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

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Abstract

Vaccines that autonomously transfer among individuals have been proposed as a strategy to control infectious diseases within inaccessible wildlife populations. However, rates of vaccine spread and epidemiological efficacy in real world systems remain elusive. Here, we investigated whether topical vaccines that transfer among individuals through social contacts can control vampire bat rabies, a medically and economically important zoonosis in Latin America. Field experiments in 3 Peruvian bat colonies which used fluorescent biomarkers as a proxy for the bat-to-bat transfer and ingestion of an oral vaccine revealed that vaccine transfer would increase population-level immunity up to 2.6 times beyond the same effort using conventional, non-spreadable vaccines. Mathematical models demonstrated that observed levels of vaccine transfer would reduce the probability, size, and duration of rabies outbreaks, even at low, but realistically achievable levels of vaccine application. Models further predicted that existing vaccines provide substantial advantages over culling bats, the policy currently implemented in North, Central, and South America. Linking field studies with biomarkers to mathematical models can inform how spreadable vaccines may combat pathogens of health and conservation concern prior to costly investments in vaccine design and testing.

Introduction

Infectious diseases of wildlife cause threats to human and animal health globally [1]. Controlling these pathogens within their natural animal hosts can offer substantial health, economic, and conservation benefits. For example, baited vaccines targeting wildlife reservoirs eliminated fox rabies from western Europe [2] and currently confine raccoon rabies to the eastern United States [3]. However, for many important wildlife diseases, delivery systems to vaccinate a sufficient proportion of host populations to control pathogens are unavailable, and direct (i.e., individual-based) vaccination is logistically prohibitive. Interventions that spread from treated to untreated individuals are increasingly used to control arthropod-borne diseases [4, 5, 6] and have been proposed as a solution to mass vaccinate wildlife since each unit of vaccine deployed would immunise multiple individuals [7, 8]. However, as seen with poliovirus eradication efforts, vaccines that sustain transmission may revert to virulent phenotypes [9], and in wildlife, vaccine shedding may have unanticipated ecological or evolutionary impacts on competing pathogens or host species [10]. Vaccines with deliberately constrained capacity to transmit are therefore currently the preferred candidates for real world applications. Encouragingly, theoretical models suggest that such weakly-transmissible vaccines consistently outperform individual-based vaccination, increasing the

potential for disease eradication [11]. Despite this theoretical promise, spreadable vaccines have only rarely been tested in natural systems (i.e., rabbit hemorrhagic disease and myxomavirus in rabbits [12]). This gap between theory and practice reflects a number of limiting factors: vaccines may be unavailable; epidemiological knowledge of the target pathogen or the dynamics of vaccine spread may be insufficient to guide deployment or predict benefits; and losses incurred under existing management strategies may be considered insufficient to warrant the real or perceived risks of novel interventions.

Vampire bat rabies (VBR), a universally lethal viral zoonosis found throughout Latin America, represents a tractable system to explore the implementation of spreadable vaccines to protect human and animal health. Where common vampire bats (*Desmodus rotundus*) routinely feed on human blood, VBR is estimated to cause up to 960 deaths/100,000 people [13]. Losses from livestock mortality exceed $50 million annually and disproportionately affect impoverished, rural communities [14, 15]. Existing management strategies have been unable to mitigate the burden of VBR. Vaccines for humans and livestock are protective, but high costs and inaccessibility to remote areas limit uptake [16]. Rabies control programs also cull vampire bats using anticoagulant poisons ('vampiricide') which are applied in topical gels that spread among bats through social contacts and are ingested during grooming (here termed 'orotopical transfer') [17]. While culling reduces bat bites on humans and livestock, effects on rabies transmission remain controversial [18, 19]. Moreover, heightened bat dispersal following culls is predicted to exacerbate VBR transmission by increasing the mixing of bat colonies, analogous to the increased transmission of bovine tuberculosis induced through effects of culling on badger home range size [20, 21]. Oral rabies vaccines that spread by the same orotopical mechanism as vampiricide offer an alternative approach. These recombinant virally-vectored vaccines can indirectly immunise untreated bats in captivity, but have never been tested in wild populations [22, 23, 24, 25]. Several unresolved questions must be answered prior to deploying vaccines for large scale bat rabies control: (1) how efficiently would vaccines transfer among wild bats?, (2) are certain demographic groups of bats especially difficult to vaccinate or especially effective disseminators of vaccines?, (3) would the resulting degree of immunisation significantly reduce rabies transmission?, and (4) would vaccines reduce human and livestock rabies risk more effectively than the current policy of culling? We address these questions by coupling field studies that used fluorescent biomarkers to quantify contact networks and orotopical transfer among wild vampire bats with mathematical models that simulated how vaccines and vampiricide, which spread by identical mechanisms, would impact the size, duration, and probability of rabies outbreaks.

Results

Biomarker transfer and ingestion shows potential for high vaccine coverage in wild vampire bats

We estimated the potential for a spreadable vaccine to transfer among bats using Rhodamine b (RB), a biomarker that when ingested leads to long-lasting fluorescence in hair follicles in diverse mammalian species [26, 27, 28]. After applying a gel-formulation of RB topically to bats in three colonies in Peru (colony sizes: 207-257 individuals, sex ratios: 43.1-50.6% male), orotopical transfer and ingestion was monitored by fluorescent microscopic analysis of hair samples collected in subsequent capture sessions, with fluorescence indicating RB consumption (Supplementary Table 1). At two sites (LMA5 & LMA6), an estimated 84 and 92% of bats ingested RB, either following topical application or transfer from treated bats (Fig. 1). The third colony (LMA12) relocated to an undocumented roost soon after RB treatment, which diminished captures during the monitoring period relative to the estimated colony size (Supplementary Table 1); consequently, the overall estimated coverage dropped to 28.8% (Fig. 1). Nevertheless, the percentage of sampled LMA12 bats at the end of the monitoring period that were RB positive (48.3%, aggregating days 24 and 25), was not statistically different from the percentages at the final capture dates in the other two colonies (58.3 and 70.0%; Chi-squared test, $\chi^2 = 3.2$, df $= 2$, p $= 0.21$). We further characterized patterns of RB uptake among demographic groups of bats. The sex ratios of transfer positive bats became slightly more male biased (3-11% increases, depending on the colony) relative to the sex ratios of bats that were treated with RB, suggesting elevated transfer to males; however these increases were not statistically significant (χ^2 tests, all p > 0.05; Supplementary Figure 1). We observed RB transfer to untreated bats in all three age classes. Across all colonies, 86.1% of sampled adults (N = 374), 75.8% of sampled juveniles (N = 33), and 94% of sampled subadults (N = 34) became RB positive through transfer during the 1 month monitoring period. Consequently, these results implied that vaccines deployed over only two days of captures (17-50% of total colony size) would yield high levels of population immunity across age classes due to orotopical transfer.

Contact heterogeneities among demographic groups of vampire bats

We next examined whether contact heterogeneities might make certain demographic groups of bats especially effective or ineffective spreaders of vaccines using ultraviolet (UV) powder marking, wherein different

age/sex groups of bats were treated with different colors of UV powder, and transfer to untreated bats was monitored over two subsequent capture nights [29, 30]. Across 3 replicate UV treatments per colony, we documented 78 instances of UV powder transfer, leading to estimated contact rates ranging from 0.23–1.25 per treated bat (Fig. 2). Male bats had significantly higher contact rates than females (Wilcoxon rank sum test, W = 91, p = 0.025; mean = 1.14 versus 0.67) and had similar rates of male-to-male and male-to-female contacts (Wilcoxon rank sum test, W = 42, p = 0.93). In contrast, females preferentially contacted other females (Fig. 2a). Transfer to juveniles could not be reliably quantified because these bats were mostly too young to forage independently and our capture method during the monitoring period required bats to fly out of roosts. Nevertheless, a single juvenile bat captured had UV transfer from a female. In contrast, transfer from juveniles to adults should have been detectable if it occurred due to the greater ease of capturing adults. However, none of the 27 marked juveniles transferred UV powder to adults. Together with our data illustrating high rates of juvenile exposure to RB, these findings suggest that vaccine deployments should target adults rather than juveniles. Targeting adults would further be logistically advantageous since it would minimize social disruption of colonies that results from entering roosts to capture juveniles.

Epidemiological models show spreadable vaccines outperform culling for rabies control

We adapted a deterministic compartmental model of VBR persistence [20] to incorporate an orotopically spread vaccine and used least-squares (Fig. 3b) to estimate expected *per capita* vaccine transfer rates from the time series of RB transfers observed in our field studies, assuming that RB transfer equated to lifelong protection. This analysis revealed that each treated bat transferred RB to 1.45—2.11 untreated individuals, up to a 2.6-fold increase in population level coverage relative to the coverage that would be expected using conventional, non-spreading vaccines (Fi.g 4b, Supplementary Figure 2 and Supplementary Table 2). We simulated the ability of spreadable vaccines to control rabies across the range of R_0 values (0.6 to 2) suggested in the rabies literature [20, 34, 35]. Applying vaccines to approximately 20% of bats vaccinated 40% of the population and reduced rabies outbreak size by 45 to 75%, depending on the assumed R_0 of rabies (Fig. 4a,b,c). However, applying vaccines to a higher proportion of bats had diminishing returns for both the proportion of the colony that was ultimately protected and for rabies control. If vaccines were applied to >30% of bats, additional reductions in rabies outbreak sizes were less than 5%, meaning a 5% increase in initial application led to less than a 5% reduction in outbreak sizes (Fig. 4d). The greatest

benefit (reduction in outbreak size relative to effort) occurred at vaccination levels below 15%.

We next compared the relative efficacy of vaccination and culling across three epidemiological scenarios [21]. representing different management strategies: (1) a 'preventative' approach, where vaccine/-vampiricide was applied to prevent VBR invasion into historically rabies–free bat populations [33, 36]; (2) a 'proactive' approach, which represented an intervention in a VBR endemic area, but in a colony that was not currently infected; and (3) a 'reactive' approach where intervention followed 60 days after a single VBR-infected bat was introduced to the colony (Supplementary Figure 4). Although we simulated outcomes across the full possible range of application effort (i.e., 0–100% of bats treated), we focused on lower application levels since capturing large proportions of bats across large geographic areas would be impractical for rabies control campaigns. Indeed, mark-recapture studies across multiple vampire bat colonies in Peru suggested that on average, <10% of colonies were captured in a single night [19]. At realistic levels of application, vaccination consistently reduced the probability of viral invasion, outbreak size, and outbreak duration more effectively than culling, regardless of whether control was preventative, proactive, or reactive (Fig. 5). Culling was only favored when at least 25% of the colony was treated, and only in reactive scenarios. However, the advantage of culling on outbreak size was relatively small – a maximum of a 20% greater reduction – relative to the larger advantages observed when vaccination was favored (up to 45% greater reduction), and differences in outbreak duration were negligible until much larger proportions of bats were culled (Fig. 5). In preventative and proactive scenarios, culling required capturing and treating much larger proportions of vampire bat populations (e.g., >60%) to match the reduction in outbreak size and duration achieved by vaccination (Fig. 5). In fact, the only discernible difference at higher application levels was a greater reduction in the duration of outbreaks by culling; however, this was due to near complete extinction of bat colonies. Even if this degree of bat culling were achievable and ethically acceptable, it may not be a favorable long-term strategy since populations that recovered from culls would be entirely susceptible to rabies, potentially causing larger future outbreaks [37].

Our *per capita* transfer rates likely represented lower bounds of vaccine and vampiricide spread since the relatively high percentage of bats initially treated with RB left few others available to be exposed via transfer in two of our colonies and relocation of the third colony reduced capture rates during the monitoring period. Indeed, some studies have suggested higher transfer rates of vampiricide [17, 39]. We therefore conducted a sensitivity analysis where both vaccines and vampiricide spread up to 10-fold

more efficiently than our RB estimates, values that exceeded the largest transfer rates suggested from vampiricide releases. Additionally, we considered transfer rates that were up to 75% less efficient than our RB estimates. This analysis demonstrated that low-level vaccination remained favored under preventative and proactive approaches even if both the vaccine and vampiricide spread up to 3-fold greater than observed in our field studies (Supplementary Figures 7-9). If both interventions spread less effectively than RB, vaccination was either superior or equivalent to culling except when large proportions of bat colonies were reactively culled (Supplementary Figure 6). Under realistic levels of application (application $\leq$ 25%), even if vampiricide spread 3-fold better than a vaccine, it was unable to outperform vaccination under preventative or proactive approaches when R_0 was less than 2. Under reactive scenarios, culling was favored if vampiricide spread 2-3-fold better than a vaccine or if VBR R_0 was 2 (Supplementary Figure 9). Given that existing oral rabies vaccines use replication-competent viral vectors with potential for lower effective doses than chemical poisons [24, 25], heightened vampiricide transfer is less likely than the converse where vaccines spread better [8, 40]. The high R_0 scenarios where culling was favored are also unlikely, as the estimated VBR R_0 is considerably lower than 2 [20]. Our results therefore support previous suggestions that culling may require near-elimination of bats to locally benefit rabies prevention [18] and reveal spreadable vaccines as efficient tools to reduce the size, duration, and probability of rabies outbreaks in Latin America.

Discussion

This study demonstrates proof-of-principle that at operationally-achievable levels of deployment and empirically-quantified rates of bat-to-bat spread, orotopical vaccines should reduce rabies transmission more effectively than culling, the current policy employed across Latin America. Since VBR persistence requires inter-colony spread for viral dispersal, even modest reductions in outbreak size are likely to have epidemiologically important impacts at the larger geographic scales over which disease control campaigns are implemented. In particular, by reducing the number of infected bats and the probability of viral invasion, vaccination of a limited number of colonies would disproportionately benefit regional rabies elimination by favoring stochastic viral extinctions. Because male dispersal spreads rabies between colonies, vaccination might further benefit from targeting male bats [33]. Although higher rates of social grooming among females was expected to undermine this strategy [39, 41], we found that males have equal

or greater inter- and intra-sex contact rates, a possible consequence of attempted mating with females or fighting among males. Importantly, because self-grooming is common [42], any vaccine transferred through these interactions would ultimately be ingested.

Designing large-scale campaigns to deploy spreadable rabies vaccines requires additional research in several areas. First, to optimize the number of vaccine doses to apply to each bat, captive and field studies should quantify individual heterogeneity in transfer rates using actual vaccines in addition to biomarkers. Second, the costs of vaccination must be estimated in economic terms in addition to the epidemiological assessment provided here. Unfortunately, vaccines are currently produced only for research and costs of large-scale production are unavailable. Third, vaccination of vampire bats without population reduction will be unacceptable to some stakeholders since uncontrolled bat depredation sustains exposures to non-rabies pathogens [43] and anemia from bites may reduce livestock productivity independently of rabies [44]. Given that culling shifts bat populations towards younger, more rabies susceptible individuals, which could enhance rabies transmission [19], future research should develop tools for reproductive suppression as an alternative to culling [45]. Finally, metapopulation maintenance of rabies provides opportunities for more efficient, epidemiologically-informed vaccination [46]. For example, vaccines might be deployed with prior knowledge of rabies presence from livestock surveillance systems (e.g., ring vaccination) or preventatively in areas where the locations and timing of outbreaks are predictable [36]. Spatially-explicit rabies transmission models will be an important next step to design these interventions, but will require a more quantitative understanding of bat dispersal than is currently available. Excitingly, once strategies are developed, the operational capacity for their implementation is already available in most Latin American countries through decades of experience with culling campaigns.

These results provide evidence that spreadable vaccines may contribute to pathogen management within wild bats. VBR provided an ideal case study because the epidemiological mechanisms underlying viral maintenance are understood and candidate vaccines are available [20, 25, 36, 47]. While the exact parameter estimates and models developed here should not be applied directly to other bat pathogens, the framework linking biomarkers to mathematical models can guide future research. For several bat pathogens of public health or conservation concern such as White Nose Syndrome, Hendra virus, and Marburg virus, epidemiological models have been proposed [48, 49, 50] and vaccines for bats either exist or have precedents encouraging their development [51, 52, 53]. In these cases, our approach could be implemented over relatively short timescales to evaluate the prospects for vaccines to aid management and

the immunological and epidemiological characteristics that would be required for success before investing resources in vaccine development. For other bat pathogens with greater uncertainty in reservoir hosts and transmission biology, such as Ebolaviruses [54], implementation will require greater fundamental knowledge of viral transmission cycles. We encourage further development of virally-vectored vaccines for bats and highlight the need to quantify their spread and efficacy in the wild.

Methods

Field studies of biomarker transfer and ingestion

Field studies were carried out between January and July 2017 in three vampire bat roosts in the Barranca (LMA5, -10.6415, -77.8160), Huaura (LMA6, -11.0555, -77.4594), and Lima (LMA12, -12.1833, -76.8500) provinces of the Department of Lima, Peru (Supplementary Table 1). Two roosts (LMA5 and LMA6) had been monitored since 2007, while the third (LMA12) was examined here for the first time [19]. All roosts were man-made tunnels that formed part of crop irrigation systems. Diurnal captures were carried out to mark bats and estimate sex ratios and colony sizes. Diurnal captures involved teams entering caves and catching bats with hand nets (BioQuip, Tropics Net). In addition, 2.5-meter mist nets (Ecotone) were placed at each end of tunnels to catch bats that attempted to escape. Diurnal capture effort was set to 1 hour across sampling dates and localities. Colony sizes were estimated using the Schnabel method [55]. Nocturnal captures were carried out in the same roosts to monitor biomarker spread. Nets placed at each roost exit were checked every 30 minutes for 4 hours per night at varying hours depending on the lunar cycle. Following removal from mist nets, bats were placed in individual cloth bags until processing. All captured bats were given an individually numbered, 4 digit incoloy wing band (3.5mm Porzana Inc.) to identify recaptures. Age was classified as juvenile, subadult, or adult based on the degree of fusion of the phylangeal epiphyses [56]. In total, we recorded 1777 captures of 709 individually-marked bats, with the average bat captured 2.39 times (range=1-9).

Studies of vaccine transfer and ingestion used RB powder (50mg) mixed with glycerine jelly (44.5ml, Carolina Biological Supply Company) and water (55.5ml) to form a gel. On days 1 and 2, RB was administered orally to confirm fluorescence in RB-treated bats (ca. 0.05ml via needle-free syringe) and applied topically (ca. 0.45ml, rubbed into the dorsal fur) to all captured bats. Uptake in un-treated bats was monitored using hair plucked from bats captured over 4-5 subsequent sessions per colony, carried

out up to 31 days after initial application (Supplementary Table 1). Hair samples were examined with a Nikon SMZ1270 microscope at 15x using a fluorescence filter with excitation wavelength 540 nm, emission wavelength 625 nm. Each sample was examined by two individuals to minimize misclassification, except at LMA12 on days 8 & 10, where only one individual examined the hair. The presence of fluorescence in hair was interpreted to indicate transfer and consumption of RB, but was not considered a quantitative measure of the volume of RB consumption. Because bats had identification tags, we were able to distinguish those that were positive due to transfer from RB treated bats ("transfer positives") from those that had RB applied by experimenters ("application positives"). Hair samples were collected under the Peruvian collection permit, 028-2017-SERFOR/DGGSPFFS and exported to the United States under export permit, 3235-SERFOR. This research was performed under approval of University of Glasgow School of Veterinary Medicine Animal Ethics Committee (Project 25A/18).

Contact heterogeneities among demographic groups of vampire bats

Powder marking was replicated 3 times per colony (total of 9 marking sessions) and bats were monitored for two nights following each marking session (Supplementary Table 1). During each session, red, green, blue, or orange UV powder (DayGlo Corp.) was rubbed into the fur of the bat across the entire body using a toothbrush, with colors dependent on age and sex. UV colors were rotated between groups at different capture dates to control for potential differences in detection probability. UV powder markings were recorded by examining each captured bat for 30s using handheld UV lights (Glowtech Ltd.) prior to removal from mist nets. After removing UV marked bats from the recaptures, directional contact rates for each sex (e.g. female-to-male contacts per marked female) were calculated using equation 1:

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

where N_{pos_X} is the number of bats of a certain sex testing positive for the UV color in question, UM_X is the number of unmarked bats of that sex captured at this time point, N_{UM_X} is the number of unmarked bats of that sex in the entire colony, and M_X is the number of initially marked bats from that sex. Example calculations are provided in the Supplementary Information (Eqs. 2 & 3).

Sex biases in UV transfer were tested by comparing all estimated rates from males to all estimated rates from females, treating each site, month, and recipient sex combination as independent observations

(N = 36). We used a non-parametric Wilcoxon rank sum test since rates were not normally distributed, even after log transformation (Shapiro-Wilk test, p = 0.01).

Parameter estimation and mathematical modeling

Per capita rates of orotopical transfer and ingestion, defined as the estimated number of bat-to-bat transfers per treated individual, were estimated using the data from our RB field study. Specifically, we incorporated a susceptible (S), application positive (A), and transfer positive (T) deterministic compartmental model (Fig. 3b) using least-squares methods in the statistical software R. A 2-day transfer period was integrated with the number of RB application and transfer positives across time to estimate the expected transfer rate of orotopical vaccines or poisons (β). A 6-day RB transfer period was also considered to examine variation in β across time (Supplementary Table 2, Supplementary Information). We assumed that successful transfer led to death in culling models and lifelong protection against VBR in vaccination models (ca. 3.5 years of protection given the lifespan of *D. rotundus*, Supplementary Table 3). Importantly, waning of vaccine-induced immunity would not alter the results shown here which focused on single outbreaks.

Mathematical models of rabies control used a stochastic model that simulated both rabies transmission and vaccine transfer. A susceptible (S), application positive (A), transfer positive (T), exposed to rabies (E), immune (I), and rabid (R) model, with a daily time-step, was simulated for 5000 iterations using a Gillespie algorithm (Fig. 3a, Supplementary Information). Following previous models of vampire bat rabies [20] and consistent with the absence of strong relationships between colony size and rabies seroprevalence [19], we utilized a frequency-dependent rabies transmission function. We used 237 bats as the colony size (the mean size from our three field sites). The base model without vaccination or culling followed the mathematical structure and parameter values used by Blackwood *et al.* [20], with the simplifications of a single infectious class and modeling a single introduction of rabies rather than sustained introductions via immigration. This model generated similar outbreak dynamics to the Blackwood *et al.* [20] model, characterized by short lived outbreaks (less than 1 year) followed by viral extinction, persistence of the bat population, and seroprevalence levels consistent with field observations, particularly at values of $R_0 >$ 0.6 (Supplementary Figure 3). Since we modelled our vaccine spread on a recombinant Raccoonpox virus-vectored vaccine that appears unlikely to spread via an infectious process (i.e., from indirectly vaccinated bats) [47], vaccines were modelled to spread only from those bats to which the vaccine was applied,

creating a single generation of transmission. Based on the very low prevalence of rabies in free-flying bats (>1%) and infrequent dispersal in vampire bats [57, 58], we simulated introduction of a single rabid bat to the population. Given that sex differences in RB transfer were non-significant and age-biased transfer was difficult to quantify due to small sample sizes in non-adult classes, we opted against more complex age and sex structured models of rabies and vaccine/vampiricide spread.

Models comparing the efficacy of vampiricide to vaccination used the same model structure with the exception that bats in the exposed class died from ingesting vampiricide, while those that consumed the vaccine were not protected (see Eqs. 8 & 9 in the Supplementary Information). This was because post-exposure vaccination has not been evaluated in bats. We generally assumed equal transfer rates of vaccines and vampiricide based on their identical mechanism of transfer; however, we relaxed this assumption in the Supplementary Information (Supplementary Figures 6-9). We also assumed that both spread over relatively short time periods since vampire bats are exceptional groomers and would quickly ingest vaccine or vampiricide [42]. Importantly, our focal vaccine remains viable over these timescales [59]. After two years (730 days) the cumulative number of newly infected bats was considered to be the outbreak size. Outbreak duration was defined as the total number of days with at least one bat in the exposed class. For preventative and proactive approaches, we quantified the probability of an outbreak as the proportion of simulations where at least 1 new bat became infected after a single rabid bat was introduced.

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Author Contributions

D.G.S., T.E.R., and J.E.O. conceived and designed the experiments; R.C.A., C.T., and J.C. performed the experiments; K.M.B. and D.G.S. analyzed the data; T.E.R., J.E.O., W.V., C.S., and N.F. contributed materials/analysis tools; K.M.B. and D.G.S. wrote the first draft of the paper and all authors contributed revisions.

Data Availability

The UV transfer and RB transfer data are available on Dryad (doi: 10.5061/dryad.64t161m). These data were used to generate Figs. 1 and 2, and Supplementary Figures 1 and 2.

Code Availability

The R scripts used to estimate RB transfer rates shown in Supplementary Figure 2 and Supplementary Table 2 and to carry out the epidemiological modeling shown in Figs. 4 and 5 and Supplementary Figures 3 and 5-9 are provided as Supplementary Software 1 and Supplementary Software 2.

Competing Interest Statement

The authors declare no competing interests.

Figure captions

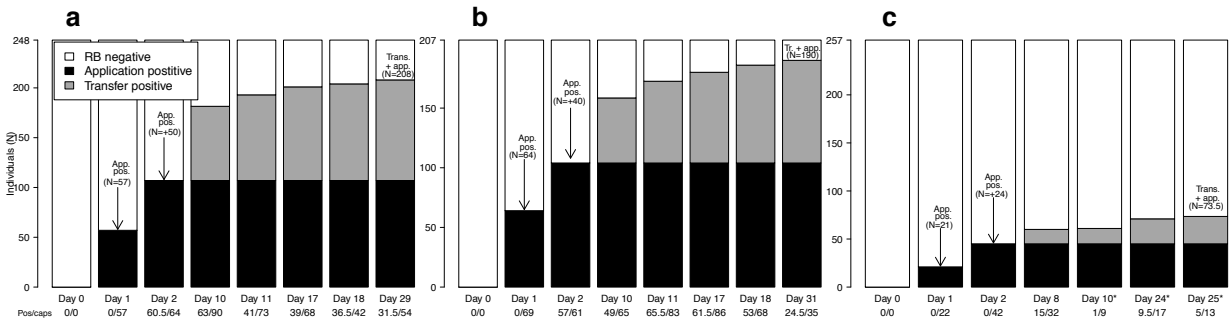

Figure 1: Transfer and ingestion of an orotopically spread gel biomarker in three vampire bat colonies. In each panel, LMA5 (**a**), LMA6 (**b**), and LMA12 (**c**), x-axes are the days since RB application with the number of transfer positive bats over total captures in subtext (Pos/caps). The y-axis is the number of bats in each colony within three categories RB negative (white), application positive (black), or transfer positive (gray). Asterisks (*) on and after day 10 from LMA12 indicate captures from the relocated roost. Data are the mean of microscopy readings from two observers, except where noted otherwise. Transfer positive bats from day 2 had RB applied and are included in the black bar to visualize the total force of application, but were included as transfers in statistical analyses.

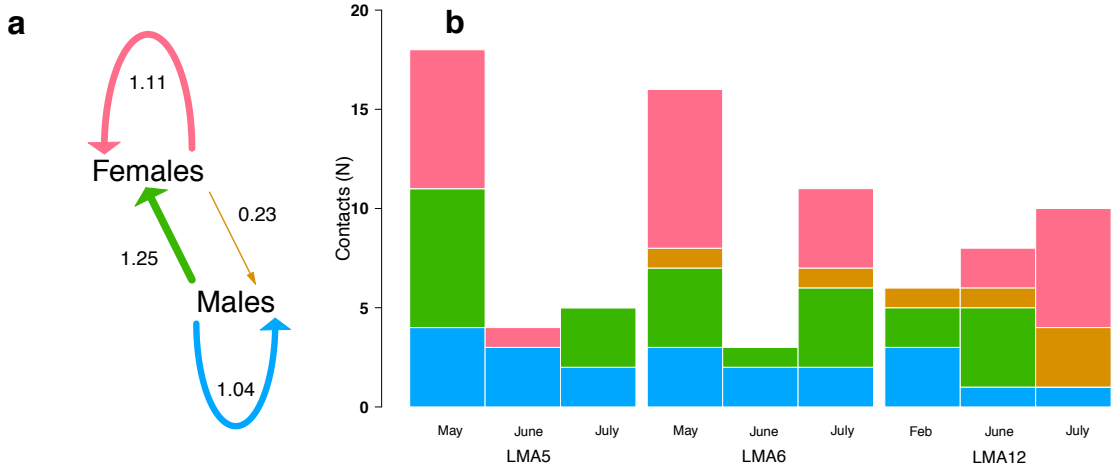

Figure 2: Bat contact heterogeneity revealed by UV powder transfers. **a,** Mean new contacts per marked bat, by sex. Arrow thickness is proportional to contact rate. **b,** Number and directionality of contacts by sex, location, and sampling date. Contacts to juveniles are not shown since the juveniles in the colonies we studied were too young to feed independently and would have been underestimated by our capture method during monitoring.

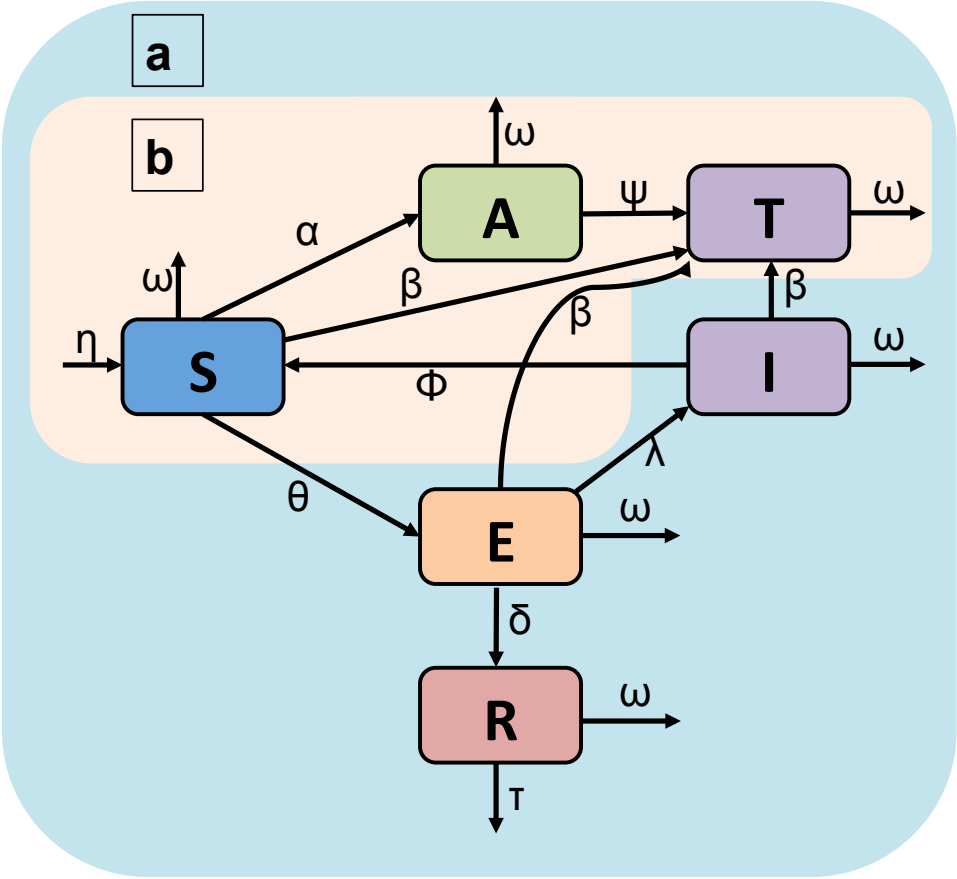

Figure 3: Dynamic models of rabies transmission and spreadable vaccination. **a,** The full model used for outbreak analyses includes orotopical transfer and rabies transmission. Classes comprise susceptible (S), application positive (A), transfer positive (T), immune (I), exposed to rabies (E), and rabid (R). **b,** The biomarker transfer model structure for fitting β. In the vaccination model, the I and T classes both provide immunity from rabies but the T class has permanent immunity. Model parameters describe rates of: natural births (η) and deaths (ω); orotopical gel application (α), persistence (ψ), and transfer (β); rabies transmission (θ); waning of immunity (ϕ); rabies induced mortality (τ); and the probabilities of succumbing to rabies (δ) or surviving (λ) following exposure. Supplementary Table 3 provides further details and references for parameter values.

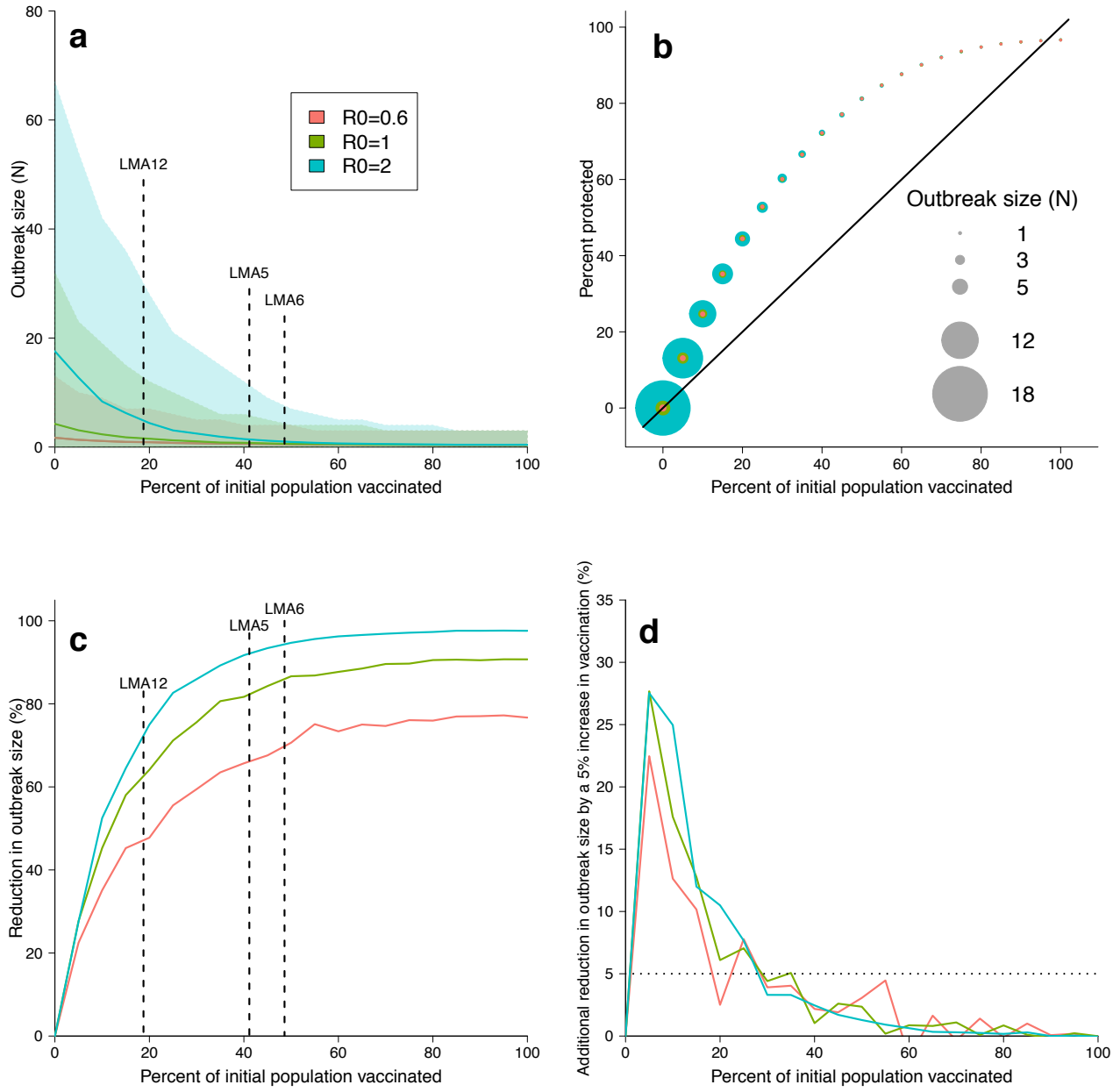

Figure 4: Simulating rabies outbreaks with vaccination. **a,** Mean rabies outbreak sizes after a single rabid bat is introduced to the colony one week following release of a spreadable vaccine. Colors represent varying degrees of rabies R_0, with 95% confidence intervals calculated from 5000 simulations. Dashed lines indicate the percent of bats that RB was applied to in our study sites. Supplementary Figure 5 shows results calculated only from simulations where outbreaks occurred. **b,** Percent of bats ultimately protected by initial vaccine release. Circle size indicates outbreak size under the three rabies R_0 values. Solid line represents the 1:1 line; points over the line represent the added benefit of vaccine transfer. **c,** Reduction in rabies outbreak size (% fewer cases) under varying initial vaccination levels and rabies R_0 values. **d,** Percent of additional rabies cases prevented by increasing the initial vaccine release effort by 5% (i.e., the rate of change in rabies reduction from the panel **c**).

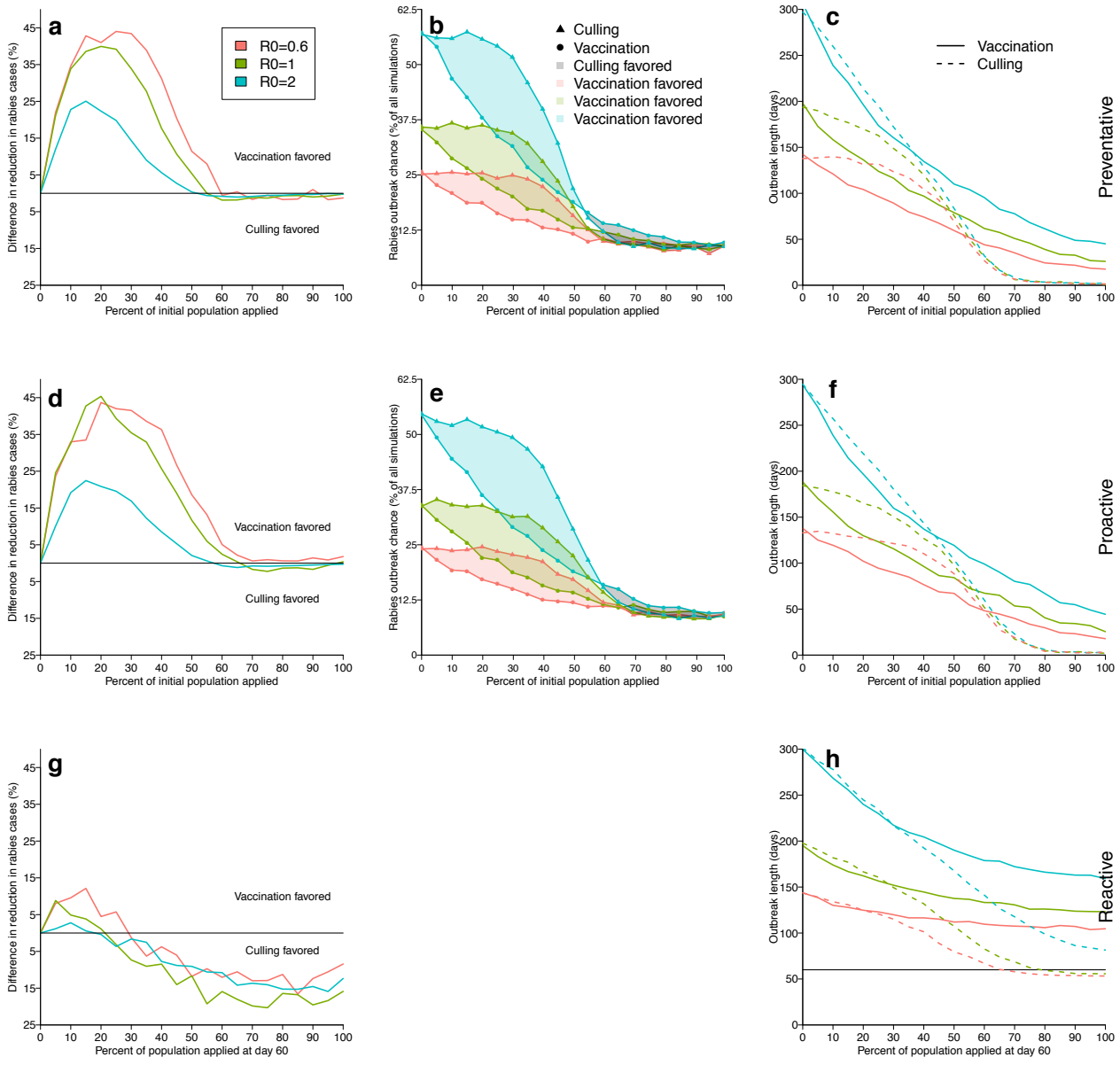

Figure 5: Comparing effects of culling and vaccination on rabies transmission. Rows group results from preventative (top), proactive (middle), and reactive (lower) strategies and columns group metrics of impacts on transmission. **a,d,g,** The difference in the reduction of rabies cases between equal levels of effort in vaccination versus culling. Values above and below 0 favor vaccination and culling, respectively. **b,e,** The probability of a rabies outbreak, defined as the percentage of simulations (N = 5000) where VBRV introduction led to onward transmission. Shaded regions represent the difference between vaccination (circles) and culling (triangles); culling is favored in grey regions and vaccination is favored in blue, green, or red regions. The probability of outbreaks was not modelled for reactive control since, by definition, outbreaks had already occurred. **c,f,h,** The duration of rabies outbreaks under vaccination and culling. The horizontal line in panel H indicates day 60, when reactive control measures were implemented. In all panels, colors correspond to different assumed R_0 values for rabies.

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

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Contents

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.

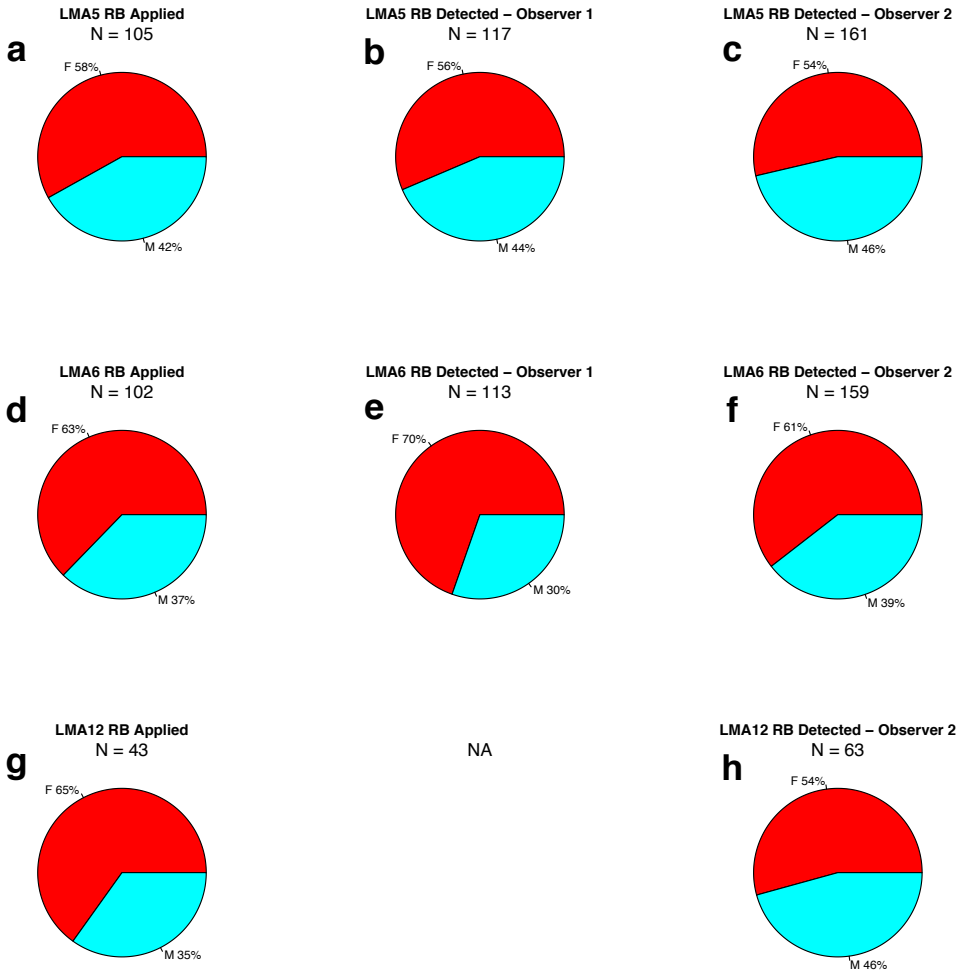
LMA5			LMA6			LMA12		
Date	Experiment	Treated/Caught	Date	Experiment	Treated/Caught	Date	Experiment	Treated/Caught
4/20/2017	RB mark	M 25/25, F 30/30, J 2/2	4/22/2017	RB mark	M 23/23, F 39/40, J 6/6	1/31/2017	RB mark	M 7/8, F 11/11, J 3/3
4/21/2017	RB mark	M 19/26, F 31/37, J 0/1	4/23/2017	RB mark	M 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	2/8/2017	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
7/23/2017	UV recapture	M 0/6, F 0/2, J 0/0	7/1/2017	UV recapture	M 0/4, F 0/8, J 0/0			
7/25/2017	UV mark	M 26/26, F 28/28, J 10/10	7/22/2017	UV mark	M 20/20, F 18/18, J 0/0			
7/26/2017	UV recapture	M 0/7, F 0/2, J 0/0	7/23/2017	UV recapture	M 0/4, F 0/6, J 0/0			
7/27/2017	UV recapture	M 0/4, F 0/4, 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:

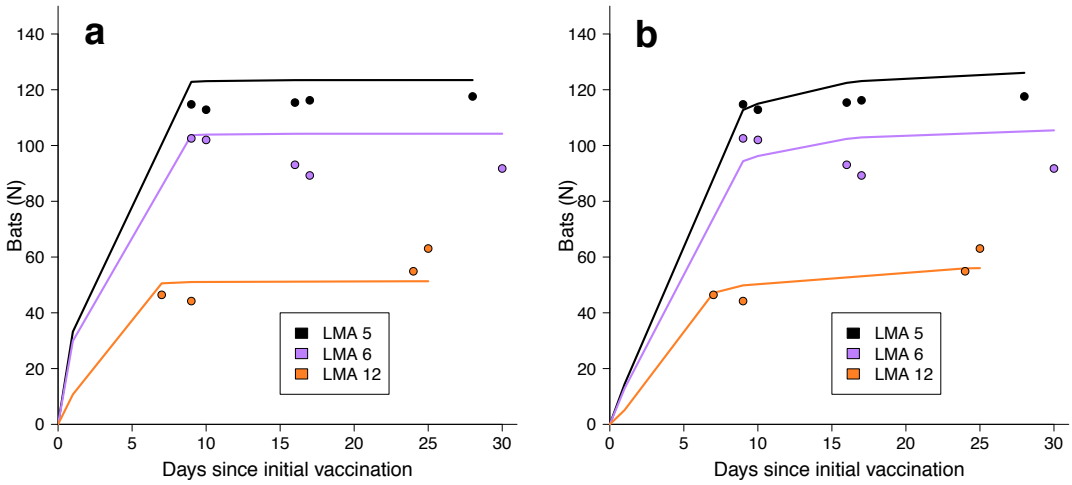
$$\text{Male-to-Male} = \frac{\frac{3}{17} * (73)}{15}. \tag{2}$$

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

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

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

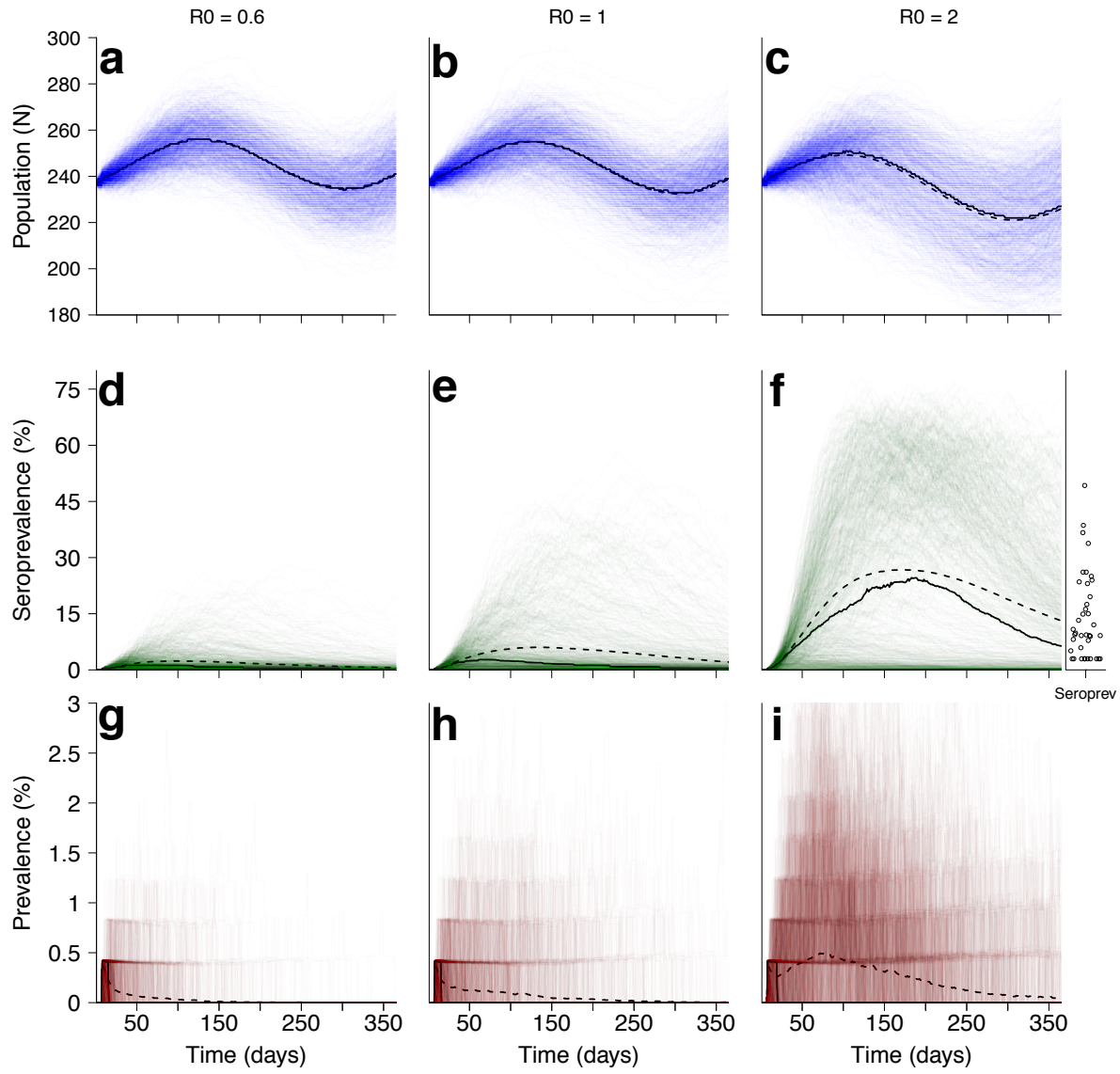
Parameters for the mathematical models were obtained from previous field or modelling studies and controlled infections in captive bats (Supplementary Table 3). Annotated R scripts for conducting mathematical modeling are provided in Supplementary Software 2. All models were implemented using the tau-leap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

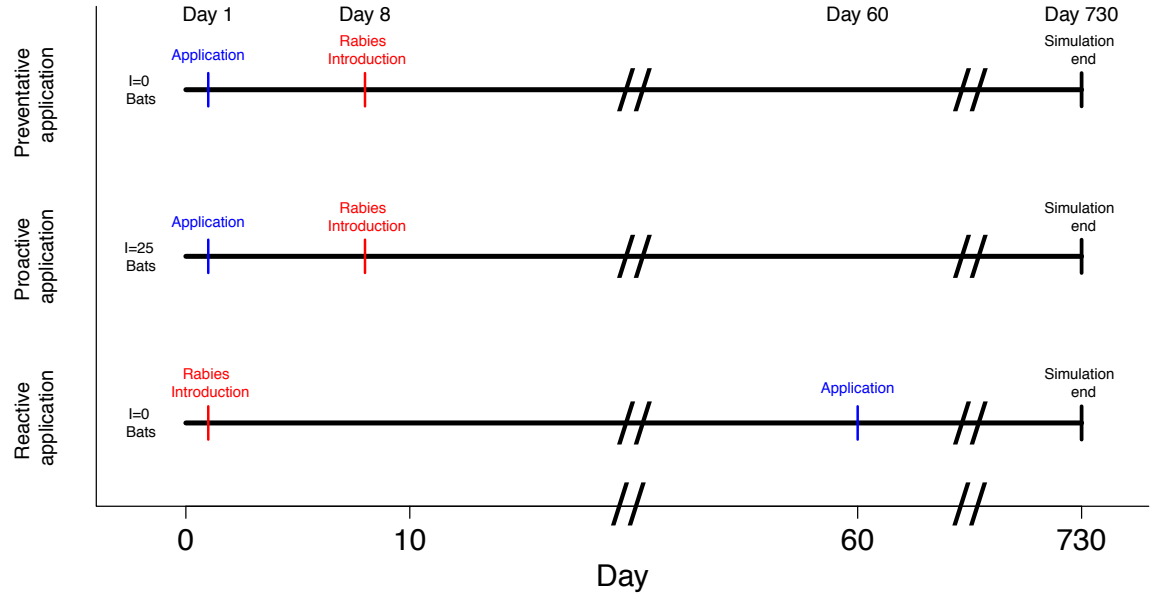
Reactive control introduced a rabid bat on day 1 and simulated orotopical application on day 60. This delay was intended to account for time that would be required for one round of transmission within the bat colony (21 day incubation period), infections in livestock to occur (21 day incubation period), 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.

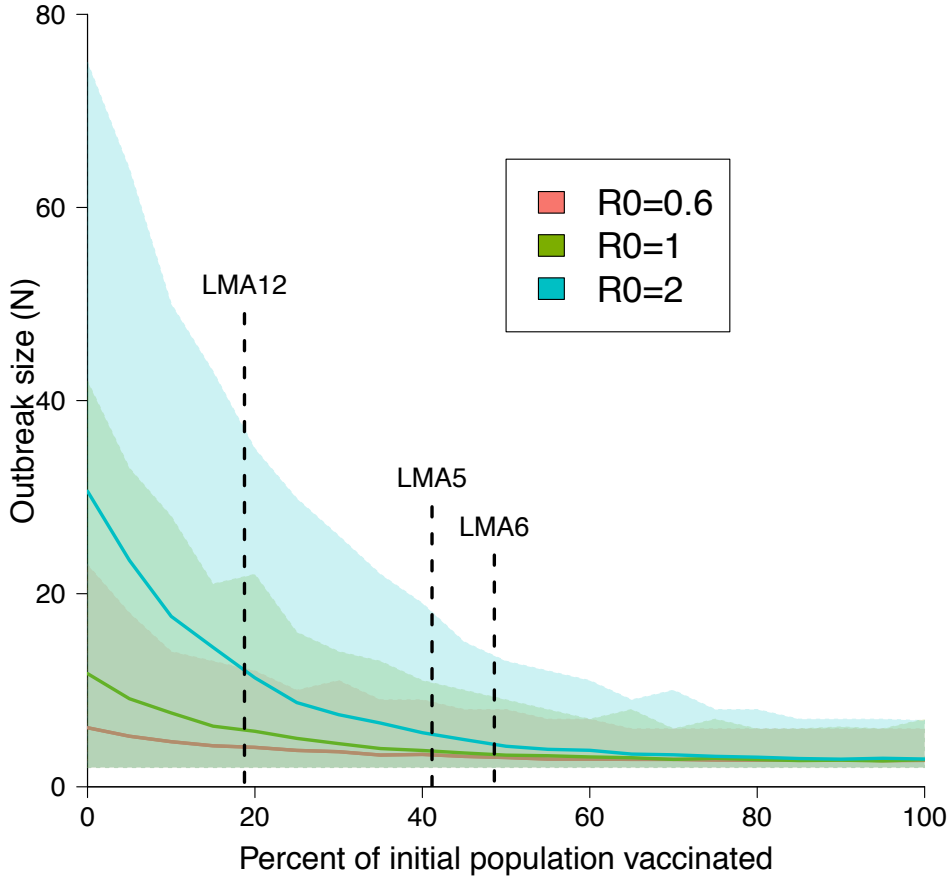
3.6 Sensitivity analysis of increased orotopical transfer of vaccines and vampiricide

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

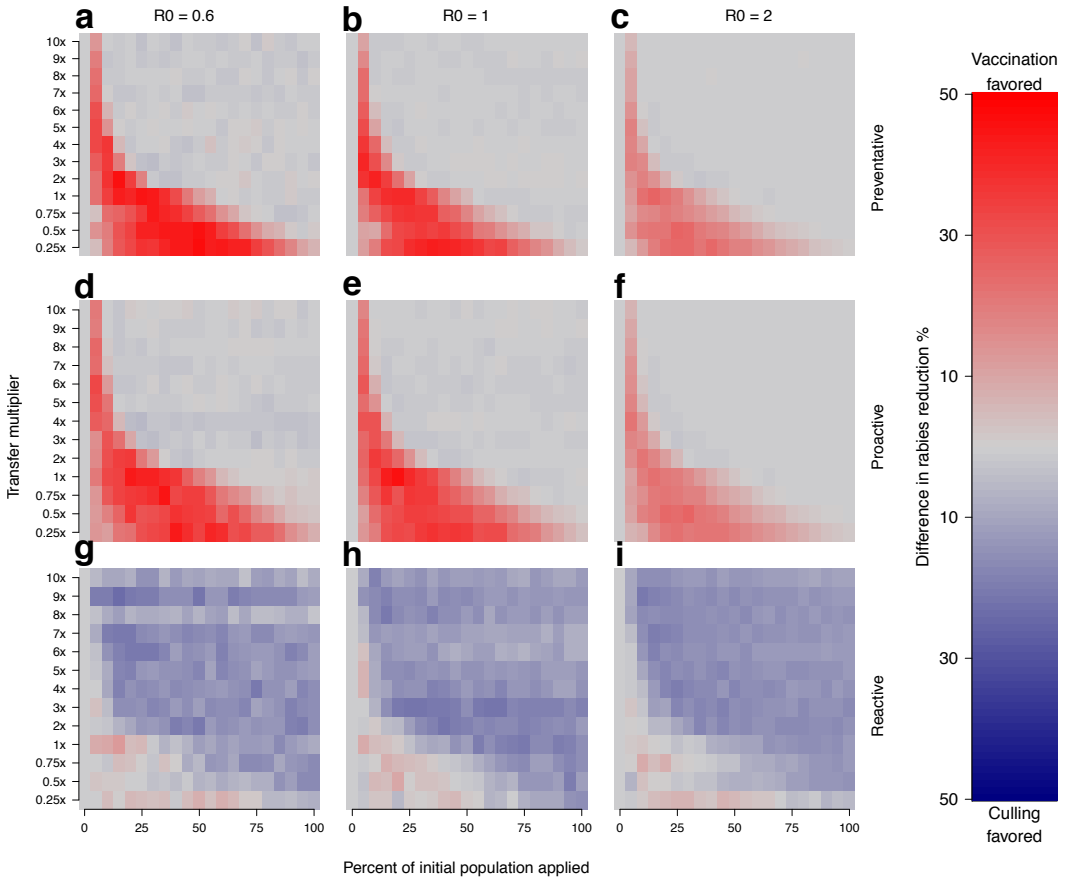


Supplementary Figure 5: Size of rabies outbreaks sizes after vaccination in simulations where rabies spread following introduction. Lines show mean rabies outbreak sizes after a single rabid bat is introduced to the colony one week following immunization. Colors represent varying degrees of rabies R_0, 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.

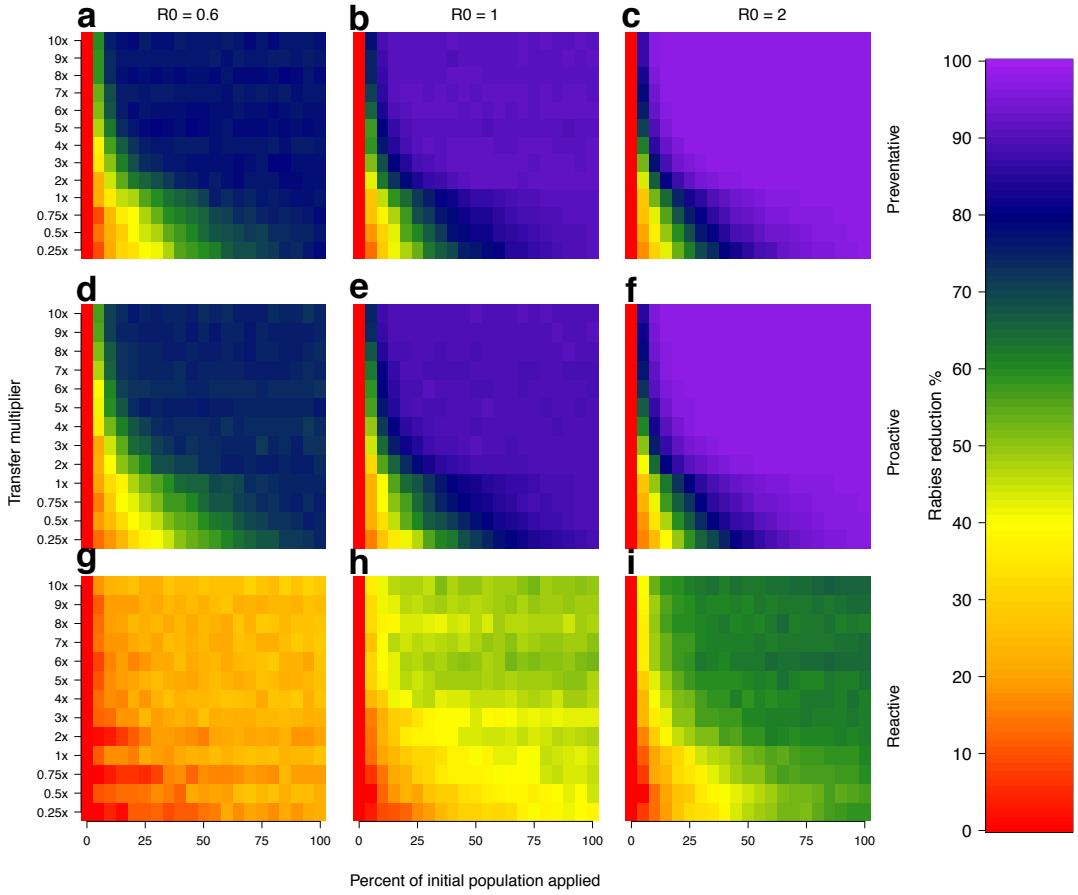
protected or culled.

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

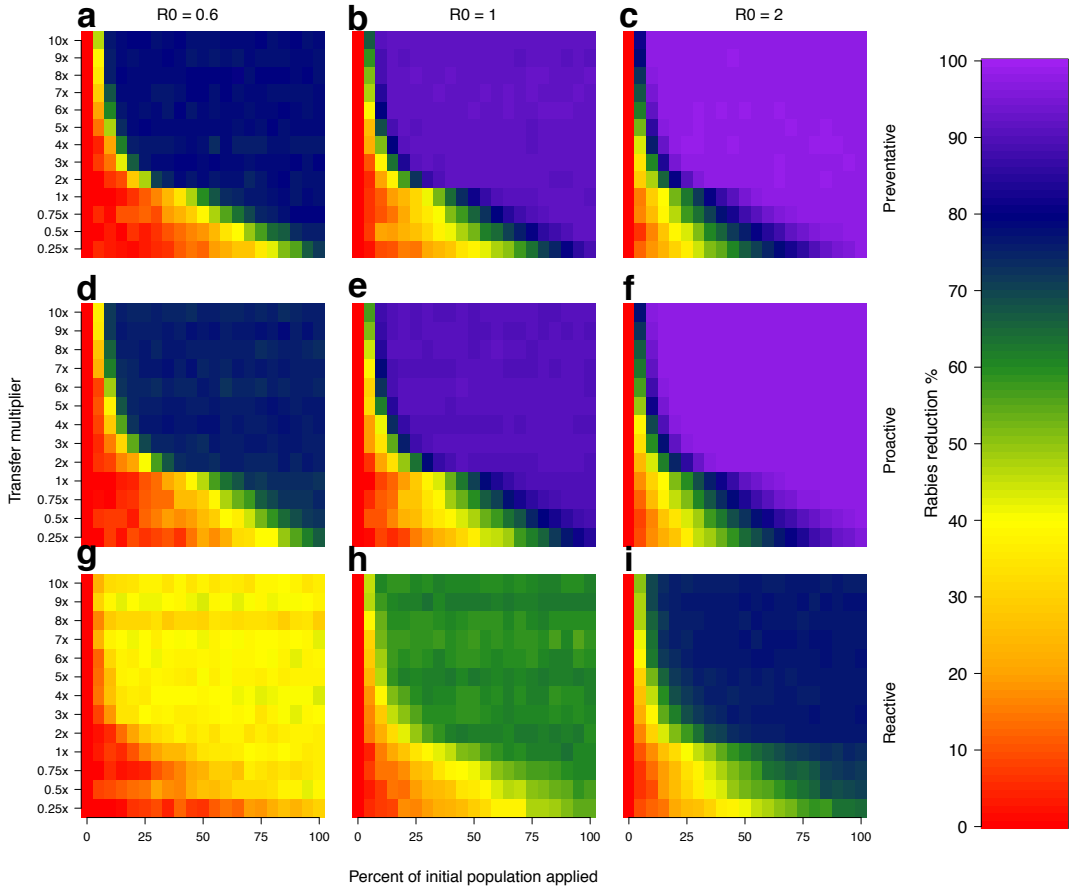
148 for both the preventative and proactive strategies, vampiricide was never more effective than vaccines at
149 reducing rabies outbreaks under realistic application levels.



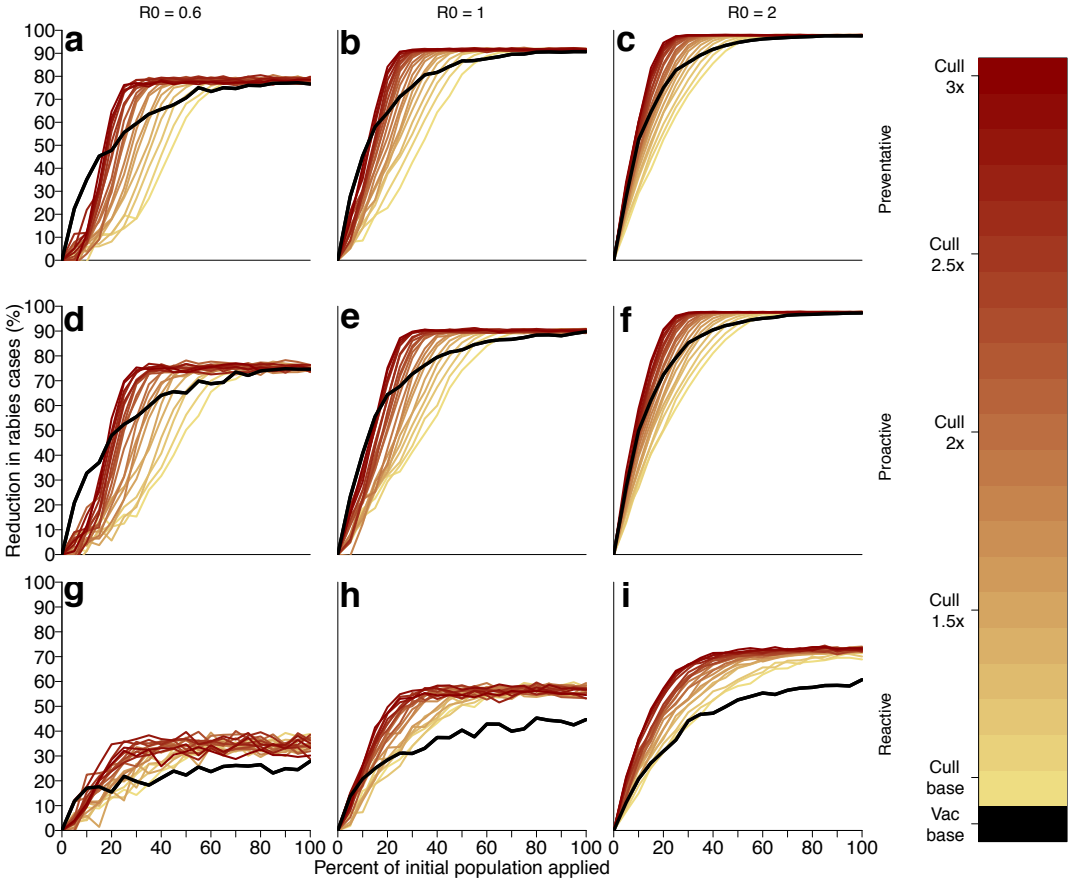
Supplementary Figure 6: Differences between vaccination and culling in the percent reduction of rabies cases compared to a no intervention scenario. The figure shows results of simulations assuming vaccines had transfer rates that were lower than (<1x), equal to (1x) or greater than (>1x) those observed 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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