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

Kevin M. Bakker, Torie E. Rocke, Jorge E. Osorio, Rachel C. Abbott, Carlos Tello, Jorge E. Carrera, William Valderrama, Carlos Shiva, Nestor Falcon and Daniel G. Streicker

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 investigate 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 three 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 showed 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 before costly investments in vaccine design and testing.
These recombinant virally vectored vaccines can indirectly immunize untreated bats in captivity, but have never been tested in wild populations\textsuperscript{22–25}. Several unresolved questions must be answered before 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 immunization significantly reduce rabies transmission\textsuperscript{14}; 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 show 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\textsuperscript{26–28}. 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 were 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 and LMA6), an estimated 84 and 92% of bats, respectively, ingested RB, either following topical application or transfer from treated bats (Fig. 1). The third colony (LMA12) relocated to an undocumentated 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 1-month 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, $\chi^2 = 3.2$; d.f. = 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 ($\chi^2$ tests; all $P > 0.05$; Supplementary Fig. 1). We observed RB transfer to untreated bats in all three age classes. Across all colonies, 73.4% of sampled adults ($\bar{\pi} = 351$, averaged across microscopy readings of independent observers), 57.5% of sampled juveniles ($\bar{\pi} = 30.5$) and 89.9% of sampled subadults ($\bar{\pi} = 34.5$) became RB positive through transfer during the monitoring period. Consequently, these results implied that vaccines deployed over only 2 d of captures (15–50% of the 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. Next, we examined whether contact heterogeneities might make certain demographic groups of bats especially effective or ineffective spreaders of vaccines using ultraviolet powder marking, wherein different age and sex groups of bats were treated with different colours of ultraviolet powder, and transfer to untreated bats was monitored over two subsequent capture nights\textsuperscript{29,30}. Across three replicate ultraviolet treatments per colony, we documented 78 instances of ultraviolet 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 female bats (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 their roosts. Nevertheless, a single captured juvenile bat had ultraviolet 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 ultraviolet powder to adults. Together with the high observed 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 the social disruption of colonies that results from entering roosts to capture juveniles.

Epidemiological models show that spreadable vaccines outperform culling for rabies control. We adapted a deterministic compartmental model of VBR persistence\textsuperscript{29} to incorporate an orotopically spread vaccine, and used least squares (Fig. 3b) to estimate the 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 (Fig. 4b, Supplementary Fig. 2 and Supplementary Table 2).

We simulated the ability of spreadable vaccines to control rabies across the range of $R_0$ (the expected number of secondary infections arising from a single viral introduction into a completely susceptible population) values (0.6–2.0) suggested in the rabies literature.20,31,32. Applying vaccines to approximately 20% of bats vaccinated 40% of the population and reduced rabies outbreak size by 45–75%, depending on the assumed $R_0$ of rabies (Fig. 4a–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 <5%, meaning that a 5% increase in initial application led to a <5% reduction in outbreak sizes (Fig. 4d). The greatest benefit (reduction in outbreak size relative to effort) occurred at vaccination levels <15%.

Next, we compared the relative efficacy of vaccination and culling across three epidemiological scenarios representing different management strategies: (1) a preventative approach, where vaccine/vampiricide was applied to prevent VBR invasion into historically rabies-free bat populations33,34; (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 d after a single VBR-infected bat was introduced to the colony (Supplementary Fig. 4). Although we simulated outcomes across the full possible range of application efforts (that is, 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 night19. 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 measures were preventative, proactive or reactive (Fig. 5). Culling was only favoured 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 favoured (up to 45% greater reduction), and differences in outbreak duration were negligible until much larger proportions of bats were culled (Fig. 5). For the preventative and proactive scenarios, culling required the capture and treatment of much larger proportions of vampire bat populations (for example, >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 favourable long-term strategy since...
populations that recovered from culls would be entirely susceptible to rabies, potentially causing larger future outbreaks.35. Our per-capita transfer rates probably represent 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.17,36. Therefore, we conducted a sensitivity analysis in which both vaccines and vampiricide spread up to tenfold more efficiently than in our RB estimates (values that exceeded the largest transfer rates suggested from vampiricide applications). Additionally, we considered transfer rates that were up to 75% less efficient than our RB estimates. This analysis showed that low-level vaccination remained favoured under preventative and proactive approaches even if both the vaccine and vampiricide spread up to threefold greater than was observed in our field studies (Supplementary Figs. 6–8). 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 Fig. 6). Under realistic levels of application (application ≤25%), even if vampiricide spread threefold better than a vaccine, it was unable to outperform vaccination under preventative or proactive approaches when R₀ was <2. Under reactive scenarios, culling was favoured if vampiricide spread two- to threefold better than a vaccine or if the VBR R₀ was 2 (Supplementary Fig. 9). Given that existing oral rabies vaccines use replication-competent viral vectors with the potential for lower effective doses than chemical poisons, heightened vampiricide transfer is less likely than the converse where vaccines spread better.1 The high R₀ scenarios where culling is favoured are also unlikely, as the estimated VBR R₀ is considerably lower than 2 (ref. 20). Our results therefore support previous suggestions that culling may require near-elimination of bats to locally benefit rabies prevention,14, and reveal spreadable vaccines as efficient tools to reduce the size, duration and probability of rabies outbreaks in Latin America.
This study provides 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 favouring stochastic viral extinctions. Because male dispersal spreads rabies between colonies, vaccination might further benefit from targeting male bats. Although higher rates of social grooming among females were expected to undermine this strategy, we found that males have equal or greater inter- and intra-sex contact rates—a possible consequence of attempted mating.

**Discussion**

Fig. 5 | Comparison of the effects of culling and vaccination on rabies transmission. a–h, Difference in the reduction of rabies cases between equal levels of effort in vaccination versus culling (a, d and g), probability of a rabies outbreak (b and e) and duration of rabies outbreaks following vaccination and culling (c, f and h) for the preventative (a–c), proactive (d–f) and reactive (g and h) strategies. In a, d and g, values above and below 0 favour vaccination and culling, respectively. In b and e, the probability of a rabies outbreak is defined as the percentage of simulations (n=5,000) where VBR virus introduction led to onward transmission, and shaded regions represent the difference between vaccination (circles) and culling (triangles); culling is favoured in the grey regions and vaccination is favoured in the blue, green and red regions. The probability of outbreaks was not modelled for reactive control since, by definition, outbreaks had already occurred. The horizontal line in h indicates day 60, when reactive control measures were implemented. In all panels, colours correspond to different assumed R₀ values for rabies.
with females or fighting among males. Importantly, because self-grooming is common38, 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 the 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 pathogens19, and anaemia from bites may reduce livestock productivity independently of rabies47. Given that culling shifts bat populations towards younger, more rabies-susceptible individuals, which could enhance rabies transmission5, future research should develop tools for reproductive suppression as an alternative to culling8. Finally, metapopulation maintenance of rabies provides opportunities for more efficient, epidemiologically informed vaccination5. For example, vaccines might be deployed with previous knowledge of rabies presence from livestock surveillance systems (for example, ring vaccination) or preventatively in areas where the locations and timings of outbreaks are predictable19. Spatially explicit rabies transmission models will be an important next step in the design of 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 as the result of 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 available38–41. 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 proposed44–46, and vaccines for bats either exist or have precedents encouraging their development47–49. 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 ebolaviruses50, 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°38′29.4″ S, 77°48′57.6″ W), Huaura (LMA6: 11°03′19.8″ S, 77°27′33.8″ W) and Lima (LMA12: 12°10′59.9″ S, 76°50′60″ W) 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 time19. All roosts were manmade tunnels that formed part of crop irrigation systems. Diurnal captures were carried out to mark the 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, 5.5-m mist nets (BioQuip; Tropics Net) were placed at each end of the tunnels to catch the bats that attempted to escape. Diurnal capture efforts were set to 1 h across sampling dates and localities. Colony sizes were estimated using the Schnabel method41. Nocturnal captures were carried out in the same roosts to monitor biomarker spread. Nets placed at each roost exit were checked every 30 min for 4 h 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, four-digit Incoloy wing band (3.5 mm; Porzana) to identify recaptures. Age was classified using the terms juvenile, subadult or adult, based on the degree of fusion of the humerus and ulna.

Characterization of transferred vaccines. Bat pathogens, the framework linking biomarkers to mathematical models. In total, we recorded 1,777 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 (50 mg) mixed with glycerine jelly (44.5 mL; Carolina Biological Supply Company) and water (55.5 mL) to form a gel. On days 1 and 2, RB was administered orally to confirm fluorescence in RB-treated bats (~0.05 mL via needle-free syringe) and applied topically (around 0.45 mL, rubbed into the dorsal fur) to all captured bats. Uptake in untreated bats was monitored using hair plucked from bats captured over 4–5 subsequent sessions (each capturing the initial applied dose). Hair samples were collected using the protocol 028-2017-SERP/DFGSPFES and exported to the United States under export permit 3235–SERFOR. This research was performed with the approval of the 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 three times per colony (for a total of nine marking sessions) and bats were monitored for two nights following each marking session (Supplementary Table 1). During each session, red, green, blue or orange ultraviolet powder (DayGlo) was rubbed into the fur of the bat across the entire body using a toothbrush, with colours dependent on age and sex. Ultraviolet colours were rotated between groups for different capture dates to control for potential differences in detection probability. Ultraviolet powder markings were recorded by examining each captured bat for 30 s using handheld ultraviolet lights (Glotech) before removal from mist nets. After removing ultraviolet-marked bats from the recaptures, directional contact rates for each sex (for example, female-to-male contacts per marked female) were calculated using equation (1):

\[
\text{Contact rate} = \frac{\text{num}_{\text{transfer}} \times \text{um}_{\text{transfer}}}{\text{M}_{\text{X}}} (1)
\]

where \(\text{num}_{\text{transfer}}\) is the number of bats of a certain sex testing positive for the ultraviolet colour in question, \(\text{um}_{\text{transfer}}\) is the number of unmarked bats of that sex captured at this time point, \(\text{M}_{\text{X}}\) is the number of initially marked bats of that sex in the entire colony, and \(\text{X}\) is the number of initially marked bats from that sex. Example calculations are provided in the Supplementary Information (equations (2) and (3)).

Sex biases in ultraviolet transfer were tested by comparing all estimated rates from males with 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 modelling. 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-d transfer period was integrated with the number of RB application and transfer positives across time to estimate the expected transfer rate of orotopical viruses or poisons (\(\beta\)). A 6-d RB transfer period was also considered to examine variation in \(\beta\) across time (Supplementary Table 2 and Supplementary Information). We assumed that successful transfer led to death in the culling models and lifelong protection against VBR in the vaccination models (\(c = 1\) for protection given the lifespan of bats) (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) and transfer-positive (T) model was used to determine compartmental model. A 2-d transfer period was integrated with the number of RB application and transfer positives across time to estimate the expected transfer rate of orotopical viruses or poisons (\(\beta\)). A 6-d RB transfer period was also considered to examine variation in \(\beta\) across time (Supplementary Table 2 and Supplementary Information). We assumed that successful transfer led to death in the culling models and lifelong protection against VBR in the vaccination models (\(c = 1\) for protection given the lifespan of bats) (Supplementary Table 3). Importantly, waning of vaccine-induced immunity would not alter the results shown here, which focused on single outbreaks.

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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., with the simplifications of a single infectious class and modelling of a single introduction of rabies rather than sustained introductions via immigration. This model generated similar outbreak dynamics to the Blackwood et al. model, characterized by short-lived outbreaks (<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 Fig. 3). Since we modelled our vaccine spread on a recombinant raccoonpox virus-vectored vaccine that appears unlikely to spread via an infectious process (that is, from indirectly vaccinated bats), 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, we simulated the 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 for an assumption of complex age- and sex-structured models of rabies and vaccine/vampiricide spread. Models comparing the efficacies of vampiricide and 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 equations (8) and (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 immunogenicity of a raccoonpox vectored rabies vaccine in the Brazilian Amazonian region of Brazil. Am. J. Trop. Med. Hyg. 55, 680–684 (1996).

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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.E.C. performed the experiments. K.M.B. and D.G.S. analysed the data. T.E.R., J.E.O., W.V., C.S. and N.F. contributed materials and/or analysis tools. K.M.B. and D.G.S. wrote the first draft of the paper. All authors contributed revisions.

Competing interests
The authors declare no competing interests.

Additional information
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Correspondence and requests for materials should be addressed to K.M.B. or D.G.S.

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Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

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- [ ] The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement

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- [ ] For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings

- [ ] For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes

- [ ] Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated

  *Our web collection on statistics for biologists contains articles on many of the points above.*

Software and code

Policy information about availability of computer code

Data collection

| Data used to collect the data | No software was used to collect the data |

Data analysis

| All code was run on R version 3.4.4 (2018-03-15) -- "Someone to Lean On", with package 'deSolve', version 1.20. Package 'Matrix' is version 1.2-12. The R scripts used to estimate RB transfer rates (Supplementary Figure 3, Supplementary Table 2) and to carry out the epidemiological modeling (Figures 4 & 5 and Supplementary Figures 5–9) are provided as Supplementary Information. |

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The UV transfer and RB transfer data are available on Dryad (doi: 10.5061/dryad.64t161m). These data were used to generate Figures 2 & 3 and Supplementary Figures 1 & 2. Additional files have also been provided with the UV transfer and RB transfer separately (Data.Figure1_RB.xls and Data.Figure2_UV.xls).
Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

| Study description | Field studies were carried out in wild vampire bat colonies in Peru to monitor the spread of two topically-applied biomarkers: a UV powder, which was intended to trace contacts among demographic groups and a rhodamine b gel that was intended to simulate the transfer and ingestion of self-spreading disease control agents. The studies were carried out in three replicate bat colonies, which ranged in size from 207 to 257 individuals (according to mark-recapture analysis). Data were used to estimate parameters that were included in mathematical models. |
| Research sample | The research sample consisted of common vampire bats (Desmodus rotundus) captured at 3 sites near Lima, Peru. Individuals were male and female bats of adult, subadult, and juvenile age. This species is the most appropriate for the research question posed which involved the dynamics of rabies transmission and control in vampire bats. The colonies we studied are of typical size, age and sex distributions for this species in Peru. The research sample is therefore designed to apply more broadly to other populations of this species. Existing data from previous field studies and experiments in captive vampire bat were used to assign parameters in mathematical modeling. The parameter values used and their associated literature references are provided in Supplementary Table 3. |
| Sampling strategy | Sample sizes for field studies depended on capture success of vampire bats, which was largely beyond our control. However, we selected highly accessible bat colonies where capture successes would be maximized and standardized capture efforts across sites. Our decision to carry out our field experiment in three replicate colonies was based on the logistical constraints of field work, since this was the maximum number of colonies that could be monitored simultaneously by a single field team working full time. |
| Data collection | Field data were collected by predominately co-authors Carlos Tello and Jorge Carrera with some early assistance from other co-authors (Bakker, Streicker, Osorio, Rocke). The rhodamine study was carried out once per bat colony since rhodamine is a long lasting marker, so transfers from repeat treatments would not be distinguishable. Rhodamine transfers were monitored by catching bats for one month after application across 4-5 sampling instances. Uptake of rhodamine was assessed by plucking hair samples from captured bats and microscopy (see below). The UV contact tracing was carried out 3 times per bat colony since powders are only detectable for several days after application. UV transfers were monitored for 2 nights after each application by inspecting bats with UV lights prior to handling (to avoid any potential contamination from gloves). Supplementary Table 1 contains a timeline of all field activities. Laboratory analysis of hair samples was carried out by co-authors Carlos Tello and Rachel Abbott. |
| Timing and spatial scale | Data were collected between January 31, 2017 and July 30, 2017 in three bat colonies in the Department of Lima, Peru. Sites were separated by 54 to 200km, a distance that is well beyond the expected dispersal ability of vampire bats, making our colonies independent. The LMA5 colony was studied between April 20, 2017 and July 27, 2017. The LMA6 colony was studied between April 22, 2017 and July 24, 2017. The LMA12 colony was studied between January 31, 2017 and July 30, 2017. |
| Data exclusions | No data were excluded. |
| Reproducibility | Each of our bat colonies is considered a replicate of the rhodamine b transfer estimate. We faced logistical challenges in colony LMA12 since a large fraction of that colony abandoned the roost site. Nevertheless we include these data in all analyses for completeness. The UV studies were repeated 3 times per bat colony (9 total). |
| Randomization | We aimed to infer rates that would be most relevant to real-world campaigns, thus randomization of biomarker application was not appropriate. Instead we apply rhodamine or UV powders to all captured individuals on designated capture days/nights. |
| Blinding | Individuals conducing the microscopy were blinded to the whether hair samples came from rhodamine treated bats. Individuals carrying out the UV detection were blinded to whether UV had been applied to those bats. |

**Field work, collection and transport**

| Field conditions | The study was carried out in the Department of Lima, Peru which is largely a subtropical coastal desert with little appreciable rainfall. Average minimum/maximum temperatures between January and July are 16-21 C and 19-27 C. |
| Location | Field studies were carried out in three vampire bat roosts in the Barranca (LMA5, -10.6415, -77.8160; elevation = 65.6 meters), Huaura (LMA6, -11.0555, -77.4594, elevation=354.7), and Lima (LMA12, -12.1833, -76.8500, elevation=202 meters) districts. |
Samples were collected under the Peruvian collection permit, 028-2017-SERFOR/DGGSPFFS and exported to the United States under export permit, 3235-SERFOR.

Disturbance to field sites was minimal, potentially including minor disturbance around roosts where nets were set up. All animals were released after sampling at the site of capture.

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**Materials & experimental systems**

| n/a | Involved in the study |
|-----|-----------------------|
| ☒  | Antibodies           |
| ☒  | Eukaryotic cell lines|
| ☒  | Palaeontology        |
| ☒  | Animals and other organisms |
| ☒  | Human research participants |
| ☒  | Clinical data        |

**Methods**

| n/a | Involved in the study |
|-----|-----------------------|
| ☒  | ChIP-seq              |
| ☒  | Flow cytometry        |
| ☒  | MRI-based neuroimaging|

Animals and other organisms

Policy information about [studies involving animals, ARRIVE guidelines](https://arriveguidelines.org) recommended for reporting animal research

**Laboratory animals**

No laboratory animals were used in this study.

**Wild animals**

Wild common vampire bats (Desmodus rotundus) were captured in Peru. Both males and females of adult, subadult and juvenile age were sampled. Bats were captured using hand nets within roosts during the day and using mist nets to capture bats exiting to forage at night. Nocturnal captures lasted from approximately 18:00 – 6:00, and nets were checked every 30 minutes. Animals were held in individual bags prior to processing, and were released in the same area where they were captured immediately after sampling and/or application of biomarkers.

**Field-collected samples**

No laboratory work was performed with field collected animals. Animals were released at the site of capture after sampling. Hair samples were collected from bats and analyzed by microscopy.

**Ethics oversight**

This research was performed under approval of University of Glasgow School of Veterinary Medicine Animal Ethics Committee, approval number, 25A/18.

Note that full information on the approval of the study protocol must also be provided in the manuscript.