Safety, immunogenicity and efficacy of intramuscular and oral delivery of ERA-g333 recombinant rabies virus vaccine to big brown bats (*Eptesicus fuscus*)

Amy T. Gilbert[1,3,5], **Xianfu Wu**[1], **Felix R. Jackson**[1], **Richard Franka**[2], **Gary F. McCracken**[3], **Charles E. Rupprecht**[4]

[1]Poxvirus and Rabies Branch, Division of High-Consequence Pathogens and Pathology, Atlanta, GA USA 30333

[2]Center for Global Health, Global Immunization Division, Atlanta, GA USA 30333

[3]Department of Ecology and Evolutionary Biology, University of Tennessee, Knoxville, TN USA 37996

[4]LYSSA LLC, Lawrenceville, GA USA

Abstract

Attenuated strains of rabies virus (RABV) have been used for oral vaccination of wild carnivores in Europe and North America. However, some RABV vaccines caused clinical rabies in target animals. To improve the safety of attenuated RABV as oral vaccines for field use, strategies using selection of escape mutants under monoclonal antibody neutralization pressure and reverse genetics defined-mutations have been used. We tested the safety, immunogenicity and efficacy of one RABV construct, ERA-g333, developed by reverse genetics for intramuscular (im) or oral (po) routes in big brown bats (*Eptesicus fuscus*). Twenty-five bats received 5×10^6 MICLD$_{50}$ of ERA-g333 im, 10 received 5×10^6 MICLD$_{50}$ of ERA-g333 po, and 22 bats served as unvaccinated controls. Twenty-one days after vaccination, 44 bats were infected im with $10^{2.9}$ MICLD$_{50}$ of *E. fuscus* RABV. We report both immunogenicity and efficacy of ERA-g333 delivered im, but no induction of humoral immunity detected in bats vaccinated po. A subset of bats vaccinated im (N=5) and po (N=3) were not challenged and none developed clinical rabies from ERA-g333. Scarce reports exist on the evaluation of oral rabies vaccines in insectivorous bats, although the strategy described here may be feasible for future studies of these important RABV reservoirs.

Keywords

bat; *Eptesicus fuscus* ; ERA; rabies; reverse genetics; vaccination

[5]Corresponding author: Amy T. Gilbert, National Wildlife Research Center, US Department of Agriculture, 4101 Laporte Avenue, Fort Collins, CO 80521, Fax: 970-266-6063, amy.t.gilbert@usda.aphis.gov.

INTRODUCTION

Rabies is a zoonotic disease caused by infection with negative-sense, single-stranded RNA viruses in the genus Lyssavirus. Rabies virus (RABV) is responsible for an estimated excess of 59,000 human fatalities each year worldwide, the majority of which are transmitted by rabid dog bites (Hampson et al. 2015). In the Americas, bats are also an important RABV reservoir for human and animal infections (Messenger et al. 2002, Schneider et al. 2009). While the common vampire bat (*Desmodus rotundus*) is the most important RABV reservoir of human and animal infections in Latin America, insectivorous bat RABV is associated with the majority of human infections as well as spillover and epizootic events in wild carnivores in Canada and the U.S. (Leslie et al. 2006, De Serres et al. 2008, Kuzmin et al. 2012). The highest diversity of sylvatic RABV reservoirs is found in the Americas, in part due to the independent circulation of RABV in multiple insectivorous bat species (Gilbert 2018). Control of RABV circulation in reservoir hosts historically focused on culling and depopulation techniques, but these methods alone were largely ineffective (Aubert 1994). The most widely employed modern method is a strategy of oral rabies vaccination (ORV) of wildlife (WHO 2018). While ORV efforts typically target wild carnivore RABV reservoirs, few studies have evaluated oral rabies vaccines in bats.

The Evelyn-Rokitniki-Abelseth (ERA) strain of RABV is an attenuated virus that was derived from the Street-Alabama-Dufferin (SAD) RABV strain. The SAD/ERA strain has been used for ORV with terrestrial reservoirs in North America and Europe (MacInnes et al. 2001, Muller et al. 2015). Cases of residual pathogenicity have been reported after the field use of the SAD/ERA strain in target and non-target animals (Fehlner-Gardiner et al. 2008, Vuta et al. 2016). The pathogenicity of the ERA strain can be significantly altered by mutations at amino acid residue 333 of the glycoprotein (Dietzschold et al. 1983). Escape mutant strains from the SAD virus strain were produced under monoclonal antibody selection pressure at position 333 and identified as SAG1 and SAG2 (Le Blois et al. 1990, Lafay et al. 1994). The SAG2 strain has also been widely used for ORV of wildlife in Europe (Mahl et al. 2014). Site-directed mutagenesis was also utilized to alter all three nucleotides of ERA at position 333 (ERA-g333) to further improve the safety profile by reducing the chance for reversion to a virulent phenotype. Experimental studies demonstrated the immunogenicity of the ERA-g333 construct in wild carnivores (Bankovskiy et al. 2008). No studies to our knowledge have examined ERA or its derivatives in bats.

The objective of this study was to evaluate experimentally the relative safety, immunogenicity and efficacy of ERA-g333, delivered by the intramuscular (im) and oral (po) routes to big brown bats (*Eptesicus fuscus*) against a lethal RABV infection.

METHODS AND MATERIALS

Animals and housing

Experimental procedures and animal care were performed in compliance with the Centers for Disease Control and Prevention Institutional Animal Care and Use Committee guidelines (protocol 1405RUPBATL). During June and September 2007, 57 big brown bats were

collected using butterfly nets from a building roost in Georgia, as authorized by Georgia Department of Natural Resources Scientific Collection Permit #29-WCH-07-54. Bats were held captive in quarantine at CDC for at least one month prior to use and marked individually with metal bands on the forearm. During quarantine and acclimation, bats were trained to eat commercial live mealworms (*Tenebrio molitor*) from petri dishes. For the duration of the experiment, individual petri dishes of water and mealworms were offered ad libitum to each cage of bats and refreshed daily. Eleven groups of bats were housed separately in 813 mm × 305 mm × 254 mm stainless steel cages, with all cages held in a room at 24-27°C and ~30% humidity. Two cages held groups of six bats (N, O), and the remaining nine cages held groups of five bats (L, M, P, Q, R, S, T, U, V; Table S1). Baseline sera were collected to screen the RABV exposure status of bats prior to vaccination.

Vaccination

The ERA-g333 vaccine used for these experiments has been previously described (Bankovskiy et al. 2008). Bats were manually restrained and administered 5×10^6 FFU of ERA-g333 (0.05mL of 10^8FFU/mL) by im or po routes, replicated as two consecutive experiments. Unvaccinated control bats were not mock-treated. Vaccine treatments were assigned to replicate cages of bats. On day 0, 25 bats were vaccinated im in the deltoid muscle and ten bats were vaccinated po by needless syringe. Seventeen bats served as unvaccinated controls and held in separate cages from vaccinated bats. Five bats served as unvaccinated and uninfected contact controls with orally vaccinated (n=1, cage N and O) and unvaccinated control bats (n=3, cage P) during experiment 1. No contact controls were held during experiment 2.

Blood samples were obtained from a peripheral wing vein during each experiment or by the intra-cardiac route for terminal exsanguination at euthanasia (Voigt and Cruz-Neto 2009), and collected in sterile heparinized microcapillary tubes. Serum was separated by low-speed centrifugation and stored at −20°C until processing. Bats were bled at similar intervals, including days 5, 14, 27, 43, 98 and 155 post vaccination (pv) during experiment 1 and days 5, 19, 34, 49, 90, and 161 pv during experiment 2.

RABV Challenge

The *E. fuscus* RABV used for challenge was collected from the salivary glands of a naturally infected big brown bat in Pennsylvania during 2006 (PAEf3684; CDC accession A06-3684), as described previously (Turmelle et al. 2010). On day 21 pv, bats were restrained manually and inoculated im into both the left and right masseter muscles with $10^{4.2}$ MICLD$_{50}$/ml of RABV in a volume of 0.05 ml. Twenty-seven vaccinates were challenged across two experiments, including 20 bats vaccinated im and seven bats vaccinated po. A total of 17 unvaccinated bats were challenged as controls across two experiments. Animals were monitored daily for clinical signs of rabies for 140 days post infection (pi).

Bats were euthanized by exsanguination under anesthesia followed by intracardiac injection of a barbiturate solution (i.e., pentobarbital sodium and phenytoin sodium), upon presentation of two or more definitive clinical signs of RABV infection (e.g., increased

aggression/reclusion, acute weight loss, ataxia, atypical vocalizations, paresis or paralysis). Brain tissue was collected and tested for RABV antigen by the direct fluorescent antibody (DFA) test, as described by Dean et al. (1996), using fluorescein isothiocyanate (FITC)-labeled monoclonal antibody conjugate (Fujirebio Diagnostics, Inc., Malvern, Pennsylvania, USA). For all rabid bats, total RNA was extracted from individual brain tissues and the RABV nucleoprotein gene was amplified and sequenced as described previously (Trimarchi and Smith 2002).

Detection of RABV neutralizing antibodies

A modified RFFIT (Jackson et al. 2008), using RABV challenge virus standard (CVS-11), was used to assay for RABV-specific viral neutralizing antibodies (RVNA) in the blood plasma of individual bats. Titers were calculated by the Reed-Muench method (Reed and Muench 1938), and were converted to international units (IU/ml) by comparison to the US Standard Rabies Immune Globulin (SRIG; Laboratory of Standards and Testing, Food and Drug Administration, Bethesda, Maryland, USA) diluted to 2 IU/ml. The RVNA titers of individual bats were determined up to a level of ~9.14 IU/mL, equivalent to 50% neutralization at the eighth serial 2-fold dilution. The minimum level of RVNA detection in the modified RFFIT was defined as greater than or equal to 0.06 IU/mL, as reported previously. In this study, values greater than or equal to 0.1 IU/ml were considered as evidence of neutralization, which was equivalent to a titer of ~1/22. Titers above the cutoff and which exhibited a four-fold increase or greater above baseline values were considered as evidence of seroconversion pv or pi.

Statistical analysis

The RVNA titers that exceeded 9.14 IU/mL were coded as 9.14 IU/mL for the purpose of geometric mean calculations. Group geometric mean titers (GMT) were calculated for different treatment groups, but excluded bats that were seropositive upon baseline sampling as well as unvaccinated control bats in cage U. The survival fraction and 95% exact confidence intervals were calculated among vaccine treatment and unvaccinated groups of RABV challenged bats. A survival analysis was also performed among challenged bats to test for homogeneity in survival curves pi, using a log-rank test on data stratified by vaccine treatment and route and the Šidák correction to adjust for multiple pairwise comparisons. SAS v.9.4 was used to perform all analyses (SAS Institute, Cary, North Carolina, USA) and significance was assessed at α=0.05.

RESULTS

A total of 57 individual big brown bats were included in the study (Table 1; Table S1). Three bats (#42, 54, 72) presented evidence of RVNA during baseline sampling and were treated as contact controls during experiment 1 (cage P). On day 98 pv of experiment 1, they were kept in the same cage, but challenged with RABV as part of experiment 2. Two other bats (#31 and 33) were used as contact controls in separate cages during experiment 1, but neither demonstrated evidence of RVNA during the study (Table S1; serology data not shown). These bats were censored during experiment 1 on days 118 (#31) and 161 (#33) pv and were not used during experiment 2.

Vaccination

In the group of 25 bats vaccinated im, three bats presented evidence of RVNA at baseline (#10, 23, 29; Table 2). Among 22 bats vaccinated im which were seronegative at baseline, a moderate proportion seroconverted by day 14-19 pv (75%, 15 of 20; Table 2), whereas evidence of seroconversion pv yet prior to challenge could not be determined for two bats (#15, 22). The RVNA GMT among 22 bats that were seronegative at baseline and vaccinated IM was 0.39 IU/mL on day 14-19pv. Although five bats did not seroconvert pv prior to challenge (#19, 20, 25, 26, 39), only two bats (#19, 25) remained seronegative pv until being censored from study on days 27 and 125 pv. Four (#14, 38, 41, 51) im vaccinates that were seronegative at baseline demonstrated RVNA as early as day 5 pv, suggestive of prior RABV exposure history. No RVNA were detected during the baseline sampling of ten bats vaccinated po and none of these bats seroconverted during the study (Table 3). No adverse events related to vaccination were noted in any subjects (e.g., reversion to virulence). Only one bat of the unvaccinated control group demonstrated RVNA during baseline sampling (#21, cage U, Table 4) and prior to challenge.

RABV Challenge

Two of the three bats (#10, 23) vaccinated im that had RVNA at baseline were challenged and both survived along with 18 bats vaccinated im that were seronegative prior to vaccination (Table 5; Figure 1). All bats that were seronegative at baseline and vaccinated im were protected against rabies infection (17 of 17, 95% CI 82-100), excluding one bat censored on day 27 pv (#19). Survival among bats vaccinated po was 57% (4 of 7, 95% CI 25-84). Three bats vaccinated po developed clinical rabies and were euthanized on days 24, 26, and 87 pi. Among unvaccinated controls, we retroactively diagnosed an outbreak of natural infection among bats in cage U and these five bats were excluded from the analysis (Supplemental Material; Table S2). The remaining 12 unvaccinated controls remained seronegative during the study, except for one bat that presented RVNA on day 6 pi, again perhaps suggestive of a prior RABV exposure history. Survival among unvaccinated controls was 44% (4 of 9, 95% CI 19-73) with a median incubation period of 26 days (range: 20-115 days), excluding two censored bats and one bat missing a diagnostic DFA result. The three seropositive contact controls from cage P survived RABV challenge during experiment 2, although there was equivocal evidence for a boosting effect of challenge on RVNA levels pi (Table 6). The im vaccinate group had greater survival compared to both the po vaccinate group (X^2=8.5, p=0.01) and unvaccinated controls (X^2=9.7, p=0.006), but there was no difference in survival between the po vaccinates and unvaccinated controls (X^2=0.5, p=0.86).

DISCUSSION

Although there are no efforts targeting control of insectivorous bat rabies in the Americas, the results of this study suggest that the ERA-g333 vaccine may be safe, immunogenic and efficacious for big brown bats, and possibly other bats, such as the common vampire bat. While the oral dose of ERA-g333 tested in this study was neither immunogenic nor efficacious, it seems plausible that the dose may have been too low in comparison to the doses typically used for oral delivery to wild carnivores (Bankovskiy et al. 2008). In one

other rabies vaccination study involving big brown bats, immunogenicity and efficacy of a different live poxvirus vaccine construct by oronasal route occurred (Stading et al. 2017) and immunogenicity of a similar poxvirus construct was also demonstrated among Brazilian free-tailed bats (*Tadarida brasiliensis*) when delivered by oronasal route (Stading et al. 2016). We had low power to detect any difference in survivorship between po vaccinates and controls, but the lack of RVNA responses among po vaccinates during experiment 1 did not compel further replication of the treatment route during experiment 2. Future studies should consider oral testing of vaccine in bats at a dose more comparable to carnivores, although vaccine delivery volume constraints of working with bats may require concentration of vaccine stock as previously reported during a study with common vampire bats (Almeida et al. 2008). Similar to observations from experimental studies with common vampire bats and more recently insectivorous bats (Almeida et al. 2005, Almeida et al. 2008, Stading et al. 2017), indirect routes of vaccination are also critical to evaluate once oral efficacy has been established, given the potential utility of social grooming oral contact behavior among gregarious bats to increase vaccination coverage beyond what can be achieved by direct delivery methods. This study suggests that live attenuated RABV constructs traditionally used for ORV of wild carnivores in North America and Europe may be efficacious for bats as well.

Other important insights regarding big brown bat RABV pathogenesis were made during the study. Firstly, several of the bats that were collected from the wild presented RVNA during baseline sampling, and several others which were seronegative upon intake appeared to develop an anamnestic response to vaccination or challenge (i.e., RVNA seroconversion within 5-6 days pv or pi). This is consistent with observation of naturally occurring abortive RABV infections in sylvatic reservoirs and demonstrates that seronegative results do not necessarily imply a naïve exposure history among wild-caught mammals, as previously reported (Turmelle et al. 2010). Secondly, naturally-acquired RVNA (range: 0.2 to greater than 9.14 IU/mL) was protective against RABV challenge in four bats. Bats may be incubating RABV infection upon collection from the wild, with potential for bat-to-bat transmission in captive settings and safety risks to humans and animals (Shankar et al. 2004, Davis et al. 2012). While we cannot conclusively establish that natural RABV transmission occurred during our study, it seems more parsimonious in comparison to a hypothesis of independent incubation of RABV infections in four bats of a single cage. The outbreak in this study was associated with an autumn collection of big brown bats, a time of year where population infection prevalence may be elevated (George et al. 2011).

In conclusion, im delivery of the ERA-g333 live attenuated recombinant RABV vaccine was safe, immunogenic and efficacious in protecting big brown bats against a lethal challenge, yet po delivery was neither immunogenic nor efficacious. However, it remains likely that a higher vaccine dose po may be effective. While broad scale delivery of vaccines to wild bats still presents logistical challenges, additional studies testing oral rabies vaccines in insectivorous and other bats are warranted, particularly for key RABV reservoir hosts.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

LITERATURE CITED

Almeida MF, Martorelli LF, Aires CC, Barros RF, Massad E. 2008. Vaccinating the vampire bat *Desmodus rotundus* against rabies. Virus Res 137:275–277. [PubMed: 18761044]

Almeida MF, Martorelli LF, Aires CC, Sallum PC, Massad E. 2005. Indirect oral immunization of captive vampires, *Desmodus rotundus*. Virus Res 111:77–82. [PubMed: 15896405]

Aubert M. 1994. Control of rabies in foxes: what are the appropriate measures? Vet Rec 134:55–59. [PubMed: 8135014]

Bankovskiy D, Safonov G, Kurilchuk Y. 2008. Immunogenicity of the ERA G 333 rabies virus strain in foxes and raccoon dogs. Dev Biol (Basel) 131:461–466. [PubMed: 18634508]

Davis A, Gordy P, Rudd R, Jarvis JA, Bowen RA. 2012. Naturally acquired rabies virus infections in wild-caught bats. Vector Borne Zoonotic Dis 12:55–60. [PubMed: 21923271]

De Serres G, Dallaire F, Cote M, Skowronski DM. 2008. Bat rabies in the United States and Canada from 1950 through 2007: human cases with and without bat contact. Clin Infect Dis 46:1329–1337. [PubMed: 18419432]

Dean DJ, Abelseth MK, Atanasiu P. 1996. The fluorescent antibody test. In: Laboratory techniques in rabies, Meslin FX, Kaplan MM, Koprowski H, editors. World Health Organization, Geneva, Switzerland, pp. 88–93.

Dietzschold B, Wunner WH, Wiktor TJ, Lopes AD, Lafon M, Smith CL, Koprowski H. 1983. Characterization of an antigenic determinant of the glycoprotein that correlates with pathogenicity of rabies virus. Proc Natl Acad Sci USA 80:70–74. [PubMed: 6185960]

Fehlner-Gardiner C, Nadin-Davis S, Armstrong J, Muldoon F, Bachmann P, Wandeler A. 2008. ERA vaccine-derived cases of rabies in wildlife and domestic animals in Ontario, Canada, 1989-2004. J Wildl Dis 44:71–85. [PubMed: 18263823]

George DB, Webb CT, Farnsworth ML, O'shea TJ, Bowen RA, Smith DL, Stanley TR, Ellison LE, Rupprecht CE. 2011. Host and viral ecology determine bat rabies seasonality and maintenance. Proc Natl Acad Sci USA 108:10208–10213. [PubMed: 21646516]

Gilbert A. 2018. Rabies virus vectors and reservoir species. Rev Sci Tech Off Int Epiz 37:371–384.

Hampson K, Coudeville L, Lembo T, Sambo M, Kieffer A, Attlan M, Barrat J, Blanton JD, Briggs DJ, Cleaveland S, Costa P, Freuling CM, Hiby E, Knopf L, Leanes F, Meslin FX, Metlin A, Miranda ME, Muller T, Nel LH, Recuenco S, Rupprecht CE, Schumacher C, Taylor L, Vigilato MA, Zinsstag J, Dushoff J, et al. 2015. Estimating the global burden of endemic canine rabies. PLoS Negl Trop Dis 9:e0003709. [PubMed: 25881058]

Jackson FR, Turmelle AS, Farino DM, Franka R, Mccracken GF, Rupprecht CE. 2008. Experimental rabies virus infection of big brown bats (*Eptesicus fuscus*). J Wildl Dis 44:612–621. [PubMed: 18689646]

Kuzmin IV, Shi M, Orciari LA, Yager PA, Velasco-Villa A, Kuzmina NA, Streicker DG, Bergman DL, Rupprecht CE. 2012. Molecular inferences suggest multiple host shifts of rabies viruses from bats to mesocarnivores in Arizona during 2001-2009. PLoS Pathog 8:e1002786. [PubMed: 22737076]

Lafay F, Benejean J, Tuffereau C, Flamand A, Coulon P. 1994. Vaccination against rabies: construction and characterization of SAG2, a double avirulent derivative of SAD Bern. Vaccine 12:317–320. [PubMed: 8178553]

Le Blois H, Tuffereau C, Blancou J, Artois M, Aubert A, Flamand A. 1990. Oral immunization of foxes with avirulent rabies virus mutants. Vet Microbiol 23:259–266. [PubMed: 2402873]

Leslie MJ, Messenger S, Rohde RE, Smith J, Cheshier R, Hanlon C, Rupprecht CE. 2006. Bat-associated rabies virus in skunks. Emerg Infect Dis 12:1274–1277. [PubMed: 16965714]

Macinnes CD, Smith SM, Tinline RR, Ayers NR, Bachmann P, Ball DG, Calder LA, Crosgrey SJ, Fielding C, Hauschildt P, Honig JM, Johnston DH, Lawson KF, Nunan CP, Pedde MA, Pond B, Stewart RB, Voigt DR. 2001. Elimination of rabies from red foxes in eastern Ontario. J Wildl Dis 37:119–132. [PubMed: 11272485]

Mahl P, Cliquet F, Guiot AL, Niin E, Fournials E, Saint-Jean N, Aubert M, Rupprecht CE, Gueguen S. 2014. Twenty year experience of the oral rabies vaccine SAG2 in wildlife: a global review. Vet Res 45:77. [PubMed: 25106552]

Messenger SL, Smith JS, Rupprecht CE. 2002. Emerging epidemiology of bat-associated cryptic cases of rabies in humans in the United States. Clin Infect Dis 35:738–747. [PubMed: 12203172]

Muller T, Freuling CM, Wysocki P, Roumiantzeff M, Freney J, Mettenleiter TC, Vos A. 2015. Terrestrial rabies control in the European Union: historical achievements and challenges ahead. Vet J 203:10–17. [PubMed: 25466578]

Reed LJ, Muench H. 1938. A simple method of estimating fifty percent endpoints. Am J Hyg 27:493–497.

Schneider MC, Romijn PC, Uieda W, Tamayo H, Da Silva DF, Belotto A, Da Silva JB, Leanes LF. 2009. Rabies transmitted by vampire bats to humans: An emerging zoonotic disease in Latin America? Rev Panam Salud Publica 25:260–269. [PubMed: 19454154]

Shankar V, Bowen RA, Davis AD, Rupprecht CE, O'shea TJ. 2004. Rabies in a captive colony of big brown bats (*Eptesicus fuscus*). J Wildl Dis 40:403–413. [PubMed: 15465706]

Stading B, Ellison JA, Carson WC, Satheshkumar PS, Rocke TE, Osorio JE. 2017. Protection of bats (*Eptesicus fuscus*) against rabies following topical or oronasal exposure to a recombinant raccoon poxvirus vaccine. PLoS Negl Trop Dis 11:e0005958. [PubMed: 28976983]

Trimarchi CV, Smith J. 2002. Diagnostic Evaluation. In: Rabies, Jackson AC, Wunner WH, editors. Academic Press, San Diego, CA, pp. 308–344.

Turmelle AS, Jackson FR, Green D, Mccracken GF, Rupprecht CE. 2010. Host immunity to repeated rabies virus infection in big brown bats. J Gen Virol 91:2360–2366. [PubMed: 20519458]

Voigt C, Cruz-Neto A. 2009. Energetic analysis of bats. In: Ecological and Behavioral Methods for the Study of Bats, Kunz TH, Parsons S, editors. The Johns Hopkins University Press, Baltimore, MD, pp. 623–645.

Vuta V, Picard-Meyer E, Robardet E, Barboi G, Motiu R, Barbuceanu F, Vlagioiu C, Cliquet F. 2016. Vaccine-induced rabies case in a cow (*Bos taurus*): Molecular characterisation of vaccine strain in brain tissue. Vaccine 34:5021–5025. [PubMed: 27576075]

World Health Organization (WHO). 2018. WHO Expert Consultation on Rabies, Third Report. WHO Technical Report Series 1012:1–195.
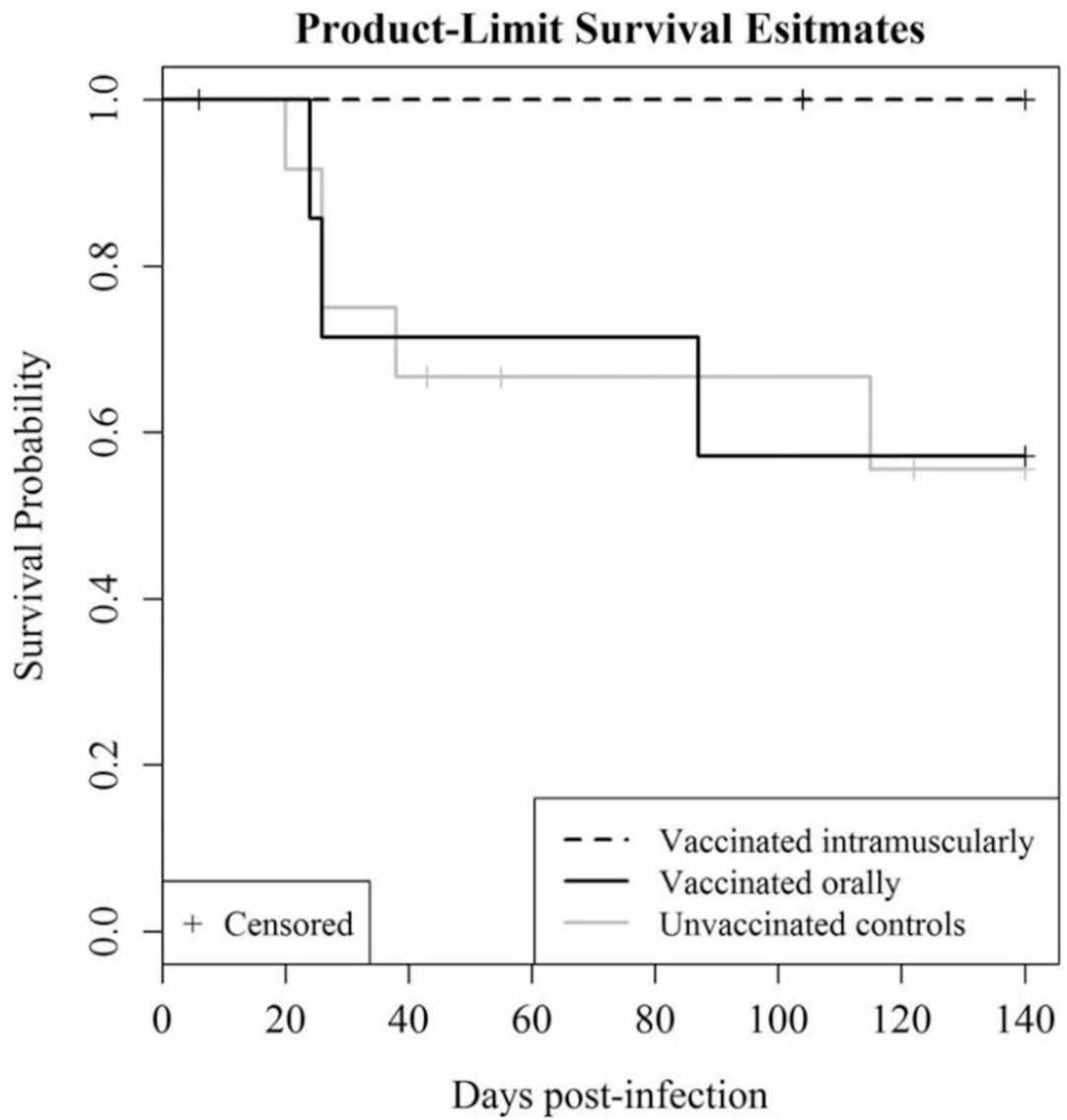
Figure 1.
Survival probabilities for vaccinated and unvaccinated bats experimentally challenged with rabies virus on day 21 post-vaccination and observed for 140 days post-infection.

Table 1

Study design of two consecutive experiments to evaluate intramuscular (IM) or oral (PO) delivery of ERA-g333 recombinant rabies virus vaccine to 57 big brown bats (*Eptesicus fuscus*).

Experiment	Number of Bats	Cages	Vaccine Routes	Vaccination Date	Infection Date (d21pv)	Experiment End Date
1	32	LMNOPQ	IM, PO	7/18/2007	8/8/2007	12/26/2007
2	25[a]	RSTUV[b]	IM	10/18/2007	11/8/2007	3/27/2008
Total	57					

IM=intramuscular, PO=per os, d=day, pv=post-vaccination

[a] Three contact control bats with pre-existing rabies virus neutralizing antibody titers were challenged with rabies virus together with other bats during Experiment 2, within their original cage P, and are only included in the tally of Experiment 1 bats.

[b] Five control bats that were experimentally challenged with rabies virus and housed in cage U were excluded from the analysis, due to an outbreak of a natural rabies virus infection within that cage.

Table 2

Rabies virus neutralizing antibody (IU/mL) detected in sera from 25 big brown bats vaccinated intramuscularly (IM) with 5×10^6 FFU of ERA-g333 recombinant rabies virus vaccine (Day 0). Twenty bats were challenged with rabies virus on day 21 post vaccination (pv), and five bats were not challenged (NI). All bats were observed for 140 days post infection. Sampling time points are labeled by pv day. Where two values are listed, bats were sampled on different days between experiments.

Cage	Vaccination Route	Challenge Dose (log MICLD$_{50}$)	Bat ID	Day 0	Day 5	Day 14/19	Day 27/34	Day 43/49	Day 90/98	Day 155/161
L	IM	2.9	39	<0.06	<0.06	0.09	0.06	0.72	0.91	n.d.
L	IM	2.9	496	<0.06	<0.06	1.62	>9.14	>9.14	5.08	n.d.
L	IM	2.9	47	<0.06	<0.06	0.14	<0.06	0.19	0.06	n.d.
L	IM	2.9	48	<0.06	<0.06	1.42	<0.06	<0.06	<0.06	0.19
M	IM	2.9	51	<0.06	0.11	1.14	0.40	1.92	3.41	2.96
M	IM	2.9	55	<0.06	<0.06	3.29	0.57	1.14	2.10	3.25
M	IM	2.9	38	<0.06	1.70	>9.14	>9.14	>9.14	8.21	n.d.
M	IM	2.9	41	<0.06	0.29	0.57	2.53	1.73	2.46	6.10
R	IM	2.9	66	<0.06	<0.06	0.40	0.64	1.48	1.34	0.07
R	IM	2.9	12	<0.06	<0.06	0.10	<0.06	<0.06	0.12	<0.06
R	IM	2.9	19	<0.06	n.d.	<0.06				
R	IM	2.9	26	<0.06	<0.06	<0.06	n.d.	n.d.	1.14	0.42
S	IM	2.9	69	<0.06	<0.06	1.62	4.57	1.91	0.14	<0.06
S	IM	2.9	10	0.21	n.d.	1.94	3.29	3.75	0.29	0.07
S	IM	2.9	20	<0.06	<0.06	0.08	0.10	0.14	<0.06	0.09
S	IM	2.9	22	<0.06	<0.06	n.d.	0.29	0.32	0.08	<0.06
V	IM	2.9	67	<0.06	<0.06	0.29	1.92	0.57	0.11	0.40
V	IM	2.9	14	<0.06	0.11	n.d.	n.d.	n.d.	n.d.	0.30
V	IM	2.9	15	<0.06	<0.06	n.d.	0.26	n.d.	0.13	0.36
V	IM	2.9	23	0.87	0.29	>9.14	>9.14	n.d.	7.49	
L	IM	NI	45	<0.06	<0.06	6.27	<0.06	0.82	0.09	0.20
M	IM	NI	50	<0.06	<0.06	0.51	0.87	0.10	0.20	0.08
R	IM	NI	28	<0.06	<0.06	0.33	0.11	0.42	0.12	0.20
S	IM	NI	29	0.52	0.19	0.47	0.85	0.24	0.43	0.22

Cage	Vaccination Route	Challenge Dose (log $MICLD_{50}$)	Bat ID	Day 0	Day 5	Day 14/19	Day 27/34	Day 43/49	Day 90/98	Day 155/161
V	IM	NI	25	<0.06	<0.06	<0.06	n.d.	n.d.	<0.06	n.d.

n.d. = not determined; insufficient blood sample from that bat-time point

Table 3

Rabies virus neutralizing antibody (IU/mL) detected in sera from 10 big brown bats vaccinated orally with 5×10^6 FFU of ERA-g333 recombinant rabies virus vaccine. Seven bats were challenged with rabies virus on day 21 post vaccination (pv), and three bats were not challenged (NI). All bats were observed for 140 days post infection. Sampling time points are labeled by pv day. Where two values are listed, bats were sampled on different days between experiments.

Cage	Vaccination Route	Challenge Dose (log MICLD$_{50}$)	Bat ID	Day 0	Day 5	Day 14/19	Day 27/34	Day 43/49	Day 90/98	Day 155/161
N	Oral	2.9	500	<0.06	<0.06	<0.06	<0.06	<0.06	<0.06	<0.06
N	Oral	2.9	52	<0.06	<0.06	<0.06	<0.06	n.d.		
N	Oral	2.9	53	<0.06	<0.06	<0.06	<0.06	<0.06	<0.06	<0.06
N	Oral	2.9	59	<0.06	<0.06	<0.06	<0.06	<0.06	<0.06	<0.06
O	Oral	2.9	44	<0.06	<0.06	<0.06	<0.06	<0.06		
O	Oral	2.9	43	<0.06	<0.06	<0.06	<0.06	<0.06		
O	Oral	2.9	40	<0.06	<0.06	<0.06	<0.06	<0.06	<0.06	<0.06
O	Oral	NI	60	<0.06	<0.06	n.d.				
N	Oral	NI	463	<0.06	<0.06	<0.06	<0.06	<0.06	<0.06	<0.06
O	Oral	NI	61	<0.06	<0.06	<0.06	<0.06	<0.06	<0.06	<0.06

n.d. = not determined; insufficient blood sample from that bat-time point

Table 4

Rabies virus neutralizing antibody (IU/mL) detected in sera from 17 unvaccinated big brown bats challenged with rabies virus on day 21 post vaccination (pv) and observed for 140 days post infection. Sampling time points are labeled by pv day. Where two values are listed, bats were sampled on different days between experiments.

Cage	Challenge Dose (log MICLD$_{50}$)	Bat ID	Day 0	Day 5	Day 14/19	Day 27/34	Day 43/49	Day 90/98	Day 155/161
P	2.9	49	<0.06	<0.06	<0.06	<0.06	<0.06	<0.06	<0.06
P	2.9	58	<0.06	n.d.	<0.06	<0.06	<0.06		
Q	2.9	32	<0.06	n.d.	<0.06	<0.06			
Q	2.9	34	<0.06	n.d.	<0.06	<0.06	<0.06		
Q	2.9	35	<0.06	n.d.	<0.06	0.13	<0.06	n.d.	n.d.
Q	2.9	36	<0.06	n.d.	<0.06	<0.06	<0.06	<0.06	<0.06
Q	2.9	37	<0.06	n.d.	<0.06	<0.06	<0.06		
T	2.9	465	<0.06	<0.06	<0.06	<0.06	<0.06	<0.06	n.d.
T	2.9	16	<0.06	<0.06	<0.06	<0.06	<0.06		
T	2.9	18	<0.06	<0.06	<0.06	<0.06	<0.06	n.d.	<0.06
T	2.9	24	<0.06	<0.06	n.d.	n.d.	n.d.		
T	2.9	27	<0.06	<0.06	<0.06	<0.06	<0.06	<0.06	
U	2.9	65	<0.06	<0.06	<0.06	<0.06			
U	2.9	11	<0.06	<0.06	<0.06	<0.06	n.d.		
U	2.9	17	<0.06	<0.06	<0.06	n.d.	<0.06	<0.06	
U	2.9	21	1.00	0.27	0.50	1.78	n.d.	n.d.	
U	2.9	30	<0.06	<0.06	n.d.	n.d.	n.d.		

n.d. = not determined; insufficient blood sample from that bat-time point

Table 5

Survival outcomes among 39 big brown bats vaccinated with ERA-g333 recombinant rabies virus vaccine by the intramuscular (IM) or oral routes (PO), or unvaccinated controls (NV). On day 21 post vaccination, all bats were experimentally challenged with $10^{2.9}$ MICLD$_{50}$ of a big brown bat rabies virus.

Experiment	Cage	Bat ID	Vaccination Route	Final Disposition[a]
1	L	39	IM	S
1	L	496	IM	S
1	L	47	IM	S
1	L	48	IM	S
1	M	51	IM	S
1	M	55	IM	S
1	M	38	IM	S
1	M	41	IM	S
2	R	66	IM	S
2	R	12	IM	S
2	R	19	IM	N
2	R	26	IM	S
2	S	69	IM	S
2	S	10	IM	S
2	S	20	IM	S
2	S	22	IM	S
2	V	67	IM	S
2	V	14	IM	S
2	V	15	IM	S
2	V	23	IM	N
1	N	500	Oral	S
1	N	52	Oral	D(24)
1	N	53	Oral	S
1	N	59	Oral	S
1	O	44	Oral	D(87)
1	O	43	Oral	D(26)
1	O	40	Oral	S

Experiment	Cage	Bat ID	Vaccination Route	Final Disposition[a]
1	P	49	NV	N
1	P	58	NV	D(26)
1	Q	32	NV	D(20)
1	Q	34	NV	D(26)
1	Q	35	NV	S
1	Q	36	NV	S
1	Q	37	NV	N
2	T	465	NV	S
2	T	16	NV	D(38)
2	T	18	NV	S
2	T	24	NV	n.d.
2	T	27	NV	D(115)

[a]N=tested negative for rabies virus infection (censored); D=died from infection with rabies virus, with incubation period in parentheses; S=survived to 140 days post rabies virus infection; n.d.=not determined, missing lab result for bat that was censored on day 51 pi

Table 6

Rabies virus neutralizing antibody (IU/mL) detected in sera from three unvaccinated big brown bats treated as contact controls during experiment 1. These bats were experimentally challenged with rabies virus on the same day as the other experiment 2 bats and were observed for 140 days post infection.

Cage	Experiment	Challenge Dose (log MICLD$_{50}$)	Bat ID	Day 0	Day 5	Day 14/19	Day 27/34	Day 43/49	Day 90/98	Day 155/161
P	1	NI	42	0.68	0.68	n.d.	0.57	0.96	0.20	
P	1	NI	72	3.01	3.23	5.45	3.46	3.67	1.17	
P	1	NI	54	6.46	6.46	>9.14	>9.14	5.45	>9.14	
P	2	2.9	42	0.20[b]	n.d.	n.d.	0.08	n.d.	n.d.	0.08
P	2	2.9	72	1.17[b]	n.d.	n.d.	2.45	n.d.	6.02	2.86
P	2	2.9	54	>9.14[b]	n.d.	n.d.	6.49	8.58	3.91	>9.14

[a] NI = not infected (during Experiment 1); n.d. = not determined; insufficient blood sample from that bat-time point

[b] the day 98 post vaccination titers for experiment 1 were used to populate the baseline values for experiment 2