PUBLIC HEALTH

Vaccination of dogs in an African city interrupts rabies transmission and reduces human exposure

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Despite the existence of effective rabies vaccines for dogs, dog-transmitted human rabies persists and has reemerged in Africa. Two consecutive dog vaccination campaigns took place in Chad in 2012 and 2013 (coverage of 71% in both years) in the capital city of N'Djaména, as previously published. 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 between dogs was interrupted for 9 months. 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 that 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 reemerged 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 Southeast 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 reintroduction requires an in-depth understanding of dog ecology (*6*), 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*, *7*–*10*). 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 small-scale study showed the feasibility of dog rabies control in an African city (*7*) with low cost of $2 to $3 per vaccinated dog (*16*). However, in some African countries, dog owners cannot afford inoculations and depend on mass vaccination campaigns that are free of cost (*9*, *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.

Previously, a citywide dog rabies mass vaccination campaign was set up in partnership with the Chadian authorities, the Institut de Recherche en Elevage pour le Développement, the Centre de Support en Santé Internationale, and the Swiss Tropical and Public Health Institute (*18*). 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 analyze the passive surveillance data taken from dogs brought to the diagnostic laboratory during this previous 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.

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RESULTS

Field data from mass vaccinations show reduced rabies incidence in dogs and humans

The vaccination campaign operations are described in detail in a previous publication (*18*) and summarized in Materials and Methods. Vaccination coverage surveys followed each sequence to assess the achieved coverage and the deficit to reach 70% target coverage. In both campaigns, 71% of all dogs were vaccinated (95% confidence interval, 69 to 76%) (*18*).

We obtained results about the weekly incidence of dogs newly infected with rabies (Fig. 1A) and the incidence of related human exposure (Fig. 1B). These data were collected through passive surveillance, that is, from dogs that were suspected to be infected and brought for testing to the rabies laboratory in N'Djaména and through collection of rabies virus strains from rabid dogs. Recorded numbers of vaccinated dogs were used for the estimation of vaccination coverage (*18*). The data suggested that mass dog vaccination campaigns in 2012 and 2013 reached sufficient coverage to interrupt transmission from January 2014 to October 2014. Per capita dog rabies incidence in the city of N'Djaména, estimated from passive surveillance, dropped from 0.33 dogs/10,000 per week before the mass vaccination campaign to 0.016 dogs/10,000 per week in 2014 (Fig. 1A). Similarly, the per capita incidence of human exposure to rabid dogs, as estimated from passive surveillance, dropped from 1/1,000,000 humans exposed to rabies virus per week before the mass vaccination to less than 0.002/1,000,000 per week in 2014, which is less than one person per year (Fig. 1B).

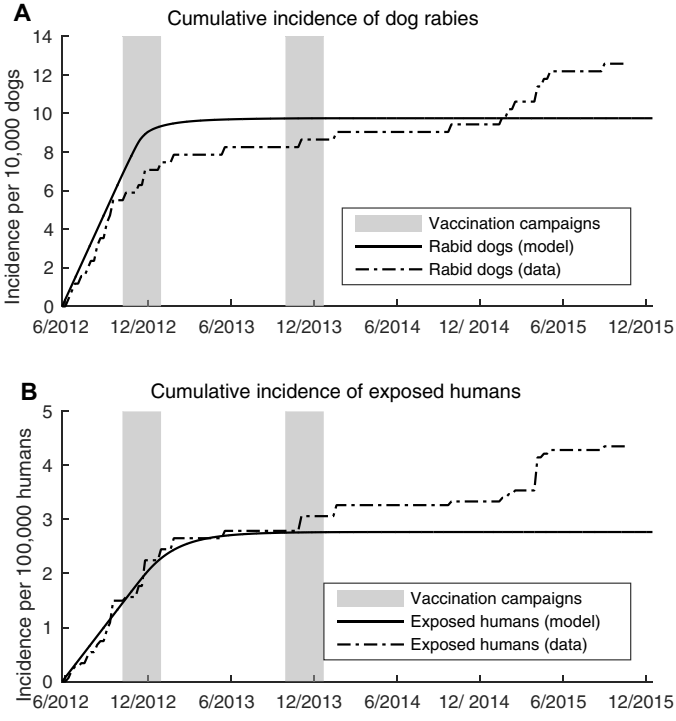

Fig. 1. Cumulative incidence of dog rabies and human exposure. (**A**) Cumulative incidence of recorded cases of dog rabies (infectious dogs) and simulated incidence of dog rabies in N'Djaména from 6 June 2012 to the end of October 2015. (**B**) Cumulative incidence of recorded human exposure to rabid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

Transmission modeling indicates extensive vaccination interrupted dog-to-dog rabies transmission

We used a deterministic, population-based model of ordinary differential equations to model rabies transmission among dogs as well as between dogs and humans (table S1). The surveillance field 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) and the threshold population density of susceptible dogs using mathematical models. 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) (*18*) 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.

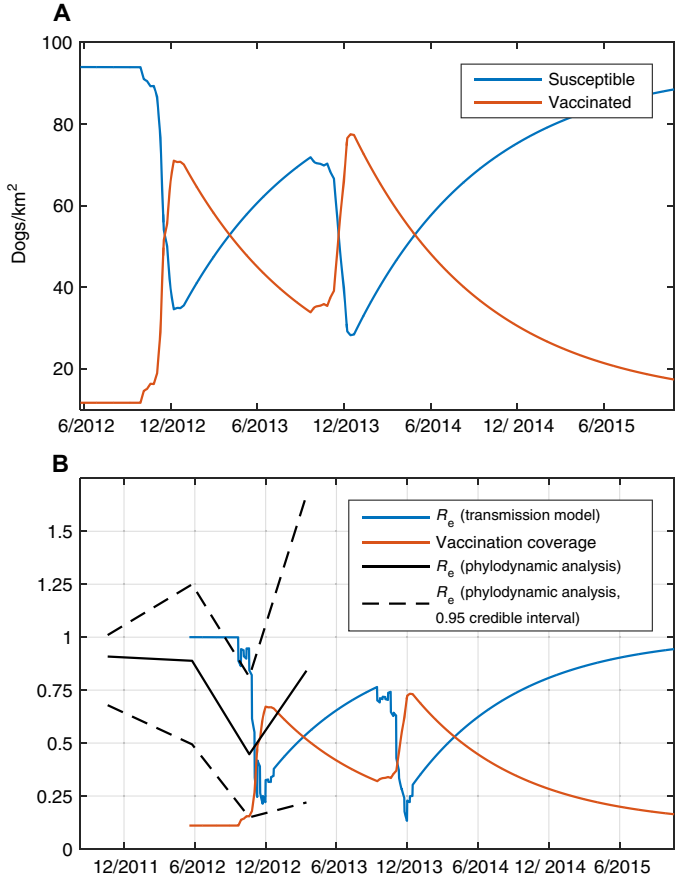

Fig. 2. Density of vaccinated and unvaccinated dogs in relation to the effective reproductive number. (**A**) Density of susceptible (blue lines) and vaccinated (orange lines) dogs against time since 6 June 2012. The solid lines show the simulated values from an ordinary differential equation transmission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

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 the current study provide a better estimate of parameter values, the threshold density of susceptible dogs, and the basic reproductive number, that is, 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 potentially more infectious in N'Djaména than previously reported (*14*). The effective reproductive number, R_e, decreased from the equilibrium value of 1 at 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 dog-to-dog rabies transmission was interrupted from early 2013 onward.

Rabies reintroduction may be due to an influx of dogs into the city from neighboring areas

Because our model did not include importation of infections, we wondered whether dog rabies cases seen from October 2014 onward (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 (ML) phylogeny of nucleoprotein sequences from rabies virus isolates collected in Chad (from N'Djaména and other regions) from August 2011 to January 2015. The dog rabies cases from 2014 onward 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*).

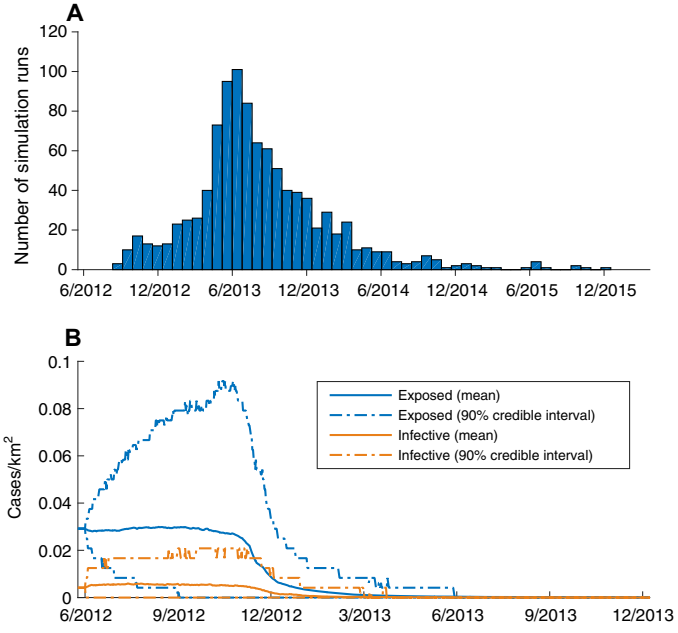

Fig. 3. Stochastic simulations of the interruption of transmission. (**A**) Distribution of the simulated expected date of interruption of transmission from 1000 simulation runs of the stochastic model of dog rabies transmission. (**B**) Mean and 90% credible interval for exposed and infectious dogs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

Transmission model inferences regarding viral dynamics are robust to parameter choice

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. Figure 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 (table S2). 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, 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.

Figure 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 the dog-to-dog and dog-to-human parameters, 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 β_{dd} and 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. S3). 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 9 months and more likely that a reintroduction occurred, either from wildlife or from dogs with ongoing transmission outside the city of N'Djaména.

Phylodynamic analyses support rabies transmission interruption by the mass vaccination campaigns

We conducted a phylodynamic analysis to estimate the transmission potential of rabies in N'Djaména. We simulated the evolutionary relationships between 29 nucleotide sequences encoding the N-protein from dog-related rabies virus isolates [collected between August 2011 and June 2013 (*10*, *20*)] under the assumption of the transmission model to estimate the viral reproductive number. Although the 95% highest posterior density (HPD) 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). This corroborated the results of the deterministic transmission model, which estimated the transmission potential from the time series of the number of weekly cases. We therefore demonstrate by two different methods, analyzing two different kinds of data, that dog rabies transmission can be interrupted by the mass vaccination of dogs in the African city of N'Djaména.

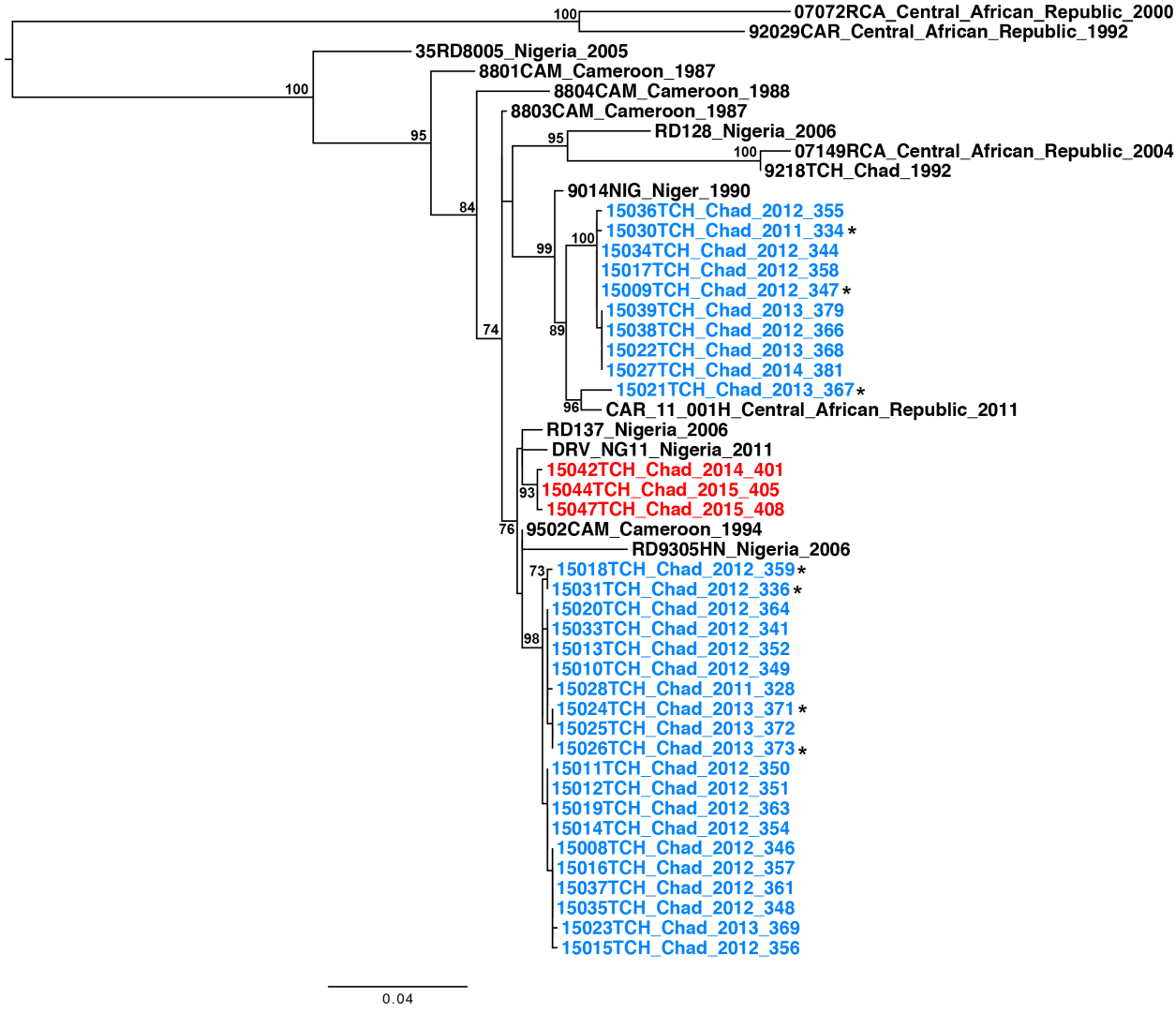

Fig. 4. Phylogeny of rabies strains isolated during and after the mass vaccination campaign. ML phylogeny of nucleoprotein sequences from rabies virus isolates collected in Chad from August 2011 to January 2015 and from sequences of previous isolates originating from Chad and from other neighboring countries. Sequences in blue were obtained from isolates collected in N'Djaména, Chad, during the period from August 2011 to January 2014, except 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 midpoint rooted for clarity only.

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 could be reduced. However, the duration of transmission interruption in our study was shorter than our model predictions presented here and in earlier work (*14*), indicating that there was likely to be a reintroduction of infection from latently infected dogs from the adjacent peri-urban areas, similar to what was reported in a recent study on rabies transmission in Bangui (*21*).

Our study suggested that urban centers may not be hotspots of dog rabies transmission, leading to spillover 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 (*10*). 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 (*22*).

There is still considerable uncertainty surrounding the role of density and spatial heterogeneity to external reintroduction in the transmission of dog rabies (*23*). 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 (*23*, *24*). Our study is limited by the low number of rabies virus strains that were isolated during the rabies mass vaccination campaigns. For this reason, the confidence intervals of the phylodynamically estimated basic reproductive number are wide.

Another limitation is that we could not identify the source of the rabies viruses that were reintroduced from outside the city. Further ongoing research will compare the isolated strains in this study with strains that will be collected countrywide.

Determining the optimal timing of vaccination campaigns in N'Djaména to maintain elimination requires knowledge of the rabies importation rate into the city. Our results are in line with a recent study in Bangui, Central African Republic, showing that rabies is continuously reintroduced in towns by human-mediated transport of dogs from surrounding peri-urban areas (*21*) and rapidly dispersed between cities (*10*). 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 dispersal of dog-associated rabies virus. Dog vaccination campaigns should also be complemented by affordable compulsory dog registration. Our study supports the need for improvements in and 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 (*25*).

In contrast to previous reports (*26*), our study suggests that mass vaccination of dogs, coupled with post-exposure prophylaxis, could be sufficient to eliminate rabies transmission in an African city, in both 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 (*27*). Dog vaccination campaigns will need to be adapted to local conditions to reach sufficient coverage (*28*, *29*), 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 toward the goal of eliminating dog rabies from Africa by 2030.

MATERIALS AND METHODS

Study design

The objective of this study was to test the hypotheses (i) that dog rabies transmission in an African city could be interrupted by the mass vaccination of dogs and (ii) that cities are potential hotspots of dog rabies transmission and reintroduction of rabies after vaccination would be slow. Given that we observed the reintroduction of dog rabies after the mass vaccination of dogs (in N'Djaména, Chad; see below), we hypothesized that rabies was reintroduced from outside the city. The research subjects were the weekly number of routinely recorded suspected rabid 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 dog suspected to be infected with rabies, information on exposed humans was collected on a routine basis. (ii) A dog rabies mass vaccination campaign was undertaken from October to December each in 2012 and 2013 (*18*). Blood was taken from 104 dogs in 2012 before the start of the mass vaccination campaign to assess the proportion of existing vaccination antibodies. The fluorescent antibody virus neutralization test was used for this purpose (*30*). Data on rabid dogs and exposed humans were collected up to the end of 2015 for this study. (iii) A mathematical model of dog-to-dog and dog-to-human rabies transmission was parameterized from the weekly number of rabid dogs and exposed humans collected before, during, and after the mass vaccination campaign. (iv) A phylogenetic and phylodynamic analysis of the rabies virus strains collected during the campaigns 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.

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 Recherche en Elevage pour le Développement by standard immunofluorescence as described in (*14*, *31*). Before 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 (ascertained by 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 (*18*). 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.). 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) (*7*). Every Friday to Sunday, 10 fixed post-vaccination teams were set up in 1 of 12 (13 in 2013) areas of the city

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 (*18*).

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

corresponding to administrative boundaries. Over the 3-day period, these teams vaccinated on average 1433 dogs (minimum, 24; maximum, 6460) in 2012 and 1709 dogs (minimum, 67; maximum, 4591) in 2013, depending on the sociocultural and ecological context of the city district (Table 1). Details of the operational performance and the results of the vaccination campaigns are published elsewhere (*18*).

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 (where the field team traveled in straight lines to count the number of owned and ownerless dogs) 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 (*18*).

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*)

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

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

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

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

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, Eq. 1 is a nonautonomous 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 ith 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 (Eq. 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*). Figure 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 (Eq. 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 show that with only background vaccination (no vaccination campaigns), when $R_c < 1$, the disease-free equilibrium point (Eq. 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. In addition, 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 it 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 Eq. 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 summarized 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 240 km^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

We 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 (Eq. 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

$$\frac{dU^{(i)}}{dt} = b_d(K_d - U^{(i)}) - \alpha_d^{-(i)}(t)U^{(i)} \quad (16A)$$

$$\frac{dV^{(i)}}{dt} = \alpha_d^{-(i)}(t)U^{(i)} \quad (16B)$$

where $U^{(i)}$ is the density of all unmarked dogs, $V^{(i)}$ is the density of dogs marked in campaign week i, and $\bar{\alpha}_d^{(i)}(t)$ 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

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

For simplicity, we ignore rabies transmission, assume that the dog population is at carrying capacity, and ignore the death of marked dogs or the loss of marking collars during the campaign week. Because the coverage assessment was conducted within 3 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

$$U^{(1)}(0) = K_d \quad (18A)$$

and from continuity

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

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

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

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

$$\alpha_0^{(1)} + \alpha_1^{(1)} = 0 \quad (19A)$$

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

The final density of dogs marked in a campaign week, $V^{(i)}$ (Eq.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 (240 km^2 in 2012 and 285 km^2 in 2013). Condition (Eq. 19) and the ordinary differential equations for the vaccination model (Eq. 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. Figure 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 1 year. Of these dogs, 58 had initial antibody titers that showed no previous vaccination and were successfully followed up over the entire year. After 1 year, 44 dogs had antibody titers above 0.5 IU, which, as a conservative estimate, we considered protective (*32*). 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 (Eq. 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 (Eq. 1) and to humans (Eq. 5) with the data for number of exposed humans.

To fit β_{dd}, we numerically simulate Eq. 1 using an adaptive step-size Runge-Kutta method and minimize the Euclidean distance between the simulated incidence of infectious dogs (from the first term of the right-hand side of Eq. 1) and the observed weekly incidence of infectious dogs in MATLAB. We assume that the probability of detecting a rabid dog is $p_d = 0.5$ so that, on average, there were twice as many rabid dogs as those detected. There are few 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 Eqs. 1 and 5 and minimizing 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%) (*33*, *34*).

Phylogenetic importation analysis

To investigate the hypothesis of a reintroduction of dog rabies 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 an 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 (*35*, *36*), 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 ML method available in PhyML 3.0 (*37*) using subtree-pruning-regrafting branch-swapping. The robustness of individual nodes on the phylogeny was estimated using 1000 bootstrap replicates and using the approximate likelihood ratio test with Shimodaira-Hasegawa–like supports (*38*). GenBank accession numbers of published sequences used in this tree are as follows: 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).

Sensitivity analysis

We conducted local and global sensitivity analysis of the control reproductive number, R_c (Eq. 10), to the model parameters (fig. S7). We used the normalized forward sensitivity index for the local analysis (*39*, *40*) at the parameter values defined in table S2 and the partial rank correlation coefficients for the global analysis (*41*), assuming that 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-induced mortality rate, μ_d, had a strong impact on the threshold for sustained transmission, R_c, whereas the other parameters had minimal impact.

Stochastic model simulations

We derived and numerically simulated a stochastic dog-to-dog transmission model based on Eq. 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 (*42*, *43*). Figure 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. Figure 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

Twenty-nine sequences of canine rabies viruses, collected between August 2011 and June 2013, were analyzed with Beast v2 (*44*). We chose a Hasegawa-Kishino-Yano model for substitutions with a relaxed log-normal clock (*45*). We assumed an exponential (0.001) prior for the mean rate, an exponential (0.3333) prior for the SD, and a log-normal (1,1.25) prior for kappa.

For the epidemiological model, we chose the birth-death skyline model (*46*). 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 (that is, every 4.8 months before 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 before 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) before the most recent sample. We ran the Markov chain Monte Carlo 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 (determined within Tracer v1.6.0, http://tree.bio.ed.ac.uk/software/tracer/), implying that we obtained substantial mixing.

To investigate sensitivity toward 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 before 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.

SUPPLEMENTARY MATERIALS

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Fig. S1. One-dimensional sensitivity analysis of simulation results on parameter values.
Fig. S2. One-dimensional sensitivity analysis of simulation results on parameter values with dog-to-dog transmission fixed as constant.
Fig. S3. Sensitivity analysis of the simulation results on the probability of detecting rabid dogs.
Fig. S4. Density of vaccinated dogs in N'Djaména in 2013 calculated on the basis of the data presented by Léchenne *et al.* (*18*).
Fig. S5. Schematic of mathematical model of rabies.
Fig. S6. Vaccination rates during the two campaigns in 2012 and 2013.
Fig. S7. Local and global sensitivity indices of the control reproductive number, R_c, to the model parameters.
Fig. S8. Sample simulation of the stochastic model, including the deterministic result.
Fig. S9. Results of the phylodynamic analysis showing median (red) and 95% HPD interval (black) for R_e through time.
Table S1. State variables of dog rabies transmission model.
Table S2. Parameters of the rabies transmission model with estimated values and sources.
RabiesData1.txt (incidence data)
RabiesData2.txt (genetic sequences of 33 dog rabies virus N-protein deposited in GenBank)

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Acknowledgments: We thank all the community leaders and vaccinators in N'Djaména. We thank T. Smith for helpful comments and discussions, and anonymous reviewers for helpful comments and suggestions. **Funding:** Funding for this study was provided by the Chadian government, the UBS Optimus Foundation (to M. Lechenne and R.M.) and the Swiss Federal Food Safety and Veterinary Office (to M. Lechenne), the European Union Seventh Framework Programme PREDEMICS (grant 278433), the European Research Council under the Seventh Framework Programme of the European Commission (PhyPD, grant agreement number 335529; to T.S.), the Wolfermann-Nägeli Foundation (to M. Lechenne), the Emilia Guggenheim Schnurr Foundation, and the Swiss National Science Foundation (grant 310030_160067/1; to M. Laager) **Author contributions:** J.Z. conceived the study. J.Z. and I.O.A. applied for funding. J.Z., M. Lechenne, M. Laager J.H., H.B., L.D., T.S., and N.C. wrote the manuscript. M. Lechenne, M. Laager, R.M., S.N., A.O., G.R., J.T., S.M., M.O., Y.M., and A.T. participated in the acquisition and analysis of data. J.Z., M. Laager, and N.C. contributed to the mathematical modeling. S.N. did all the rabies diagnostic work. A.O. coordinated the rabies mass vaccination campaign. J.Z., M. Lechenne, M. Laager, A.O., K.B., M.O., D.D.M., I.O.A, Y.M., A.T., J.H., H.B., and T.S. provided a substantial intellectual contribution. K.B., J.T., D.D. M., and I.O.A. provided administrative, technical, or supervisory support. D.D.M. took medical responsibility of the study. A.L., L.K., H.B., and L.D. participated in the analysis of molecular genetic data of the rabies viruses. N.C. supervised the mathematical modeling. **Competing interests:** The authors declare that they have no competing interests. **Data and materials availability:** The dog and human rabies incidence data and the genetic sequences of 33 dog rabies virus N-protein are available in the Supplementary Materials. The N-protein sequences are deposited in GenBank (KU564980–99, KY124532–41, MF538630–31, and MF577065) (https://www.ncbi.nlm.nih.gov/nuccore/). Requests for rabies virus strains require a material transfer agreement and should be sent to Service Naissengar, Institut de Recherche en Elevage pour le Développement, BP 433, N'Djaména, Chad (email: naissengar@gmail.com).

Submitted 16 March 2016
Resubmitted 7 December 2016
Accepted 15 August 2017
Published 20 December 2017
10.1126/scitranslmed.aaf6984

Citation: J. Zinsstag, M. Lechenne, M. Laager, R. Mindekem, S. Naïssengar, A. Oussiguéré, K. Bidjeh, G. Rives, J. Tessier, S. Madjaninan, M. Ouagal, D. D. Moto, I. O. Alfaroukh, Y. Muthiani, A. Traoré, J. Hattendorf, A. Lepelletier, L. Kergoat, H. Bourhy, L. Dacheux, T. Stadler, N. Chitnis, Vaccination of dogs in an African city interrupts rabies transmission and reduces human exposure. *Sci. Transl. Med.* **9**, eaaf6984 (2017).

Science Translational Medicine

Vaccination of dogs in an African city interrupts rabies transmission and reduces human exposure

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Sci Transl Med **9**, eaaf6984.
DOI: 10.1126/scitranslmed.aaf6984

Stemming the spread of rabies

Rabies is still a problem in some developing countries. The authors examine the impact of two previously conducted vaccination campaigns that vaccinated about 70% of dogs against rabies in N'Djaména, the capital city of Chad. Transmission modeling and phylodynamic analysis supported the idea that the sequential vaccination campaigns reduced dog-to-dog and dog-to-human rabies transmission. A phylogenetic analysis suggested that post-campaign reintroduction of rabies to N'Djaména may have occurred due to unvaccinated dogs in regions surrounding the city, rather than ongoing transmission within immunized areas in the city. Thus, mass vaccination efforts may be effective tools to stem rabies incidence in regions still afflicted by this fatal disease.