Supplementary information: Fluorescent biomarkers demonstrate prospects for spreadable vaccines to control disease transmission in wild bats

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1 Field studies of biomarker transfer and ingestion

1.1 Sampling schedule for field studies in Peru

Supplementary Table 1 describes the marking and sampling schedule for the 3 field sites we examined.

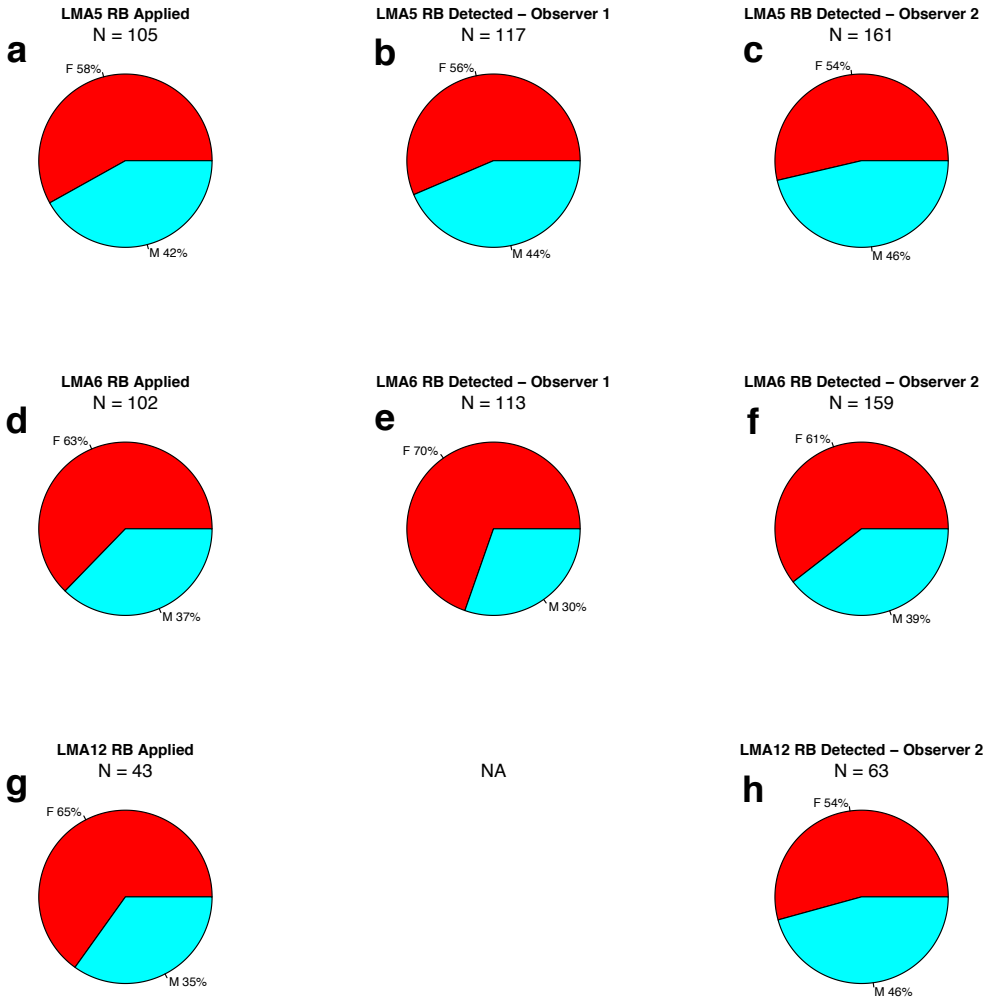
LMA5			LMA6			LMA12		
Date	Experiment	Treated/Caught	Date	Experiment	Treated/Caught	Date	Experiment	Treated/Caught
4/20/2017	RB mark	M 25/25, F 30/30, J 2/2	4/22/2017	RB mark	M 23/23, F 39/40, J 6/6	1/31/2017	RB mark	M 7/8, F 11/11, J 3/3
4/21/2017	RB mark	M 19/26, F 31/37, J 0/1	4/23/2017	RB mark	M 15/23, F 25/37, J 0/2	2/1/2017	RB mark	M 8/14, F 17/23, J 4/5
4/29/2017	RB recapture	M 0/47, F 0/36, J 0/6	5/1/2017	RB recapture	M 0/21, F 0/39, J 0/4	2/6/2017	RB recapture & UV mark	M 10/13, F 24/26, J 5/5
4/30/2017	RB recapture	M 0/29,F 0/38, J 0/3	5/2/2017	RB recapture	M 0/27, F 0/49, J 0/7	2/7/2017	UV recapture & RB recapture	M 0/13, F 0/8, J 0/0
5/6/2017	RB recapture	M 0/24, F 0/36, J 0/8	5/8/2017	RB recapture	M 0/33, F 0/46, J 0/7	*2/24/2017	RB recapture	M 0/11, F 0/3, J 0/3
5/7/2017	RB recapture	M 0/23, F 0/17, J 0/2	5/9/2017	RB recapture	M 0/23, F 0/38, J 0/7	*2/25/2017	RB recapture	M 0/10, F 0/3, J 0/0
5/18/2017	RB recapture & UV mark	M 23/24, F 27/27, J 3/3	5/22/2017	RB recapture & UV mark	M 15/15, F 16/16, J 4/4	*7/2/2017	UV mark	M 41/41, F 65/65, J 8/8
5/19/2017	UV recapture	M 0/10, F 0/14, J 0/0	5/23/2017	UV recapture	M 0/17, F 0/15, J 0/0	*7/3/2017	UV recapture	M 0/8, F 0/9, J 0/0
5/20/2017	UV recapture	M 0/7, F 0/11, J 0/0	5/24/2017	UV recapture	M 0/5, F 0/14, J 0/0	*7/4/2017	UV recapture	M 0/9, F 0/11, J 0/0
6/21/2017	UV mark	M 27/27, F 28/28, J 7/7	6/29/2017	UV mark	M 17/17, F 26/26, J 0/0	*7/29/2017	UV mark	M 45/45, F 67/67, J 11/11
6/22/2017	UV recapture	M 0/7, F 0/10, J 0/0	6/30/2017	UV recapture	M 0/5, F 0/6, J 0/0	*7/30/2017	UV recapture	M 0/11, F 0/25, J 0/0
7/25/2017	UV mark	M 26/26, F 28/28, J 10/10	7/1/2017	UV recapture	M 0/4, F 0/8, J 0/0			
7/26/2017	UV recapture	M 0/7, F 0/2, J 0/0	7/22/2017	UV mark	M 20/20, F 18/18, J 0/0			
7/27/2017	UV recapture	M 0/4, F 0/4, J 0/0	7/23/2017	UV recapture	M 0/4, F 0/6, J 0/0			
			7/24/2017	UV recapture	M 0/3, F 0/2, J 0/0			

Supplementary Table 1: Field experiment schedule in three wild vampire bat colonies in Peru. RB/UV mark indicates dates when rhodamine b or UV powder was applied to captured bats. RB/UV recapture indicates dates for RB sample collection and UV powder monitoring, respectively. The treated/caught column lists, by sex or juvenile, the number of bats treated and caught at each sampling date. Bats listed as a juvenile were not included in the male or female groupings. * Designates sampling from the relocated LMA12 roost.

2 Contact heterogeneities among demographic groups of vampire bats

2.1 Estimation of sex-specific contact rates from UV powder transfer

A worked example of Eq. 1 from the main text, using data from the May sampling at LMA6 for male transfers can be seen in Eq. 2 & 3 below. During the marking period, green UV powder was applied to 15 adult male bats. At the 24-48h recapture period, 4 of 24 captured females were green UV positive and 3 of 17 captured males were green UV positive. The estimated population size of LMA6 was 207 bats (119 females and 88 males). Since 15 males were initially marked with green UV, only 73 males had the potential to test newly positive, while all 119 females were available for green UV transfer. As



Supplementary Figure 1: Sex ratio of application positives (left column) and transfer positives results from two observers (center and right columns). While the proportion of transfer positive males increased marginally from the levels at application, differences were not statistically significant (chi-squared test: $p > 0.05$ for all comparisons).

a reminder, we have reproduced Eq. 1 from the main text;

$$\text{Contact Rate} = \frac{\frac{N_{pos_X}}{UM_X} * N_{UM_X}}{M_X}. \tag{1}$$

Male-to-female (Eq. 2) and male-to-male (Eq. 3) contact rates are therefore estimated as follows:

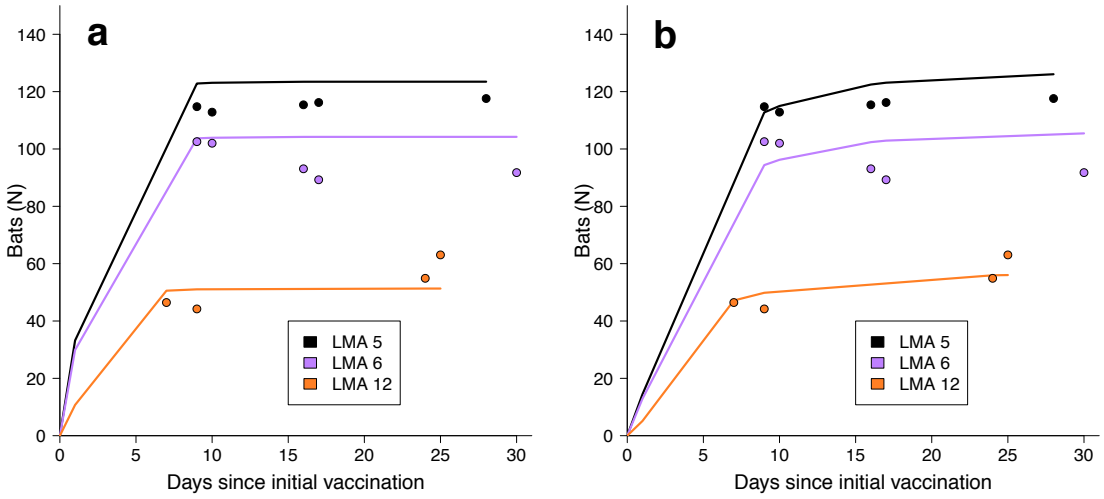
$$\text{Male-to-Male} = \frac{\frac{3}{17} * (73)}{15}. \tag{2}$$

$$\text{Male-to-Female} = \frac{\frac{4}{24} * (119)}{15}. \tag{3}$$

3 Parameter estimation and mathematical modeling

3.1 Least-squares estimation of RB transfer

A deterministic least-squares compartmental model (Fig. 3b) was used to estimate the biomarker (RB) transfer rate (Supplementary Figure 2). Specifically, we estimated this parameter using application and transfer positive time series data (i.e. the number of successful transfers over time) and the population estimates from each sampling location (Fig. 1). Transfer rates were estimated using both a 2-day and a 6-day transfer period (Supplementary Figure 2 & Supplementary Table 2). To be conservative, we used the lower mean transfer rate value (1.83) for the full outbreak model examined in the main text. The transfer parameter was estimated using least-squares in the deSolve package in R. Additional details are provided in Supplementary Software 1.



Supplementary Figure 2: Estimating the biomarker transfer parameter from field data using a least-squares fit. This parameter was estimated for both 2-day (a) and 6-day (b) transfer periods, with minimal differences in estimated transfer rate values (Supplementary Table 2). Curves were produced from plotting the model with the best-fit β values for each site.

Transfer Time	LMA5	LMA6	LMA12	Mean
2 days	2.11 *(2.10-2.12)*	1.92 *(1.92-1.94)*	1.45 *(1.41-1.48)*	**1.83**
6 days	2.24 *(2.21-2.23)*	1.99 *(1.97-2.00)*	1.74 *(1.70-1.79)*	**2.00**

Supplementary Table 2: β estimates from two different transfer periods (2 or 6 days) with 95% confidence intervals.

3.2 Mechanistic model of rabies control with spreadable vaccines

We built a stochastic susceptible (S), application positive (A), transfer positive (T), immune (I), exposed to rabies (E), and rabid (R) model (Fig. 3a) to understand how the application and transfer of a vaccine or poison would alter rabies outbreaks in bat colonies. The structure of vaccination and culling models were identical except that vaccines were not assumed to protect bats that were incubating previous rabies exposures, but vampiricide was assumed to kill incubating bats (compare Eq. 8 versus Eq. 9 below).

For both vaccination and culling models, all bats began in the S class, where they could be applied the orotopical gel at rate α, be bitten by a rabid bat at rate θ, or be exposed to the gel at rate β. Bats in all classes had a natural death rate, ω, equivalent to a lifespan of 3.5 years. Bats entered the S class through births (η, Eq. 5) or through the decay of natural immunity (ϕ), as described in Turmelle *et al.* [1] (see Supplementary Table 3 for details).

$$\frac{dS}{dt} = N\eta + I\phi - S(\alpha + \omega) - \beta\left(\frac{SA}{N}\right) - \theta\left(\frac{SR}{N}\right) \tag{4}$$

Birth rate, η, was set equal the natural death rate of 3.5 years, with a seasonal birth pulse early in the year, estimated in Blackwood *et al.* [2] and adding $3.475 * 10^{-4}$ to keep all values positive.

$$\eta = (8.4563 * 10^{-4})\cos(2\pi(t - 32.6747)/365) + 0.0003475 \tag{5}$$

Bats entered the A class through the manual application of a topical gel (α) and were able to transfer the gel for 2 days before moving into the T class at rate ψ, where they were no longer able to transfer the vaccine.

$$\frac{dA}{dt} = S\alpha - A(\psi + \omega) \tag{6}$$

Naturally immunized bats (I) arrived from the E class at rate λ. It was possible for an immune bat to re-enter the susceptible class through loss of natural immunity, ϕ. Bats in the I class were able to move to the T class through contact with the A class through gel transfer (β).

$$\frac{dI}{dt} = E\lambda - \beta\left(\frac{IA}{N}\right) - I(\phi + \omega) \tag{7}$$

All exposed bats entered from the S class after being exposed to rabies (θ). They left by developing

immunity (λ) or became rabid (δ). These values were estimated by Blackwood *et al.*, where 10% of exposed bats developed rabies and 90% acquired transient immunity [2]. In the vaccination models, vaccines were assumed not to protect bats that were exposed to rabies (E) prior to vaccination since the vaccine is a prophylactic meant to prevent infection, rather than a post-exposure prophylaxis. Instead those bats had the same probability of naturally surviving the rabies exposure and if they survived, they transferred to the T class (Eq. 8). In contrast, for the culling models exposed bats that ingested poison during the incubation period were killed regardless of whether or not they may have developed rabies (Eq. 9).

$$\frac{dE_{Vac}}{dt} = \theta\left(\frac{SR}{N}\right) - \beta\left(\frac{EA}{N}\right)\lambda - E(\lambda + \delta + \omega) \tag{8}$$

$$\frac{dE_{Cull}}{dt} = \theta\left(\frac{SR}{N}\right) - \beta\left(\frac{EA}{N}\right) - E(\lambda + \delta + \omega) \tag{9}$$

Rabid bats entered through those in the E class that developed rabies (δ) and left by dying from rabies (τ).

$$\frac{dR}{dt} = E\delta - R(\tau + \omega) \tag{10}$$

Bats entered the T class by decaying in from the applied class after two days (ψ) and from the S, I, or E classes through transfer following contact with a bat in the A class

$$\frac{dT}{dt} = A\psi + \beta\left((S + I)\frac{A}{N}\right) + \beta\left(\frac{EA}{N}\right)\lambda - T\omega \tag{11}$$

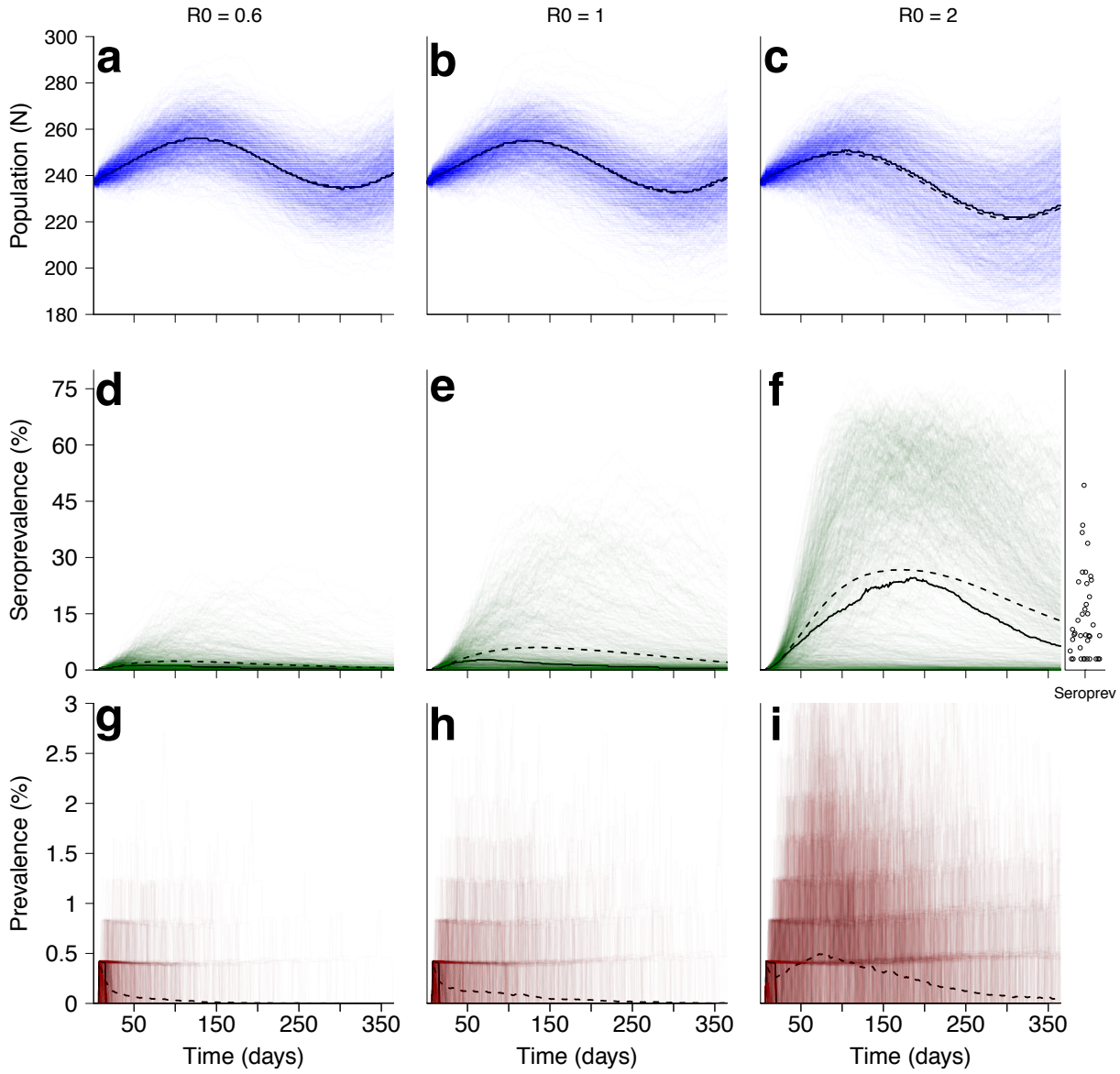
Parameters for the mathematical models were obtained from previous field or modelling studies and controlled infections in captive bats (Supplementary Table 3). Annotated R scripts for conducting mathematical modeling are provided in Supplementary Software 2. All models were implemented using the tau-leap (Gillespie algorithm) method in R.

Description	Parameter	Value	Citation/notes
Seasonal birth rate	η	See Eq. 5	[3, 4]
Applied bats	α	varies (0–100%)	NA
Bat lifespan	$1/\omega$	3.5 years	[5, 6]
Duration of orotopical gel transfer	$1/\psi$	2 days	48 hour transfer period
Immunity length	$1/\phi$	4.5 months	[2]
Vaccine & vampiricide transfer	β	0.9322064	Fitted from field data (Supplementary Figure 2)
Duration of time in rabid class	$1/\tau$	11 days	[7]
Rabies transmission rate	θ	R_0 = 0.6–2	[2, 8]
Mean time in E class	-	21 days	[7]
Develop immunity	λ	0.90	[1, 2], see text
Develop rabies	δ	0.10	[1, 2], see text

Supplementary Table 3: Parameters used in the rabies transmission and control models.

3.3 Model validation

We validated our model against previously published work examining bat population dynamics [9], seroprevalence [2], and prevalence [10] associated with rabies in bats (Supplementary Figure 3). Specifically, we expected our model to produce short-lived outbreaks that had minimal impacts on total bat population size, had moderate seroprevalence (generally, 0–40%), and were associated with a low prevalence of active infection (1%). We simulated the model for three rabies (R_0) values without vaccination or culling to demonstrate that the model generates these dynamics in the absence of interventions. Supplementary Figure 3 shows bat population dynamics, seroprevalence, and infection prevalence across 1000 simulated introductions of rabies along with the seroprevalence data from Blackwood *et al.* [2] (Supplementary Table 1, all observations of colonies with N>1) in order to compare to the range of seroprevalence values from our simulations to field observations. This model generated the expected prevalence during outbreaks and spanned the expected variation in seroprevalence. Moreover, the model generated qualitatively similar dynamics to the current understanding of rabies transmission dynamics described above.



Supplementary Figure 3: 1000 simulations of the base model (Fig. 1a) with no vaccination or culling. Top row is the colony population (start N=237), middle row is seroprevalance, and bottom row is prevalence. Columns represent simulations assuming different levels of (R_0). Colored lines indicate individual simulations with the median simulation value in a solid black line and mean simulation value as a dashed line. To the far right of the seroprevalence column are the field seroprevalence data from Blackwood *et al.* [2]

3.4 Description of timelines used to model alternative intervention strategies

We explored three control strategies for rabies outbreaks: preventative, proactive, and reactive (Supplementary Figure 4) [11]. Preventative involved applying an orotopical gel (either a vaccine or vampiricide) to bats one week before a rabid bat was introduced to the colony; proactive was the same except that 10.5% of the population (N=25 bats) were considered to be protected by previous natural exposure [2].

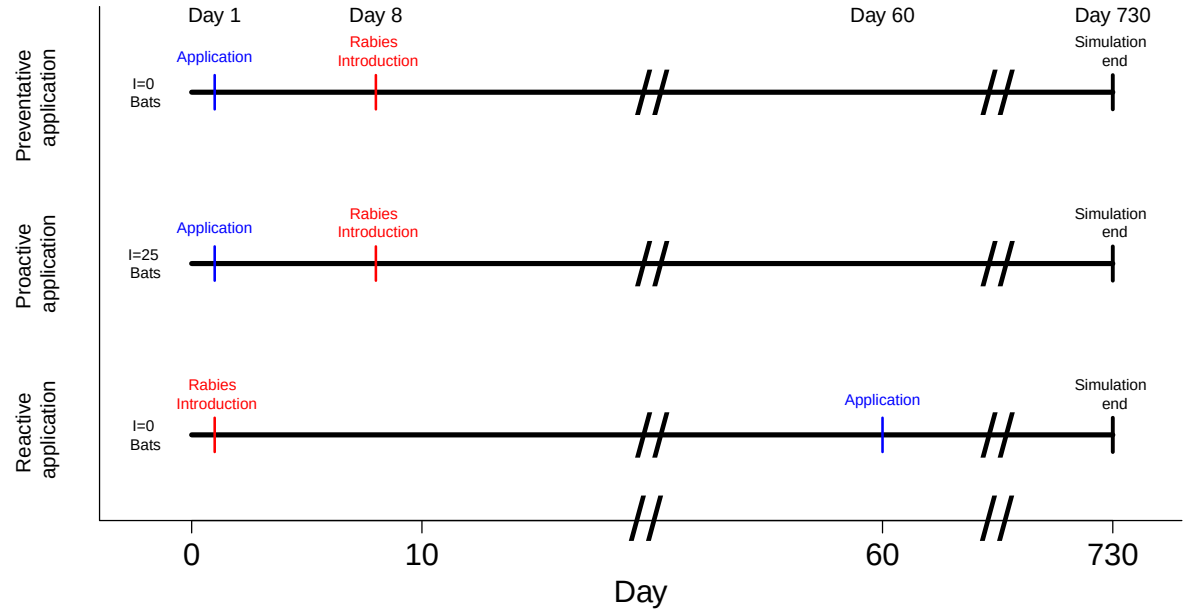
Reactive control introduced a rabid bat on day 1 and simulated orotopical application on day 60. This delay was intended to account for time that would be required for one round of transmission within the bat colony (21 day incubation period), infections in livestock to occur (21 day incubation period), be detected and be diagnosed (11 days), as well as time for logistical planning and implementation of campaigns (7 days). Reported incubation periods in livestock range from 12-40 days in experimental infections, depending on viral variant, dose, and the site of inoculation and are likely more variable in natural infections [12]. We therefore used 21 days as a conservative estimate. The delay between detection of outbreaks and laboratory diagnostics was calculated from two years of data (2013-2014) from the National Service for Agrarian Health of Peru (SENASA), which described delays ranging from 3 to 148 days (median = 11; mean = 15.02; N = 264 suspected outbreaks) [13]. Finally, our estimate of the timing of reactive control did not account for known under-reporting of VBR cases in livestock, which would further delay implementation of some campaigns [14]. Our simulations therefore represent the best-case scenario for the reactive control strategy. We expect that longer delays arising from longer incubation periods in livestock or failure to report early incidences of mortality would diminish efficacy of both intervention types since control would be implemented after rabies has naturally gone extinct from the local bat population, effectively becoming proactive control (Supplementary Figure 4).

3.5 Introductions resulting in invasion

Fig. 4 from the main text presented results from all 5000 stochastic simulations at each level of initial vaccination (N=105,000 for each R_0 value). Because we introduced only a single rabid bat to the model, many simulations, especially at lower R_0 values, resulted in stochastic die off or failure to transmit rabies from this infected bat. Supplementary Figure 5 illustrates the outbreak size of rabies when the introduced bat infected at least one other bat. Increased initial vaccine application led to smaller outbreaks.

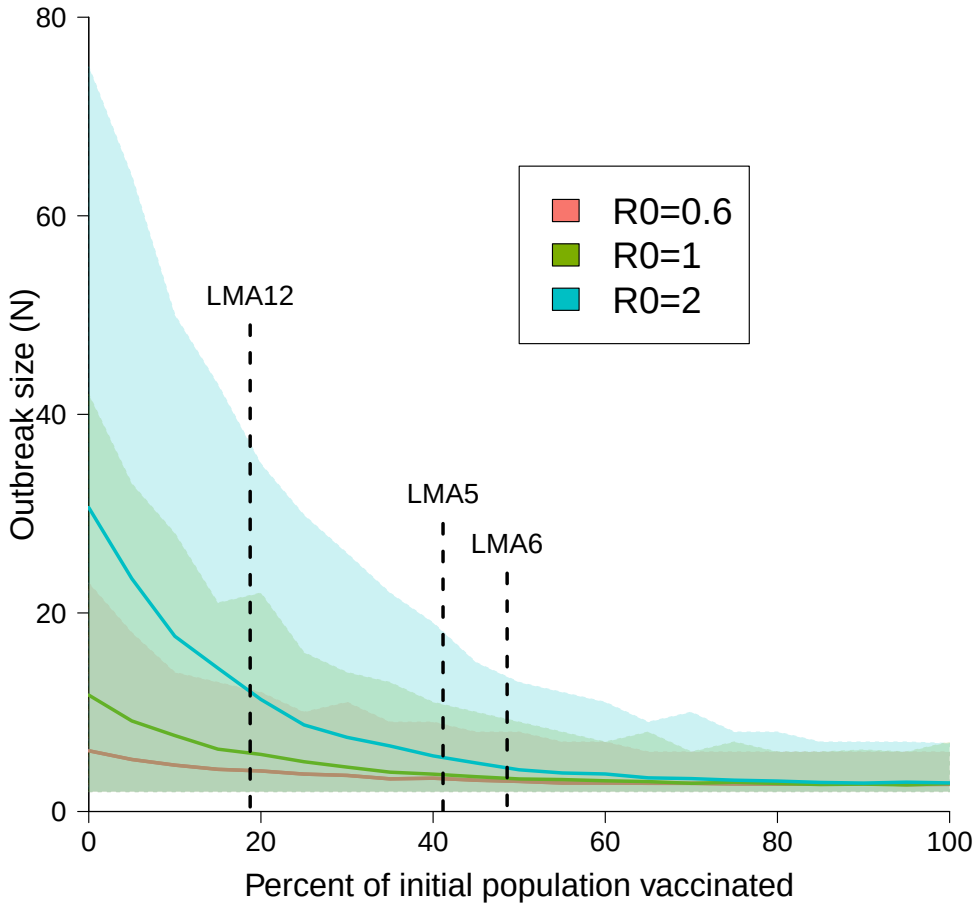
3.6 Sensitivity analysis of increased orotopical transfer of vaccines and vampiricide

Given our expectation that RB transfer rates are a lower bound on vampiricide or vaccine spread, we simulated effects of increased levels of transfer on rabies dynamics. For completeness, we also simulated decreased vaccine/vampiricide spread relative to RB. We explored values ranging from 75% less (.463 other bats) than the estimated RB value (1.85 other bats) to ten times that (18.5 other bats), which



Supplementary Figure 4: Timing of application and outbreaks for preventative, proactive, and reactive model simulations. Application indicates the date of either vaccine or vampiricide application. Values to the left of each timeline (I) indicate the number of bats assumed to have protective immunity from surviving previous natural rabies exposures

exceeds the largest reported value of vampiricide spread [15]. We simulated the percent of the colony that vampiricide or vaccine was initially applied to (0 to 100, at increments of 5%) and the RB transfer multiplier (0.25–10) for each rabies (R_0) value under each of the three intervention strategies (preventative, proactive, and reactive), 5000 times. Supplementary Figure 6 shows the difference between vaccination and culling at each point. This highlights that the benefits of preventative and proactive vaccination at low application levels hold even if both agents are far more transmissible than assumed in our main models, while culling is never favored. For the reactive strategy, vaccination was slightly favored at low levels of application when the spread was equal to, or less than our field estimate, while culling was advantageous if large fractions of bats could be captured or if agents spread twice as efficiently as our field data suggest. Supplementary Figures 7-8 show the reduction in rabies cases due to vaccination and culling, respectively. In most simulations, increasing the orotopical transfer rate above 2x past 25% initial application resulted in minimal additional reduction in rabies cases, indicating diminishing returns. This is likely because at increased application or transfer levels, most of the colony had already either been

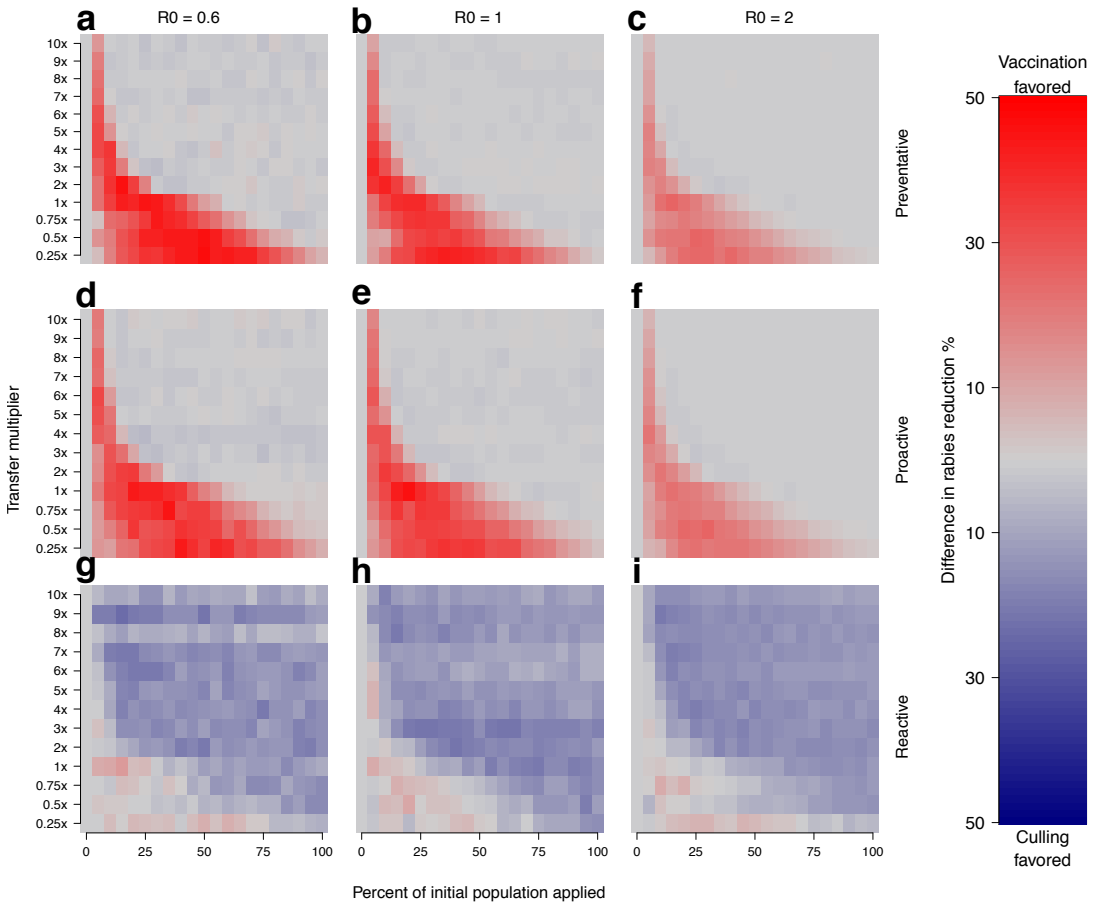


Supplementary Figure 5: Size of rabies outbreaks sizes after vaccination in simulations where rabies spread following introduction. Lines show mean rabies outbreak sizes after a single rabid bat is introduced to the colony one week following immunization. Colors represent varying degrees of rabies R_0, with 95% confidence intervals calculated from all simulations that resulted in rabies transmission. Dashed lines indicate the percent of bats that RB was applied to in each of our study sites.

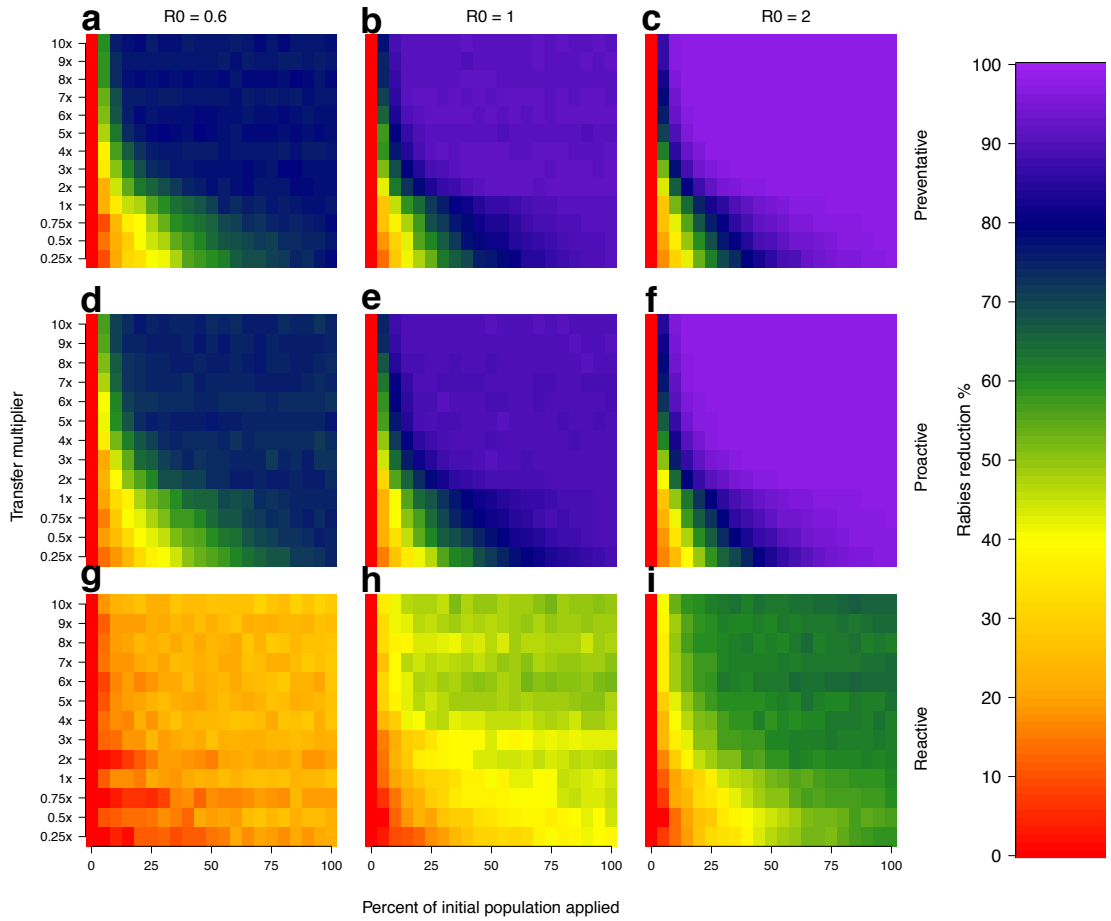
protected or culled.

Although we expected vaccines to transfer more, not less, efficiently than vampiricide (see main text), we also conducted an analysis where we allowed vampiricide to spread better than vaccines to identify how much more a transferable vampiricide would need to be to eclipse vaccination as the preferred disease control agent. Supplementary Figure 9 displays the averaged results of these simulations at each combination. At realistic application levels when rabies (R_0) was 2 or the control strategy was reactive, increasing vampiricide transfer rates showed significant additional reductions in rabies cases when compared to the empirically estimated vaccine transfer rates. Under the two lower rabies (R_0) values

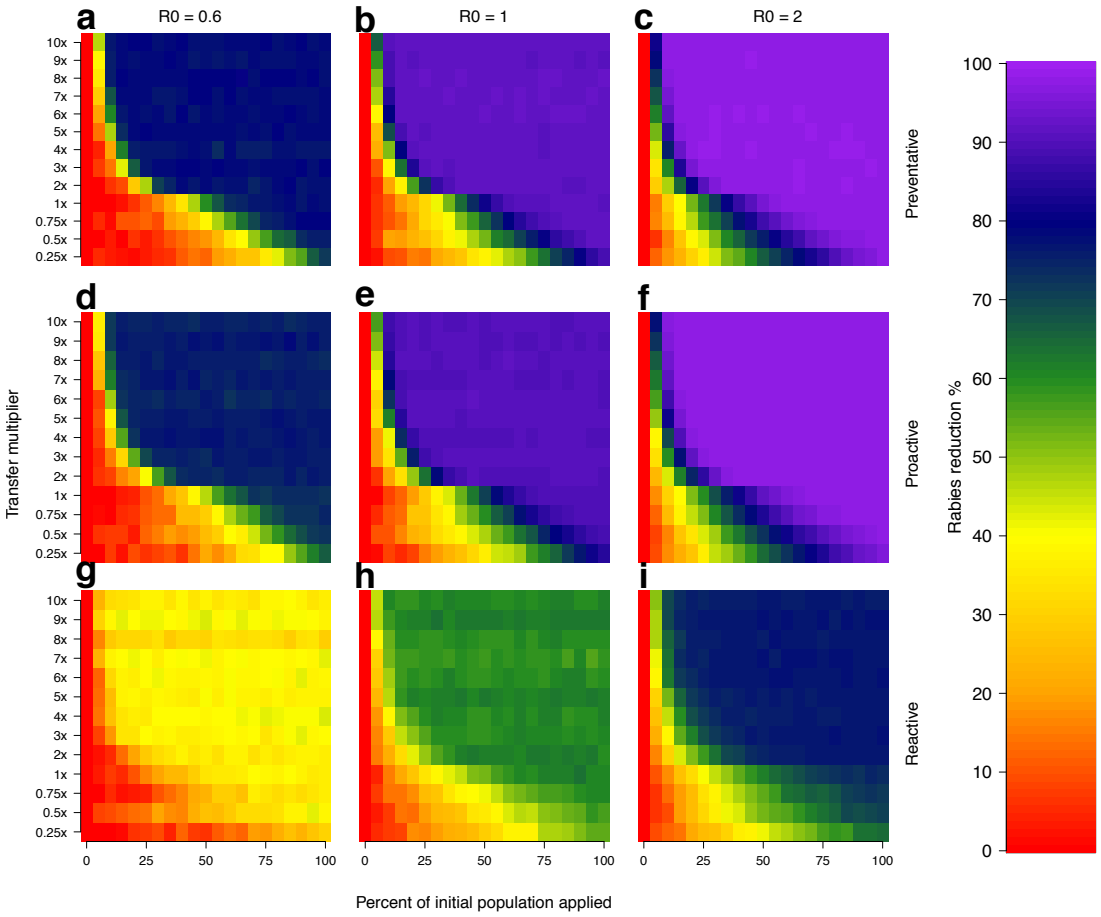
148 for both the preventative and proactive strategies, vampiricide was never more effective than vaccines at
149 reducing rabies outbreaks under realistic application levels.



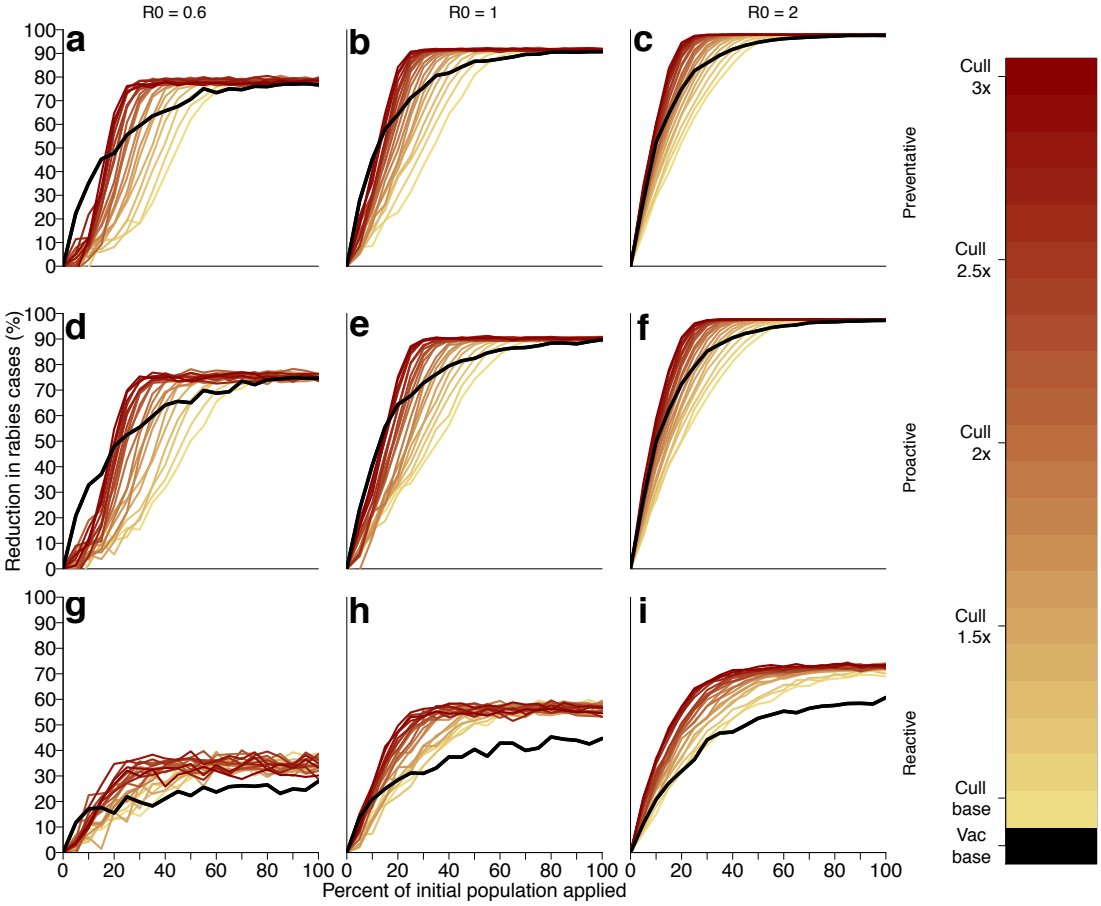
Supplementary Figure 6: Differences between vaccination and culling in the percent reduction of rabies cases compared to a no intervention scenario. The figure shows results of simulations assuming vaccines had transfer rates that were lower than (<1x), equal to (1x) or greater than (>1x) those observed in field studies with RB (1.85 transfers per treated bat) across various levels of initial application. For example, 10x indicates that both vampiricide and vaccines spread 10 times better than RB and 0.25x implies that real interventions spread only 25% as effectively as RB. Colors indicate the difference in reduction between vaccination and culling with red favoring vaccination and blue favoring culling. Results from the main text correspond to the 1x row in this figure.



Supplementary Figure 7: Simulated reduction in rabies outbreak sizes under various levels of initial vaccine application assuming vaccines had transfer rates that were lower than (<1x), equal to (1x) or greater than (>1x) those observed in field studies with RB (1.85 transfers per treated bat).



Supplementary Figure 8: Simulated reduction in rabies outbreak sizes under various levels of initial vampiricide application assuming vampiricide had transfer rates that were lower than (<1x), equal to (1x), or greater than (>1x) those observed in field studies with RB (1.85 transfers per treated bat).



Supplementary Figure 9: Simulated reduction in outbreak sizes due to vaccination (black) and culling (light yellow) at the estimated RB level and if assuming that vampiricide spreads up to 3x more effectively than a vaccine.

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