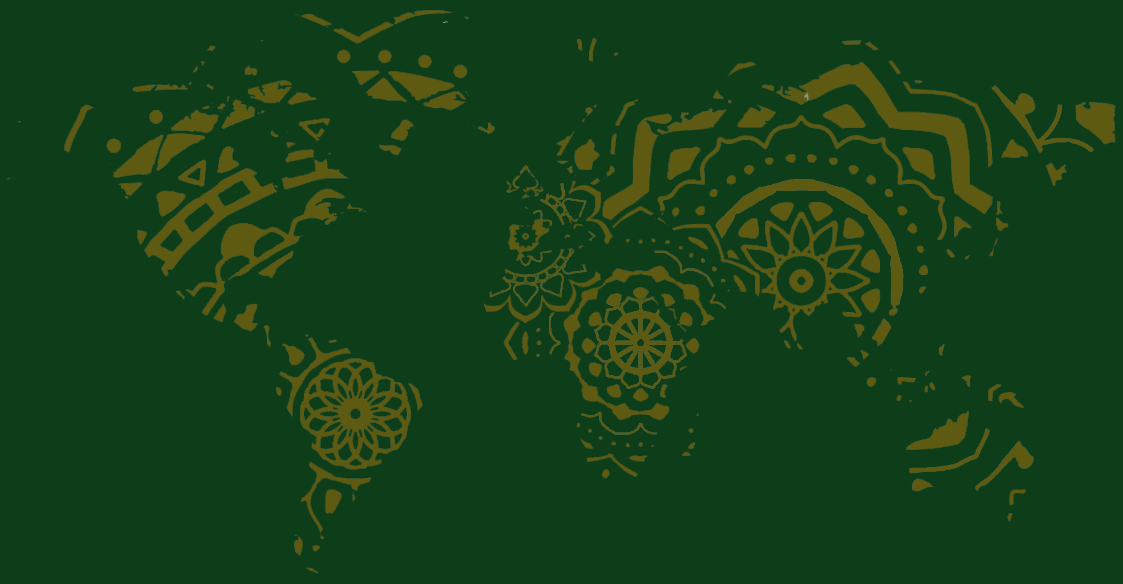
VISCERAL LEISHMANIASIS

POTENTIAL FOR CONTROL AND ELIMINATION



EPKE ANNELIE LE RUTTE

Visceral Leishmaniasis
Potential for Control and Elimination

Viscerale leishmaniasis

Mogelijkheden voor bestrijding en eliminatie

Epke Annelie Le Rutte

Visceral leishmaniasis: Potential for Control and Elimination

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Visceral Leishmaniasis
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Panta rhei

(Heraclitus, 540 - 480 B. C.)

Contents

CHAPTER 1

General introduction

1.1 Introduction to visceral leishmaniasis (VL)

The *Leishmania* genus represents one-celled parasites that are transmitted between vertebrate hosts by the bite of female sandflies. Thus far, 53 *Leishmania* species have been described, of which 31 are known to be parasites of mammals and of which 20 are pathogenic for human beings. [1] *Leishmania* parasites cause four clinical forms of disease in humans referred to as visceral, cutaneous, diffuse cutaneous, and muco-cutaneous leishmaniasis. This thesis focuses on visceral leishmaniasis (VL), which is also known as kala-azar, black fever, Dumdum fever, Assam fever, and infantile splenomegaly in various parts of the world. *Leishmania donovani* and *L. infantum* are the agents responsible for Old World VL, whereas *L. chagasi* (similar to *L. infantum*) is responsible for VL in the New World. [2] Globally around 200 million people are at risk of developing VL, mainly affecting the poorest of the poor in rural areas of tropical regions, resulting worldwide in an estimated 50,000 to 90,000 new cases of VL and 20,000 to 30,000 deaths each year. [3–6]

Approximately 166 of the more than 800 recognized sandfly species are thought to be a vector for the transmission of *Leishmania*, of which 31 in the transmission of *L. infantum* and 9 in the transmission of *L. donovani.* [2] Sandflies belonging to either *Phlebotomus spp*. (Old World) or *Lutzomyia spp*. (New World) are the primary vectors. Sandflies are tiny insects of about 1.5–3.5 mm in length, with a hairy appearance, large black eyes, long, stilt-like legs and an average lifespan of about 14 days. [7, 8] The sandflies are generally most active during twilight, evening, and nighttime hours (from dusk to dawn). It is only the female sandflies that drink blood in need of proteins to produce eggs, while male sandflies feed solely on plant sugars. [9]

Leishmania parasites are unicellular eukaryotes, with cell organelles including kinetoplasts and flagella, and they are heteroxenous, meaning that they are able to colonize two hosts. Their life cycle takes place in the phagocytes of mammals and in the intestinal tract of sandflies. [2] The parasite has two structural stages: the promastigote (infective stage) and the amastigote stage. Figure 1 shows an image of the promastigote *Leishmania* parasite together with red blood cells, taken by an electron microscope, which was co-developed by W. A. Le Rutte. [10] As presented in Figure 2, the promastigote

gets injected by the sandfly into a mammal, where macrophages and other mononuclear phagocytic cells phagocytize them. Promastigotes transform in these cells into the amastigote stage, where they multiply by simple division and proceed to infect other mononuclear phagocytic cells in the cell tissues of mammals. From there they can infect sandflies when they are ingesting infected cells during blood meals. In the sandflies, the amastigotes transform into the promastigote stage in the gut of the sandfly, before moving to the proboscis. [11] Non-vector transmission (e.g., by accidental laboratory infection, blood transfusion, or organ transplantation) is possible, but rare in humans. [12] However, in dogs, vertical transmission and transmission through blood and semen have been more widely reported. [13–15]

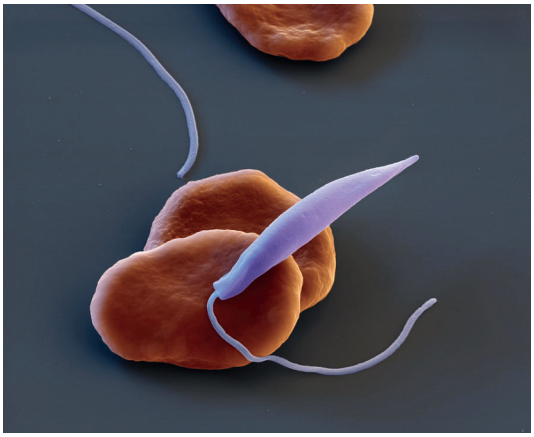

Figure 1. *Leishmania* parasite (purple) and red blood cells (red). Color-enhanced scanning electron micrograph [10] with a magnification of 5,400. *Source: Eye of Science*

Transmission of leishmaniasis can be either anthroponotic (only human and sandfly transmission) or zoonotic (human, animal and sandfly transmission). On the Indian subcontinent (ISC), where VL is caused by *L. donovan*i the infection is considered solely anthroponotic. [1] However, in Bangladesh, *Leishmania* antibodies have been found in domestic cattle and in Nepal *Leishmania* parasites have been found in cattle, buffaloes and goats, but their role as potential reservoir remains unknown. [17, 18] In the rest of the world, *L. infantum* causes zoonotic visceral leishmaniasis (ZVL) affecting mainly humans and dogs. During a large community outbreak in Madrid, hares also played a role as active reservoirs. [19] Foxes, opossums, domestic

cats and black rats have been found able to transmit *L. infantum* to sandflies, but confirmation of these hosts as primary or secondary reservoirs requires further xenodiagnosis studies at the population level. [20]

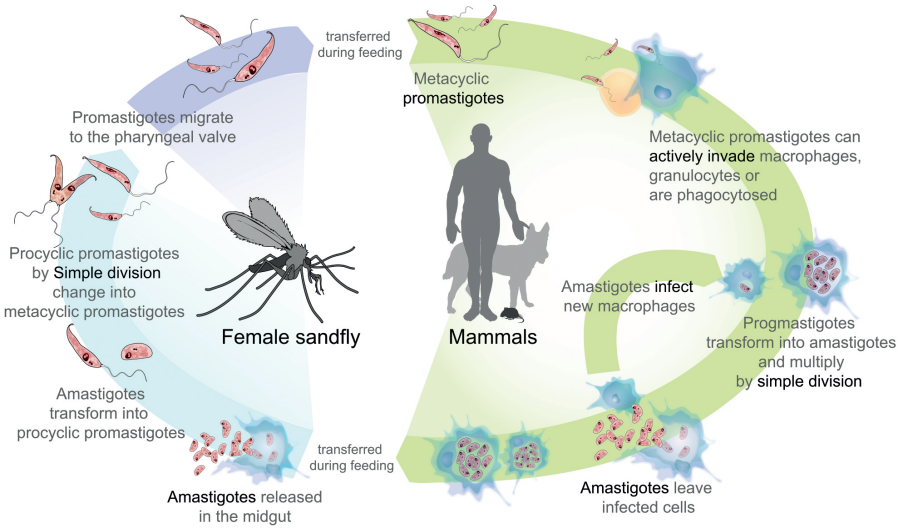

Figure 2. Schematic representation of the transmission cycle of the *Leishmania* parasite between sandflies and mammals. [16]

The first *Leishmania* parasite record was found in Burmese fossil amber, dating 100 million years back, from a *Paleoleishmania proterus* associated with a reptilian blood-filled female sandfly *P. burmitis*. The oldest *L. donovani* infection in humans has been detected in ancient Egyptian and Christian Nubian mummies dating back around 4000 years. [2] In 1903, British medical officer Willam Boog Leishman performed an autopsy on an English soldier who served in West Bengal India, and discovered new protozoan parasites from his enlarged spleen, believing they are trypanosomes. [21] In that same year, British medical officer Charles Donovan, who was serving in the Indian Medical Service and was based in Madras, confirmed that the newly discovered "leishman bodies" were the causative agent of kala-azar. [4] Kala-azar means 'black fever' in Sanskrit, because of the gray discoloration of the skin of hands, feet, abdomen and face in severe infections. [23] Donovan sent some of his slides to Ronald Ross in Liverpool, who correctly identified

the species as member of the novel genus *Leishmania*. He gave the name "Leishman-Donovan bodies", and subsequently *Leishmania donovani*, thereby equally crediting the two discoverers. [24]

Clinical symptoms of human visceral leishmaniasis

When infected with the *Leishmania* parasite, susceptible humans first develop asymptomatic infection, of which the majority recovers without ever developing clinical symptoms. The ratio of asymptomatic to symptomatic VL varies between 50:1 (*L. infantum* in Spain) to 0.6:1 (*L. donovani* in Sudan), depending on the geographic location, type of parasite and the time of outbreak. [25, 26] In this thesis we define asymptomatic infection as being tested positive for the parasite or parasite DNA, but without any sign of clinical symptoms. Well-established risk factors that influence the probability of developing symptoms include severe malnutrition, HIV co-infection, and poverty-related conditions. [27–29] VL is a systemic disease affecting various internal organs. Patients present with relatively non-specific symptoms of prolonged fever, weight loss, hepato-splenomegaly (enlarged liver and spleen, see Figure 3), anorexia, pancytopenia, anemia and wasting. [30] The blackening of the skin, that gave the disease its common name, is reserved for severe (advanced) cases of VL, although the terms kala-azar and visceral leishmaniasis often are used interchangeably. [31] When left untreated, VL is nearly always fatal, making it the world's deadliest parasitic disease after malaria. [5] Of the symptomatic individuals that are diagnosed and treated approximately 90% recover. Months to years after apparent successful treatment, some individuals develop post kala-azar dermal leishmaniasis (PKDL), a skin condition characterized by different clinical presentations from the simple hypo-pigmented macular form to more developed lesions comprising of papular or nodular lesions that appear usually on the face, upper arms and trunk (Figure 4). [32] PKDL is not a life threatening disease, but causes more of a social stigma especially when lesions present on the exposed parts of the body. [33] PKDL is mainly seen in Sudan and India, where it follows after cure in 50% and 5–10% of VL cases, respectively. [32]

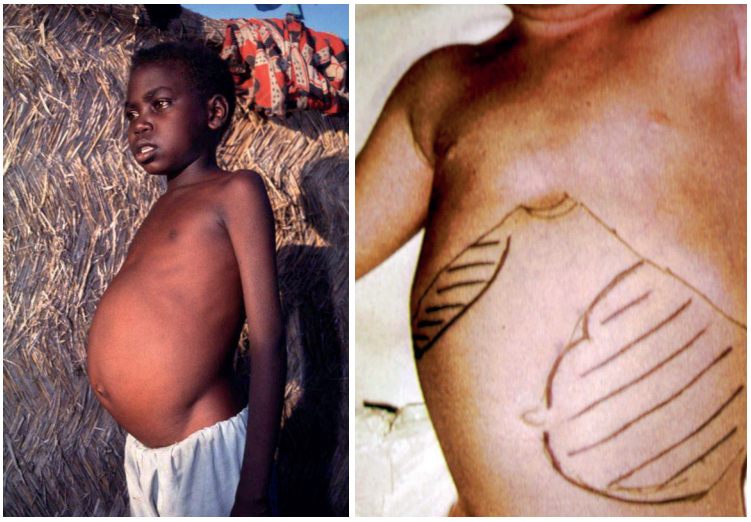

Figure 3. Children with hepato-splenomegaly caused by visceral leishmaniasis. In the right image the size of the liver and spleen are drawn on the skin, and the spleen is massively enlarged. [34]

Figure 4. Post kala-azar dermal leishmaniasis (PKDL). The left image presents papular lesions on the face in eastern Sudan. [32] The right image shows macular lesions on the forearms in Bihar, India. [Picture by author]

Clinical symptoms of canine leishmaniosis (CanL)

About 5-10% of the dogs in endemic regions that are infected with *L. infantum* develop clinical symptoms, the remaining infected dogs experience asymptomatic infection. [35] Asymptomatic dogs are likely to contribute to the transmission dynamics, as they have been found to harbour virulent and infectious *L. infantum* parasites, which they can transmit to the natural vector. [36, 37] The representation of the disease is comparable to that in humans, i.e. nonspecific, which makes it hard to diagnose. Most common clinical signs include anorexia, lymphadenopathy, alopecia, ulcerations, ocular lesions,

epistaxis, poor body condition, muscular atrophy, renal dysfunction and anaemia. CanL is usually also lethal in dogs when left untreated. [35, 38]

Spatio-temporal distribution of VL

VL can be found in more than 60 countries in the world, with a total of 200 million people at risk of becoming infected. However, more than 90% of VL cases occur in seven countries: India, Sudan, South Sudan, Ethiopia, Somalia, Kenya and Brazil. India bears the highest burden of VL cases, with Bihar state contributing 80-90% of the reported cases, here VL is caused solely by *L. donovani*. [3, 6] *L. infantum* affected regions are mainly situated around the Mediterranean Sea, North-East Africa, and Brazil where *L. infantum* is known as *L. chagasi*. [39] Figure 5 shows the distribution of parasites across the globe.

The Portuguese and Spanish colonists introduced *L. infantum* (which was later named *L. chagasi*) in Latin America around 500 years ago, where leishmaniasis became a public health problem, with currently in Brazil more than 3500 human VL cases reported annually. [40, 41]

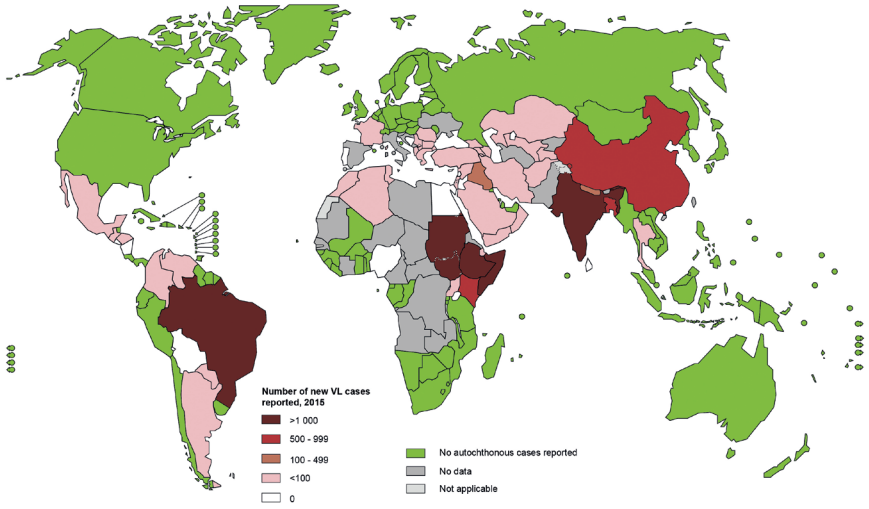

Figure 5. Map of the areas endemic for *Leishmania donovani (Indian subcontinent), L. infantum (East Africa and Europe),* and *L. chagasi (similar* to *L. infantum, South America).* Source: World Health Organization 2015.

The vertebrate hosts are believed to be mostly responsible for local VL spread, since the *Leishmania* parasites can survive in these hosts for long

periods of time, especially compared to the relatively short amount of time spent in sandflies, who only live 14 days on average and have a limited fly range of about 300 meters. [1, 2]

In several parts of Southern Europe the disease prevalence has increased fivefold during the last decade; in 2012 around 1200-2000 human autochthonous VL cases were reported due to an infection with *L. infantum* and an estimated 2.5 million dogs are infected, experiencing clinical or subclinical canine leishmaniosis. [3, 35, 41, 42, 43–47] The parasite distribution used to be solely in the South of Europe, however *Leishmania* endemic regions have been expanding to the North. Currently, both the sandfly and the parasite have reached the Swiss Alps, which were previously considered non-endemic. The same is true for France where both the sandfly and the leishmanial parasite have now crossed the Pyrenees. [35, 43] In Europe, the main drivers of transmission are the increase in sandfly distribution due to climate change and the traveling and migration of dogs, causing an increase in the public health risk of ZVL in Europe. [19, 35, 43, 44] The awareness of veterinarians and physicians of the spread of disease as well as the exact number of reported cases in these regions remains unknown.

The global cumulative trend over time of VL occurence since 1960 is presented in Figure 6, Figure 7 presents the VL incidence in India and Bihar between 1986 and 2016. In contrast to the declining trend of VL incidence on the Indian subcontinent, in Europe the disease incidence has been rising, however hardly any regular up to date figures of this trend and the geographic distribution of cases across all of Europe exist. [3, 45]

Unknown aspects of VL

Because visceral leishmaniasis has long been a neglected disease, there are aspects of the transmission dynamics that remain unknown up to now. For example, it is not well understood if people become immune after having experienced an infection with VL, and how long they would potentially be protected for. It also remains unclear whether individuals fully clear the parasite from their body after having completed successful treatment. In certain cases it seems that the disease re-occurs when the individual experiences a period of immune-suppression, for example due to an infection

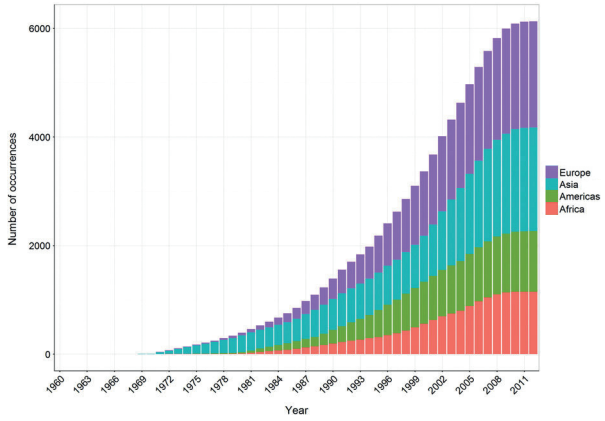

Figure 6. The cumulative number of unique visceral leishmaniasis occurrence records per year, 1960 – 2012) Adapted from [50]

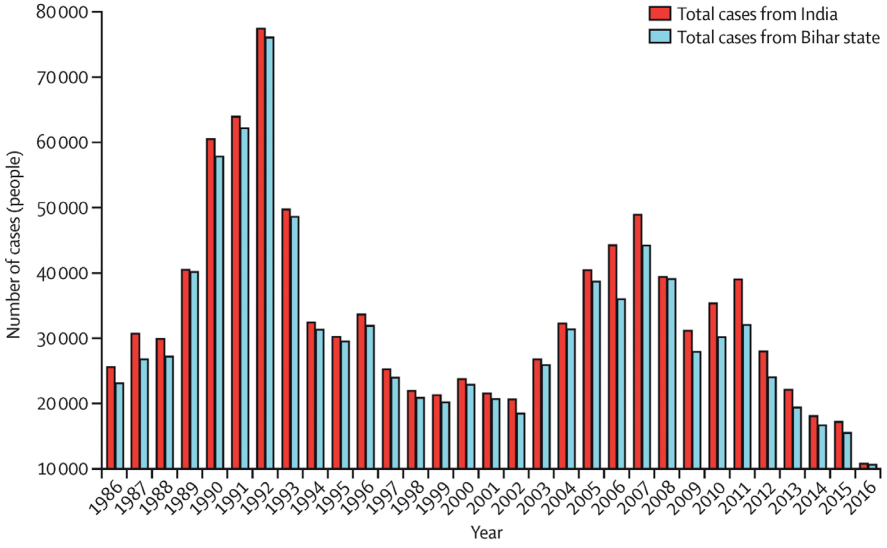

Figure 7. Numbers and trend of visceral leishmaniasis cases reported per year in India and Bihar state (1986 - 2016). Source: adapted from the National Vector-Borne Disease Control Programme, Directorate General of Health Services, Ministry of Health and Family Welfare, New Delhi, Government of India; and World Health Organization. [51]

with HIV or severe malnutrition. [46] The role that individuals with PKDL and individuals with asymptomatic infection play, potentially as a reservoir of infection, also remains undetermined, because the transmission from these individuals to sandflies has not yet been established. [47–49] The duration

of immunity, possible re-occurrence of infection and the disease reservoir are important factors regarding the transmission dynamics. Understanding their role becomes especially critical when trying to control or eliminate the disease.

1.2 Interventions for the control and elimination of VL

Diagnosis and treatment of human VL

Since the clinical features of VL are very similar to other febrile illnesses such as malaria, reliable laboratory methods are required for accurate diagnosis of infection. [52] Parasitological diagnosis remains the gold standard because of its high specificity, with this technique the amastigote forms of the parasite are detected under the microscope in tissue smears from aspirates of lymph nodes, bone marrow or spleen. [52, 53] Parasite DNA detection in the blood is also a good method of diagnosing infection, and is performed by molecular tests such as polymerase chain reaction (PCR) and real-time quantitative PCR (qPCR). To detect human antibodies against the parasite different serology tests are available, which are based on the presence of specific humoral immunity. Serological tests that are frequently used are the direct agglutination test (DAT), enzyme linked immunosorbent assay (ELISA), the indirect fluorescent antibody test (IFAT), and rapid immunochromatographic assays, such as rK39, which is available as a dipstick. [54] The leishmanin skin test (LST) detects late stage recovery with cellular immunity, and can be used as a measure for populations to detect previous exposure to *Leishmania* parasites. [43, 55]

There are different types of treatment in use, depending on the geographic region and other factors such as the presence of a cold-chain and parasite resistance. Treatments have changed over time, and previously used antimony treatments (such as sodium stibogluconate (SSG)) required daily injections for a 30-day period while being hospitalized. This treatment would pose a large burden on the infected often leading to uncompleted treatments, favoring parasite resistance, causing relapse of infection and increasing the risk of developing PKDL. [56, 57] More recently, multiple countries have registered miltefosine, amphotericin B, liposomal amphotericin B and paromomycin for the treatment of VL. [58, 59]

On the Indian subcontinent, registered treatments include single-dose liposomal amphotericin B (AmBisome®), which requires a cold-chain and miltefosine, a 28-day oral treatment but with registered treatment failures in children of up to 33%. [60–62] In Europe the WHO-guidelines suggest that in each country the first-line drug for VL treatment should be liposomal amphotericin B. Alternatively, pentavalent antimonials or amphotericin B can be used. [45]

Human vaccine development has been on-going for decades, and researchers remain hopeful that a vaccine against human VL will become available in the future. [63, 64]

Diagnosis and treatment of canine leishmaniosis (CanL)

Veterinarians use diagnostic tools comparable to those used in humans. With cytology the parasite itself can be detected, and with PCR-tests the parasite's DNA. Serological tests that detect antibodies against CanL are ELISA and IFAT. When a low antibody concentration is found the veterinarian needs to perform additional tests in order to be able to diagnose CanL. [39, 65, 66]

As with human VL, different treatments against CanL are registered which differ per region. In Europe allopurinol, meglumine antimoniate, miltefosine and paromomycin sulfate are available. [67] Since miltefosine is also used for the treatment of human visceral leishmaniasis, mass treatment of dogs is highly discouraged because of the risk of creating a strain that is resistant against miltefosine. [41] The first line treatment for CanL in Europe is maglomine antimonite or miltefosine in combination with allopurinol. Treatment will never clear the parasite from the dog, but clinical cure can be reached. Allopurinol is often prolonged for a couple of months after clinical cure to reduce the parasite load and therefore reduce the infectivity of the dog towards sandflies. [39, 65, 66, 68, 69]

There are three vaccines available of which CaniLeish® is used in Europe, and Leishmune® and Leish-Tec® in Brazil. [35, 39, 70] Vaccination does not prevent, nor protect against infection, but protects against clinical illness and reduces the infectivity of the dog. [69] Another strategy to prevent CanL is the use of domperidone, which enhances the innate cell-mediated immune

response, leading to an increase in the amount of phagocytic cells, which helps the dog fight the parasite and prevent infection. The effect after a 30-day treatment with domperidone is only temporarily, so its use should be repeated regularly during risk season. [35, 71]

Furthermore, veterinarians are supposed to advise owners who's dogs test positive for CanL, that their animal should not travel or be imported/exported to non-endemic regions. [69] In Brazil, the national control program focuses mainly on detection and culling of seropositive dogs. [72] However, this strategy has so far failed to control the disease. [73]

Vector control

Between 1960 and 1970 VL cases disappeared in India due to the collateral benefit of dichlorodi-phenyltrichloroethane (DDT) spraying by the National Malaria Eradication Programme. Withdrawal of DDT spraying in the mid-sixties resulted in building up of vector sandfly populations and since the simmering foci of VL existed, cases started reappearing in the early seventies. Since the outbreak in the 1970s, regular IRS with DDT was undertaken, of which the application method is illustrated in Figure 8. Bangladesh and Nepal opted to use pyrethroids for IRS, because of sandfly resistance to DDT in certain regions, however at that time India chose to continue the use of DDT but has recently changed fully to pyrethroids due to insecticide resistance to DDT. [47, 74] IRS is currently one of the main strategies on the ISC to control VL, but its effect on sandfly density and VL incidence remains debated. [47]

Insecticide treated bed nets (ITN) have shown to provide protection for humans from bites of *Phlebotomus orientalis* in Eastern Africa. [75] An ITN effectiveness trial in two villages along the Rahad River in 1995 showed a reduction in the number of VL cases compared with the control village. However, a more recent, large, paired cluster randomized trial in India and Nepal found no evidence that large scale distribution of long-lasting insecticidal nets provides additional protection against visceral leishmaniasis compared with existing control practice in the Indian subcontinent. [76] In contrast, insecticide impregnation of existing bed-nets in an operational research project in Bangladesh reduced VL incidence by 66.5%. [77] Most likely this has to do with differences in both sandfly and human behavior in

the different regions. Recent data suggests that for example over 88% of VL patients in Bihar, India, sleep outside for 5-8 months (Poché, unpublished data) coinciding with the May-August peak in *P. argentipes* populations.

Figure 8. Indoor-residual spraying (IRS) of insecticide. Photo credit: Rinki Deb, LSTM

Dog collars impregnated with insecticide are proven highly effective in reducing sandfly biting in dogs. [66, 79] A decrease in the risk of developing infection of 54% in dogs and consequently of 43% in children has been found in Iran. [80] Dog collars are fully effective between 1 and 34 weeks of using them, making it a highly recommended strategy in endemic regions. [66, 79]

Strategies

On the Indian subcontinent the elimination strategy combines active case detection (ACD) followed by prompt treatment with indoor residual spraying of insecticide (IRS). [81] In regions with zoonotic VL a One Health approach is essential, in which both the animal reservoir as well as the human cases are addressed. Awareness and cooperation from both veterinarians as well as physicians is crucial for such a strategy to succeed. Preventive vaccination of dogs in Brazil has led to a reduction in the incidence of canine and human VL [70] and dog collars impregnated with insecticide have proven highly effective in reducing sandfly biting in dogs, and therefore also leading to a reduction of VL in humans. [66, 79]

1.3 Targets to control and eliminate VL

In 2012 the WHO grouped 10 diseases as 'Neglected Tropical Diseases' (NTDs). These NTDs consisted of tropical infections that affect mostly the poorest of the poor in rural regions of which VL is a typical example. [82] In 2012, one in seven people on the planet were suffering from NTDs, which comprises the following diseases: schistosomiasis (bilharzia), soil-transmitted helminthes (ascariasis, trichuriasis, hookworm), onchocerciasis (river blindness), lymphatic filariasis (elephantiasis), blinding trachoma, human African trypanosomiasis (HAT), visceral leishmaniasis (VL), leprosy, Chagas' disease and dracunculiasis (Guinea worm). All diseases are helminthic infections, apart from trachoma and leprosy that are caused by bacteria.

WHO defined targets for the control and elimination of these ten NTDs to be achieved before or by 2020. The targets were published in 2012 in "Accelerating work to overcome the global impact of NTDs: a roadmap for implementation", which was followed by updated reports in 2013, 2015 and 2017. Schistosomiasis, STH, Chagas' disease, onchocerciasis and visceral leishmaniasis are targeted for *control*, lymphatic filariasis, leprosy, HAT and blinding trachoma are targeted for *elimination* and Guinea worm for *eradication*. The diseases can be categorized in two groups, the diseases that can be controlled using preventive chemotherapy (PCT; schistosomiasis, STH onchocerciasis, lymphatic filariasis and blinding trachoma) and diseases that require intensified disease management (IDM; HAT, Chagas' disease, leprosy and VL). The 2020 targets for VL are formulated by WHO as: 1) elimination of VL as a public health problem on the Indian subcontinent (ISC) by 2020, which is further defined as 'less than 1 new VL case per 10,000 population at (sub-)district level per year', and 2) 100% detection and treatment of human cases globally. [83–85, 82] The reason for the differing targets is because on the ISC, in contrast to the rest of the world, VL is solely anthroponotic and transmitted by only one vector species (*P. argentipes*), which, together with the focal nature of the disease, allows it to be targeted for elimination.

In 2012 the London Declaration was signed by more than 80 of the world's leading organizations from the public and private sector to endorse the WHO 2020 Roadmap on NTDs. The organizations included multiple pharmaceutical companies, the Bill and Melinda Gates Foundation and the World Bank among

many others. With this declaration they committed to increase research, funding, supplies and awareness to combat VL and the 9 other NTDs. [86, 87]

It would be pertinent to know what the health impact could be if the above-mentioned WHO targets that were endorsed by the London Declaration would be achieved. To quantify such a health impact, the standard unit of health measurement disability-adjusted life years (DALYs) can be used. The years lived with disability (YLDs) are a measure of the gap in healthy years of life lived by a population after implementing an intervention as compared with the normal standard without interventions. The YLDs are added to the years of life lost (YLL) in case of premature mortality to arrive at DALYs. [5] In 2015, the global burden of disease study (GBD) estimated that VL was causing a health burden of about 1.4 million DALYs globally (CI: 0.97 to 1.86) mainly consisting of YLLs.

1.4 Mathematical transmission modeling in infectious diseases research

Mathematical models quantify and simulate infectious disease dynamics by providing a framework for the biology of the disease and the underlying transmission dynamics. Interventions can be incorporated in the model, targeting different components of the disease transmission process. By quantifying the health impact of these interventions, transmission models have proven to be useful tools in aiding policy related decision-making. Mathematical models have widely been considered fundamental to understanding the dynamics of infections and populations as well as planning and assessing the efficacy of interventions by evaluating the intensity and timescale to achieve certain elimination targets and optimize elimination strategies. [88–90]

Types of transmission models

There are different types of mathematical transmission models, of which deterministic and individual-based models (IBM) are highlighted here. In deterministic compartmental models, fractions of an infinitely divisible population move between compartments or states at certain fixed rates. In IBMs, individuals are modeled separately to incorporate individual heterogeneity, and probability distributions are used instead of fixed

rates. However, IBMs are more computational intensive and included heterogeneities heavily depend on detailed and elaborate datasets, which are not always available. Both deterministic as well as IBMs are widely used in the field of infectious disease control. However, in the field of VL, mathematical models are limited, which is mostly due to the lack of a long and rich history of research as well as scarcity of data.

1.5 Data

Ideal data to model the dynamics of a vector-borne infection such as VL would consist of longitudinal datasets of disease incidence or prevalence, before and during interventions, preferably at the individual level (including data on age, sex and particular diagnostic outcomes). Vector data alongside human data from the same geographic location are essential for a transmission model to reflect accurate vector dynamics, which is especially important when interventions include vector control. When certain aspects of the disease or vector dynamics remain unknown, models can estimate such values by fitting to data.

Existing epidemiological VL datasets

The KalaNet study was designed to test the effect of insecticide-treated bed nets on VL incidence amongst 21,267 individuals in Bihar, India, and Nepal between 2006 and 2009. Sex and age-structured data on DAT and PCR prevalence, conversion and VL incidence were collected as well as infection prevalence in the sandfly population in Nepal. In this large trial the villages with bed nets and the control villages without bed nets showed a similar decline in VL incidence over time, and therefore no benefits of the use of bed nets could be determined based on this study. [76].

Between January 2012 and June 2013 non-governmental organization CARE India collected data from 6,081 VL cases in 8 (high and low) endemic districts of Bihar. The effort entailed a rapid situational assessment of VL incidence and was part of the intervention program funded by the Bill and Melinda Gates Foundation (BMGF) to gain insight in the functioning of the kala-azar elimination programme in Bihar. VL patients, whose date of diagnosis was within the reference period, reported by the state-run health facilities (block and district hospitals), were compiled and individuals were

traced and interviewed. The collected individual-level data included date of onset of symptoms, times from onset of symptoms to treatment, occurrence of a relapse, PKDL, and details of IRS spraying in the patient's house and neighborhood.

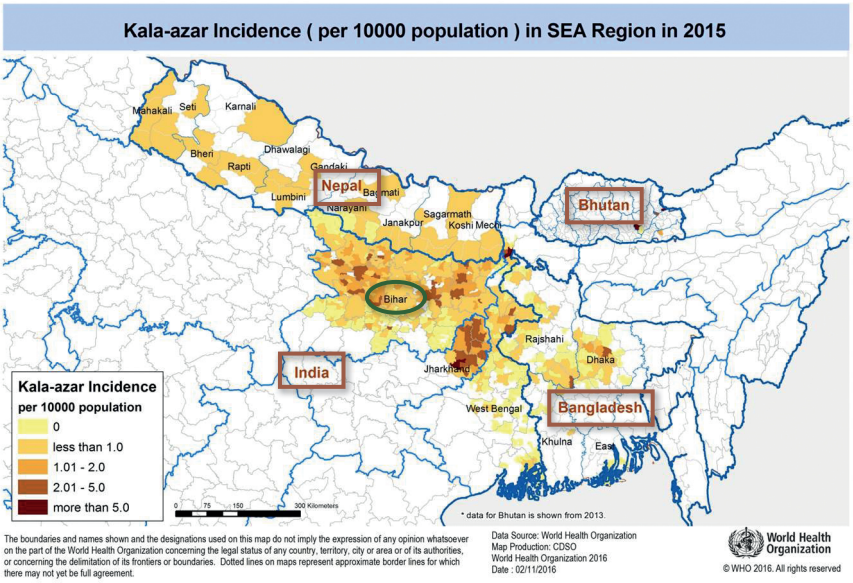

Figure 9. Kala-azar (VL) incidence per 10,000 people in India (Bihar in green circle), Nepal, Bangladesh and Bhutan in 2015. Source: WHO 2016

Countrywide data regarding VL and CanL in Europe are often irregular; focus on small study sites and often only on VL or CanL separately, even though the WHO guidelines suggest that reliable reporting and information systems are to be set up to monitor the incidence of VL and CanL. [3, 45]

1.6 Aim and research questions

Over the past years there has been a steep increase in awareness of VL; many large-scale interventions are being implemented and targets for control and elimination have been set. In this thesis the potential of reaching these targets will be explored. To achieve this, the following research questions will be addressed:

1) What is the global health impact when achieving WHOs' targets for disease elimination and control of 9 neglected tropical diseases, and in particular visceral leishmaniasis?
2) Are veterinarians in the endemic regions of Spain and France aware of the spread of zoonotic VL in Europe and do they implement the guidelines to control the disease?
3) What insights can transmission models provide regarding the feasibility of achieving the VL elimination targets on the Indian subcontinent?

1.7 Outline of this thesis

In **Chapter 2** we address research question 1 by calculating the global health impact for the ideal situation of reaching the 2020 control and elimination targets for VL and 8 other NTDs of the London Declaration. To answer research question 2, we developed an online survey for veterinarians in Spain and France to test their awareness and implementation of international intervention guidelines to control the spread of zoonotic VL in Europe, the results of which we present in **Chapter 3**. In **Chapter 4**, we present a literature review providing an overview of existing VL transmission models and parameter values to understand and quantify the disease dynamics of VL. For **Chapter 5** we develop three new VL transmission models, each with a different human reservoir of infection, which we fitted to the KalaNet dataset. With these models we quantify the effect of interventions to estimate the feasibility of achieving the WHO elimination targets on the Indian subcontinent. In **Chapter 6** we use the most plausible model from chapter 5, in which mostly asymptomatics contribute to transmission, and introduce a fourth model in which solely symptomatic individuals contribute to transmission. These models are fitted to both the KalaNet as well as the CARE dataset. The models' predictions are compared to those of another VL transmission model, to provide more robust forecasts on reaching the elimination targets on the ISC. In **Chapter 7** we draw the main policy relevant recommendations from previously published VL modeling papers and present the effect of current and alternative WHO guidelines on VL incidence to aid in prioritizing resources for control of VL on the Indian subcontinent. In **Chapter 8** we discuss the answers to the research questions, estimate the frequency and distribution of the reservoir of canine leishmaniasis in Spain

and France, present a conceptual structure for a zoonotic VL transmission model, and use our VL transmission model to explore the potential impact of human vaccines. We formulate a final conclusion and provide recommendations for future visceral leishmaniasis policy and research.

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CHAPTER 2

Concerted Efforts to Control or Eliminate Neglected Tropical Diseases: How Much Health Will Be Gained?

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The open-access web- based dissemination tool (https://erasmusmcmgz. shinyapps. io/dissemination/) contains all underlying GDB data, intermediate values, assumptions about WHO targets, and country-specific results.

Abstract

Background: The London Declaration (2012) was formulated to support and focus the control and elimination of ten neglected tropical diseases (NTDs), with targets for 2020 as formulated by the WHO Roadmap. Five NTDs (lymphatic filariasis, onchocerciasis, schistosomiasis, soil-transmitted helminths and trachoma) are to be controlled by preventive chemotherapy (PCT), and four (Chagas' disease, human African trypanosomiasis, leprosy and visceral leishmaniasis) by innovative and intensified disease management (IDM). Guinea worm, virtually eradicated, is not considered here. We aim to estimate the global health impact of meeting these targets in terms of averted morbidity, mortality, and disability adjusted life years (DALYs).

Methods: The Global Burden of Disease (GBD) 2010 study provides prevalence and burden estimates for all nine NTDs in 1990 and 2010, by country, age and sex, which were taken as the basis for our calculations. Estimates for other years were obtained by interpolating between 1990 (or the start-year of large-scale control efforts) and 2010, and further extrapolating until 2030, such that the 2020 targets were met. The NTD disease manifestations considered in the GBD study were analyzed as either reversible or irreversible. Health impacts were assessed by comparing the results of achieving the targets with the counterfactual, construed as the health burden had the 1990 (or 2010 if higher) situation continued unabated.

Principle Findings/Conclusions: Our calculations show that meeting the targets will lead to about 600 million averted DALYs in the period 2011–2030, nearly equally distributed between PCT and IDM-NTDs, with the health gain amongst PCT-NTDs mostly (96%) due to averted disability and amongst IDM-NTDs largely (95%) from averted mortality. These health gains include about 150 million averted irreversible disease manifestations (e.g. blindness) and 5 million averted deaths. Control of soil-transmitted helminths accounts for one third of all averted DALYs. We conclude that the projected health impact of the London Declaration justifies the required efforts.

Author Summary

Neglected tropical diseases (NTDs) are a group of infectious diseases that occur mostly in poor, warm countries. NTDs are caused by various bacteria and parasites, such as worms. They can either be cured or prevented through drugs and other interventions, such as control of insects that spread the infection. The London Declaration is a statement by various organizations, including the World Health Organization (WHO) and pharmaceutical companies that donate the necessary drugs. The declaration endorses targets for disease reductions by 2020, as recently formulated in the WHO Roadmap, to be achieved by rigorous application of available interventions. We explore how much health can be gained if these targets are indeed achieved. We estimate that in such case 5 million deaths can be averted before 2030 and also that huge reductions in ill health and disability can be realized. Over the period 2011–2030, a total health gain would be accomplished of about 600 million disability adjusted life years (DALYs) averted. DALYs are a measure of disease burden, consisting of life years lost and years lived with disability. This enormous health gain seems to justify similar investments as for e.g. HIV or malaria control.

Introduction

Neglected tropical diseases (NTDs) are considered a special category of infectious diseases, distinct from the major killers HIV, tuberculosis and malaria, which have been the main focus of attention and funding for developing countries over the past decades. NTDs are largely confined to (sub)tropical resource-constrained regions, where they cause substantial morbidity, disability and even mortality, as documented by the recent Global Burden of Disease (GBD) estimates [1–4], and consequently have high socioeconomic impact [5, 6]. Most NTDs are either curable or preventable, but in practice there exist major barriers to the effective implementation of control. Fortunately, international commitment to NTD control has rapidly increased in recent years. In 2012, the World Health Organization (WHO) formulated a 'Roadmap' towards ambitious control and elimination targets [7]. By endorsing the London Declaration on NTDs, several private and public

sector organizations committed to meet those targets [8]. For five NTDs—lymphatic filariasis (LF), onchocerciasis, schistosomiasis, soil-transmitted helminths (STH) and trachoma—the primary control strategy is preventive chemotherapy (PCT). For four other NTDs—Chagas' disease, human African trypanosomiasis (HAT), leprosy and visceral leishmaniasis (VL)–control programs rely on case detection with innovative and intensified disease management (IDM), sometimes in combination with other measures such as vector control. Guinea worm (dracunculiasis) is confined to just a few residual foci in Africa and close to being eradicated. For LF, trachoma, HAT and leprosy the target is elimination by 2020, and for the others it is currently control [7, 9].

The London Declaration was formulated to accelerate progress towards the WHO Roadmap targets by sustaining or expanding existing drug donation initiatives; providing funding to support NTD programs, strengthen drug distribution, and research and development; and enhancing collaboration and coordination on NTDs at (inter)national levels [8]. To further motivate and justify these efforts it is important to know their expected health gains. We therefore aim to estimate the global health impact of meeting the WHO Roadmap targets in terms of averted morbidity and mortality, expressed in years lived with disability (YLD), years of life lost (YLL), and disability adjusted life years (DALYs). YLD reflects the number of prevalent cases of each considered disease manifestation multiplied by a disease-specific disability weight between 0 (perfect health) and 1 (equivalent to death), whereas YLL reflects the number of deaths times a standard life expectancy at the age of death in years. The number of DALYs is the sum of both measures (DALYs = YLD + YLL).

Methods

Data sources

Two datasets were used in our calculations. First, the GBD 2010 estimates regarding NTDs were made available to us by the Institute for Health Metrics and Evaluation (IHME), Seattle, USA [3, 10]. Second, UNPOP demographic data and projections were obtained from the website of United Nations Department of Economic and Social affairs [11]. The GBD-2010 data consist

of three burden estimates: prevalent cases, years lived with disability (YLD) and years of life lost (YLL). These estimates were available for 1990 and 2010, per country, age group and sex. Prevalent cases were provided per disease manifestation (sequela), whereas YLD and YLL were only provided as totals per NTD. Table 1 gives an overview of all 31 sequelae considered in the GBD calculations for the London Declaration NTDs. Guinea worm was not included in the GBD study and is therefore not considered here. For STH, burden estimates were available for ascariasis, hookworm disease and trichuriasis separately. Background documents justifying and describing the underlying assumptions of the GBD estimates, including disability weights, were also kindly made available to us. GBD estimates were structured according to the following age groups: 0–6 days, 7–27 days, 28–364 days, 1–4 years, 5–9 years, . . ., 75–79 years, and 80+ years. We combined the four youngest age groups into a 0–4 years group. For irreversible sequelae (see below), the number of prevalent cases was redistributed into 1-year age groups, using a smoothing method that minimizes the squared differences between successive years, under the constraint that 5-years totals equal the available data. The demographic data were already available in 1-year age groups.

General approach

The GBD estimates of the number of prevalent cases for all 31 sequelae and 5 causes of death (HAT, VL, STH-ascariasis, Chagas' disease and schistosomiasis) in 1990 and 2010 were taken as the basis for our calculations. Estimates for other years were obtained by interpolating between 1990 and 2010, and further extrapolated until 2030, under the assumption that the 2020 WHO Roadmap targets were met and sustained beyond 2020. Health impacts were assessed by comparing the results of achieving the targets with the counterfactual, construed as the health burden had the 1990 situation continued unabated. Prevalent cases (both remaining and counterfactual) were translated to YLD and YLL, and summed to arrive at DALYs. The health impact of reaching the targets was expressed as DALYs averted over the decades 2011– 2020 and 2021–2030.

All calculations were carried out in duplicate in Microsoft Excel, and verified using R. All results (totals and country-specific values), underlying calculations and assumptions are available as an open-access web-based

Table 1. The 31 sequelae (categorized as either reversible or irreversible) and associated mortality in the Global Burden of Disease 2010 study for the ten London Declaration NTDs, except Guinea worm. The bold numbers reflect the years lived with disability (YLD) and years of life lost (YLL) for each NTD in 2010, as estimated by the GBD 2010 study [1–4]. The excess mortality rate (μ*) was chosen to reflect the severity of the sequela. The average disability weights were used to relate YLD to prevalent cases in our calculations for NTDs with multiple sequelae. Salomon et al. [24] provide more information about disability weights and lay explanations of sequelae. (a) The original GBD value for LF was 2.74 million YLD, but as Cambodia, Federated States of Micronesia, Maldives, Samoa, Sri Lanka, Togo, Tonga, Vanuatu, and Vietnam had reached elimination before 2010, their remaining burden (total of 0.04 million YLD) was removed from our calculations. (b) The GBD values for leprosy were based on a recalculation; see methods section. (c) A disability weight (DW = 0.097) for visceral leishmaniasis was needed to distinguish it from cutaneous leishmaniasis (DW = 0.013), as both were combined as leishmaniasis in the YLD values available from GBD; YLL due to leishmaniasis was assumed to be fully caused by visceral leishmaniasis.

NTD (YLD and YLL in millions from the Global Burden of Disease 2010 study)			
Sequela	**Reversible/ Irreversible**	**Excess mortality rate (μ*)**	**Average disability weight**
Lymphatic filariasis (YLD: 2.70)[a]			
Lymphedema	Irreversible	0.0	0.110
Hydrocele due to lymphatic filariasis	Irreversible	0.0	0.097
Onchocerciasis (YLD: 0.49)			
Skin disease due to onchocerciasis	Reversible	NA	0.079
Vision loss due to onchocerciasis	Irreversible	0.05	0.101
Schistosomiasis (YLD: 2.99, YLL: 0.32)			
Schistosomiasis (i.e. symptomatic infection)	Reversible	NA	0.005
Mild diarrhea due to schistosomiasis	Reversible	NA	0.061
Anemia due to schistosomiasis	Reversible	NA	0.036
Hepatomegaly due to schistosomiasis	Reversible	NA	0.012
Hematemesis due to schistosomiasis	Irreversible	0.05	0.323
Ascites due to schistosomiasis	Irreversible	0.05	0.123
Dysuria due to schistosomiasis	Reversible	NA	0.012
Bladder pathology due to schistosomiasis	Irreversible	0.05	0.012
Hydronephrosis due to schistosomiasis	Reversible	NA	0.012
STH—Ascariasis (YLD: 1.11, YLL: 0.20)			
Ascariasis infestation	Reversible	NA	0.030
Severe wasting due to ascariasis	Reversible	NA	0.127
Mild abdominopelvic problems due to ascariasis	Reversible	NA	0.012
STH—Hookworm disease (YLD: 3.19)			
Hookworm infestation	Reversible	NA	0.030
Severe wasting due to hookworm disease	Reversible	NA	0.127
Mild abdominopelvic problems due to hookworm disease	Reversible	NA	0.012
Anemia due to hookworm disease	Reversible	NA	0.032
STH—Trichuriasis (YLD: 0.64)			
Trichuriasis infestation	Reversible	NA	0.030
Severe wasting due to trichuriasis	Reversible	NA	0.127
Mild abdominopelvic problems due to trichuriasis	Reversible	NA	0.012

Table 1. Continued

NTD (YLD and YLL in millions from the Global Burden of Disease 2010 study)			
Sequela	**Reversible/ Irreversible**	**Excess mortality rate (μ*)**	**Average disability weight**
Trachoma (YLD: 0.33)			
Trachoma	Irreversible	0.05	-
Chagas' disease (YLD: 0.31, YLL: 0.24)			
Acute Chagas' disease	Reversible	NA	0.053
Chronic heart disease due to Chagas' disease	Irreversible	0.10	0.078
Chronic digestive disease due to Chagas' disease	Irreversible	0.0	0.078
Heart failure due to Chagas' disease	Irreversible	0.10	0.139
Human African trypanosomiasis (YLD: 0.01, YLL: 0.55)			
African trypanosomiasis	Reversible	NA	-
Leprosy (YLD: 0.04)[b]			
Disfigurement due to leprosy	Irreversible	0.0	-
Visceral leishmaniasis (YLD: 0.01, YLL: 3.19)			
Visceral leishmaniasis	Reversible	NA	0.097[c]

dissemination tool (https://erasmusmcmgz.shinyapps.io/dissemination/). A detailed step-wise explanation of our methodology is given below.

Trends for reversible and irreversible sequelae

Sequelae were first categorized as either reversible or irreversible (Table 1), depending on whether treatment of the underlying infection would remove the sequelae in a relatively short time, say, within a couple of years at most. For all reversible sequelae, interventions were considered to affect their prevalence, while for irreversible sequelae this was their incidence. Linear interpolation (at the log-scale for irreversible sequelae) was carried out between 1990 (or the start-year of large-scale control efforts) and 2010 for prevalence rates (i.e. the number of prevalent cases divided by population size) per sequela, country, age group and sex. Absolute numbers were then calculated from these interpolated prevalence rates, using the demographic UNPOP data. For 2020 (and beyond), WHO Roadmap targets were interpreted in terms of prevalence (for reversible sequelae) or incidence (for irreversible sequelae) levels, based on discussions with—mostly WHO—disease experts (Table 2). Trends in incidence and prevalence during the intervening years (usually 2010–2020) were obtained through linear interpolation between the 2010 levels (GBD data) and the interpreted targets. We then translated the calculated trends into absolute numbers of remaining cases using

UNPOP projections for the period 2011–2030, and compared this with the counterfactual situation of no additional control efforts, to assess the impact of meeting the targets. The counterfactual was construed as the health burden that would have been expected had the 1990 epidemiological situation (i.e. disease incidence or prevalence) continued unabated. Whenever the 2010 prevalence of a sequela exceeded that of 1990, we took 2010 as the counterfactual.

Incidence and prevalence calculations for irreversible sequelae

Interpolation for irreversible sequelae, such as blindness as a result of onchocerciasis, was carried out at the level of incidence, because even after elimination of infection these sequelae will persist until the death of the last patient. The annual incidence density λ(a,t) at age a and calendar time t of an irreversible condition is given by the following equation

$$\frac{\partial s(a,t)}{\partial a} + \frac{\partial s(a,t)}{\partial t} = -\lambda(a,t) \cdot s(a,t) + [1 - s(a,t)] \cdot \mu(a,t) \cdot s(a,t)$$

where $s(a, t)$ denotes the susceptible fraction (i.e. 1 –prevalence) of the population and $\mu(a, t)$ the excess mortality rate among those affected. In a stable endemic situation (i.e. without cohort effect, thus $\partial s(a, t) \partial t = 0$) and without excess mortality (i.e. $\mu(a, t) = 0$), $\lambda(a,t)$ can be obtained from a single crosssectional survey by taking the differences in the logarithmic age profile of the fraction susceptible. However, because the cross-sectional age profiles of GBD 1990 and 2010 for each sequela differed, we annualized the differences (on a logarithmic scale) in these profiles to obtain an estimate for $\partial s(a, t) \partial t$. We further assumed the excess mortality rate to be independent of age and calendar time, and have a pre-set value $\mu^* = 0.0$, 0.05 or 0.10, dependent on the severity of the sequela (Table 1). The value of μ^* was chosen after consultation of the disease experts and crudely reflected the mortality rates as used in the GBD calculations. The resulting incidences were calculated back to prevalences (of remaining cases) by 'exposing' cohorts to the derived age and time-specific incidence densities and excess mortality rates.

Morbidity calculations: Years living with disability (YLD)

Predicted prevalent cases for each sequela were then translated to YLD, using two matrices of multiplication factors (one for the year 1990 and one

for 2010) that we had derived from the GBD data as follows. Whenever an NTD had one sequela (e.g. trachoma), the GBD YLDs in 1990 and 2010 were divided by the number of prevalent cases in the same year to arrive at country, age and sex-specific multiplication factors that capture disability weights, the underlying case-mix (e.g. severe vs. mild disability, where applicable), and correction of burden estimates for co-morbidity, as used in the GBD 2010 study [2]. For NTDs with multiple sequelae (e.g. onchocerciasis) we followed the same procedure, but using a weight for each sequela based on an estimate of the average disability weight using GBD documentation (Table 1), because the YLD data provided by the GBD study did not separate the contributions of different sequelae. We treated all multiplication factors as constants. Remaining cases after 2010 were multiplied by the factors in the 2010-matrix, and for 1990–2010 an interpolation of the multiplication factors in both matrices was used. For counterfactual cases we used the multiplication factors in the 1990-matrix, or both matrices when 2010 was used as counterfactual (i.e. similar to the approach for remaining cases).

Mortality calculations: Years of life lost (YLL)

Regarding our mortality calculations, we first translated GBD YLLs in 1990 and 2010 to actual country, age, and sex cause-specific mortality rates, using the age and sex-specific residual life expectancies as applied in the GBD study [1]. For HAT, VL and ascariasis, where mortality is closely linked (in time) to infection prevalence, these rates were treated as prevalent cases (of reversible sequelae) as described above and back-calculated to YLLs. For Chagas' disease and schistosomiasis, where mortality is closely linked to late sequelae, we followed a different procedure. Similar to the calculation of YLDs for NTDs with multiple sequelae, we related YLLs in 1990 and 2010 to prevalent cases of selected sequelae, using a weight representing their lethality. For schistosomiasis, mortality was related to hematemesis (weight = 50), ascites (1.0) and schistosomiasis infestation (0.01). For Chagas' disease, these were heart failure (10) and chronic heart disease (1.0).

Table 2. Interpretations of WHO Roadmap targets [7] as used in our calculations. All country-specific assumptions for each NTD are provided here: https://erasmusmcmgz.shinyapps.io/dissemination/.

Lymphatic filariasis	**Target:** By 2020, 70% of countries will have been verified as free of transmission and 30% will have entered post-intervention surveillance.
	Interpretation: The PCT database [25] provides start and end years of the intervention program per country. The incidence of both chronic manifestations (lymphedema and hydrocele) is assumed to linearly decrease to zero, one year before the anticipated last treatment round in each country.
Onchocerciasis	**Target:** To eliminate onchocerciasis where feasible (without a specified target year).
	Interpretation: Interventions will continue until the end year of interventions as estimated by APOC [26]; prevalence of skin disease and incidence of vision loss reach zero two years before the end year of interventions.
Schistosomiasis	**Target:** Elimination of transmission in certain regions and countries by 2015 or 2020. Global elimination in 2025 as a public health problem. In 2020, 75% national coverage is reached in all the countries requiring preventive chemotherapy for schistosomiasis.
	Interpretation: Global elimination in 2025, therefore in all countries prevalence of reversible and incidence of irreversible sequelae will go down to zero in 2025. The general start year of interventions is 2001, the same year as the W,HA resolution on STH and schistosomiasis [27].
STH (ascariasis, hookworm disease and trichuriasis)	**Target:** 100% of countries requiring preventive chemotherapy for STH have achieved 75% national mass drug administration coverage of school-aged children (SAC) and pre-SAC by 2020.
	Interpretation: The pre-SAC and SAC (ages 5–14) will have 0% prevalence of morbidity by 2025. There will be 10% remaining prevalence of morbidity in the non-treated groups (0–4 and 15+) by the year 2025, relative to the STH level in 2010. Mortality due to ascariasis will be 0% in 2025, for all age groups. The general starting year of interventions is 2001, the same year as the WHA resolution on STH and schistosomiasis [27].
Trachoma	**Target:** Global elimination as a public health problem in 2020. All countries will have achieved the ultimate intervention goal and be free from blinding trachoma as a public health problem.
	Interpretation: Incidence of vision loss caused by trachoma will go down to zero in country specific years. Three WHO documents [7, 28, 29] provide most start years of intervention and target years of elimination.
Chagas' disease	**Target:** To eliminate transmission through blood transfusion in the America's, Europe and Western Pacificby 2015. To eliminate peri-domiciliary infestation in Latin America by 2020, but surveillance and control of oral transmission and congenital infection need to be sustained.
	Interpretation: For acute Chagas' disease the prevalence will linearly decrease to 10% of the GBD 2010 value in 2020, and remain 10% onwards. This 10% reflects remaining burden due to infections from the sylvatic cycle. For chronic heart disease, chronic digestive disease and heart failure, incidence in 2020 will be 10% of that in 2010, and remain 10% onwards. The GBD data about Chagas' disease only concern countries in Latin America, so no specific assumptions are needed for the rest of the world.
	Note: There are great concerns about the reliability of the GBD figures, as well as the feasibility of the London Declaration and associated WHO targets [30].
HAT	**Target:** Achieve elimination of >90% of foci by 2020. The global number of new cases reported annually for 2020 is <2000.
	Interpretation: There are exactly 2000 cases in 2020, and this number will subsequently decline to 0 in 2030. From 2020 onwards, all remaining cases will be detected and treated, meaning that HAT mortality is zero in 2020 and beyond. The overall number of prevalent cases in 2020 is 2.5% of the level in 2010.

Table 2. Continued

HAT	**Explanation:** In 2010, 9103 people died because of HAT according to YLL data of GBD 2010. By multiplying the number of people that died in 2010 by 3 (the burden before dying is assumed to last on average for three years in the GBD calculations) we arrive at 27,307 prevalent cases in 2010 that will eventually die because of HAT. The total point prevalence of HAT in 2010 is provided by the GBD: 36,863. This means that about 75% (27,307 out of 36,863) of prevalent cases will eventually die, whereas the remaining 25% (9,554 out of 36,863) will survive. The 9,554 surviving prevalence cases multiplied by 2 results in 19,108 new detected and successfully treated cases in 2010 (given the GBD assumption that disease lasts 6 months before treatment). Thus, on a global level there will be a decrease from 19,108 new surviving cases in 2010 down to 2000 new surviving cases in 2020, so roughly a decrease to 10% of the level in 2010. This decrease applies to the number of surviving prevalent cases, which is 25% of the total. This means that the overall number of prevalent cases will go down to 10% times 25% = 2.5% of the level in 2010, as the 75% of cases that eventually die will become 0.
	Note: The number of new detected and treated cases (19,108) and the number of new cases that will die (9,554) adds up to 28,211 new cases in 2010, which is substantially higher than what is known in WHO records [31]: 7139 new reported cases in 2010.
Leprosy	**Target:** Global interruption of transmission by 2020. Reduction of grade 2 disabilities in newly detected cases to below 1/million population at global level by 2020.
	Interpretation: The incidence of disfigurement due to leprosy has decreased in 2020 to 37% of the level in 2010, and will further reduce to 0% in 2030, in order to account for the target of global interruption of transmission by 2020.
	Explanation: According to the 2010 GBD data, the incidence of all newly detected cases was 318,876, of which 6% (19,132) had grade 2 disability [12]. This is 2.7/1 million globally. According to the WHO target, this should be reduced to 1/1 million in 2020, representing a reduction to approximately 37% of the level in 2010, or 7,086 incident cases in 2020.
	Note: See main text for our recalculations to arrive at grade 2 disability prevalences.
Visceral leishmaniasis	**Target:** On the Indian subcontinent (ISC), 1/10,000 new cases at (sub)district level per year by 2020; globally, 100% detection and treatment of VL.
	Interpretation: On ISC there will be a prevalence reduction to 5% of the 2010 situation, which will remain at 5% until 2030. Elsewhere, the prevalence of 2010 will remain unaltered. Morbidity in 2020 will have become 25% (Africa), 0.3% (ISC) and 10% (elsewhere) of the level in 2010, and remain constant thereafter.
	Explanation about prevalence on ISC: WHO reports approximately 20/10,000 new VL cases per year on ISC in 2010. Therefore, the target of 1/10,000 will be a reduction to 5% of the 2010 situation. This 5% will also apply to the prevalent cases.
	Explanation about trends in death: In 2010, 51,485 people died because of VL according to YLL data of GBD 2010. Also, worldwide there were 67,721 prevalent cases, which correspond to 270,884 new cases, given the GBD-assumed 3 month average duration of VL. Thus, in 2010 on average 19% of the people with VL died globally. According to the WHO targets, death due to VL will decrease substantially, but it will not go down to zero, as current treatment is not 100% effective [32]. We assume that in Africa 5% of the people (even though detected and treated) with VL will die in 2020, 1% of the people with VL on the Indian subcontinent, and 2% elsewhere. This means that in Africa the relative number of deaths (and also YLL) will decrease to 5/19 = about 25% of the level in 2010. On the Indian subcontinent this will be 0.05 times 1/ 19 = about 0.3% of the level in 2010. Elsewhere, this will be 2/19 = about 10% of the level in 2010. The regional differences in mortality rates were based on discussions with the disease experts and particularly reflect differences in treatment efficacy and HIV-coinfection.

Special cases

Using the above method, some irreversible sequelae—in particular for Chagas' disease and LF —showed for some countries values of $\lambda(a,t) < 0$, due to unrealistic fast declines in the GBD prevalence estimates between 1990 and 2010. Here, we chose alternative prevalences, but still within the confidence limit (Cl) provided by the GBD study, as follows. We reduced the GBD 1990 'Mean' prevalence to 0.25 'Mean' + 0.75 'Lower Cl', and we increased the GBD 2010 'Mean' prevalence to 0.25 'Mean' + 0.75 'Upper Cl'.

The GBD 2010 estimates for leprosy appeared to be mistakenly based on overall leprosy new case detection (incident cases) instead of prevalence of (irreversible) cases with leprosy grade 2 disability, on which the disability weights are based. We therefore performed a recalculation to arrive at grade 2 disability prevalences as follows. First, we took from the WHO-published global leprosy data for 2010 the proportion of newly detected cases with grade 2 disability, which was 6% [12]. Secondly, prevalence of leprosy cases with grade 2 disability in virtual birth cohorts was accrued at a rate determined by this incidence density, while assuming a steady-state until 1990 and a linear decreasing incidence to 2010. We further assumed that excess mortality due to leprosy is negligible ($\mu^* = 0.0$). These prevalence values constituted the 'GBD data' on which our calculations were based.

Results

Figure 1 shows the global trends in remaining and averted DALYs, distinguished into YLD of reversible and irreversible sequelae and YLL. According to the original GBD 2010 data (dark- colored bars), the health burden of onchocerciasis, STH, Chagas' disease, HAT and VL has clearly decreased over the period 1990 to 2010. For LF, schistosomiasis and leprosy, the absolute burden has increased, but not as fast as would be expected from the counterfactual. Thus, for these NTDs, the relative burden has decreased, when correcting for population growth. Only for trachoma (and in some countries for schistosomiasis), the GBD-estimated burden has increased faster than would be expected from the demographic trends over the period 1990– 2010.

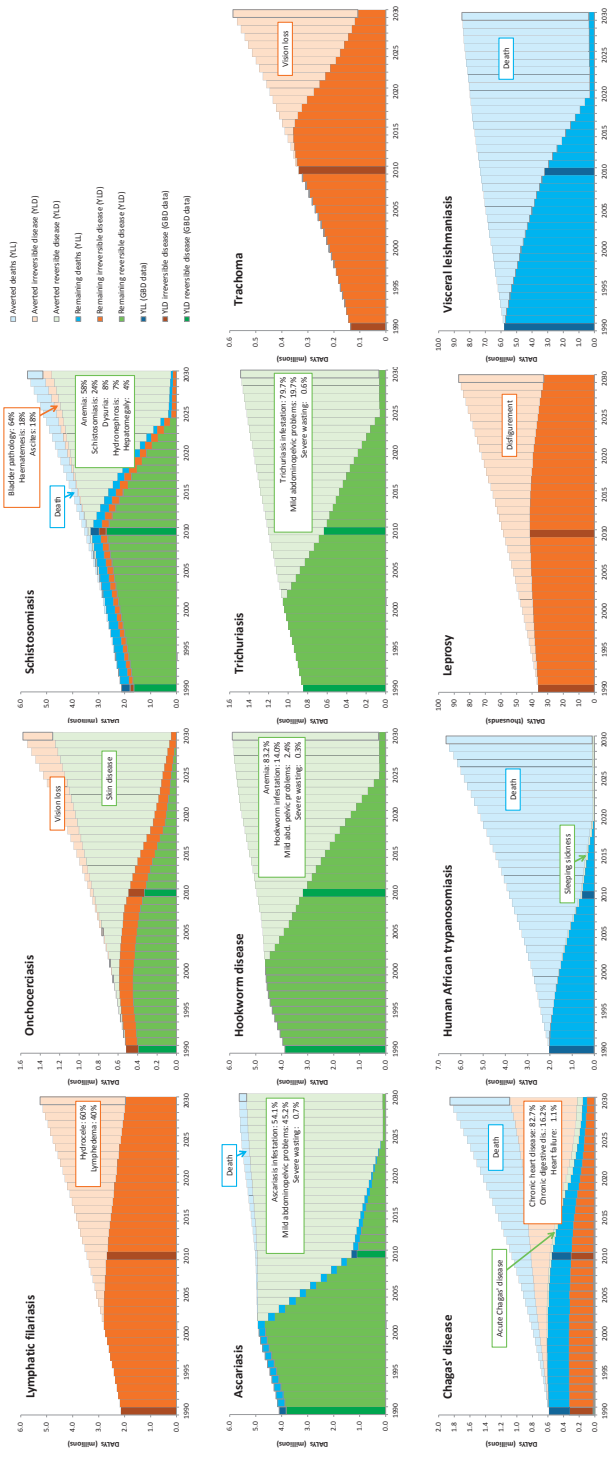

Figure 1. Global trends of remaining and averted disability adjusted life years (DALYs) from 1990 to 2030 for nine NTDs that are part of the London Declaration, with soil-transmitted helminths presented for ascariasis, hookworm disease and trichuriasis separately. DALYs are divided into years of life lost (YLL) and years lived with disability (YLD), with the latter subdivided into reversible and irreversible sequelae. Estimates for remaining DALYs were obtained by interpolating between 1990 (or the start-year of large-scale control efforts) and 2010, and further extrapolated until 2030, such that the 2020 targets of the WHO Roadmap were met. Averted DALYs were assessed by comparing the results of achieving the targets with the counterfactual, construed as the health burden had the 1990 (or 2010 if higher) situation continued unabated. The bars for 1990 and 2010 reflect the Global Burden of Disease (GBD) data [1–4] on which all calculations were based. The boxes indicate the most significant (i.e. visible) sequelae; where multiple sequelae make part of the total of reversible or irreversible disease (YLD) for an NTD, their relative contribution (%) to averted YLD in the period 2011–2030 is indicated. Underlying assumptions and more detailed results, both globally and at the country-level can be found here: https://erasmusmcmgz.shinyapps.io/dissemination/

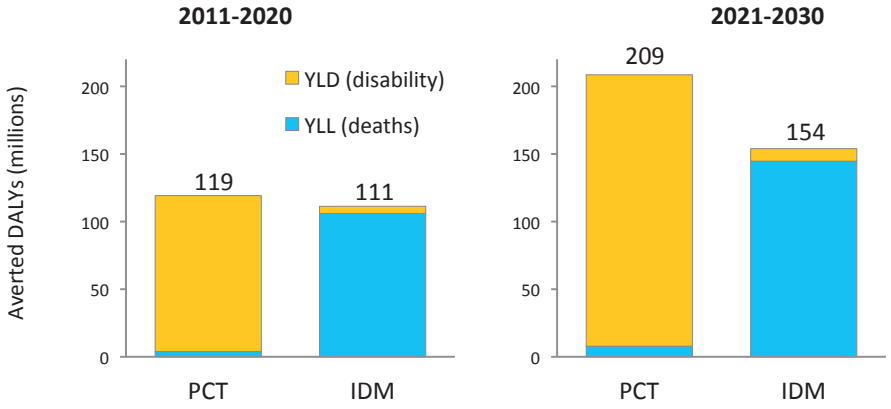

Figure 2. Estimated overall health impact of meeting the WHO Roadmap targets for nine London Declaration NTDs. Five (lymphatic filariasis, onchocerciasis, schistosomiasis, soil-transmitted helminths and trachoma) are to be controlled by preventive chemotherapy (PCT), and four (Chagas' disease, human African trypanosomiasis, leprosy and visceral leishmaniasis) by innovative and intensified disease management (IDM). Values are in millions of averted DALYs per 10-year period, subdivided into years of life lost (YLL) and years lived with disability (YLD).

Table 3. Estimated number of averted new cases of irreversible disease (in millions) when meeting the WHO Roadmap targets for London Declaration NTDs.

NTD	Sequela	2011–2020	2021–2030
Lymphatic filariasis	Lymphedema	8.4	10.3
	Hydrocele due to lymphatic filariasis	12.7	15.0
Onchocerciasis	Vision loss due to onchocerciasis	1.8	3.3
Schistosomiasis	Hematemesis due to schistosomiasis	0.1	0.4
	Ascites due to schistosomiasis	0.3	0.9
	Bladder pathology due to schistosomiasis	10.9	31.9
Trachoma	Trachoma	3.2	6.8
Chagas' disease	Chronic heart disease due to Chagas' disease	14.6	22.7
	Chronic digestive disease due to Chagas' disease	1.3	1.7
	Heart failure due to Chagas' disease	0.1	0.2
Leprosy	Disfigurement due to leprosy	1.0	1.3
Total averted new cases		54.3	94.6

Meeting the 2020 targets will lead to a substantial health-impact for all NTDs (Figure 1). It is clearly visible that reversible sequelae (green) are disappearing faster than irreversible sequelae (brown). This makes the health impact of reaching the targets for LF, trachoma and leprosy over the first two decades somewhat less spectacular compared to that for the other NTDs, of which the burden is mainly caused by reversible sequelae

or death. Another important factor determining the overall health impact is population growth and other demographic developments, as expressed by the counterfactual. NTDs that are prevalent in Asia (LF, STH, leprosy and VL) show a slower rise of the counterfactual compared to the NTDs mainly confined to Africa (onchocerciasis, trachoma and HAT) or South America (Chagas' disease).

Overall, meeting the targets of London Declaration NTDs will avert about 600 million DALYs in the two decades after 2010, nearly equally distributed between PCT and IDM-NTDs, with the former mostly (96%) attributable to averted disability, whereas the latter largely (95%) results from averted premature death (Figure 2). These health gains include about 150 million averted irreversible disease manifestations, in particular chronic heart disease due to Chagas' disease, bladder pathology due to schistosomiasis, and hydrocele and lymphedema due to LF (Table 3). In addition, approximately 5 million deaths are averted, mainly from VL and HAT, and to a lesser extent Chagas' disease (Table 4).

Table 4. Estimated number of averted deaths (in millions) when meeting theWHORoadmap targets for the five London Declaration NTDs with associated mortality.

NTD	2011–2020		2021–2030	
Visceral leishmaniasis	0.99	50.7%	1.36	47.6%
HAT	0.70	35.7%	0.99	34.6%
Chagas' disease	0.16	8.3%	0.29	10.2%
Schistosomiasis	0.08	4.2%	0.18	6.3%
STH—Ascariasis	0.02	1.1%	0.04	1.3%
Total averted deaths	1.96	100.0%	2.87	100.0%

Discussion

Of the 600 million DALYs overall averted in the period 2011–2030, in the ideal situation of meeting the WHO Roadmap targets of London Declaration NTDs, about 30 million will be realized in the year 2020, increasing to 40 million in the year 2030. This is of the same order of magnitude as the current annual health burden of any of the 'big three' infectious diseases, HIV/AIDS, TB and malaria, which accounted for about 80, 50 and 80 million DALYs, respectively, in 2010 [3]. Clearly, for these three infections elimination is a more remote

perspective than for the nine NTDs targeted by the London Declaration. Thus, the ongoing efforts to control the big three seem to justify similar investments in NTD control. In addition, it can be expected that for several of these NTDs control efforts will lead to a cessation of transmission over vast regions, after which further control can be discontinued and investments wound down adding to the value of this investment for future generations.

STH accounts for one-third (34%) of the averted DALYs, almost entirely due to avoided disability. This perhaps surprising finding can be easily explained by the wide-spread distribution of STH [13]. Importantly, approximately half (46%) of the averted STH-burden would be realized in China. This brings to the fore the sensitivity of our results to the choice of counterfactual. That is, our assumption that the situation of 1990 would continue unabated may be questioned for several countries, including China, which have experienced unprecedented economic and social development over the past decades [14]. For example, the health impact for STH would be about halved if the situation in 2010 were used as the counterfactual, as can roughly be concluded from Figure 1, but such a drastic correction would certainly not be reasonable for many endemic countries in Africa and Southern Asia. On the other hand, socioeconomic development may also have facilitated the spread of NTDs, in particular schistosomiasis, of which large outbreaks followed the construction of dams and irrigation schemes [15]. HAT perhaps follows more erratic patterns, reflecting e.g. civil unrest, war and also ecological circumstances [16], so that the year 1990 may not be representative of the actual counterfactual over 1990–2020. Trachoma and schistosomiasis showed large increases in GBD prevalence from 1990 to 2010, which may well reflect an underestimation of the 1990 burden. Consequently, this may have led to underestimating both the counterfactual and the health impact. Another potential source of underestimation of the health impact for some NTDs may be that the largest gains are achieved in the initial years of programs, followed by a slow down towards the target year, as it becomes harder to reach the more marginalized populations. Furthermore, by using a fixed excess mortality rate μ^* for irreversible sequelae (where applicable) we may have somewhat overestimated health impacts for these sequelae as treatment is likely to improve over time. However, since the remaining cases get older at the same time, possibly experiencing a higher mortality, we may have introduced some underestimation as well. Clearly, by using a uniform

methodology we have introduced (perhaps occasionally substantial) under or overestimation of NTD and country-specific results, but we are confident that the overall bias in our estimated health impact of reaching the targets will be small.

Almost half (44%) of the overall health impact is attributable to averted deaths, in particular from visceral leishmaniasis and HAT, and to a lesser extent Chagas' disease, followed by schistosomiasis and STH (ascariasis). In our calculations, we followed the GBD accounting philosophy which assigns all DALYs (i.e. residual life expectancy at the age of death) resulting from a death to the year in which it occurred [1], whereas DALYs attributable to morbidity are accrued during the years that individuals suffer [2]. Moreover, remaining life expectancies were based on the demography of Japan, according to the fundamental concept that all people are entitled to the best life expectancy in the world, irrespective of e.g. country of residence and socioeconomic status. Clearly, other methodologies might have distributed health gains differently over time.

Our calculations depend strongly on the estimates made in the GBD study [1–3]. These estimates are notably uncertain for NTDs, given the paucity of data on their geographic spread and control. Most GBD 1990 and 2010 estimates for NTDs show very wide confidence intervals, often ± 50% the mean, but sometimes with an upper confidence limit up to 5 times the mean. As a consequence, our predictions (all based on GBD point estimates) are subject to at least a similar degree of uncertainty. Also, the GBD disability weights used are still under heavy debate, such as the relatively low value for blindness as compared to itching [17]. Furthermore, our calculations are confined to the 31 sequelae considered in the GBD study, and discussions continue about whether additional sequelae need to be considered. In particular, the choice not to include so-called subtle morbidities, such as impaired cognitive development due to STH and schistosomiasis, or poor mental health from stigma and discrimination due to the disfigurements caused by LF and leprosy, is considered an important omission by many [13, 18–21]. Our results also depend upon the interpretation and formulation of the WHO Roadmap targets [7, 9], which occasionally are ambiguous. Consulting disease experts at WHO has resulted in agreement about interpretations for most NTDs, even though sometimes the targets were considered too general or utopic.

In addition to the intrinsic value of averting human suffering and death, this health impact of reaching the targets will also give rise to major economic and societal improvements, such as increased productivity and avoided (often catastrophic) out of pocket payments for treatment and care, which can be assigned monetary values. In particular, the currently ignored subtle morbidities are likely responsible for major societal impacts.

We realize that the targets are ambitious, and may for instance be jeopardized by challenges in drug distribution, disease surveillance and health care access. Also, systematic non-compliance in mass-drug administration, population groups currently not eligible for treatment, and development of drug or insecticide resistance could be serious threats, as demonstrated in a recent collection of studies by the NTD Modelling Consortium focusing on the question whether we are on track to reaching the goals [22]. Furthermore, even if the targets are reached by 2020 it is essential that control and surveillance are continued to avoid rebounding effects, certainly for those NTDs where elimination of transmission cannot be expected.

In conclusion, NTDs together constitute a major health burden, comparable to any of the three major infectious diseases HIV/AIDS, TB, and malaria. Achieving internationally agreed targets of NTD control and elimination will bring about major gains in health and reductions in human suffering. Much of this will be achieved by avoiding morbidity rather than mortality as many of the parasites involved, such as soil transmitted helminths, rarely kill their hosts. This also implies that our impact assessment depends on the valuation of health states as used by GBD, a valuation that inevitably is somewhat subjective and open to debate. We did not consider the costs involved in reaching these targets, but a recent assessment demonstrated that these are relatively modest [23], indicating that the cost-effectiveness of interventions to control NTDs will likely be high. One thing is certain however: as NTDs are disorders that disproportionately affect the poor, their control will considerably improve global equity.

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Data Availability Statement

The open-access web- based dissemination tool (https://erasmusmcmgz.shinyapps.io/dissemination/) contains all underlying GDB data, intermediate values, assumptions about WHO targets, and country-specific results.

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CHAPTER 3

Awareness and control of canine leishmaniosis in Europe: a survey among Spanish and French veterinarians

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Abstract

Zoonotic visceral leishmaniasis (ZVL) is a parasitic disease affecting dogs and humans that is transmitted by female sandflies. Over the last decade, disease prevalence has increased fivefold in parts of southern Europe, where an estimated 2.5 million dogs are infected. This increase is mainly due to an expansion in sandfly distribution due to climate change and to the greater numbers of dogs travelling between European countries. To combat the spread of ZVL in Europe, international guidelines have been drawn up that describe strategies to prevent, control and monitor ZVL. To investigate whether these strategies are being implemented in the field, we conducted an online survey among veterinarians in Spain (endemic for ZVL) and France (south: emerging; north: non-endemic). Of the 889 respondents, 459 veterinarians completed all questions. Although 60% of all veterinarians were aware of the current ZVL increase in Europe, 70% of this group were not familiar with any guidelines for controlling the disease. Most of their preventive and treatment actions were, however, in line with intervention strategies recommended by the guidelines. From the veterinarians, 76% had received no reports regarding confirmed cases of canine leishmaniosis or visceral leishmaniasis in their region or country. The fact that 88% of confirmed cases of clinical canine leishmaniosis were not reported suggests inadequate disease monitoring and evaluation. We therefore recommend that an easy-to-use and accessible international online network be developed, where both veterinarians and physicians can report confirmed cases of ZVL in dogs and humans. This is crucial for monitoring, controlling and preventing the further spread of ZVL in Europe at a regional, national and international level.

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Highlights

- We conducted an online survey among 459 Spanish and French veterinarians
- Most veterinarians are not aware of guidelines for controlling ZVL in Europe
- Most veterinarians do however apply interventions mentioned in the guidelines
- Confirmed CanL cases are not being reported or registered
- The spread of this emerging infectious disease is not being monitored or evaluated

Introduction

Visceral leishmaniasis is a major protozoal disease transmitted by the female *Phlebotomus* sandfly. In Europe, the *Leishmania infantum* parasite can cause zoonotic visceral leishmaniasis (ZVL) in both humans and animals, whereby dogs form the main reservoir. [1–3]. Between 1998 and 2013, the number of human autochthonous ZVL cases reported each year in southern Europe was around 875 [4, 5] In the last decade, the *L. infantum* prevalence has increased fivefold in several parts of southern Europe, where an estimated 2.5 million dogs are infected with *L. infantum.* These dogs have canine leishmaniosis (CanL), which can be either symptomatic or asymptomatic [6–8]. While CanL is endemic in southern Europe where the sandfly traditionally resides, not only have the numbers of cases in Mediterranean countries increased over the last decade, the distribution of cases has in this time also moved northwards into previously non-endemic regions [8–10].

This northward spread of ZVL in Europe has been attributed to multiple factors, such as the expansion of sandfly territory in the same direction, which is could be produced by global warming [11]. This has led to the expansion of sandflies from the Pyrenees further into France, and from Italy towards Germany, resulting in new environments suitable for *Leishmania* transmission [4, 12, 13]. A second explanation for the northward spread is the increase in the numbers of stray dogs and puppies being adopted and rehomed, the former often acting as reservoir of infection [14]. Both the expansion of sandfly territory and the increase in dog relocation have caused CanL to be introduced into non-endemic regions in Europe – regions that may now also be home to the vector [15]. A third possible explanation for the northward spread is the increase in the numbers of dogs being infected with CanL via other transmission routes, i.e. through vertical transmission, sexual intercourse, blood transfusion and direct dog-to-dog transmission, as has been found in parts of northern France where the vector is not currently present [15–18]. The risk of spread of *L. infantum* comes not only from symptomatic dogs recognized as having the disease; a few studies have confirmed that sandflies can also pick up the infection from asymptomatic dogs, making them a potentially persistent but invisible reservoir of infection [9, 19].

Since these factors affecting the northwards spread of *L. infantum* are not expected to change, the prevention and control of infection requires active

measures at the organizational level throughout the European Union (EU). Different sets of guidelines for the prevention, control and monitoring of the spread of *L. infantum* in the EU have been created or endorsed by the World Health Organization (WHO), the European Scientific Counsel for Companion Animal Parasites (ESCCAP), the European Food Safety Authority (EFSA), the World Organisation for Animal Health (OIE) and the Food and Agriculture Organization (FAO) [2, 20–23]. The responsibility for implementing these guidelines lies with veterinary practitioners in the field. Although the different guidelines differ slightly from one another, most of the recommendations focus on 1) increasing awareness of the presence and risk of ZVL and CanL; 2) taking preventive measures; 3) detecting and treating cases of disease; and 4) monitoring and evaluating infection. The most favourable – and possibly the only – way of restricting this zoonotic infection from spreading is thought to be through a "One Health" approach where synergism between medical physicians, veterinary practitioners, researchers, public health authorities, and politicians is central [7, 24].

In this study, we conducted an online survey among veterinarians in endemic and non-endemic countries to assess 1) the level of veterinarians' awareness of the spread of ZVL in Europe; 2) the level of their awareness of international guidelines; and 3) whether the guidelines are being used by veterinarians to control the spread of ZVL.

Methods

Study area and population

Spain was selected as an endemic region for this study because of Spain's long history with ZVL and because of the recent increase in the numbers of cases of ZVL in this country. The south of France was selected as an emerging endemic region, and the north of France as a non-endemic region. The south of Germany was also selected as a non-endemic region, but also as a region at risk due to the recent expansion of sandfly territory towards Germany. The target population were companion animal veterinary practitioners.

Survey development

An online survey (Supplementary File 1) was developed using LimeSurvey Professional (LimeSurvey, 2015). The survey had 24 questions and was categorized into three main parts. The first part contained general questions; the questions in the second part focused on awareness of the spread of ZVL in humans and dogs in the region and the international guidelines that are available; and the questions in the third part surveyed the protocols used when veterinarians suspected and confirmed ZVL cases. The survey was developed in English and translated into Spanish, French and German by native speakers of the target languages. Since the survey only included closed-ended questions, no translations were required to interpret the survey results.

Survey distribution

The survey was distributed between June and October 2016 among veterinarians in Spain, France and Germany through various online platforms, including mailing lists, websites, LinkedIn, Facebook groups and online newsletters.

Data processing

All data were anonymized and aggregated according to several different variables, including country, region and number of confirmed cases. To operationalize numerical variables, written values such as "20-30" were adapted to the average of 25, "approximately 100" was changed to the exact figure of 100 and "more than 50" was changed to 55. All answers that included percentages were removed from the survey if these could not be converted to actual numbers due to the lack of a denominator.

Statistical analysis

For the statistical analysis, R software (version 3.3.0) was used [26][26] (R Development Core Team and R Foundation for Statistical Computing Vienna Austria, 2016)(R Development Core Team and R Foundation for Statistical Computing Vienna Austria, 2016)(R Development Core Team and R Foundation for Statistical Computing Vienna Austria, 2016). To analyse associations between variables with 95% confidence, a descriptive analysis of the data was followed by t-tests and chi-squared tests.

Results

General characteristics

Between June and October 2016, 889 individuals accessed the survey. Incomplete surveys were disregarded and all 24 questions were completed by 482 veterinarians (279 from Spain, 114 from the south of France, 66 from the north of France, and 23 from the south of Germany). Due to the low numbers of German veterinarians, only the results from Spanish and French veterinarians were included in the study. In Spain, 40 out of 50 regions were represented; in France, 71 out of the total 96 departments (Figure 1A). An overview of the general characteristics of the Spanish and French veterinarians is presented in Table 1.

Veterinarians in Spain confirmed an average of 27.4 canine leishmaniosis (CanL) cases per year, compared with 6.6 in the south of France and 0.4 in the north of France, a statistically significant difference between the two countries ($p<0.01$). Figure 1B presents the geographical distribution of the average number of cases confirmed per veterinarian per year.

Of all veterinarians, 53% worked in an animal practice that covered a mix of both rural and urban areas, while 28% had only clients from urban areas, and 19% only from rural areas. Mixed (rural and urban) animal practitioners confirmed an average of 23.9 CanL cases per year, compared with 13.5 for practices in rural areas only, and 10.8 for practices in urban areas only (no statistically significant difference between rural and urban areas, $t=0.7627$; $p=0.4465$). The average duration of work experience was 16.7 years, with no difference between the regions. The distribution was normal for the range 1-30 years but skewed above 30 years.

Awareness of zoonotic visceral leishmaniasis spread and international guidelines

Of the respondents, 60% indicated that human zoonotic visceral leishmaniasis is spreading across Europe, and 62% had a similar impression regarding CanL. Veterinarians from the north of France noticed the largest increase in the number of CanL cases among their clients over the past 10 years. Awareness that CanL is involved in the spread of ZVL was significantly more prominent among Spanish (70%) than among the French veterinarians (55%) ($p<0.01$).

Table 1. General characteristics of veterinarians surveyed and their awareness of the spread of ZVL and CanL and of guidelines. Results are based on the 459 veterinarians who completed the survey, aggregated at the regional level.

	Spain	South of France	North of France	Total
Location of veterinarian	279 (61%)	114 (25%)	66 (14%)	459 (100%)
Average number of dogs suspected with CanL in the past 12 months per veterinarian	45.1	23.6	1.7	33.5
Average number of dogs confirmed with CanL in the past 12 months per veterinarian	27.4	6.6	0.4	18.3
Type of region				
Rural	37 (13%)	33 (29%)	16 (24%)	86 (19%)
Urban	83 (30%)	22 (19%)	23 (35%)	128 (28%)
Mixed	159 (57%)	59 (52%)	27 (41%)	245 (53%)
Average duration of work experience (years)	17.2	16.2	15.2	16.7
Awareness of spread of ZVL in Europe				
Yes	177 (63%)	67 (59%)	33 (50%)	277 (60%)
No	102 (37%)	47 (41%)	33 (50%)	182 (40%)
CanL plays a role in the spread ZVL				
Yes	195 (70%)	61 (54%)	36 (55%)	292 (64%)
No	84 (30%)	53 (46%)	30 (45%)	167 (36%)
CanL cases among clients in the last 10 years				
Increased	155 (56%)	58 (50%)	39 (60%)	252 (55%)
Decreased	41 (15%)	18 (16%)	0 (0%)	59 (13%)
Remained the same	83 (30%)	38 (33%)	27 (40%)	148 (32%)
Awareness of confirmed CanL and/or human ZVL cases				
No	194 (70%)	97 (85%)	60 (91%)	351 (76%)
Only CanL cases	77 (28%)	17 (15%)	6 (9%)	100 (22%)
Only ZVL cases	1 (0%)	0 (0%)	0 (0%)	1 (0%)
About both CanL and ZVL cases	7 (2%)	0 (0%)	0 (0%)	7 (2%)
Information sources CanL and or ZVL (CanL/ZVL)*				
Never heard of	(3%/ 4%)	(4%/24%)	(5%/38%)	(3%/14%)
Other veterinarians	(69%/20%)	(61%/ 5%)	(52%/11%)	(64%:15%)
Physicians	(8%/28%)	(2%/ 7%)	(2%/ 5%)	(6%/19%)
Own research	(69%/37%)	(20%/15%)	(52%/ 9%)	(61%/28%)
Study	(74%:/29%)	(59%/18%)	(58%/14%)	(68%/24%)
WHO**	(19%/28%)	(4%/ 6%)	(2%/ 5%)	(13%/19%)
EFSA**	(3%/ 4%)	(0%/ 0%)	(0%/ 0%)	(2%/ 2%)
ESCCAP**	(29%/ 7%)	(22%/ 4%)	(26%/ 2%)	(27%/ 5%)
OIE**	(19%/10%)	(16%/ 2%)	(9%/ 0%)	(17%/ 7%)
Media (television, newspaper etc)	(27%/25%)	(10%/ 9%)	(9%/ 6%)	(20%/19%)

Table 1. Continued

	Spain	South of France	North of France	Total
Mentioned during conferences	(73%/40%)	(67%/23%)	(65%/21%)	(71% 33%)
Mentioned during regular meetings	(51%/14%)	(31%/ 8%)	(9%/ 3%)	(40%/11%)
Mentioned in journals	(81%/37%)	(90%/27%)	(90%/30%)	(85%/33%)
Awareness of guidelines*				
Not aware of any	205 (73%)	71 (62%)	44 (67%)	320 (70%)
WHO	28 (10%)	24 (21%)	9 (14%)	61 (13%)
EFSA	4 (1%)	2 (2%)	1 (2%)	7 (2%)
ESCCAP	48 (17%)	15 (13%)	14 (21%)	77 (17%)
FAO	5 (2%)	10 (9%)	3 (5%)	18 (4%)
EU	14 (5%)	4 (4%)	3 (5%)	21 (5%)
OIE	15 (5%)	27 (24%)	12 (18%)	54 (12%)
Other	7 (3%)	1 (1%)	1 (2%)	9 (2%)

* Percentages do not add up to 100%, because respondents could select multiple options
**WHO: World Health Organization; EFSA: European Food Safety Authority; ESCCAP: European Scientific Counsel Companion Animal Parasites; OIE: World Organization for Animal Health

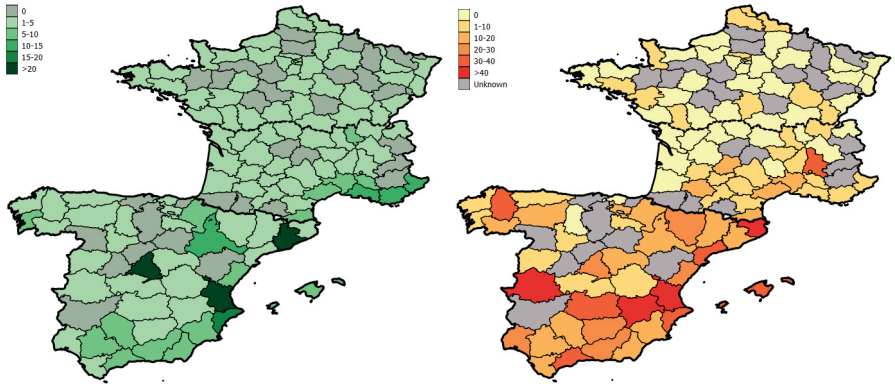

Figure 1. A. Geographical distribution of the numbers of veterinarians who participated in the survey per department in France and per region in Spain. B. The average number of confirmed cases in the last year per veterinarian per department in France and per region in Spain. The thicker black lines define the country borders and the dashed black line through the middle of France indicates our division between the north and south of France.

Nearly one third of the veterinarians who indicated seeing an increase in CanL prevalence did not indicate seeing a concomitant spread in human ZVL.

Three quarters of all veterinarians indicated that they had never received any information about CanL or ZVL cases in their region or country. In Spain, 2% of veterinarians had received reports about both CanL and ZVL, while this

figure was 0% in France. The percentage of veterinarians who indicated that they had received reports about confirmed CanL cases in their region differed per region: from 9% in the north of France to 28% in Spain. An average of 31% of veterinarians reported receiving most of the information about ZVL through scientific journals, conferences and their own research. An average of 75% of veterinarians reported receiving most of their information about CanL through conferences, scientific journals and their university training.

Between 62% (south of France) and 73% (Spain) of the veterinarians indicated not being aware of any guidelines for the control of ZVL in Europe at all (Figure 2). The ESCCAP guidelines were best known in all regions, while the OIE guidelines were best known in France. There was no correlation between the level of awareness of guidelines and the number of confirmed dogs per year (chi-squared test =5.875; p=0.3186).

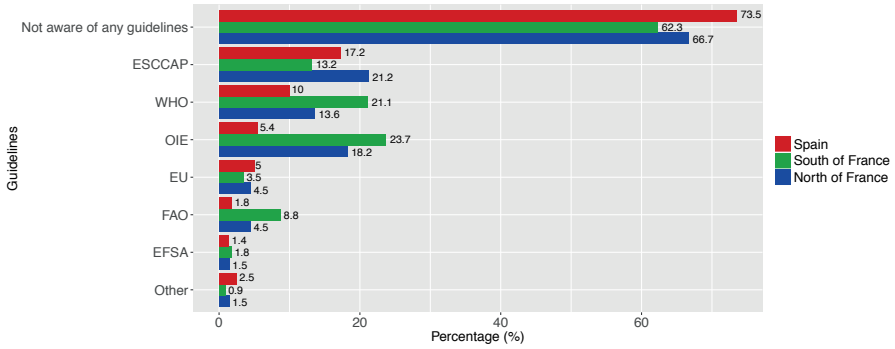

Figure 2. Percentage of veterinarians who were aware of the different guidelines, per country. Abbreviations: ESCCAP: European Scientific Counsel Companion Animal Parasites; WHO: World Health Organization; OIE: World Organization for Animal Health; EU: European Union; FAO: Food and Agriculture Organization; EFSA: European Food Safety Authority

Preventive measures

All guidelines emphasize the importance of taking preventive measures to control CanL and ZVL. Table 2 provides the survey results in terms of such preventive measures taken by veterinarians to control CanL. In Spain, 82% of the veterinarians indicated that they always recommended preventive measures to all their clients with dogs, compared with just 37% in the south of France and 0% in the north of France. However, 88% of veterinarians in the north of France indicated that they did recommend preventive measures for dogs living in an endemic region. Less than one third of the veterinarians

Table 2. Preventive measures. Results are based on the responses of 459 veterinarians who completed the survey, aggregated at regional level.

	Spain	South of France	North of France	Total
When do you recommend using preventive measures against CanL? *				
Never	2 (1%)	2 (2%)	0 (0%)	4 (1%)
If dogs are living in an endemic region	78 (28%)	63 (55%)	58 (88%)	199 (43%)
If dogs travel to an endemic region during high-risk season	70 (25%)	45 (39%)	27 (41%)	142 (31%)
If dogs travel from endemic regions to non-endemic regions	34 (12%)	12 (11%)	5 (76%)	51 (11%)
If dogs move to an endemic region for a longer period	63 (23%)	40 (35%)	32 (48%)	135 (29%)
If dogs have already been diagnosed with CanL	52 (19%)	34 (30%)	20 (30%)	106 (23%)
Always	228 (82%)	42 (37%)	0 (0%)	270 (59%)
Other	3 (1%)	2 (2%)	0 (0%)	5 (1%)
Which measures do you recommend to your clients to prevent sandfly biting and *Leishmania* infection? Please indicate with a percentage.				
Repellents or insecticides applied to the dog	96%	83%	67%	88%
Vaccination	55%	42%	10%	45%
Domperidone	44%	1.7%	1.6%	27%
If you do not recommend any preventive measures it is because: *				
You think they are ineffective	3 (8%)	0 (0%)	0 (0%)	3 (5%)
They are too expensive	4 (11%)	3 (21%)	2 (18%)	9 (15%)
You do not know where to get them	0 (0%)	1 (7%)	0 (0%)	1 (2%)
You think the risk of CanL is low	8 (22%)	7 (50%)	9 (82%)	24 (40%)
Other	21 (58%)	3 (21%)	0 (0%)	24 (40%)
Confirmed CanL cases should not be imported from endemic regions into non-endemic regions because of the public health risk.				
Agree	100 (36%)	70 (61%)	43 (65%)	213 (46%)
Disagree	179 (64%)	44 (39%)	23 (35%)	246 (54%)
Do you give information about the public health risk of CanL if the dog owner plans to travel to an endemic region with their dog during high-risk season (April - November)?				
Yes	223 (80%)	94 (82%)	59 (89%)	376 (83%)
No	56 (20%)	20 (18%)	7 (11%)	83 (18%)

* Percentages do not add up to 100%, because respondents could select multiple options

indicated recommending preventive measures for dogs travelling to an endemic region during the high-risk season between April and November.

The types of preventive measures that respondents indicated advising the most were as follows: insecticidal repellents and insecticides that can be applied to the dog (88%); vaccination of dogs (45%); and administration of domperidone to increase dogs' immunity (27%). These preventive measures are in accordance with all mentioned international guidelines. In the endemic regions, repellents were recommended in 96% of the cases in Spain and 83% of the cases in the south of France. Domperidone was not frequently recommended in France (1.7% in the south and 1.6% in the north), while in Spain it was recommended to 44% of the dog owners. Leishmania vaccination was recommended by 55% of the Spanish veterinarians, 42% by veterinarians in the south of France, and 10% in the north of France. Reasons that veterinarians in general gave for not recommending preventive measures were that they considered the risk of CanL to be low (82%) and the costs of vaccines too high (18%).

Of the Spanish veterinarians, 64% had no objection to dogs with a confirmed *L. infantum* infection travelling from endemic to non-endemic regions, in contrast with 34% of the French veterinarians. If owners indicated wanting to travel with their dog to an endemic region during the high-risk season (April – November), 83% of all veterinarians provided information about the risks of CanL, including the risks to public health.

Diagnosing and reporting cases

Table 3 provides an overview of how veterinarians indicated diagnosing and reporting cases of CanL, per region. In Spain, 93% of the veterinarians indicated having the diagnostic tools available at their practice to test for CanL, which was significantly higher than the figures in the south (71%) and the north of France (20%), a statistically significant difference between Spain and France ($p<0.01$). In Spain and the south of France, the number of confirmed cases per year was significantly higher ($p<0.01$) at veterinary clinics that had diagnostic tests available than at clinics that did not.

In the north of France, this difference was not significant ($t=0.82293$; $p=0.4203$). When the average numbers of confirmed cases in each region were compared between veterinarians who did have access to a diagnostic test and those who did not, the ration were 29.1:3.8 in Spain, 8.7:1.1 in the south of France and 0.3:0.5 in the north of France.

Table 3. Overview of diagnosis and reporting of cases. Results are based on the 459 veterinarians who completed the survey, aggregated at regional level.

	Spain	South of France	North of France	Total
Do you have CanL diagnostics available at your clinic?				
Yes	260 (93%)	83 (73%)	13 (20%)	356 (78%)
No	19 (7%)	31 (27%)	53 (80%)	103 (22%)
When do you test dogs for CanL? *				
If they have symptoms associated with CanL	247 (89%)	102 (89%)	57 (86%)	406 (88%)
If dogs have been imported from endemic regions to non-endemic regions	78 (28%)	8 (7%)	14 (21%)	100 (22%)
If dogs are being exported to non-endemic regions	34 (12%)	3 (3%)	0 (0%)	37 (8%)
If owners travelled with their dog(s) to an endemic region for a longer period during high-risk season	83 (30%)	19 (17%)	15 (23%)	117 (25%)
If another dog in the household has already tested positive	109 (39%)	53 (47%)	17 (36%)	179 (39%)
Other	100 (36%)	23 (20%)	0 (0%)	123 (29%)
When you diagnose a patient with CanL, do you inform the dog owner that it is a zoonosis?				
Yes	261 (94%)	92 (81%)	54 (82%)	407 (89%)
No	18 (6%)	22 (19%)	12 (18%)	52 (11%)
Do you report a confirmed case of CanL?				
Yes	51 (18%)	4 (4%)	0 (0%)	55 (12%)
No	228 (82%)	110 (96%)	66 (100%)	404 (88%)

* Percentages do not add up to 100%, because respondents could select multiple options

The most frequently mentioned reason for testing dogs for CanL was the presence of clinical symptoms. If dogs had been adopted from an endemic region to a non-endemic region, veterinarians in Spain recommended testing for CanL in 28% of cases; this figure was 7% for veterinarians in the south of France, and 21% for veterinarians in the north of France. If dogs tested positive for CanL, other dogs from the same household were tested for CanL in 30% of cases in Spain, 17% of cases in the south of France, and 23% of cases in the north of France.

When diagnosing a dog with CanL, an average of 89% of the veterinarians informed the dog owner that CanL is a zoonosis. The proportion of veterinarians who passed on this information was significantly higher ($p<0.01$) in Spain (94%) than in France (81% in the south and 82% in the north).

After confirming a case with CanL, 18% of the veterinarians in Spain indicated communicating this to other veterinarians – mostly to colleagues within their own clinic – compared with 4% of veterinarians in the south of France and 0% of veterinarians in the north of France. On average, 88% of the veterinarians indicated not reporting any confirmed CanL case.

Discussion

The results of our survey indicate that the majority of veterinarians are not aware of international guidelines on how to control CanL. However, most veterinarians are aware of the spread of the infection in Europe and they appear to be implementing measures similar to those recommended by the guidelines.

The distribution of the numbers of confirmed CanL cases per veterinarian per year indicated by the results of this survey is consistent with the currently recognized distribution of CanL in Europe, with Spain having the highest burden of disease, followed by the south and the north of France [8]. The interventions of veterinarians focused largely on preventive measures, such as vaccination and insect repellents. However, if the guidelines change in the future, such changes would likely reach less than 30% of the target population. Also, 76% of all veterinarians indicated that they had never received any reports regarding confirmed CanL and ZVL cases in their region or country, and 88% indicated they had never reported a CanL case that had been confirmed by them, which implies a distinct lack in the of monitoring and evaluation, which are the key areas covered by the guidelines. There is a significant gap between the data on ZVL and CanL that are publicly available and the actual numbers of cases that are being confirmed by veterinarians.

While equal efforts were put into reaching veterinarians in the three countries included in this study, the response rate from south German veterinarians was extremely low, which suggests that these veterinarians do currently not consider CanL and ZVL a priority. Due to the low number of responses from France, we decided to include their input, but consider the outcomes as if they represent a pilot study. If information about guidelines and cases is reaching only 30% of the veterinarians in Europe based on our

study, and these veterinarians are confirming an average of 18.3 CanL cases in the last year, such information will most likely reach even fewer veterinarians in regions where fewer cases are confirmed. The increasing presence of the sandfly in the south of Germany makes it therefore imperative that German veterinarians are made aware of the risk factors and that they are given advice on preventive measures [17, 27]. Such provision of information is also needed because of the possibility of dogs with confirmed *L. infantum* infection being imported into non-endemic regions, one of the major risk factors for the spread of infection into northern Europe.

Three similar survey-based studies have previously been carried out on this topic. The aim of the most recent study was to assess the management strategies of CanL by veterinarians in southwestern Europe [28]; another study assessed the management strategies of CanL-infected dogs in the highly endemic region of Madrid [29]; and the third provided a detailed picture of diagnostics and control practices in the southeast of Spain [30]. The use of preventive measures was a topic common to all three surveys and the outcomes of the above-mentioned studies are comparable to those of our study, in that they all concluded that veterinarians in endemic regions are recommending preventive measures to most of their clients. Furthermore, the findings by Bourdeau et al. (2014) that veterinarians in Spain (98%) and France (89%) are making dog owners aware of the public health risks of CanL are in line with our findings that 80% of the Spanish veterinarians, 82% of the veterinarians in the south of France and 90% in the north of France reported informing dog owners about these risks. The other surveys did not address the veterinarians' awareness of guidelines, whether veterinarians received information from governmental or official organizations about cases in their region, or if veterinarians reported confirmed cases.

While the veterinarians who participated in the current survey cannot be considered representative of all veterinarians in Spain and France due to voluntary response bias, they are likely to be more aware of ZVL in general. We are therefore almost certainly presenting an overestimation of the awareness of guidelines, of the information veterinarians receive about cases, and of the percentage of veterinarians who are informing others about confirmed cases. Nevertheless, the relatively large number of respondents, combined with their wide geographical distribution and wide range in annually confirmed cases, allowed us to interpret the data and compare variables between the

regions. However, we emphasize that our numbers still represent a very small percentage (±1%) of all registered veterinarians in both countries [31, 32]. Since dogs are the main reservoir of ZVL in Europe, this study focused on the awareness and implementation of guidelines by veterinarians. Future studies could build on this by assessing the awareness and implementation of guidelines by general practitioners in the same regions. Further explorations could focus on the implementation of the One Health approach since such collaboration between the two professions has been suggested to be a crucial aspect for controlling ZVL in both humans and animals.

Conclusion and recommendations

We recommend the creation of an easy-to-use online network where both veterinarians and physicians can report the presence of confirmed ZVL cases. An example of such an online network that is successfully being used in the United States of America is PetWare. Such a network in Europe is crucial for monitoring, controlling and preventing the further spread of zoonotic visceral leishmaniasis at the regional, national and international level.

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CHAPTER 4

Uniting mathematics and biology for control of visceral leishmaniasis

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Abstract

The neglected tropical disease (NTD) visceral leishmaniasis (VL) has been targeted by the WHO for elimination as a public health problem on the Indian subcontinent by 2017 or earlier. To date there is a surprising scarcity of mathematical models capable of capturing VL disease dynamics, which are widely considered central to planning and assessing the efficacy of interventions. The few models that have been developed are examined, highlighting the necessity for better data to parameterise and fit these and future models. In particular, the characterisation and infectiousness of the different disease stages will be crucial to elimination. Modelling can then assist in establishing whether, when, and how the WHO VL elimination targets can be met.

How mathematics can aid elimination of VL

VL (see Glossary) is a potentially fatal protozoan infection transmitted by sandflies. Individuals with acute symptoms, referred to here as patients with kala-azar (KA), show signs of fever, weight loss, splenomegaly, and anaemia; it is believed that almost all patients will die if left untreated at this stage [1]. Following recovery from KA via drug treatment, some patients go on to develop post-KA dermal leishmaniasis (PKDL), a nonfatal stage of infection with dermatological symptoms [2]. Worldwide, approximately 200 000–400 000 KA cases occur per year, the majority of which occur on the Indian subcontinent (ISC): in India, Bangladesh, and Nepal. VL on the ISC is anthroponotic (i.e., there are no non-human primary hosts), it is transmitted by just one vector species, Phlebotomus argentipes, and the burden of disease is highly localised. As a consequence, VL on the ISC is one of the NTDs that is targeted by the WHO for elimination as a public health problem (less than one new case of KA per 10 000 people per year) by or before 2017 [3]. In the rest of the world, VL is zoonotic (Box 1), limiting the possibility of elimination, and therefore the goal is 100% detection and treatment of all human cases by 2020 [4].

Within the ISC, the most affected area is the Bihar district in northern India, where VL disproportionately affects the poorest [5, 6]. Despite falling numbers of cases overall in the region [7], there remain hotspots of infection; Bihar in particular accounts for approximately 80% of reported cases on the ISC [8]. Currently, control programmes are based upon scaling up active case-detection [9] and social mobilisation [7, 10], which are both known to be beneficial in reducing VL. Another essential part of intervention programmes is vector control, usually through indoor residual spraying (IRS) [11]; however, it is not clear whether additional control measures are necessary.

To ensure the success of the interventions in reaching the WHO goals, it is vital to be able to examine critically and quantitatively the outcome of different interventions and to make quantitative assessments which will help in the fight against this disease, particularly in the context of limited resources and over a relatively short timescale. Mathematical modelling provides tools to help evaluate interventions to indicate both the intensity and timescale over which an intervention might have to be carried out. Modelling can also inform how long surveillance should be in operation before elimination can be confirmed and how elimination might be sustained. Therefore, it is

important to understand the limitations of existing models of VL on the ISC, and how better data can improve the models and generate results that are more directly useful for policy.

Insights from mathematical modelling studies are summarised here through a literature review, and the differences in results or limitations are explained such that lessons can be learnt for future models and current knowledge gaps are identified.

Current state of mathematical modelling of VL

A thorough literature review was conducted to find all mathematical transmission models of VL (further details are given in the supplementary material online). Twenty-four papers addressing relevant modelling of VL are summarised in Table 1. Of these, only seven focused on the ISC; the remainder mostly addressed transmission between dogs in Brazil or France. These zoonotic papers were included because of the cross-applicable insights that they give (Box 1). Many of the articles were by the same authors and thus there is a distinct overlap between many models. For example, three of the most recent VL modelling papers on the ISC were based on the same model [12–14]. The models were rarely validated against recent data, with the exception on the ISC being the papers by Stauch et al. [12–14] and, to some extent, Mubayi et al. [15].

The models used a range of different assumptions regarding disease progression in humans, the intensity of transmission, and the role of sandflies (discussed below). Only two studies explicitly considered spatial aspects of transmission [16, 17].

The authors modelled a range of potential control strategies to simulate the possible effects of intervention strategies. On the ISC, treatment was modelled explicitly in all but one paper (which is based on historical trends [18]) because this is the current course of action upon a diagnosis of VL. Two papers (by the same group [12, 14]) explored the impact of vector control on disease prevalence, and one article computed the cost-effectiveness of a vaccine, should one be developed [19].

Table 1. Summary of VL modeling papers.

	References																								
	Anthroponotic studies								Zoonotic studies																
	[18]	[20]	[15]	[21]	[12][a]	[19]	[13][a]	[14][a]	[22]	[23]	[24]	[25]	[26]65	[27]	[28]	[29]	[30]	[31]	[32][b]	[33][b]	[16]	[17][b]	[34]	[35]
Model structure																								
	√[c]		√		√		√	√	√	√	√	√	√	√	√	√	√	√	√	√		√	√	√
	√	√		√		√			√			√	√	√	√		√							√
Assumption																								
Asymptomatic humans					√	√	√	√																
PKDL					√	√	√	√											√	√		√		
Humans	√	√	√	√	√	√	√	√				†					†	†	√	√		√	√	
Asymptomatic dogs										√					√									√
Spatial aspects																					√	√		
Seasonality											√		√	√				√						
Interventions																								
Human treatment		√	√	√	√	√	√	√											√	√		√		
Human vaccination						√						√										√		
Vector control					√			√				√								√			√	
Dog culling															√	√	√						√	√
Dog collar																√							√	
Dog treatment												√									*		√	
Dog vaccination												√											√	
Region	India	Sudan	India	ISC	ISC	India	India	ISC	France	-	-	Brazil/ Malta	-	Brazil	Brazil	Brazil	Brazil	Marocco	Sudan	-	France	-	Brazil	Brazil

[a] Denotes studies by Stauch et al., that use the same basic model. [b] Denotes studies by ELmojtba et al. that use the same basic model. [c] √, included in model; †, dead-end hosts; *, implicitly included in other terms; -, unclear or unknown region.

Natural history of infection

An important difference between the models is the varying assumptions about how the disease progresses, including the probability of symptoms, the time between infection and symptoms, and the dynamics post-treatment. The limited knowledge about this process, also known as the natural history of the infection, will affect the interpretation of model results and, therefore, they are discussed in some detail here. There exists a general understanding of the clinical progression of VL, but few datasets can assist in quantifying the rates of progression or the probability of different events. In general, following infection, most individuals remain non-symptomatic [36] whereas a few develop KA. Those with KA have a high mortality rate in the absence of treatment, often quoted in the literature to be up to 100% within 2 years [4]. Relapses of KA sometimes occur following treatment and this can be triggered by HIV co-infection [37, 38]. After successful treatment, patients with KA recover, but this can be followed by the onset of PKDL, possibly preceded by a period of dormancy of the intracellular parasite. Unlike KA, PKDL is characterised by a nodular or papular skin rash, has no associated mortality and symptoms are dermatological. The occurrence of PKDL varies geographically: 5–10% of cases on ISC [2] and in up to 60% of cases in East Africa [39]. Approximately 10% of PKDL cases occur in individuals with no history of KA, and occasionally it occurs concurrently with KA [40, 41].

The gold standard diagnostic for KA is parasitological diagnosis of splenic aspirates, bone marrow, and lymph node samples, with decreasing sensitivity, respectively. Recently, rapid serological diagnostic tests [42, 43] mainly based on the rK39 antigen have been developed and are implemented as primary diagnostic tools on ISC because of their high sensitivity when combined with clinical symptoms. The direct agglutination test (DAT) is an alternative serological test that has been proposed as an assay to detect asymptomatic humans. Recently, it was suggested that humans that are highly reactive to DAT have a greater propensity to progress to symptomatic KA than those with lower responses [8]. The leishmanin skin test (LST) also measures serological response to infection and is seen as a marker of exposure to infection, which is important for understanding transmission but is not used in clinical practice. Currently, it is unclear whether detection of asymptomatic patients can be performed by measuring the serological response to infection alone. Therefore, molecular tests, mainly PCR, based on

the detection of parasitic DNA might be an alternative tool to detect humans who are actively infected rather than only exposed to disease [44]. It is important that the VL research community focuses on the use of diagnostic tools for the detection of asymptomatic cases such that clarity is reached on a possible algorithm to detect infectious patients who are not showing symptoms.

Modelling the natural history of infection

Most VL models are 'compartmental' models, which means that hosts pass through various 'stages' of the disease at different rates. A model can be constructed in which the assumptions are closely tied to current understanding of the biology. For example, the natural history outlined above can be represented by a flow diagram (Figure 1) with the model tracking the number of individuals in each disease stage; the different rates of moving between stages, such as the average time between KA and PKDL, are defined either directly from knowledge of the disease or by fitting the model to data. Models of VL do not include all of the known complexity of VL biology, mainly because of a lack of data to define the stages, quantify the rates of progression between stages, and chart the time-evolving distribution of the infected population between stages. Basic models including key aspects of VL transmission, guided by available data and a particular policy or research question, can yield invaluable insights.

For the ISC, the simplest model structure was the susceptible–infected–recovered (SIR) progression assumed by Dye and Wolpert [18] (Figure S1 in the supplementary material). This model was used to explain the historical disease dynamics of VL in India between 1875 and 1950. Although the model is unsophisticated and omits much of the currently known biological process of VL infection, it was effectively fitted to longitudinal incidence data, and the intrinsic processes were able to account for the timings of the long interepidemic periods that are characteristic of VL. A slightly more complex model was that of Mubayi et al. which included a more realistic latency period distribution, but did not include PKDL [15].

The probability and timing of PKDL following KA are key assumptions for interpreting surveillance data and in using models to make projections. The occurrence of PKDL is thought to be governed by several factors, including the choice of drugs and adherence to treatment [45]. Some modellers (ELmojtaba

et al. [17, 32, 33], Stauch et al. [12–14], and Lee et al. [19]) incorporated the potential progression to PKDL infection following KA in humans via the addition of another infective stage (Figure S2). This enables one not only to observe the effect of different treatment rates upon the two different types of disease but also to examine how treatments that reduce the probability of developing PKDL might affect infection dynamics and prevalence.

Inclusion of a dormancy period between KA and the onset of possible PKDL (Figure 1) was used by two groups (Stauch et al. [12–14] and Lee et al. [19]) to reflect the longer duration of this period on the ISC [45].

Although the different stages of infection are loosely based on current understanding of the clinical stages, the precise definition of each stage in the model depends upon the data used. For example, a KA case might be any KA case, or it might be a diagnosed KA case in a particular healthcare setting. Stauch et al. [12–14] particularly modelled the KALANET trial [46]; this trial evaluated the efficacy, acceptability, and cost-effectiveness of long-lasting insecticidal bed nets for the prevention of visceral leishmaniasis through a community intervention trial in the endemic Bihar focus (www.kalanet.info). Diagnosis of VL infection was made by three tests (PCR/DAT/ LST described in Table S1). The study revealed no significant impact of bed nets [47], but it suggested that incidence of asymptomatic Leishmania donovani infection in VL high-endemic foci is ninefold more frequent than incident VL disease [48].

Stauch et al., therefore, were able to partition the different stages of infection by their diagnostic status under the three tests, in addition to symptoms, and treatment. Although this method of compartmentalisation is only subtly different from that general framework described above, the stratification by different diagnostic tests structures the model slightly differently, with, for example, four different ‘recovered’ stages by diagnostic status [12–14].

Given the availability of detailed data on diagnostic outcomes, the Stauch et al. model included asymptomatic individuals who tested positive with any of the three tests but were unaware of their infection status in the absence of diagnostic tests (Figure S3). The fact that asymptomatic individuals can test positive leads to the question of their infectivity to sandflies and of their role in the transmission cycle (see below) and the specificity of the tests used.

An area of much debate in the VL research community is the existence and action of immunity, which is currently considered as a gap in our knowledge

of VL [49, 50]. The models of Stauch et al. are the only ones to feature a return to susceptibility after recovery from either KA or PKDL. Under this model, immunity from reinfection lasts approximately 307 days, which is short in comparison to the lifelong immunity assumed by other authors [12–14].

Different modellers have not only made different assumptions about the natural history of VL but, importantly, also used different parameter ranges. The huge variability in parameters and their values (Table S2) is attributable not only to the underlying model structures but also to the uncertainty in many factors in transmission and progression, such as rates or probabilities, which are either hard to measure or vary by region. Access to more detailed datasets using modern diagnostics is essential to revolutionise the study of VL transmission dynamics and the utility of models to inform policy.

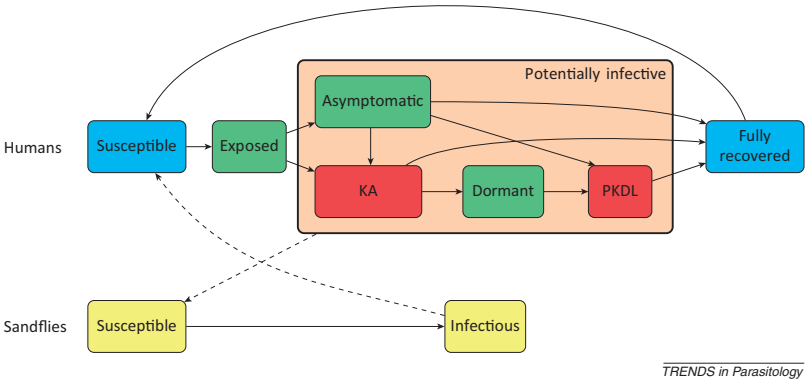

Figure 1. Compartmental diagram showing possible visceral leishmaniasis (VL) progression paths. Differently coloured boxes denote humans at various stages of VL infection: blue for no current infection, green for non-symptomatic but infected humans, and red for symptomatic individuals. Yellow boxes represent sandflies with and without infection. The orange box indicates all human stages that are infected and that might be infective to sandflies (with likely varying intensities). Broken lines indicate the direction of transmission between humans and sandflies, whereas unbroken lines show possible progression paths through different disease stages. Abbreviations: KA, kala-azar; PKDL, post-KA dermal leishmaniasis.

Transmission of VL

During the different stages of VL infection, individuals harbour different levels of the parasite in their peripheral blood and lymphatic systems [44]. Given that there is apparently no sterile cure, once someone is infected they can be infectious to sandflies throughout their lifetime, including during the dormancy period before PKDL and once PKDL develops (orange box in

Figure 2). In particular, PKDL nodules are known to contain a detectable parasitic load, implying potential significant infectivity [51].

Both asymptomatic humans (Box 2) and those with PKDL are unlikely to seek medical advice or treatment [52] owing to the costs of treatment and the fact that neither condition causes any physical limitation [53]; these individuals are therefore behaviourally distinct from those with KA. Current active case detection relies on finding KA symptoms before testing and, therefore, humans who are asymptomatic are missed. The WHO has recommended various regimens for PKDL based on geographical location, but each has a long course and is associated with toxicities [54]. In resource-poor settings, where VL is most prevalent, these long parenteral regimens invariably lead to either non-acceptance or poor compliance [55].

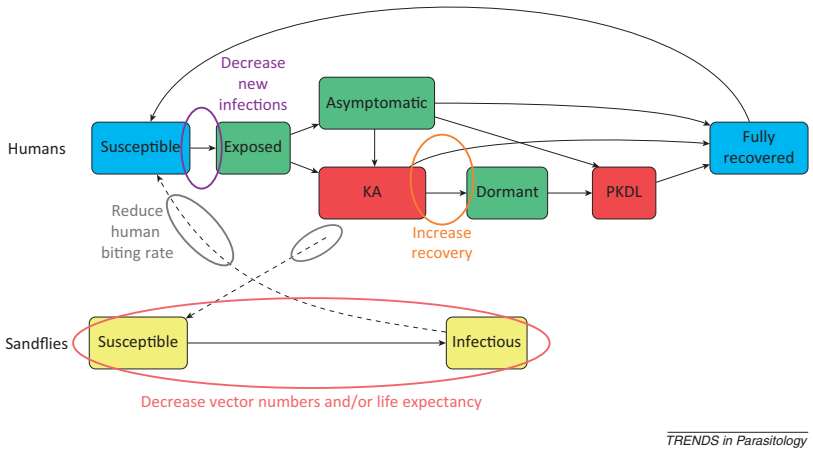

Figure 2. Main ways to interrupt the transmission cycle of visceral leishmaniasis (VL) through interventions. Orange shows the effect of standard (first- or second-line) treatment of symptomatic individuals. Pink shows the effect of vector controls. Grey shows the potential effect of bed nets. Purple shows the effect of a (potential) human vaccine or other prophylactic intervention.

Infectivity to vectors

The infectivity of humans in different disease stages was only identified in one model in which the authors explicitly modelled asymptomatic humans with a decreased infectivity within the transmission cycle [12–14] (Box 3). Currently, only two groups ([12–14] and [17, 32, 33]) have incorporated PKDL into transmission models by assuming comparable infectivity of KA- and PKDL-infected humans because of lack of evidence to the contrary. The

models with an interim dormancy period before the onset of PKDL [12–14] assumed that, during this time, the human host is not infective to sandflies, whereas the other models [17, 32, 33] assumed that individuals progress straight from KA to PKDL, and are infective to sandflies throughout.

The cost–benefit analysis model [19] included both asymptomatic humans and those with PKDL but, because a constant force of infection was used, the role of these patients towards onwards infection cannot be discerned.

These assumptions on the infectivity of asymptomatic humans and patients with PKDL are crucial in determining the estimated efficacy of increased detection and treatment of patients with KA. If there is a large asymptomatic population, as indicated in the literature [48], then they will probably lead to most of the onwards infection, even with low infectivity, as might be the case with malaria [56]. It could be argued that the decline in cases in Nepal and Bangladesh under increased active case detection suggests that the contribution from asymptomatics is small, but this depends on the timescale and acquisition of immunity as well as on the number of asymptomatic humans and the impact of IRS in interrupting transmission. Although it is unlikely that direct estimates of asymptomatic infectivity will be available soon, models could be fitted to high-quality surveillance data, giving some bounds to the contribution of different stages to transmission.

Vector biology

Sandflies are key in VL transmission, but little is known about their life cycle, biting rates, and transmission probabilities, all of which are crucial to increase understanding of transmission dynamics. Even estimates of a parameter as fundamental as sandfly life-expectancy have large confidence intervals. Whereas models typically use an estimate of 2 weeks, such estimates range from 2.4 to 16.7 days (Table S3). Likewise, the seasonal fluctuations of sandfly populations are only featured in some transmission models of zoonotic VL [24, 27, 31, 57], despite these fluctuations being one of the easier parameters to measure. In future models consideration should be made to the impact of sandfly seasonality on the ISC and how this might affect vector controls and their timing; this can be achieved by utilising frameworks from zoonotic VL models as well as other vector-borne disease models.

Effectiveness of interventions

There are several public health control measures that aim to reduce the transmission of VL [58]. Some controls, such as treatment of patients with KA, effectively speed up the progression of patients through disease stages and minimise the time that these individuals spend in the symptomatic period, potentially reducing onwards infection to sandflies. Other controls, such as insecticide spraying, aim to reduce the vector population and its effectiveness in transmitting infection. The four main parts of the transmission cycle that are affected by interventions (both current and hypothetical) are highlighted in Figure 2. Each of these interventions has been discussed within one or more of the articles found by this review, and their modelling methods and results are discussed below.

Treatment of KA

Given the high mortality rate for untreated KA, most patients are believed to seek care eventually, although the time-delay before treatment can be long [10]. There are a variety of treatments available for KA, but some studies suggest that adherence, rather than treatment type, is the key factor that might result in differing probabilities of progression to PKDL or relapse to KA [59].

In the model analysis by ELmojtaba et al. [32], both first-line treatment rate and effectiveness of preventing PKDL are varied. The authors found that, with high treatment rates alone, there was a reduction in KA cases, but, because a proportion of these treatments produced a PKDL case, infection rates were still high. Unsurprisingly, increasing both treatment and effectiveness was more efficacious at reducing transmission.

Stauch et al. [12–14] included three treatment types: first-line treatment of KA, second-line treatment following first-line treatment failure, and PKDL treatment. The authors showed that high treatment rates reduced the number of KA and PKDL cases. However, the intervention had almost no impact upon the large asymptomatic population and, consequently, transmission intensity was little affected [12].

These two groups of papers suggest that treatment alone will not be enough to truly halt the transmission of VL, and that other interventions are needed when aiming for elimination, echoing the opinion of others [7, 60].

Vaccine

There is currently no human vaccine for VL; however, advances are being made and models have been used to study the benefits of potential candidates [49, 61]. Lee et al. [19] described the possible advantages of a VL vaccine through a cost–benefit analysis. They found that even a poorly effective vaccine (25% effective – a vaccine that fully protects 25% of those immunised against infection, but makes no difference to the remaining 75%) might be cost-effective (for US$ 100 or less), whereas vaccines with higher effectiveness might even be cost-saving. For Bihar, India, in particular, this work indicated that a vaccine would be beneficial because of the high prevalence of genetic resistance to first-line treatments that has developed in this region. This model [19] used a constant (age-dependent) force of infection that did not change in response to vaccine use. Consequently, it is hard to determine what indirect effects the vaccine might have on reducing disease prevalence.

ELmojtaba et al. [17] showed that vaccination rates affected the transient dynamics through the rate of reduction of cases (in Sudan) but ultimately had little role in long-term prevalences where new cases are continuously imported. In particular, the authors noted that high levels of vaccine efficacy would be needed to reduce the equilibrium prevalence of VL significantly. These contrasting results highlight the importance of understanding the underlying assumptions of the models when interpreting their results, and that the potential impact of a future vaccine is still uncertain.

Vector control

The main intervention for vector control is IRS, which is linked to a reduction in sandfly life expectancy and to a consequent reduction in sandfly populations [11]. Lowering the total vector population size reduces the biting pressure on each human by decreasing the ratio of vectors to hosts (Figure 2). By reducing the sandfly life-expectancy, the vector is less likely to survive long enough to bite twice – once to acquire infection and again to infect a host. As the vector death rate increases, vectors spend less time infected.

Other interventions include reductions in sandfly breeding sites, which also limits population sizes, and insecticide-treated bed nets [58]. The use of bed nets decreases the contact rate between vectors and humans and, consequently, should lower transmission, not only to susceptible individuals, but also from infected ones, including asymptomatic humans and those with

PKDL. Unfortunately, the KALANET programme indicated that, despite a reduction in sandfly density, bed nets were not effective at reducing incidence in this setting and, thus, IRS remains the main control strategy [62, 63].

Given the limited data on sandfly behaviour (as mentioned above), model assumptions and outputs are variable. Stauch et al. [12] examined the effect of changing the three parameters corresponding to vector life-expectancy, vector population size, and bite rate. Each of the parameters appeared to result in a quasi-linear change in prevalence and incidence of infection. Further work by Stauch et al. [14] examined control of vector breeding sites and killing vectors directly. These authors suggested that, to break transmission, either the vector population must be reduced by 79% through the destruction of breeding sites or it must be reduced by 67% by killing adult flies, the latter effectively decreasing the life-expectancy of the sandflies in addition to suppressing their population size.

Concluding remarks

Modelling is a useful tool in informing public health control programmes, but to date there have been relatively few VL models developed. This is partly because of the status of VL as an NTD with limited data availability. Modelling results point towards IRS in particular as a highly promising strategy to control transmission of VL. However, it is difficult to determine the best way forward to achieve the goals of the WHO given the current state of knowledge. The main weakness of the current modelling literature is the lack of fitting or validation against multiple timepoint data. The one model parameterised by a modern dataset is only fitted to the cross-sectional, rather than the dynamic data [12–14]. There is an urgent need for more models of VL to be developed which can aid public health policy by analysing surveillance data and guide policy on areas where current strategies need to be improved. PKDL cases continue to occur from historic VL cases, cost-effective strategies to monitor these cases and limit onward transmission are required.

The current suite of models are also limited in terms of the technical complexity and analyses which have been performed, such as exploring asymptotic/PKDL infectivity, sandfly seasonality, age-dependent exposure, and differences (both age and gender related) in treatment-seeking behaviour. Currently no stochastic individual-based models (IBM) exist that capture the transmission dynamics of VL. Stochastic models can simulate the probability

of elimination as well as the probability of (re)-occurrence of outbreaks, both identified as crucial questions for VL on the ISC. Another technical need is for IBMs that can include more complexity including seasonality, migration and location. IBMs for other NTDs have shown to be of great value in aiding healthcare policy, such as estimating the impact of doubling the frequency of mass drug administration to accelerate elimination of onchocerciasis [64] and lymphatic filariasis [65], as well as combining various preventive and therapeutic interventions to interrupt leprosy transmission [66].

Surveillance data, together with prior experience of the epidemiology of elimination, suggest that, although there are geographical areas where the current programmes will be successful, there will be those where additional interventions are required. If goals for the ISC, as well as long-term suppression of transmission, are to be achieved, it is essential that the transmission dynamics are understood and that intervention programmes are designed to maximise the reduction in transmission and the long-term sustainability of these goals. Availability of surveillance and research data is necessary to inform future models for them to be an effective tool to support the hugely impressive efforts to control VL on the ISC. As new datasets become available through the labours of experimental and field researchers, there are now opportunities for existing model refinement and novel model exploration to address outstanding questions (Box 4). To meet the ambitious targets of the WHO, it will be essential to maintain dialogue between modellers and programme managers, policy makers, and other researchers working to combat this disease.

Glossary

Asymptomatic: patients who have active VL infection, are assumed to be infective to sandflies (Box 3), but have no symptoms of KA.

Compartmental models: models in which the population is divided into groups of people who progress through various stages of the disease, represented as boxes or 'compartments' in the model. The classic example of this is the susceptible–infected–recovered (SIR) basic epidemiological model [67] in which everyone is considered to belong to one of those three stages.

Deterministic model: these models capture average behaviour of a population and give the same outcome for a set of parameters in every simulation performed. **Dormant**: those patients in this stage are between KA and PKDL; they still harbour Leishmania parasites, but have no symptoms.

Exposed: patients who have acquired VL infection but are not yet infective to sandflies.

(Fully) recovered: patients who have previously had VL infection (of any kind) and are now parasite-free with acquired immunity.

Individual based model (IBM): a type of stochastic model where individual humans are modelled separately under an overarching set of rules. These allow for more complex simulations including differences between individual people, their movements, and geographic setting.

Kala-azar (KA): literately translated as 'black-fever', this is the acute form of visceral leishmaniasis. Patients display symptoms such as fever, weight loss, swelling of the spleen or liver, and anaemia.

Non-symptomatic: all patients with VL infection but no symptoms: includes exposed, asymptomatic, and dormant individuals (green boxes in Figure 1).

Post-kala-azar dermal leishmaniasis (PKDL): following KA, some individuals (5–10%) develop PKDL, which is characterised by a nodular or papular skin rash. It is non life-threatening.

Stochastic models: these allow for chance events as numbers of cases of disease become small (in contrast to deterministic models). Simulated disease dynamics vary every time; this allows the probability of events such as elimination or re-occurrence to be found.

Visceral leishmaniasis (VL): general term for the disease caused by Leishmania donovani (on the ISC) and L. infantum elsewhere. A VL patient refers to all individuals harbouring the parasite, including those with and without symptoms.

Box 1. What can we learn from VL in other regions?

The global picture: there are two types of parasite which cause VL: Leishmania donovani and L. infantum. L. donovani is transmitted on the ISC and East Africa and is the focus of this review. L. infantum is responsible for most other VL infection, although some countries have had outbreaks of disease due to both species [68]. The ISC has the highest number of VL cases (58% of cases in 2012), followed by East Africa (29%) and Brazil (8%) (http://www.who.int/research/en/).

(i) **Zoonotic transmission** Transmission of VL that affects animals is largely associated with L. infantum and is called zoonotic (ZVL). L donovani has been found in animals in East Africa; however, it is considered to have no animal reservoir on the ISC [69, 70]. In Brazil, high levels of infection occur in dog populations (canine or CVL), and transmission between these populations and sandflies is thought to drive VL transmission to humans.

(ii) **Targeting the animal reservoir** Where there is ZVL, the animal reservoir provides important opportunities for intervention, many of which have been modelled (see Table 1 in main text). The control measures used are largely inappropriate for consideration in the human population and include strategies such as insecticidal dog collars and culling, which reduce the force of infection towards humans. Despite the crucial differences between these two types of disease, lessons can be learnt (below), which can help in the future modelling of VL on the ISC.

(iii) **Parasite burden and transmission** Interestingly, as in humans, many PCR-positive dogs are also asymptomatic, and this has been reflected in models of CVL [23, 28, 35]. Different methods were used to compartmentalise the dog population; either from clinical signs (i.e., symptomatic/ asymptomatic) or by infectivity of dogs to sandflies (i.e., ever-infectious/never-infectious). Clinical status has been suggested as a proxy for infectivity, as it has in humans, with one study indicating that non-symptomatic infected dogs were around threefold less infectious to sandflies than infected dogs with multiple clinical signs of VL infection [28]. However, an individual dog's parasite burden also appears to be highly correlated with relative infectivity, even if asymptomatic [28, 71], and this may be a better quantitative marker for infectiousness in future models.

(iv) **Diagnostics** Modelling of CVL in Brazil [35] indicates that high-specificity diagnostics are crucial to correctly identify infectious individuals and intervene to reduce transmission. This provides a modelling framework which could be adapted to explore the issues of sensitivity/specificity of human diagnostics on the ISC.

Box 2. What is an asymptomatic VL case?

The green boxes in Figure 1 in main text identify infected individuals without symptoms. Identifying this group of non-symptomatic humans in cross-sectional or longitudinal studies is difficult because of contradictory diagnostic results [8]. For the purposes of transmission modelling, the subsets of these (termed here as exposed, asymptomatic, and dormant) are different because only those within the orange box can contribute to onwards infection.

There is ambiguity in the term 'asymptomatic' as it is used in the context of VL. It can include or exclude those humans with subsymptomatic early infection, non-symptomatic early infection, as well as post-treatment or recovery dormancy. From an epidemiological perspective, the term is used for individuals who display no outward symptoms of VL infection but do harbour parasites. For the purposes of this review and to assist in future modelling studies, the term 'asymptomatic' is used here to describe the subset of these humans capable of contributing towards transmission. Non-symptomatics are identified together with asymptomatics in some types of data collection, but in terms of transmission models they are entirely distinct or absent, with no role in transmission. This distinction is made because of the difficulty in separating exposed, uninfected, possibly seropositive, individuals from subpatent infections in cross-sectional studies because of conflicting diagnostic results (see main text). The definition of these groups by particular diagnostics is an area of ongoing research and debate, which future xenodiagnostic studies will clarify; these studies involve exposing vectors to blood containing various levels of parasites and then establishing infection probabilities.

Box 3. Relative infectivity of asymptomatic humans

In the model by Stauch et al. [12–14], PCR- and/or DAT-seropositive individuals, who are not symptomatic (termed 'asymptomatics' in the papers), included both pre-KA patients and non-KA individuals. These persons were estimated to be 40–80-fold less infective to sandflies than their KA and PKDL counterparts, depending upon their PCR/DAT status. This estimation is a result of model-fitting to data from one study (KALANET) rather than direct biological measurement.

Empirical evidence for relative infectivity of asymptomatics is sparse [72, 73], although one xenodiagnostic study [74] has enabled the assessment of the relative infectivity of humans with different parasitaemias (parasite burden) in Ethiopia. The data show similar trends to those seen in Brazilian dogs (Box 1), and the corresponding model analysis attributed between 53% and 79% of all sandfly infections to the top 3.5% most-infected people, suggesting that intervention strategies should focus on individuals with high parasitaemia. Xenodiagnostics to determine relative infectivity on the ISC have not yet been undertaken; however, the models of Miller et al. and Courtney et al. [28, 71, 74] can be used as a basis for incorporating relative infectiousness of different human disease states into models before the outcomes of future human/sandfly xenodiagnostic studies are known.

Box 4. Outstanding questions

- How infective are individuals with asymptomatic or PKDL infection?
- How large is this potential infectious reservoir? How do we detect and/or diagnose them?
- What is the best intervention or combination of interventions when targeting sustainable elimination?
- How can we establish elimination, given the potentially huge asymptomatic population?

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APPENDIX A.

Supplementary data

Supplementary data associated with this article can also be found in the online version, at http://dx.doi.org/10.1016/j.pt.2015.03.007.

This supplementary information contains a variety of more detailed information to support the main text. It is in 4 sections:

1. Literature review methodology
2. Model flow diagrams for different modelling papers
3. Further details on current and future diagnostics
4. Parameter values in different modelling papers

1. Literature review methodology

Two independent searches were conducted under this review to ensure all relevant modelling papers were found. Whilst the review focuses on the case of the ISC, other modelling papers including those pertaining to zoonotic VL were included. Papers had to contain original mathematical transmission models of VL and many exclusions were made because they were:

- models of cutaneous (rather than visceral) leishmaniasis
- experimental models
- statistical models with no mechanistic framework
- not original research articles.

Under one search the search engine Web-of-Science was used in conjunction with the search terms: ('visceral leishmaniasis' OR 'canine leishmaniasis' OR 'kala azar') AND ('mathematical model' OR 'R_0' OR 'R0' OR 'basic reproduction number' OR 'basic reproductive ratio' OR 'transmission dynamics') Each article found was checked for relevance via title and abstract screening and for those that met the criteria, the full-text was examined for eligibility.
Under the second search, Medline OvidSP, Web-of-Science, PubMed Publisher, Cochrane and Google Scholar were searched using: 'model'/de OR 'biological model'/de OR 'mathematical model'/exp OR 'disease model'/de OR 'disease simulation'/de OR 'nonbiological model'/de OR 'computer model'/de OR 'simulation'/de OR 'computer simulation'/de OR 'theoretical model'/de OR ((disease* OR biolog* OR leishman* OR comput* OR theoret* OR markov OR determin* OR stochast* OR agent* OR compartment* OR epidem* OR mathematic* OR mechanis*) NEAR/11 (model OR simulat*)):ab,ti OR ((simulat* OR microsimulat*) NEAR/3 model*):ab,ti AND ('leishmaniasis'/exp OR 'leishmania'/exp OR leishmani*:ab,ti OR 'black fever':ab,ti OR 'kala azar':ab,ti) NOT ([animals]/lim NOT [humans]/lim) Under this search, duplicates were removed and inclusions/exclusions were performed by two people, then discussed to agree the final papers.

All articles found through the searches in the different databases were compared and duplicates were removed, leaving only unique articles. The titles, abstracts and full texts of the remaining papers were examined separately by two people and were categorized in 'include' and 'exclude' files, based upon specific selection criteria, followed by a comparison of all included articles. Thorough discussions led to a final selection of 23 papers addressing relevant modelling of VL that is summarised in Table 1 of the article.

2. Model flow diagrams for different modelling papers

The complexity of compartmental, deterministic models of VL varies greatly considering how few there are.

Dye and Wolpert's [1] model was the first model of VL on the ISC and uses a basic compartmental model (Figure S1). In the following diagrams for VL progression in humans, red boxes denote those individuals who are infective to sandflies, whilst blue boxes are those considered non-infective. This type of disease progression (known as SIR) has been studied extensively within the epidemiological modelling literature [2]. Although many of the specifics of VL are not included here, Dye and Wolpert found that this type of model could still explain the inter-epidemic periods historically associated with number of KA cases.

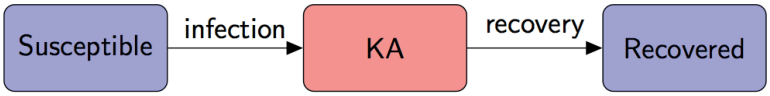

Figure S1. SIR progression model as used by Dye and Wolpert [S1]. Only individuals in the red compartment are infective and contribute to the transmission cycle.

PKDL patients are considered to contribute to the transmission cycle for VL, particularly so in the Sudan where it occurs in up to 60% [3] (rather than 10-20% [4]) of cases. In the models of ELmojtaba *et al.* [5–7], PKDL patients are explicitly included in the compartmental model as a distinct class (Figure S2). By using such a structure the authors were able to infer the relative role of PKDL upon VL transmission by exploring parameters linked to PKDL infected individuals. These included PKDL treatment and probability of developing PKDL following KA.

ELmojtaba *et al*. find that treatment of KA must be effective (at preventing PKDL) otherwise even for high treatment rates of KA, this will increase the numbers with PKDL and do little to alter the total transmission pressure; this is because PKDL patients are assumed as infective as KA patients.

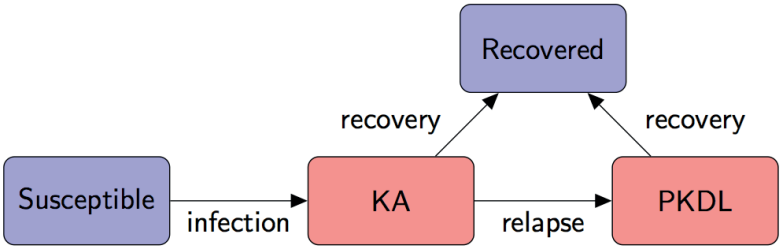

Figure S2. An amended SIR model with an additional class for individuals with PKDL as used by ELmojtaba *et al*. [5–7]. Here both KA and PKDL patients are infective and are therefore denoted by red boxes.

The model of Stauch *et al.* [8–10] is again a compartmental model, but each compartment is linked to the status of diagnostic tests. Each diagnostic test (PCR/DAT/LST) may or may not detect certain stages of VL infection within an individual and so the population is partitioned into categories using this information (Figure S3 and Table S1). The schematic of the actual model (Figure 1 in [8]) contains even more compartments, which include other factors such as first and second line treatment. However, Figure S3 shows roughly how Stauch *et al.* link the stages discussed in the main article with diagnostic test outcomes. As the model is directly influenced by diagnostic tests, and fitted to corresponding available data, the average time individuals stay recovered is just 307 days. This is considered short and is a consequence and limitation of using the LST as a proxy for acquired immunity; whilst such immunity may wane over time, it is generally thought to take much longer [11].

Unlike ELmojtaba *et al.* [5–7], Stauch *et al.* [8–10] also include a dormancy period between KA and PKDL; this is particularly relevant on the ISC where this timescale is longer (up to 3 years rather than 6 months [12]).

Stauch *et al.* conclude that asymptomatic people are key in the transmission of VL, even if they are significantly less infective to sandflies. This is due to the large numbers of non-symptomatics detected in the

population.

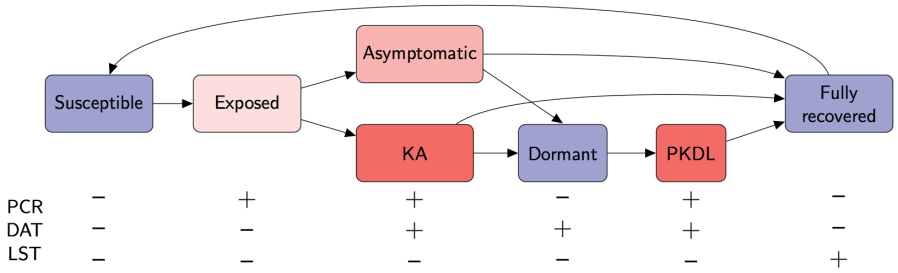

Figure S3. Multi-compartmental progression by Stauch *et al.* [8–10] in relation to diagnostic tests. In this model KA and PKDL patients are assumed to have equal infectivity (red boxes) and additionally, exposed and asymptomatic individuals also contribute to the transmission cycle with infectivity reduced by 40- and 80-fold for asymptomatic and exposed classes respectively (shown in light and very light red boxes). Individuals are assigned a class by diagnostic results and symptoms, e.g. PCR positive, DAT positive, LST negative individuals may be asymptomatic, KA or PKDL which is decided by assessing symptoms.

3. Further details on current and future diagnostics

The ability to accurately diagnose people with VL infection is highly important, not only for detection and treatment programmes, and measuring prevalence, but also for parameterising and fitting models.

Table S1 summarises the key features of diagnostic tests in particular focusing on which stages they may detect, the accuracy and the tests linked to the Stauch model.

Although some tests may detect asymptomatics, it is unclear what sensitivity and specificity values are for these individuals. Consequently all values in the table are given for KA patients on the ISC.

Table S1. Diagnostic tests used in the detection/confirmation of KA

Test	Full name	Notes	Sensitivity	Specificity
PCR*	Polymerase chain reaction	Detects early to late stage infection (including asymptomatics). More sensitive to asymptomatic infection that other tests	Sensitivity and specificity depend on tissue type ([13] gives all of these for KA patients). Example: blood 92.3% (88.4 - 94.9) [13]	63.3% (53.9 - 71.8) [13]
DAT*	Direct agglutination test	Detects late stage infection and early stage recovery	97.1% (94.9 to 98.4) [14]	95.7% (88.1 to 98.5) [14]
LST*	Leishmanin skin test	Detects late stage recovery with "cellular immunity". Measure for immune population.	Varies greatly depending on length of time since KA infection [11, 15]	
	Parasitological diagnosis, splenic aspirate	"Gold standard" test	Spleen 95%, Bone marrow 60-85% [16]	100% [16]
Rk39		Rapid diagnostic test (RDT)	97.0% (90.0 - 99.5) [17]	92.4% (85.6 - 96.8) [17]

* used in the Stauch *et al.* model [8–10]

4. Parameter values in different modelling papers

Table S2. Human parameter values used in dynamic VL models. Durations exponentially distributed unless otherwise stated

Parameter (average times given)				
Life expectancy (years)	27	68	60	40
Duration of latent period (days)	0	0	120*	72**
Duration of dormancy period (months)	-	0	-	21
Time until natural recovery from PKDL (days)	-	180	-	-
Probability of PKDL following KA	-	0.64	-	0.0315***
Time until KA-induced mortality (days)	384	91	-	152
Time from recovery until loss of seropositivity (days)	-	-	-	307
Time from KA to 1st treatment completed (days)	-	1.1 to 12.5	115*	30
Time from 1st to 2nd treatment completed (days)	-	-	-	30
Time from PKDL to treatment completed (days)	-	30	-	180
Probability of non-private treatment	-	-	0.12	-
Probability 1st treatment-induced mortality	-	-	-	0.05
Probability 2nd treatment-induced mortality	-	-	-	0.05

¢ = similar values are found in the other papers by these groups
- = not applicable for this model
* = Erlang distributed
** = this is the average time spent PCR or DAT positive before onset of symptoms and Erlang distributed
*** = either with or without second treatment and conditional upon survival

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CHAPTER 5

Feasibility of eliminating visceral leishmaniasis from the Indian subcontinent: explorations with a set of deterministic age-structured transmission models

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Abstract

Background: Visceral leishmaniasis (VL) is a neglected tropical disease transmitted by sandflies. On the Indian subcontinent (ISC), VL is targeted for elimination as a public health problem by 2017. In the context of VL, the elimination target is defined as an annual VL incidence of <1 per 10,000 capita at (sub-)district level. Interventions focus on vector control, surveillance and on diagnosing and treating VL cases. Many endemic areas have not yet achieved optimal control due to logistical, biological as well as technical challenges. We used mathematical modelling to quantify VL transmission dynamics and predict the feasibility of achieving the VL elimination target with current control strategies under varying assumptions about the reservoir of infection in humans.

Methods: We developed three deterministic age-structured transmission models with different main reservoirs of infection in humans: asymptomatic infections (model 1), reactivation of infection after initial infection (model 2), and post kala-azar dermal leishmaniasis (PKDL; model 3). For each model, we defined four sub-variants based on different assumptions about the duration of immunity and age-patterns in exposure to sandflies. All 12 model sub-variants were fitted to data from the KalaNet study in Bihar (India) and Nepal, and the best sub-variant was selected per model. Predictions were made for optimal and sub-optimal indoor residual spraying (IRS) effectiveness for three different levels of VL endemicity.

Results: Structurally different models explained the KalaNet data equally well. However, the predicted impact of IRS varied substantially between models, such that a conclusion about reaching the VL elimination targets for the ISC heavily depends on assumptions about the main reservoir of infection in humans: asymptomatic cases, recovered (immune) individuals that reactivate, or PKDL cases.

Conclusions: Available data on the impact of IRS so far suggest one model is probably closest to reality (model 1). According to this model, elimination of VL (incidence of <1 per 10,000) by 2017 is only feasible in low and medium endemic settings with optimal IRS. In highly endemic settings and settings with sub-optimal IRS, additional interventions will be required.

Background

On the Indian subcontinent (ISC), visceral leishmaniasis (VL) is caused by the protozoan *Leishmania donovani*, which is transmitted by the peri-domestic female sandfly, *Phlebotomus argentipes*. VL is a neglected tropical disease (NTD) [1] with about 300 million people at risk globally, mainly affecting the poorest of the poor in rural areas. Two thirds of the estimated global 200,000 to 400,000 new VL cases per year occur on the ISC [2]. Furthermore, over 20,000 deaths per year on the ISC are attributed to VL, making it the deadliest parasitic infection in the world after malaria [3, 4]. Humans are considered the only host for *L. donovani* on the ISC, whereas in the rest of the world VL is both anthroponotic and zoonotic, and can also be caused by *L. infantum* [3]. Only a small fraction of the people that become infected develop clinical symptoms, while most remain asymptomatic, nonetheless carrying the parasite [5]. People that develop symptoms of VL, also known as kala-azar (KA), display signs of fever, weight loss, anaemia and splenomegaly, and eventually die if left untreated [6, 7]. It is estimated that about one to five percent of successfully treated VL cases on the ISC develop post-kala-azar dermal leishmaniasis (PKDL), a self-healing skin disease which may last for several years [8–10]. *L. donovani* infection can be diagnosed by – among other methods –testing of peripheral blood for parasite DNA by means of polymerase chain reaction (PCR), and by testing for antibodies using the direct agglutination test (DAT, a marker for humoral immune response indicating current or recent infection).

Even though attention for VL has grown over the past decade, its transmission dynamics are still not completely understood. For instance, little is known about the role and duration of acquired immunity after infection, the infectiveness of different disease stages towards the sandfly, and natural sandfly behavior. The observation of low and infrequent numbers of symptomatic VL cases, which by themselves are not sufficient to sustain transmission, suggests the presence of a parasite reservoir, which is also supported by high proportions of PCR+ individuals [11]. Even though the parasite has been found in domestic animals, their role in transmission on the ISC has not been established [12], and therefore humans remain the only confirmed reservoir of the parasite on the ISC. Potential human reservoirs of infection (apart from the low number of symptomatic cases)

are asymptomatic infections, persons in whom a past infection reactivates, PKDL cases, or a mixture of these.

In 2012, WHO developed the first NTD 2020 Roadmap that contains targets for the elimination and control of VL [13]. That same year, the London Declaration was signed by several partners from the public and private sector, to support the 2020 WHO Roadmap targets through advocacy, pharmaceutical supplies and research funding [14]. On the ISC, the target is to eliminate VL as a public health problem by or before the end of 2017, where elimination is defined as an annual incidence of VL of <1 per 10,000 capita at sub-district-levels in Bangladesh and India; and at district-level in Bhutan and Nepal [15]. In the rest of the world, the WHO target is 100% detection and treatment of all VL cases. In the ideal situation of meeting the WHO targets for VL, the global impact (relative to the counterfactual had the pre-control situation in the year 1990 continued unabated) has been estimated at 2.4 million averted deaths, 140 million averted DALYs, and about 20 billion US dollars saved between 2011 and 2030 [16, 17].

The governments of the ISC-countries have committed themselves to achieving the elimination target by implementing different interventions. These are mainly focused on two approaches: (1) early diagnosis of symptomatic cases followed by effective case management, which prevents disability and death, and reduces the presence of infective individuals; and (2) vector control to reduce or interrupt transmission [3]. Indoor residual spraying (IRS) of human dwellings and cattle sheds with long lasting insecticides such as DDT is currently the most important and widely implemented form of vector control. To a lesser extent, insecticide–treated bed nets, environmental management and personal protection are also being implemented [18, 19]. Although indoor spraying campaigns on the ISC have been scaled up over the last years, not all regions have yet achieved effective IRS programs due to various challenges such as limited training of spraying teams, poor community acceptance, sandfly resistance to DDT, and the peri-domestic lifestyle of the sandfly [19–24].

Here, we focus on the following research question: is it technically feasible to achieve the WHO VL elimination targets on the ISC by 2017 with current IRS strategies and ongoing detection and treatment of cases? To this end, we upgraded the most relevant existing deterministic VL transmission model [25, 26], and developed three age-structured deterministic models representing

three potential main parasite reservoirs in humans: (1) asymptomatic cases, (2) recovered (immune) individuals in whom infection reactivates, and (3) cases of PKDL. For each model, we defined four sub-variants with different transmission dynamics: fixed or age-dependent sandfly exposure and a duration of the late recovered 'immune' stage of two or five years. All twelve models were quantified using data from the KalaNet study in Bihar (India) and Nepal [27, 28]. With the best sub-variant of each of the three models, we simulated the impact of IRS (optimally and sub-optimally implemented) on VL incidence for three endemic settings to predict the feasibility of achieving the elimination target of <1 VL case per 10,000 capita per year on the ISC.

Methods

Model structure

We developed a set of three VL transmission models, each with four sub-variants, based on the general structure of a previous model developed by Stauch and colleagues at Tuebingen University [25, 26]. In all models (see Figure 1 for schematic representation), we assume that humans are born *susceptible* and, when bitten by an infective sandfly, will move to the stage of *early asymptomatic infection*. We assume that individuals in this stage test positive for parasite DNA using PCR (PCR+), and test negative for antibodies using the direct agglutination test (DAT-). After some time, an infected person will develop antibodies and advance to the stage of *late asymptomatic infection* (PCR+/DAT+). A small fraction of cases with late asymptomatic infection will develop symptoms of VL and enter the stage of *symptomatic untreated* (PCR+/DAT+). While most symptomatic cases will require one or two treatment regimens (stages of *first-line* and *second-line treatment* (PCR+/DAT+), initiated after a detection delay) to clear infection to the extent that parasite DNA is no longer detectable (*putatively recovered* stage, PCR-/DAT+), a small fraction of untreated symptomatic cases will spontaneously clear infection and directly advance to the putatively recovered stage (i.e. non-fatal symptomatic cases that do not turn up in surveillance data because of low severity of disease) [29]. All symptomatic cases are assumed to be at excess risk of dying from VL, with the excess risk being highest in untreated cases. From the putatively recovered stage,

a small fraction of individuals may develop *PKDL* (PCR+/DAT+) from which they will eventually recover (spontaneously or with treatment; the exact mechanism of recovery is not specified in the model). However, the majority of cases in the putatively recovered stage advance to the *early recovered* stage (PCR-/DAT+), along with recovered cases of PKDL, and the majority of late asymptomatic infections that do not develop any symptoms and spontaneously clear infection to the extent that parasite DNA is no longer detectable. Eventually, individuals in the early recovered stage will lose their DAT positivity, and enter the *late recovered* stage (PCR-/DAT-), during which they are still immune to new infections. From there, individuals either lose their immunity and become susceptible again to infection through exposure to infective sandflies (model 1), or their past infection reactivates such that they re-enter the stage of early asymptomatic infection without requiring exposure to an infective sandfly (model 2). Model 2 presents a hypothetical but biologically plausible scenario, for example when individuals experience decreased immune-competence during malnutrition or co-infection (e.g. HIV) [30]. In terms of structure, model 1 is the most similar to the model by Stauch *et al* [25].

In each model, infection is transmitted between humans by bites of female sandflies (we do not consider male sandflies, which only feed on plant sugars). We define the sandfly population in terms of sandflies per human, a quantity that incorporates sandfly density, the unknown ratio of blood meals taken on human and animals, and the unknown (average) vector competence of sandflies. The sandfly population is partitioned into 3 compartments; all sandflies are born *susceptible* and after feeding on an infective human, they become *infected* with some probability depending on the infectiveness of the human stage of infection. After an incubation period, infected sandflies become *infective* and may infect susceptible humans. We assume no excess mortality among infected sandflies. IRS is assumed to reduce the sandfly density and consequently, human exposure to sandfly bites.

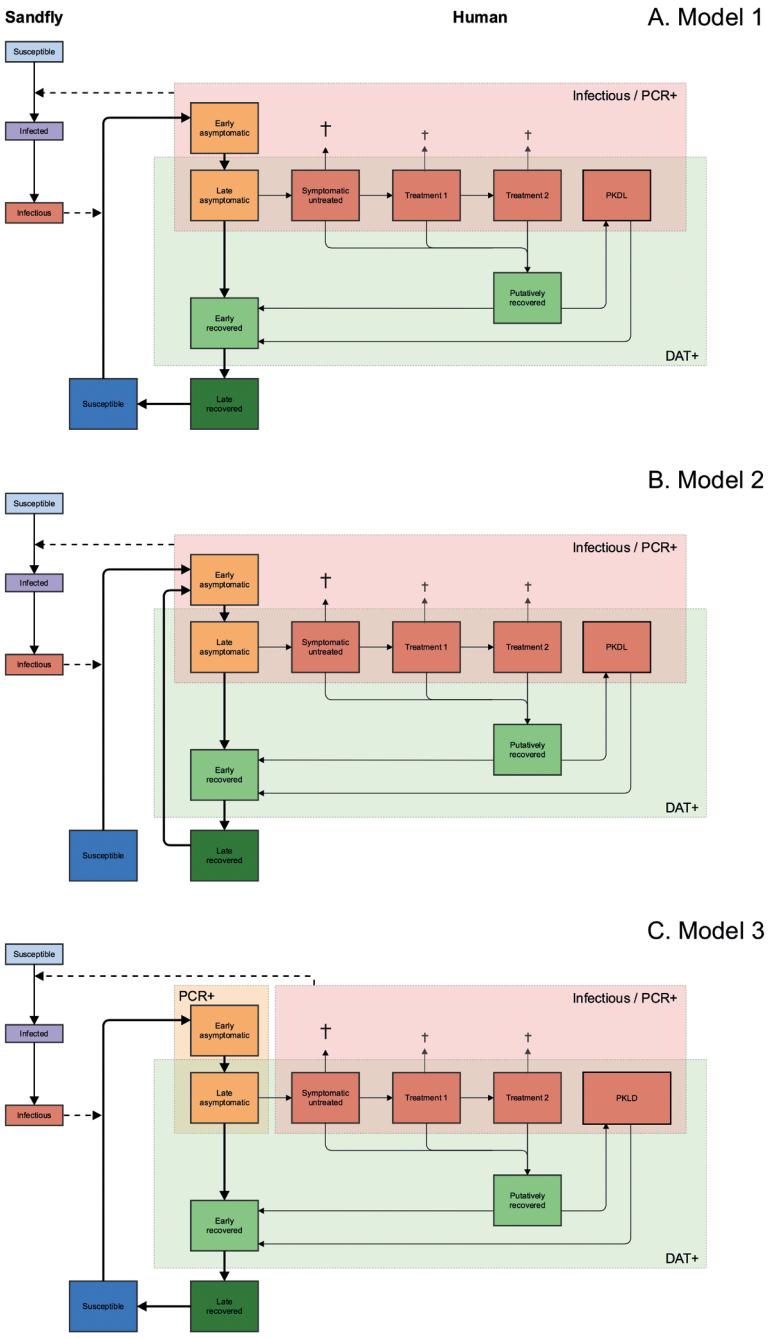

Figure 1. Schematic representation of three model structures. In model 1 (A), recovered individuals eventually lose their immunity and become susceptible again to infection through exposure to infective sandflies. In model 2 (B), recovered individuals may experience reactivation of their past infection such that they directly re-enter the stage of early asymptomatic infection without requiring exposure to infective sandflies. In model 3, which is identical in structure to model 1(C), only cases of symptomatic infection and PKDL contribute to transmission of infection, and duration of PKDL is three times as long as in model 1.

In models 1 and 2, all PCR+ human stages (asymptomatic and symptomatic infection, and PKDL) are considered to be infective towards sandflies, with early asymptomatic cases being half as infective as late asymptomatic cases (as assumed by Stauch *et al* [25]). Infectiveness of untreated clinical cases is set at 1.0, treated patients and PKDL have an infectiveness of 0.5, and that of asymptomatic cases is estimated. In model 3, which is identical in structure to model 1, only cases of symptomatic infection and PKDL are assumed to contribute to transmission [31], with PKDL having a higher (estimated) infectiveness than in models 1 and 2. Further, in model 3 we set the duration of PKDL to thrice as long as in model 1, based on expert opinion, assuming that there is a larger spectrum of PKDL severities than currently recognized, of which undiagnosed forms also contribute to transmission. Model 3 can be considered an extreme variant of model 1. A model variant in which only symptomatic human cases (VL and regular PKDL) are infective towards the sandfly, could not be fitted to data on the infection prevalence in sandflies under the assumptions of an endemic equilibrium and homogeneous mixing of human and sandfly populations (Additional File 1, section 5). This indicates that, in order to meet the infection prevalence in sandflies (Table A1-2 in Additional File 1, section 3), there has to be an additional reservoir of infection in humans that are PCR+, which could be in asymptomatic individuals (models 1 and 2), or in long lasting PKDL cases (model 3).

The transmission model was defined in terms of a system of ordinary differential equations (ODE; see Additional File 1, section 2). Hence, we assumed that all transitions between stages take place at constant rates, leading to exponentially distributed durations of stages. However, because the human demography on the ISC cannot be well approximated by the assumption of a stable human population size and exponential human survival (as applied by Stauch *et al*), we allowed for human population growth and age-specific human mortality (i.e. by stratifying the system of ODEs into annual age categories). The number of sandflies per human is assumed to be stable during human population growth and in absence of vector control.

Parameter quantification

Assumptions about human demography, excess mortality, duration of symptomatic stages of infection, and sandfly biology were based on literature

and published data sources (Table 1) [25, 32–39]. Note that for model 3, the duration of PKDL is assumed to be 15 years instead of 5 years (models 1 and 2). Next, for each model we defined four sub-variants in terms of assumptions about the duration of the late recovered stage and age-patterns in exposure to sandfly bites. The duration of the late recovered stage was chosen to be two or five years, which were reasonable values, given that the analytical solution of the system of ODEs at equilibrium showed that all three models could only support the data for durations of the late recovered stage less than seven years (Additional File 1, section 5). With regard to age-patterns in exposure to sandfly bites, we assumed that exposure is either fixed, or increases proportionally with body surface area (i.e. a linear increase in sandfly exposure between age 0 to 20 followed by a constant exposure from age 20 onwards). The latter assumption has also been previously used to model the vector-borne diseases onchocerciasis and lymphatic filariasis [40–42].

Remaining model parameters (sandflies per human, duration of asymptomatic stages of infection, infectiveness of human stages of infection, and proportion of asymptomatic infections that develop symptoms of VL) were estimated based on data from the KalaNet study, a community-based intervention trial in hyper-endemic clusters in Bihar, India, and in the Terai plains in Nepal [27, 28, 43]. The KalaNet data constitute cross-sectional information on DAT status of 21,204 individuals from three time points spanning two years, and information on incidence of VL during the entire two-year study period. For 668 individuals aged 14 and older, PCR testing was performed as well. Further, a subset of individuals were covered in consecutive cross-sectional surveys, allowing derivation of changes in PCR and DAT status. To quantify our model, we used prevalence of DAT-positivity (titre > 1:800, like Stauch *et al* [25]), PCR-positivity, PCR andDAT-positivity, incidence of VL and incidence of PCR-positivity (i.e. a change from PCR-negative to positive between two consecutive years), and the prevalence of *L. donovani* in sandflies in Nepal [43] (which in the model we take to be the proportion of sandflies that is infective, like Stauch *et al* [25]). An overview of these data is provided in Table A1-2 in Additional File 1, section 3. In the main analysis, we assume that observed levels of PCR and DAT-positivity adequately reflect prevalences of the corresponding stages of infection in our model. The importance of imperfect test sensitivity and specificity was

explored using analytical solutions of the equilibria of the system of ODEs (Additional File 1, section 5). We fitted model parameters to country-specific, population-level data, aggregated over years, villages, age, and sex. Because we used an age-structured model, we could take account of the fact that the PCR data were sampled from a sub-population aged 14 years and older, while data on DAT-positivity and VL incidence were sampled from the whole population (in contrast to Stauch *et al* [25], who analyzed the KalaNet data as one homogeneous entity).

Model parameters were fitted in two steps. First, we quantified model parameters with regard to duration of stages of asymptomatic infection, fraction of asymptomatic cases that develop VL, and the number of sandflies per human, conditional on preliminary assumption about infectiveness of human stages of infection (which is only determined by the prevalence of infection in sandflies, and can therefore be solved separately, see Additional File 1). The system of ODEs was solved numerically using the *deSolve* package [44] in R (version 3.2.0) [45], and parameters were estimated within a maximum likelihood framework (ignoring the clustered study design, just like Stauch *et al* [25]), using the BFGS algorithm from the *optim* package. Prior to every evaluation of the optimization algorithm we let the model reach equilibrium, assuming that the KalaNet data represent an equilibrium situation. Second, we analytically solved the system of ODEs with regard to infectiveness of human stages of infection and the number of sandflies per human, given data on prevalence of infection in sandflies in Nepal (for approach, see Additional File 1). The proportion of putatively recovered cases that develop PKDL was set to 5% such that the predicted PKDL prevalence for endemic villages in Nepal in models 1 and 2 was 5 per 10,000 population, which corresponds to the 4.4 to 7.8 per 10,000 that has been reported for Nepal [10]. Last, for each model we selected the best sub-variant based on the log-likelihood with regard to age-patterns in prevalence of infection markers and incidence of VL and PCR-positivity.

Predicting the impact of IRS

With each best sub-variant of model 1, 2, and 3, we simulated a high, medium, and low endemic setting, defined in terms of pre-IRS VL incidence of 20 per 10,000, 10 per 10,000 and 5 per 10,000 per year, respectively. These endemic settings were chosen given the declining trends in VL cases

Table 1. Overview of assumptions and pre-set parameters. The parameter values listed here are the same for all three models and their sub-variants, unless indicated otherwise.

Parameters	Value[a]	Source
Human birth rate (per 1000 capita, α_H)	21 (Indian crude birth rate in 2011)	[32]
Human mortality rate (μ_H)	Age-dependent (Indian mortality rates in 2011)	[33]
Average duration of late recovered stage (years, $1/\rho_{RHC}$)	2 or 5	Pre-set
Average duration of symptomatic untreated stage (days, $1/\rho_{IHS}$)	30 (fitting) and 45 (predicting)	Unpublished data
Average duration of symptomatic treatment 1 (days, $1/\rho_{IHT1}$)	30 (fitting) and 2.5 (predicting)	[34]
Average duration of symptomatic treatment 2 (days, $1/\rho_{IHT2}$)	30 (fitting) and 10 (predicting)	[35]
Average duration of putatively recovered stage (months, $1/\rho_{IHT}$)	21	[36]
Average duration of PKDL (years, $1/\rho_{IHL}$)	5 (models 1 and 2) and 15 (model 3)	Expert opinion (EH and MB)
Infectiveness of symptomatic untreated cases (p_{IHS})	1.0	Reference value
Infectiveness of patients under treatment 1 and 2 (p_{IHT1}, p_{IHT2})	0.5	Expert opinion (EH and MB)
Infectiveness of PKDL cases (p_{IHL})	0.5 (models 1 and 2 only; estimated for model 3)	Expert opinion (EH and MB)
Fraction of untreated symptomatic cases that spontaneously, putatively recover (f_H)	0.03	[25]
Excess mortality rate among untreated symptomatic cases (per day, μ_K)	1/150	Assumption
Excess mortality rate among treated symptomatic cases (per day, μ_{KT})	1/150 + 1/600 = 1/120 (fitting) and 1/150 (predicting)	[34, 35]
Fraction of failed first-line treatments (f_F)	0.05	[37]
Fraction of putatively recovered cases that develop PKDL (f_L)	0.05 (set such that models 1 and 2 predicted a prevalence of PKDL between 4.4 and 7.8 per 10,000 capita in India)	[10, 38]
Average life expectancy of the sandfly (days, $1/\mu_F$)	14	[39]
Average duration of incubation period in sandflies (days, $1/\rho_{EF}$)	5	[62]
Sandfly biting rate (per day, β)	1/4	[63]
Transmission probability sandfly to human (p_H)	1.0[b]	Reference value

[a] Parameter values marked with "fitting" only apply to the KalaNet study setting and were therefore only used when fitting the models to the KalaNet data; related to this, different parameter values were used when predicting the impact of IRS (indicated by "predicting"). [b] The probability that a susceptible person becomes infected when bitten by an infectious sandfly is assumed to be 1; potential overestimation is compensated by the estimated sandfly density per human.

and the fact that VL incidences of 20 cases per 10,000 capita per year (as observed in the KalaNet setting) are currently rarely observed [46, 47]. Each endemic setting was quantified by tuning the number of sandflies per human, assuming that transmission dynamics are in equilibrium with current detection and treatment interventions (which are slightly different from those in the KalaNet situation; see Table 1). We simulated the impact of IRS strategies as planned for India, i.e. two spraying rounds per year targeting houses and cattle sheds in endemic villages [18]. We assumed that optimally implemented IRS (*optimal IRS*) results in a continuous reduction in sandfly density of approximately 63%, given the reported reduction in sandfly density after IRS with dichlorodiphenyltrichloroethane (DDT) of 72% [48] and the assumption that rotating spraying teams continuously cover households 85%-95% of the time. Sub-optimally implemented IRS (*sub-optimal IRS*) was assumed to be half as effective due to lower continuous household coverage, sub-optimal spraying techniques and sandfly resistance to DDT [19–23], leading to a continuous sandfly density reduction of 31.5%. We interpreted the WHO elimination target in our model as an annual incidence of VL cases (receiving treatment) of <1 per 10,000 capita.

In a sensitivity analysis for predicted trends in VL incidence during IRS, we varied the values of key estimated and assumed parameter values by factors 4/5 and 5/4 (except for the number of sandflies per human, as this parameter mainly influences predicted trends in VL incidence through pre-IRS infection levels).

Results

All four sub-variants of all three models could closely reproduce the country-specific, population-level incidence and prevalence data, with deviances ranging between 2.11 and 2.61 (χ^2 degrees of freedom = 8, $p >> 0.5$). All model sub-variants estimated the duration of early asymptomatic infection (PCR+/DAT-) at around 1.1 years and the duration of late asymptomatic infection (PCR+/DAT+, excluding cases with symptoms) at just under four months. Estimates for the proportion of asymptomatically infected cases that develop VL (range 2.8–3.9%), infectiveness of early and late asymptomatic infection (0.014–0.018 and 0.027–0.035, respectively, model 1 and 2 only),

Table 2. Quantified parameter values of the twelve model variants. The colours represent the model sub-variants that best reproduced the age-structured prevalence and incidence data. See Additional File 2 for illustrations of fitting of all model variants to all data and Figure 2 for the predicted and observed age-patterns in VL incidence and DAT prevalence in India and Nepal with the selected model variants.

	Model 1				**Model 2**				**Model 3**			
Pre-set exposure to sandflies	Fixed		Age-dependent		Fixed		Age-dependent		Fixed		Age-dependent	
Pre-set duration of late recovered stage (years, $1/\rho_{RHC}$)	2	5	2	5	2	5	2	5	2	5	2	5
Duration of early asymptomatic (days, $1/\rho_{IHP}$)	383	384	382	384	390	387	392	387	384	384	383	385
Duration of late asymptomatic (days, $1/\rho_{IHD}$)	137	137	136	137	140	138	141	138	134	134	133	134
Duration of early recovered (days, $1/\rho_{RHD}$)	399	369	482	431	540	490	584	539	400	370	484	433
Fraction late asymptomatic to symptomatic (f_S)	2.91%	2.76%	3.33%	3.08%	3.63%	3.38%	3.85%	3.63%	2.91%	2.76%	3.34%	3.09%
Relative infectiveness of early asymptomatic (p_{IHP})	0.0144	0.0136	0.0144	0.0136	0.0176	0.0166	0.0150	0.0145	0*	0*	0*	0*
Relative infectiveness of late asymptomatic (p_{IHD})	0.0288	0.0273	0.0288	0.0272	0.0353	0.0332	0.0300	0.0290	0*	0*	0*	0*
Relative infectiveness of PKDL (p_{IHL})	0.5*	0.5*	0.5*	0.5*	0.5*	0.5*	0.5*	0.5*	2.72	2.70	2.32	2.32
Number of sandflies per human India ($N_{E,\text{India}}$)	0.301	1.136	0.269	0.972	0.027	0.075	0.031	0.101	0.298	1.123	0.262	0.898
Number of sandflies per human Nepal ($N_{E,\text{Nepal}}$)	0.197	0.276	0.172	0.249	0.021	0.038	0.023	0.041	0.197	0.276	0.172	0.249

**** Pre-set values (dependent on model structure)***

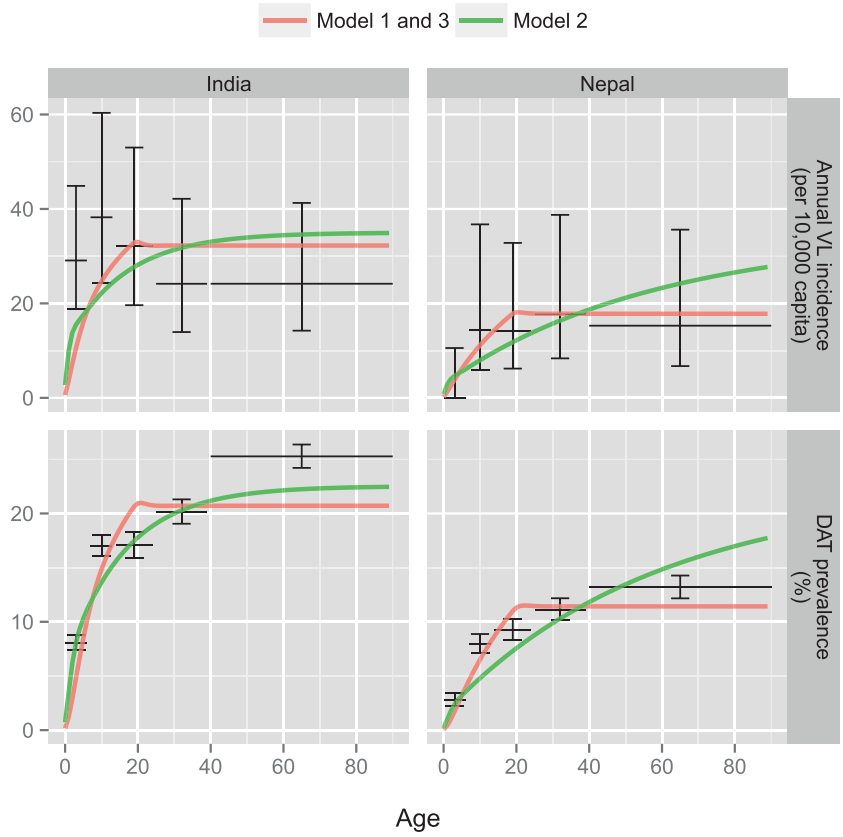

Figure 2. Predicted and observed age-patterns in VL incidence and DAT prevalence in India and Nepal. Coloured lines represent model predictions from the sub-variant of each of the three models that best fit age-patterns in human infection markers; black bullets represent the data per age group; horizontal lines indicate the age range for each data point; vertical lines represent 95%-Bayesian credible intervals, given total raw sample sizes (i.e. not accounting for clustering, see Additional File 1 for sample sizes). See Additional File 2 for illustrations of the fit of all model sub-variants to all data types.

infectiveness of PKDL (2.32–2.72, model 3 only), and duration of the early recovered stage (1.0 to 1.7 years; PCR-/DAT+, excluding putatively recovered people) slightly varied between models and sub-variants (i.e. assumptions about age-dependent exposure to sandfly bites and duration of the late recovered stage). All fitted parameter values are presented in Table 2.

Given the parameter estimates above, the most common infection history for a person to go through (susceptible, asymptomatically infected, and early recovered without ever developing VL) takes on average about 2.7 to 3.1 years (not including the duration of the late recovered stage, which we

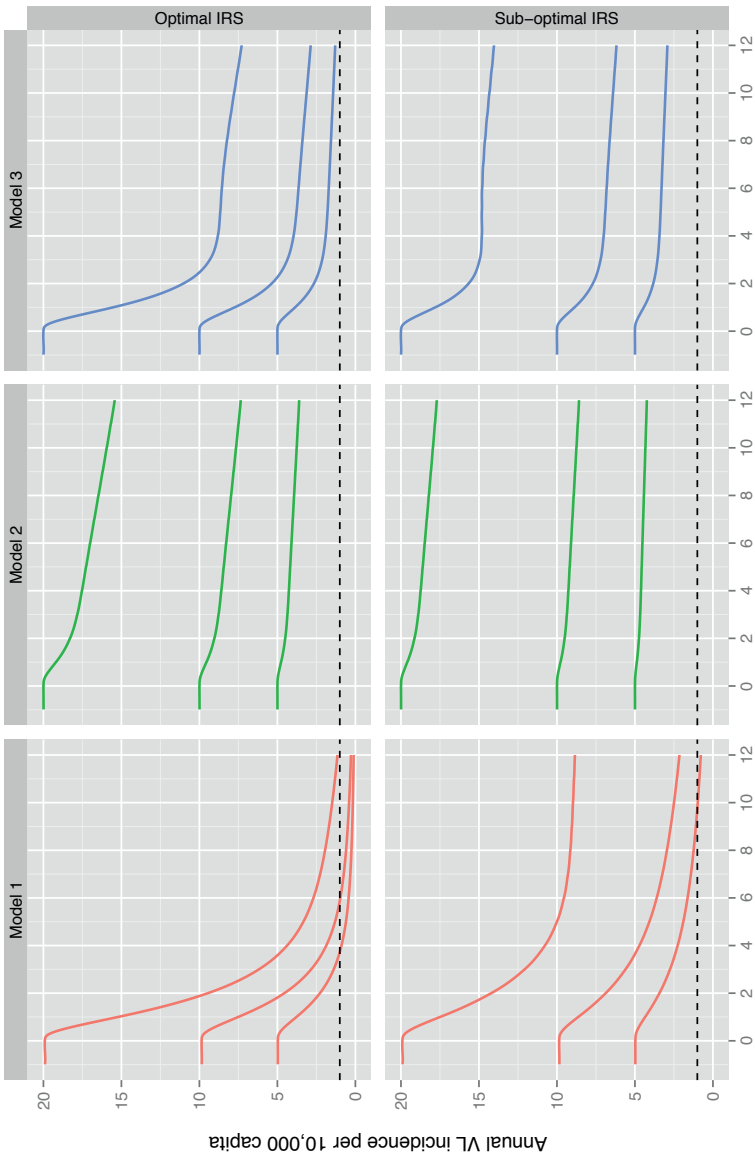

Figure 3. Predicted impact of optimal and sub-optimal IRS on VL incidence for three endemic settings. IRS is assumed to start in the year zero. Lines within plots represent different pre-IRS endemic settings (high: 20/10,000, medium: 10/10,000, low: 5/10,000); the dotted line represents the target VL incidence of <1 per 10,000 capita. Model predictions were made with the sub-variant of each of the three models that best fit age-patterns in human infection markers. See Additional File 3 for the short and long-term impact of optimal and sub-optimal IRS in low, medium, and highly endemic settings with all model sub-variants.

assume to be either two or five years). This is in line with the observation that only 6 out of 668 subjects who were tested with PCR were positive in year 1, negative in year 2, and again positive in year 3. All three models predicted that in a state of endemic equilibrium about 10% of all transmission of infection is generated by VL cases (treated and untreated). According to models 1 and 2, an additional 8% of transmission is generated by PKDL cases and the remaining 82% by asymptomatically infected cases. In model 3, 90% of transmission is generated by PKDL cases (and none by asymptomatic infections, by default).

The sub-variants of models 1 and 3 that best reproduced the age-specific data were based on the assumptions of age-dependent exposure to sandflies and a duration of late recovered stage of two years; for model 2, the sub-variant with fixed exposure to sandflies and duration of the late recovered stage of five years best fitted the data. Figure 2 illustrates the fit of the best sub-variants to the age-specific data on VL incidence and DAT prevalence, with identical fits for model 1 and 3. Fits to other data types (PCR incidence, PCR prevalence, PCR/DAT prevalence) and fits for all model sub-variants can be found in Additional File 2.

Using the best sub-variant of each model, we predicted the impact of optimal and sub-optimal IRS on VL incidence for high, medium and low endemic settings (Figure 3). Models 1 and 3 predict that optimal IRS (63% assumed reduction in sandfly density) reduces VL incidence by about 25% in the first year and by another 25% of the original incidence level in the second year after the start of IRS, irrespective of the endemicity level at equilibrium. However after two years, the predictions of model 1 and 3 diverge: in model 1, VL incidence keeps on declining due to the rapid depletion of the reservoir of infection in asymptomatically infected cases (average duration of asymptomatic infection of about 1.4 years); in model 3, the reduction in VL incidence slows down strongly after two years due to the presence of the relatively large reservoir of infection in PKDL-cases (average duration of 15 years). Model 2 predicts a relatively slow and stable decline from the start of IRS, as the decrease in sandfly density is assumed to have no influence on VL cases arising from people in whom old infection reactivates.

Model 1 predicts that about 4 to 6 years of optimal IRS will reduce the annual VL incidence in low and medium endemic settings to levels (just) under 1 per 10,000 capita. However, models 2 and 3 predict that these low

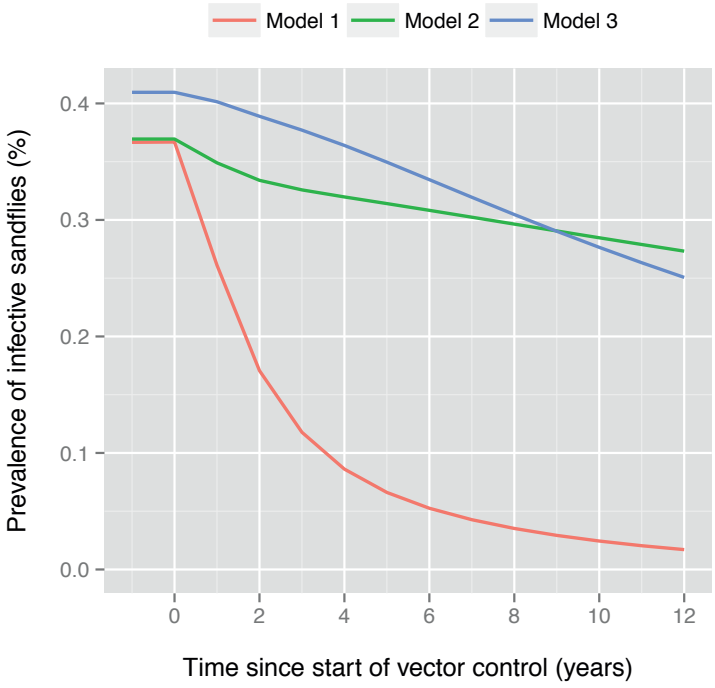

Figure 4. Predicted prevalence of infective sandflies during IRS. Pre-IRS prevalence levels of infective sandflies represent a setting with 10 annual VL cases per 10,000 capita. IRS is assumed to start in the year zero, and to be implemented optimally (63% reduction in sandfly density). The three colored lines represent the sub-variant of each of the three models that best fit age-patterns in human infection markers. See Additional File 4 for low, medium and highly endemic settings with optimal and sub-optimal IRS.

levels of VL incidence cannot even be achieved within 12 years of optimal IRS. Similarly, model 1 predicts that with sub-optimal IRS, these levels of VL incidence are only achieved after about 10 years, and only in low endemic settings. Still, when IRS is continued over an extremely long period of time (say 200 years), most sub-variants of the three models predict that optimal IRS will eventually result in elimination in all endemic settings (Additional File 3). Sub-optimal IRS will only lead to reaching the target in low and medium endemic settings, with varying durations of IRS required per model. Additional File 3 also illustrates that for model 1 (and 3 to a lesser extend), the predictions depend on the duration of the late recovered stage in high endemic settings and with sub-optimal IRS: longer (5 year) duration leads to a slower decline in VL incidence, and a faster re-occurrence of infection. For model 2, the duration of the late recovered stage on the impact of IRS

is negligible. For model 3, the deceleration of the decline in VL incidence is largely a function of the duration of PKDL. A longer duration of PKDL will generate a longer infection pressure towards the sandfly and therefore slow down the decreasing VL incidence.

Figure 4 illustrates trends in prevalence of infective sandflies (among caught sandflies) for a medium endemic setting with optimal IRS (see Additional File 4 for low and highly endemic settings). Compared to model 1, models 2 and 3 predict a relatively slow decline in prevalence of infective sandflies because of the persisting parasitic reservoirs of late recovered and PKDL cases, respectively.

Additional File 5 provides an overview of the results of the sensitivity analysis for a medium endemic setting with optimal IRS. Only the assumed effect of IRS (high and low values were 5/4 and 4/5 of the value used in the main analysis) directly influenced predicted trends without changing pre-control infection levels. The duration of IRS required to achieve the elimination target (only relevant in model 1) was most sensitive for the parameter values of the effect of IRS (4 and 9 years until elimination), the duration of the early asymptomatic stage of infection (4 and 8.5 years until elimination), and the proportion of infections that result in symptoms (4.5 and 8 years until elimination). Sensitivity of predicted trends in VL incidence during IRS were strongly associated with changes in pre-control infection levels (i.e. alternative parameter values often produced parallel trends in VL incidence). The predictions by model 3 were most sensitive to the proportion of individuals developing symptoms and PKDL, and the infectiveness and duration of PKDL (illustrated in Additional file 5). The transmission dynamics are insensitive to the assumed infectiveness of early asymptomatic cases relative to late asymptomatic cases (data not shown).

Discussion

We developed three structurally different models with different reservoirs of infection to predict the impact of IRS on VL incidence on the ISC, using the KalaNet dataset from India and Nepal to quantify transmission dynamics in each model. All three models could explain the KalaNet data equally well. However, the predicted impact of IRS varied substantially between models,

such that a conclusion about reaching the VL elimination targets for the ISC heavily depends on assumptions about the main reservoir of infection in humans: asymptomatic cases (model 1), recovered (immune) individuals in whom infection reactivates (model 2), or PKDL cases (model 3). Biologically, a mixture of the different models is most likely, but could not be quantified solely based on the KalaNet data. Still, given that the three models predict markedly different trends of VL incidence and infection in sandflies during IRS, we may be able to express preference for one of the models based on field data regarding the impact of IRS.

So far, only a limited amount of field data on the impact of IRS on VL incidence has been published [49]. Kumar *et al* report that after one year of active IRS in 19 districts of Bihar, VL incidence decreased by 49-100% in 15 districts, and VL incidence was stable or even increased in 4 districts, such that the average reduction in VL prevalence over all 19 districts was about 50%. Based on these findings we tentatively conclude that the models with the infection reservoir in asymptomatic cases (model 1) and PKDL cases (model 3) are probably closer to reality than the model with the disease reservoir in re-activating recovered cases (model 2). Although there is literature on prevalence of infection in sandflies [43, 50, 51] and the impact of IRS on sandfly density [20, 21, 52], unfortunately, there are no published data on the impact of IRS on prevalence of infection in sandflies. Such data would be very valuable to further our understanding of VL transmission dynamics, and distinguish between model 1 and 3 the model that is closest to reality. Still, as model 3 was included as an extreme variant of model 1, we consider model 1 to be the most realistic of our set of models. Currently ongoing initiatives such as the CARE project, that is taking place in Bihar India, [53] are anticipated to provide more data on the long-term impact of IRS on VL incidence and perhaps prevalence of infected sandflies in the field, which will be crucial to validate model predictions and better understand VL transmission dynamics.

The large scale implementation of IRS with DDT in India started in 2005 as part of the national VL elimination program [54], twelve years before the targeted year of VL elimination, 2017. Assuming that model 1 is closest to reality, elimination of VL (incidence <1 per 10,000 capita) is feasible in low, medium and highly endemic settings by means of about four, six and twelve years of optimal IRS, respectively. With sub-optimal IRS, which in some

settings may still be too optimistic, model 1 predicts that the elimination target can only be achieved in low endemic settings within about 10 years. Assuming that in some highly endemic areas IRS was only implemented after the release of the WHO NTD Roadmap and London Declaration in 2012, IRS would have to reduce sandfly densities by at least about 85% to achieve the elimination target in the following 5 years (by 2017). With our assumed 63% reduction in sandfly density by optimal IRS, the elimination target can be achieved within 5 years (i.e. by 2017 if IRS was only implemented in 2012) for settings with an annual VL incidence of up to about 8 per 10,000 capita. The outlook would be much poorer if IRS actually has been implemented sub-optimally. In particular for areas with highly endemic levels, a longer period and/or higher effectiveness of IRS will be required, ideally supplemented by additional interventions, certainly if the level of IRS is sub-optimal. DDT is interpreted to have an insecticidal effect on the sandfly; an insect-repellent effect would have led to a decreased biting rate, with a relatively lower impact on the transmission and VL incidence In the future, the use of DDT is expected to be phased out and replaced by synthetic pyrethroids, due to the increasing sandfly resistance to DDT [23] and its negative environmental impact [55]. In the further future, vaccination may be an important additional tool to eliminate VL on the ISC, should a vaccine become available [56, 57]. Our models provide a tool to explore the potentional impact of future vaccines and identify the target product profiles of vaccines that may achieve the elimination target.

Our study is based on the existing deterministic transmission model that was developed at Tuebingen University by Stauch *et al* [25], but we considerably improved the model in several ways. To better account for the human demography on the ISC, we added population growth and age-specific mortality. The resulting age-structured model further allowed us to better mimic age-patterns in the KalaNet data. This also allowed us to account for the fact that the PCR data in the KalaNet study were collected from a subsample of individuals aged 14 and older. Unlike Stauch *et al*, we purposely did not use data on leishmanin skin testing (LST, which was associated with the late recovered, immune stage), as these LST data did not originate from the same study area. Moreover, the fraction LST positive used and the assumption that early asymptomatic infection (PCR+/DAT-) lasts only 60 days (we estimate 1.1. year) caused the original model to predict a

very short natural history of infection; one cycle of asymptomatic infection, recovery, and loss of immunity was predicted to only take about 450 days, on average. Instead, we chose plausible values for the duration of the recovered, immune stage (two or five years, which could readily support the data as shown by the solutions to the system of ODEs in equilibrium), and used data on PCR incidence and prevalence of PCR and DAT-positivity to inform the model about the duration of the natural history of asymptomatic infection. We further improved the model by fitting our models to country-specific data (India vs. Nepal), and by taking account of the fact that the data on prevalence of infection in sandflies was only collected in Nepal.

Although our model was based on detailed field data, several uncertain factors remained. We interpreted the KalaNet dataset as if it represented an endemic equilibrium. However, in reality repeating small outbreaks of symptomatic cases have been reported to occur [58]. Whether these fluctuations are true outbreaks or simple stochastic variation remains to be clarified, which will require more modelling and detailed longitudinal data. We will investigate this in the future, using an individual-based model (based on the current study) that captures both stochastic and spatial variation. In our analyses, we assume that the KalaNet data represent an endemic equilibrium, which is reasonable given the slow transmission dynamics in all three models; this slowness is not a result of the equilibrium assumption, but due to the large and stable reservoir of infection in asymptomatic individuals (model 1), reactivating past infections (model 2), or PKDL cases (model 3). The KalaNet study included an active case-finding strategy, and although we accounted for a longer duration of the symptomatic untreated stage for our predictions, 45 instead of 30 days, the time between onset of symptoms and treatment could in certain settings be longer. This resulted in an increase in the number of predicted deaths due to VL but hardly influenced the transmission dynamics or the predicted duration until reaching the elimination target. Another potential limitation of our study is that observed levels of PCR and DAT-positivity were assumed to adequately reflect the prevalences of the corresponding stages of infection in the model. In a meta-analysis, Chappuis *et al* found that sensitivity and specificity of DAT testing for the diagnosis of VL were fairly high (about 97.1% and 95.7% respectively)" [59], but these estimates do not necessarily apply to the ascertainment of *L.donovani* asymptomatic infection, as the DAT test was

not validated as such for that purpose. Further, we interpreted the DAT data at the 1:800 titre cut-off (instead of the standard cut-off of 1:1600), which probably increased test sensitivity but decreased specificity. There is little information regarding the sensitivity and specificity of PCR, as there is no gold standard [60]. An exploratory analysis of accounting for imperfect DAT and PCR testing in fitting the KalaNet data showed that predictions for the impact of IRS only vary marginally when using realistic values of sensitivity and specificity (Additional File 1, section 5). Further, the duration of the early asymptomatic stage suggests that the development of detectable antibodies after infection requires about 1 year, which seems relatively long. However, the estimated duration of the early asymptomatic stage was only at most 7% lower when sensitivity of PCR testing was assumed to be as low as 70%. This can be explained by the fact that PCR sensitivity affects PCR prevalence and incidence in the same way (although the effect on incidence is somewhat larger due to the involvement of two measurements). Our estimate of the duration of immunity after clearance of infection (approximately 3 years, of which two year were assumed to be spent in a DAT-negative state), is very similar to that by Chapman *et al* [61], who recently analysed rK39 and LST data from Bangladesh using a Markov model. There are differences in the estimates of the duration of the of asymptomatic stage: 5 months (Chapman *et al*) and 1.5 years in this study, and the percentage of asymptomatic individuals that develop clinical symptoms: 14.7% (Chapman *et al*) and 3.3% in this study. These differences may be well explained by differences in the type of data (geographic region and type of diagnostic tests) and modelling methods used (the use of a full transmission model is the strength of the current study). Lastly, we could only estimate infectiveness of human stages of infection indirectly from the prevalence of infection in sandflies, and only after certain assumptions about the relative infectiveness of clinical cases. Ongoing xenodiagnostic studies and additional longitudinal data on the prevalence of infection in sandflies during interventions are anticipated to further inform the model regarding this aspect.

Conclusions

We conclude that several structurally different models can explain population-level data on VL transmission equally well. Consequently, the predicted impact of IRS strongly depends on assumptions about the reservoir of infection in humans. Data on the impact of IRS available so far suggest one model is probably closest to reality (model 1, where asymptomatic individuals represent the main reservoir of infection). According to this model, elimination of VL (incidence of <1 per 10,000 capita) is probably only feasible by 2017 in low and medium endemic settings with optimal IRS; in highly endemic settings and settings with sub-optimal IRS, additional interventions will be required.

List of abbreviations

DAT : Direct agglutination test
IRS : Indoor residual spraying
ISC : Indian subcontinent
KA : Kala-azar
LST : Leishmanin skin test
NTD : Neglected tropical disease
ODE : Ordinary differential equation
PCR : Polymerase chain reaction
PKDL : Post-kala-azar dermal leishmaniasis
VL : Visceral leishmaniasis
WHO : World Health Organization

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Additional to Supplementary

Additional File 1: This document provides the description, characterisation and calculations of equilibria of a system of ordinary differential equations for three VL transmission models along with data.
Additional File 2: Supplementary figure illustrating the fit of all model sub-variants to all data types (extended version of Figure 2 in the main manuscript).
Additional File 3: Supplementary figure illustrating the long-term and short-term impact of optimal and sub-optimal IRS in low, medium, and highly endemic areas for all model variants (extended version of Figure 3 in the main manuscript), as well as transmission dynamics when infection is introduced in a susceptible human population (outbreak).
Additional File 4: Supplementary figure illustrating the impact of optimal IRS on the prevalence of infective sandflies in low, medium, and highly endemic settings, according to the best sub-variant of each model (extended version of Figure 4 in the main manuscript).
Additional File 5: Supplementary figure illustrating the impact of optimal IRS on incidence of VL in a sensitivity analysis of key estimated and assumed parameter values.

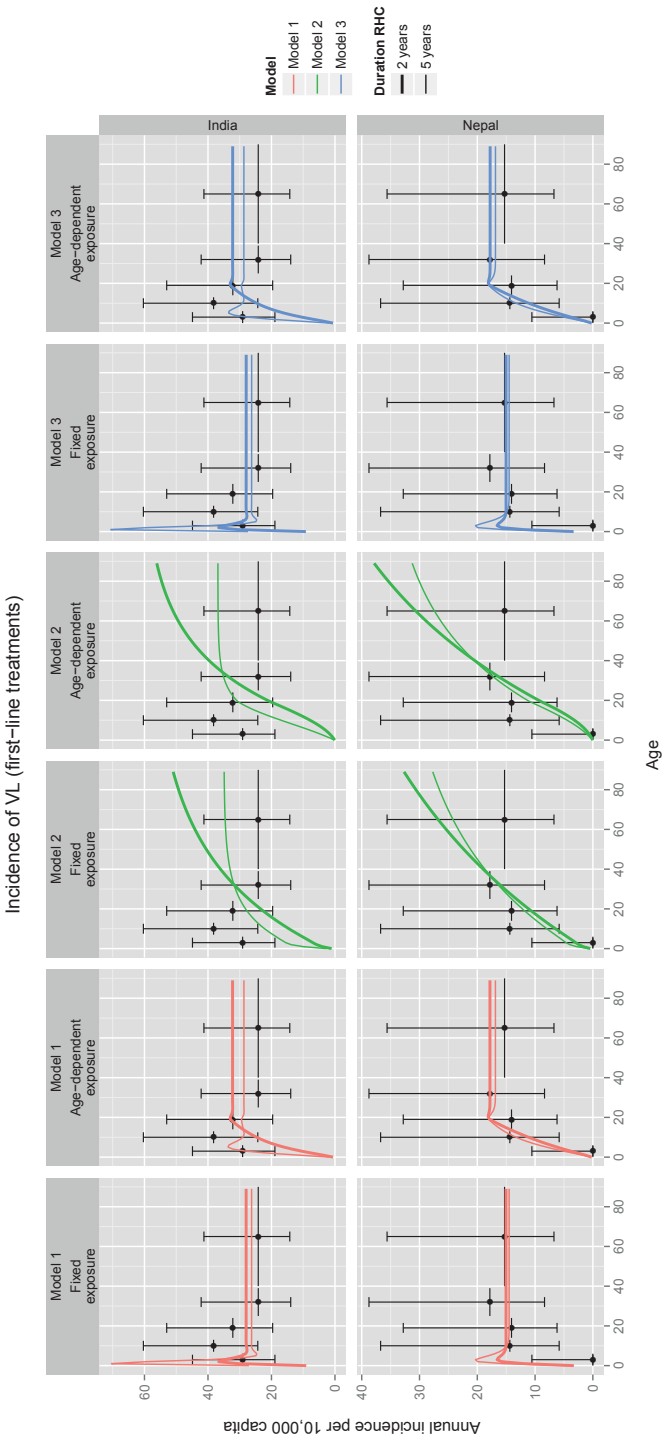
ADDITIONAL FILE 1

Description, characterisation, and calculations of equilibria for a system of ordinary differential equations for a set of VL transmission models

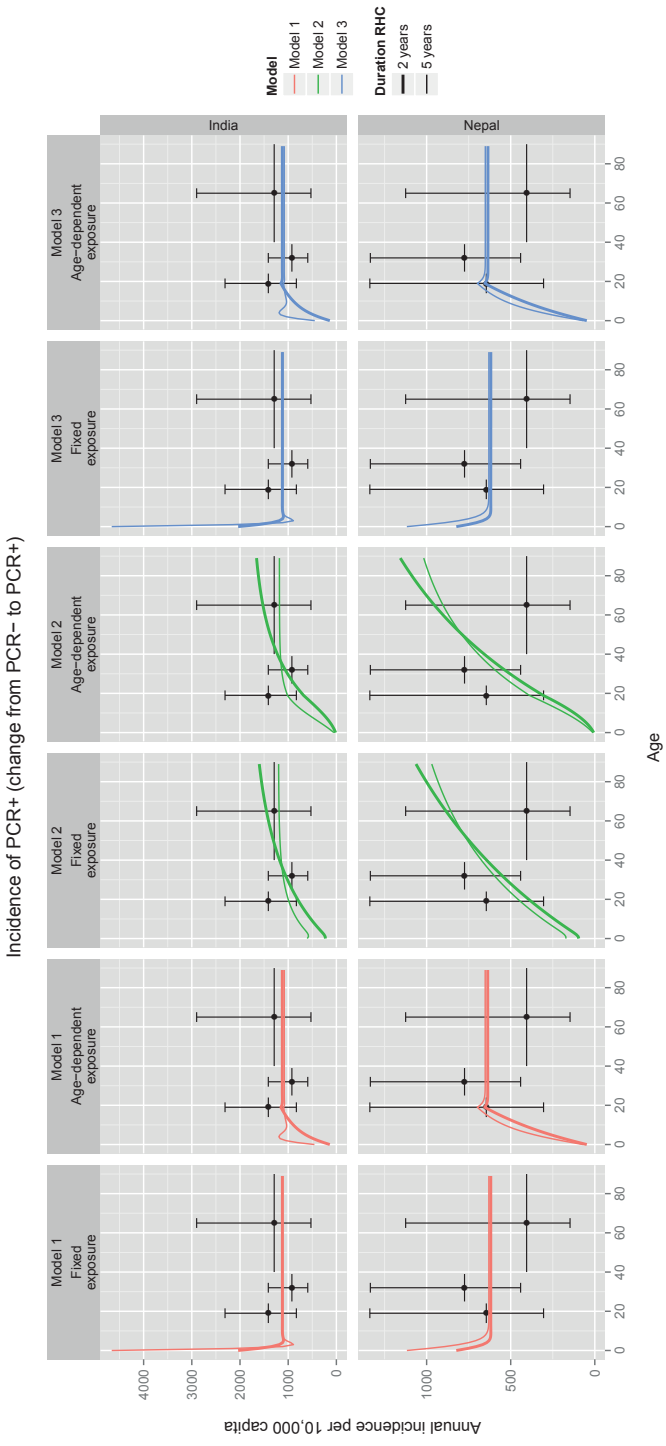
Online avaiable at https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4717541/

ADDITIONAL FILE 2

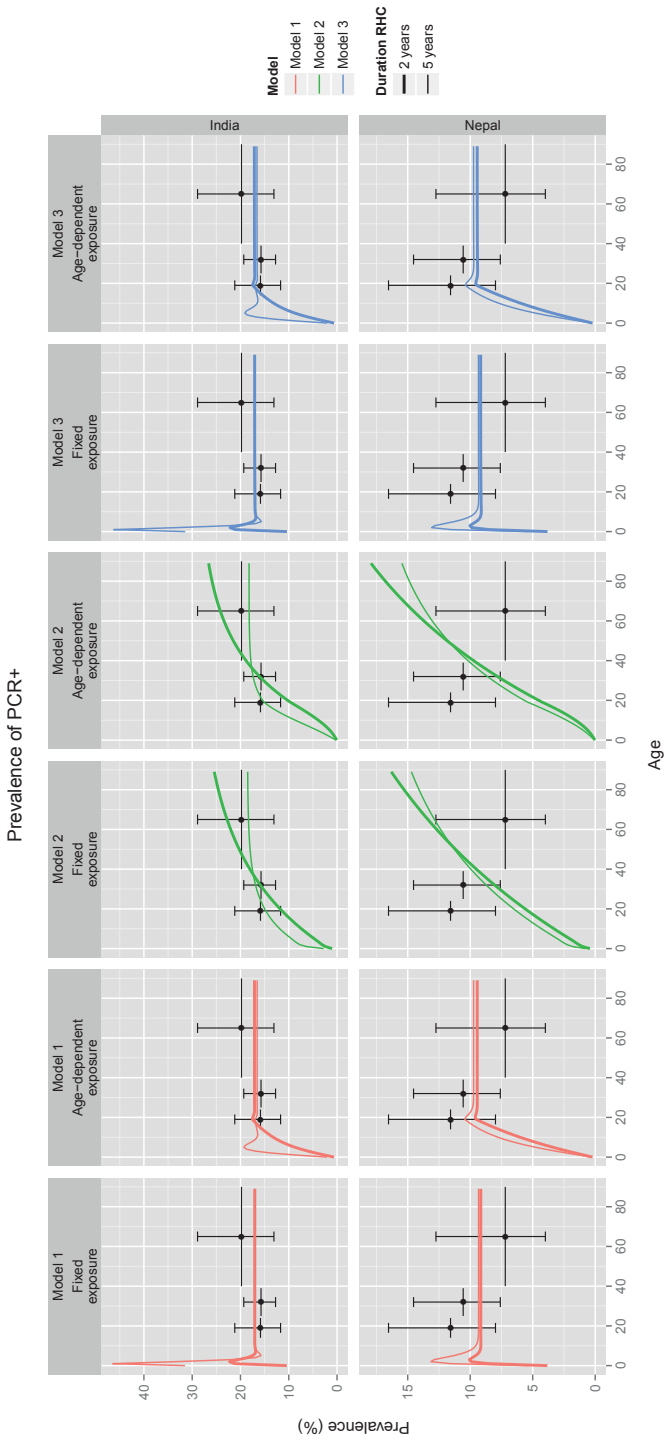
VL transmission models



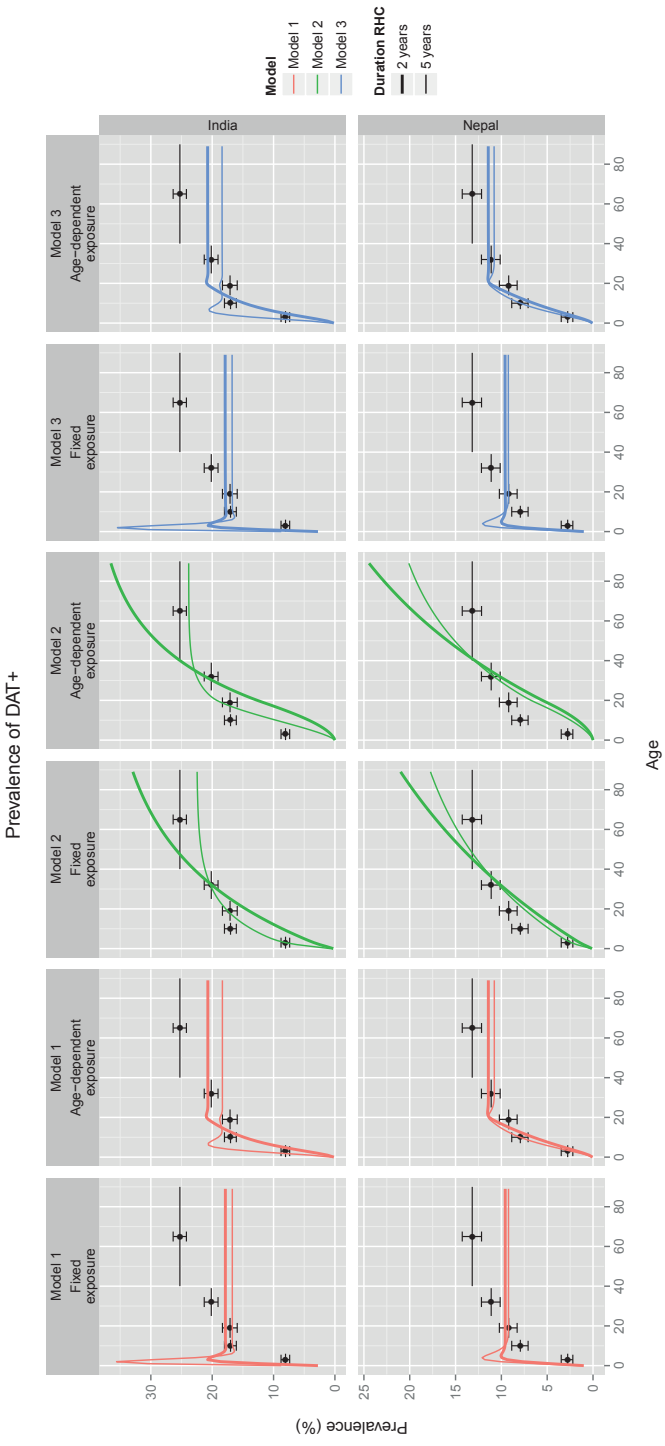
Extended figure A2-1. Incidence of VL (first-line treatments).



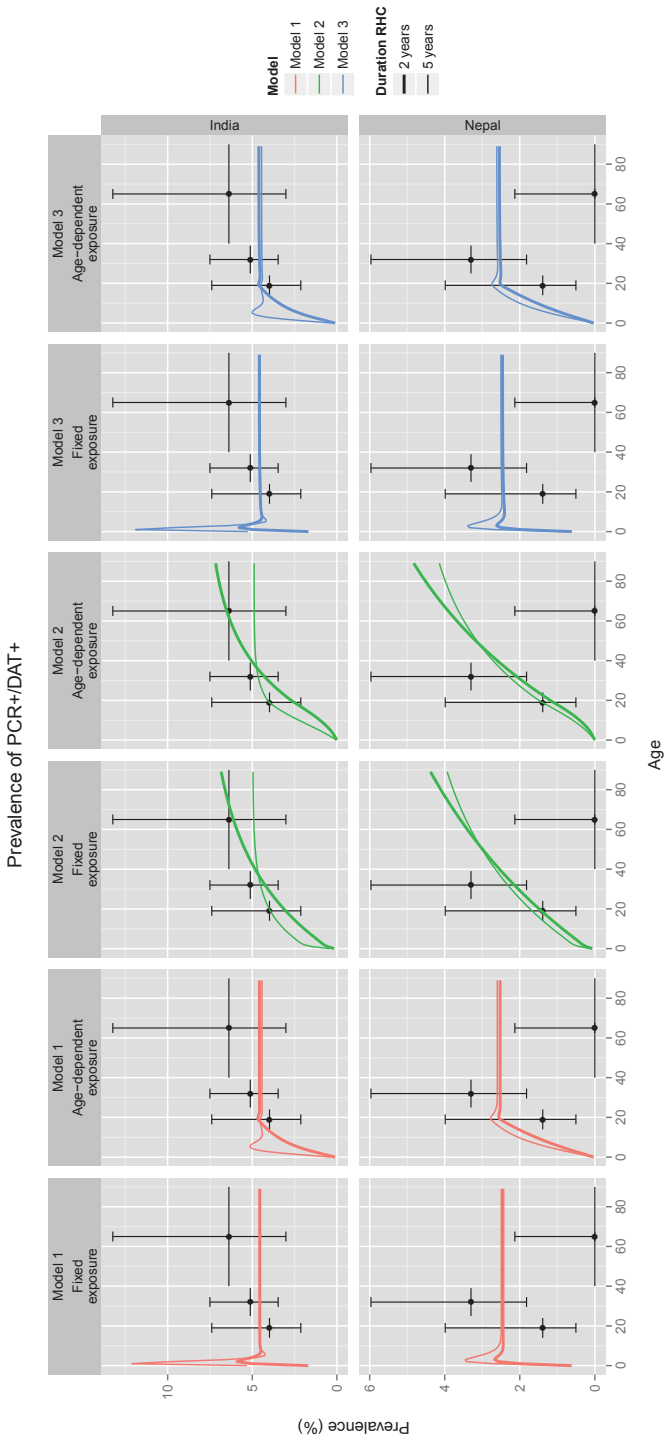
Extended figure A2-2. Incidence of PCR+ (change from PCR- to PCR+).



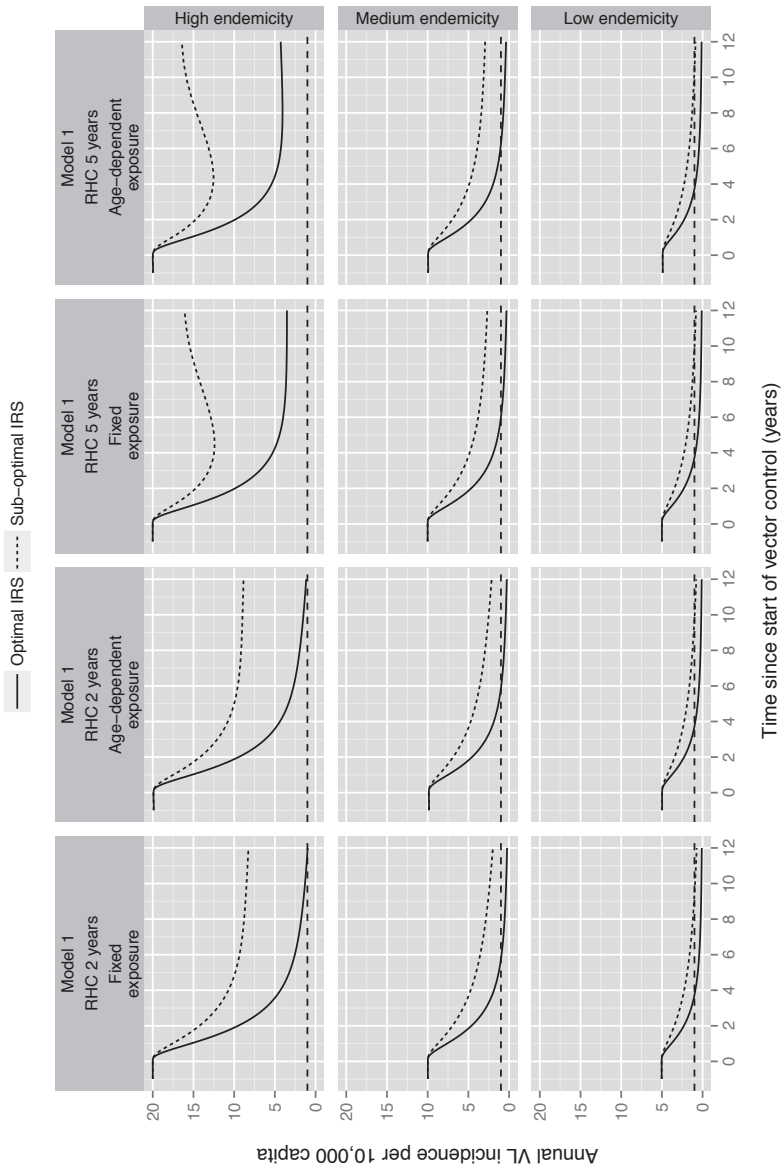
Extended figure A2-3. Prevalence of PCR+.



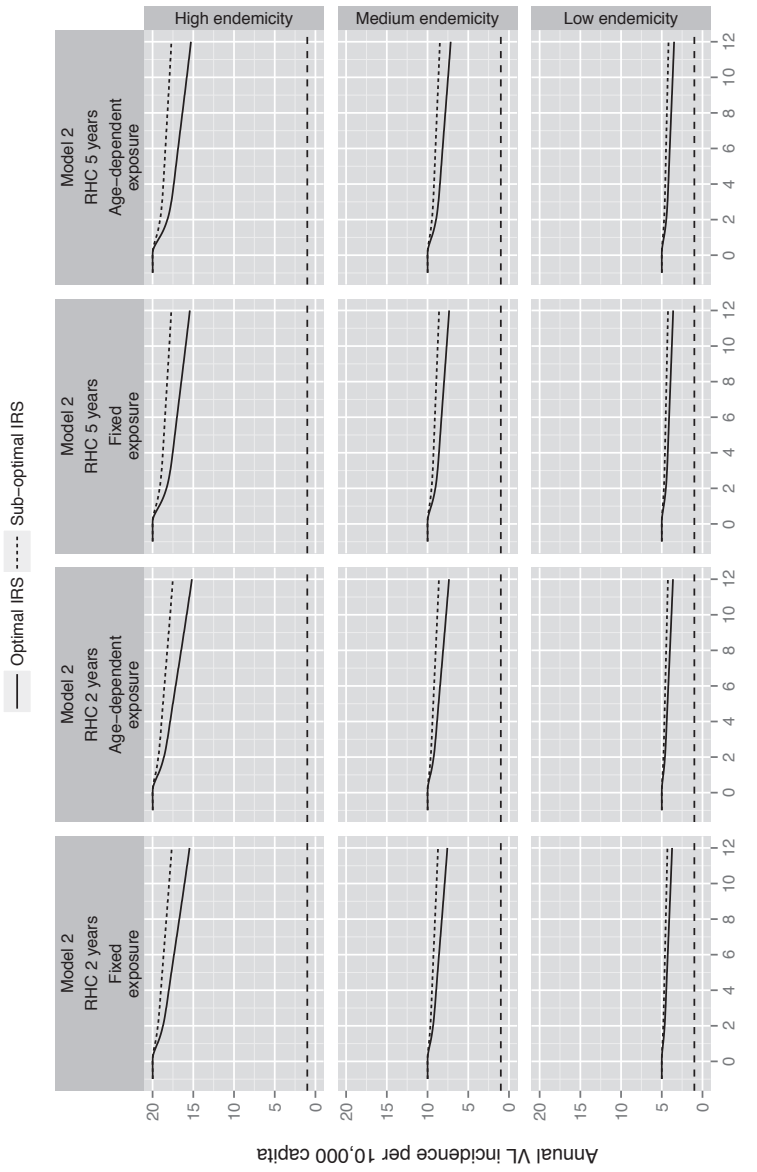
Extended figure A2-4. Prevalence of DAT+.



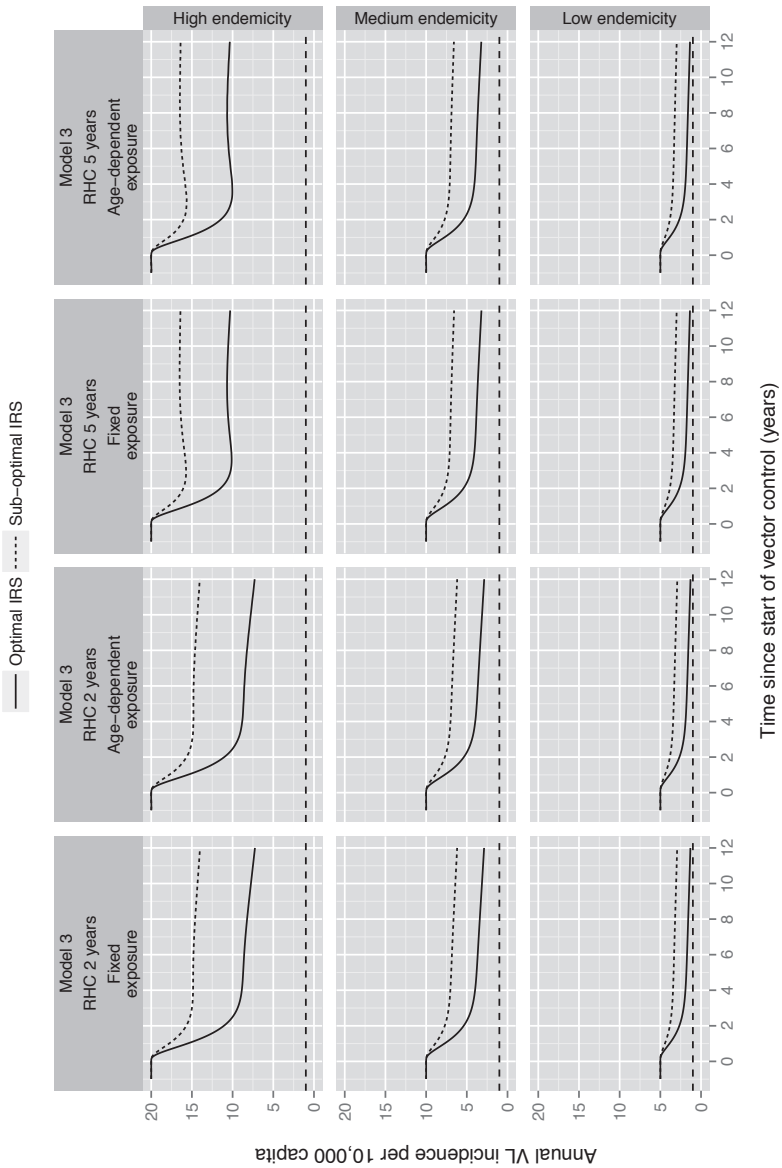
Extended figure A2-5. Prevalence of PCR+/DAT+.



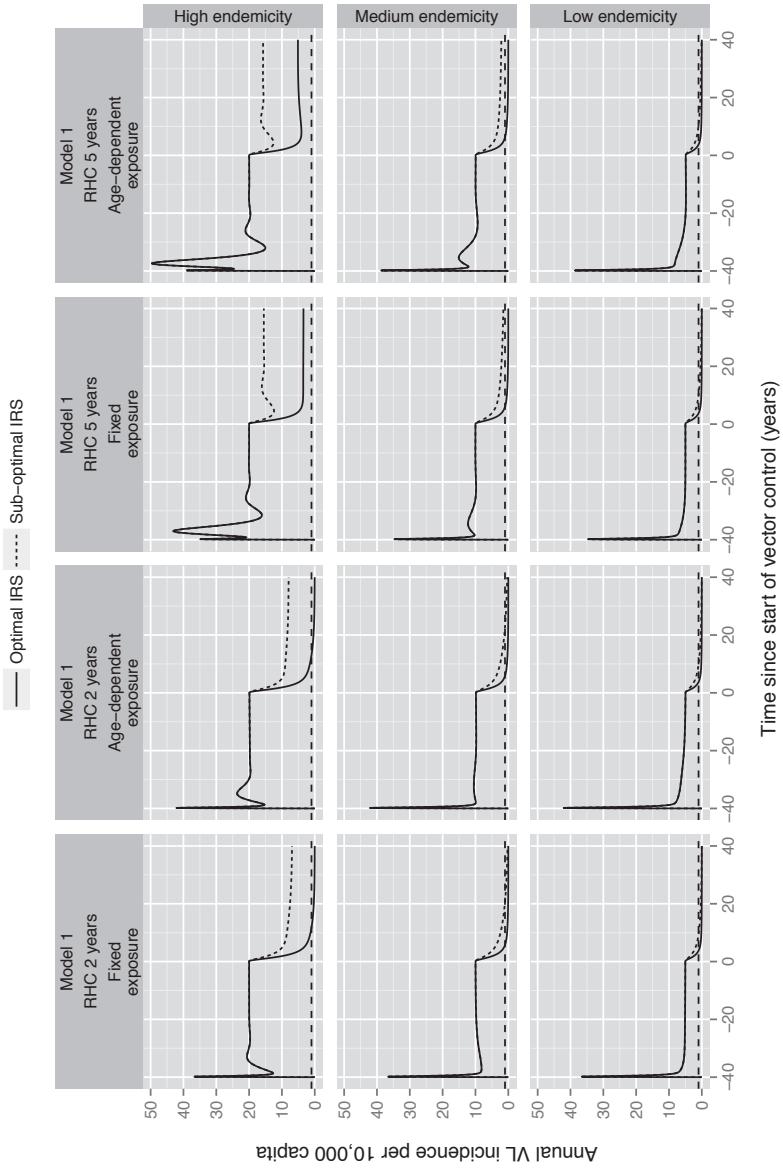
Extended figure A3-1. Annual VL incidence.



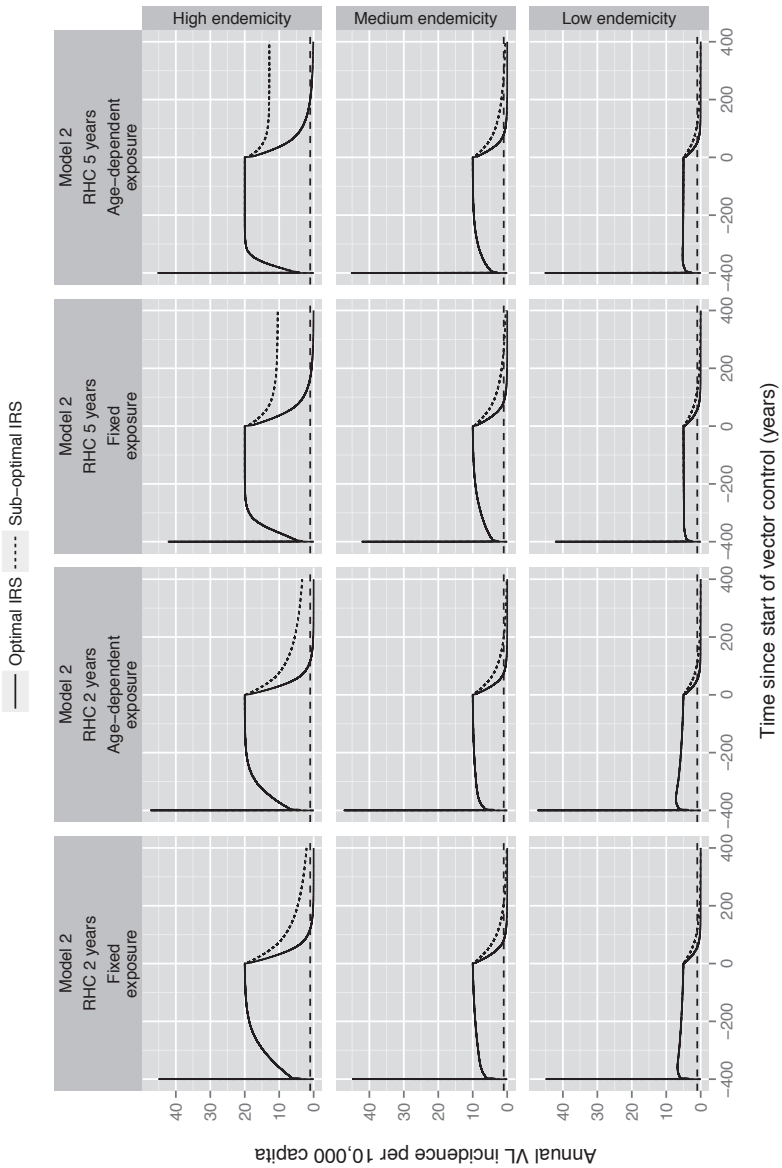
Extended figure A3-2. Annual VL incidence.



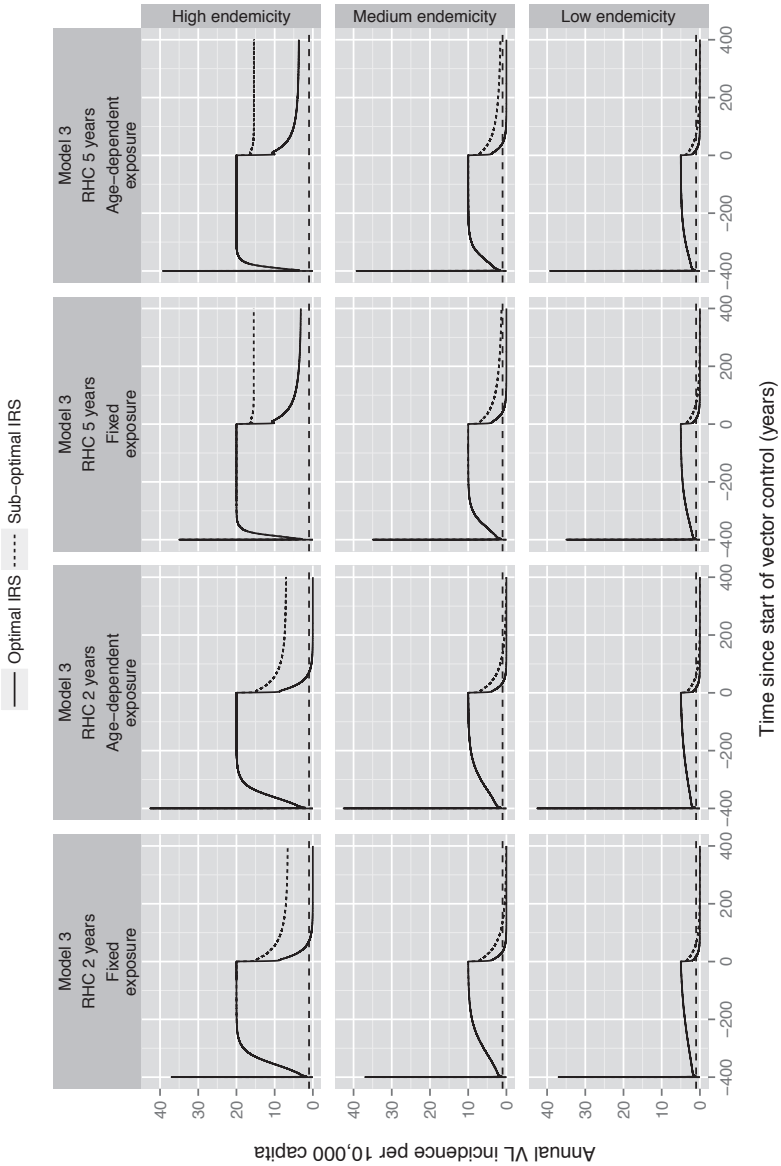
Extended figure A3-3. Annual VL incidence.



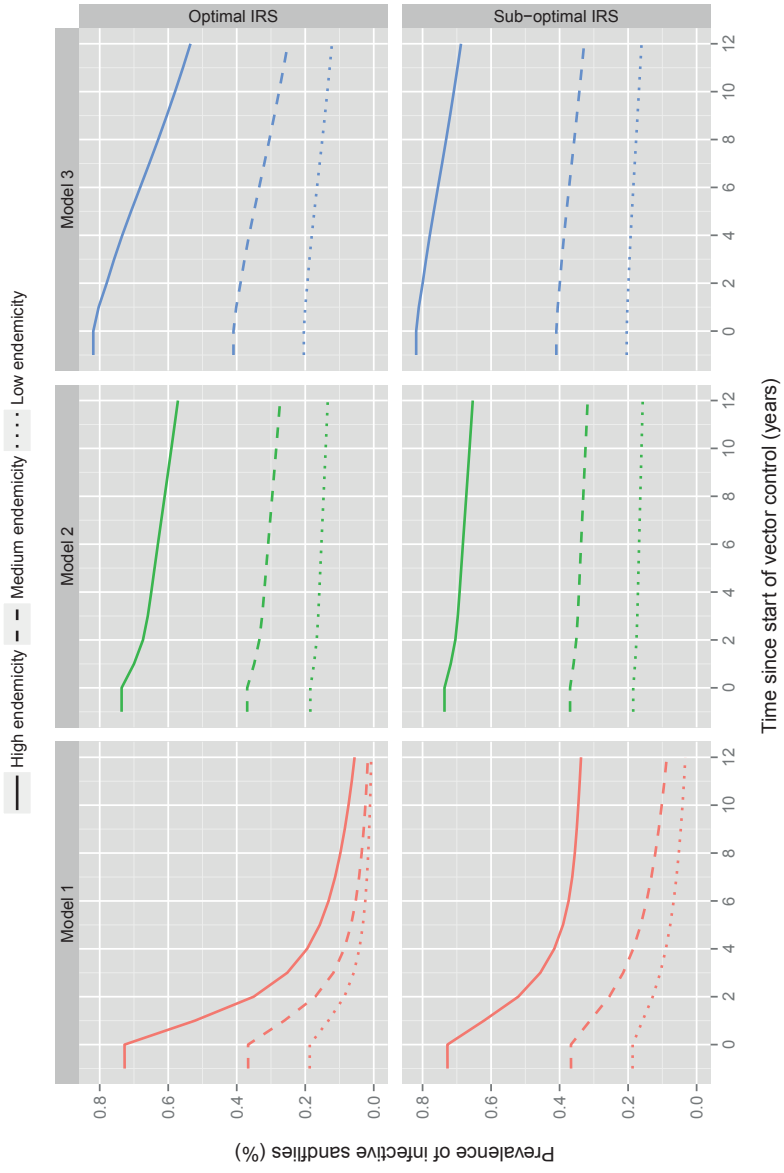
Extended figure A3-4. Annual VL incidence.



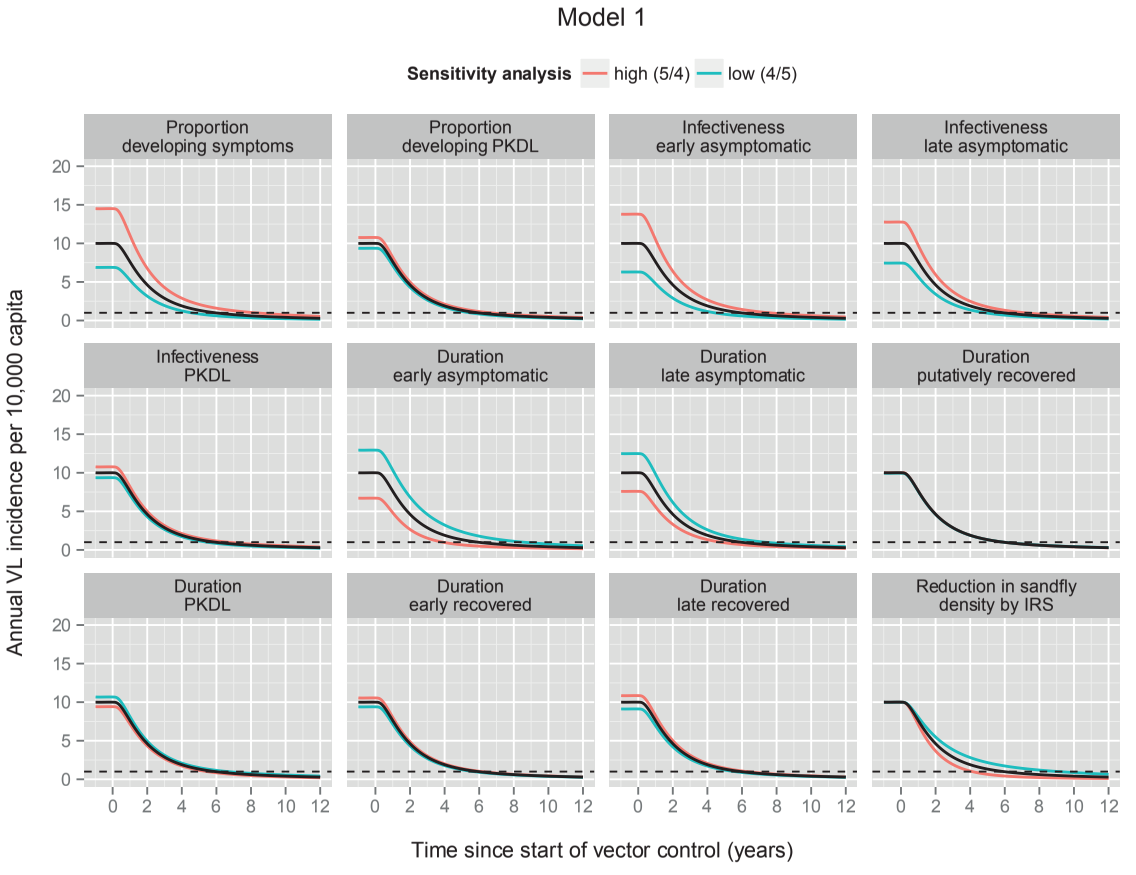
Extended figure A3-5. Annual VL incidence. .



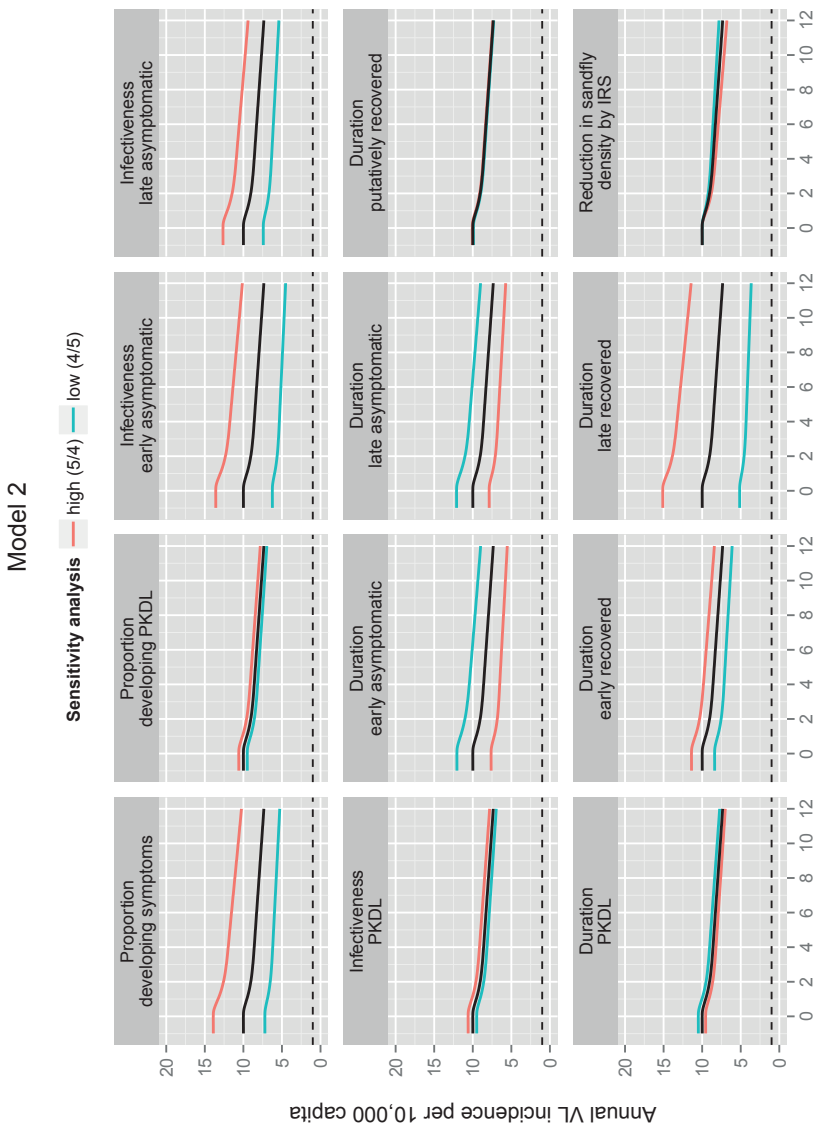
Extended figure A3-3. Annual VL incidence.



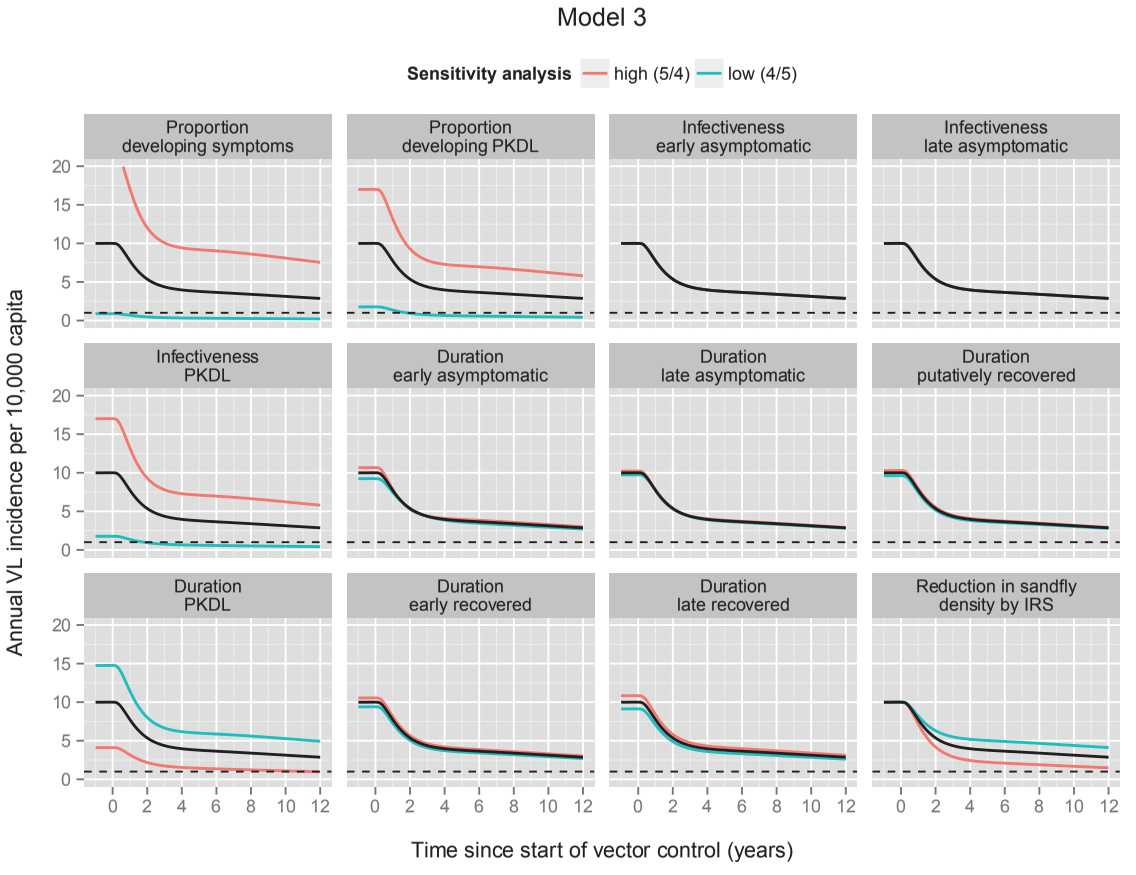
Extended figure A4. Prevalence of infective sandflies.



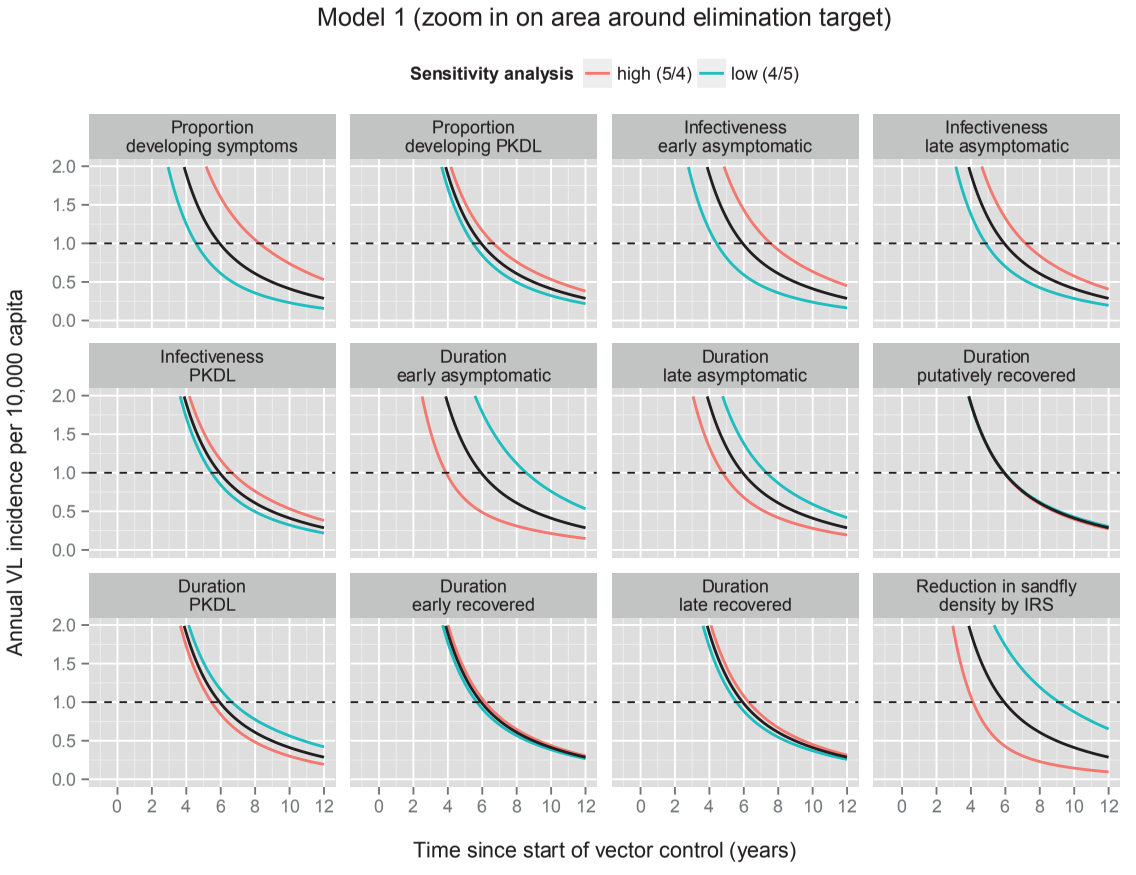
Extended figure A5-1. Annual VL incidence, sensitivity analysis. Model 1.



Extended figure A5-2. Annual VL incidence, sensitivity analysis. Model 2.



Extended figure A5-3. Annual VL incidence, sensitivity analysis. Model 3.



Extended figure A5-4. Annual VL incidence, sensitivity analysis. Model 1 (zoom in on area around elimination target).

CHAPTER 6

Elimination of visceral leishmaniasis in the Indian subcontinent A comparison of predictions from three transmission models

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Keywords: visceral leishmaniasis, kala-azar, elimination, mathematical modelling, indoor residual spraying, detection and treatment, Indian subcontinent, neglected tropical disease, sandfly, transmission dynamics, predictions

Abstract

We present three transmission models of visceral leishmaniasis (VL) in the Indian subcontinent (ISC) with structural differences regarding the disease stage that provides the main contribution to transmission, including models with a prominent role of asymptomatic infection, and fit them to recent case data from 8 endemic districts in Bihar, India. Following a geographical cross-validation of the models, we compare their predictions for achieving the WHO VL elimination targets with ongoing treatment and vector control strategies. All the transmission models suggest that the WHO elimination target (<1 new VL case per 10,000 capita per year at sub-district level) is likely to be met in Bihar, India, before or close to 2020 in sub-districts with a pre-control incidence of 10 VL cases per 10,000 people per year or less, when current intervention levels (60% coverage of indoor residual spraying (IRS) of insecticide and a delay of 40 days from onset of symptoms to treatment (OT)) are maintained, given the accuracy and generalizability of the existing data regarding incidence and IRS coverage. In settings with a pre-control endemicity level of 5/10,000, increasing the effective IRS coverage from 60 to 80% is predicted to lead to elimination of VL 1–3 years earlier (depending on the particular model), and decreasing OT from 40 to 20 days to bring elimination forward by approximately 1 year. However, in all instances the models suggest that L. donovani transmission will continue after 2020 and thus that surveillance and control measures need to remain in place until the longer-term aim of breaking transmission is achieved.

Introduction

Visceral leishmaniasis (VL), also known as kala-azar, is the world's deadliest parasitic disease after malaria [1]. From 2004–2008 there were an estimated 200,000–400,000 cases and 20,000–40,000 deaths per year globally [2]. Historically, most VL cases occur in the Indian subcontinent (ISC), where the causative parasite Leishmania donovani is transmitted by Phlebotomus argentipes sandflies and the disease is considered to infect humans only [3, 4]. However, since 2012, there has been a significant decline in the number of VL cases identified in the ISC, attributed usually to interventions and socio-economic improvements [5–7]. The World Health Organization (WHO) has targeted VL for elimination as a public health problem in the ISC by 2020 [6]. This is defined as <1 new VL case per 10,000 capita per year at sub-district (block) level. In the rest of the world, where VL is mainly zoonotic and caused by another parasite species, the WHO has not set any elimination target but aims for 100% detection and treatment of human cases. Current interventions in the ISC focus on reducing transmission through vector control, mainly by indoor residual spraying (IRS) of insecticides, and early detection and treatment of cases [8]. In 2012, the London Declaration on Neglected Tropical Diseases endorsed the WHO elimination target on VL and pledged to increase research, funding, supplies and awareness to combat this disease [9]. It has been estimated that the health and economic gains from reaching the WHO targets for VL will be enormous [10]. The governments of India, Bangladesh, Nepal, Bhutan and Thailand have signed a memorandum of understanding setting an ambitious goal of eliminating VL as a public health problem (at sub-district level in India and Bangladesh, and district-level in Nepal and Bhutan) by or before the end of 2017 [8]. Incidence of VL in Bhutan and Thailand is currently very low and limited to sporadic cases. Nepal reached the targeted low incidence level in 2014 and has sustained it for 2 years [6]. Even in Bangladesh and India the target-level incidence was reached in nearly 90% and 70% of endemic sub-districts respectively by 2015 [6]. Nevertheless, to achieve the target in the remaining endemic sub-districts in India, special attention must be paid to the state of Bihar, which borders Nepal and accounts for 60–90% of cases in the ISC [11] and about 80% of cases and 90% of deaths in India [12]. Hence, in this study we focus on the VL elimination status and control strategies in Bihar.

Mathematical models capturing disease transmission dynamics and control measures have proven to be useful tools in predicting the feasibility of achieving elimination targets with existing strategies [13–16]. Deterministic VL transmission models have been developed previously based on the KalaNet dataset from Bihar (India) and Nepal [17, 18]. More recently, Le Rutte et al. [19] published a set of 3 age-structured model variants, each with individuals from a different disease stage being the main contributors to transmission: asymptomatic individuals, previously immune individuals in whom infection has reactivated, and individuals with post-kala-azar dermal leishmaniasis (PKDL). A sensitivity analysis for the duration of immunity was included, as both the disease stage which contains the main contributors to transmission and the duration of immunity remain unknown factors in the transmission dynamics of VL [15]. Available data on the impact of IRS on VL incidence suggested that the most accurate predictions are given by the model variant in which asymptomatic individuals are the main contributors to transmission, with which elimination of VL (annual incidence rate of <1 per 10,000 per year) by 2017 was predicted to be feasible only in settings that experience optimal IRS (continuously implemented from 2012 onwards) and have a maximum baseline endemicity of 5–10 VL cases per 10,000 capita per year. In highly endemic settings (<20 VL cases per 10,000) and in settings with sub-optimal IRS that are facing challenges with IRS implementation, coverage and insecticide resistance, additional interventions will be required [19]. Chapman et al. [20] have recently estimated key epidemiological parameters for VL, including the duration of asymptomatic infection and the proportion of asymptomatic individuals who develop clinical symptoms, by fitting a multi-state Markov model for the natural history of VL to serological and case data from a highly endemic region of Bangladesh [21]. It was estimated that asymptomatic infection lasts 5 months on average and approximately 1 in 7 asymptomatic individuals progress to VL. However, the extent to which these parameters depend on geographical location, endemicity, and other risk factors remains unclear.

To improve the robustness of predictions of the impact of intervention strategies against VL it is vital to compare and combine the outcomes of different mathematical modelling approaches, as has been done previously for HIV [22–24]. Here we present the first VL modelling comparison study in which we compare the predictions from (1) the VL transmission model variant

of those developed by Le Rutte et al. [19] that provides the most accurate predictions, (2) a similar model in which symptomatic individuals are the sole contributors to transmission, and (3) a newly developed transmission model based on the simplified model of the natural history of VL presented by Chapman et al. [20]. The three models were fitted to VL case data collected by CARE India in 2012 and 2013 from 8 endemic districts in Bihar, India [25, 26], that are currently under intensive vector control with IRS. The models were compared via their predictive ability in a geographical cross-validation. The models were then used to predict whether the elimination target could be achieved in each district. We further predicted whether the elimination target could be achieved in settings with different pre-control endemicity levels, using current and improved interventions.

Methods

Mathematical models

The modelling study described in this paper was performed by two research groups: Erasmus MC, Department of Public Health in Rotterdam, The Netherlands and the Warwick Infectious Disease Epidemiology Research (WIDER) group, University of Warwick, United Kingdom.

Erasmus MC developed two VL models: model E0, in which symptomatic individuals are the sole contributors to transmission, and model E1 (the best-performing model from their recent study [19]) in which the main contributors to transmission are asymptomatic individuals. Supplementary File 4 contains guidelines to run the R-package 'VLode' of their age-structured system of ordinary differential equations for visceral leishmaniasis transmission, which is provided in Supplementary File 5. Warwick developed model W, which converts their recent Markov model of the natural history of VL [20] into a transmission model with vector population dynamics, in which asymptomatic individuals are the main contributors to transmission. The MATLAB code for Warwick model fitting and predictions is provided in Supplementary File 6. A schematic presentation of the three models (E0, E1 and W) is given in Figure 1 and the main model characteristics are listed in Table 1. The models are all deterministic compartmental transmission models inspired by an earlier VL transmission model developed by Stauch et

al. [17, 18]. In the models, susceptible humans can become asymptomatically infected when bitten by an infectious sandfly. The majority of infected humans recover without developing clinical symptoms and only a small proportion develop clinical VL. Symptomatic cases can receive one or two VL treatments, and in models E0 and E1 treated individuals can develop PKDL after some period of apparent, but not absolute recovery (this putatively recovered stage is included because some VL cases seem to harbor dormant infection after treatment which leads to this late post-treatment dermatological complication of VL [27]). Susceptible sandflies become infected when they bite infectious humans (who may include asymptomatically infected individuals, symptomatic cases, treated individuals and PKDL cases), and remain latently infected for some period before they become infectious to humans. As an important new aspect, relative to previous VL transmission models, all models presented here include seasonality in the sandfly density, an important feature in VL transmission dynamics on the ISC [28]. The differences between the models are described in detail below.

Erasmus MC models (E0 and E1)

The set of Erasmus MC VL transmission models is defined in terms of a system of ordinary differential equations (ODE) and has been previously described and fitted to data from India and Nepal [19], collected through the KalaNet study [29]. The models include population growth of both humans and sandflies (the populations are assumed to grow at the same rate in the absence of seasonality and IRS) and age-structure in human mortality and exposure to sandflies. In contrast to the Warwick model, there are compartments for early and late asymptomatic infection, and early and late recovered stage, to allow the fitting of these models to prevalence of positivity on the direct agglutination test (DAT) and/or PCR from the KalaNet study.

In this study, models E0 and E1 have been extended with a yearly seasonal pattern in sandfly density based on seasonal patterns observed in sandfly distribution studies in Bihar [30–33]. Seasonality is implemented via a stepwise function in the sandfly birth rate, which is assumed to peak during 3 months of the year (July–September) [31]. Full details of the model, including the description, characterization and calculations of equilibria of the system of ordinary differential equations along with data are provided in Additional File 1 of Le Rutte et al. [19].

Warwick model (W)

The Warwick VL model (Figure 1) is very similar in structure to the models of Stauch et al. [17, 18] and Le Rutte et al. [19], but with some key simplifications which have been made for parsimony, given the available data, and to provide a contrast to the Erasmus MC models. The main difference is that it does not include PKDL and dormant infection (in which the individual still harbors a small number of parasites but is no longer infectious) following asymptomatic infection or VL treatment. Pathways for disease progression in the models are essentially the same, but there is only one compartment for asymptomatically infected individuals instead of two (for early and late stages), and recovered individuals are not differentiated by whether they have recovered from asymptomatic or symptomatic infection, or whether they are still seropositive or not (they are all combined into one recovered class). The latter simplification is made as the model is only fitted to the CARE data, which contains no information on individuals' serological status, and because VL patients' parasite loads decrease rapidly following successful treatment [34, 35], supporting the view that sandfly infection rates from treated patients and individuals who have recovered from asymptomatic infection are both negligible, as assumed in the Stauch and Erasmus MC models. The compartmental structure of the human part of the model is inspired by that of the Markov model developed by Chapman et al. [20]. As individuals' onset-to-treatment (OT) times are included in the data, Erlang distributions (Gamma distributions with integer-valued shape parameters) are fitted to the OT time distributions for each district, and different numbers of compartments for clinical VL used for each district according to the shape parameter of the Erlang distribution [36]. The model also includes seasonality in the sandfly population via sinusoidal forcing of the sandfly birth rate, such that the sandfly population varies seasonally about a constant mean. A constant human population is assumed (so the mean sandfly-to-human ratio (SHR) remains constant), and the model does not include human age-structure. Full details of the model, including the differential equations used to describe the transmission cycle and the seasonality function, are given in Supplementary File 1 (SF1).

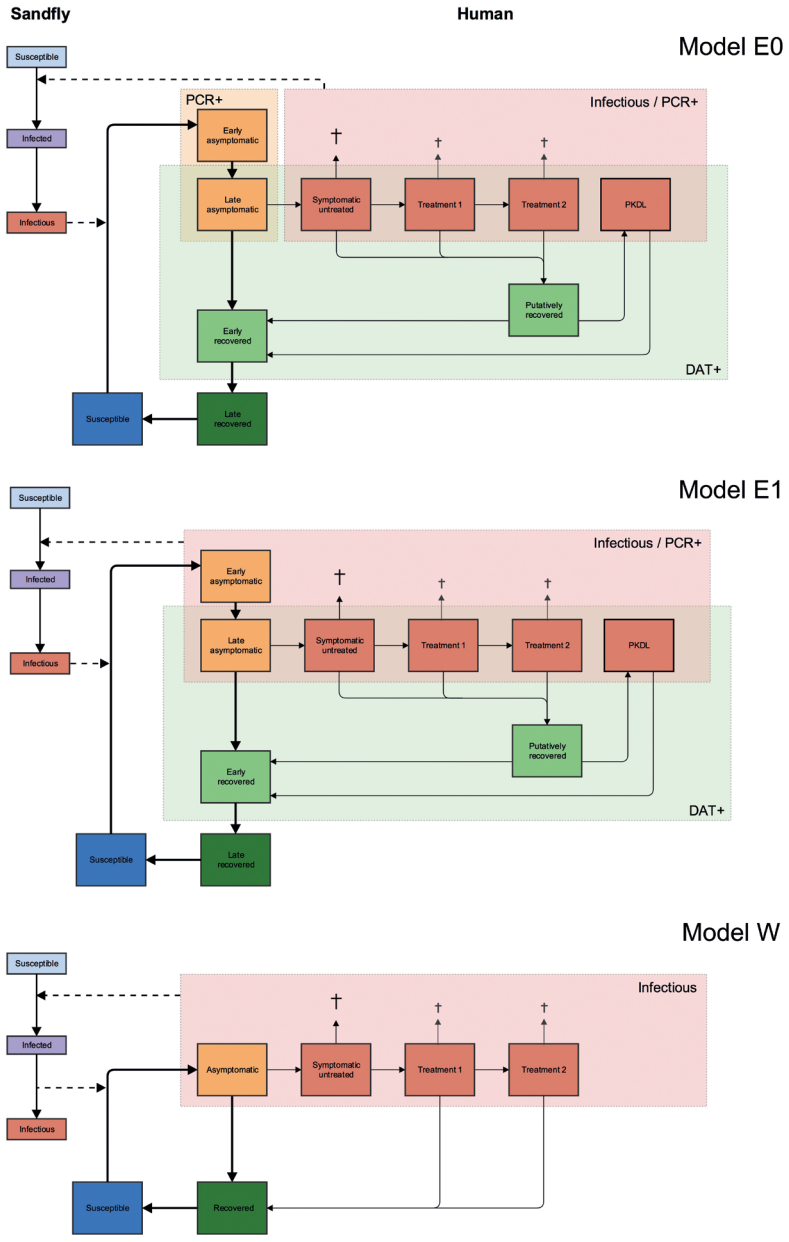

Figure 1. Schematic representation of model structures. Model E0 (Erasmus MC) assumes only symptomatic individuals (red boxes) are infectious towards the sandfly. In model E1 (Erasmus MC) asymptomatic individuals (yellow boxes) are the main contributors to transmission. The red shaded frame includes individuals that tested positive for parasite DNA on a polymerase chain reaction test (PCR+) and the green shaded frame includes individuals that tested positive for anti-leishmanial antibodies on the direct agglutination test (DAT+), obtained from the KalaNet study. In model W (Warwick University), asymptomatic individuals are the main contributors to transmission.

Table 1. Overview of the main model characteristics.

Model characteristic	Erasmus MC Rotterdam	Warwick University
Model type	Population-based (deterministic, age-structured)	Population-based (deterministic, no age structure)
Human disease states	Susceptible, early and late asymptomatically infected, symptomatically infected untreated, first-line treatment, second-line treatment, post kala-azar dermal leishmaniasis (PKDL), putatively recovered, and early and late recovered.	Susceptible, asymptomatically infected, symptomatically infected, first-line treatment, second-line treatment, recovered.
Sandfly states	Susceptible, latently infected, infectious	Susceptible, latently infected, infectious
Main contributors to transmission	E0) Symptomatic cases E1) Asymptomatic individuals	Asymptomatic individuals
Interventions considered	Vector control (IRS) and decreasing the duration of onset of symptoms to treatment (OT)	
Human demography	Per capita birth rate and age-specific mortality rates (2011)	Population based on Indian 2001/2011 census, birth and mortality rates.
Human sandfly exposure	Age-dependent, seasonal	Seasonal
Distribution of duration of states	Exponential	Exponential except for duration of symptomatic VL, for which Erlang-2 distributions were fitted to onset-to-treatment time distributions in data

Data

Both teams used longitudinal data on numbers of identified cases from the CARE study: Erasmus MC to fit the IRS efficacy (the factor that together with the IRS coverage determines the impact of IRS on the SHR) (E0 and E1) and Warwick to fit the SHR (W). Models E0 and E1 were also fitted to serological population data from the KalaNet study [29] to estimate biological parameters, and to additional epidemiological data [37] to estimate the pre-control SHR. An overview of the CARE, KalaNet and additional epidemiological data and the fitting process for each model is provided in Figure 2.

Identified cases

In 2013, CARE India collected data on 6081 VL cases in 8 districts in Bihar – Saharsa, East Champaran, Samastipur, Gopalganj, Begusarai, Khagaria, Patna and West Champaran – for an 18-month reference period for their diagnoses from January 2012 to June 2013. The cases were mainly identified from the medical records of public health facilities (primary healthcare centres,

and sub-district and district hospitals), though roughly 15% were referred by other patients, relatives, accredited social health activists and private labs and doctors/hospitals. The house of each case was visited and the VL patient or a relative was interviewed twice to obtain information about the case. Individual-level data collected included the following, all of which are used in this study: age, district, sub-district, date of onset of symptoms, date of diagnosis, times from onset to first and (if applicable) second VL treatment, type and duration of treatment, occurrence of a relapse, PKDL, and details of IRS spraying in the patient's house and neighborhood. All data were anonymized after collection. Also, data about various socio-economic factors, such as caste, house type and construction, and cattle ownership were collected. These data are not used for our study but are described in more detail by Jervis et al. [26]. Table S1 in Supplementary File 2 (SF2) shows the burden of identified VL cases for each district, and Figure S1 in SF2 the monthly numbers of onsets of VL symptoms in each district from January 2012 to June 2013. The latter is the data from the CARE study to which the three models are fitted. A seasonal pattern in onset of symptomatic VL cases can be identified, comparable to what has been found in other VL datasets [38]. Further description of the CARE data and how it is used to parameterize the models is provided in SF2.

Additional epidemiological data

The KalaNet dataset includes age-structured data on DAT and PCR prevalence and conversion (from negative to positive and vice-versa) as measured in repeated surveys as well as VL incidence data monitored continuously amongst 21,267 individuals in Bihar, India, and Nepal from 2006 to 2009, and infection prevalence in the sandfly population in Nepal [29]. PCR data were collected in a sub-population of individuals aged 15 years and older.

Longitudinal epidemiological data presented by Thakur et al. [37] on the VL case burden in the 8 CARE districts show an equilibrium situation of approximately 3 years from 2009 to 2011, before a decline in the number of cases in 2012 towards those in the CARE data (see Figure S2 in SF2). These data were therefore taken as pre-control data on the VL case burden (i.e. data from before good quality IRS implementation and reductions in delays to treatment). The average of the annual number of cases per district in 2009 and 2010 was used to estimate the pre-control sandfly-to-human ratios

for all 8 districts for models E0 and E1. Here, the model predictions for the incidence of 'symptomatic treatment 1' stage were fitted to the data (since the data consisted of numbers of treated VL cases). This was different for the CARE dataset, for which the model predicted incidence of 'symptomatic untreated' cases was fitted (as the data comprised numbers of onsets of VL symptoms). For further details see Supplementary File 2.

Parameter values that could not be taken from the CARE data, KalaNet data, Thakur data or the 2011 Indian Census were obtained from the available relevant literature.

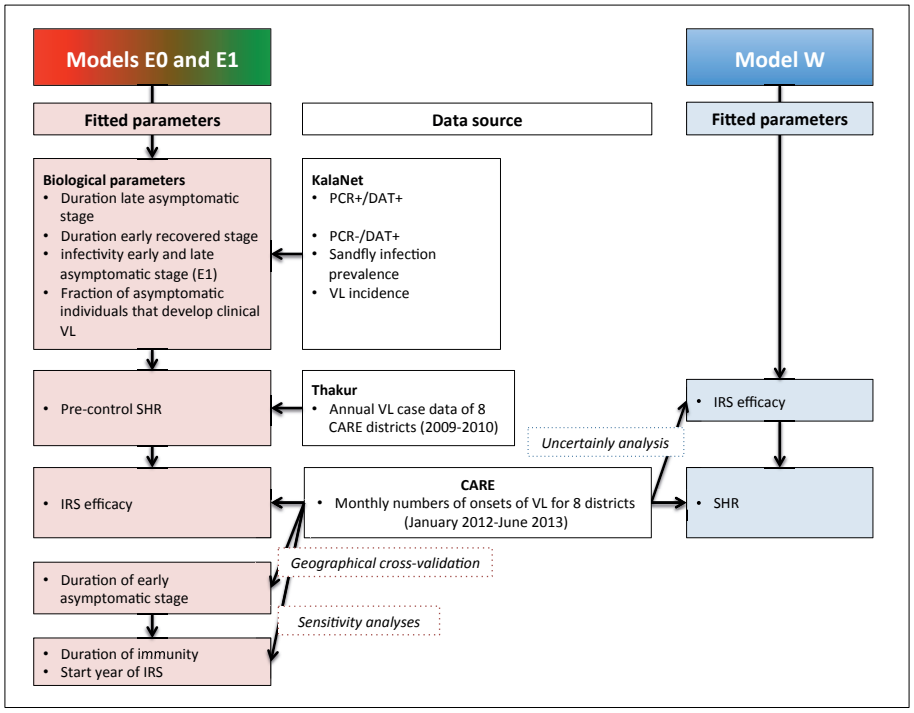

Figure 2. Schematic representation of the fitting procedure used by the two groups, representing the data in the white boxes and fitted parameters per model. SHR = sandfly-to-human ratio, IRS = indoor residual spray.

Model fitting and comparison

A geographical cross-validation approach was used to test the models' abilities to predict the monthly number of cases in each district in the CARE data over the 18-month reference period (January 2012–June 2013). This

consisted of censoring the data for one district, fitting the model to the monthly numbers of VL onsets in the other 7 districts, and predicting the trend in the monthly numbers of onsets in the censored district, and then repeating the process with a different district censored. Different approaches were used by Erasmus MC and Warwick to estimate the case numbers in the censored district. Erasmus MC (models E0 and E1) fitted the IRS efficacy jointly across the 7 uncensored districts and used this as the estimate of the IRS efficacy in the censored district together with the pre-control district sandfly-to-human ratio (SHR) estimated from the Thakur data. Warwick (model W) first estimated the IRS efficacy for all 8 districts via a parameter uncertainty analysis (see SF1), then fitted the SHRs for the 7 uncensored districts and estimated the censored district SHR by linear regression of the 2012 average district identified case burdens (Table S1 of SF2) on the fitted SHRs (see Supplementary File 3 (SF3)). Other data from the censored district that were used to predict the monthly number of onsets included the mean OT time for 2012 and 2013, and the 2012 IRS coverage level. The OT time prior to 2012 was assumed to be the same as in 2012. In all models, IRS was assumed to have started in January 2011 across all districts based on there being significant increases in the reported coverage of IRS with DDT (dichlorodiphenyltrichloroethane) between 2008/2009 and 2011/2012 (from as low as 12–17% in 2008/2009 to as high as 80–91% in 2011/2012 [39–41]). All district specific information is provided in Table S1 of SF2. The three models are compared on their ability to fit the VL case data for the 8 (censored) districts of the CARE dataset with the geographical cross-validation approach, using the deviance (twice the difference in the negative log-likelihoods of the fitted model and a saturated model that fits the data exactly) between the 18 data points per district and the model output.

One of the purposes of this study was to evaluate the predictive power of the models given surveillance data, if these were available in real time, and, vice versa, to evaluate whether routine surveillance data, together with models fitted to historic data, can provide enough information from which to infer key model parameters. Therefore two approaches were taken. Warwick only used the case data to fit their model, whereas Erasmus MC included additional data to provide more information to fine-tune key epidemiological parameters. Below we describe the specific fitting approaches of both groups, a flow diagram of which is presented in Figure 2.

Erasmus MC VL-models

Estimation of biological parameters. Model E1 has previously been fitted to the KalaNet dataset. This resulted in a relatively long estimated duration of the early asymptomatic stage of 382 days. This long duration dampens the effect of seasonal sandfly abundance on seasonality in VL incidence and therefore seems less plausible than previously thought, given the strong seasonal pattern in the newly available CARE dataset (seasonal patterns in equilibrium for different durations of early asymptomatic stage are illustrated in Figure S3 of SF3, years 2010–2011). Because the long duration of the asymptomatic stage was primarily driven by the use of both PCR prevalence and incidence data in our previous fitting exercise, here we no longer used the PCR incidence data and assumed a series of 10 pre-set values for the duration of the early asymptomatic stage (PCR+/DAT-, of between 112 and 382days), of which the longest value is the estimated duration in the previous study. The 20 sub-models (Models E0 and E1 each with 10 durations of the early asymptomatic stage) were refitted to the KalaNet dataset to re-estimate the following biological parameters: the duration of the late asymptomatic stage (PCR+/DAT+), the duration of the early recovered stage (PCR-/DAT+), the infectivity of the early and late asymptomatic stage (model E1 only), and the fraction of asymptomatic individuals that develop clinical VL (VL incidence). The age-dependent exposure to sandflies and the duration of the late recovered stage of two years (immunity) were chosen based on the conclusions from the previous paper. A sensitivity analysis was performed in this study to also explore the impact of assuming durations of immunity of one and five years. All literature based parameter values can be found in Tables 2 and 3, and all fitted parameters in Table 4 of the results section.

Sandfly-to-human ratio (SHR). The 20 sub-models were then fitted to the average VL incidence of 2009 and 2010 from Thakur et al. [37], which we interpreted as an equilibrium due to the stability of incidence over the given years in the data, to estimate the district-specific SHR in the absence of IRS.

IRS efficacy. The IRS efficacy was estimated by fitting each model to the CARE data on the trend in the numbers of cases over the 18-month period. Within the model, the IRS impact on the sandfly birth rate was defined as

the IRS efficacy multiplied by the district-specific IRS coverage rate reported in the CARE data (i.e. the sandfly birth rate was multiplied by 1 minus the aforementioned product). A sensitivity analysis for the start year of IRS was performed by fitting the model with IRS starting in 2010 and in 2012.

Parameter estimation. All parameters were estimated by maximum likelihood estimation using the BFGS algorithm from the optim package in R (version 3.3.0). The data-generating process for prevalent cases (DAT and PCR positivity) was assumed to be a binomial distribution, and that for incident VL cases (VL cases) a Poisson distribution. We calculated confidence intervals for biological parameter estimates based on the inverse of the Hessian at the global optimum (i.e. assuming a multivariate normal distribution). We further calculated the deviance between each sub-model and a hypothetical, saturated model exactly predicting the data. Based on the deviance, we selected the sub-models with the duration of early asymptomatic stage that was closest to the saturated model (i.e. lowest deviance) for both model E0 and E1. The sub-models best fitting the data were then used to generate further predictions. More details of the model fitting procedure are presented in Supplementary File 3.

Warwick VL-model

Biological parameters. Values of biological parameters such as the average duration of asymptomatic infection and duration of immunity were based on Chapman et al's modelling of the natural history of infection [20]. Other parameter values (for disease progression and sandfly bionomics) were based on estimates from previous field and modelling studies (see Tables 2 and 3). A parameter uncertainty analysis was performed to estimate the uncertainty in key transmission parameters (see below).

Sandfly-to-human ratio (SHR). Model W was fitted to the CARE data by estimating the average SHR for each district by maximum likelihood estimation. The number of new cases in each month was assumed to be Poisson distributed, and the full likelihood for each district taken as the product of the individual probabilities of the monthly numbers of cases predicted by the model.

IRS efficacy. IRS was assumed to increase the sandfly death rate by a percentage equal to the product of the IRS efficacy factor and the district IRS coverage rate from the CARE study. The model was fitted both with a fixed IRS efficacy factor (chosen based on the parameter uncertainty analysis) and assuming no impact of IRS on the sandfly density, and the goodness of fit compared using the Akaike Information Criterion (AIC = 2k − 2log(L), where k = 8 is the number of estimated parameters and L is the total likelihood for the 8 districts) to assess whether IRS may have had a significant impact on VL transmission.

Parameter uncertainty analysis. Given the high degree of uncertainty in the values of several of the model parameters, a parameter uncertainty analysis was carried out to determine 95% confidence intervals for 9 parameters: the SHR, the amplitude and phase shift of the seasonal variation in the sandfly birth rate, the proportion of asymptomatic individuals who develop clinical VL, the average duration of asymptomatic infection, the relative infectivities of asymptomatic individuals and clinical VL cases, the duration of immunity and the IRS efficacy factor (see SF1). Confidence intervals were determined by simulating the model with 300,000 parameter sets sampled from random uniform distributions for the parameters and accepting those for which the likelihood was not significantly different from the maximum likelihood according to the likelihood ratio test. Further details of the model fitting method and parameter uncertainty analysis are provided in SF1.

Predictions of future VL trends

We first predict the trends in VL incidence in the 8 CARE districts up to 2020 assuming the continuation of the district-specific 2012 intervention levels, i.e. the same IRS coverage and OT time. Then we explore hypothetical scenarios with incidence rates of 10, 5 and 2 VL cases per 10,000 capita per year, since the estimated VL endemicities at sub-district level from the CARE data in Bihar in 2012 ranged between 0 and 9.1 VL cases per 10,000 capita per year. The distribution of sub-district incidences can be found in Figure S4 of Supplementary File 2. For these hypothetical scenarios, VL incidence is predicted for the default scenario of 60% IRS coverage and 40-day average OT time, and scenarios with an increased IRS coverage of 80% and shorter average OT time of 20 days to reflect a further improvement of the control program.

Table 2. Parameter values and assumptions that are similar across all models

Parameters	Value*	Source	Reported range	Source
Human parameters				
Average duration of symptomatic untreated stage (days)	District and year specific (See SF2)	CARE data	N/A	N/A
Average duration treatment 1 (days)	28	CARE data	N/A	N/A
Average duration treatment 2 (days)	28	CARE data	N/A	N/A
Average duration of putatively recovered stage (months)**	21	[27, 42, 43]	21-36	[27]
Average duration of PKDL (years)**	5	Expert opinion and [27]	0.5-5	[27, 44]
Excess mortality rate among untreated symptomatic cases (per day)	1/150	Assumption		
Excess mortality rate among treated symptomatic cases (per day)	1/120	Assumption		
Fraction of failed first-line treatments	District-specific (See SF2)	CARE data	N/A	N/A
Fraction of putatively recovered cases that develop PKDL	2.5%	CARE data	2.4%-17%	[42, 44]
Infectivity of symptomatic untreated cases	1	Reference value	0.02-0.42	[45]
Relative infectivity of patients under treatment 1 and 2	0.5	Expert opinion	Unknown	
Sandfly parameters				
Average life expectancy of the sandfly (days)	14	[46]	10-20	
Average duration of incubation period in sandflies (days)	5	[47]	4.7-5.1	[48–50]
Sandfly biting rate (per day)	1/4	[12, 49]	1/5-1/4	[12, 46, 48, 49]
Probability of transmission from an infected sandfly to susceptible human	1	Reference value	N/A	

* Parameter values held fixed in model fitting and predictions unless otherwise stated.

** Ranges given are for average (median) duration based on the literature. Durations treated as exponentially distributed in models.

Table 3. Parameter values and assumptions that differ across the models.

Parameter	Value			Source
	Erasmus MC		Warwick	
	Model E0	Model E1	Model W	
Birth rate (per 1000/yr)	Bihar specific		District-specific (see SF2)	Indian 2011 Census
Mortality rate (per 1000/yr)	Bihar specific, age-dependent		District-specific (see SF2)	Indian 2011 Census
Sandfly birth rate	Stepwise function with 3 month peak in July - Sept		Sinusoidal function with peak in Oct-Nov	[31] (E0, E1) [34, 52, 53] (W)
Infectivity of PKDL, relative to symptomatic untreated cases	0.5	0.5	N/A	Expert opinion
Duration of immunity (years)	2 (1 and 5 in sensitivity analysis)	2 (1 and 5 in sensitivity analysis)	5	Assumption based on [19] (E0,E1) Assumption based on [20] (W)
Average duration of PKDL (years)	5	5	N/A	Expert opinion, [27]
Sandfly exposure	Age-dependent	Age-dependent	N/A	[19]
Duration of early asymptomatic stage (days)	202	202	Early + late = 150	[19] (E0, E1) [20] (W)
Duration of late asymptomatic stage (days)	69 days (fitted)	69 days (fitted)		
Infectivity of asymptomatic stage	0 (pre-set)	0.0114 (early) 0.0229 (late) (fitted)	0.025	Assumption based on [17] (W)
Fraction of (late stage) asymptomatic individuals who develop VL	0.0142 (fitted)	0.0142 (fitted)	0.03	Assumption based on [53] (W)

Results

Parameter estimation

Table 4 presents the values of all fitted parameters of models E0, E1 and W. With a duration of the early asymptomatic stage of 112 days and shorter the ability of the models E0 and E1 to reproduce the KalaNet data rapidly declined. The best fitting sub-models had a duration of early asymptomatic stage of 202 days (deviances of all 18 stable sub-models are presented in Table S3 of SF3). With model E0, the pre-control SHR ranged from 1.84 in West Champaran to 3.92 in Saharsa and with model E1 from 0.35 in West Champaran to 0.60 in Saharsa. The IRS efficacy is estimated by fitting the two models to all available CARE data points from the 8 districts simultaneously and resulted in a value of 99.9% (Model E0) and 82.9% (Model E1), which corresponds to a sandfly birth rate reduction of between 49.7% and 59.9% when multiplied by the average district IRS coverage rate of 60%. The results and interpretation of the sensitivity analyses for the duration of immunity (1 and 5 years) and the start year of IRS (2010 and 2012) are presented in Tables S4–7 of SF3. These results suggest that the duration of immunity is probably close to 2 years, as was assumed in the previous study [19]. Further, the selected start year of IRS in 2011 fitted the data best.

For model W, an average IRS efficacy value of 0.006, equivalent to an annual reduction of 9% in the sandfly density with an IRS coverage of 60%, was found to give the best fit to the data from a range of values tested in the parameter uncertainty analysis. The large discrepancy between the fitted IRS impact of models E0 and E1 (50–60%) and model W (9%) is largely due to the Erasmus MC models also including the drop from the Thakur data to the CARE data in their fitting procedure. Besides this, a greater IRS impact is also required for models E0 and E1 to reach the short term drop in cases, due to the longer duration of the asymptomatic stages (270 days in models E0 and E1 versus 150 days in model W). The district average SHRs that were estimated from fitting model W to the CARE data for each of the 8 districts individually (without censoring) are also presented in Table 4. The SHRs are positively correlated with the district average identified case burdens (compare Table 4 with Table S1 in SF2 and see Figure S1 in SF3), ranging from 0.36 in West Champaran to 0.45 in Saharsa. When model W was fitted under the assumption that IRS had no impact on incidence, the model was unable

to reproduce the drop (in all districts but West Champaran) between the first peak in the number of cases in 2012 and the second peak in 2013 (results not shown). The model only predicted a very slight drop in the number of cases in each district due to the decrease in the OT times from 2012 to 2013. Varying the change in the mean OT time for each district showed that an approximately 10-fold decrease in the time to treatment would be required to produce the drop in the numbers of cases from 2012 to 2013, a far greater decrease than observed in the actual OT times for any of the districts. The AIC value for the model with the IRS efficacy factor of 0.006 taken from the parameter uncertainty analysis was significantly lower than for the model with no effect of IRS (AIC = 1082.2 compared to AIC = 1400.8). This suggests that the drop in the numbers of cases across the districts was more likely due to decreases in the sandfly populations, which may have occurred as a result of IRS or other extraneous factors, than decreases in the OT times. However, we note that these results are dependent on the assumed infectivity of asymptomatic individuals (such that they are the main contributors to transmission) and the estimated efficacy of IRS.

Geographical cross-validation

The monthly VL case numbers for each censored district predicted by the three models in the geographical cross-validation, together with the observed case numbers from the CARE dataset are presented in Figure 3. Models E0 and E1 predict the data of censored districts Saharsa, East Champaran, Khagaria and West Champaran relatively accurately. However, in Begusarai and Patna the models both predict slightly lower numbers of identified cases compared to the data and slightly higher numbers in Samastipur and Gopalganj. For model W, the SHR for the censored district was informed solely by the average case burden in 2012 in the censored district, and therefore the predictions for the censored districts are less accurate than for models E0 and E1, which are informed by multiple data points from historical data. As expected given the simplicity of the estimation method, the fits are better when all the districts are uncensored (compare Figure 3 with Figure S2 in SF3) and the method suffers from considerable inaccuracy for the lowest burden district (West Champaran). For Begusarai, the SHR estimated from linear regression of the 2012 average district case burdens on the fitted SHRs for the other 7 districts is insufficient to give a stable endemic equilibrium (i.e. the method predicts zero cases).

Prediction of elimination

Figure 4 presents forward predictions of VL incidence up to 2020 for all 8 districts with their district-specific IRS coverage (2012) and OT time (2013). Here, the three models were fitted to all of the available CARE data. Models E0, E1 and W all show that the target incidence of <1 VL case per 10,000 capita per year will be, or has already been, reached at district-level in all districts before 2020, apart from Saharsa as predicted by models E0 and E1. In fact, for Patna and West Champaran the data already indicate an identified VL case burden in 2012 below the target of <1 new VL case per 10,000 population per year. Due to the seasonal variation in incidence, the predicted incidence for the other districts oscillates above and below 1 VL case per 10,000 capita per year before decreasing and remaining below the target. We interpreted the final point at which the model predictions pass through the target incidence line as the district-level elimination time. Models E0 and E1 predict that Begusarai, Khagaria, East Champaran, Samastipur and Gopalganj will reach the elimination target between mid-2011 (E1) and 2019 (E0). Model E0 predicts a slower trend towards elimination compared to model E1, with elimination occurring a maximum of 3 years later (Gopalganj), due to the slower impact of interventions caused by symptomatic cases (VL and PKDL) being the main contributors to transmission as opposed to asymptomatic individuals. The difference in predictions of reaching the elimination target between the shortest (142 days) and longest (382 days) duration of the early asymptomatic stage, ranges between 1 and 3 years, depending on the district, with the longest duration until elimination predicted by the model with the longest duration of the early asymptomatic stage (Figure S3 in SF3). Model W predicts that Begusarai, Samastipur, Khagaria, East Champaran and Gopalganj reached the target between April 2013 and May 2014 (in that order), and Saharsa in June 2015. The systematic difference in the pre-control endemic equilibrium between models E0 and E1 and model W is due to Erasmus MC fitting to historical annual case data from Thakur et al. [37] before fitting to the CARE data and Warwick only fitting to the CARE data.

As described above, the WHO elimination as a public health problem goal is defined as reaching the target at sub-district level, not at district level. The predicted incidences for three typical sub-districts with current and alternative intervention strategies are presented in Figure 5. In sub-districts with a pre-control endemicity level of 2/10,000, models E0 and E1

predict that elimination will be reached under the current interventions (an average OT time of 40days and 60% IRS coverage) ~1.5 years after starting IRS, whereas under the same conditions model W predicts that elimination will be reached after 3.5 years. In sub-districts with a pre-control endemicity of 10/10,000, model W predicts reaching the target incidence after 5.5 years, model E1 after 6 years and model E0 after more than 8 years from the start of IRS. Increasing the IRS coverage from 60% to 80% or halving the OT time from 40 days to 20 days brings forward elimination by 6 months to 3 years at all endemicity levels for all models (the reductions in the time to elimination being greater for the sub-districts with higher pre-control endemicities). Models E0 and E1 predict that increasing the IRS coverage to 80% will have a greater effect than halving the OT time, whereas model W predicts that halving the OT time will have a slightly greater effect. However, combining an increase in IRS coverage to 80% with a reduction in OT time to 20 days is predicted to reduce the time to elimination at all endemicity levels for all models.

Table 4. Estimated parameter values resulting from fitting to all district data without censoring

Parameter*		Erasmus MC		Warwick
		Model E0 (95% CI**)	**Model E1** (95% CI**)	**Model W**
1. Fraction of late asymptomatic individuals who develop VL (%)		1.4 (1.00- 1.84)	1.42 (1.00-1.84)	N/A
2. Duration late asymptomatic stage (days)		69 (49-119)	69 (49-118)	N/A
3. Duration early recovered stage (days)		237 (196-299)	236 (196-298)	N/A
4. Infectivity of early asymptomatic stage		0***	0.0114 ****	N/A
5. Infectivity of late asymptomatic stage		0***	0.0229 (0-0.0533)	N/A
6. District specific average sandfly-to-human ratio (SHR)	Saharsa	3.92	0.604	0.445
	East Champaran	2.20	0.392	0.381
	Samastipur	2.49	0.401	0.398
	Gopalganj	2.33	0.403	0.390
	Begusarai	2.78	0.383	0.425
	Khagaria	2.44	0.388	0.401
	Patna	2.12	0.359	0.380
	West Champaran	1.84	0.351	0.364
7. IRS efficacy *****	Employing data from all 8 districts	0.999	0.829	0.006

NB. Values for model E0 and E1 presented here are only for the duration of early asymptomatic stage of 202 days; values for the other sub-models are listed in Table S3 of Supplementary File 3.

* Parameters 1-5 were fitted to the KalaNet data (models E0 and E1), parameter 6 was fitted to the Thakur data (models E0 and E1) and the CARE data (model W), and parameter 7 was fitted to the CARE data (models E0, E1 and W).

** Confidence interval

*** Pre-set values, in model E0 asymptomatic individuals are considered not to be infective.

**** Not fitted, but calculated as half the infectivity of the late asymptomatic stage.

***** The IRS efficacy is multiplied by the district-specific IRS coverage rate to get the IRS impact on the SHR. Note, however, that the dependence of the SHR on the IRS efficacy is linear in models E0 and E1, but exponential in model W (see Additional File 1 of [20] and Supplementary File 1)

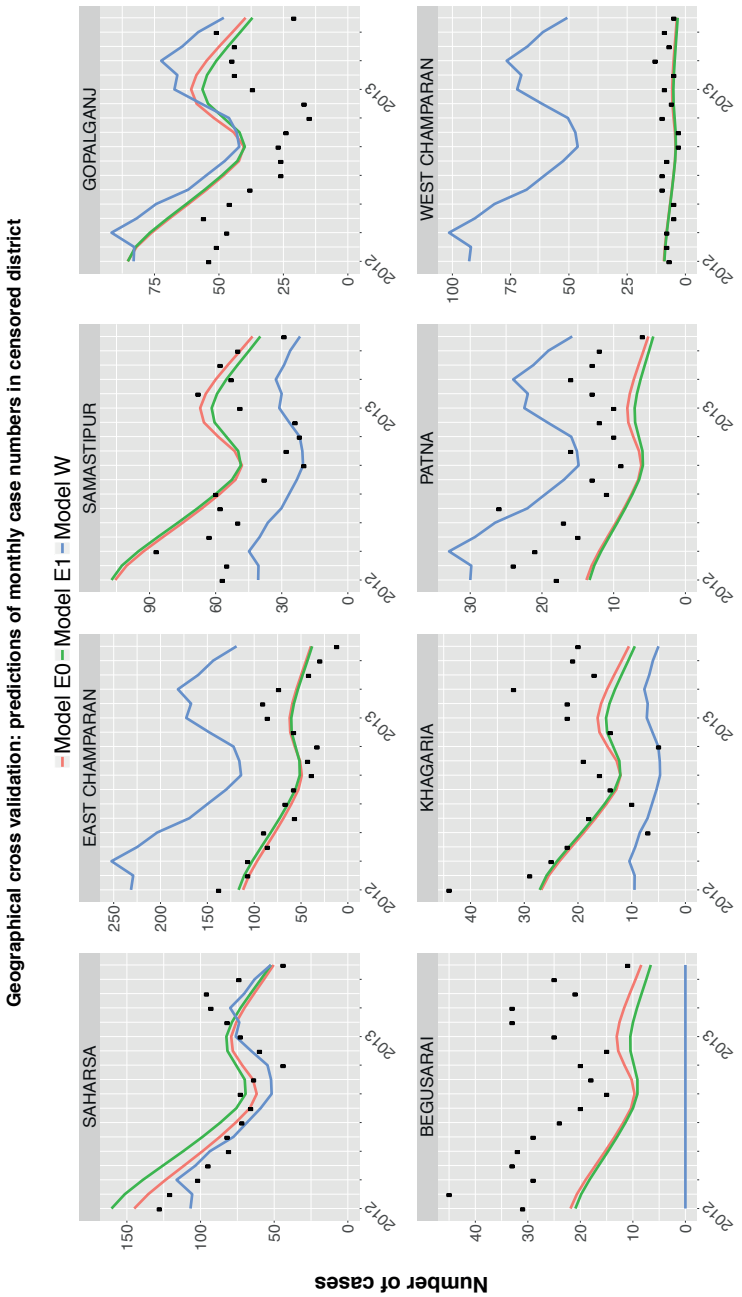

Figure 3. Geographical cross-validation of the models: predictions for the monthly number of VL cases in each district from fitting the models to the other 7 districts and using the fitted models to predict the cases in the censored district. The CARE data are presented with black dots and the lines present the predictions of models E0 (red), E1 (green) and W (blue) between January 2012 and June 2013. The prediction of 0 cases for Begusarai for model W is due to the sandfly-to-human ratio estimated from fitting to the other 7 districts giving a basic reproduction number below 1 (so that there is no stable endemic equilibrium).

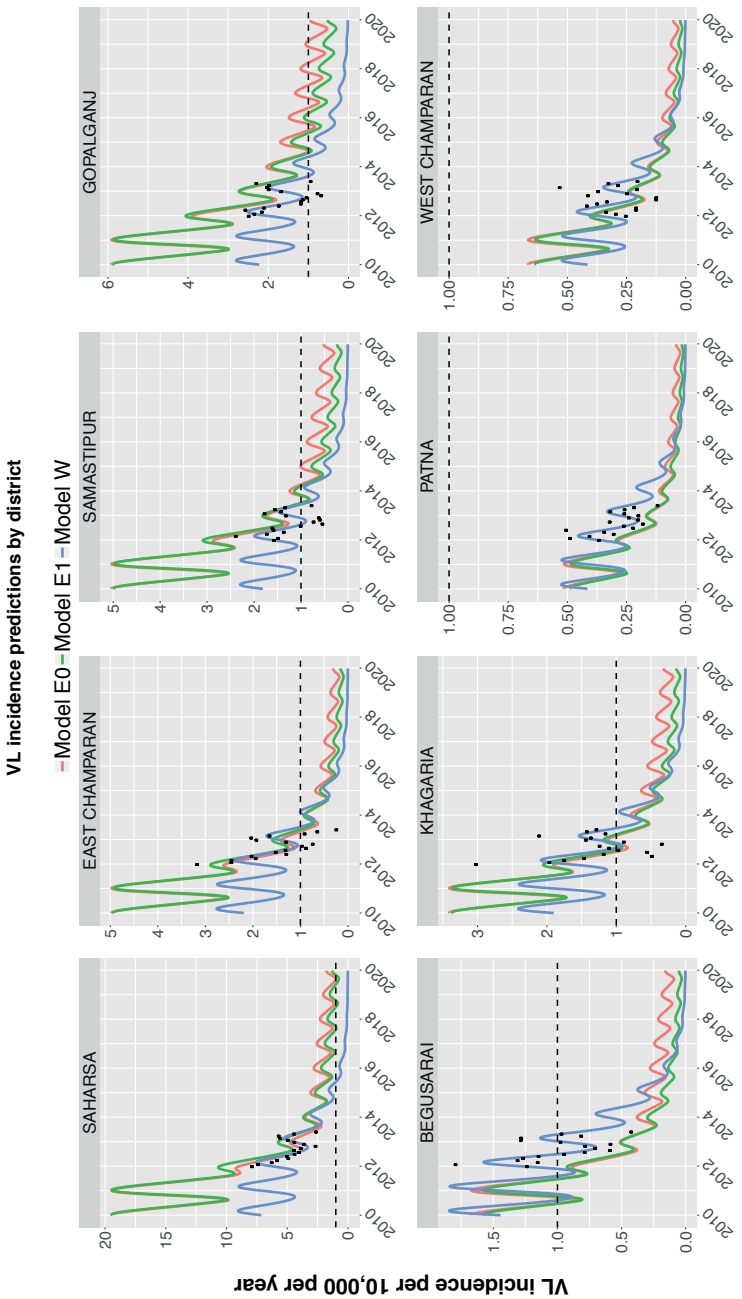

Figure 4. VL incidence predictions for each district employing all of the CARE data. The CARE data are presented with black dots and the lines present the predictions between 2010 and 2020 for each model. IRS starts in January 2011, after which IRS coverage remains constant. The difference in the pre-control endemic equilibrium between the Erasmus MC and Warwick models is due to Erasmus MC fitting to historical case data from [37] before fitting to the CARE data and Warwick fitting only to the CARE data. The black dashed line represents the WHO elimination target of <1 VL case per 10,000 population per year. The monthly predictions between January 2012 and June 2013 are also presented in SF3, Figure S2.

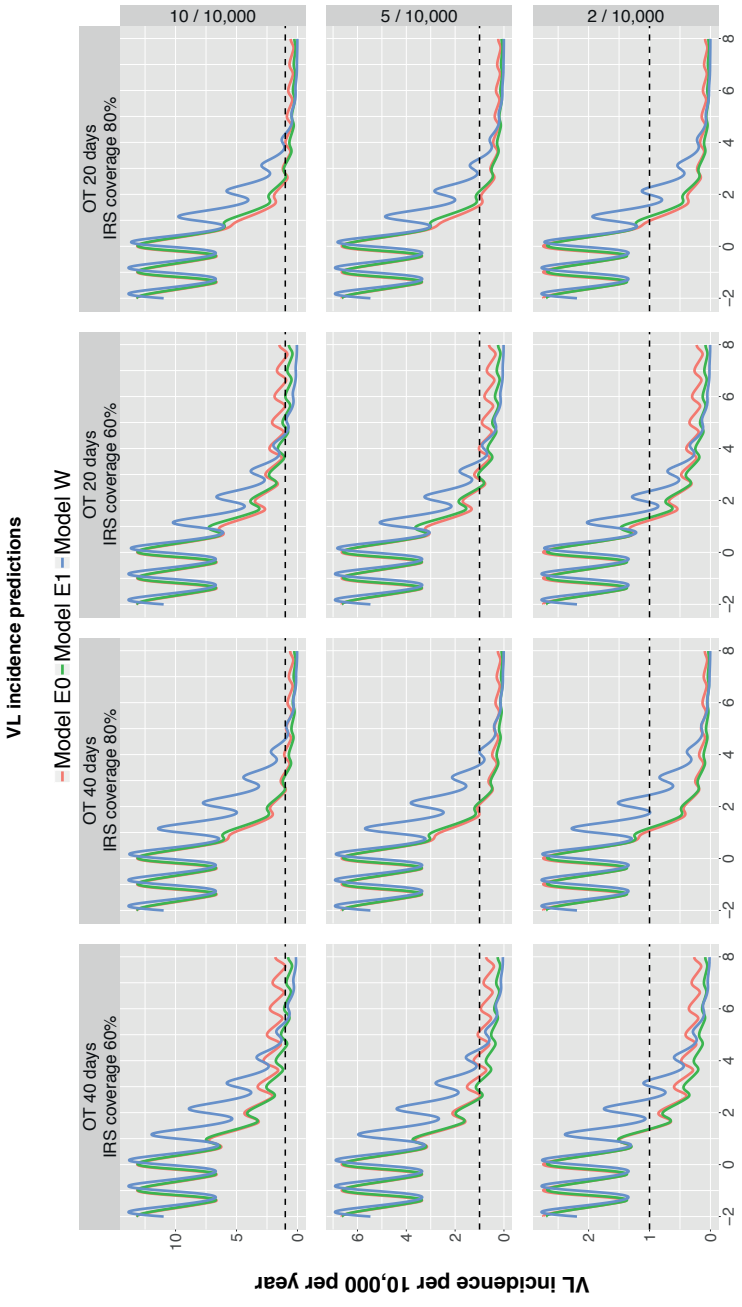

Figure 5. VL incidence predictions at sub-district level for different endemic scenarios under alternative intervention strategies. The lines present the VL incidence predictions for each model. The black dashed line represents the WHO elimination target of <1 VL case/10,000 population/year at sub-district level. Interventions start in year 0 after which they are continued at the same level. OT denotes the time between the onset of symptoms and the start of treatment, IRS coverage represents the percentage of houses sprayed, which is multiplied by a (constant) IRS efficacy of 0.999 and 0.829 in models E0 and E1 and 0.006 in model W. The 12 predictions presented here include 3 different endemic scenarios of 10, 5 and 2 cases per 10,000 population each with 4 different combinations of interventions.

Discussion

The Erasmus MC and Warwick models are quite different in their model structure, fitting methodologies and use of data, yet their long-term predictions are comparable and suggest that elimination can be reached by or shortly after 2020 even in highly endemic sub-districts (up to 10 VL cases per 10,000 capita per year pre-IRS), provided IRS started before or in 2011 with a minimum coverage level of 60% and is maintained at this level, and provided that the average onset-to-treatment (OT) time does not exceed 40 days. In settings with a pre-control endemicity level of 5/10,000, increasing the effective IRS coverage from 60 to 80% is predicted to lead to elimination of VL 1–3 years earlier (depending on the type of model), and decreasing OT from 40 to 20 days to bring elimination forward by approximately 1 year.

All VL transmission models face the challenge of describing transmission of a disease with many key factors, such as the role of immunity and the contributions of individuals in different disease stages to transmission, basically remaining unknown [14, 16, 19]. Also the impact of IRS, the main intervention strategy, on sandfly densities remains debated. Together, the KalaNet and CARE studies provide information about the prevalence of leishmania DNA and anti-leishmanial antibodies in the population, as well as detailed data on start of symptoms, detection and treatment dates of symptomatic individuals. Since both studies were conducted in the same region, biological parameters estimated from the KalaNet data were considered to be similar in the CARE study sites, but this is not necessarily the case. Also, although longitudinal, the CARE dataset only covered a time period of 18 months, which is short relative to the long duration between time of infection and cure of the disease, the time until development of PKDL and the potentially long duration of immunity. The sensitivity of the sub-district-level model predictions to the estimated reduction in the sandfly-to-human ratio due to IRS (9% for model W, 50% for model E0 and 60% for model E1) demonstrates the importance of collecting sandfly data alongside human epidemiological data to properly quantify the impact of IRS. Furthermore, the CARE dataset did not provide information about the start years of IRS per district, sandfly bionomics data from the same location and time period as the VL case and IRS coverage data, or sandfly DDT-resistance data, all important factors to estimate the impact of IRS strategies on VL incidence. Given the

relatively long duration of the asymptomatic infection stage, the duration of immunity and the role of PKDL (which could slow down the initial decline seen in the first two years of control [19]), the decline in VL incidence due to IRS is expected to be seen only months to years after the start of IRS; hence data spanning a longer time frame would be valuable for future studies.

Both model E0, in which asymptomatic individuals are not infectious, and model E1, where the main contributors to transmission are asymptomatic individuals, provided an estimate of about 200 days as the duration of the early asymptomatic stage best fitting the CARE data (i.e. 9 months when including the late asymptomatic stage). For model W, the average duration of asymptomatic infection was based on [20], where it was estimated as approximately 5 months (95% CI 4–5.5 months). This estimate was used in this paper as it is supported by the presence of a seasonal pattern in the monthly numbers of VL cases in the CARE districts and the timing of this pattern relative to observed seasonal variation in sandfly abundance in Bihar [31–33, 51]. It also agrees reasonably well with the results of the parameter uncertainty analysis for model W (the maximum likelihood estimates for the asymptomatic infection duration for the 8 districts ranged from 98 to 163 days, see SF1). Even though the main contributors to transmission remain unknown, the predictions of models E0 and E1, which both fit the data nearly equally well, provided new insights in reaching the elimination targets in both (extreme) scenarios. Model W is comparable to model E1 in terms of asymptomatics being the main contributors to transmission, but because of differences between the models in the durations of disease stages and fitting the models to different data, the fitted IRS impact for model W is much lower, leading to a slower reduction in incidence with current and improved interventions. When more information becomes available regarding the main contributors to transmission, more weight can be put on the predictions of model E0 or model E1 (or model W), which differ most in terms of long-term trends.

Another unknown aspect of the VL dynamics is the role and duration of immunity. We therefore repeated our analyses of models E0 and E1 for two alternative durations of 1 and 5 years. With a 5-year average immunity it was not possible to fit the sub-models to the data with an early asymptomatic stage duration shorter than 322 days (which was one of the 10 chosen durations). However, in our study, we interpreted the KalaNet and Thakur

data as endemic equilibriums, which is not always the case in the field when looking at longitudinal data, especially at local level. We might have arrived at a different duration of immunity had we been able to fit our sub-models to data including fluctuations in incidence over multiple years at a local level [54–56]. We also performed a sensitivity analysis of models E0 and E1 for the start year of IRS, for which no conclusive data were available. We interpreted the start year of IRS in 2011 to be the most likely scenario when compared to starting IRS in 2010 or 2012, because of the decrease in VL incidence in multiple datasets after 2011 [37, 57, 58], and since starting IRS in 2012 fitted the data poorly (see SF3).

The fitted IRS efficacy for models E0 and E1 led to a sandfly birth rate reduction between 37% in Gopalganj and 72% in Saharsa, which aligns with reported reduction in sandfly density of 72% after IRS with DDT [59]. For model W, the IRS efficacy corresponds to an annual reduction in sandfly density of 6.7% in Gopalganj and 10.7% in Saharsa. This is apparently at odds with estimates for reductions in sandfly densities from field studies [59], but could reflect poor implementation of IRS and increasing sandfly resistance to DDT [60]. However, given the correlation of the IRS efficacy factor with other parameters (e.g. the average duration of asymptomatic infection), it is also possible that the IRS efficacy factor has been underestimated due to inaccuracies in the estimates for correlated parameters. The issue of parameter identifiability is not restricted to the IRS efficacy, as the parameter uncertainty analysis for model W in SF1 shows. The average sandfly-to-human ratio and the infectivity of asymptomatic individuals are strongly negatively correlated, and the average duration of asymptomatic infection and amplitude of the seasonal forcing of the sandfly birth rate are positively correlated for most districts, which means that these parameters cannot be uniquely identified from the CARE data, which may account for some of the differences between the models' predictions. Hence, data from high quality epidemiological and entomological studies are needed alongside the CARE data to tease apart the correlation between these parameters. Using more detailed data on disease progression (e.g. longitudinal serological data for humans and infection prevalence data for sandflies) would then also improve the accuracy of model predictions (as demonstrated by models E0 and E1).

In model W it was assumed that 3% of asymptomatic individuals develop clinical symptoms even though the estimated proportion for the

dataset analyzed in [20] was 14.7% (95% CI 12.6–20.0%). This is because the maximum proportion that the CARE data can support is 7.3%, assuming an asymptomatic prevalence of approximately 1% at district level and a long asymptomatic duration of 530 days [19] (see SF1), and because more recent studies from Bihar suggest values in the region of 3–4% [53]. This difference in the estimated proportion of asymptomatics progressing to VL may reflect the different locations and time periods in which the studies were conducted – the former in Bangladesh during an epidemic, the latter in Bihar, India, 4–5 years after an epidemic [15]. However, due to the focal nature of VL it is also likely that we have significantly under-estimated local VL incidence from the CARE dataset that all three models were fitted to, because we calculated the identified case burden at district-level, such that a higher proportion of asymptomatic individuals progressing to VL is likely.

Another potential source of inaccuracy in model W is the omission of PKDL. Including PKDL in the model results in longer times to elimination, as there is a reservoir of infectious individuals with potentially long durations of infectivity. However, PKDL was excluded due to uncertainty regarding the disease history of PKDL cases and the proportion of individuals with PKDL that were actually diagnosed.

All models currently assume that there is homogeneous risk of transmission, but VL occurrence is highly spatially heterogeneous and at a scale much smaller than that of a district [61, 62]. Consequently, we are averaging the incident cases over the total population of all affected sub-districts regardless of how many of those people were at risk of infection, thus underestimating the incidence in those actually at risk. Models with finer geographic stratification (e.g. at the level of villages or even sets of households) and migration would present a more realistic framework and potentially provide an important tool for modelling elimination of infection. An individual-based model would be able to include advanced aspects such as these. Less than half of the 8885 villages and town wards in the 8 districts had a VL case during the study period. However, there may be significant transmission in villages without cases during the timeframe of the data if the asymptomatic to symptomatic ratio is as high as estimated, but this cannot be discerned from the CARE data since it contains no information on asymptomatic cases.

District-and state-level data on annual case numbers suggest that there are long-term cycles (with a period of ~15 years) in incidence, which are not produced by our models and cannot be readily explained by them either. The models all assume that the transmission dynamics were in equilibrium prior to 2011 and we did not implement an underlying mechanism for these ~15-year cycles in our models because there are no data explaining such a mechanism. However, it has been suggested that these cycles have been caused by intermittent control efforts [55]. The additional impact of IRS and reduced OT time over a decrease in incidence from being in a declining phase of a long-term epidemic cycle (as appears to be the current situation) is therefore unknown. The potential role that long-term immunity could play in the epidemic cycles, by causing rapid depletion of the susceptible pool in the population during an epidemic, which is then replenished by births in the inter-epidemic period, has also not been accounted for [63], given the assumed short durations of immunity (2–5 years). However, it remains to be shown that such a mechanism could lead to distinguishable long-term cycles at the level of districts, let alone countries and subcontinents. Longer time series, and ideally also serological and PCR data, are needed to accurately estimate duration of immunity and other key parameters.

Whilst the deterministic compartmental models presented here are suitable for understanding and predicting the longer time-scale dynamics and interactions, they are less useful for predicting the situations close to elimination or after elimination. When numbers of cases become very small stochastic effects dominate, something all three models do not account for. Given that recrudescence would have a significant public health impact if not controlled, stochastic models would be very useful. Such models could also be used to assess the effectiveness of alternative targets. For example, since VL is such a focal disease, setting targets and focusing interventions at a smaller (e.g. village) level could be a valuable next step. However, stochastic (and in particular individual-based or agent-based) models typically contain more parameters and therefore require more detailed data and/or assumptions.

Our three models differ in terms of detailed structure and parameterization. Despite these differences, the models are consistent in predicting that we are on track to reach the target elimination incidence in all 8 Indian districts – at least at district level. Several sub-districts were already below the target incidence in 2012. The 10 sub-districts with the highest

endemicity were all in Saharsa and East Champaran with ≥5 VL cases per 10,000 capita per year. These sub-districts are likely to require additional efforts, such as increased IRS coverage and reduced OT time, to reach the target incidence on time.

Capturing the disease transmission dynamics of visceral leishmaniasis in a mathematical structure is a complex challenge due to the many unknown factors regarding this neglected tropical disease [15, 64]. However, combining the outcome of different mathematical modelling approaches creates a more solid foundation from which to draw conclusions about reaching the VL elimination targets for the Indian subcontinent. We caution that a model comparison such as this can provide false confidence as there are processes which are not included in the models that might be critical. In particular, all the models assume the same underlying SIRS structure (susceptible – infected – resistant – susceptible) and none of the models includes spatial heterogeneity. Nonetheless, based on the three VL transmission models, we conclude that reaching the VL elimination target of less than 1 VL case per 10,000 capita at sub-district level before or shortly after 2020 in the Indian subcontinent seems feasible under continued interventions of indoor-residual spraying and early detection and treatment of cases.

Supplementary Files

Supplementary File 1: Warwick model description and parameter uncertainty analysis
Supplementary File 2: Data description
Supplementary File 3: Fitting procedure and Erasmus MC sensitivity analyses
Supplementary File 4: Description to Supplementary File 5.
Supplementary File 5: R-package 'VLode' of age-structured system of ordinary differential equations for visceral leishmaniasis transmission developed by Erasmus MC
Supplementary File 6: MATLAB code for Warwick model fitting and predictions

Author contributions

Oversaw the data collection: SD, IC, SS, ECH and MCB. Conceived and designed the study: EALR, LACC, LEC, SJ, GFM, TDH and SJdV. Cleaned the data: LACC and SJ. Performed the analysis: EALR, LACC, LEC and SJ. Wrote the manuscript: EALR and LACC. Helped with interpretation of the data: MK, AD, TM, IC, GFM, SS, TDH and SJdV. Provided feedback on the modelling and reviewed the paper: LEC, GFM, TM, SS, TDH and SJdV.

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SUPPLEMENTARY FILE 1

Warwick visceral leishmaniasis transmission model

Online available at https://ars.els-cdn.com/content/image/1-s2.0-S1755436516300792-mmc1.pdf

SUPPLEMENTARY FILE 2

Further description of CARE data and parameter estimation

Spatial, seasonal and annual variation in identified VL case numbers

The burdens of identified VL cases in 2012 in the 8 districts in the CARE dataset are given in Table S1. These have been calculated from the data by dividing the number of identified cases in each district by the total population of its *sub-districts* that had at least 1 case between January 2012 and June 2013. The sub-district populations are taken from the 2011 Census of India and population growth has been taken into account using the average yearly percentage population growth per district, derived from the change in the district populations between the 2001 and 2011 censuses [1]. The identified VL case burden in 2012 varied significantly by district, ranging from 0.29 cases per 10,000 capita per year for West Champaran to 5.03/10,000/year for Saharsa, though for most districts it was between 1 and 2 cases per 10,000/year. Figure S1 shows the monthly numbers of onsets of VL symptoms from January 2012 to June 2013 for each of the 8 districts. There is clear seasonal variation in the numbers of cases in the five highest burden districts (Saharsa, East Champaran, Samastipur, Gopalganj and Khagaria), with an annual peak between January and April. This seasonal variation in case numbers has also been observed in other studies [2, 3] (though from monthly numbers of diagnoses rather than onsets of symptoms), and is almost certainly driven by seasonal variation in sandfly abundance. The lag(s) between the peak(s) in the sandfly population (believed to be in June-August and October-November [4–6]) and that in the incidence may therefore hold valuable information about the duration of asymptomatic infection prior to clinical VL. In all districts but West Champaran, the numbers of cases decreased between January to June 2012 and January to June 2013.

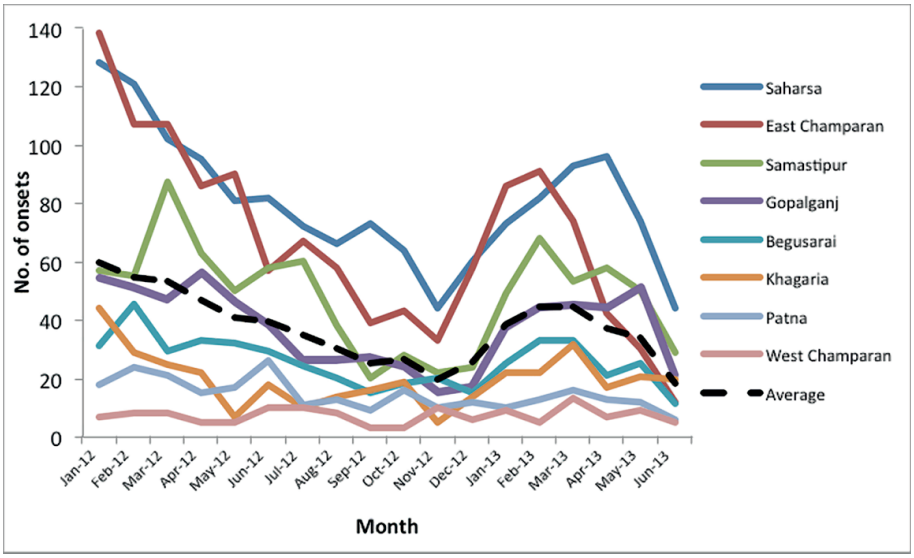

Figure S1. Monthly numbers of onsets of symptomatic VL cases for districts in CARE data. Dashed line shows average monthly number of cases across the districts.

Official data on the annual numbers of VL cases in the 8 districts, collected by Thakur *et al* between 2006 and 2011, show a period of relatively constant numbers of cases between 2009 and 2011 (inclusive). This period of relative equilibrium is followed by a sharp decrease in incidence from 2011 to 2012 in the four districts with most cases (Saharsa, East Champaran, Samastipur and Gopalganj) (Figure S2). The districts with lower numbers of cases – Begusarai, Khagaria, Patna and West Champaran – also show a decrease in cases, but less steep. All 8 districts experienced a smaller decrease in the following period between 2012 and 2013. The numbers of cases in each district in 2012 recorded in the CARE data agree well with the official figures as presented by the State Surveillance Unit of the State Health Society Bihar [7] except for Samastipur, for which the number from the CARE data appears to be a considerable underestimate. Figure S3 shows the monthly numbers of VL diagnoses for all of Bihar from 2009 to 2012, which as in the CARE data have a clear seasonal pattern, with a peak between March and May (which is later than the peak in onsets due to the delay between onset and diagnosis). These data also show that the seasonal pattern in Bihar at state level was different for 2012, with a smaller peak in cases than in previous years followed by a large decrease in incidence over the rest of the year. This corresponds well with the general downward trend in cases at district level

Table S1. Overview of CARE data and Census data (2001, 2011), input for geographical cross validation.

District	Number of identified VL cases *		Population size**		Annual percentage growth rate (%) ***	Burden of identified cases (cases per 10,000 population per year)#	Mean duration onset of symptoms to treatment (OT) (days)		Fraction T1 to T2##	Level of IRS coverage(%) ###	Start year of IRS
	2012 (Jan-Dec)	2013 (Jan-June)	2012	2013	2001-2011	2012	2012	2013	2012	2012	
Saharsa	988	462	1963972	1998338	2.34	5.03	33.3	34.2	6.4%	72.46	2011
East Champaran	884	335	5289197	5392537	2.61	1.67	53.5	44.1	7.3%	63.16	2011
Samastipur	562	307	4401084	4476786	2.30	1.27	38.2	34.2	14.0%	56.68	2011
Gopalganj	427	242	2625987	2660501	1.76	1.63	47.5	42.8	21.9%	44.42	2011
Begusarai	311	148	3039108	3093054	2.37	1.02	21.6	22.5	10.8%	62.09	2011
Khagaria	223	134	1730364	1764943	2.67	1.29	36.6	32.9	14.5%	58.14	2011
Patna	192	70	5949098	6044879	2.15	0.32	44.9	43.0	9.4%	58.70	2011
West Champaran	83	48	2879280	2935300	2.60	0.29	64.6	60.6	6.4%	53.66	2011
Total / Average	3670	1746	2787809	2836634	2.35	1.57	42.5	39.3	11.3%	58.66	

* Numbers include 37 individuals with estimated onset dates between 1st January 2012 and 30th June 2013 from known diagnosis dates and mean district onset-to-diagnosis times

** Estimated population size of affected sub-districts at 1st of July 2012 and 1st of April 2013.

*** District level population growth rates, calculated from the Indian census data 2001-2011

Calculated as the total number of cases per district in 2012 divided by the population size of all affected sub-districts on the 1st of July 2012 * 10,000

T1 = treatment 1 and T2= treatment 2

Calculated as the average of the percentage of patients houses that were sprayed in 2012 and the percentage of houses that were sprayed in the tola of patients in 2012

in 2012 observed in the CARE data, but suggests that the 2012 CARE data may not be representative of the `typical' annual seasonal pattern.

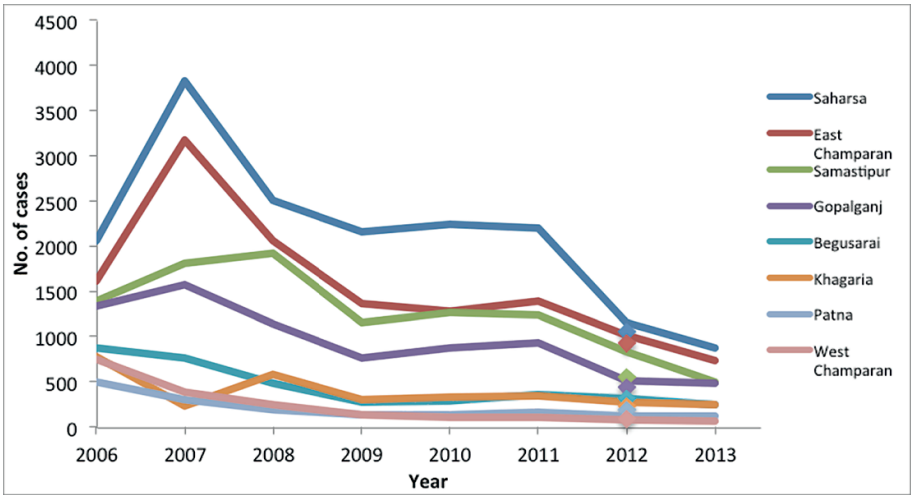

Figure S2. Yearly numbers of cases in 8 districts in Bihar from 2006 to 2011 from Thakur *et al* [8] **in 2012 from** [7] **and in 2013 from** [9]. Dots represent the 2012 CARE data (onset of symptoms) which is for most districts lower compared to the data presented here by Thakur *et al*.

Data cleaning

The data were extensively cleaned to correct inconsistencies in dates reported by patients or their relatives (such as patients reporting being diagnosed before their onset of symptoms). Dates were corrected by crosschecking with all available information on durations of illness, dates of diagnosis and treatment for VL, and treatment prior to diagnosis for each case; and dates were only changed when there was sufficient information to be confident of the correct date. The self-reported dates of onset of symptoms and times between onset of symptoms and start of treatment were used to fit the models. The monthly number of onsets per district was used as the incidence of 'symptomatic untreated' individuals in all models. Although the diagnosis dates are in theory more reliable (as more than half were taken from medical records), they include delays from onset to diagnosis, which differ between individuals and between districts. Hence the onset dates more accurately reflect, and enable fairer comparison of, seasonality in incidence across districts. Nevertheless, the diagnosis dates were used to correct inconsistencies in the onset dates.

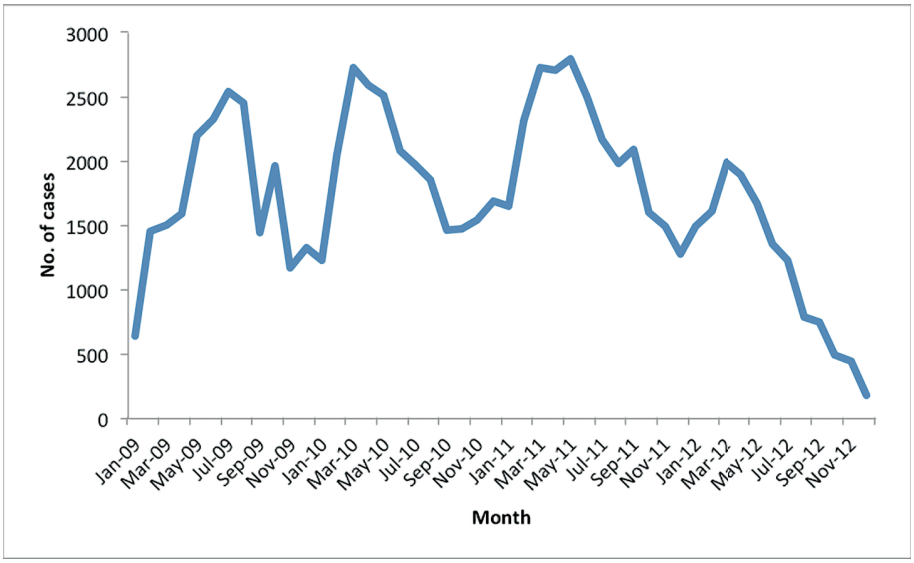

Figure S3. Monthly numbers of cases in CARE study districts from 2009-2013 from Annual Communicable Disease Surveillance Report, 2012, Bihar [7]

There were 43 individuals in the data without an onset day or month, 42 of whom had a diagnosis date. The onset dates of the 42 individuals were estimated by subtracting the mean onset-to-diagnosis time for their district (for their year of diagnosis) from their diagnosis dates. Thirty-seven of the estimated onset dates fell between 1st January 2012 and 30th June 2013, and hence were included in the monthly numbers of onsets for each district (and in the figures in Table S1).

Onset-to-treatment time

Times between onset of symptoms and start of treatment varied significantly between districts with means ranging from 21.6 days in Begusarai to 64.6 days in West Champaran in 2012 (Table S1). The mean onset-to-treatment times decreased from 2012 to the first half of 2013 by 2 to 9 days for all districts apart from Saharsa and Begusarai, where the mean times both increased slightly. In both the Erasmus MC and Warwick models, this is accounted for by a step change in the mean duration of symptoms at the start of 2013.

Treatment duration

The majority of VL cases were treated with Miltefosine and the reported duration of 1st- and 2nd-line treatments was 28-30 days for most individuals, corresponding to taking a capsule daily or receiving daily injections for 4 weeks to a month. The average 1st and 2nd treatment durations are therefore taken as 28 days in all models.

Fraction of VL cases progressing to 2nd treatment

Across all 8 districts, the average proportion of VL patients who received a 2nd treatment was 11.3%. However, at district level the proportion ranged from 6.4% for Saharsa and West Champaran to 21.9% for Gopalganj. Hence we use the district-specific proportions in fitting the models.

PKDL

The data include some cases of PKDL, but the numbers per district are very low, so have not been used to fit the PKDL incidence in the Erasmus MC models (PKDL is not included in the Warwick model). Approximately 2.5% of all the VL cases in the data developed PKDL, which is comparable to estimates from other field studies [10]. This value is therefore used to determine the flow between 'putatively recovered' to 'PKDL' in the Erasmus MC models, both for the fitting and the predictions.

IRS coverage

IRS coverage was calculated at district level from the CARE VL dataset and is based on the percentage coverage of spraying in 2012 in the house and/or neighborhood of the VL case. (Table S1) This approach is likely biased as only a subpopulation was interviewed in the CARE survey, however it represents the percentage coverage in VL endemic regions, which is what is being modeled in this study. Data on IRS coverage in the general population are unavailable for these years. For fitting the models, IRS at the calculated coverage levels was assumed to have started in 2011. When more detailed data become available in the near future, this can be included. A constant multiplication factor (representing the IRS efficacy), which was fitted, was used to convert the percentage coverage of IRS to the rate of reduction in sandfly density in the models.

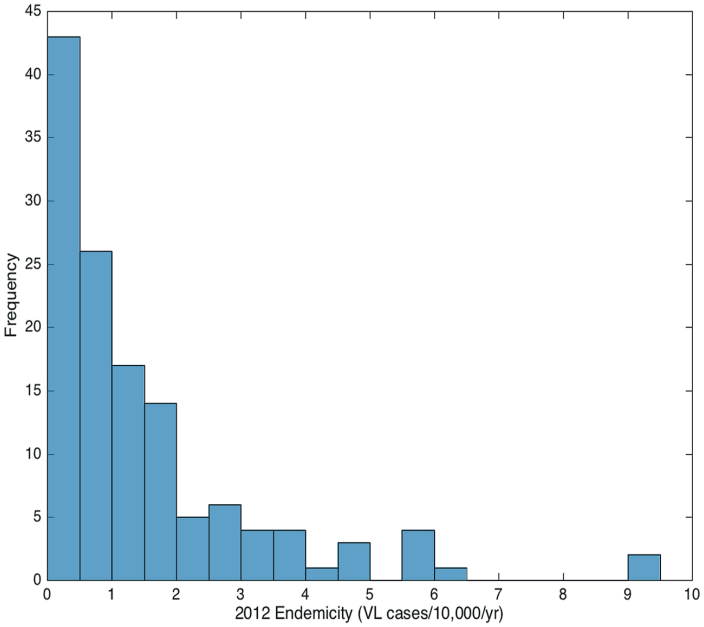

Figure S4. Distribution of 2012 sub-district endemicities for CARE districts

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SUPPLEMENTARY FILE 3

Geographical cross-validation of models, Erasmus MC sub-model selection, and sensitivity analyses

Cross-validation method

We tested the models' ability to reproduce censored data by means of a geographical cross-validation. The cross-validation was performed using a leave-one-out approach at the level of districts. Iteratively, the monthly number of VL onsets from January 2012 to June 2013 for one district were censored, and the model fitted to the data for the other 7 districts to estimate the IRS efficacy factor (Erasmus MC models, E0 and E1) or linear association between the district sandfly-to-human ratios (SHRs) and average 2012 case burdens from which the SHR for the censored district was estimated (Warwick model, W). The estimated IRS efficacy or SHR based on the 7 uncensored districts was used to estimate the monthly numbers of VL cases for the censored district. The mean onset-to-treatment times for 2012 and 2013, and 2012 IRS coverage level of the censored district were used in the simulation for the censored district. The ability of each model to reproduce the censored data was expressed in terms of the deviance of the model from the data summed over the eight iterations of censoring (Table S1). The model deviance is defined as twice the difference between the negative log-likelihood (LL) of the fitted model and the negative LL of a saturated model (i.e. one that fits the data exactly), as is presented for each model in Table S1. The lower the deviance, the closer the predictions of the model are to the data.

Although the geographical cross-validation enabled the predictive power of the models to be tested, fitting the models to the data from all 8 districts leads to better estimation of the fitted parameters. Therefore the models were also fitted to all districts without censoring, and these fitted parameter values were used for future predictions.

Table S1. Model deviances for censored districts in geographical cross-validation. Values for models E0 and E1 are based on the selected sub-models with an early asymptomatic stage duration of 202 days, which were associated with the lowest deviances. Deviances of all other sub-models are presented in Table S3.

Censored district	Model E0	Model E1	Model W
Saharsa	60	95	39.1
East Champaran	89	88	1516.2
Samastipur	182	176	214.7
Gopalganj	195	176	263.3
Begusarai	181	239	N/A
Khagaria	67	78	332.0
Patna	84	100	69.6
West Champaran	31	36	1655.9
Total deviance	890	988	4090.8

Models E0 and E1

Estimation of IRS efficacy

The geographical cross-validation was performed for each of the 18 sub-models (models E0 and E1 each with nine different assumptions about the duration of the early asymptomatic stage of VL). The district specific SHR was fitted to the Thakur data [1] for all 18 sub-models, to arrive at a pre-IRS equilibrium. Then the IRS efficacy parameter was fitted to the CARE data using the monthly numbers of VL onsets for the 7 uncensored districts via a maximum likelihood approach, assuming the data are Poisson-distributed. The estimated IRS efficacy was then used to predict the number of cases in the censored district for which the deviance per sub-model was calculated. Table S3 presents the deviances for all the 18 sub-models for the 8 iterations of censoring and also when fitting to all districts simultaneously. For both models E0 and E1, the sub-model with a duration of the early asymptomatic stage of 202 days best reproduced the censored data. These models were used for further simulations and forward predictions. Table S2 shows the deviances when all models are fitted to all of the CARE data.

Table S2. Deviances of models E0, E1 and W when employing all available CARE.

District	Model E0	Model E1	Model W
Saharsa	60.2	87.6	34.5
East Champaran	88.8	88.0	107.1
Samastipur	163.5	160.2	48.1
Gopalganj	183.2	163.8	32.3
Beghusarai	180.3	216.8	16.7
Khagaria	66.7	75.7	50.5
Patna	83.9	95.5	17.0
West Champaran	31.1	35.7	19.7
Total deviance	857.7	923.3	325.9

Sensitivity analyses

The deviances of the 18 sub-models in the sensitivity analyses of different average durations of immunity of one and five years are presented in Tables S4 and S5 respectively. The deviances of the 18 sub-models in the sensitivity analyses of different start year of IRS, 2010 and 2012, are presented in tables S6 and S7 respectively.

Model W

Estimation of sandfly-to-human ratio

The sandfly-to-human ratio in the censored district was estimated from the fitted SHRs in the other 7 districts by least squares linear regression of the average uncensored district identified case burdens in 2012 (see Table S1 in Supplementary File 2) against the fitted SHRs (Figure S1).

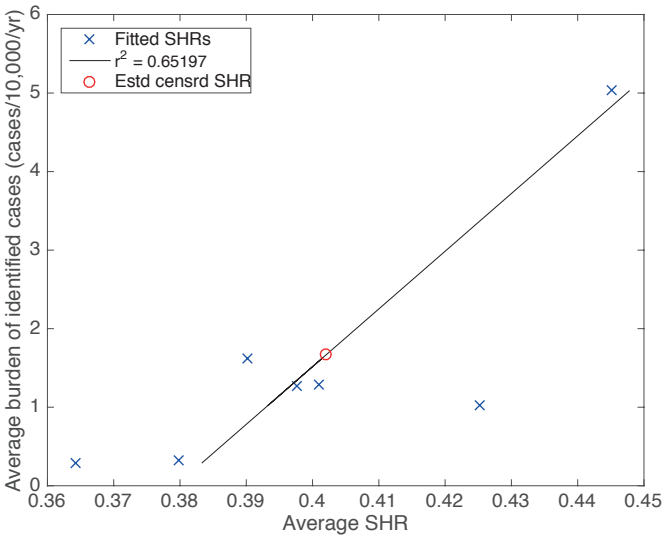

Figure S1. Example of estimation of average sandfly-to-human ratio (SHR) for censored district using fitted SHRs and average identified VL case burdens in 2012 for 7 uncensored districts. Censored district is East Champaran.

Results

Figure 2 in the main text presents the estimated monthly numbers of cases in each censored district for the 3 models against the actual numbers from the data. Figure S2 in this document shows the estimated monthly numbers of cases for all districts employing all of the CARE data. For all models, the estimated numbers match the data relatively well across all the districts, although the models do not capture the extremities of the seasonal variation, in particular in the 4 districts with the highest number of cases (Saharsa, East Champaran, Samastipur and Gopalganj).

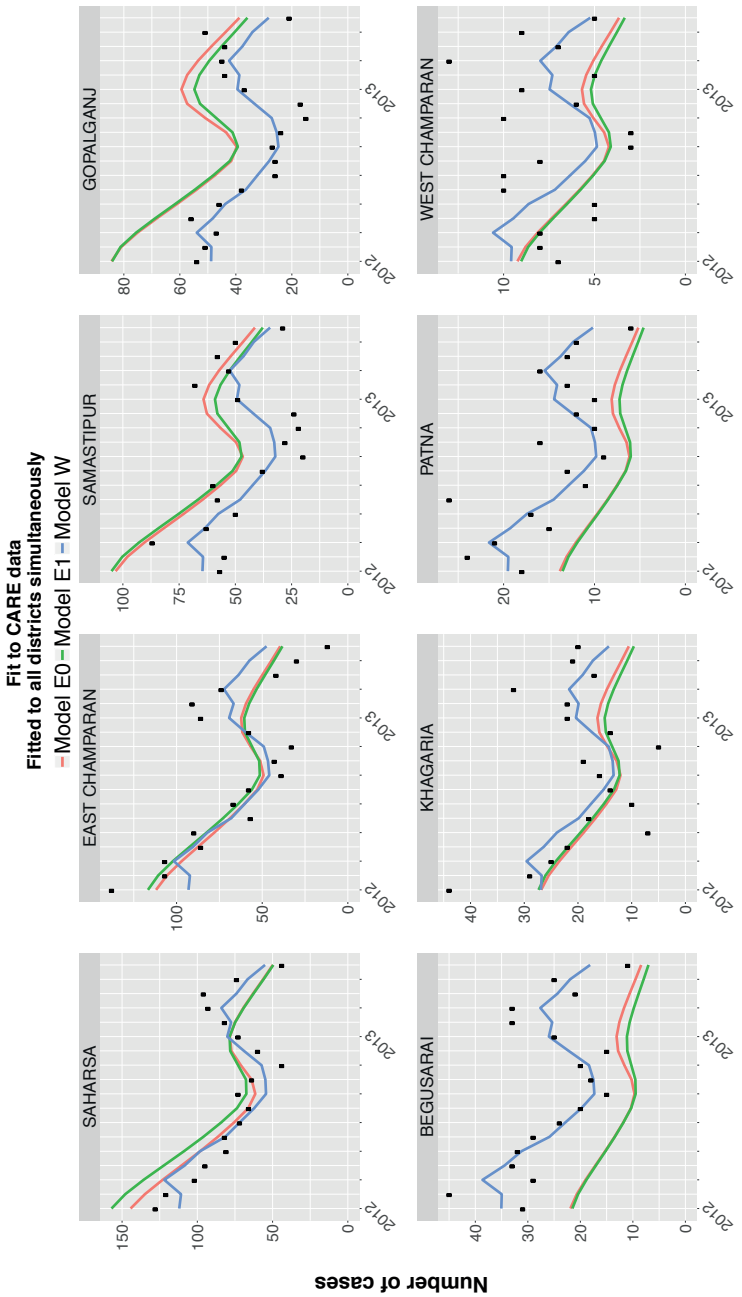

Figure S2. Estimated monthly numbers of cases from the models when fitted to all districts without censoring. Black dots show monthly numbers of cases from the CARE data.

Models E0 and E1 sensitivity analysis

Duration of immunity

When assuming a duration of immunity of one year, the models also fitted the data closely, but with a slightly higher overall deviance compared to the default duration of two years. When fitting the models with a duration of immunity of five years, extreme sandfly-to-human ratios were required to compensate for the corresponding relatively small population of susceptible individuals, and for the same reason a relatively long duration of the early asymptomatic stage (> 292 days) was required (Table S5) and only possible in model E0 which has a faster disease progression.

Start year IRS

In the sensitivity analysis for the start year of IRS control, both models E0 and E1 were able to predict the CARE data of the censored districts with IRS starting in January 2010 resulting in similar deviances compared to the default start year of 2011. Much lower IRS efficacy was needed to arrive at the CARE data due to the longer period of application of IRS between the pre-control equilibrium (fitted to Thakur *et al.*) and the start of the CARE data (Table S6). Fitting the models with a start year of IRS in January 2012, however, gave a much poorer fit to the CARE data (Table S7), and the maximum IRS efficacy of 100% was required in all the geographical cross-validations to reproduce the steep decrease in cases between 2012 and 2013 in the CARE data and between the Thakur and CARE data.

Predictions

The predictions by models E0 and E1 for the 9 durations of the early asymptomatic stage are presented in Figure S3. The difference in predictions of reaching the elimination target between the shortest (142 days) and longest (382 days) duration of early asymptomatic stage, ranges between 1 and 3 years, depending on the district, with the longest duration until elimination predicted by the model with the longest duration of early asymptomatic stage.

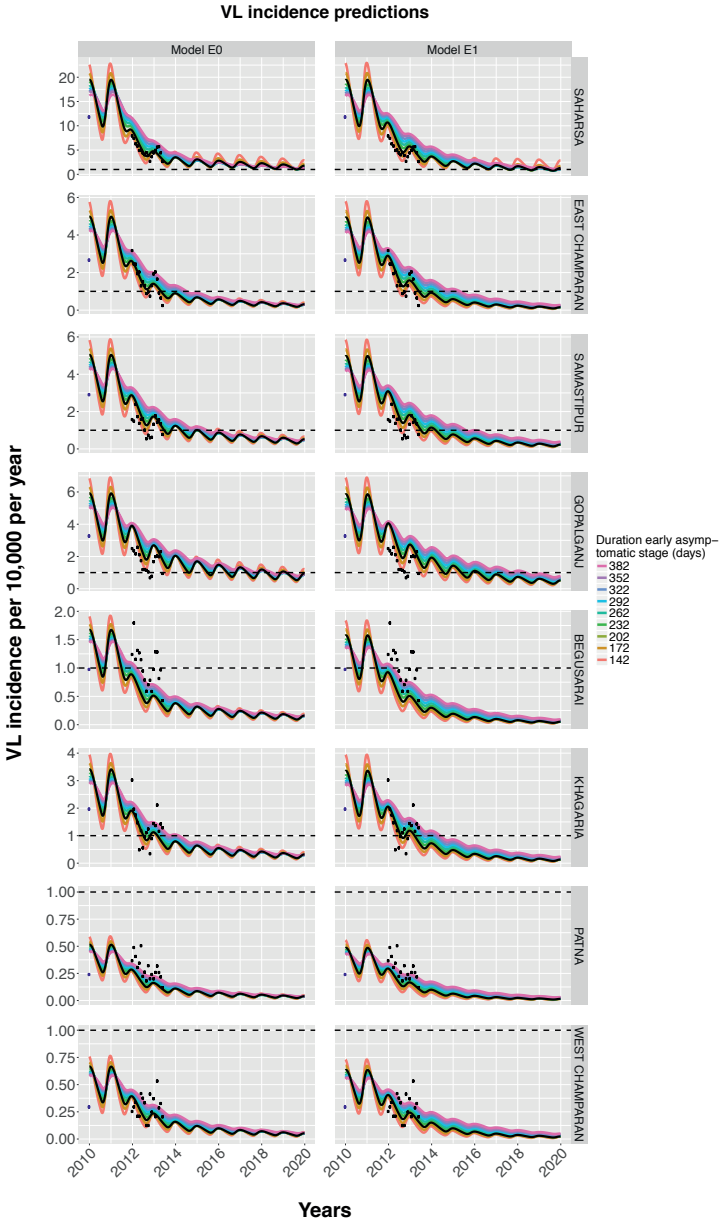

Figure S3. Estimated VL incidence for all Erasmus MC sub-models when fitted to all districts without censoring. The blue dot in 2010 represents the 2009-2010 average yearly incidence level in the data presented by Thakur *et al* [1], which was taken as the pre-control equilibrium incidence. The Thakur data consist of numbers of individuals that were treated for VL, which was linked to the 'treatment 1' stage in the model. The black dots show the monthly incidence by onset of symptoms from the CARE data. The black incidence lines overlay the lines of the best fitting sub-models, which is the sub-model with the early asymptomatic stage duration of 202 days in both models E0 and E1. The black dashed line represents the WHO elimination target.

Table S3. Deviances of Erasmus MC sub-models with start year of IRS in 2011 and duration of immunity of 2 years. Deviances of the sub-models when fitted to all districts simultaneously (^{A}) are presented in the first column 'All districts'. Listed left of this column is the IRS efficacy based on the simultaneous fit to all districts. The deviances for the 8 censored districts are presented in the other columns, and are added together in the last column, which is listed as 'Total deviance', the deviance in this column that is closest to zero (shaded row) indicates the best-performing sub-model for models E0 and E1. The abbreviations used for the districts are as follows: SAH for Saharsa, ECH for East Champaran, SAM for Samastipur, GOP for Gopalganj, BEG for Begusarai, KHA for Khagaria, PAT for Patna and WCH for West Champaran.

Model	Duration early asymptomatic stage (days)	IRS efficacy (All districts)	Deviance									
			All districts	Geographical cross-validation								
				Without SAH	Without ECH	Without SAM	Without GOP	Without BEG	Without KHA	Without PAT	Without WCH	Total deviance
E0	382	1.000	1933	470	361	514	414	53	76	23	22	1933
	352	1.000	1687	376	301	452	377	63	70	26	21	1688
	322	1.000	1450	286	243	390	339	75	65	32	21	1450
	292	1.000	1234	204	189	327	299	92	61	39	22	1233
	262	1.000	1050	134	143	266	259	115	60	50	23	1050
	232	1.000	916	84	107	210	219	144	61	64	26	916
	202	0.999	858	60	89	182	195	181	67	84	31	890
	172	0.948	878	69	83	178	191	212	69	99	35	936
	142	0.905	1021	130	98	192	197	241	76	119	43	1096
E1	382	1.000	1670	395	293	423	348	86	72	32	22	1670
	352	1.000	1443	304	236	361	308	104	69	39	22	1443
	322	1.000	1238	221	184	298	266	128	68	49	24	1238
	292	1.000	1077	154	141	238	223	160	71	63	27	1077
	262	1.000	988	114	115	199	191	203	79	83	33	1017
	232	0.913	951	102	102	187	183	230	79	92	34	1010
	202	0.829	923	95	88	176	176	239	78	100	36	988
	172	0.749	928	104	76	168	171	254	79	112	40	1003
	142	0.675	1030	162	76	171	173	285	86	134	47	1133

Table S4. Deviances of Erasmus MC sub-models with start year of IRS in 2011 and duration of *immunity of 1 year*. Columns and abbreviations as in Table S3.

Model	Duration early asymptomatic stage (days)	IRS efficacy (All districts)	Deviance									
			All districts	Geographical cross-validation								
				Without SAH	Without ECH	Without SAM	Without GOP	Without BEG	Without KHA	Without PAT	Without WCH	Total deviance*
E0	382	1.000	2086	476	461	539	413	55	99	20	22	2086
	352	1.000	1725	388	391	445	321	45	84	19	32	1725
	322	1.000	1504	277	273	411	305	101	63	40	32	1504
	292	1.000	1220	207	194	329	274	71	61	59	26	1220
	262	1.000	1135	132	132	281	282	125	59	90	34	1135
	232	1.000	877	83	107	219	229	107	64	46	23	877
	202	0.997	884	62	90	206	196	182	70	95	24	924
	172	0.944	890	66	82	192	199	195	69	108	35	946
	142	0.897	985	108	96	211	201	231	70	91	38	1046
E1	382	1.000	6151	462	157	915	770	1644	142	2024	37	6151
	352	1.000	1655	313	322	132	169	210	70	76	362	1655
	322	1.000	1970	258	211	101	117	325	69	35	857	1973
	292	1.000	2123	143	182	228	254	35	94	1117	70	2124
	262	1.000	2048	129	169	107	119	144	70	1287	55	2079
	232	0.944	2166	104	103	269	277	340	90	1012	79	2274
	202	0.849	947	86	88	213	225	232	79	49	43	1016
	172	0.739	1087	108	105	283	114	421	112	24	37	1204
	142	0.659	1185	133	73	177	173	310	111	73	221	1270

* Values of 'Total deviance' and deviance of 'All districts' are similar when the fitted IRS efficacy for both the cross-validation as well as the fitting to all districts arrives at a value of 1.0.

Table S5. Deviances of Erasmus MC sub-models with start year of IRS in 2011 and duration of *immunity of 5 years*. Columns and abbreviations as in Table S3.

Model	Duration early asymptomatic stage (days)	IRS efficacy (All districts)	Deviance									
			All districts	Geographical cross-validation								
				Without SAH	Without ECH	Without SAM	Without GOP	Without BEG	Without KHA	Without PAT	Without WCH	Total deviance
E0	382	1.000	21225	2727	3930	3964	2981	1242	1069	3357	1954	21225
	352	1.000	21872	2662	3893	4055	3028	1354	1089	3743	2048	21872
	322	1.000	21146	2506	3649	4024	3031	1300	1032	3632	1972	21146
	292	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	262	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	232	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	202	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	172	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	142	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
E1	382	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	352	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	322	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	292	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	262	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	232	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	202	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	172	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	142	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

Table S6. Deviances of Erasmus MC sub-models with *start year of IRS in 2010* and duration of immunity of 2 years. Columns and abbreviations as in Table S3.

Model	Duration IHP (days)	IRS efficacy (All districts)	Deviance									
			All districts	Geographical cross-validation								
				Without SAH	Without ECH	Without SAM	Without GOP	Without BEG	Without KHA	Without PAT	Without WCH	Total deviance
E0	382	1.000	904	54	159	178	186	175	73	81	26	933
	352	0.959	890	51	157	171	181	195	75	88	27	944
	322	0.916	875	49	154	165	175	197	74	91	27	931
	292	0.875	861	50	150	158	169	199	73	95	28	921
	262	0.839	852	55	146	151	163	202	72	100	30	917
	232	0.806	855	70	142	145	157	207	71	106	32	930
	202	0.779	887	103	143	143	153	216	72	115	35	979
	172	0.760	988	173	155	150	154	232	76	130	41	1111
	142	0.753	1246	332	199	178	168	264	88	156	52	1435
E1	382	0.878	940	65	155	165	169	240	82	96	29	1002
	352	0.821	929	64	151	160	165	243	81	98	29	993
	322	0.766	916	64	146	154	161	247	80	101	30	983
	292	0.712	903	65	140	146	156	252	80	105	31	975
	262	0.661	892	71	132	138	150	258	79	110	32	972
	232	0.612	890	87	124	130	144	268	79	117	34	983
	202	0.566	914	123	117	123	139	283	80	128	38	1030
	172	0.525	1004	211	119	119	135	309	85	147	44	1168
	142	0.491	1272	438	148	129	139	359	101	180	56	1552

Table S7. Deviances of Erasmus MC sub-models with *start year of IRS in 2012* and duration of immunity of 2 years. Columns and abbreviations as in Table S3.

Model	Duration IHP (days)	IRS efficacy (All districts)	Deviance									
			All districts	Geographical cross-validation								
				Without SAH	Without ECH	Without SAM	Without GOP	Without BEG	Without KHA	Without PAT	Without WCH	Total deviance
E0	382	1.000	5729	2022	1223	1303	849	46	202	32	52	5729
	352	1.000	5524	1949	1169	1263	828	42	194	29	50	5524
	322	1.000	5293	1865	1108	1218	804	38	185	26	48	5293
	292	1.000	5037	1772	1039	1168	778	34	176	23	46	5037
	262	1.000	4751	1669	962	1112	748	31	165	20	44	4751
	232	1.000	4433	1552	874	1049	714	30	154	18	42	4433
	202	1.000	4085	1424	775	979	675	31	142	17	41	4085
	172	1.000	3713	1286	665	901	633	38	130	20	41	3713
	142	1.000	3349	1144	548	821	589	57	120	28	43	3349
E1	382	1.000	5506	1993	1168	1236	811	37	190	26	46	5506
	352	1.000	5303	1918	1114	1197	790	34	183	23	44	5303
	322	1.000	5074	1833	1053	1152	765	33	175	21	43	5074
	292	1.000	4818	1738	984	1102	737	32	166	18	41	4818
	262	1.000	4532	1633	905	1045	705	32	157	16	39	4532
	232	1.000	4217	1516	816	980	667	35	148	16	38	4217
	202	1.000	3879	1392	717	908	624	43	139	18	38	3879
	172	1.000	3537	1265	610	830	576	61	132	24	40	3537
	142	1.000	3250	1153	505	752	525	96	132	41	47	3250

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SUPPLEMENTARY FILE 4

Age-structured system of ordinary differential equations for visceral leishmaniasis transmission by Erasmus MC

Steps:

- R-package ‘VLode’ version 0.2.0
- Download the Supplementary File 5.tgz file.
- install.packages(devtools)
- library(devtools)
- setwd([“*path where ‘Supplementary File 5.tgz’ -file is located”*])
- install(“VLode”)
- library(VLode)
- ?VLode for examples and execution

SUPPLEMENTARY FILE 5

R-package 'VLode' of age-structured system of ordinary differential equations for visceral leishmaniasis transmission developed by Erasmus MC.

http://www.sciencedirect.com/science/article/pii/S1755436516300792?via%3Dihub

SUPPLEMENTARY FILE 6

MATLAB code for Warwick model fitting and predictions.

http://www.sciencedirect.com/science/article/pii/S1755436516300792?via%3Dihub

CHAPTER 7

Policy recommendations from transmission modelling for the elimination of visceral leishmaniasis in the Indian subcontinent

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Keywords: visceral leishmaniasis; Indian subcontinent; transmission modelling; WHO guidelines; elimination

Running title: Insights for VL control from modelling

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40-word summary:

Using transmission models we predict visceral leishmaniasis incidence in the Indian subcontinent under WHO-recommended interventions. All except high pre-control endemicity areas are predicted to reach the 2020 elimination target, but the growing susceptible population presents a risk of large outbreaks.

Abstract

Background: Visceral leishmaniasis (VL) has been targeted by the World Health Organization (WHO) and five countries in the Indian subcontinent for elimination as a public health problem. To achieve this target, the WHO has developed guidelines consisting of four phases of different levels of control activities, based on vector control through indoor-residual spraying of insecticide (IRS) and active case detection (ACD). Mathematical transmission models of VL are increasingly used for planning and assessing the efficacy of interventions and evaluating the intensity and timescale required to achieve the elimination target.

Methods: This paper draws together the key policy-relevant recommendations from recent transmission modelling of VL, and presents new predictions for VL incidence under the control interventions recommended by the WHO using the latest transmission models.

Results: The model predictions suggest that the current WHO guidelines should be sufficient to reach the elimination target in areas that had medium VL endemicities (up to 5 VL cases per 10,000 population per year) prior to the start of interventions. However, additional interventions, such as extending the WHO attack phase (intensive IRS and ACD), may be required to bring forward elimination in regions with high pre-control endemicities, depending on the relative infectiousness of different disease stages.

Conclusions: The potential hurdle that asymptomatics, and in particular post-kala-azar dermal leishmaniasis cases (PKDL), may pose to reaching and sustaining the target needs to be addressed. As VL incidence decreases, the pool of immunologically naive individuals increases, which creates the potential for new large-scale outbreaks.

Introduction

Visceral leishmaniasis (VL), also known as kala-azar, is a neglected tropical disease (NTD) caused by protozoan *Leishmania* parasites transmitted by female *Phlebotomine* sandflies. Only a small proportion of infected individuals develop clinical symptoms, which include prolonged fever and an enlarged liver and spleen. VL is generally considered fatal if left untreated [1, 2]. After recovery and, more rarely, after asymptomatic infection, individuals can develop post-kala-azar dermal leishmaniasis (PKDL), a skin rash involving macular, papular or nodular lesions [3]. Individuals with PKDL are thought to contribute to transmission [3–6].

The largest burden of VL is in the Indian subcontinent (ISC), where transmission is considered solely anthroponotic. In 2005, India, Nepal, and Bangladesh instituted a programme to eliminate visceral leishmaniasis as a public health problem by 2017 [7]. In 2014, Bhutan and Thailand joined the commitment and the target was set for 2020 [8]. The elimination target is annual incidence of <1 VL case per 10,000 inhabitants for three consecutive years per subdistrict/district (defined in each region) [2]. The programme has four phases: a pre-control 'preparatory phase', a 5-year attack phase designed to bring the incidence below 1/10,000/year by 2017, a consolidation phase where incidence is kept below the target for three years, and a maintenance phase to ensure sustainable reductions in incidence beyond 2020 [9]. These phases entail different levels of control activities including active case detection (ACD), and vector control through indoor-residual spraying of insecticide (IRS).

Mathematical transmission models are increasingly used for planning and assessing the efficacy of interventions for VL, although challenges remain due to key biological uncertainties in its transmission dynamics [10–13]. This paper draws together the key policy-relevant recommendations from recent transmission modelling of VL, and presents new predictions of the impact of the interventions in each WHO phase on VL incidence. We also explore alternative durations of these phases, to aid prioritisation of resources for VL control in the ISC.

Overview of recent VL modelling and key policy-relevant outcomes

There are several published models of VL transmission dynamics [10, 11]. Those which are focused on the ISC have been particularly influenced by the work of Stauch et al [14, 15]. More recently, modelling groups from Erasmus MC and Warwick University have been performing transmission modelling and quantitative analyses in this area [16–18], and we use these models in this paper. We first describe these models and highlight the main uncertainties in VL transmission dynamics.

Description of transmission models and key knowledge gaps in VL dynamics

Figure 1 illustrates the basic structure of the Erasmus MC (E0, E1) and Warwick models (W0, W1), with the main differences between models highlighted. The models are deterministic, and were parameterised using different data, but have both undergone geographical cross-validation against data on over 5000 VL cases from 8 endemic districts in Bihar collected by CARE India [19] (see [18] for full model descriptions and sensitivity analyses).

Attempts to model VL transmission and control have highlighted the importance of certain parameters that are highly variable or remain largely unknown [11, 12, 20]. These parameters can be distinguished according to whether they relate to human aspects of infection, sandfly bionomics, or intervention efficacy. Regarding the human aspects of infection, the key unknown parameters are the duration of asymptomatic infection, the proportion of asymptomatic individuals who develop clinical symptoms, the relative infectiousness of asymptomatic individuals, VL and PKDL cases, and the duration of acquired immunity. Therefore, the models make different assumptions about these aspects of the natural history.

The Erasmus MC models [17, 18] consist of a set of age-structured model variants based on different assumptions about where the main reservoir of infection lies; namely, solely in symptomatic individuals (VL and PKDL; model E0), or mainly in asymptomatics (model E1). Other variants, with the main reservoir of infection in previously immune individuals in whom infection re-activates or PKDL cases, have also been explored [17]. The models were parameterised with age-structured data on approximately 21,000 individuals included in the KalaNet bednet trial in India and Nepal [21].

In the original Warwick model (model W1) [18] asymptomatic individuals constitute the main reservoir of infection, and PKDL cases are not included in the transmission dynamics. Certain parameters, such as the durations of asymptomatic infection and immunity, are based on estimates from modelling of the natural history of VL, using annual serological test and skin test results from a detailed epidemiological study in a high-endemicity setting in Bangladesh [16, 22]. Here, we introduce one new model variant (model W0), which is comparable to model W1 except that only symptomatic individuals contribute to transmission, as in model E0.

Using these models, and other statistical approaches, attempts have been made to estimate the average durations of asymptomatic infection and immunity. Le Rutte *et al* [18] reported that asymptomatic infection (defined by PCR positivity without symptoms) lasts approximately 10 months (95% CI 8-14 months) based on the KalaNet data. Chapman *et al* [16] concluded that the asymptomatic stage (defined by rK39 ELISA positivity and leishmanin skin test negativity without symptoms) lasts approximately 5 months (95% CI 4-5.5 months), based on the Bangladesh data. The percentage of asymptomatic individuals that develop VL was estimated by Le Rutte *et al* at approximately 1.5% [18], whereas Chapman *et al* estimated it at approximately 15% [16]. These estimates may reflect potentially realistic possibilities at both ends of the spectrum [20, 23], and mainly differ because the models had different structures and were fitted to datasets from settings with different endemicities and diagnostics [18, 23].

Since there is no definitive data on the duration of immunity to VL after (asymptomatic and symptomatic) infection, in a recent model comparison study [18] it was assumed to last 2 years (by Erasmus MC), based on fitting of the models to the KalaNet and CARE data [17, 18], and 5 years (by Warwick) based on previous modelling of the natural history of VL [16]. However, the potential implications of some individuals developing lifelong immunity to disease remain to be explored.

The relative infectiousness of individuals in different infection states remains debated [12]. Despite limited data and reliance on strong underlying assumptions, the modellers estimated the infectiousness of asymptomatics from case incidence data as about 2.5% that of symptomatic cases [18]. In models E0 and E1, PKDL cases are assumed to be half as infectious to sandflies as active VL cases, which is considered conservative based on available data

[24]. Further, we note that all three lesion types contain detectable parasite loads [25], and have been shown to transmit to sandflies in historical and recent xenodiagnosis studies [5, 6]. Ongoing xenodiagnosis studies will provide more direct evidence of the relative infectiousness of asymptomatic individuals and PKDL cases [6, 26]. Detailed longitudinal follow-up studies are required to provide further insight into the progression of asymptomatic individuals to clinical disease and the duration of immunity.

Also, little is known about the bionomics of *P. argentipes* sandflies. All four models assume the same parameter values for sandfly bionomics [18]. Models E0 and E1 treat exposure to sandflies as age-dependent to explain observed age patterns in seroprevalence and VL incidence [17, 18]. The relationship between sandfly and host densities, prevalences of infected and infectious flies, host biting preferences, time and location of transmission, and *P. argentipes* life expectancy remain largely uncertain [11, 12].

Policy-relevant insights from recent VL modelling

Modelling has shown that reducing time to diagnosis, and subsequent treatment, can lead to a dramatic reduction in incidence of VL cases [18, 27]. The impact of early identification and treatment of VL cases, e.g. during fever before the onset of VL-specific symptoms, was explored by Medley *et al* in a model that assumes that clinical VL cases are significantly more infectious than asymptomatic or pre-clinical cases [27]. The results highlighted the importance of the timeliness of diagnosis, suggesting that a diagnostic capable of targeting earlier treatment need only be 30% sensitive to have a significant impact. However, such a diagnostic would need to be highly specific to justify VL treatment using current drugs. In this regard, it has been shown that individuals with high initial antibody levels, and those who seroconvert to high antibody levels, are more likely to develop clinical VL than individuals who are seronegative, or who do not seroconvert between tests [16, 23]. However, the specificity of using high-titre seropositivity or seroconversion to identify progressors to VL is low [23].

The impact of IRS was first studied in a model in which asymptomatic individuals are the main drivers of transmission [14]. This suggested that a large reduction in sandfly density via IRS (of around 60-70%) is needed to achieve elimination. Le Rutte *et al* later demonstrated that reducing cases below 1/10,000/yr could be feasible with optimal IRS (63% continuous

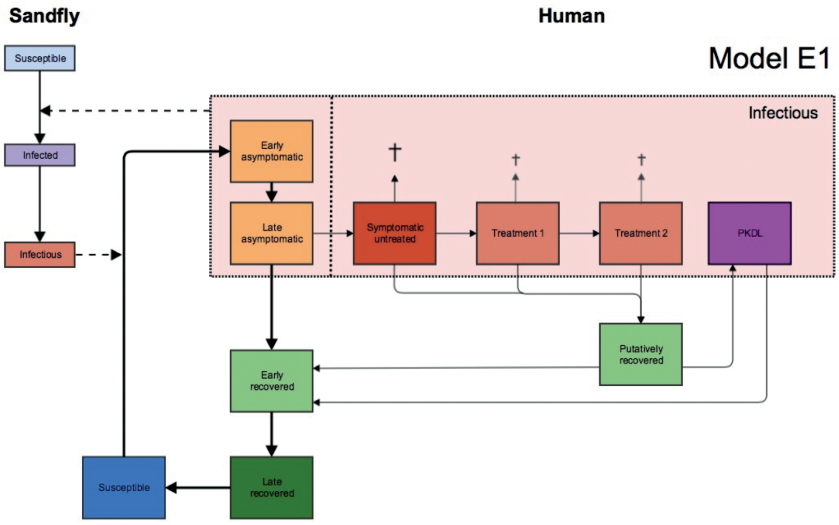

Figure 1. Schematic presentation of the structures of model E1 and the related models E0, W1 and W0. Model W1 is similar to model E1, but has one combined compartment for asymptomatic individuals (yellow), and one combined compartment for recovered individuals (green), and no PKDL (purple). For models E1 and W1, asymptomatic individuals (yellow compartments) are the main contributors to transmission. Models E0 and W0 have the same structures as models E1 and W1 respectively, but asymptomatic individuals do not contribute to transmission. All four models have different durations of infection stages from fitting to data, which are listed elsewhere [18]. Indoor-residual spraying reduces the populations of the sandfly compartments, and active case detection leads to a shorter duration of the symptomatic untreated state (dark red) in all models.

reduction in sandfly density) in low-endemic and medium-endemic settings (≤10/10,000/yr at baseline). In higher endemicity areas, additional interventions were advised [17].

Recent modelling suggests that if asymptomatic individuals are the main contributors to transmission (models E1 and W1), the continuation of combined IRS and ACD at current levels (60% IRS coverage and 40-day average onset-to-treatment time) should be sufficient to reach the elimination target by 2020 for subdistricts with a pre-control endemicity ≤10/10,000/yr [18]. However, if transmission is caused solely by symptomatic individuals (model E0), the models suggest the target will be reached years later, due to transmission being maintained by a remaining pool of VL and PKDL cases with long infectious periods. The Erasmus MC and Warwick models gave discrepant results on whether increasing IRS coverage from 60% to 80% or halving the average onset-to-treatment time from 40 to 20 days reduced incidence more rapidly. These discrepancies

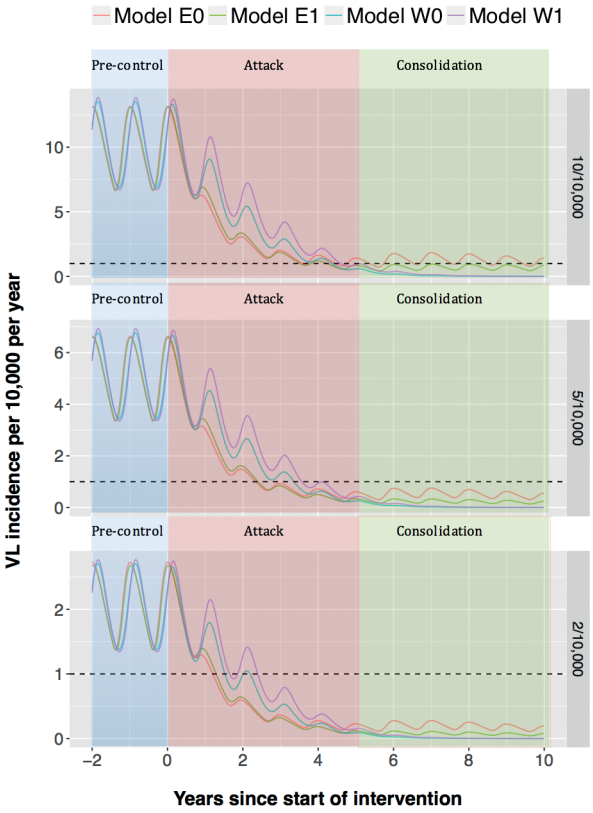

Figure 2. VL incidence during the WHO pre-control phase (< year 0), attack phase (years 0-5), and consolidation phase (year 5 onwards) for three different pre-control endemicity levels (2, 5 and 10 cases per 10,000 people/yr), as predicted from four transmission models. Oscillations are due to the seasonal pattern in incidence caused by seasonal variation in the sandfly population.

are largely due to different estimated IRS efficacies and parameterisation of asymptomatic infection (relative infectivities and durations), and inclusion of PKDL (in terms of the long-term predictions). All models agreed that a combination of increasing IRS coverage and reducing onset-to-treatment times would lead to the target being reached most quickly, and that these two interventions together would be sufficient to achieve the elimination target in all settings.

Impact of the different phases of the WHO guidelines on VL incidence

With the 4 transmission models (models E0, E1, W0 and W1) we predict the effect that the different WHO phases have on VL incidence over time

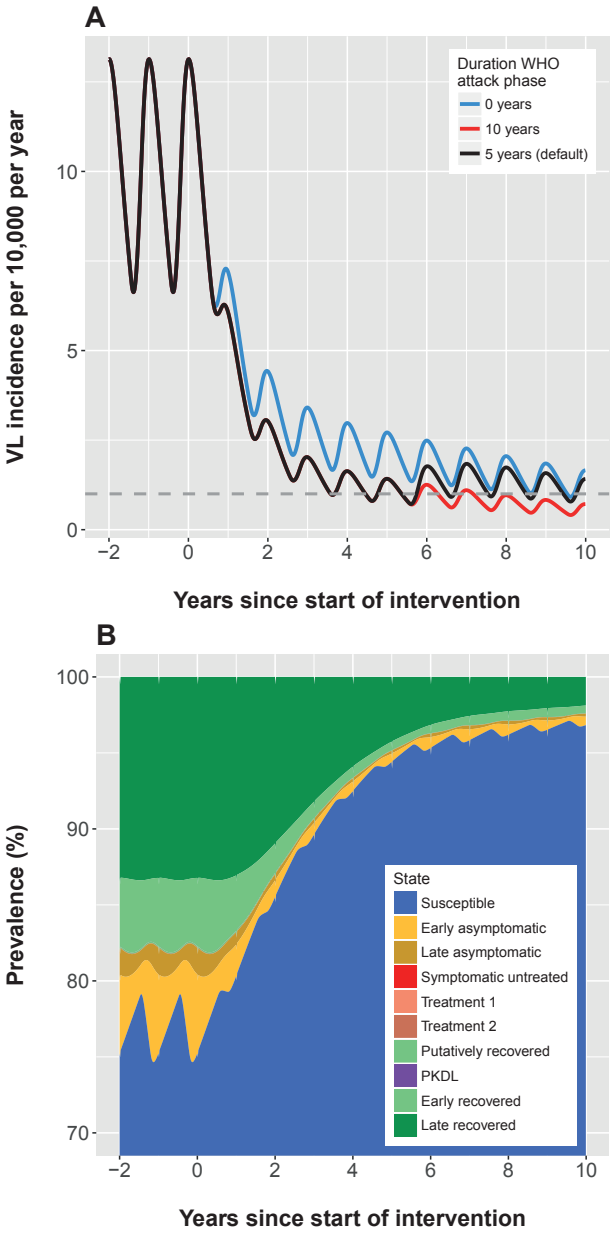

Figure 3. ***A,*** **Predictions from model E0 for the default duration (5 years) and 2 alternative durations (0 years and 10 years) of the attack phase for a setting with a high pre-control endemicity level of 10 cases/10,000 peolpe/year.**
B**, Stacked line chart of distribution of infection states over time for model E0 in the same high endemic setting as *A*, with the default 5-year attack phase starting in year 0, followed by the consolidation phase.** Supplementary Files 1 and 2 include the Figures with the predictions and distribution for all models.

for subdistricts with high (10/10,000/yr), medium (5/10,000/yr) and low (2/10,000/yr) pre-control endemicity levels. During the pre-control phase without IRS and active case detection (ACD), an average 'onset-to-treatment' (OT) time of 60 days is assumed [19, 28]. In the attack phase, active case detection is assumed to reduce the OT to 45 days, combined with 100% IRS coverage as mentioned in the guidelines. We interpret this 100% to be comparable to the maximum IRS coverage so far achieved in Bihar, which is equivalent to 67% of all households being sprayed (53% households fully sprayed and 29% partially sprayed (53% + 29% /2 = 67%)) [29]. The "limited IRS" in the consolidation phase [9] is interpreted here as 2/3 of the IRS coverage in the attack phase, combined with "intensified ACD" leading to a shorter OT of 30 days.

Figure 2 shows that the attack phase brings down the incidence quickly, which is then sustained by the consolidation phase. If the IRS efficacy is relatively low (models W0 and W1), reducing the IRS coverage after 5 years does not appear to have a big impact on incidence, since incidence decreases to very low levels in most settings after 5 years of ACD and intensive IRS. Only model E0 in the highly endemic pre-control setting (10/10,000/yr) suggests that the target will not be reached within 10 years with the current strategy.

We also explored alternative durations of the attack phase for the three pre-control endemicity levels, as presented in Figure 3A for model E0 (see Supplementary Figure 1 for outcomes for the other models). Increasing the duration of the attack phase in high pre-control endemicity settings from 5 to 10 years brings forward the elimination target – by at least 5 years for this model (E0), but less so for others. Doing this for a medium endemicity setting gives hardly any additional benefit in the short term and does not result in reaching the target earlier. In contrast, for low endemicity settings, all models predict that leaving out the attack phase entirely, and starting with the consolidation phase, at worst leads to a minor increase in time to elimination. Adjusting the length of the attack phase to the pre-control endemicity level may therefore lead to more effective use of limited resources.

The distribution of the different disease states of model E0 over time is presented in Figure 3B, which emphasizes the large susceptible population (blue) that accumulates when nearing and sustaining elimination, posing a risk factor for (re-)introduction and recrudescence of infection.

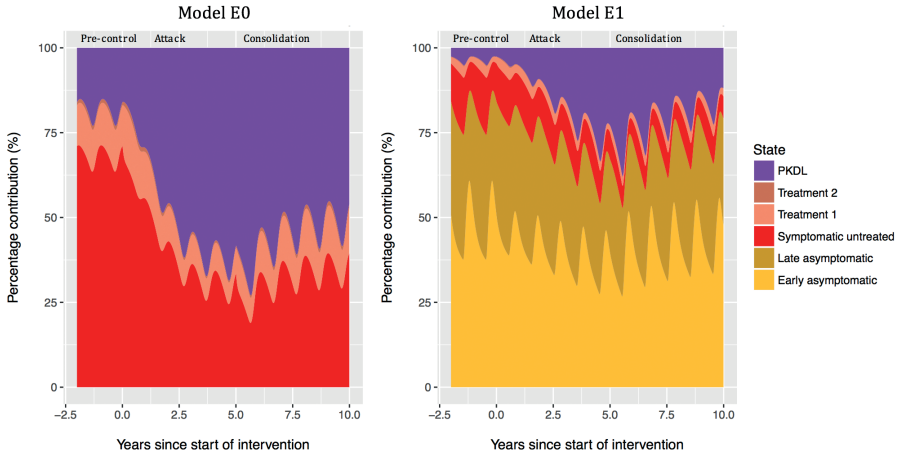

Figure 4. Relative contribution of different disease states to VL transmission over time during the WHO interventions. In model E0 (left), only symptomatic individuals (VL and PKDL) contribute to transmission. In model E1 (right), asymptomatic individuals are the main contributors to transmission. Both graphs are for a 10/10,000/yr pre-control endemicity setting with 5-year attack phase followed by the consolidation phase. Assumptions: 2.5% of treated VL cases develop PKDL, PKDL lasts for 5 years on average, PKDL cases are half as infectious as active VL cases. Supplementary File 3 contains the Figures with the relative contribution of different disease states for all models.

Role of PKDL in maintaining transmission

The potential role of PKDL in maintaining transmission as VL incidence decreases is illustrated by Figure 4, which shows the change in the contribution of different disease states to transmission during the WHO phases for models E0 and E1. The relative contribution of PKDL increases as the elimination target is approached. After 5 years of the attack phase, about 70% (Model E0) or 25% (Model E1) of the infection pressure to sandflies comes from PKDL cases. Both models show that the role of PKDL increases when nearing elimination [4, 12]. Active detection and treatment of PKDL cases would thus be a promising additional tool to speed up and sustain elimination, but diagnosis of PKDL remains a challenge [30, 31].

Discussion

Our models suggest that the four-phase intervention strategy, as described in the current WHO guidelines, is likely to be sufficient to reach the elimination target in areas that had low to medium VL endemicities (≤5 cases/10,000

people/year) prior to the start of interventions, and for higher pre-control endemicities an extended attack phase (intensive IRS and ACD) may be required. Whether IRS or reducing onset-to-treatment time is the more effective intervention depends on the relative infectiousness of asymptomatic and symptomatic individuals, and the efficacy of IRS; both still important gaps in knowledge. If most asymptomatic individuals are infectious to sandflies (even if only 1/80th as infectious as symptomatic individuals [18]) and their duration of infection is as long and their rate of developing VL as low as estimated, then they will act as the main source of transmission. In this case, increasing IRS coverage will cause a greater reduction in transmission than reducing delays to treatment, provided IRS is effective in killing sandflies [14]. However, if asymptomatic individuals are not infectious to sandflies, or only a very small proportion of them are, so that clinical cases drive transmission, then reducing delays to treatment will lead to a greater decrease in incidence [27]. These varying assumptions, some of which are covered by the different sub-models, influence the time taken to reach elimination.

In the model outputs, the decrease in VL incidence is solely attributable to the impact of interventions. However, the impact of the main interventions on VL incidence, or, in the case of IRS, even on vectorial capacity, varies significantly between studies [32–35]. Reported VL incidence in the Indian subcontinent has declined considerably since 2011, from approximately 37,000 cases to just 6,500 in 2016 [36]. The decline is likely multifactorial; some have attributed it to improved vector control and others to reductions in delays to treatment [37, 38]. However, other factors that should be borne in mind include a possible natural cycle of VL in the community and the effect of herd immunity [1, 39], alongside a decrease in risk factors for developing the disease, such as malnutrition.

The models reveal the potential hurdle that asymptomatics and in particular PKDL cases may pose to reaching and sustaining the target, which is currently not addressed in the elimination target or strategy. As already mentioned by the WHO [9], we also recommend including PKDL cases in the VL elimination strategy and target, for which a combined detection strategy, for example together with leprosy, may offer an efficient and sustainable solution. Adding PKDL in the elimination target, however, requires some empirical threshold, which has not yet been established [9].

Current models have proven to be useful tools for evaluating broad-scale trends in VL incidence under different assumptions about the underlying biology. As incidence falls, the influence of stochastic effects will increase, so a stochastic individual-based model will be required to predict the probability of true elimination or resurgence in the maintenance phase, and will be included in future studies. When incidence decreases further, different (largely unknown) aspects of the transmission become increasingly important, such as the highly focal nature of the disease, the constant migration of individuals, and potential 'super-spreading' of infection by HIV-coinfected patients [40].

Conclusion

Modelling analyses suggest that the current WHO strategic guidance seems adequate to reach the target of elimination of VL as a public health problem in areas that had medium pre-control endemicities (up to 5/10,000/yr) before active case detection and high-coverage IRS began, but that additional interventions may be required in areas with higher pre-control endemicities, such as a longer duration of the attack phase. Asymptomatics and PKDL cases pose a potential threat to reaching and sustaining elimination, leading to ongoing 'hidden' transmission for several years after reaching the target. This needs to be addressed in the elimination target and strategy. Also, the increasing pool of susceptibles that forms as VL incidence decreases may be a source of new large-scale epidemics.

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SUPPLEMENTARY FILE 1

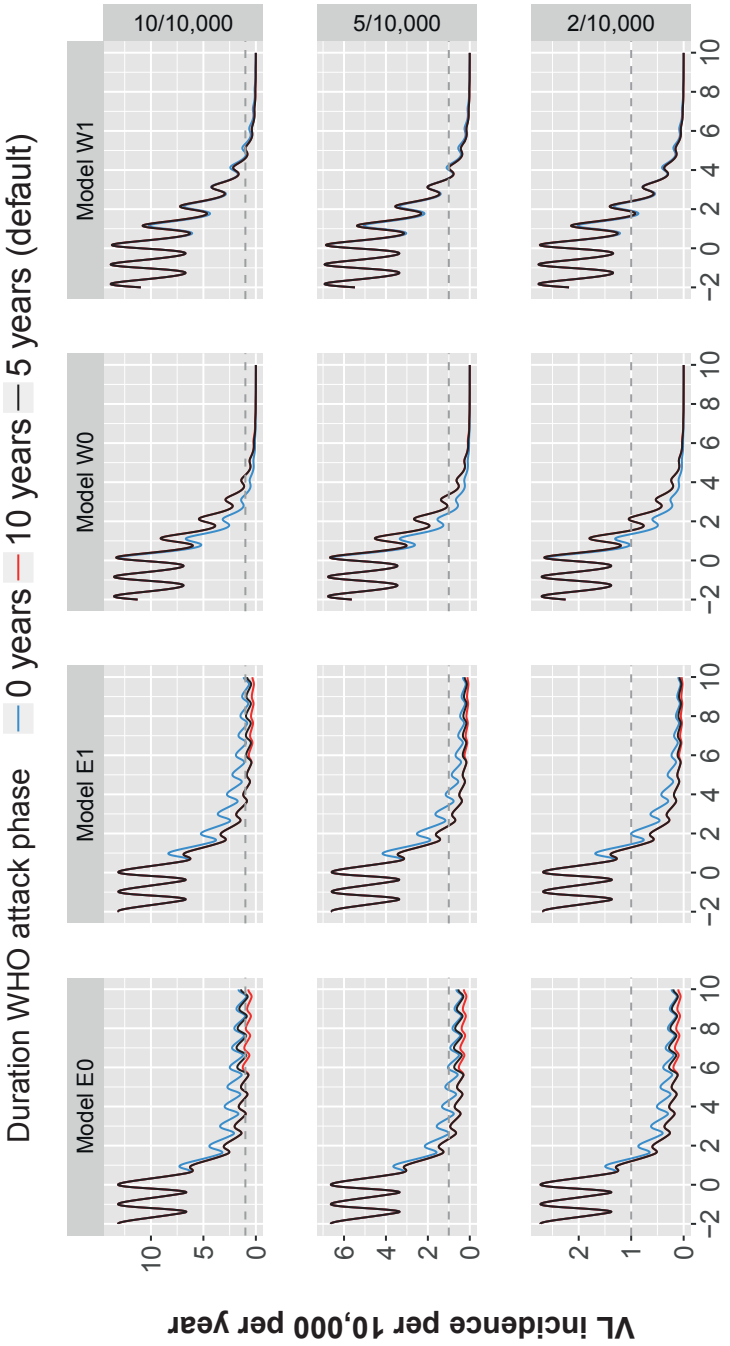

Figure S1. Predictions from all 4 models for the default duration (5 years) and 2 alternative durations (0 years and 10 years) of the attack phase for settings with a high, medium and low pre-control endemicity levels.

SUPPLEMENTARY FILE 2

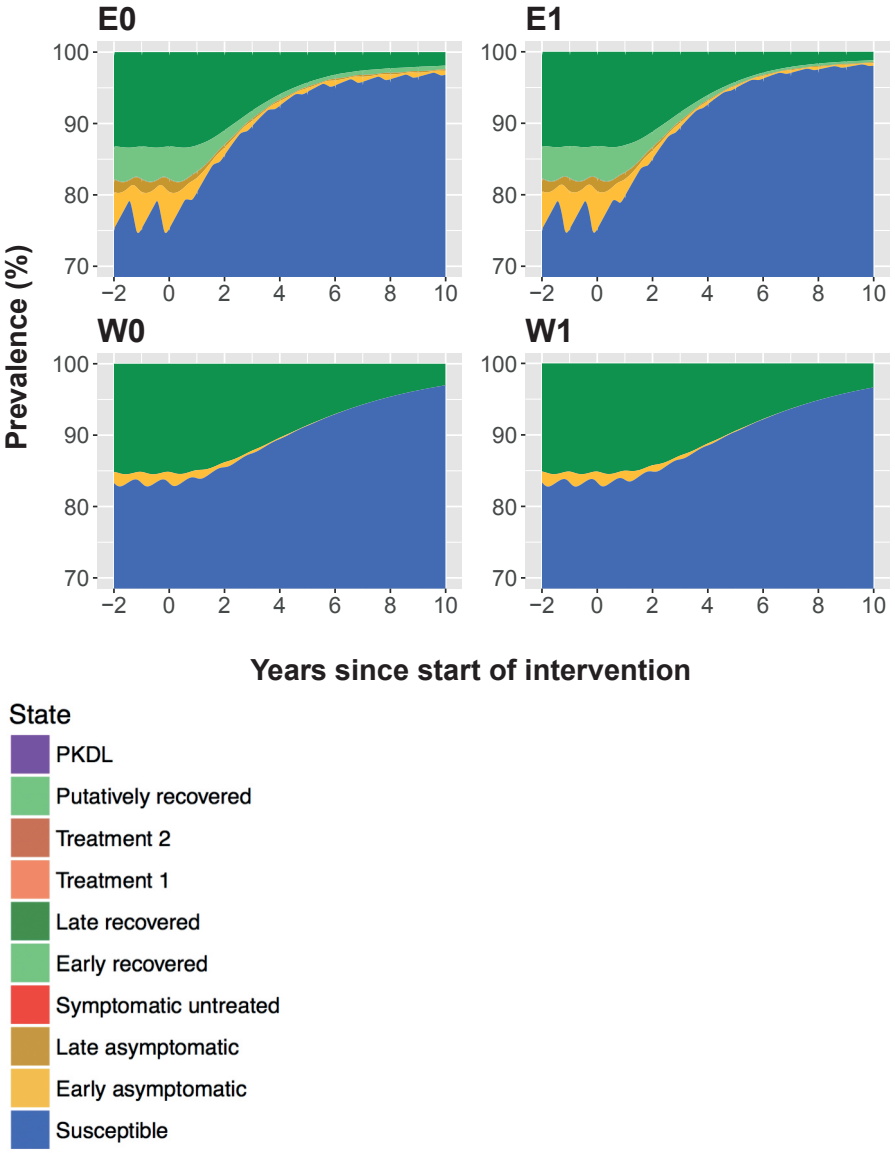

Figure S2. Stacked line chart of distribution of infection states over time for models E0, E1, W0 and W1 for a setting with a high pre-control endemicity level of 10 cases/10,000 people/ year. The default 5-year attack phase starts in year 0, followed by the consolidation phase between year 5 and 10.

SUPPLEMENTARY FILE 3

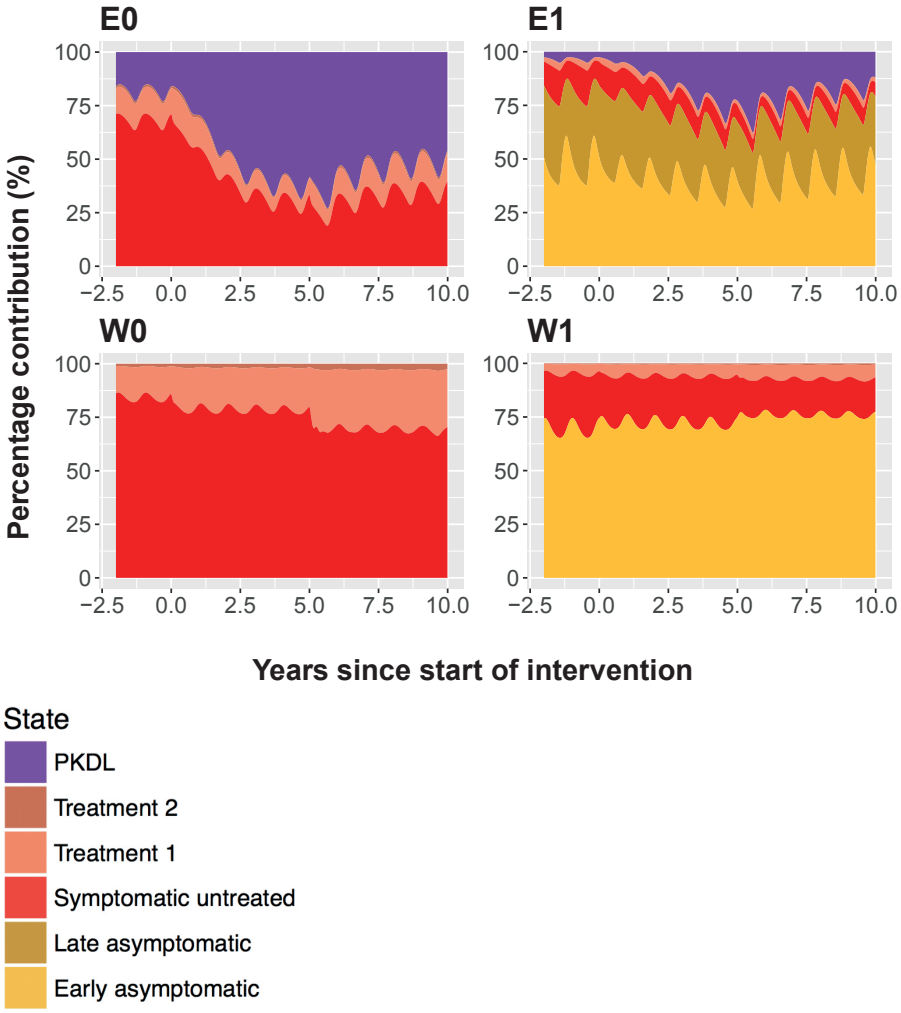

Figure S3. Relative contribution of different disease states to VL transmission over time during the WHO interventions. In models E0 and W0 (left), only symptomatic individuals (VL and PKDL(model E0)) contribute to transmission. In models E1 and W1 (right), asymptomatic individuals are the main contributors to transmission. All graphs represent a 10/10,000/yr pre-control endemicity setting with 5-year attack phase followed by the consolidation phase. Assumption for PKDL: 2.5% of treated VL cases develop PKDL, PKDL lasts for 5 years on average, PKDL cases are half as infectious as active VL cases.

CHAPTER 8

General discussion

8.1 Answers to research questions

Research question 1: What is the global health impact when achieving WHO's disease elimination and control targets for 9 NTDs and in particular visceral leishmaniasis?

An enormous global health and socio-economic impact can be achieved by reaching the WHO control and elimination targets for 9 NTDs of the London Declaration, including visceral leishmaniasis, justifying the financial efforts that are required to reach these targets.

In **Chapter 2** [1] we estimated that in the ideal situation of achieving the WHO control and elimination targets of all 9 NTDs the global health impact between 2011 and 2030 would be nearly 600 million averted disability adjusted life years (DALYs). Nearly a quarter of these averted DALYs are attributed to reaching the global visceral leishmaniasis (VL) targets (140 averted DALYs, Table 1). The number of averted deaths due to VL in the period of 2011 and 2030 is estimated at just under 2.5 million, which is half of the averted deaths of all NTDs together (Table 1).

VL health gains in India

The Global Burden of Disease study (GBD) reported that 59% of the global DALYs due to VL in 2010 came from India, where the WHO elimination target is formulated as <1 VL case per 10,000 capita, at sub-district level per year by 2020. The calculations that we described in **Chapter 2**, interpolate prevalences and DALYs between 1990 and 2010 and extrapolate towards the targets up to 2030, taking into account demographic trends. According to these calculations, achieving the WHO targets in India would lead to 90 million averted DALYs and 1.6 million averted deaths between 2011 and 2030 compared to the situation in which the 1990 situation would have remained unabated. Two thirds (67%) of the global health gains due to the control of VL will be achieved in India, and 9% in East Africa (Table 1). The remaining prevalent cases, deaths, YLDs and YLLs (DALYs) and the estimated health gains in India between 1990 and 2030 are shown in Figure 1.

Table 1. Health impact between 2011-2030 when achieving the WHO elimination targets for all 9 NTDs including VL

NTD	Region	Averted DALYs (millions)			Averted deaths (millions)
		YLD	YLL	DALYs	
All*	World	328.1	264.9	592.9	4.8
VL	World	0.1	140.1	140.2	2.4
VL	India	0.1	90.1	90.2	1.6
VL	East Africa**	0.0	13.8	13.8	0.21

* Onchocerciasis, lymphatic filariasis, soil-transmitted helminthes, schistosomiasis, blinding trachoma, human African trypanosomiasis, Chagas' disease, leprosy and visceral leishmaniasis.
** Sudan, South Sudan, Ethiopia. YLD = years lived with disability. YLL = years of life lost.

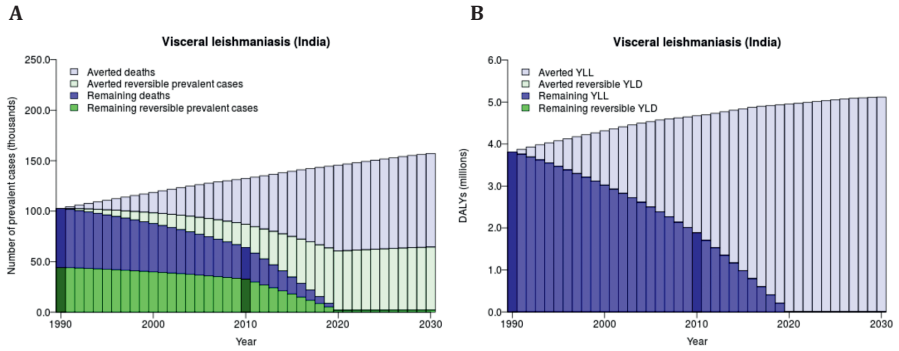

Figure 1. Health impact of reaching the WHO VL elimination targets in India. (**A**) Trends of remaining and averted prevalent cases and deaths between 1990 and 2030. (**B**) Trends of remaining and averted DALYs (YLDs + YLLs) from 1990 to 2030. Note that the remaining YLDs (green) cannot be seen, because of their relatively small contribution to the DALYs. The darker bars for 1990 and 2010 reflect the Global Burden of Disease (GBD) data [61–64] on which all calculations were based.

Critical appraisal

The large burden of disease in the counterfactual situation is in most cases caused by the decision to choose 1990 as the reference year. We assumed that without any interventions the prevalence of the disease would have remained equal from the reference year onwards. The number of averted cases, which we calculated as the counterfactual minus the number of remaining cases, could be very high in settings due to high counterfactual values, for example due to population growth as was often the case in developing countries. Between 1990 and 2010 both the Indian population as well as the Indian economy experienced major changes. It could be questioned whether for India the reference year of 1990 is representing a realistic scenario, since economic progress has also caused part of the decrease in the NTD-prevalence due to

better health circumstances and therefore completely halting interventions might not ever lead to the 1990 prevalence levels again. In collaboration with experts however, we agreed that in the majority of the scenarios the prevalence levels could potentially return to the 1990 pre-control levels when halting interventions, as often the poorest of the poor are affected by NTDs and they have benefitted disproportionally less from economic progress. [2] We have therefore decided to take a universal approach, but in some settings (including VL in India) this could certainly have caused counterfactual trends being too high, causing an overestimation of the health impact.

The large contribution of VL to the total number of averted DALYs for all NTDs, is caused by the lethal aspect of this infection in relatively young people, leading to high numbers of years of life lost (YLL). The YYLs form the major part of the averted DALYs, which is especially the case for VL (Table 1). In our study the YLLs are calculated as the number of years between the age at the time of the person's death and a persons' maximum potential age, using the global maximum life expectancy based on individuals from Japan. These YLLs are then being attributed to the year of the persons' death, following the approach of the Global Burden of Disease (GBD) study, which forms the basis of our calculations. An alternative approach, however, would be to take into account country-specific life tables, and spread out the impact of the death over the expected remaining years of that persons' life, would he/she not have died. The health impact, had we used this alternative approach, would only be about 17% of the health impact using our default method, which is a considerable difference (Table 2). When calculating the economic impact of a person's death, it was decided in a recent study to use this alternative approach by attributing the economic impact to each year the person cannot attend work up to their country-specific life expectancy. [3]

Table 2. Health impact between 2011-2030 when achieving the WHO elimination targets for VL using alternative YLL calculations.

NTD	**Region**	**Averted DALYs (millions)** Our original calculations in brackets		
		YLD*	YLL	DALYs
VL	World	0.1	23.7 (140.1)	23.8 (140.2)
VL	India	0.1	16.2 (90.1)	16.3 (90.2)

* YLDs are similar for both methods

Furthermore, our calculations are based on the 1990 and 2010 prevalence data from the GBD-study. Over the past years, the Institute for Health Metrics and Evaluation has released new GBD values for 2013, 2014 and 2015 [4, 5]. Updating our study using these data would provide more insight in the actual trends towards the targets and therefore more accurate estimations of the averted health impact when achieving the WHO control and elimination targets. The 2015 GBD data for VL are on average 17% lower than our extrapolated values (Table 3). The data from GBD are therefore closer towards the WHO targets than our extrapolated values, from which we could conclude that we are on track towards achieving the WHO targets, would our linear extrapolation be a reasonable method. If we would include the new 2015 GBD data in our study, the overall health impact of achieving the targets would become slightly higher. However, the choice of counterfactual obviously has a much larger impact on the estimated health impact.

Table 3. GBD estimates for VL in 2015. In brackets our extrapolated values for 2015. [1, 5]

NTD	Region	New GBD 2015 estimates (our 2015 extrapolation)	
		DALYs in millions	Deaths in thousands
VL	World	1.4 (1.8)	24.2 (29.8)
VL	India	0.8 (1.0)	15.4 (16.7)

Economic impact

Globally eliminating and controlling VL has, besides the enormous health impact as described above, also an impact on the productivity of individuals, households, communities, and countries. [6] Based on the health impact calculations that are presented in **Chapter 2**, the related economic impact and equity in distribution of health gains have also been calculated. [2] In this section, these findings for the control and elimination of VL are presented and interpreted.

Lenk *et al* estimated how much of the income loss faced by individuals affected with VL would be avoided by reaching the WHO targets, based on the number of averted cases and deaths from **Chapter 2**. [7] A literature search resulted in 10 studies with quantitative data on productivity loss due to VL and 14 studies with quantitative data on out-of-pocket payments that are caused from direct and indirect costs related to infection with VL. The results from the literature search, which was part of the analysis by Lenk *et*

al, resulted in an average annual productivity loss in India of 100% when left untreated for VL and 20% when treated. [7] When calculating the global productivity loss due to VL, productivity loss was only applied to individuals over 15 years of age and included both productivity loss due to disease and productivity loss due to premature mortality. For these calculations the economic impact caused by YLLs was spread out over the remaining life years would the person not have died, which is the same methodology as described earlier in the critical appraisal section and in Table 2. Because VL affects the poorest of the poor in low and middle-income countries (LMIC), the GDP per capita of the lowest quintile was used as a proxy for income. [8] The total economic benefit from globally averted productivity loss in the period 2011-2030 when reaching the WHO targets for VL was calculated to be 21.3 billion I$[1] (Table 4), of which 15.1 I$ (70%) are from reaching the targets in India.

The out-of-pocket payments (OPPs) for an individual with VL in India were found to be $354.75 [9]. According to literature, 80% of all patients were being treated in India [10–12], which was in this study assumed to remain constant until 2030. The percentage of patients paying for treatment was assumed to linearly reach $0.00 in 2030, considering universal health coverage. The total economic benefit from globally averted OPPs for VL added up to 270 million IS$ for the period 2011-2030 (Table 4).

Table 4. Total economic benefit from averted productivity loss and averted out-of-pocket payments (OPPs), base case estimates and 2.5th and 97.5th percentiles. [7]

Region	**Averted productivity loss** I$ - International dollars (***billions***)		**Averted OPPs** ***I$ - International dollars (millions)***	
	2011-2020	2021-2030	2011-2020	2021-2030
VL disease	I$ 0.1	I$ 0.1	I$ 130 [60 – 190]	I$ 140 [60 – 220]
VL deaths	I$ 7.9	I$ 13.2	NA	NA
Total	I$ 8.0 [5.1 – 11.7]	I$ 13.3 [8.5 – 19.4]	I$ 130 [60 – 190]	I$ 140 [60 – 220]

WHO has estimated the cost of the required investments to achieve the targets for VL. A crude estimate of the relationship between these necessary investments to reach the WHO targets and the economic benefits that are listed in Table 4 result in a net return on investment (ROI). It was calculated

1 An international dollar (I$) would buy in the cited country or countries a comparable amount of goods and services a U.S. dollar would buy in the United States. [60]

that for VL, the net benefit[2] was US$ 3.5 for every dollar invested in the period 1990-2030. [Adapted from [13]] Hence, it was considered that the financial efforts that are required to reach the WHO VL targets are justified.

Equity

Stolk *et al* categorized the 1990 and 2010 DALYs from the GBD data and extrapolated the 2020 DALYs from **Chapter 2** by income group, to gain inside in inequalities between affected countries. [2] Figure 2 presents the absolute burden in DALYs for 1990, 2010, and 2020 by income group, based on the World Bank classification. [14] India is classified by the World Bank as being a lower-middle income country and holds the largest VL burden. The global health burden resulting from VL declined between 1990 and 2010 by 45%, but the reduction was only 15% in the low income countries compared to 67% in upper-middle income countries, explaining the disproportionate concentration of the burden and interventions in the poorest countries (Table 5). Between 2010 and 2020 a further 90% reduction of DALYs is expected, would the targets for VL be met, with again the smallest gain for the low-income countries, however decreasing the burden with 78%. Meeting the WHO 2020 targets would lead to considerable improvement of global equity, supporting the pro-poor designation of public policies against NTDs. [1, 2]

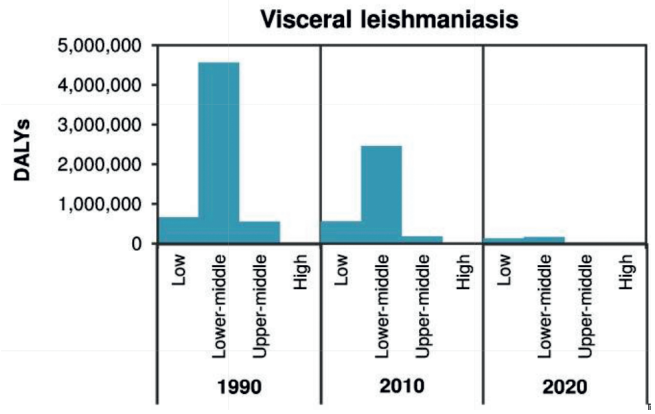

Figure 2. Burden of disease by country income group for visceral leishmaniasis in 1990, 2010 and 2020.

2 Only investments in individual management of VL were included, as well as active case finding and vector control (only in areas of the Indian subcontinent that are not co-endemic with malaria).

Table 5. Burden of VL by income group in 1990, 2010 and 2020, and relative change in 1990–2010 and 2010–2020.

Income group	Burden of disease in DALYs, in thousands (%)			Relative change in disease burden[a]	
	1990	2010	2020	1990-2010	2010-2020
Total	5,770 (100%)	3,198 (100%)	305 (100%)	-45%	-90%
Low	659 (11%)	560 (18%)	125 (41%)	-15%	-78%
Lower-middle	4,558 (79%)	2,455 (77%)	159 (52%)	-46%	-94%
Upper-middle	548 (9%)	179 (6%)	20 (7%)	-67%	-89%
High	4 (0%)	4 (0%)	0 (0%)	-	-

[a] Not calculated for an income group if the 1990 share of the disease burden was <1% in that income group.

Research question 2: Are veterinarians in the endemic regions of Spain and France aware of the spread of zoonotic VL in Europe and do they implement the guidelines to control this disease?

Even though veterinarians are not aware of the number of cases in their region and the presence of guidelines, they do apply most of the recommended intervention measures, but hardly any monitoring and evaluation takes place.

In **Chapter 3** we reported that in Spain and France, 60% of the 459 veterinarians that participated in our online survey are aware of the spread of visceral leishmaniasis in Europe. 70% of them are not aware of any guidelines, but they do apply most preventive, detection and treatment measures that are recommended in the guidelines, since these consist of the main interventions available for the control of leishmaniasis. However, 76% of all veterinarians included in the study have never received any reports regarding confirmed zoonotic visceral leishmaniasis (ZVL) cases in their region or country and 88% never reported a case that had been confirmed by them, leading to a lack of monitoring and evaluation, which is one of the key topics of the guidelines. Currently there is no infrastructure available through which veterinarians can report confirmed cases.

Voluntary participation bias

The link to the survey was distributed through email, websites and online newsletters and was open for 4 months. Therefore, we only reached veterinarians with (regular) internet access. Voluntary participation in the

full 24 question-survey, without an incentive was required for veterinarians to be included in the study, leading to voluntary response bias. Consequently, motivated veterinarians who are potentially more aware of the disease, because they diagnose more cases, would be more likely to fill out such an extensive survey. However, when we analyzed how many dogs were diagnosed with canine leishmaniasis per veterinarian per year, this varied widely and nearly 20% (n=83) of all respondents diagnosed zero cases per year, with an average of 27.4 cases, and a maximum of 700 (n=1). The low awareness of guidelines and little reporting of cases among this potentially highly motivated group of veterinarians is therefore extra alarming.

Only 23 veterinarians (4.5%) from Germany responded to our survey, whom we interpreted to be less interested and aware of the disease, based on the effect of voluntary response bias. We highlight in **Chapter 3** that this is an unsatisfactory finding, due to the presence of sandflies in the South of Germany and the enormous inflow of dogs and puppies from endemic countries such as Greece, Italy and Spain. [15, 16]

We have developed a parallel survey for physicians / general practitioners also focusing on the distribution of infection, awareness of guidelines and interventions regarding human VL. Equal effort was put in reaching out to them through a similar amount of institutions that were asked to distribute the survey on behalf of us. This survey was also translated to Spanish, French and German. However, even after multiple reminders, only one physician opened the survey, and did not complete any questions. This extremely poor response highlights the potential lack of awareness for this topic within this profession, perhaps because physicians mainly consider leishmaniasis to be an animal disease. However, this lack of awareness poses a substantial risk on human public health and a strong collaboration between both professions, with awareness of the guidelines at both ends, is crucial to be able to quickly monitor and counter outbreaks of infection.

Control and monitoring of infection

We assume it to be likely that there is a significant gap between the data on VL and canine leishmaniasis that are publicly available and the actual number of cases that are being confirmed, because of the lack of monitoring and evaluation based on the survey data. **Chapter 8.2** addresses this issue through an additional survey among veterinarians in Spain and France to

obtain their CanL incidences. These incidences are then extrapolated to gain insight in the distribution and magnitude of the CanL reservoir in these countries.

Through the survey presented in **Chapter 3** we were only able to identify symptomatic cases that were diagnosed by veterinarians. It is estimated that only 5-10% of all infected dogs show clinical signs. [17] Symptomatic stray dogs are also not included in our figures. The actual reservoir of infection in asymptomatic dogs (who have proven capable of transmitting the parasite [18]) is many times larger, emphasizing the importance of preventive measures. From our study we learned that prevention measures are mainly applied by veterinarians that are aware of the spread of disease, emphasizing the need for monitoring, evaluation and communication to veterinarians about the existence of cases in their area. Currently only 24% of the veterinarians receive any type of updates.

To our knowledge there is currently no mathematical model for the transmission of zoonotic visceral leishmaniasis in the Mediterranean region. Such a model would be useful to estimate the impact of different interventions on the incidence of CanL or VL. In **Chapter 8.3** we propose a structure for such a zoonotic VL transmission model for this region.

In recent years there has been a lot of attention for the global control and elimination of VL. [19, 20] The majority of funding and research has been focused on the Indian subcontinent (ISC), which holds the largest numbers of VL cases, and currently also shows the steepest decline in cases. [21] Recent outbreaks of VL in Southern Europe and Eastern Africa have however increased awareness for VL in regions outside the ISC. The current increase and expansion of ZVL in Europe has caused it to be classified there as an 'emerging zoonotic disease' and WHO created guidelines for its control. However, the awareness in 'the field' of veterinarians and physicians needs to increase, so that prevention and control measures are implemented and the monitoring and evaluation of both human and canine cases to control the spread of zoonotic VL is intensified. A One Health approach, in which both physicians and veterinarians are involved will be crucial to prevent the infection from spreading further. [22]

Research question 3: What insights can transmission models provide regarding the feasibility of achieving the VL elimination targets on the Indian subcontinent?

Transmission models have shown to be useful tools to simulate and compare the impact of different types of interventions on VL incidence, and generally support the idea that achieving the VL elimination targets seems feasible using current interventions in regions with a pre-control endemicity level of 10 or less VL cases per 10,000 population per year.

In **Chapter 4** we provide an overview of the existing mathematical models capturing VL transmission dynamics, highlighting the scarcity of VL models. In **Chapter 5** we introduce three newly developed VL sub-models, which we based on the existing model by Stauch and colleagues [23–25], with three different assumptions about the human reservoir of infection. These sub-models, which we fitted to Indian and Nepalese data, were used to provide insight into the impact of optimal and sub-optimal indoor residual spraying of insecticide (IRS) on VL incidence. We conclude, with the model with the main reservoir of infection in asymptomatic individuals, that elimination of VL (<1 new VL case per 10,000 capita per year at sub-district level by 2017 on the ISC) is only feasible in settings with a pre-control endemicity level of 10 VL cases per 10,000 people per year or less, with optimal IRS. In settings with higher pre-control endemicities or with sub-optimal IRS, additional interventions are required.

In **Chapter 6** we use our sub-model with the main reservoir of infection in asymptomatic individuals (E1) and introduce an additional sub-model (E0), in which solely symptomatic individuals are infectious. These models are compared to a VL model from Warwick University (W), by fitting all three models with a geographical cross-validation after which we compare the simulated impact of IRS and active case detection (ACD) on VL incidence. All models suggest that the WHO elimination target on the ISC is likely to be met before or close to 2020 in sub-districts with a pre-control incidence of 10 VL cases per 10,000 people per year or less, with current interventions (60% IRS coverage, and ACD leading to an average duration between onset of symptoms and start of treatment of 40 days).

Chapter 7 presents the policy relevance of all previously mentioned findings and we calculate the impact of the WHO 'attack and consolidation phase' using our models E0, E1 and models W0 and W1 from Warwick University. The WHO attack phase includes intensive IRS and regular ACD, during the preceding consolidation phase IRS is less intensive and ACD efforts increase. We conclude that the interventions of a 5 year attack phase followed by the consolidation phase as recommended in the WHO guidelines, should be sufficient to reach the elimination target in areas that had VL endemicities up to 10/10,000/year prior to the start of interventions. In regions with a higher pre-control endemicity of 15/10,000/year, additional interventions are required, such as a longer duration of the WHO attack phase, to bring forward elimination. In regions with a very high pre-control endemicity of 20/10,000/year, the current recommended duration of the attack phase of 5 years in not sufficient to reach the target of less than 1/10,000/year within a time period of 30 years. In these regions a 20 year attack phase (model E0) or a continuous attack phase (model E1) is required to bring the incidence below 1/10,000/year, which is illustrated in Figure 3.

The contribution of PKDL to transmission in combination with the large population of susceptibles when nearing elimination can cause a potential hurdle in reaching and sustaining the VL elimination target.

In **Chapter 8.4** we have used models E0 and E1 to explore the potential impact of an alternative intevention that is currently under development, a human VL vaccine.

When nearing elimination, many other aspects that are not included in the models can play an important role and change the feasibility of achieving the targets. One aspect is the highly focal nature of disease geographically, in many studies spatial clustering of cases is found, whereas in the current models we are assuming a homogeneous spread of infection. [26, 27] Such clusters could have very high incidences, while the target of 1 VL case per 10,000 population per year can still be achieved at sub-population level. Another aspect is the increasing incidence of HIV in India. Between 2011-2013 a cohort of 2077 VL patients aged ≥14 years were screened in Bihar, of which 5.6% were HIV positive. [28] It has been suggested that these co-infected cases might be highly infectious 'super spreaders' of the infection. Another factor that might play a role when nearing elimination is the losing interest of funders. Currently in the guidelines as well as in our models the

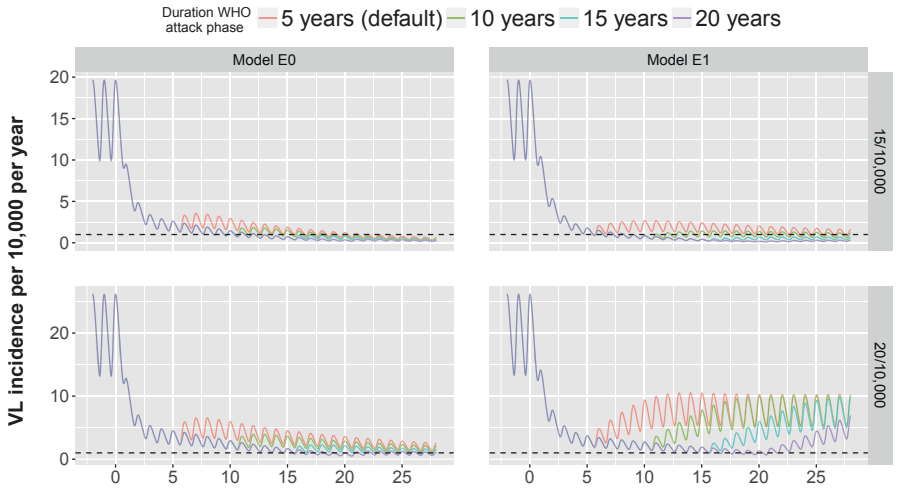

Figure 3. VL incidence predictions for alternative pre-control endemicities of 15 and 20 cases per 10,000 population per year using model E0 (only symptomatic individuals contribute to transmission) and model E1 (asymptomatic individuals are main contributors to transmission). Alternative durations of the attack phase of 10, 15 and 20 years were simulated. The black dashed line represents the WHO elimination target of 1/10,000/year.

interventions are in place continuously, but this might be too optimistic for all scenarios. The recent decline in VL cases has been speculated to be also related to a long-term epidemic cycle of VL with a duration of approximately 15 years at country level, that is currently in a declining phase. Such cycles are not included in current models, in which the decrease in incidence is solely attributable to interventions. Would these cycles exist, a future increase in cases is expected, which would influence future predictions that are currently not accounted for by the models. However, the explanation of these cycles as being due to presence and absence of interventions [29] would be reflected by the current interpretations of the models. Lastly, our simulations do not include any potential increase in insecticide resistance over time causing the impact of IRS to decrease, which is considered constant in our models. If insecticide resistance increases, our simulations are too optimistic. [30]

The 4 models that we present in **Chapters 5, 6** and **7** are basically extreme variations of the same structure. In model E0 solely symptomatic individuals (symptomatic untreated, treatment 1, treatment 2 and PKDL) are infectious towards the sandfly. In model E1 asymptomatic individuals constitute the main reservoir of infection. Model E2 assumes lifelong immunity, and immune

individuals can reactivate the disease by becoming asymptomatically infected again. In model E3 the reservoir of infection is, similarly to model E0, solely in symptomatic individuals, but with an extremely important role of individuals with PKDL. We took these assumptions regarding the reservoir of infection because of the remaining knowledge gaps in this field. A combination of these models would most likely be closest to reality, but the subdivision between them is hard to estimate with current scientific evidence. In order to be able to make such a distinction, results of ongoing xenodiagnosis studies are important, as well as on other information such as the durations of PKDL and immunity.

All models presented in **Chapters 5**, **6** and **7** are deterministic compartmental models. These models are based on the susceptible – infected – recovered (SIR) framework, where groups of individuals move between compartments or states at certain fixed rates. In these deterministic models, the durations of states are usually exponentially distributed, which often does not reflect the actual distributions of durations as found in nature. With exponential distributions most individuals will be in a certain state shorter than the average duration, whereas a few individuals will experience extremely long durations on the other hand. This could influence the simulations, for example at the long term when infectious PKDL cases are around for many years in this model. Also, deterministic models are not suitable to estimate the chance (%) of a scenario to lead to exactly zero VL cases. Since all rates are fixed, identical simulations will always lead to identical output, and no element of chance is included. Stochastic individual-based models (IBMs) can have the same structure of states, but here, due to the stochastic nature, random chance is incorporated. IBMs allow for different types of distributions of duration of states, which improves the representation of the dynamics. The probability of elimination of infection or recrudescence of infection can be estimated for a given population, both very relevant issues regarding the potential of future VL control and elimination. Individuals are modeled separately, which allows for more heterogeneity in individual features, such as HIV-VL coinfection, compliance to screening and potential vaccination strategies and immigration of individuals.

We concluded that with sufficient efforts the 1/10,000 target could be achieved in most settings. This low level incidence can be sustained when interventions remain in place continuously, however, it is most likely that

once the targets have been achieved, intervention efforts will lessen and funding will decrease. Since elimination of transmission is currently not the target, the continuation of interventions will be crucial to counteract new outbreaks of infection. New strategies are to be designed that are both feasible to sustain over long periods of time as well as sufficient to prevent flare-up of infections. Such strategies could include VL and PKDL awareness programs for local health facilities, availability of diagnostic tests, regular fever camps where individuals with fever get tested for malaria and VL among other diseases, combined active case finding programs for skin diseases, such as PKDL and leprosy, and adequate re-active screening and IRS control after a new VL or PKDL case has been identified.

The contribution of the VL transmission models presented in this thesis, to science and policy is diverse but still very modest. They have improved our way of thinking about VL transmission dynamics and have quantified the effect of different interventions on incidence, in particular the feasibility of achieving the WHO 2020 elimination targets on the ISC. Even though certain aspects of the transmission dynamics remained unknown, useful conclusions could be drawn, and progress has been made as we described in **Chapters 4, 5, 6** and **7**. Being part of the NTD Modelling Consortium has definitely accelerated this process. [31] In the future, the VL transmission models need to keep being updated as soon as new information becomes available, and continuing progression is required in order for the models to remain of use in the future of VL control and elimination.

8.2 Frequency and distribution of canine leishmaniasis in Spain and France

In the case of zoonotic visceral leishmaniasis (ZVL) in Europe, humans are not the main reservoir of the infection. The underlying canine reservoir drives the infection, and controlling this reservoir might be equally or even more important to be able to control the disease in humans. In the 'Strategic Framework for Leishmaniasis Control in the European Region' [32], the World Health Organization (WHO) acknowledged the emergence of *Leishmania* in Europe as a serious public health issue and emphasizes the importance of monitoring and control of the disease. Up to date knowledge regarding the

magnitude of the incidence of canine leishmaniasis (CanL) in Europe is essential, but as we concluded in **Chapter 3**, this remains largely unknown. With this additional analysis, we aim to provide an insight into the incidence and the geographical distribution of the canine reservoir of ZVL in Spain and France.

The veterinarians that responded to our survey from **Chapter 3** were approached for a short follow-up survey, and organizations that had spread our first survey were asked to also distribute the link to this additional survey on our behalf. From the beginning of February until the end of March 2017, 413 veterinarians opened the survey, of which 280 completed all seven questions, their responses are presented in Table 6, aggregated at country level. The geographic distribution of veterinarians covered 80 different regions, 30 out of the 48 provinces in Spain and 50 out of the 96 departments in France. The longitude and latitudes of the locations of veterinarians were uploaded in Geographic Information System qGIS. Figure 1 shows the frequency and distribution of veterinarians that completed our survey.

We calculated the incidence of confirmed CanL cases per veterinarian using the following formula:

$$Incidence = \frac{Number\ of\ reported\ new\ cases\ of\ CanL\ per\ year}{(Dogs\ seen\ weekly\ \cdot workweeks\ per\ year)/dog\ visits\ a\ clinic\ per\ year} \cdot 1000$$

The average calculated incidence was 22 confirmed CanL cases per 1000 dogs per veterinarian per year. Spain had significantly higher incidences (33/1000/yr) compared to France (6/1000/yr), which ranged between 0-284/1000/yr in Spain and 0-69/1000/yr in France, reflecting high heterogeneity between veterinarians. In Figure 2 we link the incidence to the geographic distribution of the veterinarians. The highest incidences of CanL reported in our survey are situated in southeastern Spain and along the Mediterranean coast, but veterinarians are reporting cases up to the most northern departments of France.

Table 6. Results from the survey among 280 veterinarians, aggregated at national level.

Survey question		France	Spain	Total
1. Location of veterinarian		108 (39%)	172 (61%)	280 (100%)
2. Average number of dogs seen per veterinarian, per week		64.5	51.8	56.7
3. Average number of dogs with confirmed CanL diagnosis in the past 12 months per veterinarian		4.7	14.9	10.9
4. Number of veterinarians who diagnosed leishmaniasis in other animal species in the past 12 months				
	Cat	1 (1%)	7 (4%)	8 (3%)
	Ferret	0 (0%)	1 (1%)	1 (0%)
	No	107 (99%)	164 (95%)	279 (97%)
5. Total number of cats diagnosed with leishmaniasis in the past 12 months		2	8	10
6. Number of veterinarians who have been aware of human VL cases in their area in the past 10 years				
	Yes	13 (12%)	49 (28%)	62 (22%)
	No	95 (88%)	123 (72%)	218 (78%)
7. Total number of human cases in their area veterinarians have been aware of in the past 10 years		20	108	128

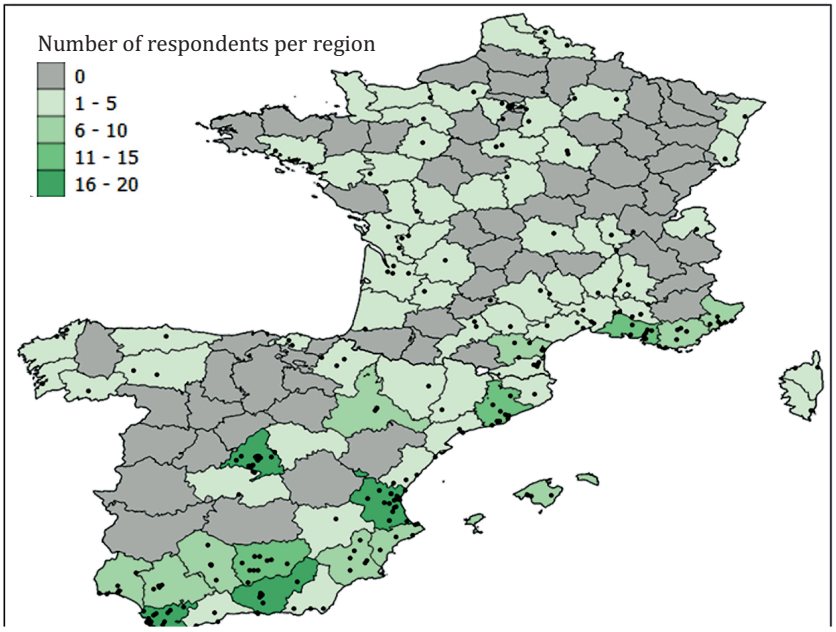

Figure 4. The frequency and distribution of veterinary survey respondents. The different shades of green correspond to the number of veterinarians per department (France) or province (Spain). The black dots represent each respondent.

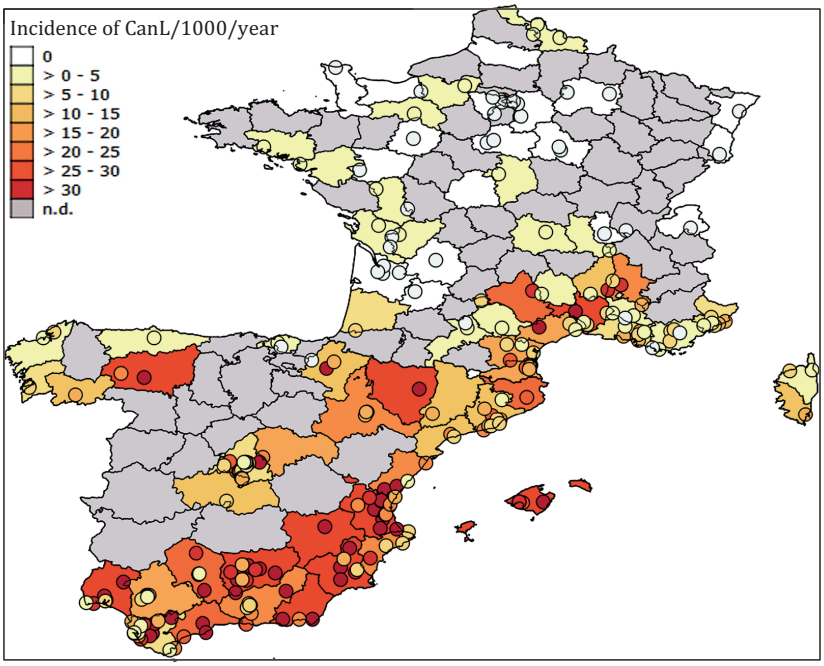

Figure 5. Distribution and average incidence of canine leishmaniasis per province (Spain) and department (France). The coloured dots each represent calculated incidence per veterinarian.

Interpolation of the data to estimate the incidence of CanL in Spain and France in regions that we had no respondents from, was performed using Inversed Distance Weighted (IDW) interpolation. IDW estimates a value at unknown points, using the weighted average of the available values at points nearby. IDW is based on the assumption that points located nearer to each other are more related than distant points. The interpolated incidence of the canine leishmaniasis reservoir in France and Spain is illustrated in Figure 3.

The distribution of incidence is comparable to what has been found in other studies, with higher incidences in Spain and around the Mediterranean coast and lower incidences in France, following the distribution of the sandfly. [33, 34] As far as we know, the magnitude of the reservoir in northeastern France has not previously been documented.

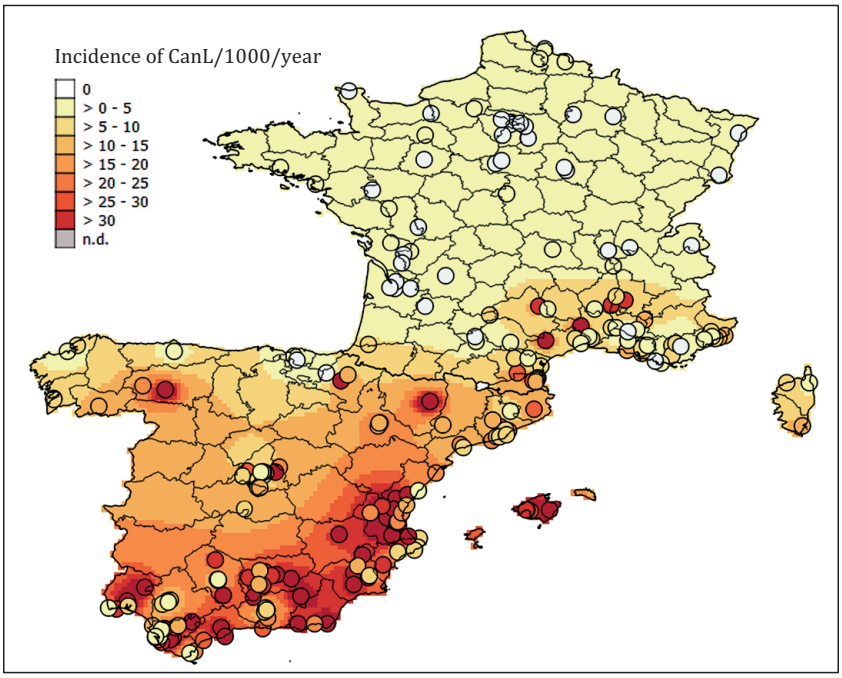

Figure 6. Interpolation of the incidence of canine leishmaniasis per 1000 dogs per year using inversed distance weighting (IDW). The coloured dots each represent calculated incidence per veterinarian.

Based on this survey, it remains unclear whether the dogs have been infected locally or whether they have been imported from an endemic region. This map does provide insight where infected dogs have been diagnosed, and in combination with the increasing sandfly distribution, it shows where preventive measures should be highly recommended.

This survey only provided insight in the incidence of symptomatic CanL cases, but it is important to realize that only 5-10% of the dogs in endemic regions develop clinical signs, the remaining dog population infected with leishmaniasis experiences subclinical infections. [17] Asymptomatic dogs have been found to be highly competent to transmit *L. infantum*, and therefore we are looking at the tip of the iceberg of the disease reservoir in dogs. [18] The current risk factors (expansion of the sandfly territory and the large flow of traveling and migrating dogs and puppies) are not likely to decline in the near future. Therefore, monitoring of at least the clinical ZVL cases combined

with adequate preventive measures are highly recommended in all regions of Spain and France.

8.3 Conceptual mathematical transmission model for zoonotic VL

Mathematical transmission models can be useful tools to estimate the impact of current and alternative interventions on disease incidence. Such insights can guide policy makers on how best to interrupt the emerging trend of infection, and how best to control the disease, as we present in **Chapter 7** for anthroponotic infection of VL on the Indian subcontinent. A mathematical VL transmission model with an animal reservoir has been developed for a setting in Bihar, India [35], but no models have been fitted to the European setting as far as we know. Incorporating an animal reservoir in the Erasmus MC models presented in **Chapters 5, 6,** and **7**, would require detailed data on the VL / CanL ratio and the canine, sandfly and human transmission dynamics. In contrast to the models used in **Chapters 5, 6,** and **7**, in these transmission dynamics dogs are the main reservoir of infection and humans are potentially 'dead-end hosts'. [REF] Figure 1 shows an image of what such a model structure could look like, but the actual development of this model highly depends on the data available. In this potential model dogs are born susceptible, after which they become asymptomatically infected when bitten by an infectious sandfly. Most dogs will recover, but about 5-10% will develop clinical symptoms, first being symptomatic untreated for a certain duration of time, which is most likely region specific, after which they become symptomatically infected under treatment or experiencing excess mortality when left untreated. After treatment the dogs recover and experience a certain duration of immunity, after which they will become susceptible again. The human transmission cycle is similar to that presented in models E0, E1, E2 and E3, however it has been simplified to 6 stages; susceptible, asymptomatic, symptomatic, putatively recovered, PKDL and recovered. The duration of disease states and their relative infectivity to the sandfly can be informed by xenodiagnoses data, diagnostic tests and longitudinal follow up studies.

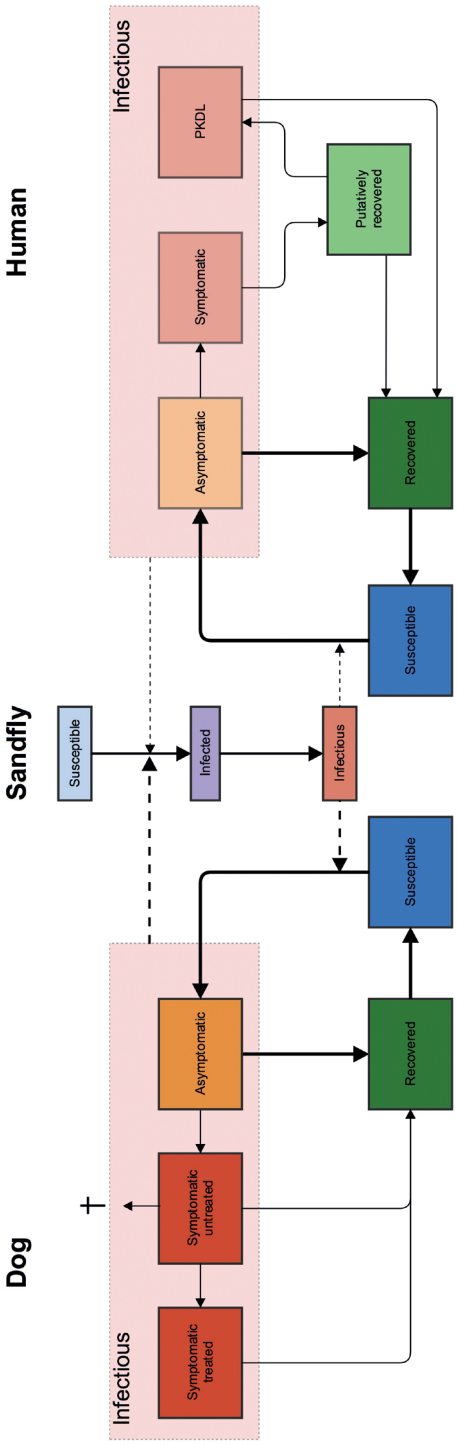

Figure 7. Schematic representation of a potential model structure of zoonotic visceral leishmaniasis (ZVL), in which dogs are the main contributors to transmission.

Besides the dogs, in Figure 7 we do not include any other animal reservoir because even though the infectivity of dogs has been widely researched, the relative abundance and infectivity of other (wildlife) species or even stray dogs remains speculative, but potentially plays an important, region specific, role as well. [36, 37]

Would we develop the zoonotic VL transmission model presented in Figure 1, different strategies for the control of ZVL could be implemented such as detection and treatment of dogs (and humans), vaccination of dogs, and vector control.

8.4 Model explorations of the potential impact of VL vaccines

Potential role of vaccines in VL control

Current interventions to control and eliminate VL consist of diagnosis and treatment of cases, often in combination with vector control. In regions that are nearing or sustaining the elimination target, the expected large susceptible population could pose a big risk for new outbreaks as we visualised in figure 4 of **Chapter 7**. Asymptomatic individuals and PKDL cases could still harbour the parasite for a long time after the last symptomatic case has been treated. In this situation, vaccines would be an interesting option to prevent recrudescence of infection. Also, in regions such as East Africa, where detection and treatment strategies are sometimes not sufficient in reaching 100% of the cases, as is targeted for by WHO, a vaccine program could be an effective intervention to reduce the number of cases. (1)

Vaccines have already played an important role in the control of canine leishmaniasis, through three types of effects: reducing the parasite load in the blood, reducing the development of symptoms, and reducing the risk of death. (2,3) At the population level these canine vaccines have proven to be effective in reducing disease incidence in both dogs (directly) and humans (indirectly). (4,5) Although the development of human VL vaccines has been ongoing for decades, no vaccine is available yet. (6,7) However, the evidence for the scientific feasibility of an effective vaccine for VL in the future is strong, given the promising results from experimental human VL vaccine trials. (6,8–10) This is further supported by the abovementioned successful

development and implementation of canine vaccines. (3,11) Using a decision analytic model it has been estimated that a future effective vaccine would be cost-effective when used at large scale. (12)
Mathematical transmission models have proven to be useful tools to gain an insight into the possible health impact of a future vaccine and to aid in defining an ideal target product profile (TPP), for example in the field of malaria. (13–15) Here, we implement multiple effects of potential VL vaccines into our set of established deterministic VL transmission models, model E0, in which symptomatic individuals drive transmission and model E1, in which asymptomatics drive transmission, which are further described in **Chapters 6** and **7**. With these models we estimate the potential impact of these vaccine effects on VL incidence in an anthroponotic setting.

Overview of VL vaccine candidates and their effects
Currently there are trials ongoing with multiple VL vaccine candidates, of which we selected 5, based on a paper from a VL vaccine expert meeting in Rockville, USA, that we attended in September 2015 at the National Institute of Allergy and Infectious Diseases: adjuvant Leish-F3, LEISHDNAVAX, Ad5-A2/rA2 Prime / Boost, ChAd63-KH, and genetically modified live attenuated whole parasite. (1) Some of these vaccines have multiple effects, such as protecting against infection, decreasing the infectiousness of an individual, and reducing the development of symptoms. We decided to model these properties separately to provide an insight into the independent health impacts. Table 7 provides an overview of the effects that these vaccines are suggested to have. Vaccine effect 2 was separated for asymptomatic individuals and all PCR-positive individuals, since it is still unknown to what extent asymptomatics are infectious towards the sandfly.

Implementation and simulation of vaccine effects
Figure 8 is a schematic representation of model E1 in which the numbers are related to the vaccine effects that are listed in Table 7. Vaccine effect 1, protection against infection, is implemented in our model through a reduction of development of infection, and is therefore comparable to the impact of IRS from **Chapters 5, 6 and 7.** Because the percentages of reductions of developing infection, infectivity and the proportions of individuals that develop clinical VL and PKDL are not yet well understood (vaccine effects 1-4),

Table 7. Overview of VL vaccine candidates and their type of effect regarding the VL natural history

Vaccine effect number	Type of effect	Vaccines	References
1	Protection against infection	- Adjuvant Leish-F3 - LEISHDNAVAX - Ad5-A2/rA2 Prime / Boost	(16) (17) (18)
2a	Asymptomatics half as infectious (model E1 only)	- Adjuvant Leish-F3 - LEISHDNAVAX - Ad5-A2/rA2 Prime / Boost	(16) (17) (18)
2b	All PCR-positive individuals half as infectious	- Adjuvant Leish-F3 - LEISHDNAVAX - Ad5-A2/rA2 Prime / Boost	(16) (17) (18)
3	Reduced development of symptoms	- Adjuvant Leish-F3 - LEISHDNAVAX - Ad5-A2/rA2 Prime / Boost	(16) (17) (18)
4	Reduced development of PKDL	- ChAd63-KH	ISRCTN07766359
5	Development of immunity	- Whole parasite	(19–21)

we have chosen reductions of an arbitrary 50%. For vaccine effects 3 and 4, the flow towards clinical VL and PKDL respectively is halved, leading to more people flowing through the other arrow that is leaving the compartment.

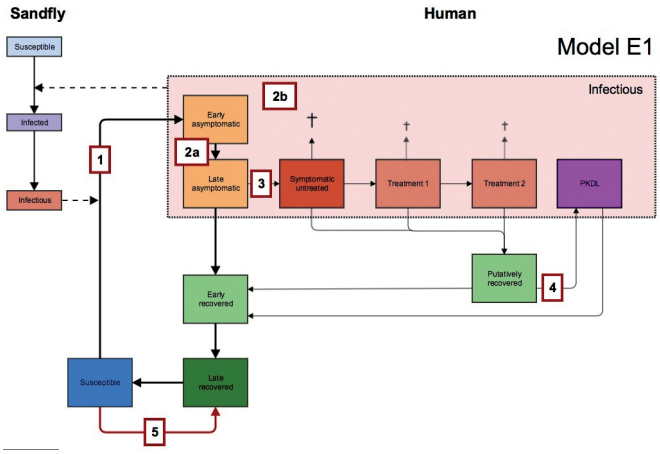

Figure 8. Schematic presentation of model E1, with numbers related to different types of vaccine effects that are implemented in the models (Table 7). Model E0 is similar in structure; only early and late asymptomatic individuals are not infectious towards to sandfly. (1) Vaccinated individuals are 50% less likely to get infected, (2a) Early and late asymptomatics become half as infectious, (2b) All disease states become half as infectious, (3) Vaccinated individuals are 50% less likely to develop symptoms, (4) Vaccinated individuals are 50% less likely to develop PKDL, and (5) Vaccinated individuals become immediately immune.

With vaccine effect 5, we selected a full effect of 100% development of immunity, but experimented with vaccinating 100% and 50% of the population. The duration of immunity after vaccination is assumed similar to the duration of immunity after having experienced infection, i.e. 2 years. For the simulations of vaccine effects we assume that they apply to everyone involved, all ages and sexes. We also assume that the vaccine effects are in place constantly from the start of the intervention, only for vaccine effect 5, after which vaccinated individuals develop immunity, we experimented with alternative durations where the susceptible population becomes immune once, or 5 years in a row, simulating repeated yearly vaccination rounds.

Predictions of the impact of vaccines on VL incidence

The impact of the 5 separate vaccine effects on VL incidence is illustrated in Figure 9. Vaccine effect 1, in which vaccinated individuals become 50% less likely to get infected, leads to a steep decrease in VL incidence, reducing the incidence from 5/10,000/year at equilibrium pre-control to below 1/10,000/year in about 2.5 years (model E1) and about 8 years (model E0). Model E0 shows a slower decline, because in this model the infection pressure comes solely from symptomatic individuals, so there is a delay before this infection state is affected by the vaccination, compared to model E1 in which asymptomatic individuals are the main contributors to transmission, and are directly affected by this vaccine effect.

Vaccine effect 2a, reduction of infectivity of asymptomatic individuals, only applies to model E1, and also leads to a steep decline in incidence, since in this model asymptomatics are the main contributors to transmission. However, here symptomatic individuals still contribute as usual, leading to a steeper decline for model E1 when implementing vaccine effect 2b. Vaccine effect 2b, reduction of infectivity in all infected individuals by 50%, has obviously a similar impact as vaccine effect 1, protection against infection, when selected percentages are similar.

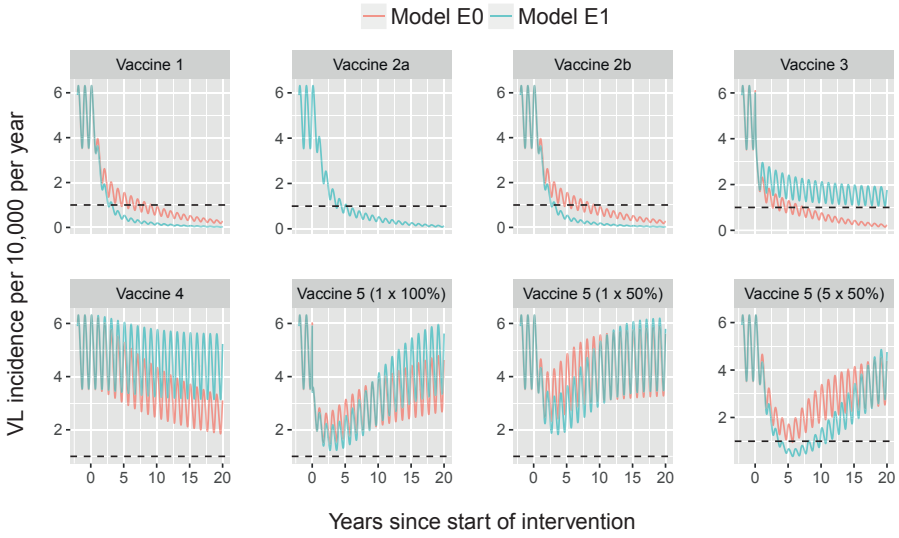

Figure 9. The impact of different vaccine effects on VL incidence using models E0 and E1 in a setting with a pre-control endecmicity of 5/10,000/year. Vaccine effects are in place continuously from year 0 onwards, unless for vaccine effect 5, which is administered once or yearly for five years in a row. The different vaccine effects are explained in Table 7 and illustrated in Figure 8. The black dashed line represents the WHO elimination target of 1/10,000/year.

Vaccine effect 3, halving the chance of developing symptoms, has a considerable impact on transmission, especially when assuming that only symptomatic individuals are infective (Model E1). Vaccine effect 4, after which individuals are 50% less likely to develop PKDL, has the least impact on transmission. As expected with this effect, the larger impact is seen with model E0, in which PKDL plays a more prominent role in the transmission dynamics, which we visualized in figure 5 of **Chapter 7**.

Vaccine effect 5, development of immunity, causes the most rapid decrease in incidence, since the pool of susceptible individuals is completely removed at once. We additionally explored the effect of yearly vaccinating half the population 5 years in a row, which shows that regular vaccinations are required to sustain the impact as is presented in Figure 9.

Using a PKDL vaccine in combination with current interventions

Vaccine effect 4, after which vaccinated individuals are 50% less likely to develop PKDL, had showed the least impact by itself, but could potentially be used in combination with, or after indoor-residual spraying of insecticide

(IRS) and active case detection (ACD) have brought the number of cases down to the level of elimination as a public health problem. Figure 10 shows the impact on VL incidence of decreasing the development of PKDL with 50% and 100% for a setting with a pre-control endemicity level of 5/10,000/year using 2 different strategies. Firstly, we present the default scenario (red line) without the vaccine, in which a 5-year WHO attack phase (intense IRS and ACD) is followed by 5 years of the WHO consolidation phase (IRS and intense ACD) after which the interventions return to the pre-control scenario. The VL incidence will return to the pre-control equilibrium over time.

In the first vaccine strategy (green line), the PKDL vaccine is introduced at the start of the consolidation phase, the consolidation phase ends again after 5 years, but the PKDL vaccine remains in place continuously. In the second vaccine strategy (blue line) the PKDL vaccine is already introduced at the start of the attack phase, after which it remains in place continuously. In the two scenarios with the PKDL vaccine (green and blue lines) a new, much lower, equilibrium will be reached. For the vaccine with a 50% decrease in PKDL development a new equilibrium around the target of 1/10,000/year is reached as simulated by Model E0. When assuming an effect of 100% protection from developing PKDL, model E0 suggests that vaccinating only could keep the incidence below 1/10,000/year after the active and consolidation phase have brought the incidence down.

However, in settings with a pre-control endemicity of 10/10,000/year, even a vaccine with 100% protection against development of VL will not be enough to prevent VL incidence to increase again substantially after quitting IRS and ACD (figure not shown).

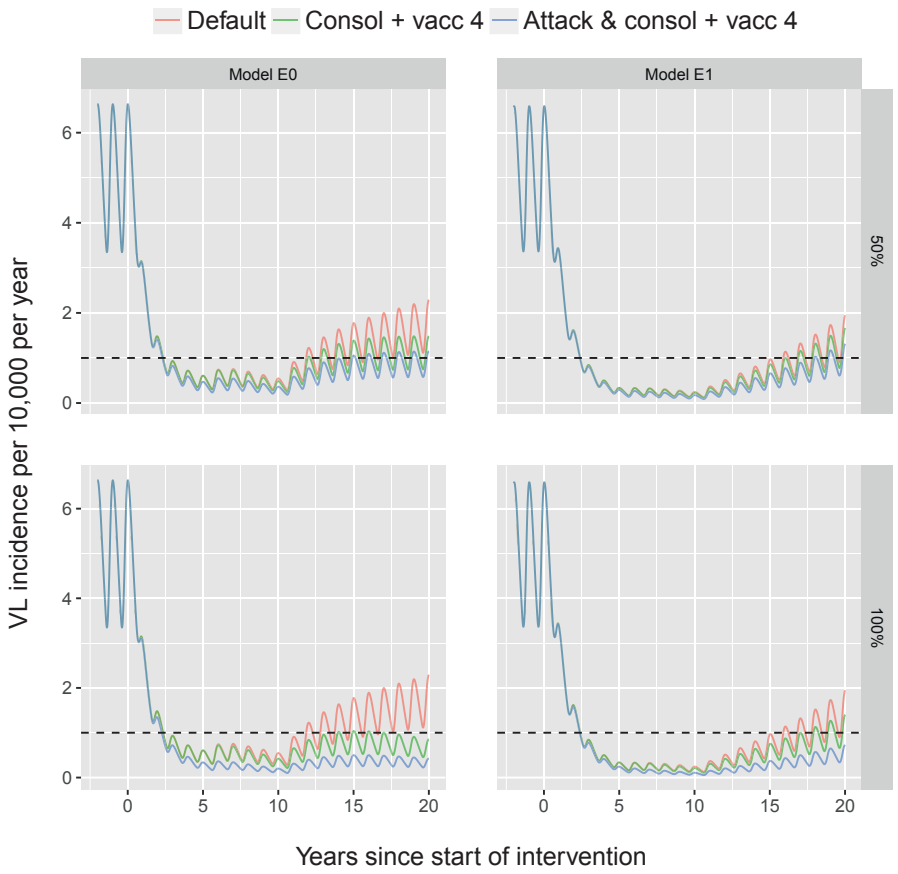

Figure 10. Strategies of combining vaccine effect 4. Top row: vaccine effect with *50%* protection against the development of PKDL, bottom row: vaccine effect with *100%* protection against the development of PKDL. Both vaccine effects have been combined with the WHO attack and consolidation phase for a setting with a pre-control endemicity level of 5/10,000/year. The default strategy is visualized with the red line (top and bottom row identical), in which 5 years of attack phase are followed by 5 years of consolidation phase, after which interventions are halted in year 10. For the green line, the PKDL vaccine is introduced during the consolidation phase, which continues after the consolidation phase has ended at year 10. For the blue line, the PKDL vaccine is already introduced at the start of the attack phase, continues during the consolidation phase and is continued when IRS and ACD are halted. Left figures show the simulations for Model E0 (in which solely symptomatic individuals contribute to transmission), the right figures show the simulations for Model E1 (in which asymptomatic individuals constitute the main reservoir of infection. The black dashed line represents the WHO target of 1/10,000/year.

Discussion of VL vaccination

The potential target product profile (TPP) for a VL vaccine depends on whether you are looking at the individual level or at the population level, our simulations focus only on the impact of vaccines at population level. The TPP

also depends on whether you are looking at a 'stand-alone' vaccine or one to be used in combination with other strategies. The ideal TPP for a stand-alone VL vaccine, based on our rough preliminary simulations that are presented in Figure 9, consist of the protection of individuals against developing infection and/or decreasing the individuals' infectivity. However, the assumed degree to which individuals are protected, and to which extent the parasite load is decreased by the vaccine highly influences this impact. In this study we have simulated the vaccine effects separately, but in reality the Adjuvant Leish-F3, LEISHDNAVAX and Ad5-A2/rA2 Prime / Boost vaccines are expected to have multiple effects (effects 1, 2 and 3). However, the relative contribution of each vaccine effect may differ per vaccine, therefore the three vaccines can have a varying impact on VL incidence.

The potential implementation feasibility of a vaccine also plays an important role when defining an ideal vaccine strategy. Vaccinating an entire population or even half of the population requires a tremendous effort, both logistically as well as financially. Vaccinating VL cases when they present themselves for treatment, as is the case for vaccine effect 4 (which protects individuals against the development of PKDL), would potentially be more feasible. Adding a vaccine strategy to an already existing approach would enlarge the chance of successful implementation.

Our deterministic model is a powerful tool to simulate and compare the effects of different interventions on VL incidence. However when incidences get very low, below the 1/10,000/year elimination target, chance elements become more important as well as individual features, both of which are not incorporated in deterministic models. Another aspect of deterministic models, as discussed in **Chapter 8.1**, is that the duration of states are exponentially distributed, which often does not reflect the actual distributions of durations as found in nature. Therefore, certain individuals experience extremely long durations, influencing the simulations, for example at the long term when individuals have a duration of infectious PKDL for many years in the model. Individual-based models (IBM) allow for different types of distributions of duration of states, which improves the representation of the dynamics. The probability of elimination of infection and the probability of recrudescence of infection can be estimated using an IBM, both very relevant issues regarding the potential of future VL control and elimination. In a deterministic model, in contrast to an IBM, the incidence would always return to the pre-control

equilibrium when all interventions are halted, albeit at different speeds. When using an IBM, individuals are modeled separately, which allows for more heterogeneity in individual features, such as compliance to potential vaccination strategies. In our deterministic model we vaccinate a random 50% of the population, not taking into account any systematic non-compliance, which has shown to be of large impact when looking at for example modeling of interventions of helminth infections. (22)

The way we model the vaccine effects in this study is very simplistic. We alter the rates in the model at a very sudden moment in time applying this simultaneously to all individuals involved. However, vaccinated individuals should ideally move to different, additional, compartments in the model, after which they experience a different history of infection. In such a model you would have vaccinated an unvaccinated individuals living besides each other, both influencing the transmission dynamics differently. Our current method reflects a simplification of these processes. Currently we also assume that (besides vaccine effect 5) 100% of the individuals in the endemic setting that we simulate are vaccinated and that the vaccine effects occur in 50% of the people. Therefore we are most certainly overestimating the health impact, as 100% vaccine coverage would be unlikely in these settings. However, this allows us to interpret the maximum potential that vaccines could have on a population and to compare the impact of different vaccines. In remote settings vaccinating half of the population would be much more feasible compared to 100% coverage. When vaccinating a sub-population, ideally the campaign should focus on the population that is at highest risk for leishmania infection, i.e. children and young adults, and/or specific sub-populations, such as migrant workers in East Africa. (23) Something we currently do not include in the model, and for which an IBM would be the best tool.

Based on these very rough preliminary analyses we conclude that the impact of vaccines that protect individuals for 50% against developing infection and decrease the individuals' infectivity by 50% show most potential in reducing the incidence at population level. However, combining a vaccine with current strategies, such as vaccinating VL cases against the development of PKDL when they receive VL treatment, could also be a worthwhile strategy. More advanced models and simulations are required to provide a more realistic insight in the impact that vaccines and vaccine strategies could have on VL incidence. Would any of the simulated vaccines

become available, insights into the implementation feasibility, coverage levels, costs and selection of target populations are of great importance before any policy recommendations can be made.

8.5 Conclusions and recommendations

Conclusions

1. The substantial global health and socio-economic impact gained by reaching the WHO elimination and control target for visceral leishmaniasis, justifies the required financial efforts.
2. Hardly any monitoring and evaluation takes place on canine leishmaniasis in Spain and France.
3. Elimination of VL as a public health problem on the Indian subcontinent is feasible with the recommended WHO strategies in regions with a medium to high pre-control endemicity level, but the reservoir of infection influences the duration to reaching this target and in extremely high endemic regions additional efforts will be necessary.

Recommendations for policy

1. The substantial health and socio-economic benefits of VL control and elimination are powerful arguments for advocacy purposes.
2. Europe should have an easy online network where both veterinarians and physicians report the presence of confirmed zoonotic VL cases.

Recommendations for scientific research

1. Further research of the contribution of PKDL towards the overall transmission dynamics of VL is essential, especially now that elimination targets are in sight.
2. A more detailed understanding of the transmission dynamics of VL will aid policy makers in the next phase of reaching and sustaining the elimination targets on the Indian Subcontinent, for which a stochastic individual-based transmission model would be an ideal tool.

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CHAPTER 9

Summary

9.1 English summary

The overall aim of this thesis was to provide an insight into the potential health gain of the control and elimination of visceral leishmaniasis.

In **Chapter 1** we provided a general introduction to visceral leishmaniasis (VL). VL is a zoonotic vector-borne disease that is caused by a single-celled parasite and transmitted by the sandfly, affecting mainly humans and dogs. VL is globally the deadliest parasitic infection after malaria. The World Health Organization (WHO) has targeted this neglected tropical disease (NTD), together with 8 other NTDs, for global control or elimination by 2020. On the Indian subcontinent (ISC), where VL is considered to solely infect humans, the target is elimination of VL as a public health problem, which is defined as less than one VL case per 10,000 population per year at sub-district level. Here, the disease incidence has decreased over the past years, among others due to interventions such as indoor residual spraying of insecticide (IRS) and active case detection (ACD). In the rest of the world, where VL is also zoonotic, 100% detection and treatment of cases is targeted for. In other regions, such as the Mediterranean, an increase in cases and outbreaks has been experienced over the past years both in humans and dogs.

To research the potential of reaching the WHO control and elimination targets for VL, the following research questions were addressed:

1) What is the global health impact when achieving the WHOs' targets for disease elimination and control of 9 neglected tropical diseases, and in particular visceral leishmaniasis?
2) Are veterinarians in the endemic regions of Spain and France aware of the spread of zoonotic VL in Europe and do they implement the guidelines to control this disease?
3) What insights can transmission models provide regarding the feasibility of achieving the VL elimination targets on the Indian subcontinent?

In **Chapter 2** we answered research question 1, by estimating the global health impact between 2011 and 2030 in the ideal situation of achieving the WHO control and elimination targets of all 9 NTDs. We based our calculations on country, sex and age specific data of all 9 NTDs in 1990 and 2010 from the

Global Burden of Disease Study. We interpolated the data linearly between these years and further extrapolated between 2010 and the WHO targets up to 2030. The potential health impact was calculated by comparing the results of achieving the targets with the counterfactual, which we defined as a continuation of the percentage prevalence of 1990. These calculations resulted in nearly 600 million averted disability adjusted life years (DALYs) globally, of which nearly a quarter could be attributed to reaching the targets for VL (140 averted DALYs). The number of averted deaths due to VL in the period of 2011 and 2030 was estimated at just under 2.5 million, which accounts for half of the averted deaths of all NTDs together.

In **Chapter 3** we focused on the Mediterranean region, where the main reservoir of infection is in dogs. Here, both the sandfly territory and the infection are moving northwards due to climate change and migration of dogs and puppies from Southern to Northern European countries. To address research question 2, we developed an online questionnaire for veterinarians in Spain and France, to test their awareness and implementation of international intervention guidelines to control the spread of zoonotic VL in Europe. 456 veterinarians completed the survey from which we learned that 70% of them were not aware of any guidelines, nevertheless applying most preventive, detection and treatment measures that are recommended in the guidelines. However, 76% of all veterinarians from this study had never received any reports regarding confirmed zoonotic VL cases in their region or country and 88% never reported a case that had been confirmed by them, leading to a major lack of monitoring and evaluation, which is one of the key topics of the guidelines.

Based on previously published literature, we provided an overview of the existing mathematical VL transmission models in **Chapter 4**, and highlighted the scarcity of VL models. We also described the natural history of infection to inform the structure and parameterization of models, while underlining the remaining knowledge gaps, including the infectivity of individuals in different disease states and therefore the main reservoir of infection. We concluded that there was an urgent need for more VL transmission models to be developed to aid public health policy by analysing surveillance data and guiding policy regarding the impact of different intervention strategies on VL incidence.

For **Chapter 5**, we developed a mathematical model to simulate the transmission dynamics of human VL on the ISC, which we fitted to Indian and Nepalese data. We created three sub-models, which differ regarding the reservoir of infection since this remains unknown; in model E1 asymptomatic individuals constitute the main reservoir of infection, model E2 assumes the reservoir to be in immune individuals that can reactivate the disease, and in model E3 the reservoir of infection is solely in symptomatic individuals, but mainly in individuals with PKDL. With these three sub-models, we estimated the impact of indoor residual spraying of insecticide (IRS) on VL incidence and concluded that, with the model with the main reservoir of infection in asymptomatic individuals (E1), elimination of VL (<1 new VL case per 10,000 capita per year at sub-district level by 2017 on the ISC) would only be feasible in settings with a pre-control endemicity level of 10 VL cases per 10,000 people per year or less, with optimal IRS. In settings with higher pre-control endemicities or with sub-optimal IRS, additional interventions would be required.

In **Chapter 6,** we have used the most plausible sub-model from **Chapter 5**, with the main reservoir of infection in asymptomatic individuals (E1), and introduce an additional sub-model, in which solely symptomatic individuals are infectious, model E0. These models are compared to a VL model from Warwick University, model W, by fitting all three models using a geographical cross-validation, after which we compare the simulated impact of IRS and active case detection (ACD) on VL incidence. All models suggest that the WHO elimination target on the ISC is likely to be met before or close to 2020 in sub-districts with a pre-control incidence of 10 VL cases per 10,000 people per year or less, with current interventions (60% IRS coverage, and ACD leading to an average duration between onset of symptoms and start of treatment of 40 days).

In **Chapter 7**, we drew the main policy relevant recommendations from previously published VL modeling studies (including those described in **Chapters 5** and **6**) and presented the effect of current and alternative WHO guidelines on VL incidence. The WHO guidelines formulated that a five years of an 'attack phase' is followed by a minimum of three years of a 'consolidation phase', both phases consisting of different intensities of IRS

and ACD. We simulated this strategy to be sufficient to reach the elimination target in areas that had medium VL endemicities (up to 5 VL cases per 10,000 people per year) prior to the start of interventions. However, we found that additional interventions would be required in regions with higher pre-control endemicities, such as a longer duration of the WHO attack phase (intensive IRS and ACD), to bring forward elimination. We presented new insights into the potential contribution of PKDL to transmission, which, in combination with the large population of susceptibles when nearing elimination, can cause a potential hurdle in reaching and sustaining the VL elimination target.

Finally, in **Chapter 8**, we discuss the answers to the research questions and present three additional analyses. At first, we developed a secondary follow-up survey among veterinarians in Spain and France. Based on this survey we could estimate the magnitude and geographical distribution of canine leishmaniasis incidence. We also extrapolated these data to the entire two countries by using an inversed distance weighting technique. We concluded that the distribution of incidence was comparable to what has been found in other studies, with higher incidences in Spain and around the Mediterranean coast and lower incidences in France. However, as far as we know, the magnitude of the reservoir in northeastern France has not previously been documented. Secondly, we present a conceptual structure for a zoonotic VL transmission model, which could be a useful tool in estimating the impact of different interventions on the incidence of zoonotic VL. However, the significance of such a tool highly depends on available data regarding these transmission dynamics and the effects of interventions. Thirdly, we have used our two VL transmission models from **Chapters 6** and **7**, to explore the potential impact of human vaccines that are currently under development. Based on very rough preliminary analyses we conclude that the impact of vaccines that protect individuals for 50% against developing infection and decrease the individuals infectivity by 50% would be most effective in reducing the incidence at population level when used as stand-alone tools. A vaccine protecting VL cases to develop PKDL could potentially be a valuable tool to sustain low levels of incidence after IRS and ACD control. We ended the discussion with the following conclusions and recommendations:

Conclusions

1. The substantial global health and socio-economic impact gained by reaching the WHO elimination and control target for visceral leishmaniasis, justifies the required financial efforts.
2. Hardly any monitoring and evaluation takes place on canine leishmaniasis in Spain and France.
3. Elimination of VL as a public health problem on the Indian subcontinent is feasible with the recommended WHO strategies in regions with a medium to high pre-control endemicity level, but the reservoir of infection influences the duration to reaching this target and in extremely high endemic regions additional efforts will be necessary.

Recommendations for policy

1. The substantial health and socio-economic benefits of VL control and elimination are powerful arguments for advocacy purposes.
2. Europe should have an easy online network where both veterinarians and physicians report the presence of confirmed zoonotic VL cases.

Recommendations for scientific research

1. Further research of the contribution of PKDL towards the overall transmission dynamics of VL is essential, especially now that elimination targets are in sight.
2. A more detailed understanding of the transmission dynamics of VL will aid policy makers in the next phase of reaching and sustaining the elimination targets on the Indian Subcontinent, for which a stochastic individual-based transmission model would be an ideal tool.

9.2 Nederlandse samenvatting

Het doel van dit proefschrift is inzicht geven in de potentiële gezondheidswinst van het bestrijden en elimineren van viscerale leishmaniasis.

In **Hoofdstuk 1** geven we een introductie over viscerale leishmaniasis (VL). VL is een zoönotische infectieziekte die wordt overgedragen door de zandvlieg en veroorzaakt door een eencellige parasiet die voornamelijk mensen en honden infecteert. VL is na malaria wereldwijd de dodelijkste parasitaire infectie. De Wereldgezondheidsorganisatie (World Health Organisation, WHO) heeft voor 2020 doelen opgesteld voor het wereldwijd bestrijden of elimineren van VL en acht andere verwaarloosde tropische infectieziekten (Neglected Tropical Diseases, NTDs). Op het Indiase subcontinent (ISC), waarvoor wordt verondersteld dat VL alleen bij mensen voorkomt, is het doel: eliminatie van VL als volksgezondheidsprobleem. Dit wordt gedefinieerd als minder dan één VL-geval per 10.000 mensen per jaar. In de rest van de wereld, waar VL ook zoönotisch is, is het doel: 100% detectie en behandeling van VL-gevallen. Op het ISC is de incidentie van de ziekte de afgelopen decennia afgenomen, onder andere door interventies zoals het binnenshuis sprayen van langdurig actieve insecticide (indoor residual spraying, IRS) en het actief opsporen van VL-gevallen (active case detection, ACD). In andere regio's, zoals het Middellandse Zeegebied, is er de afgelopen periode een toename van incidentie waargenomen, zowel bij mensen als bij honden.

Om de haalbaarheid van de WHO-doelstellingen te onderzoeken, hebben we de volgende onderzoeksvragen opgesteld:

1) Wat is de wereldwijde gezondheidswinst van het behalen van de WHO-doelstellingen, in casu het elimineren en bestrijden van negen verwaarloosde tropische infectieziekten, en in het bijzonder van viscerale leishmaniasis?
2) Zijn dierenartsen in de endemische gebieden van Spanje en Frankrijk zich bewust van de verspreiding van zoönotische VL in deze landen en implementeren ze de richtlijnen om deze ziekte onder controle te houden?

3) Welke inzichten kunnen transmissiemodellen geven met betrekking tot de haalbaarheid van het bereiken van de VL-eliminatie-doelstellingen op het Indiase subcontinent?

In **Hoofdstuk 2** beantwoorden we onderzoeksvraag 1 door een schatting te maken van de wereldwijde gezondheidswinst tussen 2011 en 2030 in de ideale situatie waarbij alle WHO-bestrijdings- en eliminatiedoelstellingen worden gehaald voor alle negen NTD's. We hebben onze berekeningen gebaseerd op land-, geslacht- en leeftijdsspecifieke data van de negen NTD's in 1990 en 2010 van de Global Burden of Disease Study. Vervolgens hebben we de data lineair geïnterpoleerd tussen deze jaren en daarna geëxtrapoleerd van 2010 tot het WHO-doel en verder tot 2030. De potentiële gezondheidswinst hebben we berekend door de resultaten van het bereiken van de doelen te vergelijken met die van het scenario waarbij de situatie na 1990 constant zou zijn gebleven. Deze berekeningen resulteerden in een gezondheidswinst van bijna 600 miljoen te voorkómen 'verloren gezonde levensjaren door ziekte' (disability adjusted life-years, DALY's) wereldwijd, waarvan bijna een kwart kon worden toegeschreven aan het behalen van de doelen voor VL (140 miljoen voorkomen DALY's). We hebben het aantal voorkómen doden door VL in de periode tussen 2011 en 2030 geschat op iets minder dan 2,5 miljoen, wat neerkomt op ongeveer de helft van de te voorkómen doden van alle NTD's tezamen. We concluderen dat de grote wereldwijde gezondheidswinst voor het bestrijden en elimineren van viscerale leishmaniasis de vereiste investeringen rechtvaardigt.

In **Hoofdstuk 3** hebben we ons geconcentreerd op het Middellandse Zeegebied waar het overgrote deel van het reservoir van de infectie zich in honden bevindt. Zowel het territorium van de zandvlieg als de infectie spreidt zich naar het noorden uit vanwege klimaatveranderingen en de migratie van honden en puppy's vanuit Zuid- naar Noord-Europese landen. Om onderzoeksvraag 2 te beantwoorden hebben we een online vragenlijst voor dierenartsen ontwikkeld om hun bewustzijn en implementatie van de internationale interventierichtlijnen voor het bestrijden van leishmaniasis in Europa te onderzoeken. 456 dierenartsen hebben de vragenlijst volledig ingevuld. 70% gaf aan zich niet bewust te zijn van enige leishmaniarichtlijnen, desalniettemin passen ze bijna alle preventieve,

detectie- en behandelingsmaatregelen die in de richtlijnen geadviseerd worden toe. Echter, 76% van alle dierenartsen van deze studie heeft nooit een rapport van een bevestigd zoönotisch VL-geval uit hun regio ontvangen en 88% heeft nooit een geval dat door hen gediagnosticeerd was gerapporteerd. Er blijkt dus een groot gebrek aan monitoring en evaluatie te zijn, wat een risico vormt voor de volksgezondheid, en één van de belangrijke onderwerpen van de richtlijnen is.

In **Hoofdstuk 4** geven we, gebaseerd op eerder gepubliceerde literatuur, een overzicht van de bestaande wiskundige transmissiemodellen van VL, waaruit een gebrek aan VL-modellen naar boven kwam. We beschrijven daarnaast ook het natuurlijk verloop van de infectie om zo de structuur en modelparameters te kunnen vaststellen, waarnaast we ook de resterende gaten in de kennis zichtbaar maken, zoals de besmettelijkheid van individuen in verschillende stadia van de infectie, en daarmee het reservoir van de infectie. We concluderen dat het van groot belang is om meer VL-transmissiemodellen te ontwikkelen om zo het volksgezondheidsbeleid te kunnen ondersteunen. Dit kan door middel van het analyseren van epidemiologische datasets en het geven van beleidsadvies met betrekking tot de impact van verschillende interventiestrategieën op de incidentie van VL.

Voor **Hoofdstuk 5** hebben we een wiskundig model ontwikkeld om de transmissiedynamiek van humaan VL op het ISC te simuleren. We hebben dit model gekwantificeerd op Indiase en Nepalese epidemiologische datasets. We hebben drie submodellen gecreëerd, die verschillen met betrekking tot het reservoir van infectie, dat tot heden onbekend is. In model E1 vormen asymptomatische individuen het reservoir van infectie, in model E2 nemen we aan dat de infectie kan re-activeren in immune individuen en in model E3 is het reservoir van infectie alleen te vinden in symptomatische gevallen en voornamelijk in mensen met post-kala-azar dermal leishmaniasis (PKDL), een complicatie van VL. Met deze drie submodellen hebben we de impact van binnenshuis sprayen met langdurige insecticiden (indoor residual spraying of insecticide, IRS) op de VL-incidentie geschat. We concludeerden dat met het model waarvan het reservoir van infectie zich in asymptomatische individuen bevindt (model E1), eliminatie van VL (minder dan één nieuw VL-geval per 10.000 mensen per jaar op subdistrictsniveau voor 2017 op het

ISC) haalbaar is in settings met een eerder endemiciteitsniveau van tien VL-gevallen per 10.000 mensen per jaar of minder en in combinatie met optimale IRS. In settings met eerder hogere endemiciteiten of met sub-optimale IRS zullen aanvullende interventies nodig zijn.

In **Hoofdstuk 6** hebben we het meest plausibele submodel van **Hoofdstuk 5** gebruikt (model E1), waar het reservoir zich hoofdzakelijk in asymptomatische individuen begeeft, en introduceren we een aanvullend submodel, model E0, waarin alleen symptomatische individuen (VL en PKDL) infectieus zijn. Deze modellen zijn vergeleken met een VLmodel van Warwick University (UK), model W, door middel van een geografische kruisvalidatie. Daarna hebben we de voorspellingen van de drie modellen vergeleken waarbij we de impact simuleren die IRS en ACD (active case detection) hebben op de VL-incidentie. Alle modellen wijzen erop dat het waarschijnlijk is dat het WHO eliminatiedoel voor het ISC gehaald kan worden vóór of rondom 2020 in subdistricten met een pre-control incidentie van tien VL-gevallen per 10.000 mensen per jaar of minder. Dit in combinatie met de huidige interventies (60% IRS dekkingsgraad en een niveau ACD dat leidt tot een duur tussen de start van symptomen en de behandeling van veertig dagen). Het moment van het behalen van eliminatie hangt af van het reservoir van infectie.

In **Hoofdstuk 7** beschrijven we de belangrijkste beleidstoepassingen van eerder gepubliceerde VL-modellen-studies (waaronder die beschreven in **Hoofdstukken 5** en **6**) en presenteren we het effect van de huidige en alternatieve WHO-richtlijnen op de incidentie van VL. De WHO-richtlijnen schrijven voor dat een vijf jaar durende 'attack phase' gevolgd wordt door een minimaal drie jaar durende 'consolidation phase'. Beide fases bestaan uit verschillende intensiteiten van IRS en ACD. Uit onze simulaties bleek dat de huidige strategie voldoende is om het eliminatiedoel te behalen in gebieden met gemiddeld hoge VL-endemiciteit (tot vijf VL-gevallen per 10.000 mensen per jaar) voorafgaand aan de start van de interventies. Echter, we vonden ook dat aanvullende interventies nodig zijn in gebieden met hogere pre-control endemiciteiten, zoals een langere duur van de WHO 'attack phase' (intensieve IRS en ACD), om eliminatie eerder te kunnen bereiken. Daarnaast geven we inzicht in de potentiële bijdrage van mensen met PKDL aan de overdracht

van de besmetting, die, in combinatie met de groeiende populatie vatbare individuen rondom eliminatie, een potentieel gevaar kan vormen voor het bereiken en behouden van het VL-eliminatiedoel.

Uiteindelijk, in **Hoofdstuk 8**, bespreken we de antwoorden op de onderzoeksvragen en presenteren we drie extra analyses. Ten eerste hebben we een vervolgvragenlijst ontwikkeld voor dierenartsen in Spanje en Frankrijk. Op basis van deze vragenlijst konden we de omvang en geografische verspreiding van leishmaniasis bij honden schatten. We hebben deze data vervolgens geëxtrapoleerd over de volledige oppervlakte van de twee landen door middel van een Inversed Distance Weighting methode. We hebben geconcludeerd dat de verspreiding van incidentie vergelijkbaar is met wat eerder over deze landen gepubliceerd is, met hogere incidenties in Spanje en rond de Middellandse Zee en lagere incidenties in Frankrijk. Echter, voor zover wij weten is de omvang van het reservoir in Noordoost-Frankrijk niet eerder gedocumenteerd. Ten tweede presenteren we een conceptuele structuur voor een zoönotisch VL-transmissiemodel, wat een bruikbaar hulpmiddel zou kunnen zijn om de impact van verschillende interventies op de incidentie van zoönotisch VL te schatten. Echter, het belang van een dergelijk hulpmiddel hangt grotendeels af van de beschikbare data op het gebied van de transmissiedynamiek en het effect van interventies. Ten derde hebben we onze twee VL-transmissiemodellen uit **Hoofdstukken 6** en **7** (model E0 en E1) gebruikt om de potentiële impact van vaccins die op dit moment in ontwikkeling zijn te berekenen. Gebaseerd op ruwe voorlopige analyses concluderen we dat de impact van vaccins die individuen voor 50% beschermen tegen het ontwikkelen van infectie en die 50% reductie veroorzaken in de infectieusiteit van individuen, het meest effectief zijn in het terugbrengen van de incidentie op populatieniveau als ze worden gebruikt als enige interventie. Een vaccin dat mensen met VL beschermt tegen het ontwikkelen van PKDL zou potentieel een waardevol hulpmiddel kunnen zijn om incidentie na IRS- en ACD-interventies laag te houden. We sluiten de discussie af met de volgende conclusies en aanbevelingen:

Conclusies

1. De aanzienlijke wereldwijde gezondheids- en sociaaleconomische impact die bereikt wordt door het behalen van de WHO bestrijding- en

eliminatiedoelstellingen voor viscerale leishmaniasis, rechtvaardigt de benodigde financiële inspanningen.

2. In Spanje en Frankrijk vindt nauwelijks monitoring en evaluatie plaats van het vóórkomen én voorkómen van leishmaniasis bij honden.
3. Eliminatie van het volksgezondheidsprobleem van VL op het Indiase subcontinent is haalbaar met de huidige WHO-strategieën en in gebieden met een gemiddeld tot hoog pre-control endemiciteitsniveau, maar het reservoir van infectie beïnvloedt de duur tot het behalen van de doelstellingen. In extreem hoog endemische gebieden zijn aanvullende inspanningen noodzakelijk.

Aanbevelingen voor beleid

1. De aanzienlijke gezondheids- en sociaaleconomische voordelen van het bestrijden en elimineren van VL rechtvaardigen nader onderzoek.
2. Europa zou een gemakkelijk online netwerk moeten hebben waar zowel dierenartsen als huisartsen hun bevestigde zoönotische VL-gevallen kunnen rapporteren.

Aanbevelingen voor wetenschappelijk onderzoek

1. Verder onderzoek naar de bijdrage van PKDL aan de totale VL-transmissiedynamiek is essentieel, vooral nu de eliminatiedoelen in zicht zijn.
2. Een gedetailleerder begrip van de transmissiedynamiek van VL zal beleidsmakers helpen om de volgende fase van het behalen en behouden van de eliminatiedoelen op het Indiase subcontinent vorm te geven. Een stochastisch individual-based model zou daarvoor het ideale hulpmiddel zijn.

CHAPTER 10

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Lieve Chaï, 2 zomers 5 weken vanuit huis werken in vaders kantoor zijn een zegen geweest. De rust die je uitstraalde, hebben geleid tot ultieme focus en het kunnen afzonderen van alles dat er niet toe deed. De zomers waar mam elke dag een lunch van Ottolenghi maakte, pap een extra scherm scoorde zodat ik goed kon werken, en we aan het einde van de dag in het zwembad lagen met een ultiem gevoel van vrijheid, hebben ertoe geleid dat ik met opperste focus onder andere hoofdstuk 6 heb kunnen schrijven, als ook het begin van dit dankwoord.

Lieve Beppe en Grootpapa, ondanks dat jullie hier niet meer fysiek aanwezig zijn, geven jullie proefschrift en veterinaire bul die boven mijn bureau hangen, mij dagelijks inspiratie en bevestiging om het door mij ingeslagen pad met vertrouwen en grootste inzet te vervolgen.

Lieve Opa en Oma, graag had ik nog zoveel met jullie willen delen, maar jullie inzet, het serieus nemen van je taken en jullie gepassioneerde liefde voor de Chaï leven dagelijks voort in mijn daden.

Allerliefste pap en mam. Als er twee mensen op de wereld zijn zonder wie ik dit niet had kunnen doen, dan zijn jullie het. Voor jullie onvoorwaardelijke enthousiasme, trotsheid en bevlogen betrokkenheid ben ik in-en-in dankbaar. Dit boekje is voor jullie.

Lieve Willem Adriaan, Wal, van jou heb ik geleerd om te relativeren en af en toe ook goed te chillen. Ik geniet altijd met volle teugen van de momenten die we delen.

Lieve Philippe. Jouw support de laatste jaren was episch, van hoe jij trots op feestjes aan mensen bleef uitleggen hoe belangrijk het uitroeien van tropische infectieziekten was en me kwam ophalen op Schiphol 's avonds laat als ik weer van een meeting terugkwam, tot het lachen om mijn R-scherm waarvan jij stiekem dacht dat ik geheime onderzeeboten aanstuurde. Ik vind het heel bijzonder dat je altijd zo trots bent en mij het allerbeste gunt. Dat gun ik jou ook, en daarom verheug ik mij op een waanzinnige toekomst samen vol joie de vivre, wat het leven ons ook mag brengen.

Dan ook nog even Gijs. Dankzij jou waren thuiswerkdagen een feest en was thuiskomen na een lange dag, waar jij spinnend op mij stond te wachten, hartverwarmend. Het maakt nederig en doet je realiseren, dat het doctoraat, hoe groot ook de eer van het behalen ervan, een door mensen bedacht principe blijft, en dat liefhebben en zorgzaam zijn voor de mensen, dieren en de natuur om je heen altijd boven alles staat.

CHAPTER 11

About the author

11.1 Curriculum vitae

Epke Annelie Le Rutte was born on May 13th, 1987 in Delft, the Netherlands. She attended RSG Lingecollege in Tiel at the age of 12 and at the age of 15 she moved to the United Kingdom to continue the last 3 years of her high school education at Reed's School in Cobham, Surrey. She graduated in 2005 after which she moved back to the Netherlands to pursue her dream of studying veterinary medicine like her grandmother.

During her studies at Utrecht University Epke developed a great interest in veterinary public health and continued with a master in Livestock and Veterinary Public Health, with a minor in Research. She was given the opportunity to perform her 8-month master research project at the Food and Agriculture Organization of the United Nations in Rome, Italy, where she studied the transmission dynamics of 40 human and veterinary pathogens. She continued her education by taking courses at Harvard School of Public Health in Boston, USA, increasing her knowledge in epidemiology, and vector-borne and zoonotic infectious diseases from a human public health point of view.

In 2013 she was offered a job in the infectious disease control team at the Department of Public Health of Erasmus MC, Erasmus University Rotterdam, the Netherlands. Where, after having graduated in Utrecht as a veterinarian with distinction, she further embarked on her path of infectious disease control with a main focus on visceral leishmaniasis (VL). During her PhD she developed a profound interest in mathematical models, which she used to capture the transmission dynamics of VL and simulate the impact of different interventions on incidence to ultimately inform policy. During this time, she has been an invited speaker at a variety of expert meetings and conferences around the globe. She enjoyes supervising master students with their research, coordinates the Tropical Medicine course at Erasmus MC, teaches various lectures, and has created the Tropical One Health workshop bringing together veterinary and medical students to spark a multidisciplinary

approach in zoonotic infectious disease control. Epke is also a certified yoga teacher, teaching vinyasa en yin yoga during the weekend.

After obtaining her PhD, Epke is looking forward to continue her scientific career in the field of tropical infectious disease control combining both her veterinary and public health education.

11.2 List of publications

Published

*Le Rutte EA**, Chapman LAC*, Coffeng LE, Jervis S, Hasker EC, Dwivedi S, Karthick M, Das A, Mahapatra T, Chaudhuri I, Boelaert MC, Medley GF, Srikantiah S, Hollingsworth TD, de Vlas SJ: **Elimination of visceral leishmaniasis in the Indian subcontinent: a comparison of predictions from three transmission models**. *Epidemics* 2017, **18**:67–80.

Le Rutte EA, Coffeng LE, Bontje DM, Hasker EC, Ruiz Postigo JA, Argaw D, Boelaert MC, De Vlas SJ: **Feasibility of eliminating visceral leishmaniasis from the Indian subcontinent: explorations with a set of deterministic age-structured transmission models**. *Parasit Vectors* 2016, **9**:24.

Hollingsworth TD, Adams ER, Anderson RM, Atkins K, Bartsch S, Basáñez M, Behrend M, Blok DJ, Chapman LAC, Coffeng L, Courtenay O, Crump RE, de Vlas SJ, Dobson A, Dyson L, Farkas H, Galvani AP, Gambhir M, Gurarie D, Irvine MA, Jervis S, Keeling MJ, Kelly-Hope L, King C, Lee BY, *Le Rutte EA*, Lietman TM, Ndeffo-Mbah M, Medley GF, Michael E, et al.: **Quantitative analyses and modelling to support achievement of the 2020 goals for nine neglected tropical diseases**. *Parasit Vectors* 2015, **8**:630.

de Vlas SJ*, Stolk WA*, *Le Rutte EA**, Hontelez JAC, Bakker R, Blok DJ, Cai R, Houweling TAJ, Kulik MC, Lenk EJ, Luyendijk M, Matthijsse SM, Redekop WK, Wagenaar I, Jacobson J, Nagelkerke NJD, Richardus JH: **Concerted Efforts to Control or Eliminate Neglected Tropical Diseases: How Much Health Will Be Gained?** *PLoS Negl Trop Dis* 2016, **10**:e0004386.

Stolk WA, Kulik MC, *Le Rutte EA*, Jacobson J, Richardus JH, de Vlas SJ, Houweling TAJ: **Between-Country Inequalities in the Neglected Tropical Disease Burden in 1990 and 2010, with Projections for 2020**. *PLoS Negl Trop Dis* 2016, **10**:e0004560.

Rock KS, *Le Rutte EA*, de Vlas SJ, Adams ER, Medley GF, Hollingsworth TD: **Uniting mathematics and biology for control of visceral leishmaniasis**. *Trends Parasitol* 2015, **31**:251–259.

* These authors have contributed equally

In press

Jervis S, Chapman LAC, Dwivedi S, Karthick M, Das A, *Le Rutte EA*, Courtenay O, Medley GF, Banerjee I, Mahapatra T, Chaudhuri I, Srikantiah S, Hollingsworth TD: **Variations in VL burden, mortality and the pathway to care within Bihar, India: Hotspots and the role of socio-economic differences**. *Parasit Vectors* 2017

Under review

*Le Rutte EA**, Chapman LAC*, Coffeng LE, Ruiz Postigo JA, Adams ER, Boelaert MC, Hollingsworth TD, Medley GF, de Vlas SJ: **Policy recommendations from transmission modelling for the elimination of visceral leishmaniasis in the Indian subcontinent.**

Le Rutte EA#, van Straten R, Overgaauw, PAM: **The control of zoonotic visceral leishmaniasis in Europe: a survey among Spanish and French veterinarians VL in Europa.**

Lenk EJ, Redekop WK, Luyendijk M, Fitzpatrick C, Niessen L, Stolk WA, Tediosi F, Rijnsburger AJ, Bakker R, Hontelez JA, Richardus JH, Jacobson J, *Rutte EA Le*, Vlas SJ de, Severen JL: **The socioeconomic benefit to individuals of achieving the 2020 targets for four neglected tropical diseases controlled or eliminated by innovative and intensified disease management (human African trypanosomiasis, leprosy, visceral leishmaniasis and Chagas**.

* These authors have contributed equally

11.3 PhD Portfolio

Name PhD-candidate:	Epke Annelie Le Rutte
University:	Erasmus University Rotterdam, Netherlands
Faculty:	Medical Center
Department:	Department of Public Health
PhD-period:	July 2013 – July 2017
Promotor:	Prof. dr. Jan Hendrik Richardus
Co-promotor:	Dr. Sake J. de Vlas

Oral Presentations

Conference/meeting, "*presentation title*"	**Location**	**Date**	**Workload**
WHO expert meeting, "*WHO targets for the control and elimination of VL*"	Geneva, Switzerland	Oct 2013	24 hours
WHO expert meeting, "*WHO targets for the control and elimination of HAT*"	Geneva, Switzerland	Oct 2013	24 hours
BMGF expert meeting: "*Health impact calculations: interpretation of the WHO elimination targets*"	Geneva, Switzerland	Feb 2014	24 hours
Bill and Melinda Gates Foundation (BMGF), Visceral Leishmaniasis Diagnostics Modeling Consortium, "*The impact of different diagnostics for VL, a modeling point of view*"	Seattle, USA	July 2014	24 hours
International Congress of Parasitology, Chagas meeting: "*Modeling Chagas' disease?"*	Mexico City, Mexico	Aug 2014	24 hours
PKDL-meeting, "*Role of PKDL in VL transmission and elimination*"	Istanbul, Turkey	Dec 2014	24 hours
BMGF Final presentation, "*Health impact and demo of interactive online tool*"	Seattle, USA	Jan 2015	24 hours
WHO Kala-azar elimination program partners meeting: "*Potential VL incidence trends towards elimination*"	Geneva, Switzerland	Feb 2015	24 hours
Annual NTD Modeling Consortium Technical Meeting, "*modeling VL*"	Warwick, UK	Mar 2015	24 hours
ASTMH "*Feasibility of eliminating visceral leishmaniasis from the Indian subcontinent*"	Philadelphia, USA	Oct 2015	24 hours
Annual NTD Modeling Consortium Technical Meeting, "*How to define 'true' elimination - Deterministic versus a stochastic individual-based approach*"	Warwick, UK	Apr 2016	24 hours

Oral Presentations

Conference/meeting, "*presentation title*"	**Location**	**Date**	**Workload**
Annual NTD Modeling Consortium Technical Meeting, "*VL modeling plans*"	Warwick, UK	Apr 2016	24 hours
Joint spring meeting of the Belgian Society of Parasitology & Protistology (BSPP) and the Netherlands Society of Parasitology (NVP) "Vectors & Parasites", "*Feasibility of eliminating VL on the Indian Subcontinent*"	Rotterdam, Netherlands	May 2016	8 hours
COR-NTD, "*Impact of ramping up case detection – get large surge in cases, but how large will it be, what is the impact on transmission - Visceral leishmaniasis*"	Atlanta, USA	Nov 2016	16 hours
ASTMH, "*Definitions and feasibility of elimination of visceral leishmaniasis*"	Atlanta, USA	Nov 2016	24 hours
MGZ seminar, "*Feasibility of eliminating visceral leishmaniasis*"	Rotterdam, NL	Nov 2016	8 hours
Section meeting infectious disease control, "*Visceral leishmaniasis: transmission model comparison*"	Rotterdam, NL	Dec 2016	8 hours
Annual NTD Modeling Consortium Technical Meeting, "*Clustering of visceral leishmaniasis*"	Warwick, UK	Mar 2017	8 hours
Annual NTD Modeling Consortium Technical Meeting, *"Insights from modelling on VL elimination policy"*	Warwick, UK	Mar 2017	8 hours
AC Migratory Health (EMC) "*Visceral leishmaniasis, potential for control and elimination*"	Rotterdam, NL	Apr 2017	8 hours
World Leish, "*Elimination of visceral leishmaniasis on the Indian subcontinent: a comparison of multiple transmission models*"	Toledo, Spain	May 2017	24 hours

Oral Presentations

Conference/meeting, "*presentation title*"	**Location**	**Date**	**Workload**
World Leish, "*The control of zoonotic visceral leishmaniasis in Europe: a survey among Spanish and French veterinarians*"	Toledo, Spain	May 2017	24 hours
Leishmaniasis Modelling & Economics workshop, "*The impact of interventions on future incidence: visceral leishmaniasis transmission models"*	York, UK	Oct 2017	24 hours
ECTMIH, "*Visceral leishmaniasis transmission models: the impact of interventions*"	Antwerp, Be	Oct 2017	24 hours
MGZ Seminar, "*Geographic analyses of visceral leishmaniasis: Control and incidence of zoonotic VL in Europe & Spatiotemporal patterns of anthroponotic VL in India*"	Rotterdam, NL	Oct 2017	8 hours
ASTMH, "*Visceral leishmaniasis: challenges when nearing elimination*"	Baltimore, USA	Nov 2017	24 hours

Poster presentations

Conference, "*poster title*"	**Location**	**Date**	**Workload**
ASTMH, "*The Global Health Impact of VL and HAT when Reaching the 2020 WHO Control and Elimination Targets*"	New Orleans, US	Oct 2014	24 hours
ECTMIH, "*Feasibility of eliminating visceral leishmaniasis from the Indian subcontinent*"	Basel, Switzerland	Sep 2015	24 hours
BSP, One Health congress, "*Human African Trypanosomiasis*"	London, UK	Sep 2015	24 hours
ESCCAP, Vector-borne diseases symposium, "*Control and awareness of the spread of zoonotic VL in Europe among veterinarians*"	Granada, Spain	Oct 2016	24 hours
World Leish, "*The potential impact of visceral leishmaniasis vaccines: explorations with different deterministic age-structured transmission models*"	Toledo, Spain	May 2017	24 hours
ECTMIH, "*Incidence and geographical distribution of canine leishmaniosis in Spain and France*"	Antwerp, Be	Oct 2017	24 hours
ECTMIH, "*The potential impact of visceral leishmaniasis vaccines: explorations with different deterministic age-structured transmission models*"	Antwerp, Be	Oct 2017	24 hours
ASTMH, "*Incidence and geographical distribution of canine leishmaniosis in Spain and France*"	Baltimore, USA	Nov 2017	24 hours
ASTMH, " *The potential impact of visceral leishmaniasis vaccines: explorations with different deterministic age-structured transmission models*"	Baltimore, USA	Nov 2017	24 hours

Teaching			
Course, *"Topic"*	**Location**	**Date**	**Workload**
Tropical Animal Health, lecture, "*Human African trypanosomiasis*"	Faculty of Veterinary Medicine, Utrecht University	Sep 2013	8 hours
Tropical Animal Health, lecture, "*Zoonotic visceral leishmaniasis*"	Faculty of veterinary medicine, Utrecht University	Sep 2014	8 hours
Supervisor Community Project	Erasmus MC, Rotterdam	2015	24 hours
Tropical Animal Health, lecture, "*Zoonotic visceral leishmaniasis*"	Faculty of veterinary medicine, Utrecht University	Sept 2015	8 hours
VO 1, Bachelor year 1, lecture, "*Advisering voedingsgewoonten*"	Erasmus MC, Rotterdam	June 2015	8 hours
Examination of bachelor essays	Erasmus MC, Rotterdam	2015	16 hours
Supervisor Community Project	Erasmus MC, Rotterdam	2016	
Supervisor master research project veterinary student "*VL and immunity in East Africa*"	Erasmus MC Rotterdam	Jan- May 2016	125 hours
Coordinator of STOLA tropencursus	Erasmus MC Rotterdam	March-June 2016	30 hours
Tropical Animal Health, lecture, "*Visceral leishmaniasis control*"	Faculty of veterinary medicine, Utrecht University	Sept 2016	8 hours
Supervisor master research project veterinary student "*Zoonotic VL in Europe*"	Erasmus MC Rotterdam / Utrecht University	April – August 2016	125 hours
VO 1, Bachelor year 1, lecture, "*Advisering voedingsgewoonten*"	Erasmus MC Rotterdam	Sept 2016	8 hours
Coordinator of STOLA tropencursus	Erasmus MC Rotterdam	Oct – Dec 2016	30 hours
Coordinator tropical One Health Workshop for veterinary and medical students	Erasmus MC Rotterdam / Utrecht University	Oct 2016	40 hours
Supervisor master research project veterinary student "*Geographical analysis of VL incidence at hamlet level in India"*	Erasmus MC Rotterdam / Utrecht University	Oct 2016 – Feb 2017	100 hours
Supervisor master research project medical student "*Incidence and distribution of zoonotic VL in Spain and France*"	Erasmus MC Rotterdam / Utrecht University	Jan – April 2017	75 hours

BHU 1 day course, lecture, "*Mathematical modeling of VL*"	Banaras Hindu University, Varanasi, India	April 2017	16 hours
Coordinator of STOLA tropencursus	Erasmus MC Rotterdam	March – June 2017	30 hours
Coordinator tropical One Health Workshop for veterinary and medical students	Erasmus MC Rotterdam / Utrecht University	Oct 2016	30 hours
Tropical Animal Health, lecture, "*Visceral leishmaniasis*"	Faculty of veterinary medicine, Utrecht University	Sep 2017	8 hours
VO, Bachelor year 3, lecture, "*Advisering voedingsgewoonten*"	Erasmus MC Rotterdam	Oct 2017	8 hours
Coordinator of STOLA tropencursus	Erasmus MC Rotterdam	Oct – Dec 2017	30 hours
STOLA tropencursus, lecture, "*Bestrijding en eliminatie van verwaarloosde tropische infectieziekten*"	Erasmus MC Rotterdam	Dec 2017	8 hours

Teacher trainings	**Location**	**Date**	**Workload**
Didactietraining: 0.5 day	Erasmus MC	June 2015	8 hours
Teach the teacher: 2 day workshop	Erasmus MC	Oct 2015	16 hours
Workshop: "Hoorcollege geven"	Erasmus MC	March 2016	8 hours
Workshop: "Tentamenvragen maken"	Erasmus MC	April 2016	8 hours
Teaching video analysis	Erasmus MC	Oct 2016	8 hours
Yoga Foundation	Saktiisha	July-Oct 2015	50 hours
Ashtanga Yoga intensive teacher course	Delight Yoga	April 2016	50 hours
Yoga Teacher Training Module I	Balanzs	Oct-Dec 2016	100 hours
Yoga Teacher Training Module II	Balanzs	Feb-May 2017	100 hours

Certificates / courses	Location	Date	Workload
Deel-certificate BKO	Erasmus MC	2015	48 hours
Scientific integrity	Erasmus MC	2015	8 hours
10-week writing course: English biomedical writing and communication	Erasmus MC	2015	84 hours
Mentoring Programme MGZ workshop	Erasmus MC	2016	4 hours
RYT-200 Registered Yoga Teacher Yoga Alliance	Balanzs, The Hague	2017	200 hours
Introduction to Geographic Information System (QGIS)	Erasmus MC	2017	4 hours
QGIS and infectious disease analyses	Institute of Tropical Medicine, Antwerp	2017	40 hours

Other activities	Location	Date	Workload
President of Promeras, representing all 1,000 PhD-candidates at Erasmus MC	Erasmus MC	2014 -2016	80 hours
Representative Junior (JVO) of the infectious disease section	Erasmus MC	2014 - 2017	120 hours
Section discussion meeting organizer	Erasmus MC	2014 -current	120 hours

Memberships	Date
American Sociaty of Tropical Medicine and Hygiene (ASTMH)	2013 -2017
British Society of Parasitology (BSP)	2014 - 2015
Nederlandse Vereniging van Tropische Geneeskunde (NVTG)	2014 -2017
Nederlandse Vereniging van Parasitologie (NVP)	2015-2017