stueng Treng	3	0	2	177	425	4	0	1	289	423
KH20 Svay Rieng	191	244	280	1396	2049	226	233	270	1857	2993

KH21 Takeo	946	794	794	2092	4575	1017	1213	1213	2891	6650
KH22 Otdar Meanchey	1	1	13	448	1445	2	0	10	571	2919
KH23 Kep	5	11	11	147	164	9	8	8	185	208
KH24 Pailin	3	0	23	214	280	5	0	15	241	303
KH25 Tbong Khmum	NA	NA	NA	NA	NA	186	305	2161	2281	5248

Table ST4: Predicted patients and accessibility to PEP centers in the 21 simulations of Scenario 5 and comparison with Scenario 2.

Scenario 2 represents the current situation in Cambodia with three PEP centers available in Phnom Penh, Battambang and Kampong Cham. Scenario 5 includes 21 models that each add one center from the 21 remaining provinces to the three in existence. Scenario 5 was used to identify the best future location for adding new centers.

	Predicted patients	Difference in PEP patients from Scenario 2	Population in districts with a median travel time to a PEP center below 60min	Difference in population from Scenario 2
Scenario 2	29,950	NA	5,386,549	NA
KH01 Banteay Mean Chey	32,494	2,544	5,889,612	503,063
KH04 Kampong Chhnang	31,158	1,208	5,584,194	197,645
KH05 Kampong Speu	31,546	1,596	5,488,476	101,927
KH06 Kampong Thom	31,591	1,641	5,529,896	143,347
KH07 Kampot	31,768	1,818	5,767,358	380,809
KH08 Kandal	31,062	1,112	5,386,549	0
KH09 Koh Kong	30,240	290	5,414,599	28,050
KH10 Kratie	31,066	1,116	5,477,115	90,566
KH11 Mondul Kiri	30,143	193	5,399,510	12,961
KH13 Preah Vihear	30,509	559	5,422,345	35,796
KH14 Prey Veaeng	31,242	1,292	5,704,213	317,664
KH15 Pursat	31,034	1,084	5,749,240	362,691
KH16 Ratanakiri	30,518	568	5,439,867	53,318
KH17 Siem Reap	33,286	3,336	6,053,755	667,206
KH18 Preah Sihanouk	30,784	834	5,512,641	126,092
KH19 Stueng Treng	30,457	507	5,421,975	35,426
KH20 Svay Rieng	32,300	2,350	6,069,565	683,016
KH21 Takeo	32,376	2,426	5,890,556	504,007
KH22 Otdar Meanchey	30,760	810	5,452,168	65,619
KH23 Kep	31,231	1,281	5,601,545	214,996
KH24 Pailin	30,528	578	5,458,289	71,740

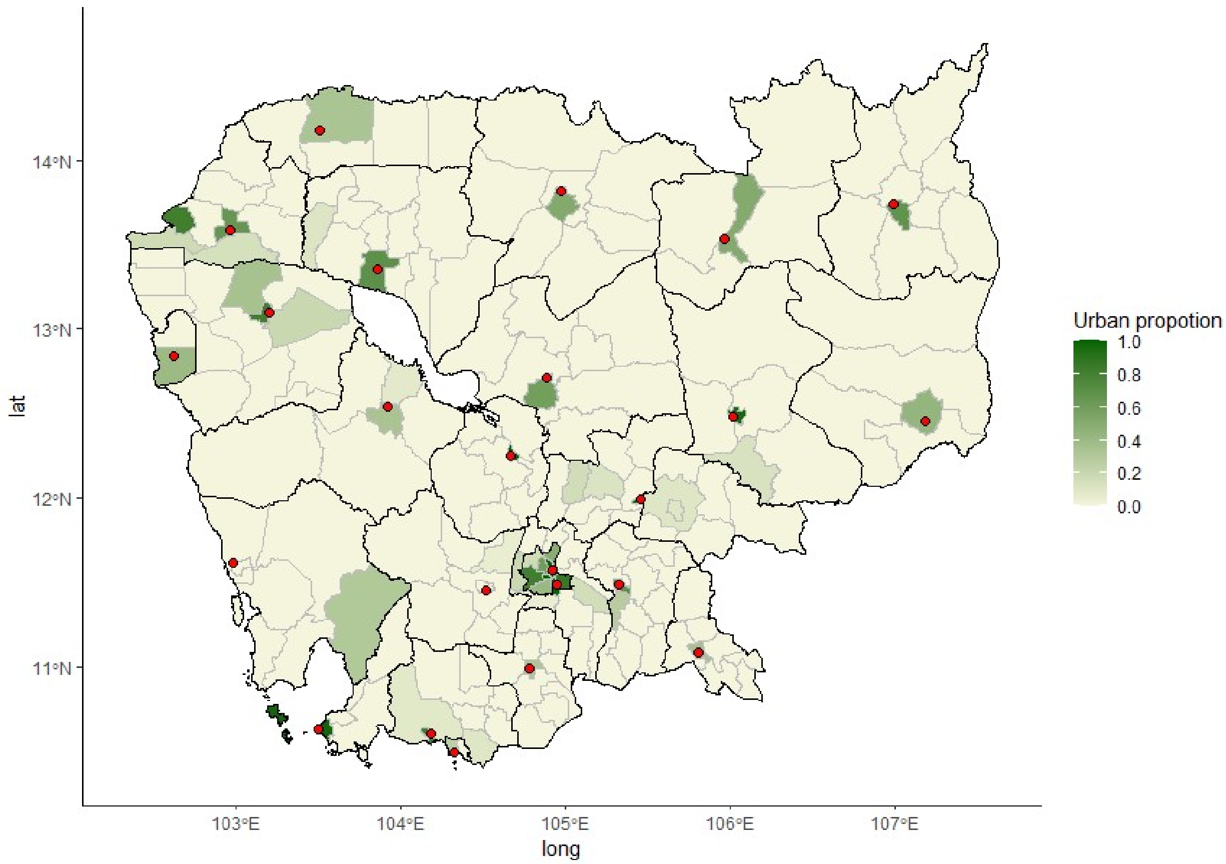
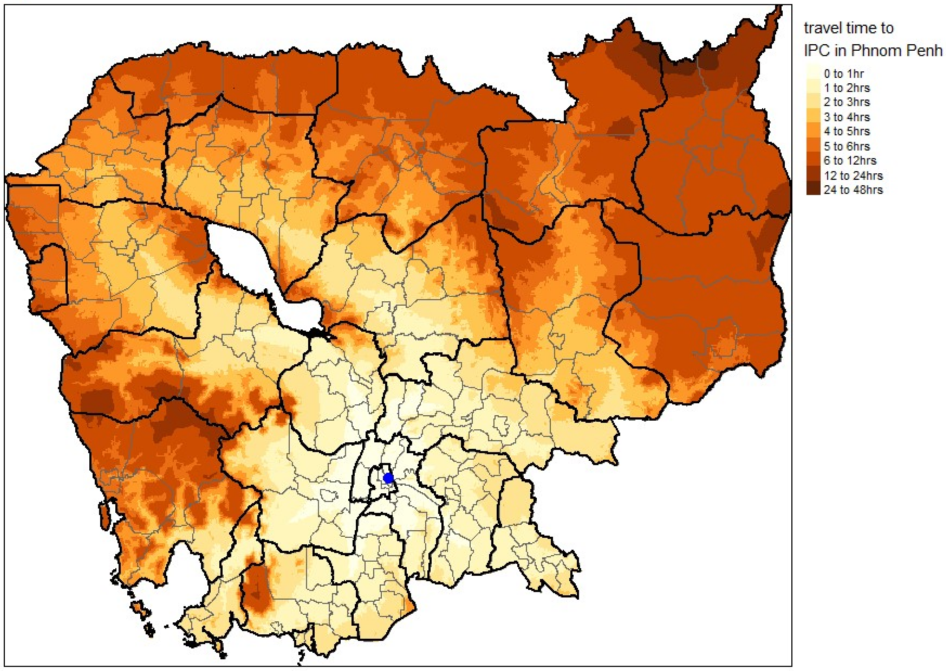


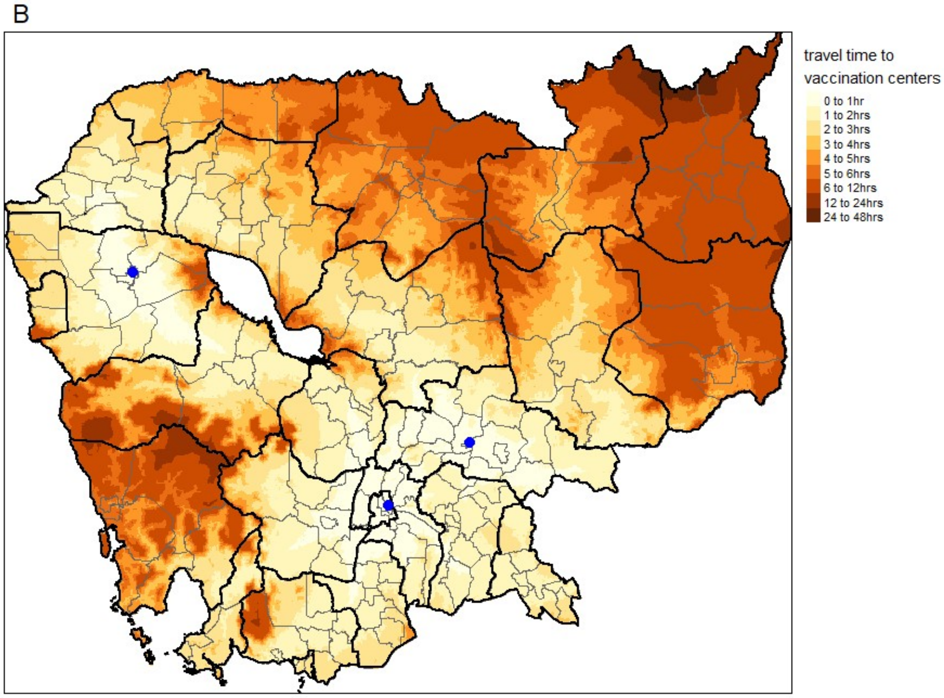
Figure SP1. Urban population proportion by district for the year 2016.

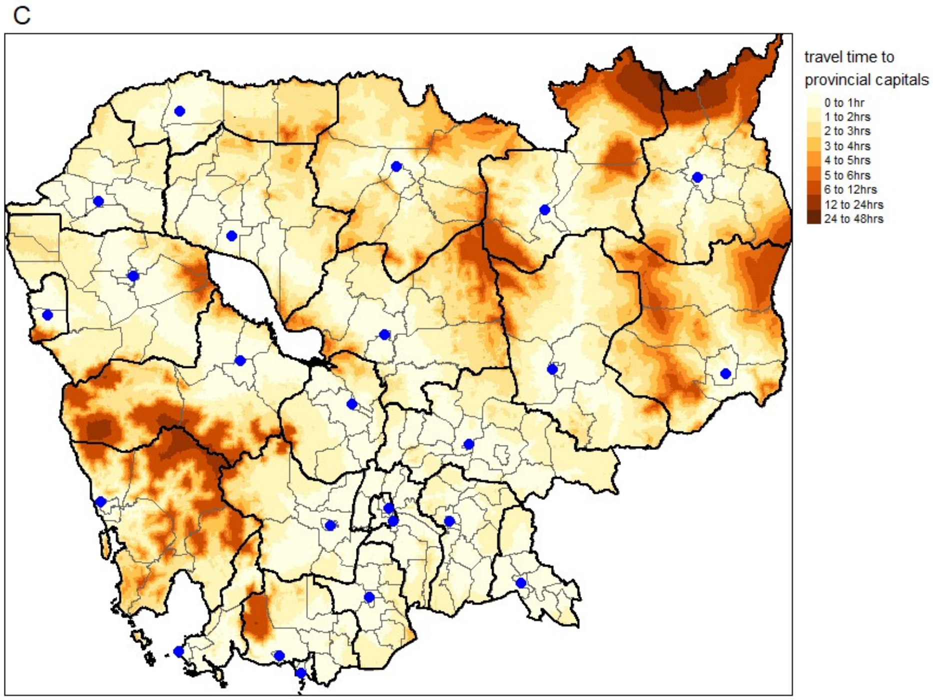
Estimates based on linear projections of the urban and rural populations by district from the 1998 and 2008 census. Red dots represent the location of provincial capitals. Most urbanized districts in Cambodia are where provincial capitals are located.

A


travel time to
IPC in Phnom Penh
0 to 1hr
1 to 2hrs
2 to 3hrs
3 to 4hrs
4 to 5hrs
5 to 6hrs
6 to 12hrs
12 to 24hrs
24 to 48hrs

B


travel time to
vaccination centers
0 to 1hr
1 to 2hrs
2 to 3hrs
3 to 4hrs
4 to 5hrs
5 to 6hrs
6 to 12hrs
12 to 24hrs
24 to 48hrs



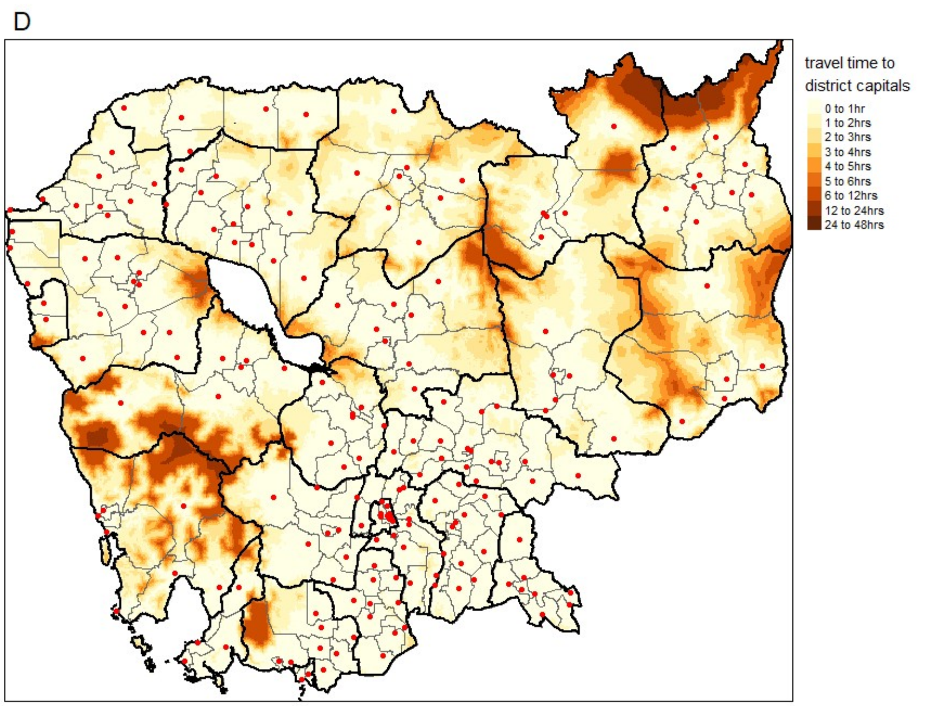


Figure SP2 A, B, C & D. Accessibility maps.

Travel time to point rasters for (A) IPC in Phnom Penh as was the case up to 2017, (B) all three current vaccination centers including the one in Battmabang opened in 2018 and the one in Kampong Cham opened in 2019,(C) all provincial capitals in Cambodia based on the 2010 administrative break-down and (D) all district capitals. Blue dots represent the points of interest for each maps: vaccination centers or provincial capitals.

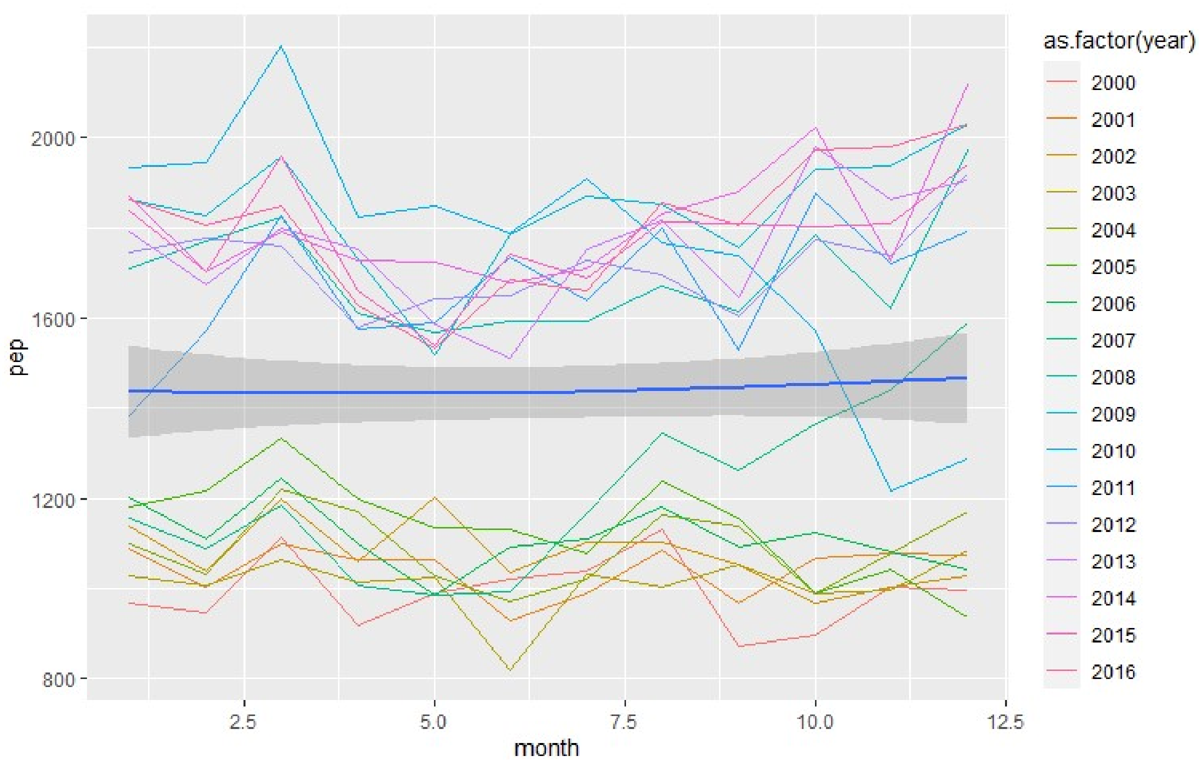


Figure SP3. Monthly distribution of patients by year with genelarized additive model (GAM) smoothed curve.

GAM curve is represented with a thick blue line and uncertainty shading.

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CHAPTER2: Analysis of predictors of rabies-positive biting animals in Cambodia using spatio-temporal Bayesian regression modelling

Abstract

Cambodia is endemic with rabies, a fatal zoonotic viral disease transmitted through dog bites. The Pasteur Institute of Cambodia is the main institution in charge of rabies prevention and surveillance in the country. Its main tool for prevention is post-exposure prophylaxis (PEP) for bite victims. Allocation of specific PEP regimen is done based on information collected by IPC doctors from patients regarding themselves, the attack, and the attacking animal. Furthermore, a small proportion of patients bring animals for testing, 60% of which test positive for rabies. Using the data collected from patient interviews, we use a Bayesian spatio-temporal regression model to identify predictors of a rabies positive animal in the goal of providing information that could help with allocation of PEP resources. We identified a number of variables associated with test results. Notably non-owned animals, a large number of bite victims, and unprovoked attacks were all predictive of a positive test. A suspected rabies status assigned by doctor based on animal symptom description was also highly predictive of a rabies test. Furthermore, we identified three Provinces of Cambodia with higher odds of positive tests: Kampong Cham, Kandal and Kampong Thom. This information could help allocate limited PEP resources, though this study showed IPC already a strong protocol to identify patients exposed to a rabies suspect dog.

1. Introduction

Rabies is a fatal zoonotic viral disease which can infect most mammalian species and is responsible for the deaths of nearly 60,000 people per year globally and billions of dollars in costs due to health expenses and loss of livestock [1–3]. The vast majority of human deaths result from exposure through dog bites in developing countries in Africa and Asia where dogs are typically free-roaming [1]. Once symptoms appear, rabies is nearly always fatal [4,5]. Cambodia, like most countries in Southeast Asia, is endemic with canine mediated rabies. It is estimated that around 800 people die of rabies each year, giving it one of the highest rates in the world at 6 per 100,000 [6]. Cambodia has a relatively large owned but mostly free-roaming dog population with dog-to-human ratios ranging from 1 dog for 2.8 people to 1 dog for 4.8 people [6–9]. Survey studies have reported bite rates of 1.1 to 4.8 bites per 100 person-years depending on the province, which was extrapolated as 375,000 to 600,000 bite injuries nationally per year [7,9,10].

Despite the prospective negative outcome following a bite, post-exposure prophylaxis (PEP) has proven a very effective tool in preventing disease even when administered post-exposure, before symptoms start [11]. PEP consists of the immediate administration of human rabies immune globulin (HRIG) followed by a condensed 1-week vaccine multi-site regimen of 3 doses as recommended by WHO [11–14]. In Cambodia, until 2018, three non-private entities offered PEP: the Pasteur Institute of Cambodia (IPC) and the National Institute of Public Health clinic in Phnom Penh, and the Angkor Hospital for Children in Siem Reap [15,16]. Together these centers administered some 25,000 PEP regimens per year, 85% of which were at IPC [16,17]. Some private clinics also offer PEP across Cambodia, but numbers and reliability are not confirmed. IPC opened in 1995 and started distributing PEP in 1998, initially for free and with a fee since 2010 [15,16]. In order to expand capacity, IPC opened two new centers, a first in Battambang Province in July 2018 and a second in Kampong Cham Province in March

2019 [18,19]. However, concurrent to these openings, in February 2019, a video of a young Cambodian girl dying of rabies was widely distributed on social media leading to a surge in demand for PEP [20,21]. In 2019, IPC recorded nearly 78,700 PEP patients, stretching limited PEP resources beyond normal capacity and causing long waiting times for patients [22]. This exemplifies how limited PEP resources can quickly be overwhelmed. Despite this, the number of patients coming to IPC remains much lower than the estimated number of dog-bites in Cambodia described above. This demonstrates the need for increased communication and access to PEP, and the ability to identify bite victims most at risk of rabies to better allocate limited supplies of HRIG and vaccine.

When PEP patients present to IPC, an initial assessment of rabies risk is done on arrival through a questionnaire focusing on the characteristics of the injury, the attack, and the animal perpetrating it. Patients exposed to animal saliva will all receive the vaccine, however HRIG is allocated based on wound severity and when the animal is suspected of being rabid. When available, the animal is tested for rabies using direct fluorescent antibody test (FAT) to confirm suspicion. The location of the attack is collected at the province level. Human cases are rarely recorded. With just 1.5% of PEP patients bringing an animal for testing, only a few hundred heads are tested every year with around 60% of them being positive [6]. Through statistical modelling, we can identify animal and attack characteristics that are predictive of a rabid animal with the aim of guiding PEP resource allocation to patients that are at greater risk. Furthermore, whilst anecdotal testing of animals is not sufficient to establish a true canine burden of disease, which would rely on population level field investigations such as serological surveys or active contact tracing [23,24], we can infer risk of exposure in patients geographically and identify high-risk areas by taking into account spatio-temporal distribution and auto-correlation of tested animals in the model structure. Similar studies have been conducted in Thailand and Vietnam [25–27], but using a spatio-temporal Bayesian framework with a spatial-autocorrelation matrix we can estimate a more complete geographical distribution of risk including in areas with little or no data available.

The goals of this study are twofold. First, we aim to identify predictors of a biting animal being rabid in order to inform on the individual exposure risk of patients presenting for PEP following an interaction with an animal. Second, in parallel with a project seeking to predict PEP needs geographically by adjusting for accessibility to PEP centers, we aim to identify high-risk areas in Cambodia where future PEP centers would be beneficial and where canine vaccination campaign efforts should focus.

2. Methods

2.1 Data collection and laboratory diagnosis

Data for the years 2000 through 2016 were provided by IPC. When patients present at IPC for PEP following an incident with an animal a document is completed in four main sections. Three of these consist of questions that are asked to the patient regarding: 1) patient demographic characteristics, 2) characteristics of the exposure and wound, and 3) animal characteristics (sup document SD1). The fourth section includes information on prior vaccination history and the progress of the current PEP regimen, which is completed during follow-up visits. In the animal characteristics section, a question on the animal's health appearance is completed by the doctor conducting the questionnaire based on a set of secondary questions focusing on specific symptoms, the status of the animal post-attack, the spontaneity of the aggression, and the number of victims. This aggregate question is used to determine if a dog is suspected rabid and guides the allocation of HRIG at the start of the PEP regimen (sup document SD2).

In some cases, patients bring with them the head of the attacking animal. Animals are tested at IPC using FAT [6,28]. This method uses polyclonal or monoclonal FITC-conjugated antibodies to detect the presence of viral antigens in brain tissue with fluorescence microscopy. It can only be performed post-mortem. It is the WHO and OIE recommended gold standard with sensitivity and specificity nearing 99% [29,30].

2.2 Data management

Inconclusive tests were excluded from analysis. Predictor variables followed five broad groups. The first was location and time, including the province in which the attack took place, year, month and number of days from accident to consultation. Cambodia's administrative divisions have changed significantly during our study period, including the division of Kampong Cham province in 2013, creating the new 25th province of Tboung Khmum. For this study, we applied the provincial boundaries prior to 2013 for the whole time period, keeping the number of provinces at 24, and adding Tbong Khmum data to Kampong Cham. The second group of variables involved the characteristics of the victim, i.e., sex and age groups. The third group described the characteristics of the attack: exposure type (binary: bite or other such as scratch and licking contact) and number of known victims. The third group was the characteristics of the wound: severity of the wound, location of wound, and number of wounds. The last group included characteristics of the animal: species (dog, cat, other domestic, and wildlife), spontaneity of aggression (provoked or not), health appearance (healthy or sick) and ownership status (owned or not).

A number of variables were categorized as these were strongly skewed, with a few outliers. Age was categorized into eight groups: small children (0 to 5 years), children (6 to 14 years), young adults (15 to 24 years), and then in 10 year increments up to a 65 years or more category. The highest recorded age was 88. The number of lesions ranged from 0 to 23 with a median of 2, and it was decided to categorize it into 5 groups (1, 2, 3, 4, 5 or more lesions). Similarly, the number of victims ranged from 1 to 15 with a median of 1, and it was decided to categorize it into 4 groups (1, 2 or 3, 4 or 5, 6 or more victims). Finally, time from accident to consultation ranged from 0 to 59 days, with a median of 1 and was categorized into 1 day, 2 or 3 days, 4 or 5 days and 6 or more days.

For wound location, the data initially recorded nine different locations. However, as numerous patients had multiple wounds and these were often in different locations, this lead to a large number of different mixed location categories. To account for this, wound location was divided into five dummy binary variables: head & neck, trunk & genitals, arm, hand & fingers, leg, and foot.

2.3 Statistical analysis

Exploratory univariate analysis was done using generalized linear mixed model (GLMM) with the R package "lme4" [31]. These univariate analyses were used to explore the relationships between each potential predictor and rabies test outcome, and guide categorization of continuous variables. Province was included as a random intercept. The Akaike Information Criterion (AIC) for model fit was used to determine if continuous or categorized variables were more appropriate for the following multivariate model selection.

To be able to incorporate conditional auto-regressive structures for the spatial random effect as well as time, a Bayesian framework using Integrated Nested Laplace Approximations (INLA) was used for our multivariate model building. As not all provinces in Cambodia had tested animals, this method has the benefit of being able to obtain a random effect estimate and posterior probability for provinces without any data-points, thanks to the correlation with neighboring provinces. Month was used as a cyclic auto-correlated random effect to capture the potential impact of seasonality. The spatial auto-correlation of provinces used a rook contiguity matrix where edges are considered for contact but not corners. Equal weight was assigned to all contacts between provinces. Default non-informative priors were used in this model. Default priors for the hyperparameter τ of the temporal random effect follows a Log-Gamma distribution with parameters of 1 and 0.00005. For the hyperparameters of the spatial random effect, default priors are a Penalized Complexity (PC) prior with parameters 1 and 0.01 for τ_1 and a PC prior for precision with parameters 0.5 and 0.5 for τ_2. Year was also tested as a non-cyclic auto-correlated random effect but was eventually included in the model as a fixed effect as there was no evidence of yearly temporal trends in the proportion of positive tests. Model selection was performed with a two-way stepwise selection process from an empty model using the Deviance Information Criterion (DIC) and the Watanabe-Akaike Information Criterion (WAIC) to assess model fit. Data analyses were done using the package R-INLA [32–35]. As our model results clearly indicated that the "animal health status" variable had a major influence on model outputs, a second multivariate model was selected excluding this variable. From here, the model with the health variable will be referred in the text to as Model 1 whilst the one without it as Model 2.

2.4 Fit and validation

For both multivariate Models 1 and 2, we obtained fitted probabilities for an animal testing positive for rabies. The R package "pROC" was used to generate Receiver Operating Characteristic curves (ROC curves) to evaluate the models' predictive performance and select an optimal prediction probability threshold [36]. The area under the curve (AUC) was generated to see how predictive each model was as a whole. Then, the optimal prediction threshold was selected by maximizing the sum of sensitivity and specificity using Youden's J statistic. The prediction threshold is the value above which a fitted probability is considered a positive result and below which it is considered a negative result. For the selected threshold, we produced model performance statistics, including: Sensitivity, Specificity, Positive Predictive Values (PPV) and Negative Predictive Values (NPV).

2.5 Predictions

Bayesian models allow us to infer predictions in all observations including incomplete ones. Thus, we were able to apply this model to the full patient dataset, including patients that did not have a tested animal nor complete information for the exposure variables, to predict the probability that the patient was attacked by a rabid animal. Predicted probabilities were obtained by fitting a new model that included all observations without an outcome, allowing it to compute the median fitted probability of the biting animal being rabid, with a 95% credibility interval (95% CI). Then applying the prediction threshold selected with the ROC curve, this probability was used to infer a binary outcome (rabid vs not rabid). The prediction threshold was applied to the median predicted probability and the 95% CI bounds to obtain an estimate of the sum of patients exposed to a rabid animal and the interval of that sum. This was done for both Models 1 and 2.

All analysis and data management was done in R studio version 4.0.3 [37].

3. Results

3.1 Descriptive

In total 294,040 patients came to IPC Phnom Penh for PEP from 2000 to 2016. Of these, 4,515 patients (1.5%) brought an animal for testing. Six test results were inconclusive and were discarded from the analysis, leaving 4,509 test results to be analyzed, 60.5% (2,726) of which were positive. Of these animals, 98.5% (4,442) were dogs, 60.5% (2,686) of which were positive. Seven of Cambodia's 24 provinces accounted for 93.0% (4,192) of tested animals (Fig 1). These were concentrated in close proximity to the capital Phnom Penh, where IPC is located with numbers ranging from 443 to 728 tested animals (table SP1). These seven provinces contained 92.5% (2,521) of positive animals and 95.7%

(281,490) of PEP patients. However, the rate of testing varied greatly between them, with 9.9% of patients coming from Kampot Province bringing an animal for testing compared to 0.3% in Phnom Penh. The percentage of positive animals varied from 52% to 56% in five of these seven provinces, whilst the provinces of Kandal and Kampong Cham had noticeably higher values at 72% (526/728) and 73% (474/653) respectively. In 13 provinces, the number of tested animals varied from two to 106 with wider ranges of positive rates from 25% to 100%. Finally, four provinces had no animals tested for the period 2000 to 2016.

The number of tested animals was relatively stable from 2000 to 2006, ranging between 143 and 212. It then increased quickly from 2007 to a peak of 471 in 2009 before gradually dropping back to 202 by 2016. This peak is particularly noticeable in Kandal Province as can be seen in Figure 1. The proportion of positive dogs remained stable over time, however, with 11 of 17 years having values between 58% and 63%, and the rest ranging from 50% to 75% (Fig 2).

3.2 Model 1 results

Numerous variables proved to have a significant association with the rabies test outcome in univariate models. As most of these are described later in the multivariate results and had similar results, only major differences with multivariable results will be described.

In Model 1 we selected four animal related variables (health appearance, spontaneity of aggression, ownership status, species), two attack and wound related variables (number of victims, number of lesions), and finally month and year. Detailed results are presented in table 1. As expected, animals designated as appearing sick had significantly higher odds of being rabid compared to healthy ones (OR=533.5, 95% CI=380.7-766.4). To a lesser degree, feral or wild animals also had significantly higher odds of testing positive compared to owned animals (OR=19.1, 95% CI=10.0-38.4).

Conversely, provoked aggressions were significantly less likely to be associated with a positive rabies test compared to spontaneous ones (OR=0.33, 95% CI=0.25-0.45). Cats had lower odds of testing positive compared to dogs (OR=0.06, 95% CI=0.02-0.16). Interestingly, wild animal species (which has to be differentiated from feral domestic animals and could include domesticated wildlife) had significantly lower odds of being positive in the univariate model (OR=0.10, 95% CI=0.01-0.81), but this became non-significant in the multivariate model 1.

Table 1: Multivariate model result summaries for the Bayesian spatio-temporal logistic regression. *Model 1 is the model, which included animal health aspect in the selection process. As this variable is a decision from doctors based on an aggregate of other primary data points and was highly predictive, Model 2 was selected removing this variable from the selection process.*

Variable	Category	Number of tested animals	Number of positive animals	Percentage of positive animals	Odds ratio (Model 1)	95% credibility interval	Odds ratio (Model 2)	95% credibility interval
Total	NA	4,509	2,726	60.5	NA	NA	NA	NA
Animal								
Animal health aspect	Healthy	1,786	150	8.4	ref	-	Removed from selection	
	Sick	2,723	2,576	94.6	**533.46**	**380.66 to 766.37**		
Aggression	Spontaneous	3,405	2,190	64.3	ref	-	ref	-
	Provoked	1,102	536	48.6	**0.33**	**0.23 to 0.45**	**0.49**	**0.41 to 0.58**
Animal ownership	Owned	4,037	2,276	56.4	ref	-	ref	-
	Feral or wild	471	450	95.5	**19.11**	**9.96 to 38.37**	**20.33**	**13.08 to 33.58**
Animal species	Dog	4,422	2,686	60.7	ref	-	ref	-
	Cat	45	12	26.7	**0.06**	**0.02 to 0.16**	**0.17**	**0.07 to 0.37**
	Livestock	35	27	77.1	0.51	0.17 to 1.95	1.73	0.74 to 4.46
	Wild	7	1	14.3	0.22	0.00 to 36.37	**0.07**	**0.01 to 0.47**
Victim								
Sex	Male	2508	1570	62.6	Not selected		ref	-
	Female	2001	1156	57.8			**0.82**	**0.71 to 0.94**
Age categories	0 to 5	854	440	51.5	**Not selected**		**0.83**	**0.68 to 1.01**
	6 to 14	1550	884	57.0			ref	-
	15 to 25	583	402	69.0			**1.77**	**1.40 to 2.25**
	26 to 35	485	324	66.8			**1.64**	**1.28 to 2.11**
	36 to 45	425	279	65.6			**1.61**	**1.24 to 2.10**
	46 to 55	336	216	64.3			**1.57**	**1.18 to 2.11**

	56 to 65	182	110	60.4			1.25	0.87 to 1.82
	66 or above	94	71	75.5			**2.10**	**1.23 to 3.71**
Attack and wounds								
Days from accident to consultation	1 day	3096	1965	63.5	Not selected		ref	-
	2 or 3 days	980	510	52.0			**0.59**	**0.49 to 0.70**
	4 or 5 days	281	159	56.6			**0.63**	**0.47 to 0.85**
	6 or more days	152	92	60.5			0.71	0.48 to 1.06
Number of victims	1	2,441	1,226	50.2	ref	-	ref	-
	2 or 3	1,430	981	68.6	**1.90**	**1.42 to 2.54**	**2.30**	**1.96 to 2.70**
	4 or 5	383	304	79.4	**3.93**	**2.36 to 6.58**	**4.59**	**3.44 to 6.19**
	6 or more	252	214	84.9	**4.55**	**2.31 to 9.04**	**5.64**	**3.79 to 8.60**
Number of lesions	1	550	335	62.0	ref	-	ref	-
	2	3,256	1,968	60.4	**1.60**	**1.04 to 2.47**	0.92	0.72 to 1.18
	3	426	265	62.2	1.30	0.72 to 2.34	0.88	0.63 to 1.22
	4	172	103	59.9	1.61	0.76 to 3.43	0.70	0.45 to 1.09
	5 or more	112	55	49.1	0.44	0.18 to 1.12	**0.36**	**0.21 to 0.60**
Wound on hands or fingers	no	3379	1997	59.1	Not selected		ref	-
	yes	1127	728	64.6			**1.75**	**1.46 to 2.12**
Wound on arms	no	4239	2546	60.1	Not selected		ref	-
	yes	267	179	67.0			**1.66**	**1.21 to 2.29**
Wound on legs	no	3510	2137	60.9	Not selected		ref	-
	yes	996	588	59.0			**0.82**	**0.68 to 0.99**
Wound on trunk and genitals	no	4088	2497	61.1	Not selected		ref	-
	yes	418	225	54.5			**0.79**	**0.62 to 1.02**
Time								
Year	2000	143	97	67.8	ref	-	ref	-
	2001	156	103	66.0	0.59	0.22 to 1.60	0.99	0.53 to 1.83
	2002	214	160	74.8	0.79	0.31 to 2.02	1.29	0.71 to 2.32
	2003	154	84	54.5	**0.21**	**0.09 to 0.54**	0.68	0.37 to 1.24
	2004	212	130	61.3	**0.28**	**0.11 to 0.68**	0.95	0.54 to 1.69
	2005	153	88	57.5	**0.31**	**0.12 to 0.79**	0.73	0.40 to 1.32

	2006	175	89	50.9	**0.25**	**0.10 to 0.65**	**0.54**	**0.30 to 0.96**
	2007	268	157	58.6	0.96	0.38 to 2.43	0.85	0.49 to 1.48
	2008	412	257	62.4	0.94	0.39 to 2.24	0.82	0.48 to 1.38
	2009	471	291	61.8	0.92	0.38 to 2.19	0.97	0.57 to 1.63
	2010	357	180	50.4	0.45	0.18 to 1.12	**0.52**	**0.30 to 0.89**
	2011	332	183	55.1	0.44	0.17 to 1.12	0.71	0.41 to 1.22
	2012	362	225	62.2	0.64	0.25 to 1.63	0.97	0.56 to 1.67
	2013	344	215	62.5	**2.55**	**1.06 to 6.14**	0.95	0.55 to 1.64
	2014	320	198	61.9	1.84	0.75 to 4.47	0.92	0.53 to 1.60
	2015	234	147	62.8	1.50	0.55 to 3.97	1.03	0.58 to 1.83
	2016	202	122	60.4	1.23	0.43 to 3.43	0.85	0.47 to 1.55

For the attack related variables, the odds of positive test increased with the number of victims, ranging from an OR of 1.9 with two or three victims to 4.6 with six or more victims compared to the reference of one victim, with all ORs being significantly different from the reference. For the number of lesions, two, three or four lesions had similarly higher odds of positive test, but only the two lesion category was significantly higher than the reference of one lesion (OR=1.6, 95% CI=1.04-2.47) in Model 1. This differed from the univariate model where having two, three, or four lesions all had non-significant odds ratios just below one. The five or more lesions category had lower odds of a positive test but this was non-significant in Model 1 despite being significant in the univariate model (OR=0.52, 95% CI=0.34-0.80).

Though not individually significant, the month random effect in the INLA model showed a seasonal trend with the months of January through April having slightly above average odds of positive tests, whereas those from July through October had slightly lower odds. However, these values were extremely close to one. In the univariate model, this seasonal trend was much clearer. Using January as the reference, February was the high point (OR=1.01) followed by odds ratios decreasing to a low in July (OR=0.66, 95%CI=0.49-0.88) before gradually re-increasing up December (OR=0.92). For the year variable, results varied from the univariate as to which years were significantly different from 2000, but at no level was there a clear long-term temporal trend.

When looking at results for the spatial random effect, we observed that Kandal and Kampong Cham had odds of positive tests that were much above average, with Banteay Meanchey, Phnom Penh and Svay Rieng having the lowest values (Fig 3A). Unsurprisingly, the uncertainty levels are lowest in the seven provinces with most tested animals as described in section 3.1. (Fig 3C). Kampong Thom and Kampong Chhnang also had a higher numbers of tested animals (106 and 71, respectively) and have relatively low uncertainty compared to the rest of the provinces where data were scarce.

3.3 Model 2 results

When removing the animal health variable, the other variables previously selected for Model 1 were retained for Model 2. A number of other variables were added in Model 2 including the sex and age of the patients, the time from accident to consultation, and four of the wound location dummy variables (hand and fingers, arm, leg, and trunk and genitals). For the variables that were retained from Model 1, estimates were mostly similar as can be seen in table 1 and so will not be described in detail. The main difference was in the number of lesions with two, three or four lesions having non-significantly lower odds of positive tests and the five or more lesions category having significantly lower odds of a positive rabies test (OR=0.36, 95%CI=0.21-0.60) similar to what was observed in the univariate result.

For the newly added variables in Model 2, results were again mostly similar to univariate results. Women had lower odds of being bitten by a positive animal (OR=0.82, 95%CI=0.71-0.94). Using the children aged 6 to 14 as reference, we can see that all adult age groups had higher odds of positive animal tests with the highest values observed in the young adult (ages 14 to 25, OR=1.77, 95% CI=1.40-2.25) and elderly groups (ages 66 or above, OR=2.10, 95% CI=1.23-3.71). Young children (5 years or less) had even lower odds of the bite coming from a rabies positive animal, though this was borderline significant in Model 2 whilst significant at the univariate level. Both being bitten in the hands and in the arm had significantly higher odds of a positive rabies test (OR=1.75 and 1.74 respectively) while being bitten in the legs or trunk and genitals had lower odds (OR=0.82 and 0.79 respectively) with trunk and genitals being borderline significant. At the univariate level, the leg and foot variables were not significant, whereas the head and neck variable was (OR=0.63, 95% CI=0.51-0.78).

For the spatial random effect, the results from Model 2 most closely resembled the results observed in a univariate INLA model that only included the spatial random effect. In Model 2, we observed four provinces (Kandal, Kampong Cham, Kampong Thom and Battambang) as having noticeably above average odds of positive animals whilst another six had below average odds (Kampong Speu, Kampot, Takaeo, Phnom Penh, Prey Veng and Svay Rieng) (Fig 3B). The uncertainty was very similar to what was observed in Model 1 (Fig 3D).

3.4 Model fit

Model 1 yielded a highly predictive model when including the animal health aspect variable, with an area under the curve above 0.97 (table 2), which is clearly illustrated with the ROC curve being nearly at a right angle in the top left corner and the distribution of fitted probabilities forming two clearly separate peaks (Fig 4A and 5A). Accordingly, Model 1 showed strong test performances once a probability threshold was selected with specificities and sensitivities close to 0.94. Similar observations can be made with the predictive values, confirming the highly predictive nature of this model. The selected probability threshold was 0.61(table 2).

Table 2: ROC curve analysis outputs and model predictive performance. *As the dog health aspect has a very high odds ratio in the model, it was decided to compare the model performance to the performance of that variable alone in assessing the rabies status of an animal.*

Model	Area Under the Curve (AUC)	Predicted Probability Threshold	Specificity	Sensitivity	Negative Predictive Value (NPV)	Positive Predictive Value (PPV)
Model 1	0.973	0.613	0.937	0.946	0.919	0.958
Dog health aspect variable (not in a model)	na	na	0.918	0.945	0.916	0.946
Model 2	0.825	0.611	0.791	0.703	0.635	0.837

For comparison, the most influential variable in the model (animal's health appearance as assessed by IPC doctors based on the victim's description of symptoms) was used as a simple binary test and compared to the actual test results. This yielded test performances that were very close to Model 1, with similar sensitivity, NPV and PPV, but slightly lower specificity.

Model 2 had comparatively lower but still high predictive performances with an AUC of 0.82 and specificity and sensitivity of 0.79 and 0.70 respectively (table 2). The lower predictive ability can be seen in the ROC curve, which is further from the top left corner and the distribution of fitted probabilities in positive and negative animals overlapping significantly (Fig 4B and 5B). In this model, the PPV of 0.84 was noticeably higher than the NPV of 0.64.

3.5 Model predictions

Of 294,040 patients, 8,186 had a fitted probability above the prediction threshold obtained from the ROC curve for Model 1. This represents 2.8% of PEP patients potentially exposed to rabies. If we use the 95% credibility interval of the fitted probability in relation to the prediction threshold, we obtained a range of 7,182 to 10,556 (2.4 to 3.6%). Predictions were relatively stable over the study period, with a range 1.4 to 3.8% of PEP patients each deemed being possibly exposed to a rabid animal. Geographically, in four of the seven provinces with the most tested animals (Kampong Cham, Kampong Speu, Prey Veaeng and Takeo), the proportion varied from 6.1% (765/12,507) to 8.0% (792/9,870). Values varied more in the other three provinces with Phnom Penh and Kandal having lower values at 0.8% (1,194/158,009) and 2.8% (1,676/60,267), respectively, and Kampot being high at 12.0% (534/4,465) (table SP1). In the remaining 17 provinces, 929 of 12,465 patients (7.5%) were predicted exposed, ranging from 2.5% (1/40) in Stueng Treng to 41.7% (5/12) in Kep.

In Model 2, predictions were much less precise with wider ranges for the fitted probabilities and thus predicted numbers of exposed patients. We predicted 66,495 (22.6%) patients potentially exposed to a rabid animal with a range of 12,500 to 198,459 (4.3% to 67.5%), suggesting that many individuals had very wide ranges in fitted probabilities. Over the study period, we saw more variability compared to Model 1 with 9.8% to 44.7% of patients exposed to rabies in any given year. In the seven provinces where most animals were tested, the predictions followed the odds ratios observed for the spatial random effect. Kampong Cham and Kandal Provinces had high rates of predicted exposed patients (47.8% and 44.2% respectively or 10,982/22,809 and 26,125/60,267) and the other five provinces had lower rates ranging from 11.5% (18,116/158,009) in Phnom Penh to 20.2% (901/4,465) in Kampot. The remaining 17 provinces had a predicted 33.7% (4,082/12,465) of patients exposed to rabies.

4. Discussion

4.1 General observations

From 2000 to 2016, the number of PEP patients presenting at IPC has gradually increased. This was initially accompanied by an increase in animal testing, peaking in 2007-08 when a study to improve rabies diagnostics was set up by IPC, before dropping again. However, during this time period, the proportion of animals testing positive remained relatively constant, around 60%. This is similar to what was observed in other studies in neighboring Thailand which saw stable and high rates of rabies positive animals over long periods of time, even as the number of tested animals and thus diagnosed animals dropped [25,38]. This provided strong evidence that rabies remains endemic and a serious public health concern in the region, though the data from Thailand is from an earlier period, and this has been accompanied by a drop in human cases thanks to intervention both at the dog and human level [39]. Without similar human data in Cambodia, animal testing, even if based on voluntary reporting, is an important indicator of disease presence and evolution.

4.2 Animal and attack

By far, the strongest association in our models with a rabies positive test was the animal health status assigned by the doctor interviewing newly presenting PEP patients, based on symptoms and behaviors described by the patient. It is impressive to note that this variable identified the presence or absence of disease correctly in 93.4% of the animals that were tested. This has led to the model that included this variable to be barely superior to the doctor's assessment of the health of the attacking animal, and removing this variable led to a model with a lower predictive performance. This suggests that patients are very proficient at correctly identifying and reporting symptoms and behaviors associated with a rabid animal. Unfortunately, not all indicators used to assign this health status are individually recorded in the patient surveys and it could be useful for future studies to record these individually to identify which are most predictive of a positive animal. This is in agreement with the very high awareness of the disease's existence in dogs reported in field surveys conducted in the provinces of Kandal in 2009 and Phnom Penh and Siem Reap in 2010, ranging from 80 to 96% depending on the location and the nature of the question [7,8]. In the first of these studies, specific symptoms were also correctly identified by a majority of respondents.

Two other animal characteristics associated with positive results were the spontaneity of the aggression and ownership status. Unsurprisingly, unprovoked aggression was more likely to come with a positive test than provoked aggression. This is to be expected as aggressiveness is one of the oldest and most commonly described behavioral symptoms in rabid animals [40–42], especially in dogs, which comprised the majority of tested animals. Aggression also had a strong correlation with positive tests in a similar study conducted in Thailand that focused specifically on animal symptoms [25]. Furthermore, as the number of victims increased we saw a significant increase in the likelihood of a positive rabies test, which likely stems from the fact that a rabid animal is more likely to attack individuals at random and un-provoked, whereas healthy animals will target aggression to the individual responsible for the provocation.

When looking at ownership status we must first note that nearly 90% of tested animals were owned. Furthermore, 98% of all PEP patients reported that the attacking animal was owned, regardless of whether the animal was tested, indicating that stray dogs are uncommon in Cambodia, as has also been observed in demographic studies [9]. Animals for which the owner was not identified were much more likely to be positive. However, as very few owners confine or vaccinate their dogs [8,9], it is unlikely that any sort of direct owner intervention restricting exposure caused this effect. More likely, as rabid animals present erratic behavior once symptoms, start they stray away from home and attack in locations other than where they could have been identified by their owner.

The vast majority of tested animals were dogs (98%), leaving us with very small sample sizes with regard to other species, making it difficult to reach definitive conclusions. Despite this, cats were significantly less likely to test positive, and this has also been seen in two studies from Thailand, where around 15% of cats where positive compared to more than 50% of dogs, with larger numbers of cats tested [25,38]. Although the results observed for livestock were not significantly different, we observed a higher positive rate in livestock. Similarly, 73% of cattle in one of the Thai studies tested positive, though this was also a small sample [25]. One should note our livestock category is not species-specific since this information was not systematically collected. Anecdotal evidence in some patient surveys where animals were not tested suggests that the most common livestock species leading to an IPC visit were swine, followed by bovines and rabbits. Though this doesn't allow for definitive conclusions on livestock rates, it does provide evidence that rabies presence is not negligible in livestock species which should be considered more often for testing and in terms of public awareness campaigns.

4.3 Wound

Although wound severity is used as one of the main indicators to guide PEP allocation and regimen according to WHO guidelines [14], this variable saw no evidence of association with the rabies test outcome of the animal. Nevertheless, it should remain an important factor in PEP allocation as it relates more to the transmission mechanism than the probability of the animal being rabid. Severe or deep wounds have increased risk of transmission should the animal be rabid as the initial steps of transmission require the transfer of saliva into muscle tissue where the virus will replicate before moving to the nervous system [1,4]. The number of lesions was counterintuitive and results varied between models. Model 1 suggests that 2, 3, and 4 wounds had higher odds of positive rabies tests, which would seem to follow the logic that a more randomly aggressive animal would bite multiple times. However, Model 2 and the univariate results appeared to show an opposite trend. These might be due to unaccounted for confounders that are someone adjusted for when including the animal health status variable.

As has been observed in many other studies, the most common wound locations in IPC patients were feet (34%), legs (28%), and hands and fingers (19%), with all other locations being below 10% [26,27,43,44]. This is expected as lower limbs are the lowest and thus most exposed parts of our body to a potential bite, whereas hands serve as our primary means of defense should an animal attack. Less intuitive is the evidence showing that wounds to the hands and arm were more likely to be associated with a rabid animal than wounds to the feet and legs. We would expect the opposite, with lower limbs being easy targets of unprovoked aggression, and upper limbs being the instrument of provocation in cases of provoked aggression. On the other hand, head and neck wounds, which are often used as an indicator for PEP allocation as these form the shortest path of transmission to the brain should exposure happen, showed no significant association with rabies status of the animal.

4.4 Patient demographics

Women where significantly less likely to have been bitten by a rabid animal, and similarly they represented less than half (48%) of PEP patients. This might be linked to occupational exposure, with men more likely to be working in fields where encounters with unknown dogs might happen.

Nearly half (49%) of patients at IPC throughout our period were children under the age of 14, which comprised 34% of the population in the 2008 census [45]. This trend has also been observed in other countries [26,27,43]. However, we observed that children were also significantly less likely to have been attacked by a rabid animal compared to the adult age groups. This could be due to two reasons. Firstly, children might be more likely to provoke a healthy dog through playfulness and lack of awareness of the dog's potential reaction at home, whilst being less likely to be exposed to an unknown dog outside. This could also explain that children are more likely to be bitten overall and so come to IPC for PEP. Secondly, the risk perception threshold of a parent is often lower for one's child than his or herself, meaning that adults are possibly more likely to bring their child in for lower risk situations. This could also explain the over-representation of children as PEP patients.

4.5 Spatio-temporal distribution and predictions

Three provinces of the eight with more than 100 tested animals showed evidence of higher odds of animals testing positive: Kandal, Kampong Cham, and to a lesser extent, Kampong Thom. This led to these three provinces having the highest rates of exposed patients in predictions using Model 2. In this model, Phnom Penh both had noticeably lower odds of positive tests and lower rates of predicted exposed patients. On the other hand, in Model 1, despite the odds ratios mostly following a similar pattern, predictions were heavily influenced by the spatial distribution of the animal health status variable. Thus, Kampot and Kampong Speu provinces, which had the highest rates of sick looking animals, were predicted to have the highest rates of exposed patients. Kandal Province, despite still having one of the highest odds ratios, had some of the lowest predicted exposed patients. Phnom Penh remained the province with the lowest predictions.

We observed no long-term temporal trends over the study period. We did observe a seasonal effect, with the winter months of December to February having higher odds of positive animals at the univariate level. However, this effect seemed to be much reduced once other variables were included in multivariate models, suggesting some of these variables accounted for part of the seasonal factor.

4.6 Limitations

One of the main limitations of this study came from the biased nature of the data resulting from passive voluntary reporting. Two forms of selection biases were observed. Firstly, PEP patients coming to IPC were biased by their accessibility to IPC as has been demonstrated in chapter 1. Thus, patients and the animals they bring for testing are not a representative sample of the nation as a whole. Secondly, when patients do come to IPC, the perceived severity of the attack influences the act of bringing an animal for testing. Overall, 1.5% of patients brought an animal for testing. However, when stratifying, we observed higher rates of tested animals for select categories in a number of variables, leading to biased selection. These categories primarily related to perceived severity: animal health status (34.8% tested in sick animals), species (4.6% in livestock, 0.4% in cats), number of victims (increase from 1.1 to 7.2% with the categories), number of lesions (increase from 1.1 to 5.1% with the categories) and severity of wound (2.4% in severe wounds).

Our models were also highly influenced by a single variable, and removing this variable led to a much less statistically robust model with very different and more uncertain predictions. Furthermore, this variable was an aggregate of a number primary variable which were unfortunately, not all available to us for modelling individually. Questions regarding individual symptoms of animals were not recorded in the dataset, whilst the question on the post-attack status of the animal was not usable as it is directly correlated to the fact the dog is available for testing. We conclude that though these models were good at identifying specific variables associated with rabies test outcomes, they were limited in terms of predicting exposure in patients without a tested animal. This resulted in fitted probabilities that either mostly aligned with the distribution of the animal health variable or had very large credibility intervals when that variable was absent, leading to a wide range of predicted exposed patients.

5. Conclusion

We provide strong evidence that rabies remains a major public health concern in Cambodia, despite the difficulty establishing a direct human burden of rabies in Cambodia. In an effort to establish predictors of rabies positive tests to guide PEP allocation, we observed that IPC already has a robust and highly sensitive and specific protocol to identify animals suspected of being rabid. Although we did identify specific predictors, these only marginally improved the performance of the protocol already used by IPC doctors. We also established a geographical distribution of the risk of rabies exposure. Notably Phnom Penh, which had the lowest uncertainty, had much lower risk of rabies exposure compared to other more rural provinces. Amongst provinces with large numbers of tested animals, we mostly saw a North-South divide, with coastal southern provinces less at risk and provinces north of Phnom Penh more at risk. However, the geographically biased nature of the data limits our interpretation of the spatial distribution of rabies. With new centers being opened by IPC in Kampong

Cham and Battambang Provinces, there will likely be an improvement in the reach of animal testing in Cambodia leading to better identification of high-risk areas.

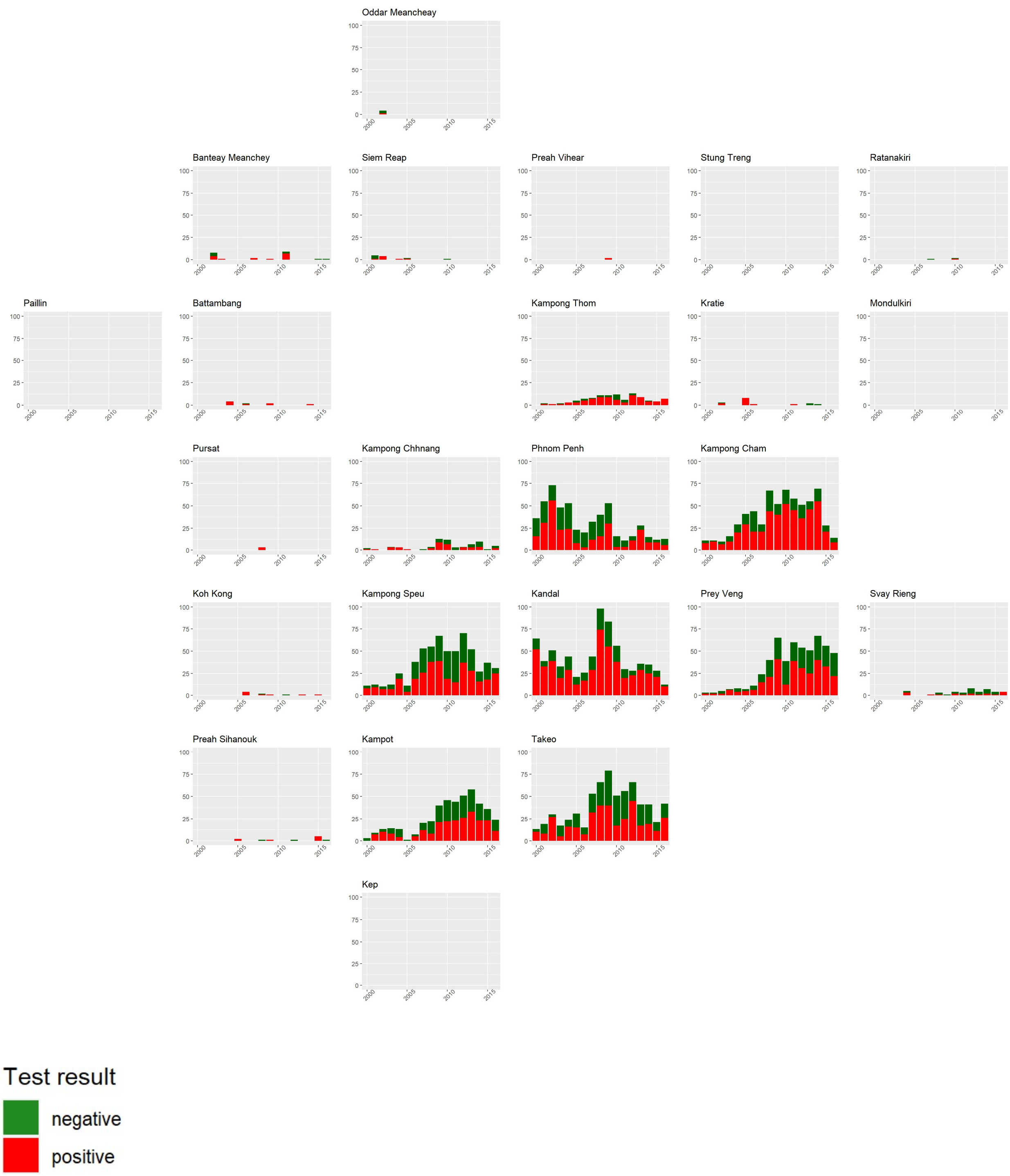


Figure 1: Number of tested animals by province and year in Cambodia from 2000 to 2016.

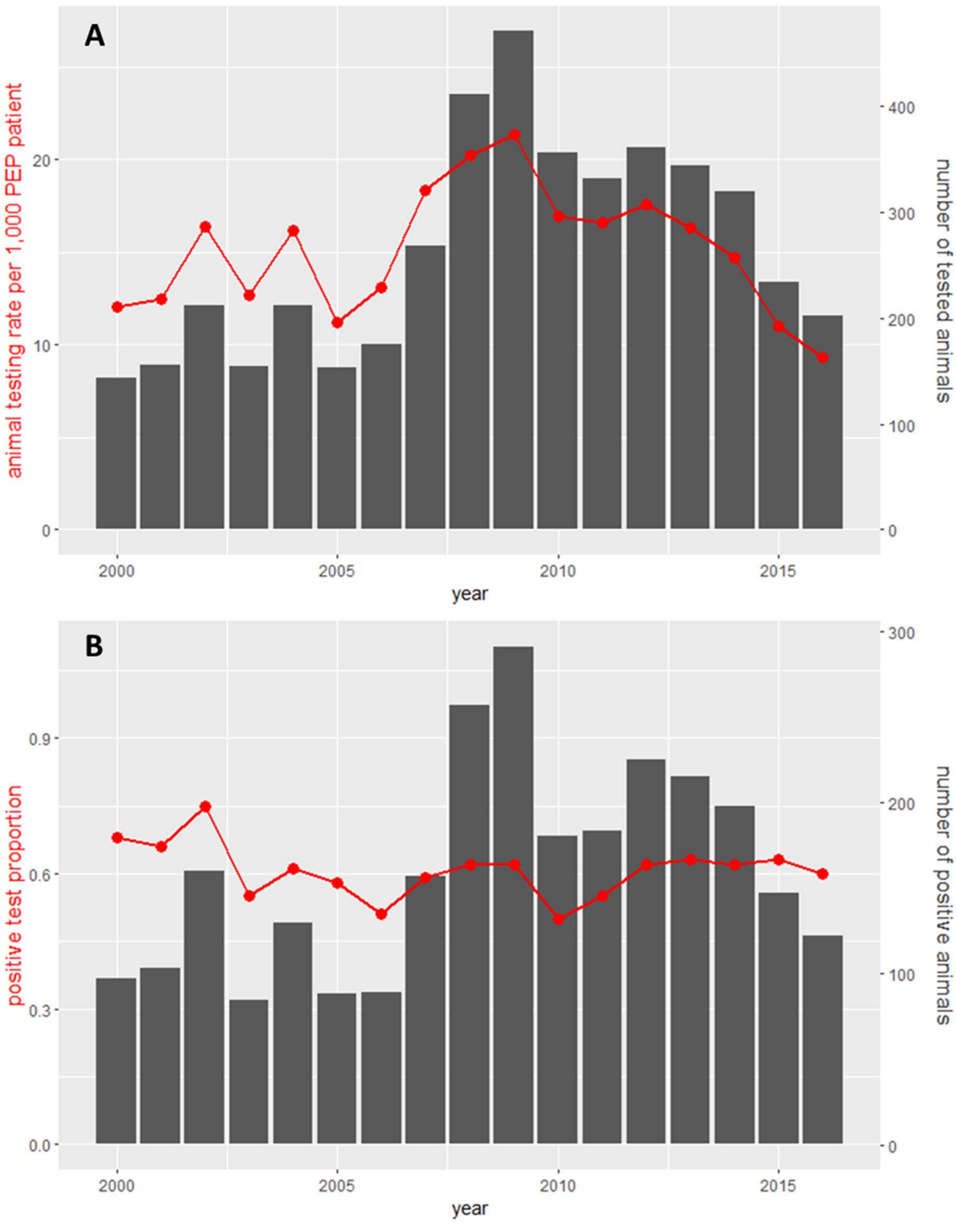
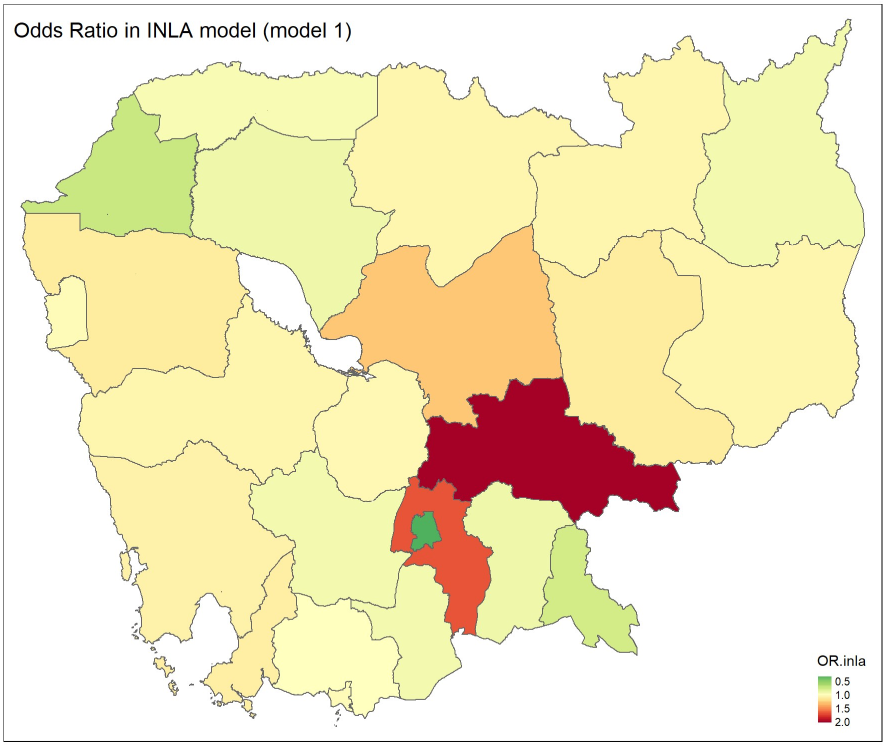
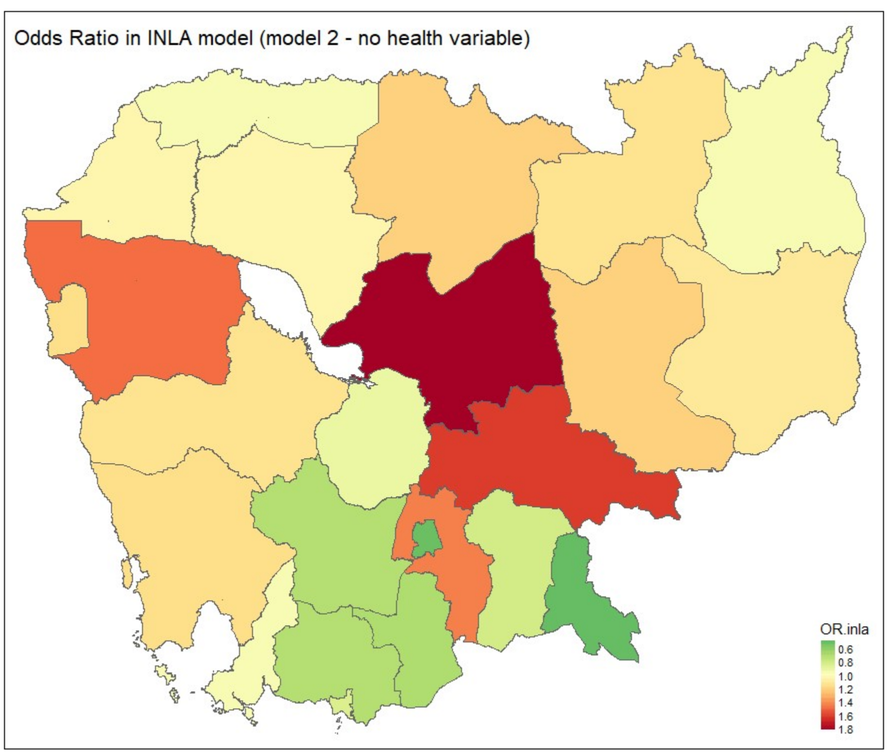
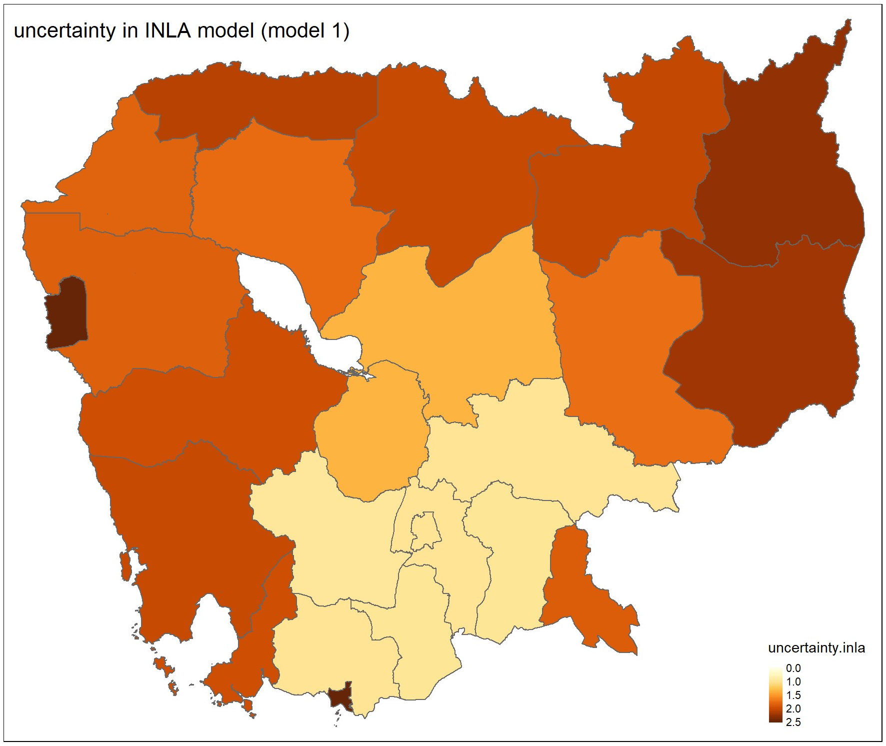


Figure 2: Number of animals tested (2A) and positive for rabies (2B) in Cambodia from 2000 to 2016.

Red lines represent the rate of testing amongst patients (2A) and the proportion of positive tests (2B)


Odds Ratio in INLA model (model 1)
OR.inla
0.5
1.0
1.5
2.0


Odds Ratio in INLA model (model 2 - no health variable)
OR.inla
0.6
0.8
1.0
1.2
1.4
1.6
1.8


uncertainty in INLA model (model 1)
uncertainty.inla
0.0
1.0
1.5
2.0
2.5

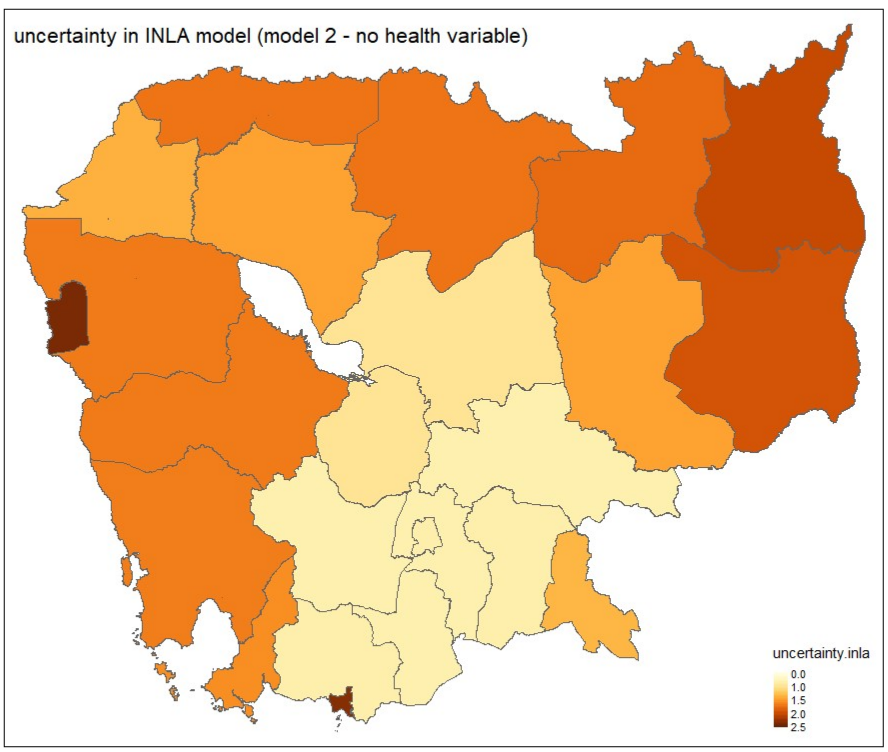
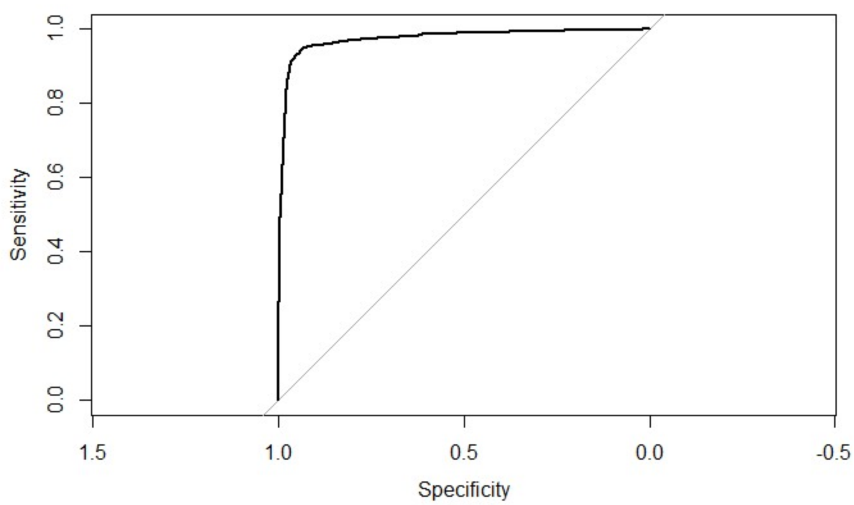

uncertainty in INLA model (model 2 - no health variable)
uncertainty.inla
0.0
1.0
1.5
2.0
2.5

Figure 3A, B, C & D: Maps of provincial odds ratios and uncertainty for the Bayesian spatio-temporal logistic regression models. *(A) Odds ratios for the model, which includes the animal health aspect in the selection process. (B) Odds ratios for the model without the animal health aspect. (C & D) Uncertainty for the odds ratios in map A & B respectively. Uncertainty is calculated as the difference between the 97.5 percentile and the 2.5 percentile of the non-exponentiated coefficients for each province.*



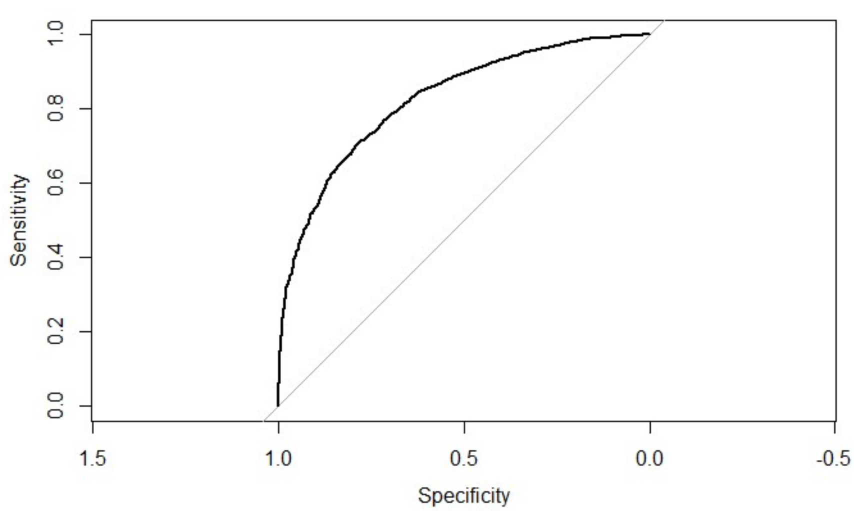


Figure 4A & B: ROC curves for the INLA models when including the animal health aspect variable (A) or removing it (B)

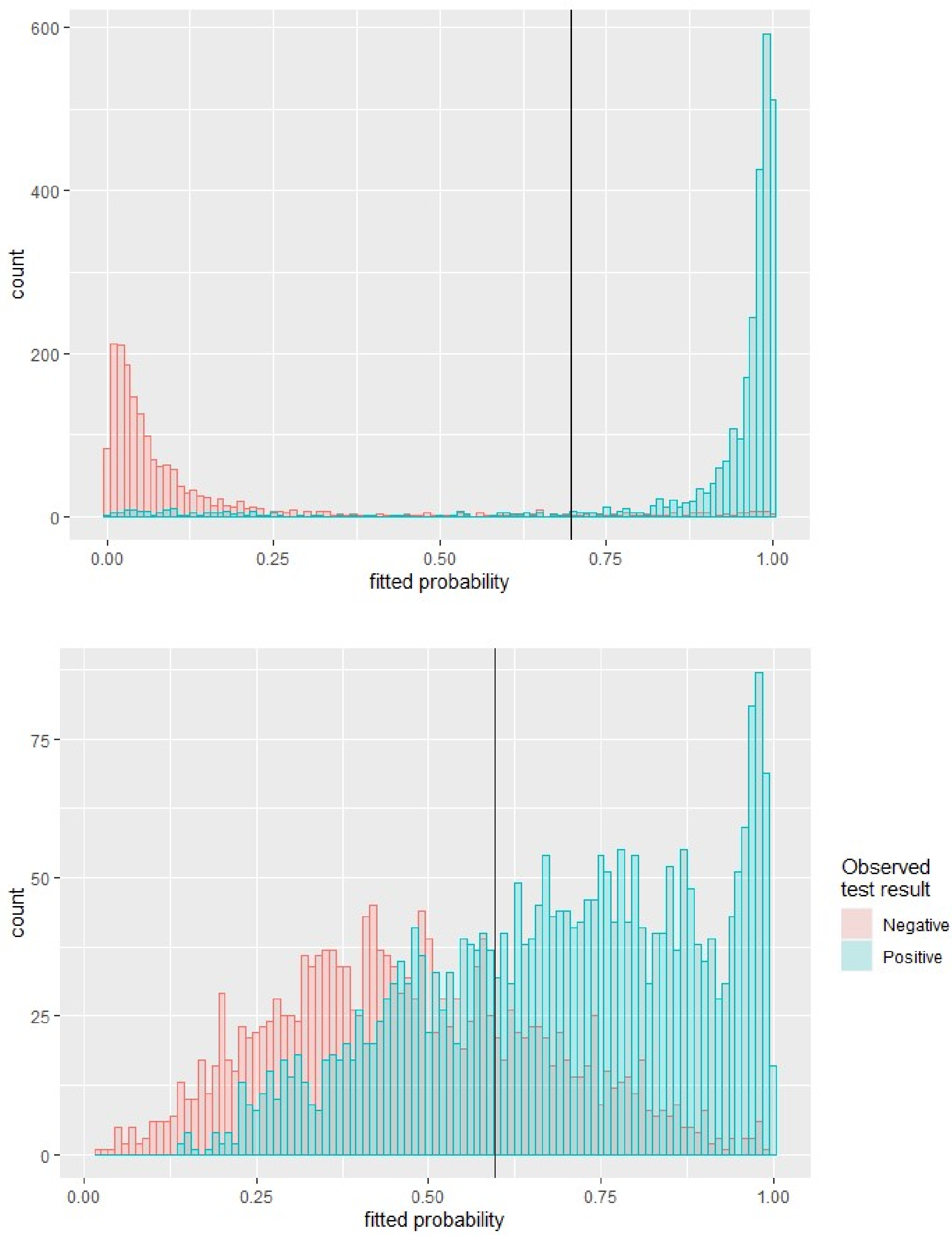


Figure 5A & B: Histogram of fitted probabilities for the INLA models with the animal health variable (A) or without it (B). *The dark vertical line represents the prediction threshold selected from the Youden statistic in the ROC curve analysis. Any value to the right of that line would be predicted as positive, any value below would be predicted as negative. The colors of the bars represents the observed real test results. Thus, blue bars to the left of the line would be false negatives whereas red bars to the right of the* *line are false positives. We can see that plot A is highly predictive with little overlap whereas there is much more overlap in plot B.*

PREVENTION ANTIRABIQUE

INSTITUT PASTEUR DU CAMBODGE

Médecin ____________________________

Sticker

Patient

Sexe 1[_] masculin 2[_] féminin

Grossesse 1[_] oui 2[_] non

Terme

1[_] 1er 2[_] 2nd 3[_] 3e trimestre

Age ______ ans ou ______ mois

Nationalité 1[_] cambo. 2[_] étrangère

Téléphone ______________________

Email ______________________

Voyageur 1[_] oui 2[_] non

Date d'exit du Cambodge ___________

Prise en charge initiale du patient

Soins locaux 1[_] oui 2[_] non

Sérum antitétanique 1[_] oui 2[_] non

Vaccin antitétanique 1[_] oui 2[_] non

Vaccination antérieure contre la rage

1[_] oui 2[_] non Année _________

Sérum antirabique 1[_] oui 2[_] non

Date ____________ Poids _______ Kg

Raison 1[_] tête d'animal positive

2[_] animal suspecté

3[_] plaie profonde

4[_] autre ___________________

Vaccin antirabique 1[_] oui 2[_] non

Si non, raison ______________________

Date J3 Animal 1[_] abatttu

________ 2[_] mort spontané

3[_] disparu 9[_] NA

Date J7 Animal 1[_] abatttu

________ 2[_] mort spontané

3[_] disparu 9[_] NA

Caractéristiques de l'accident

Date ______________________

Province ______________________

Mode d'exposition

1[_] morsure 2[_] griffure 3[_] léchage

4[_] morsure et griffure

5[_] contact avec cas humain

6[_] personnel médical 7[_] préexpo-

8[_] demande particulière

Surface 1[_] peau saine 2[_] Peau excoriée

3[_] muqeuse

Gravité 1[_] Superficielle 2[_] Profonde

Saignement 1[_] oui 2[_] non

Suture 1[_] oui 2[_] non

___ points 1[_] partielle 2[_] complète

Interposition vêtement 1[_] oui 2[_] non

Nombre de lésions _______________

Localisation des lésions principales

Loc- 1. __________________________

Loc- 2. __________________________

Loc- 3. __________________________

Caractéristique de l'animal et test laboratoire

Espèce 1[_] chien 2[_] chat 3[_] singe

9[_] autre ______________________

Agression 1[_] spontanée 2[_] provoquée

Aspect 1[_] sain 2[_] malade

Appartenance 1[_] avec propriétaire

2[_] sans propriétaire 3[_] animal sauvage

Nombre de victime ___________

Récupérabilité 1[_] abattu 3[_] disparue

2[_] mort spontanée 4[_] accessible

Animal testé 1[_] oui 2[_] non

Ce patient 1[_] index 2[_] secondaire

ID code du patient index __________________

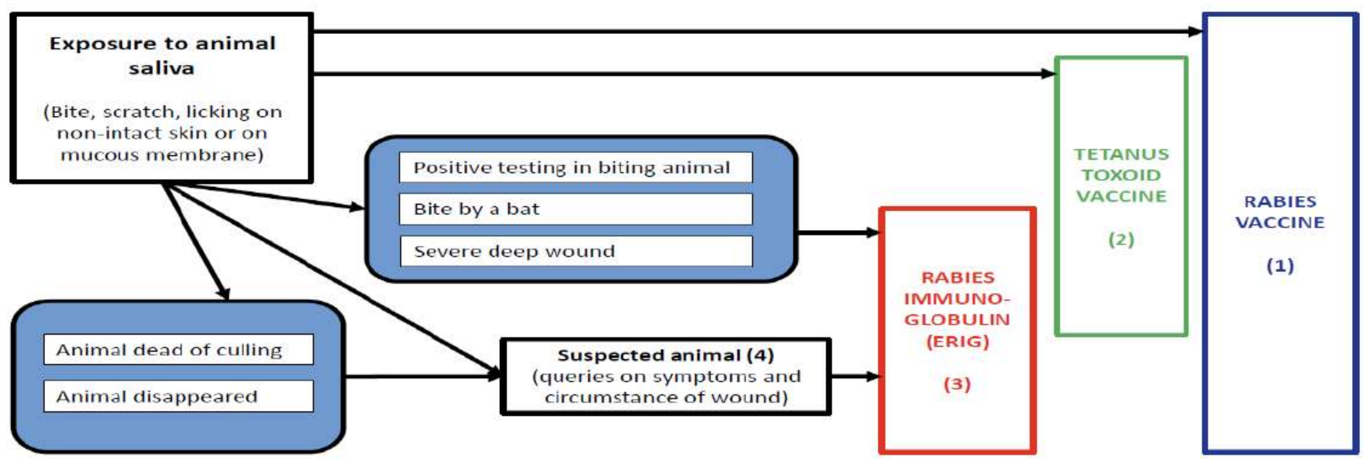
(sticker)

Supplementary Document SD1: Questionnaire completed by IPC doctors when interviewing PEP patients on their first visit (document provided by IPC).

Status of animal	Monitoring	Signs in animals Sick/suspected
Accessible	Evaluating the circumstance of animal at time of bitten → **Check signs** Observation animal for 10 days	1- In a state of illness - Drop tail - Hyper salivation, -Not recognize the owner -Loss of orientation -Abnormally aggressive -Bite even objects, paralysis 2- Spontaneous aggression 3- Wounded multiple victims (human and animal)
Dead of culling (slaughter)	Evaluating the circumstance of animal at time of bitten → **Check signs** If animal head available for lab testing → **Treatment bases on lab result** of rabies analysis If animal head not available → **Treatment bases on circumstance of animal**	
Animal disappeared	Evaluating the circumstance of animal at time of bitten → **Check signs**	
Dead of illness	**Considering suspicious rabies animal**	

Revision on
23 Oct 2020

Diagram summarizing Prescription of Rabies Post-Exposure Prophylaxis (PEP) at the Rabies Prevention Center, Institut Pasteur du Cambodge (IPC)



Supplementary Document SD2: Decision tree to assess the rabies status of the biting animal and to inform the allocation of HRIG for PEP patients (document provided by IPC).

Table SP1: number of tested animals per province and predicted number of exposed patients. The totals do not sum up to the overall as number of tested dogs (4,509) and PEP patients (294,040) as two dogs and 85 patients did have the attack location recorded.

Province	Tested Animals	Positive Animals	Percentage	PEP patients	Predicted Exposed (%) Model with Dog health	Percentage	Predicted Exposed (%) Model without Dog health	Percentage
TOTAL	4,507	2,726	60.5	293,955	8,185	2.8	66,495	22.6
KH01 Banteay Mean Chey	23	15	65.2	229	49	21.4	91	39.7
KH02 Battambang	9	8	88.9	507	47	9.3	260	51.3
KH03 Kampong Cham	**653**	**474**	**72.6**	**22,809**	**1,444**	**6.3**	**10,982**	**47.8**
KH04 Kampong Chhnang	71	39	54.9	4,143	207	5.0	833	20.9
KH05 Kampong Speu	**611**	**334**	**54.7**	**9,870**	**792**	**8.0**	**1,718**	**17.4**
KH06 Kampong Thom	106	83	78.3	3,432	239	7.0	1,947	56.7
KH07 Kampot	**443**	**236**	**53.3**	**4,465**	**534**	**12.0**	**901**	**20.2**
KH08 Kandal	**728**	**526**	**72.3**	**60,267**	**1,676**	**2.8**	**26,125**	**43.3**
KH09 Koh Kong	10	8	80.0	291	36	12.4	109	37.5
KH10 Kratie	16	12	75.0	545	57	10.5	198	34.3
KH11 Mondul Kiri	0	-	-	46	4	8.7	10	21.7
KH12 Phnom Penh	**544**	**285**	**52.4**	**158,009**	**1,194**	**0.8**	**18,116**	**11.5**
KH13 Preah Vihear	2	2	100.0	67	7	10.4	23	34.3
KH14 Prey Veaeng	**548**	**306**	**55.8**	**12,507**	**765**	**6.1**	**2,467**	**19.7**
KH15 Pursat	3	3	100.0	435	52	12.0	140	32.2
KH16 Ratanak Kiri	3	1	33.3	66	11	16.7	14	21.2
KH17 Siem Reap	13	7	53.8	356	73	20.5	146	41.0
KH18 Preah Sihanouk	11	8	72.7	482	66	13.7	149	30.9
KH19 Stueng Treng	0	-	-	40	1	2.5	10	25.0
KH20 Svay Rieng	44	18	40.9	1,738	70	4.0	142	8.2
KH21 Takaeo	**665**	**360**	**54.1**	**13,563**	**851**	**6.3**	**2,134**	**15.7**
KH22 Otdar Meanchey	4	1	25.0	42	3	7.1	9	21.4
KH23 Kep	0	-	-	12	5	41.7	4	33.3
KH24 Krong Pailin	0	-	-	34	2	5.9	8	23.5

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CHAPTER3: Modelling of rabies in five villages in Cambodia using agent-base models

Abstract

Rabies is fatal zoonotic disease that is endemic in Cambodia. Its reservoir lies in free-roaming dog populations and is most commonly transmitted through a bite. In Cambodia, to date, rabies prevention in humans focuses on providing post-exposure prophylaxis to bite victims to prevent disease from becoming symptomatic after exposure. However, the most cost-effective way to control rabies is through sustained mass canine vaccination. Cambodia has not yet implemented mass vaccination, though pilot vaccination campaigns have been done to inform future efforts. In this study, we used demographic and spatial data from these pilot campaigns to construct a spatially explicit agent based model in five villages of Kandal Province. Our aim was to use this model to study the impact of population turnover on vaccination coverage and measure the effectiveness of sustained annual vaccination drives on rabies transmission. We observed that vaccination drops by 40% one year after initial vaccination. However, annual vaccination with a target coverage of 70% maintained the average vaccination coverage over six years at 65% and reduced the basic reproduction number (R_0) under one with every infection scenario we tested. On the other hand, 8% of simulations with this coverage led to outbreaks of 10 dogs or more in the worst-case infection scenario. A target coverage of 90% allowed reducing the number of simulations with outbreaks of 10 dogs or more to below 1% in this same worst-case scenario. We provide evidence of the need to vaccinate dogs regularly due to the quick population turnover, but also that annual vaccination of 70% of more is sufficient to control rabies in this setting.

1. Introduction

Rabies is a fatal zoonotic viral disease estimated to cause around 59,000 deaths yearly mainly in developing countries in Asia and Africa [1,2]. In these settings, most cases in humans are caused by dog bites, with free-roaming dog population being a viral reservoir. Despite its fatal outcome once symptoms start, tools to prevent rabies in humans and dogs are highly effective, even after exposure in the form of pre-exposure and post-exposure prophylaxis (PrEP & PEP). PEP is the main tool used in humans to prevent disease after a bite has occurred but before symptoms start. However implementation of canine vaccination is considered to be the most cost-effective approach to controlling rabies when well implemented, and is the only tool capable of eradicating it [3–6]. When well implemented, vaccination campaigns are highly effective, however in the absence of a sustained commitment these efforts can fail in achieving long-term disease control [3,7–10]. Failure to achieve long-term control is often due, to the high turnover of the canine populations in developing countries, leading to a quick drop of immunity if initial efforts are not continued. It is also due to the lack of local knowledge of population and disease dynamics, leading to implementation of control measures that are not adapted to the actual situation [7,11–14]. In that sense, dog ecology studies and disease dynamics models are two key tools that allow to forecast and strategize the impact of human interventions prior to their application [3].

Intervention and prevention of rabies rely heavily on knowing the main dynamics of disease, which are highly variable between different settings. Modelling is one of the most powerful tools to help mimic and understand these dynamics under specific epidemiological settings. However, nothing has yet been published on the rabies cycle in Cambodia and very little in the rest of continental Southeast Asia [15]. Most models in continental Asia focus on China and rely exclusively on compartmentalized models [16–25]. Such models usually assume random mixing of the population and often do not account for how local spatial characteristics and population distribution can impact individual interactions in a population and how the virus is transmitted, though some do include long distance disease spread. However certain models using contact networks have been used to model spatially explicit models [26–28]. Agent base models are another approach that allows to model spatially explicit events. Furthermore, by parameterizing at the individual level, agent-based model also have the ability to take into account specific parameters for many population subgroups, such as age-specific parameters, without having to create a large number of specific categories or complex equations as we might have in a compartmentalized model. To our knowledge, only one other agent based model study has been conducted to model the hypothetical introduction of rabies in rabies-free Dingoes in Norther Australia [29]. Further studies have used ABMs to model canine populations and other diseases such as canine distemper virus [30–32].

Cambodia is a rabies endemic country in Southeast Asia. It is estimated to have one of the highest burdens in humans with an estimated 800 deaths a year [33]. It was also the site of early canine vaccination attempts in 1934 under the direction of the then Pasteur Institute of Saigon, now the current Pasteur Institute of Ho-Chi-Minh city in Vietnam [34]. Today, the main institution in charge of rabies prevention and control in Cambodia is the Pasteur Institute of Cambodia (IPC), based in Phnom Penh. However the main tools currently in use are PEP regimens for bite victims and education campaigns, with no large scale implementation of canine vaccination to date [35,36]. Nevertheless, there have been recent efforts to initiate vaccination programs and data collection with the goal of informing future implementation of vaccination campaigns. In 2017 and 2018, a pilot vaccination campaign was conducted in two provinces of Cambodia which collected detailed information on dog demographic parameters and was accompanied by follow-up visits to measure the evolution of the population and it's vaccination status [37]. The data collected from this study is to serve as the basis for multiple modeling exercises both at the village and country level to inform future implementation of vaccination strategies. The aim of this specific study is to use agent-base modelling to observe rabies transmission dynamics at the village level and the impact of population turnover on vaccination coverage using data collected in the field.

2. Methods

2.1 Model type and study area

We created a spatially explicit agent-based model of the dog population, rabies transmission and impact of vaccination in five villages in Kandal province in Cambodia. These are located in the Chey Thum commune of the Ksach Kandal district and are composed of Chey Touch, Ta Koat Lech, Ta Koat Keut, Chey Thom, Chrey Loas (Fig 1). Spatial data for these villages was obtained from openstreet map and formatted in R version 4.0.3 [38,39]. These villages were selected as four of them were part of a pilot canine vaccination campaign in which detailed demographic data for the dog population was collected [37]. The fifth village, Chey Thom, was selected due to its spatial proximity and connection to the other four. These villages are also representative of most villages in South-East Cambodia, around the upper section of the Mekong river delta where the majority of the Cambodian population is located. They are surrounded by rice patties that flood during the rainy season, and are connected by levy roads usually flanked by irrigation canals when not flooded. Houses are typically wooden structures on stilts to protect them from flooding. Notably, this also allows animals to roam freely under and around the houses (Fig 2).

2.2 Dog population

Population parameters were sourced from data of the field study described by Chevalier et al. 2021 [37], and when not available, from other published literature on dog populations in developing countries, as described in table 1. As we had dog population totals for only four of five villages, the population of Chey Thom was assumed to be 300 as the number of houses in that village was similar to the other two larger villages, which had a population of 309 and 383 respectively. For other parameters, we used the aggregated values for all villages sampled in Kandal province as opposed to looking at village specific parameters. The total of 1,343 dogs were randomly allocated to a buffer of 10m around buildings identified as houses or religious buildings. The first location reflects the fact that the vast majority of dogs are owned and home bound, serving as guard dogs, as described in Chevalier et al. 2021 [37].The second location was also selected as religious buildings are a common location to leave unwanted puppies and where they will be fed.

Table 1: Parameters for model

Parameter	Value	Source
Demographics		
Population size	Ta Koat Lech=210 Ta Koat Keut=383 Chey Touch=309 Chrey Loas=141 Chey Thom=300 (assumed)	[37]
Age distribution (in days)	Gama(shape=0.6518617346, rate=0.0008779193) Bounded between 0 and 6935 days (19 years)	derived from [37] by applying a Gama distribution to the collected ages of individual animals
Sex Distribution	55%	[37]
Age Specific Mortality (per year)	0 to 1 = 68% 1 to 2 = 47% 2 to 3 = 42% 3 to 4 = 39% 4 to 5 = 36% 5 to 6 = 35% 6 to 7 = 33% 7 to 8 = 31% 8 to 9 = 30% 9 to 10 = 29% 10 to 11 = 28% 11 to 12 = 27% 12 to 13 = 26% 13 to 14 = 25% 14 and above = 25%	derived from [37] age distribution by assessing the drop in numbers from one age group to the next
Birth Season	From October to February	[40–46]

Reproduction probability	females under 1 year: 0% females 1 to 6 years: 65% females 6 years or more: 15%	[47]
Litter size	random number between 1 and 4	calibrated to maintain stable population
Disease		
Probability of new introduction	$Pintro_t = 1/(2*365*N_t)$ N_t=population at time t	Calculated to average one every two years
Infectious distance	100m, 200m, 500m	first value from [48] next two values tested as sensitivity analysis
Probability of infectious contact	numerous values between 0.0005 and 0.04 depending on the simulation run	defined by authors
Incubation period	Gama(1.08549, 0.04920) bounded between 0 and 365 days	[48]
Symptomatic period	Gama(2.83, 0,91936) bounded between 0 and 10 days	[48]
Vaccination		
Vaccination target	Defined at the start based on scenario vaccination target Vaccination scenarios: 0%, 30%, 40%, 50%, 60%, 70%, 80%, 90%	defined by authors

2. 3 Age structure and mortality

Age distribution was obtained from the data of Chevalier et al. 2021 for Kandal province [37]. A Gama distribution of shape 0.652 and rate 0.000878 was fitted on the recorded ages using the R package "fitdistrplus" [49]. This distribution was then used to randomly allocate ages in days to dogs at the beginning of each simulation. Mortality was derived from this distribution by calculating the proportional difference in population from one age to the next, so as to maintain a stable age distribution over time. High mortality in the first year was confirmed in other field studies often seeing between 60 and 70% mortality [42,43,48].

2.4 Reproductive parameters

Sex distribution was also derived from the field data, with approximatively 55% of dogs being female. Based on a number of papers looking at the demographics of dogs in India, we assumed a seasonal reproductive cycle with births occurring over five months, between October and February [40–46]. However, we assumed that the probability of birth was uniform during these months, as we had no data that allowed to infer a distribution over time, even though it is likely that probability would peak in the middle of that time period. Information on the probability of birth for a female in a year was much more variable and we selected values from Gsell et al. 2012 [47]. To simplify these observations, we assumed that females in their first year did not give birth. From one to six years old, the probability was 65%, and beyond six, it was 15%. For litter size, we chose a random number between one and four, with a uniform probability of selection. We obtained this value by calibrating the population model, prior to introducing disease, so as to keep the average population stable over time. This was a smaller litter size compared to what was observed in field studies [40,43,44,47,48]. Location of newborn puppies was randomly distributed in the 10m buffer around houses and religious buildings. Sex was randomly allocated based on the proportion of 55% mentioned above.

2.5 Disease parameters

As there is no published information on the prevalence of canine rabies in Cambodia, beyond passive surveillance testing of some of the dogs responsible of bite attacks, it was decided to start each model rabies free, and assume a disease introduction in randomly selected dogs at random times throughout each simulations. The probability of a dog becoming the index case followed the formula:

$$P_{intro(t)} = \frac{1}{2 * 365 * N_t}$$

Where $P_{intro(t)}$ is the probability of a dog becoming an index case at time t, and N_t is the population size at time t. This equation was selected to select one index case in the population on average every two years. This approximates an introduction from an unknown source once every two years. Index cases were selected as infectious directly and so had no incubation period. To allow most outbreaks to end before the simulation ended the introductions of new index cases were stopped one year prior to the end of the simulation time.

Due to hardware limitations, it was decided not to explicitly model dog movements, as this proved too computationally intensive in small-scale simulations and difficult to parametrize appropriately when considering rabid dog behavior. Thus, all dogs were immobile, including rabid animals. However, to model the spread over a distance, a contact radius was defined around rabid dogs that is larger than the actual distance at which contact does occur between two dogs. This allowed simulating the distance a dog might move before biting another. We took as our base the assumption a radius of 100m from Hampson et al. 2009 and also tested two other values for this parameter of 200m and 500m [48]. The probability of infectious contact was uniform within the radius. From the model's mechanics, this probability should be understood as the probability that a rabid dog infects a specific dog present in the infection radius. This means that the probability of infecting any dog was cumulative based on the number of dogs in the radius. As there is no recorded values for the probability of infectious contact within a given radius but there are many publication which have inferred the basic reproduction number (R_0) from observed epidemiological data or transmission models, we chose to test a range of contact probabilities within a radius and select those that yielded R_0 values similar to what was found in the literature. R_0 is a key measure in infectious disease transmission and is defined as the number of secondary cases issuing from an index case in a fully susceptible population. An R_0 below ones indicates a disease cannot spread [50]. Based on literature we aimed to model a range for R_0 of between 1.0 and 2.0 [3,27,28,48,51,52]. The range of probabilities we tested were 0.0005, 0.001, 0.0025, and then from 0.005 to 0.03 in increments of 0.03. The lower values were selected after testing the initial range of 0.005 to 0.3, which resulted in R_0 much higher than targeted for the 500m infection radius.

With an agent-based framework where individual dogs are modelled, we could specifically identify the secondary cases infected directly by the index case. Thus, our computation of R0 was calculated directly as the sum of secondary cases in a simulation divided by the sum of index cases, or the average number of secondary cases per index case. A similar approach was used in Durr et al. 2015 [27]. Given the low frequency of index case introduction and the low R_0 values observed for rabies, we assumed that outbreaks would be mostly short and separated in time, meaning most index case would enter a fully naive population, making our computation representative of the definition of R_0 as the number of secondary cases issuing from a single case in a fully susceptible population.

Two other key disease parameters were used, the length of the incubation period and the length of the symptomatic period. Both of these were sourced from Hampson et al. 2009 and followed bounded Gama distributions [48]. The incubation period had a distribution of shape 1.0855 and rate 0.0492, and was bounded between 0 and 365 days. The infectious period had a distribution of shape 2.830 and rate of 0.919 and was bounded between 0 and 10 days. The outcome following the infectious period was always assumed to be death.

2.6 Vaccination

We tested various levels of vaccination coverage on the long-term population immunity coverage and the infectious disease outcome. We set the vaccination to be on the first day of the month of March. This month was selected as it is on the one hand when the pilot vaccination study was conducted and also at the end of our modelled birth season, when the population reaches its peak, and so when the most dogs are available for vaccination in our model. This also had the benefit that no females were expected to be pregnant anymore at this time, as pregnant females are sometimes not considered for vaccination[46]. However, we did not limit the age of vaccine recipients as even though in practice it might be avoided to vaccinate puppies below four months of age, WHO recommends vaccinating all dogs regardless of age [53]. Follow-up vaccination campaigns were considered once a year at the same date, with the objective of returning the population to the target coverage of a given scenario. Given the frequency of vaccination we did not model waning immunity as few dogs should have experienced this by the time the next vaccination campaign occurred assuming and average immunity length of 2 years as mentioned in Hampson et al. 2007 and that the re-vaccination effort would also re-vaccinate animals that had been previously vaccinated [51]. We also did not account for vaccinating incubating animals and those cases, if they happened, were left to incubate and become symptomatic.

For the vaccination scenarios, we modelled these based on the 100m infection radius only. However, we did test a range of probability of infectious contacts that were informed by the previous set of simulations for that infection distance. Thus, we modelled a range of probabilities from 0.015 to 0.04 in increments of 0.005. For the vaccination target, the rate of vaccination in the population directly after each campaign, we used a control of 0% and then tested values from 30% to 90% in increments of 10%. From the model's mechanics perspective, this translated in the probability of any dog in the population to be vaccinated on vaccination day. Thus, vaccination coverage was distributed at random throughout the population, regardless of demographic characteristics and spatial location.

2.7 Simulation strategies

Due to the potential long incubation time of the disease and the need to model scenarios in a stable population, especially to measure the impact of population turnover on vaccination coverage, the model was conducted over six years with a daily time step. In addition, to be able to start vaccination scenarios with coverage fully attained, the overall simulations were started in March, the month selected for vaccination. Furthermore, this was also when Chevalier et al. conducted their first visit when the initial demographic data was collected, thus, it seemed appropriate to start the model at the same season as that of the input data collection date.

As briefly explained above we ran two sets of simulations. The first focused on transmission parameters and tested three different infection radii and nine different probabilities of infectious contact for a total of 27 combinations of the two. The second set of simulations focused on vaccination scenarios and involved six different probabilities of infectious contact and eight different vaccination target values for a total of 48 combinations. For each combination of parameters, we ran 1,000 six-year simulations leading to a total of 75,000 simulations. Numerous output parameters were collected for each simulation the most important of which were: R_0, the mean outbreak size, the population size at the end of the period, the vaccination level one year after initial vaccination and mean vaccination coverage over time. Mean vaccination rate over time was calculated as the sum of vaccinated dog-days divided by the sum of total dog-days.

Modelling was done using the GAMA platform version 1.8.1 and out data analysis was conducted using R version 4.0.3 [38,54].

3. Results

3.1 Dog population

The random spatial distribution of the dog population in our model appeared similar to the recorded location of dogs from Chevalier et al. 2021, and is relatively evenly distributed around the households of the villages (Fig 3). In the absence of disease or with low transmission parameters, the population remained stable over time but we observed seasonal cycles in the population size and mean age (Figure 4). Population was at its peak at the end of the birth season and then declines until the start of the next with an average low near 1,000 animals compared to an average high close to the inputted starting population of 1,343. The average age followed the same seasonal cycle with age diminishing when new puppies were born and increasing during the rest of the year as mortality was higher in the first year of life. As expected based on the input, sex distribution remained stable over time.

3.2 Rabies outbreak measures

On average, we observed just over two index cases per five-year simulation, although this number was lower in the simulations that resulted in complete population collapse due to high mortality from disease as this eventually led to early termination of the simulation. Due to the stochasticity in disease introduction, 12.5% of simulations did not introduce an index case. As this introduction was not linked to disease or vaccination parameters in the model, they were evenly distributed amongst the different scenarios and thus did not create any sort of bias. These observations were removed when analyzing the results as no outbreaks could be measured

Our two main measures of disease transmission were R_0 and mean outbreak size (Fig 5A and 5B). With the 100m infection radius, the mean R0 increased from 0.04 to 1.70 (median 0.00 to 1.75) between the lowest and the highest contact probabilities. Contact probabilities between 0.015 and 0.03 led to R_0 values that were between the range of 1 and 2 we were looking for to model later vaccination steps. The maximum observed R_0 was 14. With the 200m radius, the mean R_0 increased from 0.10 to 6.33 (median 0.00 to 4.67) with two contact probabilities (0.005 and 0.01) leading to a mean R0 in the 1 to 2 range (1.00 and 1.93 respectively). The maximum observed R0 was 47. Finally the 500m infection radius lead to much higher values with the mean R_0 increasing from 0.26 to 21.5 (median 0.00 to 17) with a single contact probability (0.0025) leading to a mean R_0 in the required range (1.53). Individual values for this radius varied between 0 and 124. The overall relationship between R_0 and probability of infectious contact appeared mostly linear (Fig 5A). Outbreak size followed a similar trend to R_0 (Fig 5B). However, with the largest R_0 values we observe a plateau effect, which is due to the collapse in population from high disease mortality, causing an absence of future susceptible animals. This population collapse can be seen in figure 5C where we observe large population reductions with the 200m radius and probability of contact above 0.01, or with a radius of 500m and probability of contact of 0.005 and then near total population collapse with probabilities of contact of 0.01 and above.

In the second set of simulations, which focused on the 100m infection radius only, we observed a range in mean R0 from 1.06 to 2.74 in the absence of vaccination (table 2). This lead to a range in mean outbreak size from 5.4 to 53.5, in line with what we observed in the previous set of simulations. However, it is notable that the median values were much lower. This is due to the very skewed nature of the data with a large portion of simulations leading to no outbreak or outbreak of very small sizes compared to a few outbreaks with very large values. With a contact probability of 0.015, we observed that 21.9% (191/874) of simulations had no outbreaks. Even with the highest contact probability of 0.04, we observed 7.0% (61/870) of simulations had no outbreaks. The largest outbreak size was 120 for the lowest contact probability of 0.015 and 528 for the highest contact probability, 0.04.

Table 2: Outcome measures for different infectious contact probabilities with an infection radius of 100m and annual vaccination target rate required to control disease for that probability.

Infection parameters prior to vaccination						
Infection probability	0.015	0.020	0.025	0.030	0.035	0.040
Mean initial R0	1.06	1.35	1.83	2.06	2.49	2.74
Mean initial outbreak size	5.4	9.6	19.9	28.3	43.5	53.5
Median initial outbreak size	1.3	2.0	3.7	7.5	15.3	23.5
Vaccination target required to reach R0<1						
Vaccination target	30%	30%	50%	60%	70%	70%
R0 post-vaccination	0.68	0.99	0.92	0.95	0.88	0.91
Mean outbreak size post-vaccination	1.5	3.2	2.0	1.8	1.4	1.8
Median outbreak size post-vaccination	0.5	1.0	1.0	1.0	0.8	1.0

3.3 Impact of vaccination

The first goal of the vaccination simulations was to assess how population turnover impacted the vaccination level in the population. Due to the annual nature of the vaccination program, we observed a cyclicity in the number of vaccinated animals. An example simulation with a vaccination target of 70% in the absence of disease is presented in Fig 6. We observed a sharp increase in the number of vaccinated animals on each vaccination day, and a steady decrease in between vaccination days as dogs die throughout the year (Fig 6B). We also observed that the seasonal dog population cycles impacted the vaccination rate within a given year with the rate not following a similar linear decrease (Fig 6A). Vaccination was conducted just after the end of the birth season, so during the 7 months that followed, the vaccination rate remained stable as no new susceptible animals were added to the population and the mortality was the same in vaccinated and un-vaccinated dogs in the absence of disease. However, once births would start in October, we observed a sharp drop in the vaccination rate as the number of new susceptible puppies quickly increased.

With the different simulation scenarios, we observed a drop of 38.5% to 39% in the proportion of vaccinated dogs one year after initial vaccination occurred regardless of the initial vaccination target. As an example, for the commonly used 70% vaccination target value, this represented a drop down to 42.8% of animals vaccinated one year later. In comparison to disease parameters, the variation around this mean was much less, and symmetrical as can be seen in Fig 7A. However as the drop in vaccination coverage mainly happened over a few specific months, the average coverage over the whole simulation was only 6 to 8% below the initial target. This means that for a 70% target with annual re-vaccination, the average vaccination rate over 6 years was 64.9% (Fig 7B).

Vaccination had a visible impact on infectious disease outputs as can be seen in table 2 and Figure 8. In every infection scenario, a target of 70% vaccinated was sufficient to reduce both the mean and median R_0 below one. If the initial mean R_0 was two or below (contact probability of 0.030), then a

60% vaccination target rate was sufficient in all cases. With a 70% target vaccination rate, 48.5% (422/871) of simulations had no outbreak with the lowest infectious contact probability of 0.015, going down to 25.3% (220/869) with the highest infectious contact probability of 0.040. In comparison, for these extremes we observed 0.006% (5/871) to 7.6% (66/869) of simulations having outbreaks of five animals or larger. The maximum outbreak size went from 8 to 41 in these cases with increasing infectious contact probability. With a 90% vaccination target, 71.1% (620/872) of simulations lead to no outbreak at the lowest contact probability, and 46.5% (409/879) of simulations did so with the highest contact probability. The percentage of outbreaks of five or more dogs went from 0% with the lowest contact probability to 0.009% (8/879) with the highest. The maximum outbreak sizes increased from 3 to 11 in this case. These levels of vaccination ensured minimal outbreak sizes that would not go beyond 1 to 3 symptomatic cases on average. We observed a near linear relationship between the vaccination target and R0 at every tested level of the infectious contact probability.

4. Discussion

Our model was a first step in providing information to guide future vaccination efforts in Cambodia. We provide insight on how the specific demographic structure of a local dog population impacts vaccination coverage and its effectiveness when also taking into account the spatial distribution of the population. When using a 100m infection radius, a contact probability between 0.015 and 0.03 was associated with a mean R_0 between one and two, which is the typical range for rabies. In the specific context of our model's mechanics, this contact probability should be described as the probability that a dog living in a 100m radius of a rabid dog's location is infected. Our model also found that with and R_0 between one and two, we observed highly skewed outbreak sizes, with a large portion of simulations having no outbreaks at all or very small outbreaks. This is consistent with other small scale spatially explicit models [26].

Vaccination is a key method to control rabies. Our results are consistent with what has been modelled in other studies, showing that 70% is sufficient on average to reduce R_0 below one, thus theoretically stop disease transmission [48,52,55–58]. However, as we've seen, and as has been discussed in other papers, 70% vaccination coverage does not stop all outbreaks from happening [29]. In our model we observed with 70% vaccination still resulted in disease transmission in a majority of simulations, and though these were mostly small (below five animals), a few involved up to several dozens of animals. 90% vaccination target ensured a much higher chance of reducing the risk of a major outbreak.

This model also exemplified the need for sustained vaccination program. Within a year of vaccinating, vaccination coverage had dropped by nearly 40% due to the high population turnover. This was not even taking into account waning immunity. However, if high coverage is achieved at each visit, annual re-vaccination is likely sufficient as the drop in coverage is influenced by the annual reproduction cycle, and in our case, with vaccination happening at the end of the five month long birth season, coverage had not yet dropped a half-year after vaccination. However, this might not hold true if vaccination occurs at a different moment in the year, or if reproductive cycle are not as clearly seasonal as defined in our model. With the current model, annual vaccination with a target of 70% yielded an average vaccination rate of 65% in the population over time. This is much more optimistic than was observed in a model where similar vaccination settings led to a maintained herd-immunity of 20-45% [59].

As with most models, our model relied heavily on assumptions for parameters and individual behavior, which brings a number of limitations. One of the main limitations in our design of a spatial explicit model was that we did not explicitly model dog movements. Beyond the technological constraints individual movements had on the model, there are inherent difficulties in modelling animal movements at a fine spatial scale. It requires detailed information on dog movements that were not available for Cambodia. Such data has been collected in studies in different settings, but dog roaming behavior and range can be very specific to the local village structure, how dog management is conducted making it difficult to transfer such behavior to another setting [60–66]. Furthermore, there are no such data for rabid dogs which are obviously key to model rabid transmission and whose neurological symptoms induce behavior changes that likely significantly impacts how the animal moves, responds to its surroundings and enters in contact with other dogs. In that sense, local short-term healthy dog movements are of much lower relevance for disease transmission. Consequently, mimicking rabid dog movement to a 100m radius made it impossible to model scenarios where a sick animal might travel longer distances.

The way our model was built, with agents being fixed and movements inferred by a contact radius, ensured that population density was key in transmission as an infected immobile dog requires a neighboring dog within the radius to transmit disease. Furthermore, since the contact probability is from the perspective of the recipient and not the infectious dog, the chance of dogs an infectious dog to transmit disease cumulates with the number of susceptible dogs in the radius. Thus, outbreak size in our model is dependent on having population clusters. It might also seem intuitive that dog density might be related to transmission, however other studies have demonstrated that maintenance and transmission of rabies in a dog population is not related to dog density [67,68].

Two disease parameters, which we did not include in the model, were the pre-symptomatic or prodromal period when it has been shown dogs can already be sheading virus, and the proportion of dogs suffering from furious rabies. We did not consider these parameters as the literature referring to their values was limited to a few experimental studies or [69–72], or in the case of the furious proportion, observational studies that contradict experimental results [73]. Furthermore, the experimental nature of these studies made it even more difficult to infer real world impact of these stages of disease in terms of natural transmission and we suspected that transmission parameters inferred from epidemiological studies that observed rabies outbreaks and transmission most likely came from symptomatic furious animals. Difficulties in parametrizing the disease form, furious or paralytic, has been described in other modelling papers as well [26,29]. Thus, we modelled transmission following and simple two-step (incubation and symptomatic period) as we did not have enough information to quantify how different stages of the disease would impact transmission differently compared to what has been measured.

Our population model relied exclusively on births and deaths to modify the population structure, as we had no specific to accurately depict other ways dogs enter or exit the population. As described in Chevalier et al. 2021, a number of dogs are obtained through gifts, and these animals are not necessarily small puppies. As a consequence, the introduction of new dogs might not be as clearly seasonal as observed, which might impact vaccination coverage, causing it to drop quicker than observed.

We had no local data that allowed for validation of the rabies situation in these villages. From surveillance data provided by IPC (ref chapter 1), we identified 94 PEP patients coming from Chey Thom commune between 2013 and 2016, of these 77 came from the five villages under study. Unfortunately, no dogs were tested in this location. One final and more general limitation to constructing small scale, locally specific is that outputs themselves can become highly specific and not easily generalizable.

As a whole, our model provides information on the level and frequency of vaccination required to prevent rabies outbreaks in the event of introduction to a Cambodian village. It also takes into account how the demographic structure of a small-scale dog population influences the maintenance of vaccine coverage over time. With the intent of guiding future vaccination campaigns in Cambodia, further model developments should also include human outcome estimates and cost-effectiveness measures whilst varying the vaccination strategies in terms of frequency, period of vaccination and method of vaccination such as providing door-to-door, central-point vaccination campaigns or targeting specific subgroups, as has been done in other studies [52,74,75].

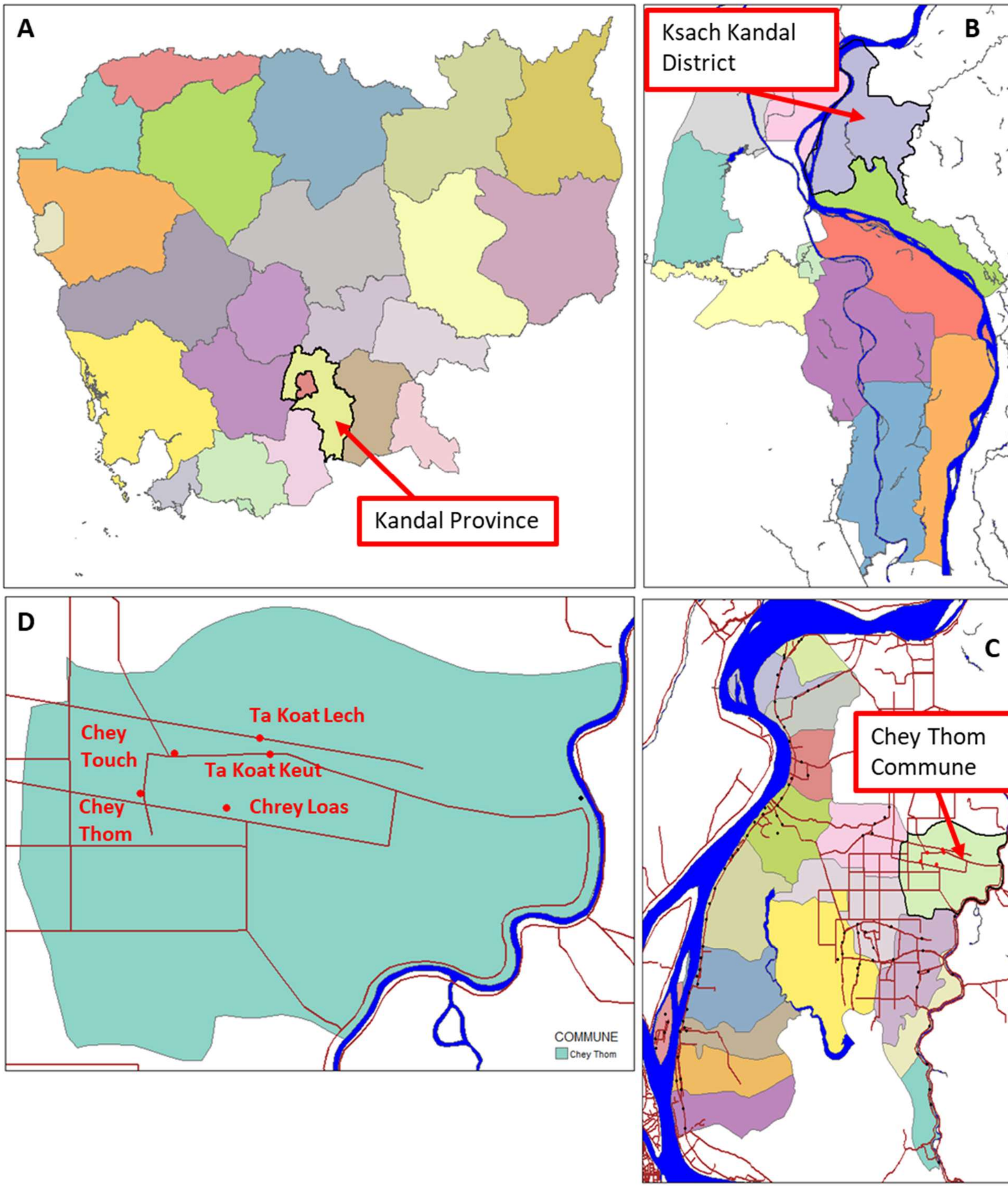

Figure 1: Location of the study Area.

The study areas was composed of five villages from the the Chey Thom commune of the Ksach Kandal district within the Kandal province of Cambodia.

Figure 2: Houses and dogs in Chey Tounch village.

Houses are often on stilts to avoid flooding during the rainy season. This allows dogs to take shelter or roam under them, thus they are often not obstacles for movement. They are usually separated by fences, but as can be seen these are often damaged, allowing dogs to move freely.

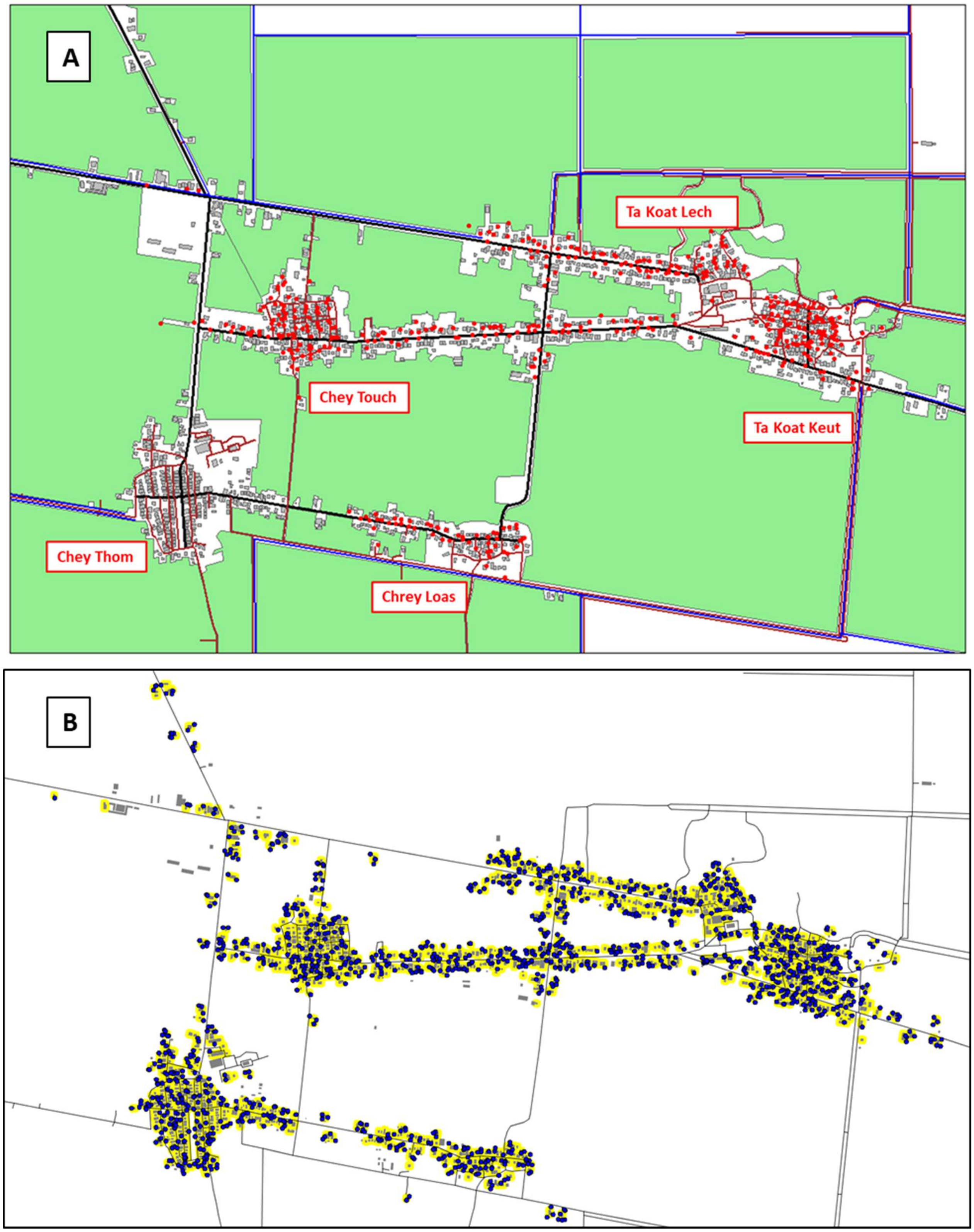

Figure 3: Distribution of real dogs (4A) and randomly distributed dogs (4B) in the commune of Chey Thom.

We can notice the lack of sampled dogs in Chey Thom village in the South-West in figure 4A. In figure 4B, the yellow area represents the 10m buffer around houses and religious building in which dogs were randomly placed.

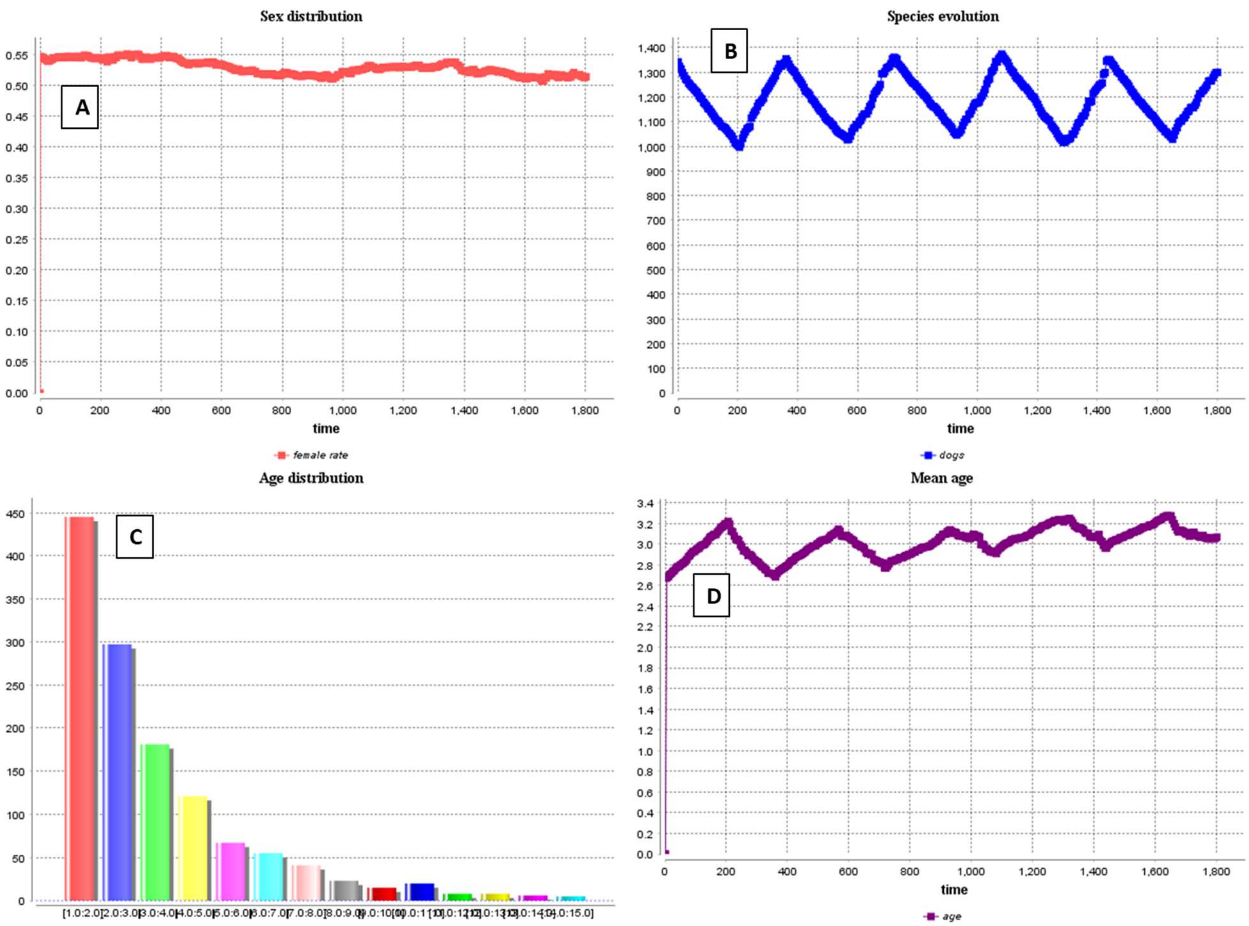

Figure 4: Population parameters of five years in an example simulation in the absence of disease transmission

Figure 4A shows the proportion of females in the population, which stays mostly constant. Figure 4B shows the total number of animals over time. This follows a seasonal cycle, increasing during birth seasons and reducing in-between. Figure 4C shows the population age distribution in years at the end of the simulation. Figure 4D shows the mean age over time. This also follows a cyclical pattern, dropping when new births occur and re-increasing in-between birthing seasons as puppies have much higher mortality.

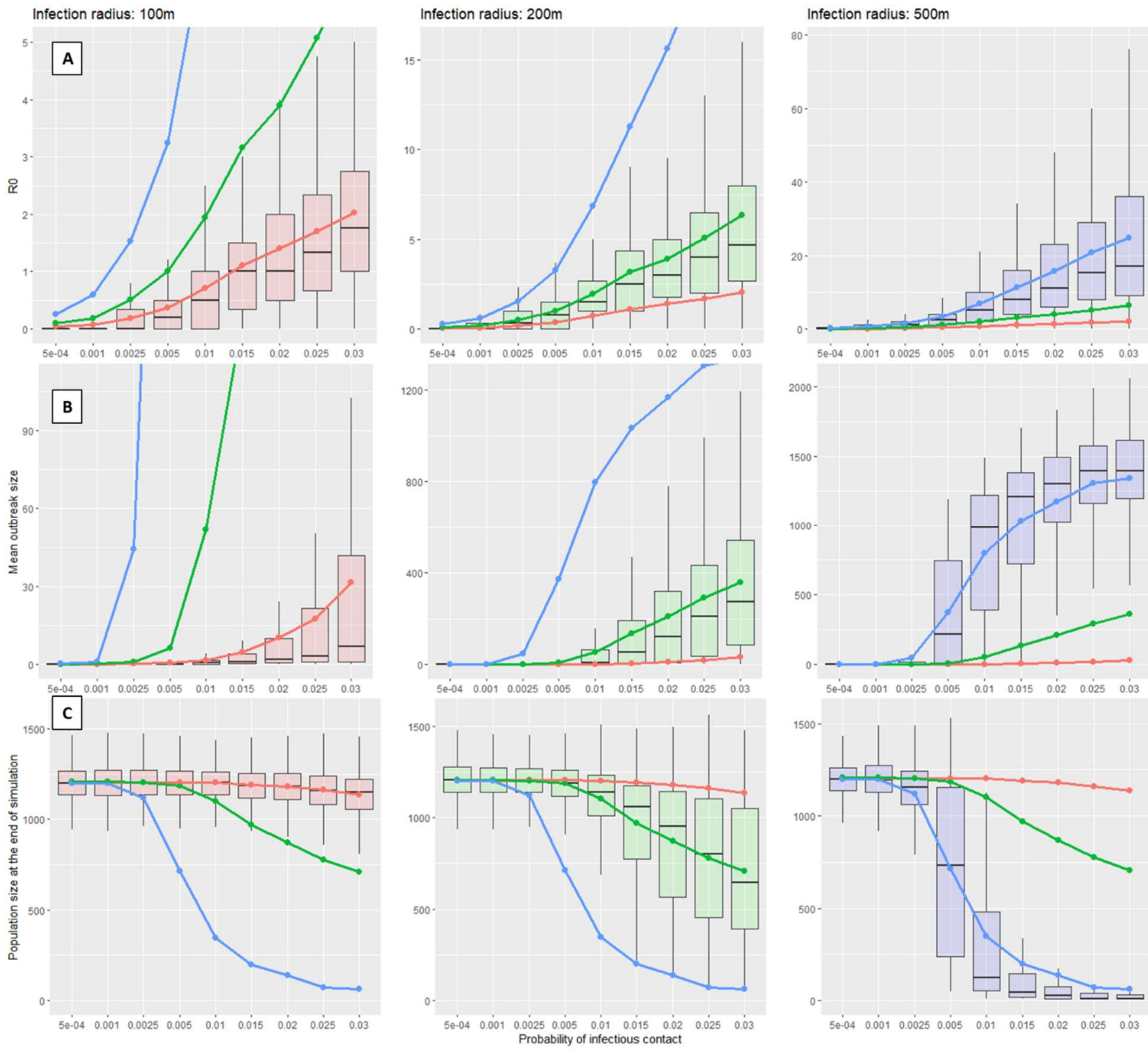

Figure 5: Boxplot and means for output values for the first set of simulations for different infection radii and probabilities of infectious contact.

Three output values are presented: R0 (5A), outbreak size (5B) and population size at the tend of the simulation (5C). The points and lines represent the means for the selected values. As boxplots varied dramatically in range depending on the infection radius, it was necessary to rescale between sets of boxplots. Thus to be able to compare with different scales, the mean graphs for each infection radius was kept in all plots, with the red being 100m, green being 200m and blue being 500m.

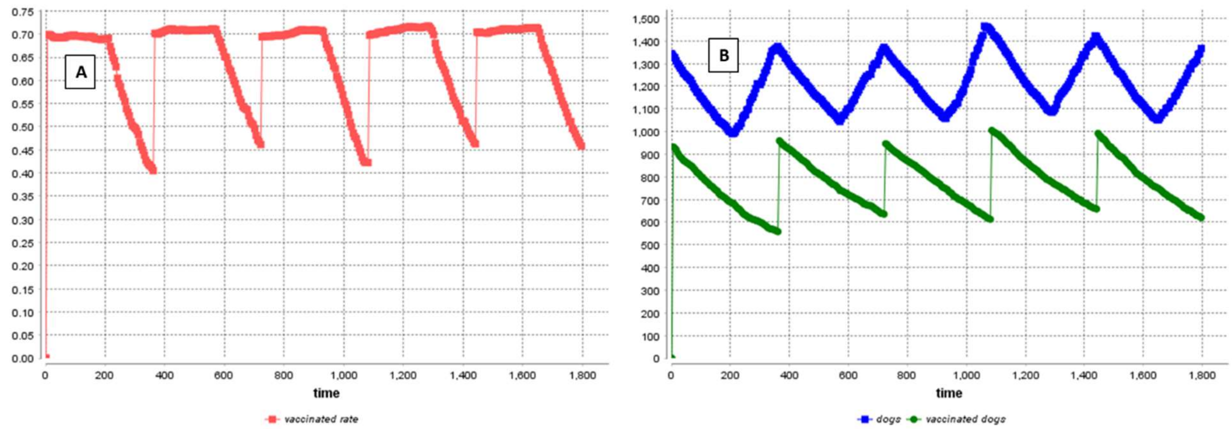

Figure 6: Vaccination rate and numbers over a five-year simulation with a target rate of 70% and in the absence of disease

Figure 6A shows the evolution of the proportion of vaccinated animals over time. Figure 6B shows the number of vaccinated animals (green) compared to the total population (blue) over time.

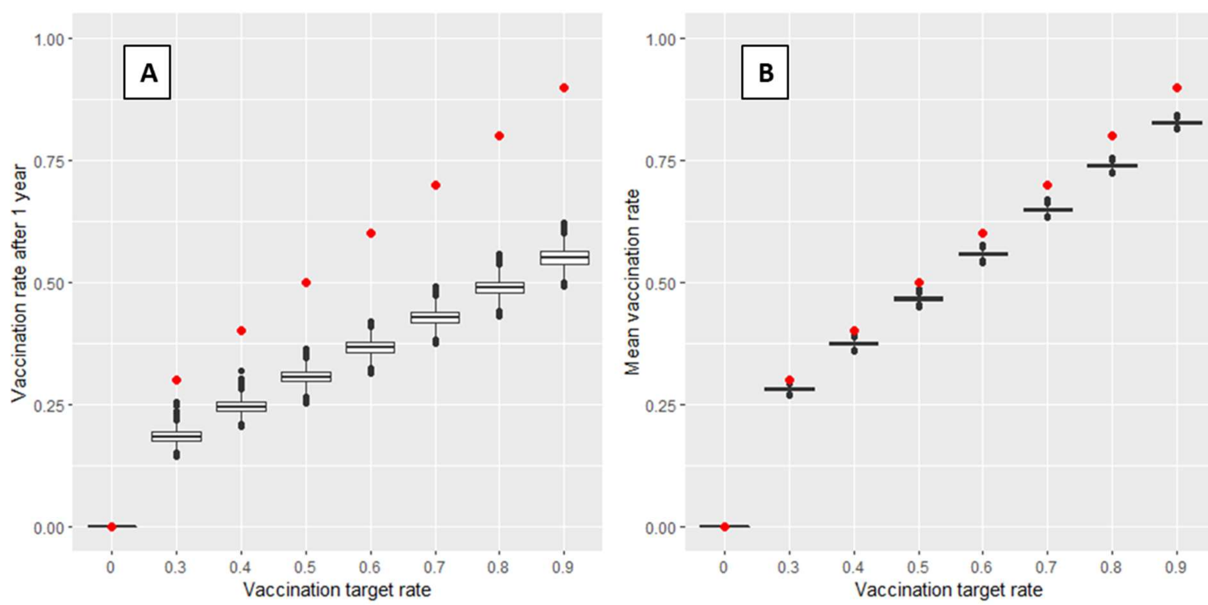

Figure 7: Distribution of the proportion vaccination 1 year after initial vaccination (7A) and of the mean vaccination rate over 6 years (7B) in the absence of disease.

The red dots represent the value of the initial vaccination target.

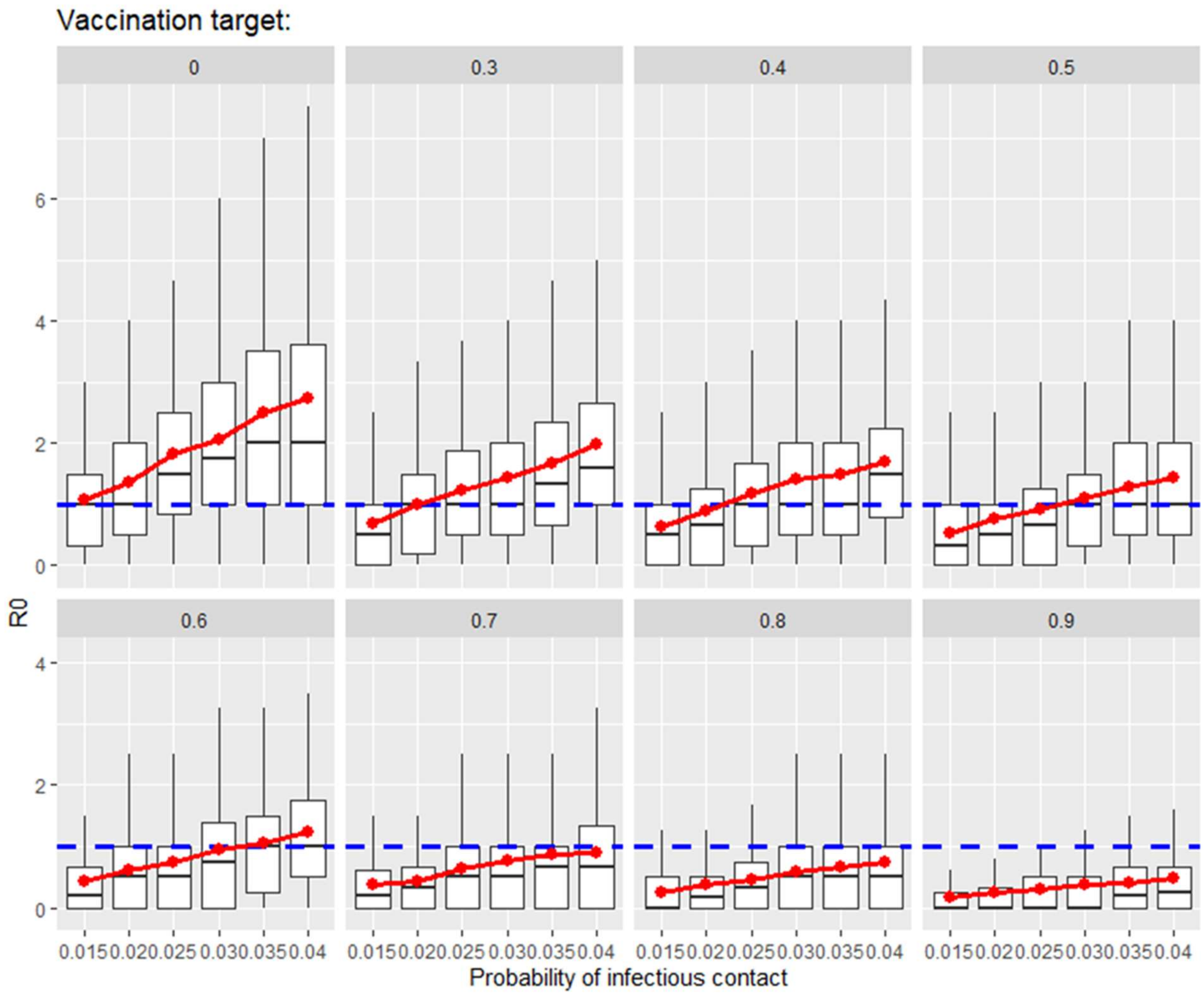

Figure 8: Impact of vaccination on R0 with different probabilities of infectious contact.

The blue hashed line represents and R0 value of 1, under which we need the R0 to be to stop an outbreak.

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CONCLUSION

Cambodia remains endemic with canine rabies, though there are ongoing efforts from the IPC to improve and expand control and prevention by increasing access to PEP and investigating the feasibility and requirements of mass canine vaccination. Through these three chapters we explored different tools to help inform future intervention in Cambodia. Chapters one and two used surveillance data and Bayesian regression modelling to inform on the geographical need for new PEP centers as well as guide allocation of PEP resources. Chapter three used a rabies transmission model at a small spatial scale in five villages of Cambodia to inform vaccination strategies.

In chapter one we observed that the rate of PEP patients in the population was strongly associated with the travel time to the PEP center in Phnom Penh using a Bayesian Poisson regression with INLA. An increase in one hour of travel time leading to a drop of 70% to 80% in the rate of PEP patients. The models predicted that increasing the number of PEP center locations would increase the number of yearly PEP patients. We identified five locations where new centers would be most beneficial in terms of improving accessibility to PEP for bite victims, thus increasing the expected number of PEP patients the most. These were located in the provinces of Banteay Meanchey, Siem Reap, Takeo, Kampot and Svay Rieng.

In chapter two, we used a Bayesian spatio-temporal logistic regression with patient interview data to identify predictors of positive rabies test. From 2000 to 2016, just above 2,500 animals, mostly dogs, were tested at IPC, with 60% returning positive. Positive dogs were associated with being ownerless, having attacked unprovoked and having attacked multiple victims. We aimed to use such data to inform allocation of PEP resources such as HIRG towards at risk patients without an associated tested animal, however we found that outcome variable from the protocol in place at IPC to define rabies suspect animals was more predictive than any other variable in our model. We also identified three provinces were odds of a positive test were higher than average: Kandal, Kampong Cham and Kampong Thom.

In Chapter three, we used agent-based modelling to establish a spatially explicit rabies model in five villages from Kandal Province. This model was parametrized with location specific demographic data collected during pilot vaccination campaigns and disease transmission data from published observational and modelling studies. We established a range of contact probabilities within a 100m that would be compatible with the R_0 range commonly described in the literature rabies, between one and two. Within this range we observed that a target vaccination rate of 70% was sufficient to reduce R_0 below one with every tested infection scenario, though it did not prevent outbreaks from taking place in all simulations with the more aggressive transmission scenarios. However, we also observed that annual vaccination is necessary as the quick population turnover led to a sharp drop in coverage after one year.

Expansion of PEP is key in reducing human mortality from rabies and should be accompanied by information campaigns for the broader public indicating what actions should be taken in the event of exposure, as well as what can be done at home to prevent exposure in the first place. However, the critical tool in long-term rabies control is expansive and sustained canine vaccination. Dogs are the main reservoir of rabies and controlling the disease at their level is the most cost-effective approach to rabies prevention and control. To be effective, canine vaccination must have high coverage and be sustained with repeated annual drives as long as rabies is present in the country, and the region as a whole.