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Modelling canine leishmaniasis spread to non-endemic areas of Europe

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SUMMARY

Expansion of sandflies and increasing pet travel have raised concerns about canine leishmaniasis (CanL) spread to new areas of Europe. This study aimed to estimate the probability of CanL introduction and persistence following movements of infected dogs. Stochastic modelling was used to estimate the probabilities of (1) CanL infection during travels or imports of infected dogs (P_{inf} and P_{infCA}, respectively), (2) CanL persistence in a dog network with sandflies after introduction of an infected dog (P_{per}), and (3) persistence in a CanL-free region ($P_{per\ region}$) for N dogs moving between endemic and free regions. Different mitigation measures (MMs) were assessed. P_{inf} [7·8%, 95% predictive interval (PI) 2·6–16·4] and P_{per} (72·0%, 95% PI 67·8–76·0) were reduced by use of repellent, vaccine, prophylactic medication, and insecticide, in decreasing order of effectiveness. Testing and exclusion of positive dogs was most effective in reducing $P_{per\ region}$ for a small N. The spread of CanL to CanL-free areas with sandflies is thus likely, but can be reduced by MMs.

Key words: Canine leishmaniasis, diagnostic test, insecticide, mitigation, prophylactic medication, repellent, stochastic model, vaccination, vector-borne disease.

INTRODUCTION

Canine leishmaniasis (CanL) is a zoonotic parasitical infection of dogs caused by *Leishmania* ssp. and transmitted by infected phlebotomine sandflies [1]. In dogs, CanL causes chronic infection that may progress to a clinical stage and can be fatal if untreated [1]. The infection can be transmitted to humans causing visceral or cutaneous leishmaniasis, and it is the second most important protozoan infection after malaria [2].

In Europe, CanL is endemic in the Mediterranean basin but has been reported more frequently in northern latitudes of Europe where sandflies were previously thought to be absent or present only in very low densities [3]. In CanL-free areas of Europe, seropositive dogs have been associated with imported dogs or dogs returning from endemic areas [3–5].

Clinical trials have been used to evaluate the individual effect of mitigation measures (MMs) to control CanL infection [6–8]. In a simulation model, Dye [9] also evaluated the relative reduction in CanL incidence by varying the (unknown) efficacy of different MMs.

Compartmental deterministic models have helped in understanding CanL transmission in dogs [9–11] and evaluating MMs in endemic areas [1, 9]. Nonetheless, stochastic models can more realistically model disease transmission and persistence in small populations such as contact networks of dogs, where the effect of randomness largely influences the variability of the results

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[12]. Stochastic models also facilitate the modelling of uncertainty in key parameters.

The objectives of this study were: (1) to estimate the probability of CanL persistence in a previously CanL-free area with competent vectors following the introduction of infected dogs and (2) to evaluate the effectiveness of repellent use, vaccination, prophylactic medication, insecticide use, or test and exclusion of infected animals in reducing the probability of introduction and persistence of CanL in a free area.

METHODS

The overall probability of CanL persistence in a previously CanL-free area was estimated using a model with three main simulation steps. In the first step, two disease introduction pathways were considered: imports of infected dogs from endemic areas (P_{infCA}), and infection of dogs during travels to endemic areas with their owners (P_{inf}). In the second step, CanL transmission in a previously free area in the presence of sandflies was simulated to estimate the probability of persistence (P_{per}) in a hypothetical independent contact network of dogs (Fig. 1). Finally, the joint uncertainty distributions for P_{per}, P_{inf} and P_{infCA}, were used in a third step to simulate the probability of persistence in at least one independent contact network in a previously CanL-free region with sandflies ($P_{\text{per region}}$).

P_{inf} and P_{per} were both modelled using an individual-based stochastic model of CanL transmission in a contact network of dogs. A contact network of dogs represented a group of dogs that interact and may be able to transmit CanL to each other through sandflies (e.g. dogs in a neighbourhood, or a dog park [12]). The infection state of dogs in the contact network was followed-up individually for the duration of the simulation. Dogs could be:

Susceptible (S): uninfected with *Leishmania* but susceptible to infection;

Latent (L): exposed but not yet infectious (unable to transmit the infection to other S dogs);

Infectious Sub-Clinical (I): able to transmit *Leishmania* to S dogs but not showing clinical signs of CanL;

Infectious Clinical (C): able to transmit *Leishmania* and with clinical signs of CanL;

Resistant (R): exposed to *Leishmania* and having developed an immune response (via effective vaccination or curative treatment) preventing them from becoming infectious.

It was assumed that there are no naturally resistant dogs. C dogs were more likely to be diagnosed and treated against CanL than I dogs, and were thus modelled separately. Dogs becoming R remained in that state for the duration of the simulation [1, 13]. A dog's probability of death was age-dependent. A proportion of dogs were replaced with S dogs after a period of time, creating new S introductions into the contact network (see detailed description of model in the Supplemental material).

Transmission from infectious to susceptible dogs occurred via sandfly bites, and was modelled using vectorial capacity (VC), representing the number of secondary cases resulting from an infectious case in time t in a fully susceptible population [9]:

$$\text{VC} = m\alpha^2 \text{e}^{-\mu l}/\mu, \quad (1)$$

where m is the number of female sandflies per dog, α is the number of female sandfly bites per day, μ is the daily mortality rate of female sandflies, and l is the latency period of *Leishmania* in sandflies ($l = 1/\tau$, the transition rate from latent to infectious, assuming an exponentially distributed latent period in the sandfly). VC was assumed to be independent of the prevalence in the sandfly population [14]. Sandflies were assumed to be present in CanL-free areas for 90 days per year, during which time transmission could thus occur.

A two-dimensional modelling approach [15] was used to report the uncertainty in P_{inf} and P_{per} in the form of 95% predictive intervals (95% PI). The model was implemented in MS Excel 2010, using the Monte Carlo simulation @RISK 6·0 add-in (Palisade Corporation, USA).

Probability of introducing an infected dog into a non-endemic area (P_{infCA} and P_{inf})

Commercial dog movement records were unavailable, so it was assumed that all dogs from endemic areas were equally likely to be imported. Thus, P_{infCA} corresponds to the true prevalence of CanL infection in endemic areas, which was estimated using a Bayesian latent class model [16] based on the IFAT seroprevalence estimates obtained from three cross-sectional studies [17–19].

P_{inf} was estimated using the individual-based stochastic model previously described (see Supplementary material) with short simulation periods representing households travels to endemic areas. Up to five dogs travelling per household were simulated. As the number

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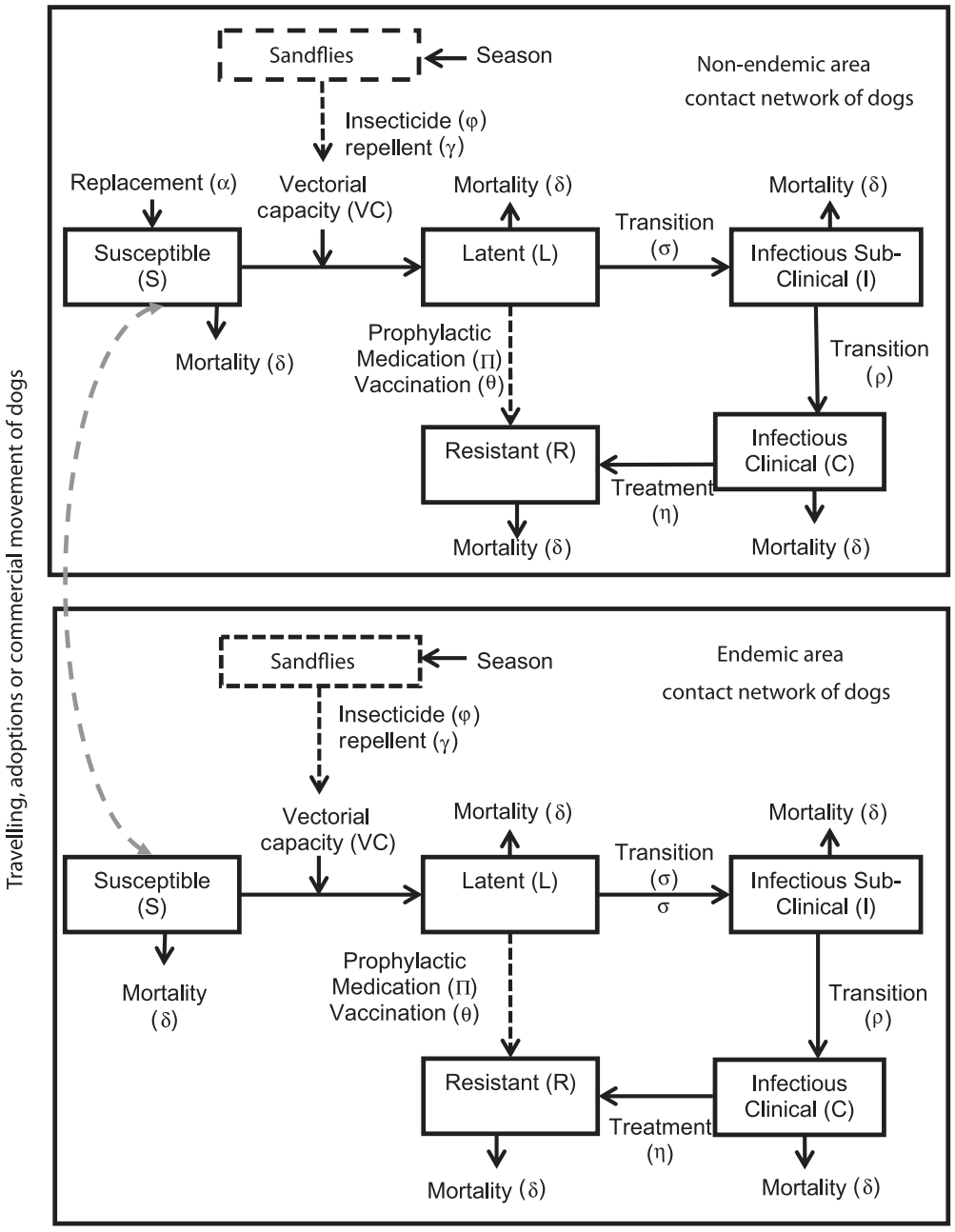

Fig. 1. Schematic representation of the model of canine leishmaniasis transmission and movement of dogs between endemic and CanL-free areas of Europe. Solid black boxes represent infection states of the dog population. Solid arrows indicate transitions between infection stages. Dashed black boxes represent the sandfly population. Dashed black arrows represent mitigation measures. Grey dashed arrow represents movement of dogs between endemic and non-endemic areas of Europe.

of travelling dogs did not affect the results and conclusions, only results for one dog per household are reported here. Given CanL's long incubation period, it was assumed that the number of infected dogs in the endemic area remained constant for the duration of the household trip, so CanL infection and progression was only simulated for the travelling dog. The dog's infection state was evaluated daily for the duration of the trip, and recorded at the end of the simulation period. A transmission season of 150 consecutive days per year represented the period when sandflies were present in endemic areas and transmission occurred [20, 21]. P_{inf} was calculated as the proportion of iterations where the travelling dog became infected.

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Probability of persistence in a previously CanL-free area with competent vector (P_{per})

The individual-based stochastic model was also used to estimate P_{per}. In this case, the spread of CanL was modelled within an independent contact network of dogs with seasonal presence of sandflies for 90 consecutive days. Simulations started with the introduction of an infected dog in the contact network, and CanL transmission was simulated for 3 years. Given the slow progression of the disease, the model was simulated in weekly steps and the daily parameters for transmission and VC (Table 1) were converted to weekly parameters. CanL infection was considered persistent in the network if $\geqslant 1$ L, I or C dogs (other than the initially introduced dog) remained at the end of the simulation period. P_{per} was calculated as the proportion of iterations that led to persistence of CanL within the contact network.

Probability of persistence within a region ($P_{per\ region}$)

$P_{per\ region}$ was estimated using equation (2):

$$P_{per\ region} = 1 - \left(1 - P_{per}\right)^{N_{inf}}, \quad (2)$$

where N_{inf} is the number of infected dogs introduced into the CanL-free areas given by P_{inf} or P_{infCA} (depending on whether the dogs were travelling to endemic areas or imported from endemic areas, respectively).

It was assumed that a proportion of the total number of dogs introduced into CanL-free areas (following commercial imports, adoptions, individual purchases from endemic areas, or travels to endemic areas) were infected with CanL, and that a proportion of those infected may transmit the infection to other dogs in their contact network and generate persistence in the previously CanL-free area.

Mitigation measures

Vaccination, prophylactic medication, repellent use, insecticide use, and diagnostic test and exclusion were modelled alone or in combination. Test and exclusion was only considered for $P_{per\ region}$, whereas the others affected P_{inf} and P_{per}. The effect of MMs was modelled using the product of their level of use and their efficacy. The level of use represented the proportion of dogs treated with MMs and the efficacy represented the proportion of dogs on which it had its intended effect – with the exception of insecticide use, as discussed later. Efficacies were estimated from published data (Table 1), while different levels of use were tested via scenario analysis.

Vaccination and prophylactic medication prevented infection and therefore transitioned dogs from the S to the R state (Fig. 1). When used on S dog(s), repellents reduced the transmission of CanL by decreasing the sandfly biting rate α [equation (1)] proportionally to their efficacy, thus reducing VC. When used on I or C dog(s), repellents reduced the number of I and C dogs in the network proportionally to its efficacy (Fig. 1).

Unlike other MMs, insecticides were applied to the environment and therefore affected the entire contact network rather than individual dogs. Thus, the level of insecticide use corresponded to the proportion of contact networks on which insecticides were used, and insecticide efficacy corresponded to the proportional reduction of the sandfly density within the contact network where applied.

Dogs imported from endemic areas were tested for infection and positive dogs were denied entry (excluded). Test and exclusion use was the proportion of imported dogs that were tested, whereas efficacy was the sensitivity (Se) of the diagnostic test (i.e. probability that an infected animal is positive by the test).

It was assumed that when used, MMs were applied regularly following instructions/recommendations and thus remained efficacious during the simulation period. The effectiveness of MMs was measured in terms of the proportional reduction in the mean P_{inf} and P_{per}.

Scenario analysis

For P_{inf} and P_{per}, the effectiveness of MMs was evaluated in 20% increments of levels of use (from 0% to 100%). The combined effect of pairs of MMs was implemented for high (80%) and medium to low (40%) levels of use. Levels of use and efficacies were assumed to be independent between MMs. The following combinations were assessed: vaccination and repellent, vaccination and insecticide, repellent and prophylactic medication, repellent and insecticide, and insecticide and prophylactic medication. Vaccination and prophylactic medication were not combined as they are both aimed at inducing resistance: they were modelled using the same mechanism and the difference in their effectiveness depends on efficacy only, and it was assumed they are unlikely to be used in combination on a dog.

For $P_{per\ region}$, test and exclusion was individually evaluated when used in 0%, 50% and 100% of the dogs moved to CanL-free areas, and also combined with repellent and vaccination (at 0% and 80% use in dogs).

$P_{per\ region}$ was calculated for 10, 100 and 5000 dogs travelling to or imported from endemic areas. The

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Table 1. *List of parameters used to model the transmission of canine leishmaniasis in a non-endemic area following the introduction of an infected dog with competent vector and the transmission of canine leishmaniasis to susceptible dogs traveling to endemic areas*

Description	Parameter [ref.]	Probability distribution	Parameter distribution	Descriptive statistics
Transmission parameters				
Time to transition from Latent to Infectious (days)	σ [21]	Weibull	Shape: 1·34 Scale: 396·95	Mean: 364·5 s.d.: 274·8
Time to transition from Infectious Sub-Clinical to Infectious Clinical (days)	ρ [21]	Weibull	Shape: 4·3 Scale: 233	Mean: 212·1 s.d.: 55·7
Vectorial capacity				
Number of female sandflies per dog (n)	m [23]	Gamma	Shape: 866 Scale: 0·0019	Mean:1·6 s.d.: 0·06
Number of female sandfly bites per day (n)	α [24]	Normal	μ: 2·03 σ: 0·29	—
Transition rate from latent to infectious sandflies (n/day)	τ [24]	Normal	μ: 6·5 σ: 0·53	—
Daily mortality rate of female sandflies (n/day)	μ [24]	Empirical samples with equal weight	21 days 42·5 days	n.a.
Characteristics of environment				
Prevalence of infectious dogs in endemic areas (%)	[25–27]	Mixture of two equally weighted betas	Beta (alpha: 30, beta: 1048), Beta (alpha: 20, beta: 400)	Mean: 0·03 s.d.: 0·005 Mean: 0·05 s.d.: 0·01
Prevalence of CanL-infected dogs in endemic areas (%)	[17–19]	Posterior Bayesian estimation of true prevalence	n.a.	n.a.
Day of the year travelling		Uniform	Min: 0 max: 365	Assumes that travel can happen any day with equal chance
Travelling days	[22]	Empirical (histogram frequencies)	1–3 days: 20·7% 4–7 days: 38·1% 8–14 days: 6·3% 15–28 days: 11·1% 29–91 days: 3·6% 95–365 days: 0·2%	n.a.
Mitigation measures				
Vaccine use (%)	θ (Scenario analysis)	Fixed	n.a.	n.a.
Vaccine efficacy (%)	[7]	1 – Log-normal	μ: −2·43 σ: 1·04, truncated max:1	—
Repellent use (%)	γ (Scenario analysis)	Fixed	n.a.	

Table 1 (*cont.*)

Description	Parameter [ref.]	Probability distribution	Parameter distribution	Descriptive statistics
Repellent efficacy (%)	[6]	1 – Log-normal	μ: −3·14 σ: 0·33	—
Prophylactic medication use (%)	π (Scenario analysis)	Fixed	n.a.	n.a.
Prophylactic medication efficacy (%)	[28]	1 – Log-normal	μ: −1·44, σ: 0·45, truncated: max: 1	—
Insecticide use (%)	ϕ (Scenario analysis)	Fixed	n.a.	n.a.
Insecticide efficacy (%)	[8]	1 – Normal	μ: 0·41, σ: 0·079	—
Diagnostic test sensitivity (%)	[29]	Beta	Alpha: 10 Beta: 9	Mean: 0·53 s.d.: 0·11
Treatment efficacy (%)	η [30, 31]	Mixture of two equally weighted betas	Beta (alpha: 12 beta: 8) Beta (alpha: 33, beta: 10)	Mean: 0·6 s.d.: 0·11 Mean: 0·77 s.d.: 0·06
Dog's life				
Mortality rate (n/days)	δ [32]	Weibull	Shape: 42·47 Scale: 4468·8	Mean: 4410·5 s.d.: 131·0
Age (days)	[25]	Log-normal	μ: 1898 σ: 1241	—
Time to replacement (days)	[33]	Pert	Min: 3 Max: 3650 Mode: 120	—
Probability of replacement (%)	κ [33]	Beta	Alpha: 52 Beta: 53	Mean: 0·5 s.d.: 0·05
Transmission period				
Endemic area Non-endemic area	[20, 21]		150 days 90 days	n.a.

n.a., Non-available.

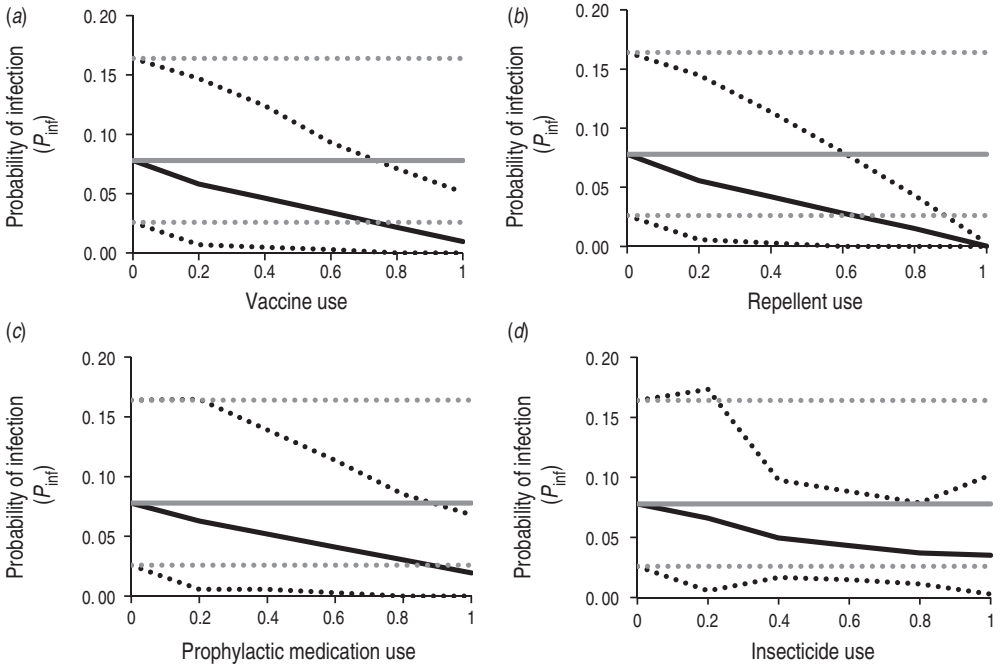

Fig. 2. Probability of infection [mean (solid line) and 95% predictive interval (dotted line)] of a dog after a trip to a CanL endemic area (P_{inf}), by proportions of use of mitigation measures (black lines) compared to no use of mitigation measures (grey lines). (*a*) Vaccine use, (*b*) repellent use, (*c*) prophylactic medication and (*d*) insecticide use.

number of dogs needed to reach a $P_{per\ region}$ of 100% was also calculated.

Sensitivity analysis

A sensitivity analysis using the conditional effect of the 2·5th and 97·5th percentiles of input parameters on the mean outputs (P_{inf} and P_{per}) was reported.

Validation

Two cohort studies were used to validate P_{inf} estimation. In these studies, a cohort of CanL-free dogs was introduced into an endemic area in southern Italy [19] and southern France [18], and dogs were physically examined and sampled every 1–3 months to evaluate the clinical signs of CanL and positivity to tests (PCR and IFAT). The incidence rate ratio (IRR) was used to compare P_{inf} to the results of these field studies. As the information available on CanL outbreaks or the presence of the vector in non-endemic areas is scarce, it was not possible to validate P_{per} estimates.

RESULTS

In the absence of MMs, the mean probability that a dog travelling to endemic areas became infected, P_{inf}, was 7·8% (95% PI 2·6–16·4), and the mean probability of importing an infected dog from endemic areas, $P_{infCA,}$ was 10·7% [95% credibility interval (CrI) 1·9–22·4]. Vaccinating 100% of travelling dogs decreased P_{inf} by 88·5%. By contrast, low levels of vaccine use (i.e. 20%) only reduced P_{inf} by 25·6% (Fig. 2*a*). Similarly, repellents used in all travelling dogs reduced P_{inf} by 99·6% while P_{inf} decreased by only 28·2% when used in 20% of travelling dogs (Fig. 2*b*). When prophylactic medication was used in 100%, 80%, and 20% of dogs, P_{inf} decreased by 75·6%, 61·5% and 19·2%, respectively (Fig. 2*c*). P_{inf} decreased by 55·1% when insecticide was used in all endemic areas (Fig. 2*d*). All combinations of MMs with high level of use (80%) reduced P_{inf} to $\leqslant$2·5%. The most effective was the combination of vaccination and repellent, which reduced P_{inf} by 94·9%, compared to a reduction of 78·2% and 80·7%, respectively, when used alone. At 40% use, the combinations of vaccination and repellent, and repellent and prophylactic medication were the most effective, reducing P_{inf} by 45·6% and 47·7% compared to individual use of 40% for vaccine and prophylactic medication, respectively.

The mean number of secondary cases a week after the index case became infectious was 0·32 (range 0–3) with seasonality (Fig. 3*a*) and 1·32 (range 0–5) without seasonality (i.e. uninterrupted transmission,

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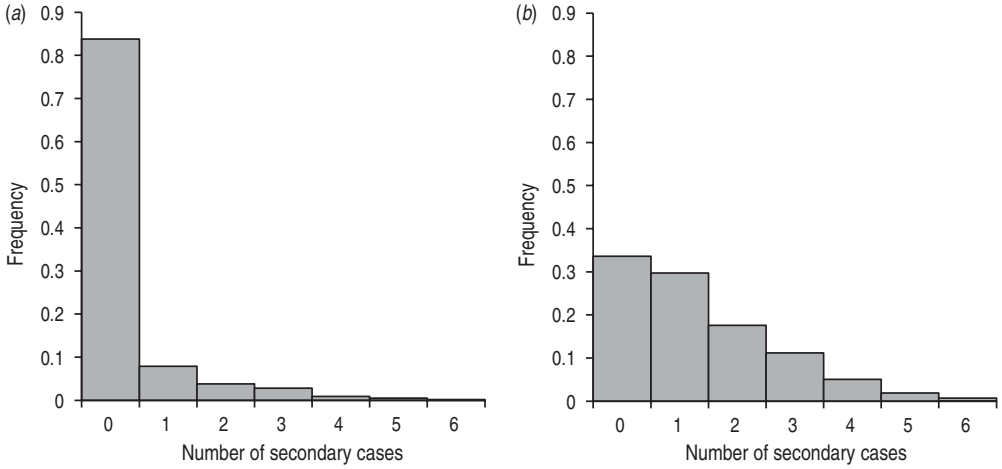

Fig. 3. Distribution of the number of secondary of canine leishmaniasis cases at the end of the first week after the introduction of an infectious dog in a non-endemic area with competent vectors when (*a*) seasonality was implemented, and (*b*) no seasonality was included.

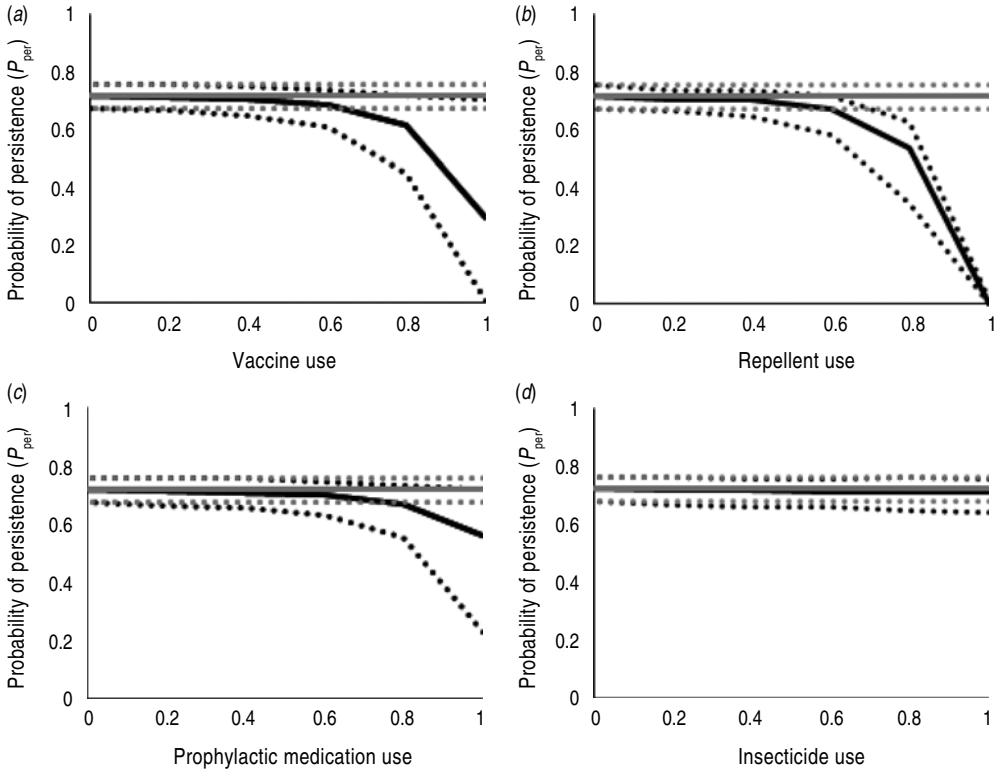

Fig. 4. Probability of persistency (P_{per}) [mean (solid line), 95% PI (dotted line)] following the introduction of a CanL-infected dog into a non-endemic area with competent vector, by proportions of use of mitigation measures (black lines) compared to no use of mitigation measures (grey lines). (*a*) Vaccine use, (*b*) repellent use, (*c*) Prophylactic medication and (*d*) insecticide use.

Fig. 3*b*). When no MMs were used, the mean P_{per} following the introduction of an infectious dog was 72·0% (95% PI 67·8–76·0). When vaccination, repellent, and prophylactic medication were used on all dogs, and insecticide in the environment of all contact networks, P_{per} decreased by 58·3%, 99·6%, 21·7% and 1·8%, respectively (Fig. 4). When repellent was used in combination with vaccination, prophylactic

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Table 2. *Effect of different levels of testing and exclusion and mitigation measures on the probability of CanL persistence in at least one network of a non-endemic region ($P_{per\ pegion}$), for different numbers of dogs travelling to endemic areas*

Mitigation measure	Test and exclusion level (%)	Number of dog travelling to endemic areas		
		10	100	5000
None	0	40·2% (0–0·98·1)	97·5% (70·3–100)	100% (100–100)
None	50	32·9% (0–95·2)	93·8% (0–100)	100% (100–100)
None	100	20·1% (0–92·9)	86·8% (0–100)	100% (100–100)
Vaccination at 80%	0	10·7% (0–71·8)	56·6% (0–100)	95·3% (0–100)
Vaccination at 80%	50	7·1% (0–66·0)	47·7% (0–99·5)	95·3% (0–100)
Vaccination at 80%	100	4·6% (0–58·3)	35·4% (0–99·8)	95·1% (0–100)
Repellent at 80%	0	9·0% (0–74·3)	53·7% (0–100)	97·0% (0–100)
Repellent at 80%	50	6·1% (0–72·4)	45·1% (0–99·4)	97·0% (0–100)
Repellent at 80%	100	3·8% (0–68·6)	32·3% (0–96·9)	96·8% (0–100)
Repellent and vaccination at 80%	0	1·1% (0–21)	9·4% (0–59·8)	77·5% (0–100)
Repellent and vaccination at 80%	50	0·6% (0–16·4)	7·0% (0–50·4)	74·6% (0–100)
Repellent and vaccination at 80%	100	0·4% (0–0)	4·4% (0–40·7)	68·0% (0–100)

medication or insecticide at 80% usage each, P_{per} decreased by 76·9%, 67·1% and 36·1%, respectively, compared to no MMs. P_{per} also decreased when use of insecticide was combined with vaccination (22·2%) or prophylactic medication (12·5%). Combining MMs at 40% usage further reduced P_{per} by only 2–7% compared to using each MM separately.

In the absence of control measures, high $P_{inf,}$ P_{infCA} and P_{per} resulted in a high $P_{per\ region}$. A $P_{per\ region}$ of 100% was reached for 170, 240 and 350 dogs returning from travel to an endemic area when 0%, 50% and 100% of the dogs were tested and excluded if positive, respectively. The effectiveness of test and exclusion in reducing $P_{per\ region}$ was high when a very small numbers of dogs (e.g. 10) were returning from endemic areas, but quickly decreased and was almost zero for large numbers of travelling dogs (e.g. 5000, Table 2). The use of both repellent and vaccination in 80% of travelling dogs, in addition to test and exclusion of infected dogs on 100% of dogs returning to CanL-free areas decreased $P_{per\ region}$ by 98·0%, 94·9% and 32·0% for 10, 100 and 5000 travelling dogs, respectively, compared to test and exclusion only (Table 2).

Twenty, 30 and 80 dogs imported (commercial imports, adoptions or individual purchases) from endemic areas resulted in $P_{per\ region}$ of 100% when 0%, 50% and 100% of the animals were tested and excluded if positive, respectively (Table 3). The reduction of $P_{per\ region}$ from test and exclusion quickly diminished as N increased, and was almost zero for large numbers of dogs (e.g. 5000) moved into CanL-free areas (Table 3).

When testing and exclusion of positives dogs was used on 0%, 50% and 100% of imported dogs in combination with repellent use in 80% of dogs in contact networks in non-endemic areas, $P_{per\ region}$ reached 100% when 270, 350 and 600 dogs were imported from endemic areas, respectively. Similarly, when testing and exclusion of positive dogs was used on 0%, 50% and 100% of imported dogs in combination with vaccination of 80% of dogs in contact networks

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Table 3. *Effect of different levels of testing and exclusion and mitigation measures on the probability of CanL persistence in at least one network of a non-endemic region ($P_{per\ region}$), for different numbers of dogs imported from endemic areas (commercial imports, adoptions, individual purchases)*

Mitigation measure	Test and exclusion level (%)	Number of dogs imported or adopted from endemic areas		
		10	100	5000
None	0	92·2% (0–100)	100% (100–100)	100% (100–100)
None	50	86·5% (0–100)	100% (100–100)	100% (100–100)
None	100	28·8% (0–97·5)	89·0% (0–100)	100% (100–100)
Vaccination at 80%	0	45·3% (0–96·4)	94·6% (46·8–100)	100% (100–100)
Vaccination at 80%	50	36·2% (0–94·6)	91·8% (0–100)	100% (100–100)
Vaccination at 80%	100	23·8% (0–89·7)	84·5% (0–100)	100% (100–100)
Repellent at 80%	0	51·2% (0–99·0)	96·1% (59·0–100)	99·9% (100–100)
Repellent at 80%	50	41·7% (0–98·0)	94·1% (0–100)	100% (100–100)
Repellent at 80%	100	27·7% (0–95·1)	88·2% (0–100)	100% (100–100)
Repellent and vaccination at 80%	0	26·7% (0–83·2)	84·0% (17·0–100)	100% (100–100)
Repellent and vaccination at 80%	50	20·7% (0–76·2)	77·8% (0–100)	100% (100–100)
Repellent and vaccination at 80%	100	13·3% (0–64·2)	65·5% (0–100)	100% (100–100)

in non-endemic areas, $P_{per\ region}$ reached 100% for 320, 450 and 710 imported dogs from endemic areas, respectively (Table 3). When testing and exclusion of positive dogs was used on 0%, 50% and 100% of imported dogs in addition to the combined use of vaccination and repellent at 80% each, a $P_{per\ region}$ of 100% was reached for 770, 1000 and 1700 imported dogs from endemic areas, respectively (Table 3).

P_{inf} was most influenced by the day of the year travelling (transmission season), followed by the number of travelling days and CanL prevalence in the endemic area (Fig. 5*a*). The most influential parameter for P_{per} was the day of the year when the index case dog became infectious (transmission season), followed by the efficacy of MMs, VC and infection transition parameters (Fig. 5*b*).

After adjusting for the time of exposure and transmission period, the IRRs predicted by the P_{inf} module were comparable to those from the cohort studies used for validation [20, 21]. Oliva *et al.* [21] reported an incidence rate of 0·036 cases/dog per week, which is not significantly different to the 0·051 cases/dog per week incidence estimated by the model (IRR 0·71, 95% CrI 0·38–1·29). Simolarly, Dye *et al.* [20] reported an incidence of 0·028 cases/dog per week, which was not significantly different from the 0·025 cases/dog per week predicted by the model (IRR 1·1, 95% CrI 0·68–1·79).

DISCUSSION

The scenarios evaluated suggest that the probability of introducing CanL-infected dogs to CanL-free regions can be high and, furthermore, the probability that these dogs will transmit the infection within their contact networks can also be high. These probabilities may seem high at first inspection but are plausible as dogs exhibit a prolonged infectious period which is often sub-clinical, allowing them to spread the disease for a very long time period [1, 9] before they are identified as infected. Nonetheless, several MMs can reduce these probabilities.

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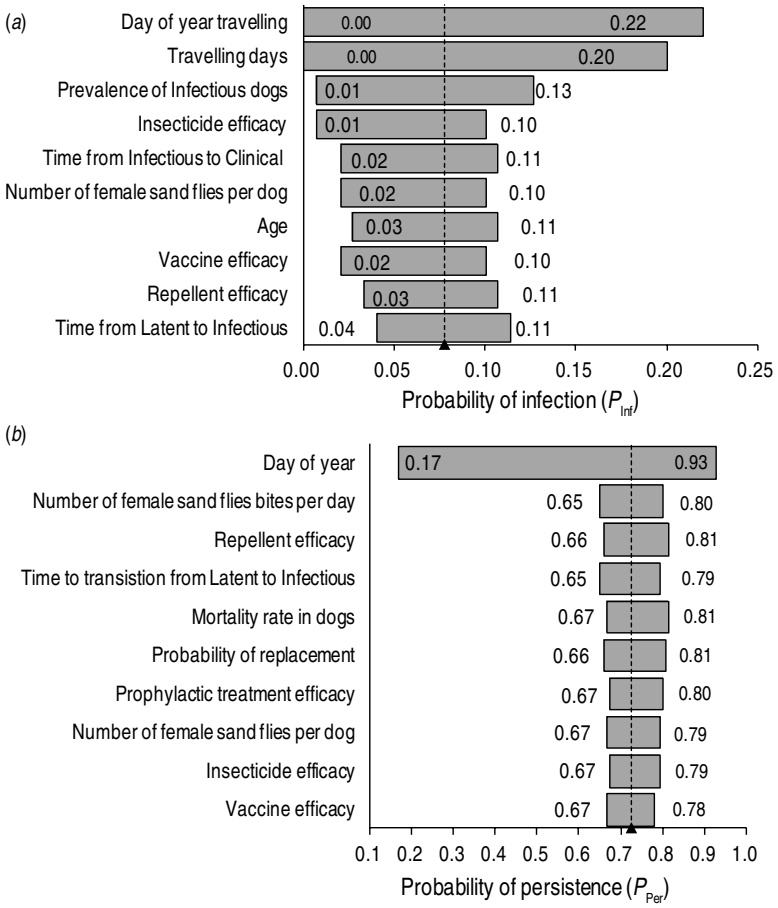

Fig. 5. Sensitivity analysis using the conditional effect of the 2·5th and 97·5th percentiles of input parameters on the (*a*) mean probability of infection of a dog after a trip to a CanL-endemic area (P_{inf}) and (*b*) mean probability of persistency following the introduction of a CanL-infected dog into a non-endemic area with competent vector (P_{per}).

Several key assumptions were made for this modelling work, one of the most important being that dogs become resistant after effective treatment. Although it is possible that some clinical dogs return to the clinical stage 6–24 months after treatment [1, 13], it was assumed that dogs with a history of clinical infection would be monitored on a regular basis for seroconversion and clinical signs, and therefore treated as needed within a short period [1]. P_{per} may have been underestimated if R dogs can transition back to I after treatment or after losing vaccine immunity.

Information on the number of networks, size of networks, and movement of dogs between endemic and non-endemic areas within Europe was unavailable. Therefore, the estimation of the probability of persistence in a region ($P_{per\ region}$) relied on simplified assumptions such as independence between networks, which may not reflect reality. It was also assumed that dogs moved between networks independently (i.e. any contact network has the same chance of 'receiving' one or more infected imported dog). $P_{per\ region}$ may have been overestimated if this assumption is unwarranted. Nonetheless, these assumptions can be easily relaxed to estimate $P_{per\ region}$ as more detailed information becomes available.

VC represents the potential of sandflies to transmit leishmaniasis between dogs and it is independent of the prevalence of *L. infantum* infection in sandflies [14]. VC was a parsimonious choice used to avoid modelling the transmission of *L. infantum* between sandflies, which occurs on a faster time-scale than in dogs, and because few sandflies live long enough to acquire infection [9]. The density of vectors depends on environmental factors such as temperature and humidity which are highly variable throughout the year. Given the lack of information on such heterogeneous parameters, the density of sandflies within the contact network was assumed to be constant throughout the breeding season; however, the length of the breeding season (transmission period) was assumed to be 150

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days in endemic areas and 90 days in non-endemic areas, to reflect shorter summers of northern non-endemic areas of Europe. This assumption may have overestimated the initial size of the outbreak because VC may take a few weeks to reach stability at the start of the short breeding season in non-endemic areas. By contrast, by assuming a single introduction of an infected dog in a network instead of a continuous inflow of dogs, P_{per} may have been underestimated. As P_{per} is measured as the probability of at least one new infected dog at the end of the simulated 3-year period, it is unlikely that the above assumption affected this parameter, nor the relative effectiveness of the different MMs.

In the present study, dogs travelling to endemic areas were exposed to sandflies only for relatively short travel periods [22]. However, after adjusting for the exposure period, the model-predicted incidence agreed with that from field studies where cohorts of CanL-free dogs were introduced into endemic areas in southern Italy [21] and southern France [20], and their infection status measured at the end of the sandfly breeding season, suggesting that the model predictions were valid against two independent datasets not used to estimate model parameters.

When no MMs were implemented, both P_{inf} and P_{per} were high (means of 7·8% and 72·0%, respectively) resulting in a $P_{per\ region}$ of 100% even for small numbers ($n = 20$) of dogs moved to a CanL-free region with competent vectors. CanL-infected dogs exhibit a prolonged infectious period (median ~200 days, [9]) which is often sub-clinical, allowing them to silently spread the disease for extended time periods, especially since no culling of infectious dogs was considered in the model. Moreover, $P_{per\ region}$ reports the probability of persistence of CanL in at least one dog in at least one contact network in a region, therefore this parameter does not readily relate to the actual disease prevalence in the region when CanL is introduced, and this prevalence may range from extremely low to extremely high.

The most effective MM in reducing P_{inf} and P_{per} was repellent either used individually or in combination with other MMs, followed in decreasing order of effectiveness by vaccine, prophylactic medication and insecticide. Although the ranking of MMs was similar for P_{inf} and P_{per}, the differences between MMs were more important for P_{per} than P_{inf}. P_{inf} was modelled to estimate the probability that one susceptible dog, protected with a certain MM and travelling to an endemic area, will become infected. By contrast, P_{per}, was modelled to estimate the probability that one infected dog introduced in a network of susceptible dogs, all protected with a certain MM, will lead to a persistent infection of at least one of these dogs in the network. The larger efficacies of the MMs observed when P_{per} was modelled may be explained by the longer modelling time for P_{per} than P_{inf}. For each time step, there was a probability that a susceptible dog becomes infected, which was dependent on the effectiveness of the MMs applied to the susceptible dogs, thus the longer the modelling period, the larger the differences in the overall probability that the travelling dog becomes infected. Furthermore, for P_{inf}, the MMs were applied to only one dog, with the exception of insecticide use, while for P_{per} the MMs were applied to all dogs in the network, thus the efficacy of the MMs was amplified for P_{per}.

The main mechanism of action of repellent use is to avoid sandfly bites, which reduces the VC by several-fold. Vaccination and prophylactic medication both reduce CanL transmission by inducing resistance and the slight difference in their effectiveness is due to their different efficacies. Insecticide use was shown to be the least effective MM in reducing P_{inf} and P_{per}. By contrast, in an earlier CanL modelling article, Dye [9] concluded that insecticide use was more effective than vaccination. However, Dye [9] modelled the disease in an endemic area, and the effect of the insecticide as a percentage of change in the mortality rate of sandflies, whereas in the present study P_{per} was estimated in a previously CanL-free area and insecticide use was modelled as decreasing the vector density proportionally to its efficacy [8].

Test and exclusion of positive dogs moving into non-endemic areas was effective for low numbers of dogs, but its benefits diminished as the number of dogs moved to CanL-free areas increased. The main drivers of these results were the high P_{per}, the relatively high prevalence of CanL in endemic areas (P_{infCA} range 2·0–22·6%), and the low sensitivity (52·6%, 95% CrI 30·8–74·0) of the diagnostic test used to model the test and exclusion policy. Furthermore, it was assumed that the dogs imported were a random sample from the dog population in the endemic area. However, CanL prevalence in commercial dogs (P_{infCA}) may be lower than that of the general population since they may be younger animals and commercial breeding facilities may regularly use vaccination or repellent, or may also house dogs indoors, further reducing the exposure to sandflies, in

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which case the results of the import pathway may be overestimated and more comparable to those of the travelling pathway with MMs implemented. Tests with higher sensitivity would be more effective at reducing $P_{\text{per region}}$, although as N increases some infected animals will inevitably go undetected and enter the non-endemic region.

The day of year when the trip to an endemic area was made, followed by the travelling length were the greatest drivers of P_{inf}. The first parameter was assumed to randomly occur at any time of the year, although travelling to endemic areas may be concentrated during certain periods of the year. The transmission of CanL depends on the presence of the vector and is thus seasonal [21]. In addition, longer trips increase the time of exposure to infected vectors.

The most influential parameter for P_{per} was the time of the year when the index case dog became infectious. Naturally, if no vectors were present at that time, transmission was delayed until the next vector breeding season started, and could be avoided altogether if the index case dog was removed or died.

In conclusion, the model results suggest that the introduction of infected dogs in previously CanL-free regions with competent sandflies can result in a high probability of CanL persistence in the absence of MMs. The best mitigation options evaluated were: prevention of infection during travel (i.e. using repellent, vaccination, or prophylactic medication), and test and exclusion of infected dogs coming from endemic areas. The testing and exclusion policy had a high impact only for small numbers of dogs moved between endemic and free areas. The most effective measures to reduce the probability of CanL infection during travel to endemic areas and CanL persistence in a network of dogs was the use of repellent, followed closely by vaccination and prophylactic medication.

SUPPLEMENTARY MATERIAL

For supplementary material accompanying this paper visit http://dx.doi.org/10.1017/S0950268814002726.

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DECLARATION OF INTEREST

None.

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