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Overline: Epidemiology

One sentence summary: A citywide dog vaccination effort in Chad reduced the local spread of rabies from dogs to humans.

Abstract

Despite the existence of effective rabies vaccines for dogs, dog transmitted human rabies persists in Africa. Two consecutive dog vaccination campaigns in Chad in 2012 (dog vaccination coverage: 72%) and 2013 (coverage: 70%) interrupted rabies transmission for nine months in N'Djaména, the capital city. We developed a deterministic model of dog-human rabies transmission fitted to weekly incidence data of rabid dogs and exposed human cases in N'Djaména. Our analysis showed that the effective reproductive number, that is, the number of new dogs infected by a rabid dog fell to below one through November 2014. The modeled incidence of human rabies exposure fell to less than one person per million people per year. A phylodynamic estimation of the effective reproductive number from 29 canine rabies virus genetic sequences of the viral N-protein confirmed the results of the deterministic transmission model, implying that rabies transmission was interrupted after the vaccination campaign. However, new dog rabies cases appeared earlier than the transmission and phylodynamic models predicted. This may have been due to the continuous movement of rabies-exposed dogs into N'Djaména from outside the city. Our results show that canine rabies transmission to humans can be interrupted in an African city with currently available dog rabies vaccines, provided the vaccination area includes larger adjacent regions and local communities are informed and engaged.

Introduction

Dog rabies has been eliminated in large parts of the industrialized countries in Europe and North America. In the last few decades, a concerted effort by South and Central American countries has reduced dog rabies transmission close to elimination (*1*). Despite the existence of effective vaccines for dogs, dog transmitted human rabies persists and has even re-emerged in Asia and Africa where still more than 59,000 people die annually from this preventable disease. The largest part of the burden is borne by India followed by Africa, China and South East Asian countries (*2*). Because of rabies' low propensity to transmit secondary infections beyond a bitten individual, it appears feasible to eliminate dog-mediated human rabies through the mass vaccination of dogs (*3, 4*). However, reaching this goal in partnership with the World Health Organization (WHO), the Food and Agriculture Organization of the United Nations (FAO), the World Organization for Animal Health (OIE) and the Global Alliance for Rabies Control (GARC, www.rabiesalliance.org) requires a rigorous scientific approach (*5*).

Reaching sufficient coverage to interrupt dog rabies virus transmission and prevent re-introduction requires an in-depth understanding of dog ecology, dog-human interactions, and the social and cultural determinants of vaccine acceptability, as well as the effective deployment of vaccines with a highly sensitive surveillance system (*4, 6-9*). It requires scientists to closely collaborate with authorities and communities as partners in a transdisciplinary way between human and animal health (*11, 12*). Concomitant mathematical and economic frameworks can yield new insights into fundamental properties of pathogen transmission (*13*) and comparative

cost-effectiveness (*14*) but do not explain sufficiently how this effectiveness can be achieved (*15*).

In 2003, a smallscale study showed the feasibility of dog rabies control in an African city (*6*) with low cost of US$ 2-3 per vaccinated dog (*16*). However, in some African countries, dog owners cannot afford anti-dog rabies inoculations and depend on mass vaccination campaigns that are free of cost (*8, 17*). Analysis of pre- and post-vaccination rabies cases and economic data showed that a single simulated dog vaccination campaign was able to interrupt transmission and was less costly than human post-exposure prophylaxis (*14*). A proof of the feasibility of dog rabies elimination in an African city would have far reaching consequences for a regionally concerted effort to eliminate rabies in Africa.

A city-wide dog rabies mass vaccination campaign was set up in partnership with the Chadian authorities, the Institut de Recherches en Elevage pour le Developpement (IRED), the Centre de Support en Santé Internationale (CSSI) and the Swiss Tropical and Public Health Institute (Swiss TPH)(*23*). The Chadian government paid for the costs of personnel and logistics and a philanthropic donor paid for the costs of dog vaccines and research. Passive dog rabies and human exposure surveillance started before the campaigns and is still ongoing. Here, we analyse the passive surveillance data of dogs brought to the diagnostic laboratory from this prior work using mathematical transmission models and phylodynamic analyses of dog-related rabies virus. We investigate the impact of the vaccination campaigns for interrupting transmission and the potential for maintaining elimination.

Results

Mass vaccination and field data

The vaccination campaign operations are described in detail in a previous publication (*23*) and summarized in the materials and methods. Vaccination coverage surveys followed each sequence to assess the achieved coverage and the deficit to reach 70% target coverage (*23*). In 2012, 72% of all dogs were vaccinated (95% confidence interval 69-76%) and in 2013 70% were vaccinated (95% confidence interval 69-75%).

Our results are based on data obtained about the weekly incidence of dogs newly infected with rabies virus(Fig. 1A) and the incidence of related human exposure (Fig. 1B). These data were collected through passive surveillance, i.e. by suspected dogs that were brought for testing to the rabies laboratory in N'Djaména and through collection of rabies virus isolates from rabid dogs throughout the vaccination campaign, described above. Recorded numbers of vaccinated dogs were used for the estimation of vaccination coverage (*23*). These data were used to estimate the effective reproductive number R_e (the number of new rabid dogs infected by one rabid dog at any time, accounting for immunity and interventions, estimated from the transmission model) and the threshold population density of susceptible dogs using mathematical models. The data suggested that mass dog vaccination campaigns in 2012 and 2013 reached sufficient coverage to interrupt rabies transmission from January 2014 to October 2014. Dog rabies incidence in the city of N'Djaména, estimated from passive surveillance, dropped from 0.33 rabid dogs / (10 000*week) prior to the mass vaccination campaign to 0.016 rabid dogs / (10 000 *week) in 2014 (Fig. 1A). Similarly, the incidence of human exposure to rabid dogs, estimated from passive surveillance,

dropped from one human exposed to rabies virus / (1 000 000*week) prior to the mass vaccination to less than 0.002 / (1 000 000*week) in 2014, which is less than one person per year (Fig. 1B).

Transmission model

We used a deterministic, population-based model of ordinary differential equations to model rabies virus transmission amongst dogs as well as between dogs and humans (Table S1). The transmission model showed that between the two campaigns in 2012 and 2013, effective recorded vaccination coverage decreased from a peak of 67% (December 2012) (*23*) to a trough of 33% (October 2013), assuming an exponential distribution for the persistence of immunity, which was estimated from 105 immunized dogs undergoing repeated serological measurements. This represents a 51% relative coverage loss (Fig. 2A). The model suggested that population replacement by the birth of susceptible dogs accounted for 29% of the relative coverage loss, whereas individual dog immunity loss accounted for 22% of this relative coverage loss.

The effectively vaccinated surface area in our campaign of 240 km^2 (2012) was much lower than the 770 km^2 assumed in an earlier simulation (*14*). The empirical data from this study provides a better estimate of parameter values, the threshold density of susceptible dogs and the basic reproductive number, i.e. the number of secondary infections resulting from a typical case in a completely susceptible population, as $R_0 = 1.14$, instead of $R_0 = 1.01$. This means that rabies is more infectious in N'Djaména, than previously reported (*14*). The effective reproductive number, R_e, decreased from the equilibrium value of 1 from the start of the first vaccination campaign and remained below 1 through November 2014, implying that the conditions for rabies virus persistence were not maintained since the start of the vaccination campaigns. Simulations of a

deterministic ordinary differential equation model (Fig. 1), fitted to rabies case data from N'Djaména, and a stochastic extension (Fig. 3) suggested that rabies transmission was interrupted from early 2013 onwards.

As our model did not include importation of infections, we wondered whether dog rabies cases seen from the October 2014 onwards (Fig. 1A) were due to imported cases (with subsequent local transmission) rather than sustained ongoing transmission from the end of 2013 or the beginning of 2014. To test this hypothesis, we performed a maximum likelihood phylogeny of nucleoprotein sequences from rabies virus isolates collected in Chad (from N'Djaména and other regions) from August 2011 to January 2015. Indeed, the dog rabies cases from 2014 onwards were phylogenetically distinct from those previously circulating in N'Djaména (Fig. 4)). We therefore suspected that domestic dogs from surrounding peri-urban and rural areas were the more likely source of reinfection rather than ongoing transmission in dogs or wild animals. Consistent with this, only dog-related rabies virus strains and no wildlife-related strains were found in a previous rabies virus phylogenetic analysis performed in N'Djaména (*19*).

Sensitivity analysis

We performed sensitivity analysis to determine whether the simulation results were robust compared to our estimates of parameter values. Figure S1 shows simulation results of the density of infectious dogs over 6 years allowing for uncertainty in each of the parameter values (varied one at a time). In each simulation run, the dog transmission rate, β_{dd}, was refitted for that set of parameter values. The results were robust to uncertainty in the parameter values and except for low vaccine efficacy values, the simulations predicted that transmission would be interrupted

after the first campaign. Fig. S2 shows a similar sensitivity analysis of the simulated number of infectious dogs but with a fixed value for the dog transmission rate, β_{dd}, estimated from the baseline set of parameter values (tableTS2). The ranges of the parameter values were greater than in Fig. S1 and the results showed the importance of that parameter on the expected number of rabid dogs over time. Most parameters had little effect, but similar to the sensitivity analysis for R_c, high values for the carrying capacity of dogs and the probability of an exposed dog developing rabies and low values for the rabies induced death rate led to a high number of infectious dogs.

Fig. S3 shows the simulated densities of infectious dogs and exposed humans depending on the probability of detection of infectious dogs, p_d, and of exposed humans, p_h, used to fit β_{dd} and β_{hd}, respectively. Low values of these detection probabilities result in higher numbers of infectious dogs and exposed humans, leading to higher estimates for the dog to dog, β_{dd}, and dog to human, β_{hd}, transmission parameters. The results indicated that the simulation data were robust regarding these detection probabilities unless the probabilities were very low and that underreporting of rabies cases was unlikely to have a substantial effect on our results (Fig. S 3). This in turn suggested that underreporting of cases did not play a large role in the persistence of rabies transmission. Even accounting for heterogeneity in underreporting, it was unlikely that unreported transmission persisted for nine months and more likely that a re-introduction occurred, either from wildlife or from dogs with ongoing transmission outside the city of N'Djaména.

Phylodynamic analysis

29 nucleotide sequences encoding the N-protein from dog related rabies virus isolates collected between August 2011 and June 2013 were sequenced as previously described (*9*, *24*). Although

the 95% highest posterior density intervals were wide because of the small number of sequences, the effective reproductive number estimated from the genetic data showed the same pattern as the R_e estimated from the incidence data (Fig. 2B). We demonstrate thus by two different methods, transmission modelling and a phylodynamic analysis that dog rabies transmission can be interrupted by the mass vaccination of dogs in N'Djaména.

Discussion

The models and data presented here show that the period with no rabies transmission in N'Djaména was longer after mass vaccination campaigns than in the absence of such campaigns, suggesting that dog rabies virus transmission in this African city could be interrupted and consequently human rabies exposure reduced. However, the duration of interruption of transmission in our study was shorter than our model predictions presented here and in earlier work (*14*), indicating that there was likely to be a re-introduction of infection from wildlife or latently infected dogs from the adjacent areas, similar to what was reported in a recent study on rabies transmission in Bangui (*26*).

Our study suggested that urban centres may not be hotspots of dog rabies transmission leading to spill over cases in rural areas, as previously thought. Dog rabies transmission is ongoing in peri-urban and rural African areas and is likely to be continuously transmitted into urban areas through human-mediated transport of dogs (*9*). Sustainable elimination of dog rabies therefore will require action over a much larger geographical area. We have proposed a development impact bond financing scheme for dog rabies elimination in the entire country of Chad (*27*).

There is still considerable uncertainty surrounding the role of density and spatial heterogeneity and external re-introduction in the transmission of dog rabies (*28*). A meta-population or contact network modeling approach may better represent the observed heterogeneity of the dog population in N'Djaména (Fig. S4). Further research is needed to assess how dog density and the spatial heterogeneity of dog populations influences the dynamics of dog rabies elimination (*28, 29*).

Determining the optimal timing of vaccination campaigns in N'Djaména to maintain elimination would require better knowledge of the rabies importation rate into the city. Our results are in line with a recent study from Bangui, Central African Republic showing that rabies is continuously re-introduced in towns by human related transport of dogs from surrounding peri-urban and rural areas (*26*) and rapidly dispersed between cities (9). Therefore, we suggest that dog rabies control in African cities should be planned for larger areas, including suburban and rural areas, and be coordinated regionally between neighboring countries for effective elimination of dog rabies in Africa (*1*). In particular, movement of dogs with or without their owners should be restricted to limit the rapid dispersal of dog associated rabies virus. Dog mass vaccination campaigns should also be complemented by affordable compulsory dog registration. Our study further supports the need for an improvement and a reinforcement of rabies surveillance in rural and more remote areas to achieve inclusive and comprehensive rabies reporting that can then be used to guide vaccination decisions. New rapid tests for rabies could be used in a decentralized manner and may enable collection of data about rabies epidemiology in remote locations (*30*).

In contrast to previous reports (*31*), our study suggests that mass vaccination of dogs, coupled with post-exposure prophylaxis, could be sufficient to eliminate rabies transmission in an

African city, both in dogs and humans, as long as vaccination is extended to a larger area beyond the city itself. In the long term, eliminating the infectious rabies reservoir in dogs will be more cost-effective than perpetual post or pre exposure prophylaxis in humans(*32*). Dog vaccination campaigns will require a regional approach similar to the well-coordinated dog rabies control efforts among Latin American countries (*1*). The recent creation of the Pan African Rabies Control Network (PARACON, paracon.rabiesalliance.org) is an important first step towards the goal of eliminating dog rabies from Africa by 2030.

Supplementary materials

Supplementary Figures

Supplementary Figure 1: 4 One -dimensional sensitivity analysis of simulation results on parameter values. The plots show simulations of the density of infectious dogs over 6 years (300 weeks) where all parameters are fixed at values described in table S2 except for the parameter being varied and βdd. The x-axis shows the time in weeks and y-axis shows the value of the parameter (in its corresponding units). The colour of each pixel represents the density of infectious dogs. The horizontal red lines correspond to the parameter values in table S2 and the solution plotted in Fig. 1A and Fig. 2A.

Supplementary Figure 2: One-dimensional sensitivity analysis of simulation results on parameter values. The plots show simulations of the density of infectious dogs over 6 years (300 weeks) where all parameters are fixed at values described in Table 3 except for the parameter being varied (β_dd is fixed at 0.0292). The x-axis shows the time in weeks and y-

axis shows the value of the parameter (in its corresponding units). The colour of each pixel represents the density of infectious dogs.

Supplementary Figure. 3: Sensitivity analysis of the simulation results on the probability of detecting rabid dogs. (A) The simulated density of infectious dogs depending on the detection probability of rabid dogs, p_d, over time. The x-axis corresponds to time (measured in weeks) and the y-axis to the detection probability. The colour of each pixel corresponds to the density of infectious dogs. (B) The endemic equilibrium value for density of infectious dogs (in the absence of vaccination campaigns) depending on p_d. The x-axis corresponds to to the detection probability, p_d and the y-axis (and colour of the pixel) correspond to the endemic equilibrium density of infectious dogs.

Supplementary Figure. 4: Density of vaccinated dogs in N'Djaména in 2013 calculated based on the data presented in Léchenne et al. 2016. Black dots indicate the locations of the fixed vaccination posts. It is assumed that dogs diffuse from these locations after vaccination in a homogeneous way. We used a diffusion kernel prediction map with a bandwidth of 1040 m (which is the diameter of a circle of 0.86 km2, the area per post of 331 posts in a total area of 285km2). The water surface was included as a barrier function.

Supplementary Figure. 5: Schematic of mathematical model of rabies. Birth and death rates of humans and dogs are not shown.

Supplementary Figure 6: Vaccination rates during the two campaigns in N'Djaména, Chad a) in 2012 and b) in 2013. The weeks are labelled starting from 4 June 2012.

Supplementary Figure 7: Local and global sensitivity indices of the control reproductive number, R_c, to the model parameters.

Supplementary Figure 8: Sample simulation of the stochastic model showing the density of exposed and infectious dogs with the simulation results of the deterministic model and the observed number of infectious dogs.

Supplementary Figure 9: Results of the phylodynamic analysis showing median (red) and 95% HPD interval (black) for R_e through time. Solid lines correspond to the constant sampling proportion assumption, and dashed lines to the changing sampling proportion assumption. Blue points indicate the change of R_e and sampling proportion. We plot the R_e estimate for each interval at the midpoint of the interval, and interpolate linearly in between.

Supplementary Tables

Supplementary Table 1: State variables of dog rabies transmission (model)

Supplementary Table 2. Parameters of the rabies transmission model with estimated values and sources. Most parameters have the same value as in the previous model (14), but some have been updated from more recent publications or from new data from the current study (as described in the section on parameter estimation).

Data files

- RabiesData1.txt (incidence data)
- RabiesData2.txt (genetic sequences of 33 dog rabies virus N-protein deposited in GenBank)

Materials and Methods

Study design

The objective of this study was to test the hypotheses a) that dog rabies virus transmission in an African city can be interrupted by the mass vaccination of dogs and b) that cities are hotspots of dog rabies virus transmission and that re-introduction would be slow. As we observed the re-introduction of dog rabies after the mass vaccination of dogs, we hypothesized that rabies virus was re-introduced from the outside of the city. The research subjects were the weekly number of routinely recorded rabies suspected dogs and the number of exposed humans per rabid dog.

The design of the present study is composed of four main components covering the city of N'Djaména, Chad i): An ongoing passive dog rabies surveillance system. Suspected rabid dogs (dead or alive) were brought to the rabies diagnostic laboratory. No active collection of

suspected dogs was done. For every rabies suspected dog, information on exposed humans was collected on a routine basis. ii): A dog rabies mass vaccination campaign was done from October to December 2012 and 2013 (*23*) Blood was taken from 104 dogs in 2012, prior to the start of the mass vaccination campaign to assess the proportion of existing vaccination antibodies. The Fluorescent antibody virus neutralization test (FAVN) was used for this purpose (*33*) . Data on rabid dogs and exposed humans was collected up to the end of 2015 for this paper. iii) A mathematical transmission model of dog to dog and dog to human rabies transmission was parametrized from the weekly number of rabid dogs and exposed humans collected under i) prior, during and after the mass vaccination campaign (ii). iv) A phylogenetic and phylo-dynamic analysis of the rabies virus strains collected under i) was used to assess their genetic closeness and to estimate the basic reproductive number of the rabies transmission in dogs independently from the mathematical transmission model (iii).

Surveillance of dog rabies and human exposure

Passive routine dog rabies surveillance started on 4 June 2012 and is currently ongoing in N'Djaména at the Institut de Recherches en Elevage pour le Développement by standard immunofluorescence as described in (*14, 18*) . Prior to the mass vaccination campaign, the average weekly incidence of dog rabies was of 0.33 dogs per 10,000. For every laboratory-confirmed rabid dog, on average 1.6 humans were reported to be exposed (from questioning the dog owner) leading to a weekly incidence of 0.11 per 100,000 people.

Dog rabies mass vaccination campaign 2012 and 2013

A citywide mass dog vaccination campaign including all 10 districts of N'Djaména took place in 2012 and was repeated in 2013. In both campaigns, the objective was to vaccinate 70% of the total dog population of N'Djaména with the dog rabies vaccine Rabisin™ (Merial Inc. Lyon, France). The vaccination campaigns began in the first week of October 2012 and 2013 and lasted for a total of 13 weeks until the first week of January of the next year. Vaccination took place only on Friday to Sunday due to availability of staff and participation of the public during these days (as evaluated in previous studies) (*6*). Every Friday to Sunday, ten fixed post vaccination teams were set up in one of 12 (13 in 2013) areas of the city corresponding to administrative boundaries. Over the three day period, these teams vaccinated on average 1,433 (min. 24; max. 6,460) dogs in 2012 and 1,709 dogs (min. 67; max. 4,591) dogs in 2013, depending on the socio-cultural and ecological context of the city district (Table 1). Details of the operational performance and the results of the vaccination campaigns are published elsewhere (*23*) .

Coverage assessment

A coverage assessment was carried out each week after vaccination in the previously vaccinated area Fig. S4. Vaccination zones and their analysis perimeter corresponded in most cases to a district. The coverage assessment was composed of a household survey in randomly selected geographical locations within the analysis perimeter to estimate the proportion of owned vaccinated dogs. In addition, random transects were carried out with a car in the same zone to estimate the dog density in the street and the proportion of ownerless dogs. Data from both studies were then combined in one Bayesian statistical model as reported elsewhere (*23*) .

Description of mathematical model of dog-dog and dog-human transmission

We use a deterministic population based model of ordinary differential equations extended from a previously published model for dog to dog rabies transmission (*14*),

$$\frac{dS_d(t)}{dt} = b_d N_d(t) + \lambda_d V_d(t) - r_d \beta_{dd} S_d(t) I_d(t) - \left(\nu_d \alpha_d(t) + m_d + \gamma_d N_d(t)\right) S_d(t), \quad (1a)$$

$$\frac{dE_d(t)}{dt} = r_d \beta_{dd} S_d(t) I_d(t) - \left(\sigma_d + \nu_d \alpha_d(t) + m_d + \gamma_d N_d(t)\right) E_d(t), \quad (1b)$$

$$\frac{dI_d(t)}{dt} = \sigma_d E_d(t) - \left(\mu_d + m_d + \gamma_d N_d(t)\right) I_d(t), \quad (1c)$$

$$\frac{dV_d(t)}{dt} = \nu_d \alpha_d(t)\left(S_d(t) + E_d(t)\right) - \left(\lambda_d + m_d + \gamma_d N_d(t)\right) V_d(t), \quad (1d)$$

where the state variables and parameters are defined in Tables S1 and S2 respectively. The total dog population size is,

$$N_d(t) = S_d(t) + E_d(t) + I_d(t) + V_d(t), \quad (2)$$

and the density dependent death rate is,

$$\gamma_d = \frac{b_d - m_d}{K_d}, \quad (3)$$

where K_d is described in Table S2 and b_d is required to be greater than m_d. We note here that we assume density-dependent transmission and that in general, (1) is a non-autonomous model where $\alpha_d(t)$ varies with time,

$$\alpha_d(t) = \alpha_d^* + \alpha_0^{(i)}(t) + \alpha_1^{(i)}(t) e^{-\varphi t}, \quad (4)$$

where α_d^* is the (assumed) constant background vaccination rate, $\alpha_0^{(i)}(t)$ and $\alpha_1^{(i)}(t)$ are campaign-dependent vaccination values for the i^{th} week, and φ is a saturation parameter. Outside of the campaigns, $\alpha_d(t) = \alpha_d^*$. We further restrict the values of $\alpha_0^{(i)}$ and $\alpha_1^{(i)}$ to ensure that $\alpha_d(t)$ is continuous so that the system for rabies transmission (1) has a unique solution that exists for all time.

We similarly use an ordinary differential equation model for dog to human transmission based on (*14*) ,

$$\frac{dS_h(t)}{dt} = b_h N_h(t) - \beta_{hd} S_h(t) I_d(t) + a_h E_h(t) - m_h S_h(t), \quad (5a)$$

$$\frac{dE_h(t)}{dt} = \beta_{hd} S_h(t) I_d(t) - (a_h + \sigma_h + m_h) E_h(t), \quad (5b)$$

$$\frac{dI_h(t)}{dt} = \sigma_h E_h(t) - (m_h + \mu_h) I_h(t), \quad (5c)$$

where the total human population size is,

$$N_h(t) = S_h(t) + E_h(t) + I_h(t), \quad (6)$$

σ_h is the rate of progression from the exposed to the infectious state depending on the site of the bite,

$$\sigma_h = \frac{P_2 P_6}{i_{head}} + \frac{P_3 P_7}{i_{arm}} + \frac{P_4 P_8}{i_{trunc}} + \frac{P_5 P_9}{i_{leg}}, \quad (7)$$

a_h is the abortive rate of progression from the exposed back to the susceptible state,

$$a_h = \frac{P_2(1 - P_6)}{i_{head}} + \frac{P_3(1 - P_7)}{i_{arm}} + \frac{P_4(1 - P_8)}{i_{trunc}} + \frac{P_5(1 - P_9)}{i_{leg}}, \quad (8)$$

and the probabilities of biting different parts of the body (P_2 through P_5), the probabilities of subsequent progression to rabies (P_6 through P_9), and the average time to do so ($1/i_\xi$ where ξ is head, arm, trunk or leg), are described in more detail in the previous formulation of the model (*14*). Fig. S5 shows a schematic of the model system. We note that the dynamics for rabies transmission in humans is dependent on rabies transmission in dogs but the transmission in dogs is independent of transmission in humans.

Mathematical analysis

In the absence of vaccination campaigns ($\alpha_d(t) = \alpha_d^*$), the autonomous mathematical model for rabies transmission in dogs (1) has a trivial disease-free equilibrium point,

$$S_d = \frac{(b_d + \lambda_d)K_d}{b_d + \lambda_d + \nu_d \alpha_d^*}, \quad (9a)$$

$$E_d = 0, \quad (9b)$$

$$I_d = 0, \quad (9c)$$

$$V_d = \frac{\nu_d \alpha_d^* K_d}{b_d + \lambda_d + \nu_d \alpha_d^*}. \quad (9d)$$

The control reproductive number for the dog rabies model is the number of dogs that one newly introduced rabid dog would infect, assuming no disease in the population (with only background vaccination),

$$R_c = \frac{r_d \beta_{dd} \sigma_d K_d}{(\sigma_d + \nu_d \alpha_d^* + b_d)(\mu_d + b_d)}. \quad (10)$$

We omit the mathematical details here but can show that with only background vaccination (no vaccination campaigns) when $R_c < 1$, the disease-free equilibrium point (9) is locally asymptotically stable and when $R_c > 1$, the disease-free equilibrium point is unstable and there exists a locally asymptotically stable endemic equilibrium point where rabies persists in the population. Additionally, if there is no background vaccination, the control reproductive number reduces to the basic reproductive number,

$$R_0 = \frac{r_d \beta_{dd} \sigma_d K_d}{(\sigma_d + b_d)(\mu_d + b_d)}.$$

At any time, t, allowing for vaccination campaigns, the effective reproductive number, $R_e(t)$, represents the expected number of new infections caused by one infectious dog,

$$R_e(t) = \frac{r_d \beta_{dd} \sigma_d S_d(t)}{(\sigma_d + \nu_d \alpha_d(t) + m_d + \gamma_d N_d(t))(\mu_d + m_d + \gamma_d N_d(t))}. \quad (11)$$

If we assume that the total dog population is at carrying capacity (which is reasonable because the density of rabid dogs is low so has a minimal impact on the population density of dogs), the effective reproductive number simplifies to,

$$R_e(t) = \frac{r_d \beta_{dd} \sigma_d S_d(t)}{(\sigma_d + \nu_d \alpha_d(t) + b_d)(\mu_d + b_d)}. \quad (12)$$

The threshold density of susceptible dogs at which transmission occurs, S_d^* is the density at which $R_e = 1$. From (12), outside of vaccination campaigns this is,

$$S_d^* = \frac{(\sigma_d + \nu_d \alpha_d^* + b_d)(\mu_d + b_d)}{r_d \beta_{dd} \sigma_d}. \quad (13)$$

The threshold vaccination coverage reached in a campaign to eliminate transmission, ψ^*, is given by,

$$\psi^* = 1 - \frac{1}{R_c}, \quad (14)$$

when background vaccination takes place outside the campaign. Equivalently this is,

$$\psi^* = \frac{K_d - S_d^*}{K_d}. \quad (15)$$

After the vaccination campaigns, the coverage of protected dogs decreases exponentially due to population loss of susceptible dogs (proportionally $b_d/(\lambda_d + b_d)$: 57% for parameter values in table S2) and due to loss of vaccine efficacy (proportionally $\lambda_d/(\lambda_d + b_d)$: 43% for parameter values in table S2).

Parameter estimation

The values for most parameters are taken from the previous model (*14*) except where new published results or new data have allowed for revised values. The parameter values and their sources are summarised in table S2. The birth and death rates of dogs were calculated as in previous work but the carrying capacity of dogs was revised to reflect a total population of 25,103 dogs in an area of 240km^2 as estimated in the 2012 coverage assessment. The vaccination rate of dogs and the transmission rates from dogs to dogs and dogs to humans were estimated as described below.

Dog vaccination rate

The baseline study found that 12% (n=105) of all owned dogs had antibodies (and so could be considered effectively vaccinated), implying that there was some ongoing background vaccination outside of the two campaigns conducted in 2012 and 2013. The coverage assessment estimated that for every 10 owned dogs, there was one unowned dog. Assuming that the background vaccination rate was constant and the proportion of vaccinated dogs was at equilibrium (9), with demographic and other vaccination parameters as in table S2, the per capita background vaccination rate was 2.96×10^{-3}/week.

The number of dogs marked as vaccinated in each campaign is shown in Table 1. We estimated the vaccination rate parameters, $\alpha_0^{(i)}$ and $\alpha_1^{(i)}$ using a simple model of vaccination for each campaign,

$$\frac{dU^{(i)}}{dt} = b_d\left(K_d - U^{(i)}\right) - \overline{\alpha}_d^{(i)}(t)U^{(i)}, \quad (16a)$$

$$\frac{dV^{(i)}}{dt} = \overline{\alpha}_d^{(i)}(t)U^{(i)}, \quad (16b)$$

where $U^{(i)}$ is the density of all unmarked dogs, $V^{(i)}$ is the density of dogs marked in campaign week i, and $\overline{\alpha}_d^{(i)}(t)$ is the rate of marking dogs during campaign week i. We define time, t, as varying from 0 at the start of each campaign week to 1 at the end of each campaign week.

The coverage assessment could only determine whether dogs were marked as vaccinated or not and did not determine the immune status of dogs. We therefore ignore the efficacy of the vaccination and do not consider background vaccination because these dogs would not be marked as campaign-vaccinated dogs, so

$$\overline{\alpha}_d^{(i)}(t) = \alpha_0^{(i)}(t) + \alpha_1^{(i)}(t)e^{-\varphi t}. \quad (17)$$

For simplicity we ignore rabies virus transmission, assume the dog population is at carrying capacity, and ignore the death of marked dogs or the loss of marking collars during the campaign week. Since the coverage assessment was conducted within three days of the vaccination campaign, these assumptions are reasonable. We assume that the markings from the 2012 campaign do not last until 2013 so for both campaigns, the initial density of unmarked dogs is equal to the carrying capacity,

$$U^{(1)}(0) = K_d, \qquad (18a)$$

and from continuity,

$$U^{(i)}(0) = U^{(i-1)}(1) \quad \text{for } i > 1. \qquad (18b)$$

The initial density of dogs marked during a campaign week is zero,

$$V^{(i)}(0) = 0 \quad \text{for } i \geq 1. \qquad (18c)$$

We fix $\varphi = 100$. To ensure that $\alpha_d(t)$ is continuous, we set

$$\alpha_0^{(1)} + \alpha_1^{(1)} = 0, \qquad (19a)$$

$$\alpha_0^{(i)} + \alpha_1^{(i)} = \alpha_0^{(i-1)} + \alpha_1^{(i-1)} e^{-\varphi} \quad \text{for } i > 1. \qquad (19b)$$

The final density of dogs marked in a campaign week, $V^{(i)}(1)$ is set equal to the number of marked dogs estimated from the coverage assessment for that week (Table 1) divided by the campaign area for that year (240km^2 in 2012 and 285km^2 in 2013). Condition (19) and the ordinary differential equations for the vaccination model (16) with its boundary conditions provide two sets of equations for each campaign week. For other parameter values as provided in table S2, we numerically simulate the vaccination model using an adaptive step-size Runge-Kutta method (ode45) and then use a root-finding algorithm (fzero) to calculate $\alpha_0^{(i)}$ and $\alpha_1^{(i)}$ (in MATLAB, version 8.5) for each campaign week. Fig. S6 shows the final estimated vaccination rates during the two campaigns.

Rate of loss of vaccine immunity

In 2012, before the vaccination campaigns were conducted, a total of 105 dogs in N'Djaména were tested for antibody titers, vaccinated and then followed up over a period of one year. Of these dogs, 58 had initial antibody titers that showed no previous vaccination and were successfully followed up over the entire year. After one year, 44 dogs had antibody titers above 0.5 IU, which, as a conservative estimate, we considered protective (*34*). We calculated the rate of loss of vaccine decay, λ_d, assuming exponential decay and a relative value of 0.76 after 52 weeks.

Rabies transmission rates

The number of rabid dogs and exposed humans recorded per week since 4 June 2012 are shown in the additional file RabiesData1.txt. Recording of both human and dog cases is ongoing but the analysis only included cases until the end of October 2015. We divide the numbers of dogs and humans by the area estimated in the coverage assessment of the 2012 vaccination campaign (240 km^2) to provide the densities of rabid dogs and exposed humans.

We first fit the dog to dog transmission rate, β_{dd}, for the model with transmission only in dogs (1) with the data for the number of rabid dogs with other parameter values as described in table S2 and the vaccination rate as described above. We then use this value for β_{dd} to estimate the dog to human transmission rate, β_{hd}, for the full model with transmission between dogs (1) and to humans (5) with the data for number of exposed humans.

To fit β_{dd}, we numerically simulate (1) using an adaptive step-size Runge-Kutta method and minimise the Euclidean distance between the simulated incidence of infectious dogs (from the first term of the right hand side of (1c)), and the observed weekly incidence of infectious dogs in

MATLAB. We assume that the probability of detecting a rabid dog, $p_d = 0.5$ so that on average there were twice as many rabid dogs as those detected. There is little data on this parameter but our sensitivity analysis showed that unless p_d is very low, the estimated values for β_{dd} did not change much (Fig. S3). We assume that the initial condition for the ordinary differential equations in June 2012 is at the unique endemic equilibrium (with $\alpha_d(t) = \alpha_d^*$) and the dog density is at carrying capacity.

We similarly fit β_{hd} by numerically simulating (1) and (5) and minimising the Euclidean distance between the simulated density of exposed humans, $E_h(t)$, and the observed density of exposed humans on a weekly time step in MATLAB. Here we assume perfect detection of exposed humans ($p_h = 1$) and that the initial condition in June 2012 is at the unique endemic equilibrium with a population density of humans of 4833 humans/km^2 (from a total population size of 1.16 million in 2012 estimated from the 2011 population size of 1.079 million using a growth rate of 7.5%) (*35, 36*).

Phylogenetic importation analysis

To investigate the hypothesis of a reintroduction of dog rabies virus in N'Djamena from outside of the city after the vaccination campaigns, we performed a phylogenetic analysis using the previously described 29 complete nucleoprotein sequences of rabies virus isolates collected between August 2011 and January 2014, with the inclusion of one supplementary sequence of a isolate collected during this period (GenBank accession number KY124541), in addition to the sequences of the three first isolates collected in the city after this period (from February 2014 to January 2015, GenBank accession numbers MF538629-31) and to published available sequences from Chad (n=1) and from neighboring countries (n=14). Using jModelTest2 (*37, 38*), the best-fit model of nucleotide substitution according to the Bayesian Information Criterion was the general

time reversible model with proportion of invariable sites plus gamma-distributed rate heterogeneity (GTR+I+Γ4). A phylogenetic tree was then estimated using the maximum likelihood (ML) method available in PhyML 3.0 (*39*) utilizing SPR branch-swapping. The robustness of individual nodes on the phylogeny was estimated using 1,000 bootstrap replicates and using the approximate likelihood ratio test (aLRT) with SH-like supports (*40*).

Sensitivity analysis

We conducted local and global sensitivity analysis of the control reproductive number, R_c, (10) to the model parameters (Fig. S7). We used the normalized forward sensitivity index for the local analysis (*41*, *42*) at the parameter values defined in table S2 and the partial rank correlation coefficients for the global analysis (*43*), assuming all parameters were uniformly distributed in the intervals: $r_d \in [0.049,1]$, $\beta_{dd} \in [0.00292,0.0614]$, $K_d \in [10.5,221]$, $\sigma_d \in [0.0239,0.504]$, $b_d \in [0.0013,0.0273]$, $\mu_d \in [0.123,2.59]$, $\nu_d \in [0.094,1]$ and $\alpha_d^* \in [0.000296,0.00622]$. Both the local and global analysis showed that the probability of developing rabies, r_d, the transmission rate, β_{dd}, the carrying capacity, K_d and the rabies virus induced mortality rate, μ_d, had a strong impact on the threshold for sustained transmission, R_c, while the other parameters had minimal impact.

Stochastic model simulations

We derived and numerically simulated a stochastic dog to dog transmission model based on (1), with the master equation,

$$\frac{dP(n,t)}{dt} = \sum_i [\, W_i(n|m_i)P(m_i,t) - W_i(m_i|n)P(n,t)], \qquad (20)$$

where n is any state of the system at time t and W_i are the transmission rates deduced from the parameters in table S2, using the Gillespie algorithm with the tau-leaping simulation method (*44*, *45*) . Fig. S8 shows a sample stochastic simulation of the density of exposed and infectious dogs with the corresponding simulation of the deterministic model and the underlying data for the number of infectious dogs. Fig. 3B shows that the mean of 500 simulation runs of the stochastic model declines after the first vaccination campaign in a similar manner to the deterministic model.

Phylodynamic analysis

29 sequences of canine rabies viruses, collected between August 2011 and June 2013, were analysed with Beast v2 (*25*). We chose a Hasegawa-Kishino-Yano (HKY) model for substitutions with a relaxed log-normal clock (*46*). We assumed an exponential (0.001) prior for the mean rate, an exponential (0.3333) prior for the standard deviation, and a log-normal $(1,1.25)$ prior for kappa.

For the epidemiological model, we chose the birth-death skyline model (*47*) . We used a log normal $(0,1)$ prior for the effective reproductive number, R_e, and allowed R_e to change in January 2013, August 2012, and April 2012, i.e. every 4.8 months prior to the last sample in June 2013. We assumed a uniform prior on the interval $(9.44,9.5)$ for the dog removal rate (corresponding to an expected infection time of exposed and active rabies between $(1/9.5,1/9.44)$ years, which is about 1.1 months. The sampling probability of a rabid dog was assumed to be 0 prior to the first sample, and uniform on $(0.4,0.6)$ between the first and last sample. The time of the initial case in that transmission chain was assumed to be a uniform prior on $(0,20)$,

prior to the most recent sample. We ran the Markov chain Monte Carlo (MCMC) simulations for 10^9 steps. We neglected the first 10% of the states as a burn-in period. The effective sample size of all parameters was 350 or higher, implying that we obtained substantial mixing.

In order to investigate sensitivity towards our assumption of a constant sampling proportion, we performed a second analysis allowing the sampling proportion to change at the same time points as when the R_e changes. As above, sampling was assumed to be 0 prior to the oldest sample. Further sampling was assumed to be uniform on $(0.2,0.6)$ in each interval (compared to uniform on $(0.4,0.6)$ above. As shown in fig. S9 the results do not change qualitatively.

Limitations of the study

The study is limited by the low number of rabies viruses that were isolated during the rabies mass vaccination campaign. For this reason, the credibility intervals of the phylodynamic estimation of the basic reproductive number are wide. Another limitation is that we could not clearly identify the source of the rabies viruses that were re-introduced from outside the city. Further ongoing research will relate the rabies viruses collected in N'Djamena in this study with strains that will be collected countrywide.

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Acknowledgements:

We thank all the community leaders and vaccinators in N'Djaména. We thank Thomas Smith for helpful comments and discussions, and anonymous reviewers for helpful comments and suggestions. There is no paid or unpaid consulting and there are no patents related to this work.

Funding:

Funding for this study was provided by the Chadian government, the UBS Optimus Foundation and the Swiss Federal Food Safety and Veterinary Office (FFSVO), the European Union Seventh Framework Programme PREDEMICS (grant #278433), the Wolfermann-Nägeli Foundation, the Emilia Guggenheim Schnurr Foundation and the Swiss National Science Foundation (grant#: 310030_160067 / 1).

Support was provided by the UBS Optimus Foundation (M.Le, R.M.), the Swiss Federal Food Safety and Veterinary Office (FFSVO) (M.Le.), the Wolfermann-Nägeli Foundation (M.Le.), Swiss Nationals Science foundation grant number 310030-160067 (M. La.),. and the European Research Council under the Seventh Framework Programme of the European Commission (PhyPD: grant agreement number 335529)(T.S.).

Requests for rabies virus strains require a Material Transfer Agreement and should be sent to Service Naissengar Institut de Recherches en Elevage pour le Développement, BP 433, N'Djaména, Chad email : naissengar@gmail.com

Author contributions:

J.Z.: conceived the study, J.Z. and I. A. O. applied for funding. J.Z., M. Le, M. La. J.H., A. L., H. B., L. D., T.S., N.C. wrote the manuscript. M.Le., M.La., R.M., S.N., A.O., G.R., J.T., S.M., M.O., Y.M., A.T. participated in the acquisition and analysis of data. J. Z., M.La. N.C. contributed to the mathematical modelling. S. N. did all the rabies diagnostic work. A.O. coordinated the rabies mass vaccination campaign. J.Z., M. Le, M. La. A.O., K. B., M.O., D.D.M., I.A.O., Y.M., A.T. J. H., A.L., H. B., T.S., provided a substantial intellectual contribution. K.B., J.T. D.D.M., I.A.O. provided administrative, technical or supervisory

support. D.D.M. took medical responsibility of the study A.L., L.K., H.B., L.D., participated in the analysis of molecular genetic data of the rabies viruses. N.C.: Supervised the mathematical modelling.

Competing interests:

None declared

Data and materials availability:

The dog and human rabies incidence data and the genetic sequences of 29 dog rabies virus N-protein are available in the Supplementary materials. The N-protein sequences are deposited in GenBank (https://www.ncbi.nlm.nih.gov/nuccore/) under the accession numbers KU564980-99 and KY124532-40.

- RabiesData1.txt (incidence data)
- RabiesData2.txt (genetic sequences of 29 dog rabies virus N-protein deposited in GenBank)

Tables

Table 1. Number of dogs vaccinated in each week of the vaccination campaigns. The campaign in 2012 started on 8 October 2012 (week 19) and in 2013 started on 30 September 2013 (week 70), as described in (*23*).

Vaccination Week	Vaccinated dogs (2012)	Vaccinated dogs (2013)
1	834	722
2	181	468
3	376	330
4	24	434
5	793	67
6	2901	1173
7	6460	928
8	1393	4215
9	3074	4372
10	1698	3424
11	311	4591
12	385	979
13	209	525

Figures

Fig. 1 (A) Cumulative incidence of dog rabies cases and human exposure. Cumulative incidence of recorded cases of dog rabies (infectious dogs) and simulated incidence of dog rabies in N'Djaména from 6 June 2012 to the end of October 2015. (B) Cumulative incidence of recorded human exposure to rabid dogs and simulated incidence of human exposure to rabid dogs in N'Djaména from 6 June 2012 to the end of October 2015.

Fig. 2 (A) Density of vaccinated and unvaccinated dogs in relation to the effective reproductive number. Density of susceptible (blue lines) and vaccinated (red lines) dogs against time since 6 June 2012. The solid lines show the simulated values from an ordinary differential equation transmission model from June 2012 to October 2015. (B) The effective reproductive number, R_e, and vaccination coverage against time. The solid red line shows the vaccination coverage and the solid blue line shows the effective reproductive number – both estimated from the ordinary differential equation transmission model. The black line is the median R_e obtained from the phylogenetic sequencing data, with upper and lower 95% credible intervals as black dashed lines.

Fig. 3 (A) Stochastic simulations of the interruption of transmission. Distribution of the simulated expected date of interruption of transmission from 1000 simulation runs of the stochastic model of dog rabies virus transmission. (B) Mean and 90% credible interval for exposed and infectious dogs from 500 runs of the stochastic model.

Fig. 4: Phylogeny of rabies viruses isolated during and after the mass vaccination campaign. Maximum likelihood phylogeny of nucleoprotein sequences from rabies virus isolates collected

in Chad (from N'Djaména and other regions) from August 2011 to January 2015, and from sequences of previous rabies virus isolates originated from Chad and from other neighboring countries. Sequences in blue were obtained from isolates collected in N'Djaména, Chad, during the period from August 2011 to January 2014, excepted for the sequences with an asterisk which correspond to isolates collected outside of N'Djaména or without any precise origin (for one isolate) during the same period. Sequences in red are those obtained from isolates collected in N'Djaména from February 2014 to January 2015. Only bootstrap values > 70 are indicated on selected nodes. A scale indicating genetic distance is presented by the horizontal bar. The tree is mid-point rooted for clarity only. GenBank accession numbers of published sequences used in this tree are: EU853590 (07072RCA), EU853651 (07149RCA), EU038107 (35RD8005), KT119773 (8801CAM), KX148243 (8803CAM), U22635 (8804CAM), EU853654 (9014NIG), KX148208 (92029CAR), KT119779 (9218TCH), KT119784 (9502CAM), KF977826 (CAR_11_001h), KC196743 (DRV_NG11), EU038108 (RD128), EU038096 (RD137) and EU038093 (RD9305HN).