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Alternative transmission pathways for guinea worm in dogs: implications for outbreak risk and control

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ABSTRACT

Guinea worm (*Dracunculus medinensis*) has exerted a high human health burden in parts of Africa. Complete eradication of Guinea worm disease (dracunculiasis) may be delayed by the circulation of the parasite in domestic dogs. As with humans, dogs acquire the parasite by directly ingesting infected copepods, and recent evidence suggests that consuming frogs that ingested infected copepods as tadpoles may be a viable transmission route (paratenic route). To understand the relative contributions of direct and paratenic transmission routes, we developed a mathematical model that describes transmission of Guinea worm between dogs, copepods and frogs. We explored how the parasite basic reproductive number (R_0) depends on parameters amenable to actionable interventions under three scenarios: frogs/tadpoles do not consume copepods; tadpoles consume copepods but frogs do not contribute to transmission; and frogs are paratenic hosts. We found a non-monotonic relationship between the number of dogs and R_0. Generally, frogs can contribute to disease control by removing infected copepods from the waterbody even when paratenic transmission can occur. However, paratenic transmission could play an important role in maintaining the parasite when direct transmission is reduced by interventions focused on reducing copepod ingestion by dogs. Together, these suggest that the most effective intervention strategies may be those which focus on the reduction of copepods, as this reduces outbreak potential irrespective of the importance of the paratenic route.

1. Introduction

Many infectious diseases are caused by parasites with complex life cycles that ultimately infect their definitive host, in which they reproduce, following development in one or more species (Poulin, 2011). Some parasites with complex life cycles additionally use paratenic hosts that harbour a parasite, although parasite development does not occur. Utilising this transmission route can potentially increase parasite genetic diversity, as well as promoting transmission via geographic dispersal and trophic transmission (Antonovics et al., 2017). Overall, the plurality of transmission opportunities is potentially adaptive, increasing parasite survival and the probability of completing its life cycle (Auld and Tinsley, 2015; Parker et al., 2015; Antonovics et al., 2017). While alternative transmission routes are recognised for many parasites, our understanding of their relative importance in emergence and spread is incomplete. Further, the existence of alternate transmission pathways presents challenges to any control efforts, since targeting only one pathway will not block transmission entirely. Outbreak control may not be optimally targeted to the most important transmission routes and removal of some transmission options may even be accompanied by parasite life-history adjustments to make better use of remaining options (Poulin, 2003). Consequently, quantifying the relative contributions of alternative transmission pathways is of central importance in predicting and managing the spread of multi-host parasites.

Guinea worm (*Dracunculus medinensis*) is a nematode parasite that has caused widespread human suffering over centuries,

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especially in particular regions of Africa (Hopkins et al., 2017). Since human exposure typically occurs through drinking water containing *Dracunculus*-infected copepods, eradication efforts have largely focused on providing filtration and clean water for consumption, and education on parasite biology and how to limit the risk of infection (Hopkins et al., 2017). Overall, these intervention strategies have brought Guinea worm close to eradication, reducing the number of human cases by approximately 99% from highs of 3.5 million in 1986 (Hopkins et al., 2017) to 27 cases in 2020 (WHO Collaborating Center for Research Training and Eradication of Dracunculiasis, 2021). However, complete eradication of the parasite has been complicated by the incidence of Guinea worm infections in non-human animals with domestic dogs as the most frequently infected (Eberhard et al., 2014; Molyneux and Sankara, 2017).

Similar to human hosts, dogs are a definitive host for the parasite (Eberhard et al., 2014). As in humans, it has been assumed that the plausible infection route for dogs is ingestion of copepods infected with Guinea worm larvae when visiting water bodies. However, tadpoles that ingest infected copepods can maintain Guinea worm L3s in their tissues and even through metamorphosis, and can then cause infection in definitive dog hosts when dogs consume parasite-infected adult frogs; in other words, frogs can act as paratenic hosts for the parasite (Eberhard et al., 2014, 2016; Cleveland et al., 2019; Garrett et al., 2020). Since most efforts to reduce dog exposure to Guinea worm have focused on reducing larval releases into the waterbody (e.g. tethering of dogs to remove access to water bodies), there is concern that paratenic transmission via consumption of infected adult frogs could compromise eradication efforts.

Transmission via paratenic hosts has been observed in other trematode and nematode parasites, e.g. *Alaria americana* and *Skrjabingylus nasicola* (Fernandes et al., 1976; Weber and Mermod, 1985); however, the relative importance of the paratenic transmission route compared with transmission from intermediate hosts is generally lacking (Médoc et al., 2011). Moreover, since paratenic hosts remove the parasite from the environment and thus could reduce their direct ingestion by definitive hosts, they could increase or decrease overall transmission efficiency depending on the relative probabilities of encounter and infection. Mathematical models provide useful tools for assessment of the contributions of multiple transmission pathways to outbreak risk, pathogen persistence and control. Past modelling studies of zoonotic pathogens have demonstrated that alternative transmission pathways influence the size and duration of avian influenza outbreaks (Rohani et al., 2009), can 'repair' chains of direct transmission of Ebola virus broken by interventions reducing direct transmission events (Vinson et al., 2016), and that ignoring these pathways can result in underestimates of the basic reproductive number of waterborne diseases including cholera and giardiasis (Tien and Earn, 2010).

Past modelling studies of infectious diseases in humans or dogs, including Guinea worm, have attempted to estimate the basic reproductive number (R_0), integrated seasonal dynamics using an agent-based modelling approach, and developed network models to target domestic dogs for interventions (Ghosh et al., 2018; Wilson-Aggarwal et al., 2019; Perini et al., 2020). The results of these studies support the tethering of dogs, isolation of infected individuals, and the treatment of contaminated water sources to reduce Guinea worm transmission (Ghosh et al., 2018; Perini et al., 2020). However, failure to assess the contributions of paratenic transmission could impede the development of intervention and control strategies.

To investigate the role of the paratenic hosts in transmission of Guinea worm to domestic dogs, we developed a model tracking Guinea worm infection in copepods, frogs and dogs at a local (village level) scale at which dogs plausibly utilise water bodies for drinking. The model contrasts three scenarios for the role of frogs in transmission: (i) 'no frogs', frogs are removed as a disease-control intervention or frogs play no role as predators or hosts; (ii) frogs act as predators that consume copepods but do not contribute to onward transmission of the parasite; and (iii) frogs act as both predators and hosts that contribute to parasite transmission. We parameterized the model, guided by literature and expert opinion on the Guinea worm system in Chad (Ghosh et al., 2018; Cleveland et al., 2019; Garrett et al., 2020). To determine how paratenic hosts influence parasite emergence potential, we calculated the parasite basic reproduction number (R_0), a quantity that integrates abundance and interactions of all host types to quantify how many new infections will arise following a complete parasite life cycle (Joseph et al., 2013; Mihaljevic et al., 2014; O'Regan et al., 2015). We co-varied parameters corresponding to key quantities amenable to manipulation by public health interventions, to determine their net effect on R_0. We interpreted our findings in the context of how the efficacy of intervention strategies targeting different actors in the system (copepods, frogs or dogs) may be affected by paratenic transmission.

2. Materials and methods

2.1. Model formulation

2.1.1. Demography and predation

We modelled local infection dynamics (i.e. at the scale of a village) as a system of coupled ordinary differential equations, where each equation described the number of hosts of each species (copepods, tadpoles, frogs and dogs, denoted by respective subscripts *C,T,F*, and *D*) by their infection status (susceptible, exposed or infectious, denoted *S*, *E* and *I* respectively; Eqs. 1–3; Fig. 1). For simplicity, we assumed susceptible individuals of

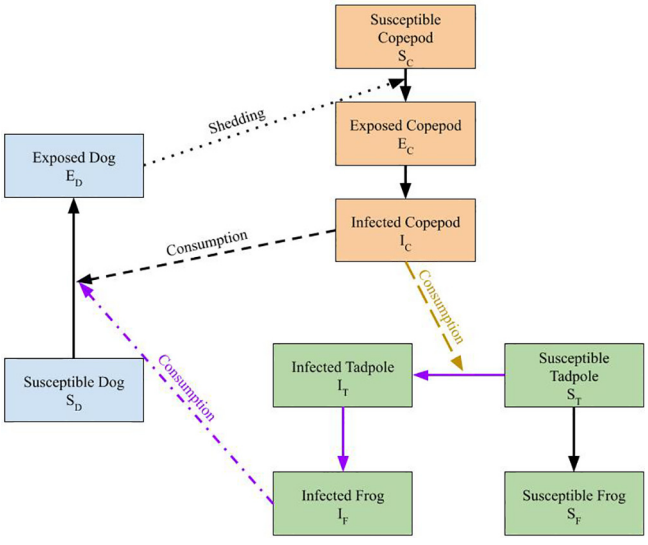

Fig. 1. The compartmental model for local Guinea worm transmission. Solid arrows represent the movement of individuals from one compartment to another, which could be due to a change in infection status or a change in life stage (i.e. metamorphosis of tadpoles into frogs). Dashed arrows represent consumption of individuals, while dotted arrows represent the release of L1s which leads to the infection of copepods. Dash-dot arrows represent mechanisms that are turned off in the model when frogs only act as predators ("no frog-dog trans."). Long-dash arrows represent the mechanism that is turned off when there are no frogs in the system ("no frogs").

species i (=C,T,D) recruited to the population at rate λ_i and experienced a natural per capita mortality rate μ_i; additionally, tadpoles developed into frogs at per capita rate ψ_T that we assumed was not affected by tadpole infection status. Predation rates were described by simple mass-action terms. Copepods experience predation by dogs at per capita rate $d_D V_D N_D$, where $d_D$represents the average number of visits made by a dog to a water body per day, V_D represents the dog-copepod encounter rate per visit, and N_D is the total number of dogs. Copepod predation by tadpoles occurs at per capita rate $a_T N_T$, and frog predation by dogs at per capita rate $B_{DF} N_D$.

2.1.2. Parasite lifecycle

After initial ingestion by dogs, parasite larvae must develop into adult worms and mate, which takes an average of $\frac{1}{\psi_D}$ days, after which adult female worms release larvae into the water source in an instantaneous event. Each larval release event results in a total of N_{shed} copepods acquiring larvae, and infected copepods experience a higher mortality rate (μ_{IC})compared to their uninfected counterparts. After $\frac{1}{\psi_C}$ days in copepods, *Dracunculus* develop into a third larval stage, at which point copepods ingested by dogs and tadpoles cause infection with respective probabilities p_{TC} and p_{DC}. Frogs infected as tadpoles and subsequently predated by dogs cause infection with probability p_{DF}.

Copepods (first intermediate host)

$$\frac{dS_C}{dt} = \lambda_C - \mu_{SC} S_C - N_{shed} \psi_D E_D - d_D V_D N_D S_C - a_T N_T S_C \quad (1a)$$

$$\frac{dE_C}{dt} = N_{shed} \psi_D E_D - \mu_{IC} E_C - \psi_C E_C - d_D V_D N_D E_C - a_T N_T E_C \quad (1b)$$

$$\frac{dI_C}{dt} = \psi_C E_C - \mu_{IC} I_C - d_D V_D N_D I_C - a_T N_T I_C \quad (1c)$$

Tadpoles and frogs (predator and paratenic host)

$$\frac{dS_T}{dt} = \lambda_F - \mu_{ST} S_T - \psi_T S_T - p_{TC} a_T S_T I_C \quad (2a)$$

$$\frac{dI_T}{dt} = p_{TC} a_T S_T I_C - \psi_T S_T - \mu_{IT} I_T \quad (2b)$$

$$\frac{dS_F}{dt} = \psi_T S_T - \mu_{SF} S_F - B_{DF} N_D S_F \quad (2c)$$

$$\frac{dI_F}{dt} = \psi_T I_T - \mu_{IF} I_F - B_{DF} N_D I_F \quad (2d)$$

Dogs (definitive host)

$$\frac{dS_D}{dt} = \lambda_D - \mu_D S_D + \psi_D E_D - p_{DF} B_{DF} S_D I_F - p_{DC} d_D V_D S_D I_C \quad (3a)$$

$$\frac{dE_D}{dt} = p_{DF} B_{DF} S_D I_F + p_{DC} d_D V_D S_D I_C - \psi_D E_D \quad (3b)$$

2.2. Model analysis

2.2.1. Quantifying outbreak potential

We calculated the parasite basic reproduction number, R_0, defined as the number of secondary cases in the definitive host resulting from a single index case introduced to a wholly susceptible population; values of $R_0 > 1$ thus represent conditions in which sustained transmission is likely to occur. We calculated R_0 by analytically or numerically, solving for the dominant eigenvalue of the next-generation matrix (Dobson, 2004; Keeling and Rohani, 2008; O'Regan et al., 2015) evaluated at the disease-free equilibrium of Equations 1–3:

$$R_0 = \begin{bmatrix} 0 & \frac{N_{shed}\psi_D}{\psi_C+\mu_{IC}+a_T K_T+d_D V_D K_D} & \frac{N_{shed}\psi_D\psi_C}{(\mu_{IC}+a_T K_T+d_D V_D K_D)(\psi_C+\mu_{IC}+a_T K_T+d_D V_D K_D)} & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 \\ \frac{p_{DC} d_D V_D K_D}{\psi_D+\mu_D} & 0 & 0 & \frac{p_{FC} a_T K_T}{\psi_T+\mu_{IT}} & \frac{p_{FC} a_T K_T \psi_T}{(\psi_T+\mu_{IT})(B_{DF} K_D+\mu_{IF})} \\ 0 & 0 & 0 & 0 & 0 \\ \frac{p_{DF} B_{DF} K_D}{\psi_D+\mu_D} & 0 & 0 & 0 & 0 \end{bmatrix}_{\lambda_{max}} \quad (4)$$

2.2.2. Transmission scenarios

We derived R_0 based on three scenarios describing how frogs influence transmission processes. The first assumed that frogs are absent (or, equivalently, that copepod consumption by tadpoles is negligible) so that frogs play no role in transmission ("no frogs"). The second assumed tadpoles predate copepods but do not contribute to onward transmission ("no frog-dog transmission"). This was achieved by setting the probability of successful transmission from frogs to dogs to zero ($p_{DF} = 0$). The third model assumed that dog consumption of frogs is a viable transmission route ("full").

2.2.3. Parameterization and sensitivity analysis

Parameter values and ranges found in Table 1 were taken from published peer-reviewed articles or from Guinea worm expert suggestions (Cleveland et al., 2019; Garrett et al., 2020). Where this was not possible, we used expert opinion to set plausible ranges and subjected these to sensitivity analysis.

We performed extensive sensitivity analyses to understand which combinations of parameters maximise outbreak potential for the three transmission scenarios. In order to obtain a baseline for understanding how variation in dog population size among villages and water visitation behaviour across seasons influences transmission risk, we first derived an analytical expression for R_0 in the absence of frogs. Next, for each transmission scenario we explored how four parameters that represent actionable interventions affect R_0: (i) the number of dogs that visit the waterbody (K_D), which could be modified by the culling of dogs or chaining them to prevent waterbody visitation and consumption of frogs; (ii) the daily visitation rate of dogs to the waterbody (d_D), which could be modified by providing alternative water sources; (iii) the number of copepods infected per larval release event (N_{shed}), which could be affected by treating the waterbody with an insecticide; and (iv) the number of tadpoles in the waterbody (K_T); which could be modified by direct removal of adult frogs and, in effect, tadpoles.

Finally, we conducted a global sensitivity analysis by simultaneously co-varying model parameters across their plausible ranges and computing R_0 for each transmission scenario. We sampled 10,000 parameter combinations using Latin Hypercube sampling from the parameter distributions found in Table 1. The net effect of each parameter on R_0 was visualised with a trendline generated using a general additive model in the "stat_smooth" function in R (V 3.6.1).

Table 1
The parameters used in the model described by Eqs. (1)–(3).

Parameter	Meaning	Range	Mean [S.D.]	Sampling Distribution	Source
λ_i	Birth rate of host i	–	–	–	–
$\frac{1}{\mu_D}$	Dog lifespan (days)	1095–2555	1825 [365]	normal	Guagliardo et al., 2021
$\frac{1}{\psi_D}$	Dog incubation period (days)	300–420	360 [22.5]	normal	Onabamiro 1956
p_{DF}	Probability of successful transmission from frogs to dogs	0–1	0.5 [0.1]	normal	
B_{DF}	Dog consumption rate of frogs	0.0002–0.0442	0.02 [0.01]	normal	
p_{DC}	Probability of successful transmission from copepods to dogs	0–1	0.1 [3]	log-normal	
d_D	Number of visits to water source (per day)	0–20	15 [3]	normal	
V_D	Copepod encounter rate by dogs	10^{-4}-0.05	−12 [3] (0.0015)[b]	log-normal	
λ_F	Birth rate of tadpoles by adult frogs	–	–	–	
$\frac{1}{\mu_{ST}}$	Susceptible tadpole lifespan (days)	–	–	–	
$\frac{1}{\mu_{IT}}$	Infected tadpole lifespan (days)	42–63	52.5 [3.5]	normal	
$\frac{1}{\psi_T}$	Metamorphosis period of tadpoles (dogs)	70–50	62 [5]	normal	(Babošová et al., 2018)
p_{TC}	Probability of successful transmission from copepods to tadpoles	0–1	0.5 [0.1]	normal	
a_T	Consumption rate of copepods by tadpoles	0–2	0.05 [0.5]	normal	
$\frac{1}{\mu_{SF}}$	Susceptible frog lifespan	–		–	–
$\frac{1}{\mu_{IF}}$	Infected frog lifespan	1825–5475	3650 [730]	normal	
λ_C	Birth rate of susceptible copepods	–	–	–	–
$\frac{1}{\mu_{SC}}$	Susceptible copepod lifespan	–	–	–	–
N_{shed}	Number of susceptible copepods that become infected during adult worm larval release events	0–60,000	25,000 [10,000]	normal	
$\frac{1}{\psi_C}$	Incubation period of copepods	20–14	17 [1]	normal	(Bimi, 2007; Muller, 1972)
$\frac{1}{\mu_{IC}}$	Infected copepod lifespan	1–75	60 [10]	normal	(Hopp et al., 1997)
K_D	Number of dogs in village	0–500	0.77206 [0.02347] [a]	neg. binomial	
K_T	Number of tadpoles in water source	0–200	100 [25]	normal	

[a] The size and probability of the negative binomial distribution.
[b] The value was set for Figs. 1 and 2, which is the mean of 10,000 simulations.

2.3. Data accessibility

R code used to generate Figs. 2–4 is provided online as electronic supplementary material at https://doi.org/10.6084/m9.figshare.c.4846551.v1.

3. Results

3.1. Effects of dog numbers and waterbody visitation on outbreak potential (no frogs)

We analytically obtained the expression for R_0 in the "no frogs" transmission scenario as the dominant eigenvalue of a system of equations (1) and (3), when $N_T = N_F = 0$:

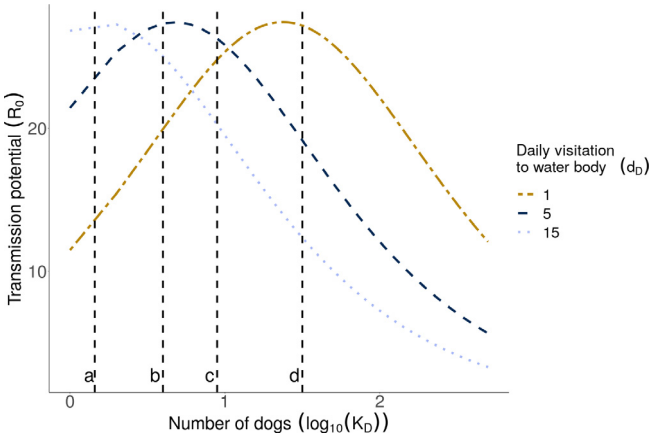

Fig. 2. The reproductive number of Guinea worm (R_0) as a function of the number of dogs in a village (K_D) and the daily dog visitation frequency to the village water body (d_D; line colour). Results are shown for the transmission scenario when frogs are absent. Each dashed line (a-d) represents cases where rank orders of R_0 values in relation to the dog visitation rate change with increasing dog population size. All other parameters in the model are held at the mean values found in Table 1.

$$R_0 = \sqrt{\frac{\psi_C}{\psi_C + \mu_{IC} + d_D V_D K_D} * \frac{1}{\mu_{IC} + d_D V_D K_D} * N_{shed} * \frac{\psi_D}{\psi_D + \mu_D} * p_{DC} d_D V_D K_D} \quad (5)$$

In this expression, parameters corresponding to dog population size (K_D) and per capita water body visitation rate (d_D) appear in both the numerator and denominator, resulting in non-monotonic relationships between outbreak risk, dog population size and water-seeking behaviour (Fig. 2). Outbreak risk (R_0) tends to increase with dog population size and visitation rates when these quantities are low, reflecting increased encounters between susceptible dogs and *Dracunculus*-infected copepods. However, at relatively high dog population sizes or visitation rates, the relationship between R_0 and these quantities reverses due to rapid depletion of infected copepods (because they are consumed before the parasite completes the extrinsic incubation period, or through wasted transmission events where too many infected copepods are ingested by a small number of dogs).

3.2. Effects of key control parameters across transmission scenarios

We next characterised the effect of four key parameters on R_0 (dog population size and visitation rate, the number of infected copepods produced by a larval release event, and the number of tadpoles in the waterbody); these parameters influence the outbreak potential for at least two of the three transmission scenarios, have reasonably well estimated ranges, and have been (or could be) manipulated by public health interventions.

The effects of dog population size (K_D, Fig. 3A) and visitation rate (d_D, Fig. 3B) on R_0 vary quantitatively and qualitatively across the three transmission scenarios. The presence of frogs always reduces transmission potential relative to the frog-free model, due to the increased overall predation risk of copepods before they become infectious. When frogs act as predators but not paratenic hosts, frogs can shift the relationship between R_0 and K_d (or d_d) from mostly negative (in the absence of frogs) to mostly positive, since more dogs (or more dog visits) increases the chance that

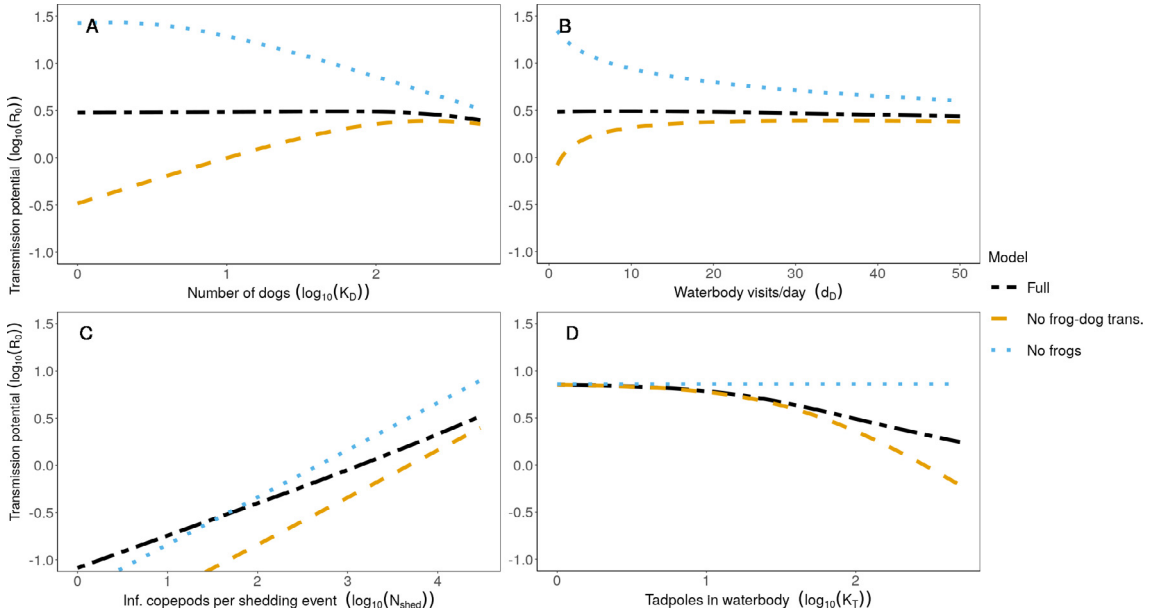

Fig. 3. The reproductive number of Guinea worm (R_0) as a function of the number of dogs (K_D; A), the daily visitation rate to the waterbody by dogs (d_D; B), the number of infected copepods resulting from one larval event (N_{shed}; C), and the number of tadpoles in the waterbody (K_T; D). Across the panels, patterns represent each transmission scenario: two-dash line represents the model where frogs are predators and paratenic hosts ("full" model); dashed line represents frogs acting as only predators and not hosts ("no dog-frog transmission"), and dotted line represents the model where frogs are neither predators nor hosts ("no frogs). All model parameters are held at the mean values found in Table 1. Inf., infected.

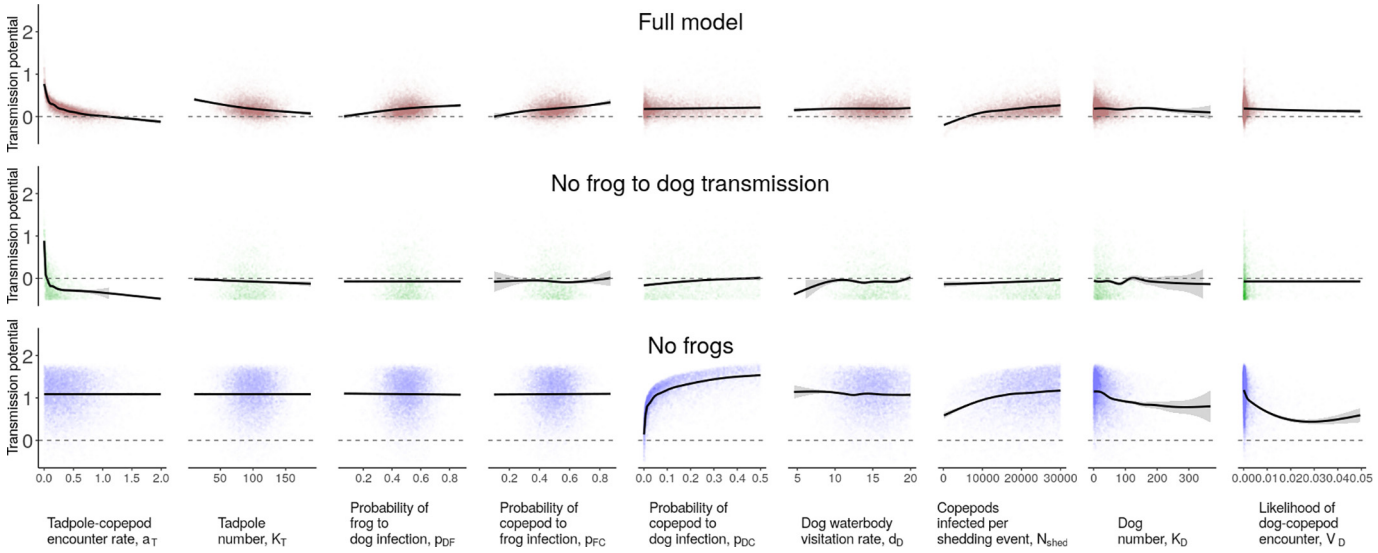

Fig. 4. Global sensitivity of the reproductive number of Guinea worm (R_0) to key model parameters (columns) and different transmission scenarios (no frogs, tadpoles predate copepods with no onward transmission, frogs act as paratenic hosts). Within each plot, a coloured dot represents one parameter combination, and the black line illustrates the trend in the relationship between R_0 and the focal parameter, averaged over all parameter combinations. The trend line was generated using the "stat_smooth" function in R (V 3.6.1).

infected copepods will be ingested by the definitive host than removed by a non-competent predator. When frog-to-dog transmission occurs, frogs can reduce the overall sensitivity of R_0 to dog numbers and visitation rates: when direct dog-copepod encounters are rare (low K_D and/or d_D), frog-dog transmission buffers the negative effects of tadpole consumption of copepods on direct transmission; when dog-copepod encounters are frequent, the maintenance of parasites in frogs reduces dog–dog 'competition' for infected copepods.

Across all models, increasing the number of copepods infected per larval release event (N_{shed}) always increases R_0 (Fig. 3C). The sensitivity of R_0 to N_{shed} is similar when frogs are absent or act as predators only, with frogs reducing transmission potential for any given value of N_{shed}. When frogs are paratenic hosts, paratenic transmission increases transmission potential relative to the absence of frogs at low values of N_{shed} (because dog-frog encounter is more likely than dog-copepod encounter), but reduces transmission potential at high values of N_{shed} (when paratenic transmission is less efficient than direct copepod-dog transmission). When tadpoles are present in waterbodies, R_0 decreases with increasing tadpole density (K_T) because predation of copepods by tadpoles always reduces the efficiency of direct copepod-dog transmission (Fig. 3D); paratenic transmission reduces the sensitivity of R_0 to K_T relative to when tadpoles act as copepod predators only.

3.3. Global sensitivity analysis

We quantified the relationships between individual parameters and R_0 when all model parameters are simultaneously varied (Fig. 4). Overall, these simulations confirmed that (i) outbreak potential is generally highest in the absence of frogs; (ii) outbreaks are highly unlikely if tadpoles act only as copepod predators; (iii) sustained transmission may be possible when frogs act as paratenic hosts; (iv) paratenic transmission tends to dampen the sensitivity of R_0 to parameters relevant to control. Together, these suggest that reducing tadpole densities via the removal of adult frogs would likely increase the overall outbreak risk in areas where tadpoles are ecologically significant copepod predators, but that reducing dog consumption of adult frogs could reduce outbreak risk.

When frogs are both predators and hosts (Fig. 4 "full model") the number of tadpoles (Fig. 4 K_T) and the tadpole-copepod encounter rate (Fig. 4 a_T) have a negative relationship with R_0. This is due to tadpoles ingesting many copepods, reducing the number of direct dog-copepod infection events that can occur. When frogs act only as predators in the system ("no frog to dog transmission"), these parameters (K_T and a_T) lead to a decrease in R_0 as frogs are dead-end hosts that consume copepods limiting dog-copepod transmission events. When frogs are not present in the system ("no frogs"), these parameters have no effect on R_0 (as they are set to zero). Across all three models, increasing the number of dogs (Fig. 4 K_D) results in a decrease in R_0, particularly in already high populations. Similarly, increasing the likelihood of a dog-copepod encounter (Fig. 4 V_D) leads to a decrease in R_0. This is due to competition between dogs for infected copepods. When the dog-copepod encounter likelihood (Fig. 4 V_D) increases, then already infected dogs are consuming infectious copepods, reducing the potential for transmission events to susceptible dogs.

Increasing the probability of infection via the paratenic or direct routes (Fig. 4 p_{DF}, p_{FC}, and p_{DC}) always leads to an increase in R_0. This trend is most strongly seen with the probability of copepod-to-dog infection (Fig. 4 p_{DC}) when frogs are absent. Increasing the number of infected copepods per larval release event (Fig. 4 N_{shed}) results in an increase in R_0 across all models because more infected copepods in the water body reduces dog–dog and tadpole-dog competition for copepods. Not included in Fig. 4, there was little effect of consumption of frogs by dogs (B_{DF}) on R_0 across all model assumptions.

4. Discussion

Many pathogens have multiple transmission modes, and understanding the interactions between the transmission routes may be key to predicting and preventing outbreaks. Here we derived expressions for the basic reproduction number for Guinea worm transmission in dogs, incorporating the potential for paratenic transmission via frogs in addition to the direct dog-to-copepod transmission route. R_0 took a range of values, but the highest values occurred when dog populations are large and waterbody visitations are high, which would occur when dogs have unrestricted

roaming. We found that adding paratenic hosts into transmission models reduced overall transmission potential compared with models including only the direct transmission route. Further, paratenic transmission had the potential to flip the relationship between definitive host density and R_0 from a positive to negative, depending on whether paratenic hosts acted primarily as diluters (by removing infectious stages from the environment) or prolonged the lifespan of the parasite outside of the definitive host. In the Guinea worm system, this suggests that the success of interventions targeted at dogs or frogs is highly dependent on the role that frog and tadpole populations play in parasite transmission, and more generally suggests that disease control strategies targeted at a single host could have unintended consequences when transmission routes interact.

The non-linear relationship between dog numbers, behaviour and outbreak risk has implications for predicting both transmission potential across villages with different dog population sizes and mobilities and seasons, and for the effectiveness of intervention strategies. For example, R_0 is maximised at different dog population sizes depending on the frequency of dog visits to water bodies. If visitation rates are low, the chances that most infected copepods are ingested prior to becoming infectious (or by relatively few dogs) is low, meaning that R_0 increases with dog population size, thus suggesting that efforts to reduce dog population size could reduce transmission. However, when visitation rates are relatively high (e.g. because most dogs are free-roaming, or during hot or dry conditions when water availability is scarce), R_0 decreases with dog population size, meaning that efforts to reduce dog population size would likely increase transmission risk.

Contrary to studies which demonstrate that multiple transmission routes can increase parasite transmission and prevalence (Rohani et al., 2009; Majewska et al., 2019), we found that adding a paratenic host typically reduces transmission potential, especially when the paratenic host is an efficient predator of infectious copepods and when ingestion of paratenic hosts by the definitive host rarely results in transmission. In this case, the presence of frogs dilutes the encounter rate of dogs and copepods, similar to 'friendly competition' among *Daphnia* where fungal spores are depleted by non-susceptible species (Hall et al., 2009). If the efficiency of paratenic transmission is relatively high, the benefits of encounter-dilution are eroded (Buck and Lutterschmidt, 2017). Further, when infected copepods are scarce, paratenic hosts could be important for maintenance of the parasite, by allowing the parasite to persist for longer outside of definitive hosts. These findings underscore the importance of incorporating ecological interactions in multi-host parasite systems, especially when these result in antagonistic interactions between transmission modes.

Our study adds to the growing body of theory demonstrating that the relationship between (definitive) host density and transmission potential is not uniformly positive and may depend on the ecological or environmental context. Previous research has shown, for example, that for generalist vector-borne parasites, increases in host community size can at first impede transmission by reducing the vector-to-host ratio but ultimately augment transmission as competent host species join the community assembly (Vinson and Park, 2019). Research on host culling to control directly transmitted parasites has demonstrated that reduction in host density can promote transmission if timing of the cull removes predominantly recovered, immune individuals followed by demographic introduction of susceptible recruits (Choisy and Rohani, 2006). In the Guinea worm system these drivers can be seasonal (variation in water body visitation) or ecological (competition for parasites among definitive hosts, or between definitive and paratenic hosts).

The influence of paratenic hosts on parasite transmission also extends to control efforts in the Guinea worm system. Reductions in the number of dogs may not reduce transmission, especially when frogs are absent, as the non-monotonic relationship between dog population size and R_0 means that culling or confining dogs to households in high dog population areas may increase overall transmission, while reductions in the smaller sized populations may still offer effective control measures. Effective interventions include those aimed at reducing copepod populations via the larvicidal treatment of water bodies; however, this is contingent on the frog population being unaffected by these treatments (Rwakimari et al., 2006; Junges et al., 2017; Ghosh et al., 2018). Chemical control of copepods that simultaneously causes high tadpole mortality could compromise control efforts if tadpoles primarily remove infected copepods; this is worth further empirical study.

Potentially, combining restraining dogs and reducing copepod population abundance may provide a better outcome for parasite elimination. Further, the presence of frogs as predators consistently leads to the lowest values of R_0, as frogs consumed the infectious copepods but were dead-end hosts leading to lower direct copepod-dog transmission opportunities. Schistosome transmission has been observed to be negatively impacted by predation effects from prawns, which reduced the intermediate host abundance and lifespan (snails), and pointing towards a potential biocontrol strategy (Sokolow et al., 2015). Further, Gonzalez Engelhard et al. (2021) recently investigated fish as a paratenic route in Guinea worm transmission, suggesting that this route is plausible. To reduce transmission, they propose that dogs should not be provided with feeding opportunities of fish (i.e. discarded fish entrails) together with a method of control of the copepod population (Gonzalez Engelhard et al., 2021). In the Guinea worm system, accounting for frog populations not only improves understanding of natural variation in transmission across regions but could be developed into an active control strategy.

However, should there be an intervention that completely stops the direct consumption pathway between copepods and dogs, there may be some sustained transmission with paratenic hosts. For example, providing drinking water for dogs in households may reduce instances of dogs drinking from natural water bodies, but they may still consume frogs. Frog-to-dog transmission events may repair broken chains of direct copepod to dog transmission. Buffering of one transmission route by another has been noted for other pathogens, such as ebolaviruses, where models have demonstrated that an alternative sexual transmission pathway is able to repair broken chains of direct transmission (Vinson et al., 2016). For avian influenza viruses, environmental transmission is thought to help local outbreaks begin and extend their duration, even when direct transmission is the primary mechanism (Rohani et al., 2009). While not captured explicitly in our model, it should be noted that if copepods are distributed heterogeneously in the water body, for example due to localised larval recruitment and limited water circulation, the extent to which dogs drink water where larvae are present or absent could modify the strength of these effects. Therefore, the role of paratenic hosts must be judged carefully when thinking holistically about the Guinea worm system.

While the model presented demonstrates nuanced effects of alternative transmission pathways on parasite transmission, there are other factors that are likely to be important in understanding local and regional patterns of Guinea worm incidence. For example, during the rainy seasons, flooding events can connect local populations, allowing parasite transmission at broad spatial scales (Sreenivasan et al., 2017), and paratenic hosts. We have demonstrated the relative effects that paratenic hosts can have on the transmission of a complex life-cycle parasite. These effects are underscored by the ecological and epidemiological roles that the paratenic host populations have in the host community and, therefore, a full understanding of these roles is crucial in the

implementation of effective interventions. With our current understanding of paratenic hosts for Guinea worm, we propose that the most effective and feasible interventions should aim to reduce copepod populations.

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