### Title

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### Journal

American Journal of Tropical Medicine and Hygiene, 96(4)

### ISSN

0002-9637



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### Publication Date

2017-04-01

### DOI

10.4269/ajtmh.16-0745

Peer reviewed

*Am. J. Trop. Med. Hyg.*, 96(4), 2017, pp. 835–841
doi:10.4269/ajtmh.16-0745


# *Grammomys surdaster*, the Natural Host for *Plasmodium berghei* Parasites, as a Model to Study Whole-Organism Vaccines against Malaria

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*Abstract.* Inbred mice are commonly used to test candidate malaria vaccines, but have been unreliable for predicting efficacy in humans. To establish a more rigorous animal model, we acquired African woodland thicket rats of the genus *Grammomys*, the natural hosts for *Plasmodium berghei*. Thicket rats were acquired and identified as *Grammomys surdaster* by skull and teeth measurements and mitochondrial DNA genotyping. Herein, we demonstrate that thicket rats are highly susceptible to infection by *P. berghei*, and moderately susceptible to *Plasmodium yoelii* and *Plasmodium chabaudi*: 1–2 infected mosquito bites or 25–100 sporozoites administered by intravenous injection consistently resulted in patent parasitemia with *P. berghei*, and resulted in patent parasitemia with *P. yoelii* and *P. chabaudi* strains for at least 50% of animals. We then assessed efficacy of whole-organism vaccines to induce sterile immunity, and compared the thicket rat model to conventional mouse models. Using *P. berghei* ANKA radiation-attenuated sporozoites, and *P. berghei* ANKA and *P. yoelii* chemoprophylaxis vaccination approaches, we found that standard doses of vaccine sufficient to protect laboratory mice for a long duration against malaria challenge, are insufficient to protect thicket rats, which require higher doses of vaccine to achieve even short-term sterile immunity. Thicket rats may offer a more stringent and pertinent model for evaluating whole-organism vaccines.

## INTRODUCTION

After decades of malaria vaccine development, a highly efficacious vaccine to prevent infection remains a top priority. Inbred mice have long been the animal model most commonly used to test candidate vaccines, yet have failed to predict outcomes in humans. For example, BALB/c mice develop sterile immunity against *Plasmodium berghei* (*Pb*) infection after a single immunization with 1,000 *Pb* ANKA radiation-attenuated sporozoites (RAS),[1] and 90–100% protection against *Plasmodium yoelii* (*Py*) with very few *Py*RAS (750 given intravenously × 3 doses).[2] In comparison, humans that received much higher doses and numbers of immunizations have achieved inconsistent levels of protection.[3–6]

Consideration of this difference led Chatterjee and others to investigate *Pb* in African woodland thicket rats (TRs) or *Grammomys surdaster*, a natural host–parasite combination. In this model, infection evolves in a chronic manner, similar to human infection and in contrast to infection in laboratory mice that frequently results in high parasite loads. Their studies suggested that TRs are highly susceptible to infection and are more difficult to protect via RAS immunization, comparable to what is observed in humans with *Plasmodium falciparum*.[3,7] Herein, we seek to establish a thriving colony of TR, confirm previous findings, and test whether TRs are susceptible to other prominent strains of rodent malaria, including *Py* and *Plasmodium chabaudi chabaudi* (*Pcc*) strains. We also characterize whether RAS immunization or chemoprophylaxis vaccination (CVac) confer protection against various rodent malaria strains in TR. Finally, we confirm that TRs are a more stringent animal model than laboratory mice and therefore more pertinent to humans than are laboratory mice.

## MATERIALS AND METHODS

**Rodent parasites.** *Plasmodium berghei* ANKA and *Py* 17XNL stabilates (cryopreserved, frozen, blood-stage parasites) were stored at the National Institutes of Health (NIH) Laboratory of Malaria Immunology and Vaccinology (LMIV), the *Pb* NK65 stabilate was obtained from the NIH Laboratory of Malaria and Vector Research (LMVR), and the *Pcc* AS and *Pcc* CB stabilates were obtained from Jean Langhorne, Division of Parasitology, Medical Research Council National Institute for Medical Research, London, United Kingdom.

**TRs and mice.** African woodland TRs were originally captured in the wild in the south of the Democratic Republic of Congo, Africa, obtained from Department of Parasitology of Institut National de Recherche Biomédicale (INRB), and bred under pathogen-free conditions at NIH/National Institute of Allergy and Infectious Diseases (NIAID)/LMIV. TRs are outbred and housed as couples or harem for life. The offspring were weaned and removed from parents 21–28 days postdelivery and housed separately. Laboratory mice (C57BL/6, BALB/c, and CD1) were obtained from NIH-approved vendors. All breeding procedures and experiments were done in accordance with LMIV 1E ASP approved by the Institutional Animal Care and Use Committees.

**Identification of TRs.** African woodland TRs were identified using four specimen heads and other body parts. DNA was extracted from biopsies taken from an external ear pinna from each, and 1,141 base pairs of mitochondrial DNA sequence were determined for each sample as previously described.[8] Further identification was made by standard measurements of skulls and teeth, and by direct visual comparison with study skins and skulls in the National Museum of Natural History (NMNH).

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**Mosquitoes.** Three- to ten-day-old female *Anopheles stephensi* mosquitoes were obtained from the LMVR. The mosquitoes were infected by allowing them to feed on a donor mouse (BALB/c, CD1, or C57BL/6 mouse) during blood-stage parasitemia. The donor mouse selected for the feed exhibited gametocyte counts of 1+ or 2+ (one or two gametocytes per field for up to 10 fields) and a parasitemia level of less than 10%. The infected mosquitoes were reared at 24°C and 80% relative humidity for the *Py* strain, 26°C and 80% relative humidity for the *Pcc* strains, and 19–21°C and 80% relative humidity for the *Pb* strain. The infected mosquitoes were maintained on water containing 10% Karo syrup. Sporozoites (SPZ) were ready to use from 14 to 21 days post blood meal (dpb) for *Py* and *Pcc* strains, and from 18 to 25 dpb for the *Pb* strain.

**Radiation and dissection of infected mosquitoes.** Infected mosquitoes were irradiated or attenuated with 10,000–15,000 rads (100–150 Gy) using a cesium irradiator. The irradiated or nonirradiated mosquitoes were placed in a freezer (–20°C) for 3–5 minutes to immobilize them. The immobilized mosquitoes were washed in a petri dish containing 70% ethanol for 1 minute and were dried using a Kim wipe (Kimberly-Clark Europe Limited, Reigate, Surrey, United Kingdom) and transferred to another petri dish containing 1× phosphate-buffered saline (PBS) or E199. Mosquitoes were dissected at room temperature and salivary glands were removed using a dissecting microscope (Zeiss Stemi 2000-C [Edmund Optics, Barrington, NJ] or Olympus SZ61 [Tokyo, Japan]), 3/10-mL insulin syringes (U10029G1/2), and forceps. One hundred pairs of dissected glands were put into a Protein LoBind 1.5-mL Eppendorf tube (Hamburg, Germany) containing 100 µL of dissecting medium, 5% normal mouse serum in PBS or E199 medium. After dissection, 0.2 mL of dissection medium was added to increase the total volume to 0.3 mL. A 1-mL syringe with a 26G needle attached was used to triturate and release the SPZ from the glands. The total volume of processed salivary gland was increased to 0.5 or 1.0 mL, depending on the expected SPZ yield per mosquito. SPZ were counted using a Phase contrast microscope (Nikon ECLIPSE E200 [Tokyo, Japan] or Zeiss Axiostar Plus [Gottingen, Germany]) and a disposable hemocytometer. Ten microliters of medium containing SPZ per sample were loaded in the hemocytometer and the SPZ concentration per milliliter was determined.

**Infectious dose titrations in TR.** Using the nonirradiated SPZ concentration per milliliter, the highest infectious dose was calculated and serial dilutions (1:2) were made to titrate the amount of SPZ needed per TR (400, 200, 100, 50, and 25). TR, C57Bl/6, and BALB/c mice were infected with each dose titration using *Pb* ANKA SPZ, *Pb* NK65 SPZ, *Py* 17XNL SPZ, *Pcc* CB, and *Pcc* AS. Each infectious SPZ dose was administered in 200-µL dose volume intravenously via the tail vein. Daily blood smears were prepared on a slide and stained with 10% Giemsa to determine blood-stage parasitemia levels by microscopy.

Each TR was exposed to 5–8 infected mosquitoes at a time, and mosquitoes were replaced only once if they did not probe or feed. Each mosquito pint was fitted with a net screen through which the mosquitoes probed or bit for a blood meal. The test mosquitoes in the pints were immobilized in a –20°C freezer for 3 minutes and then examined under a dissecting microscope. The mosquitoes with blood meals were dissected, and only those with SPZ in their salivary glands were recorded as administering an infectious bite. The percent of infected mosquitoes used or the prevalence of infection of the mosquitoes used for the experiments ranged from 70% to 90%, depending on the batch.

**RAS immunization and homologous challenge.** Using *Pb*RAS, groups of 10 C57BL/6 mice and 10 TRs were immunized three times with $10^4$ *Pb*RAS at 3- to 4-week intervals, and another group of 10 TRs was immunized three times total with $10^5$ *Pb*RAS for the first dose, and $5 \times 10^4$ *Pb*RAS for the second and third doses. Groups of five naïve C57Bl/6 mice and five TRs were used as controls. Four to five weeks after the last immunization, groups received an intravenous homologous challenge of 1,000 *Pb*SPZ.

**CVac and challenge.** Using the nonirradiated SPZ concentration per milliliter, the vaccination dose under chemoprophylaxis (pyrimethamine [PYR]) was calculated. PYR drug was administered orally via acidified drinking water from 5 hours before nonirradiated SPZ injection until up to 42 hours after nonirradiated SPZ injection. PYR drug water was then replaced with normal acidified drinking water. The following PYR drug stock concentration was prepared: 14 mg of PYR/mL of dimethyl sulfoxide with the stock diluted 100 times in acidified drinking water (1:100 = 3 mL of stock PYR: 297 mL of acidified drinking water). For intraperitoneal drug treatment, PYR stock concentration was diluted 100 times (1:100 = 100 of stock PYR: 9900 of PBS) and 200 µL of diluted PYR was administered intraperitoneally at the following time points: 20, 27, and 42 hours after nonirradiated SPZ injection or vaccination. After each vaccination, blood smears were collected on day 7 and 14 to check for blood-stage infections. Four to five weeks or 16 weeks after the last immunization, groups received an intravenous homologous challenge of 200 *Py*SPZ.

## RESULTS

**TRs are identified as *G. surdaster*.** To establish a more rigorous animal model for testing malaria vaccines, we acquired African woodland TRs of the genus *Grammomys* (Figure 1), the natural hosts for *Pb*. TRs were transferred from a colony at INRB in Kinshasa to establish a breeding colony at NIAID in Rockville, MD. TRs used in this study were originally captured in the wild at Fungurume (200 km from Lubumbashi) and Lumata (50 km from Lubumbashi) in

FIGURE 1. Photos of thicket rat (TR; *Grammomys surdaster*). Color photos of African woodland TR originally captured in the wild in the south of Democratic Republic of Congo, Africa, obtained from Department of Parasitology, Institut National de Recherche Biomédicale, and bred under pathogen-free conditions at National Institutes of Health/National Institute of Allergy and Infectious Diseases/Laboratory of Malaria Immunology and Vaccinology.

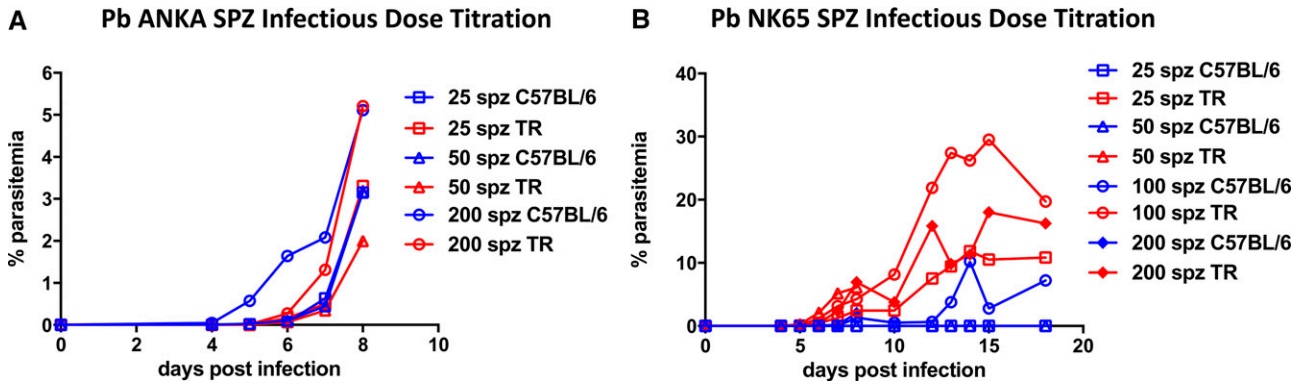


FIGURE 2. Thicket rats (TRs) are susceptible to infection with two strains of *Plasmodium Berghei* (*Pb*). (**A**) Dose-dependent parasitemia in three mice and three TRs inoculated with 25, 50, or 200 *Pb* ANKA sporozoites (SPZ). TRs were treated with chloroquine on day 6 after SPZ inoculation. (**B**) Parasitemia in four C57BL/6 mice and four TRs inoculated with 25, 50, 100, or 200 *Pb* NK65 SPZ. Each line represents one animal.

the Katanga Province, south of the Democratic Republic of Congo, and have been maintained in a breeding colony at INRB, before transfer and rearing for these studies at LMIV. The areas where TRs were originally captured correspond with the regions where *Pb* strains were first isolated from *G. surdaster* and *Anopheles dureni*.[9,10]

Animals were identified as *G. surdaster* by mitochondrial DNA genotyping and by skull and teeth measurements. All mitochondrial sequences from four independent animals were identical. When blasted to NCBI nonredundant protein database (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastp), the top (best) hit was to accession number AF141218.1, for cytochrome B from a *Grammomys* species from Tanzania with 93% identity. The second best hit was to accession number EU349747.1 for *G. surdaster* with 86% identity. Comparatively, the percent identity to laboratory mice and laboratory rats was 83%, with approximately 880 other NCBI protein sequences of cytochrome B being more similar to TR than laboratory mice or rats. The methods used to identify our TR are previously described.[11] Further identification was made by standard measurements of skulls and teeth taken with digital calipers accurate to 0.02 mm and compared with those in Table 2 of Dieterlen and others[12] and the measurements listed in Happold and others.[13] Species determinations were made by direct visual comparison with study skins and skulls in the NMNH. The skulls best match *Grammomys dolichurus* among the species represented in the NMNH collection, which is synonymous with *G. surdaster* by current taxonomy. This identification is consistent with current understanding of species distributions and definitions within *Grammomys*.[12,13]

TABLE 1
TR infection with *Plasmodium berghei* ANKA by mosquito bite

| | TR | Fed mosquitoes | Fed mosquitoes with sporozoites | Parasitemia |
|---|---|---|---|---|
| Experiment 1 | 1 | 5 | 1 | Positive |
| | 2 | 3 | 2 | Positive |
| | 3 | 0 | 0* | Positive |
| Experiment 2 | 1 | 3 | 1 | Positive |
| | 2 | 2 | 2 | Positive |
| | 3 | 0 | 0* | Positive |
| | 4 | 1 | 0 | Negative |

TR = thicket rat.
*Denotes mosquitoes that likely probed without taking a blood meal.

**TRs are susceptible to infection by rodent malaria species.** To establish reliable sporozoite doses to cause parasitemia, we first tested the susceptibility of TR to infection by parasites used in rodent malaria models. As *Pb* is commonly used in C57BL/6 mice, we dissected *Pb* ANKA and *Pb* NK65 salivary gland SPZ and compared their infectivity in TR to that in C57BL/6 mice. Initial infections determined that *Pb* ANKA was lethal to TR, and subsequent infection experiments required chloroquine cure once the blood-stage infection was imminent. Using dose titrations of between 25 and 200 SPZ injected intravenously, as few as 25 *Pb* ANKA (Figure 2A) or *Pb* NK65 SPZ (Figure 2B) resulted in patency; mice and TRs appeared to have similar susceptibility to *Pb* ANKA, whereas susceptibility to *Pb* NK65 was as high or higher in TR than in mice. To assess infection by natural transmission, TRs were exposed to *A. stephensi* mosquitoes infected with *Pb* ANKA. Four of four TRs that provided blood meals to one or two sporozoite-infected mosquitoes became infected (Table 1). Two out of the three TR that did not provide blood meals to sporozoite-infected mosquitoes, nevertheless became infected presumably because they were probed by sporozoite-infected mosquitoes that did not take a blood meal. As few as one infected bite was sufficient to induce parasitemia.

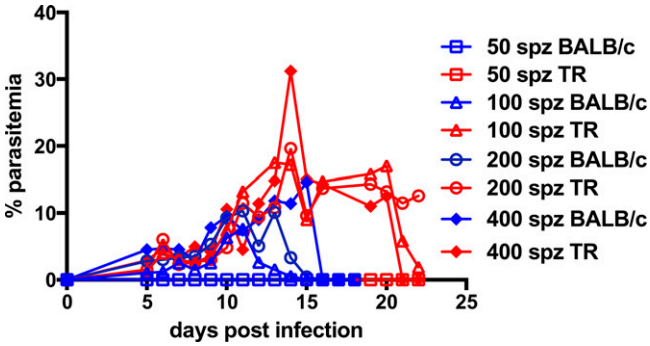


FIGURE 3. Thicket rats (TRs) are susceptible to infection with *Plasmodium yoelii* (*Py*) 17XNL. Parasitemia in four BALB/c mice and four TRs inoculated with 50, 100, 200, or 400 *Py* 17XNL sporozoites (SPZ). Mice self-cured by day 16 postinfection, whereas TR also self-cured by day 23 postinfection. Each line represents one animal.

TABLE 2
TR infection with *Plasmodium yoelii* 17XNL by mosquito bite

| TR | Fed mosquitoes | Fed mosquitoes with sporozoites | Parasitemia |
|---|---|---|---|
| 1 | 2 | 1 | Positive |
| 2 | 5 | 3 | Negative |
| 3 | 3 | 1 | Positive |
| 4 | 3 | 3 | Negative |

TR = thicket rat.

Next, *Py* 17XNL was used to infect TR and BALB/c mice. Salivary gland SPZ were dissected and injected intravenously in a dose range of 50–400 SPZ per animal. TRs were easily infected by *Py*, though not with the same sensitivity as seen with *Pb*, with 100 or more *Py* SPZ resulting in patency in both animal models (Figure 3). Interestingly, while mice and TR self-cured, TR appeared to require ~1 week longer to clear parasitemia when SPZ were injected intravenously. We also confirmed infection by the natural route; two of four TRs that provided blood meals to sporozoite-infected mosquitoes became infected. As few as two mosquito bites were sufficient to result in patency (Table 2).

Finally, we confirmed TR were susceptible to *Pcc* CB (Figure 4A, Supplemental Figure 1) and AS (Figure 4B, Supplemental Figure 2) by both intravenous injection and mosquito bite (Table 3). Two of four TRs that provided blood meals to sporozoite-infected mosquitoes became infected, and as few as three infectious mosquito bites were sufficient to result in patency. In each mosquito bite experiment, all mosquitoes that probed took a blood meal except for one feeding study in which *Pb* ANKA-infected mosquitoes probed without taking a blood meal; in this study, TRs were not fully anesthetized and thus not completely still during mosquito exposure, thus preventing the mosquitoes from taking a blood meal.

**Whole-sporozoite vaccines have greater efficacy in laboratory mice than TRs.** We assessed the potential of TRs as a model to study whole-organism vaccines. C57BL/6 mice were 100% protected (10/10) against challenge with 1,000 *Pb*SPZ (Figure 5) after immunization with a total of $3 \times 10^4$ *Pb*RAS administered in three doses ($10^4$ *Pb*RAS per dose) at 3- to 4-week intervals. In contrast, this regimen protected 0% of TRs against challenge with 1,000 *Pb*SPZ. A higher immunizing dose of $2 \times 10^5$ *Pb*RAS administered in three doses at 3- to 4-week intervals ($10^5 + 5 \times 10^4 + 5 \times 10^4$) protected 20% of TRs from challenge with 1,000 *Pb*SPZ (Figure 5).

We next tested whole-sporozoite immunization of TRs against *Py* using nonattenuated SPZ administered under drug cover. Because we wanted to limit parasite development to the liver, we used PYR, which has been shown to be a successful approach with *Pb* in C57BL/6 and BALB/c mice.[14] Using a CVac approach, we again observed differences between laboratory mice and TRs. After three doses of 3,000 *Py* 17XNL SPZ (*Py*SPZ) under PYR drug cover at 3- to 4-week intervals, 75% of BALB/c mice versus 0% TRs were protected from challenge with 200 *Py*SPZ (Table 4). After three doses of $10^4$ *Py*SPZ under PYR treatment at 3- to 4-week intervals, 100% of mice were protected but only 30% of TRs were protected from challenge with 200 *Py*SPZ 5 weeks postvaccination (Figure 6A). When challenge with 200 *Py*SPZ was delivered 15 weeks postvaccination, 90% of the mice were protected and none of the TRs were protected (Figure 6B).

## DISCUSSION

Laboratory mice infected with rodent *Plasmodium* species such as *P. berghei*, *P. yoelii*, and *P. chabaudi* have been commonly used as proxies to investigate malaria in vivo. These models have been essential in developing an understanding of the mechanisms of infection in a mammalian host, the progression of the disease, host immune responses, and parasite genetics.[15] Rodent infection models have also been instrumental in drug and vaccine development. However, many study results have been difficult to translate into clinical applications in humans, owing in part to the physiological and genetic differences of the host and parasite species used. In particular, vaccine studies in current mouse models of malaria have not been reliably predictive of human efficacy; laboratory mice are generally much easier to protect than humans. Although

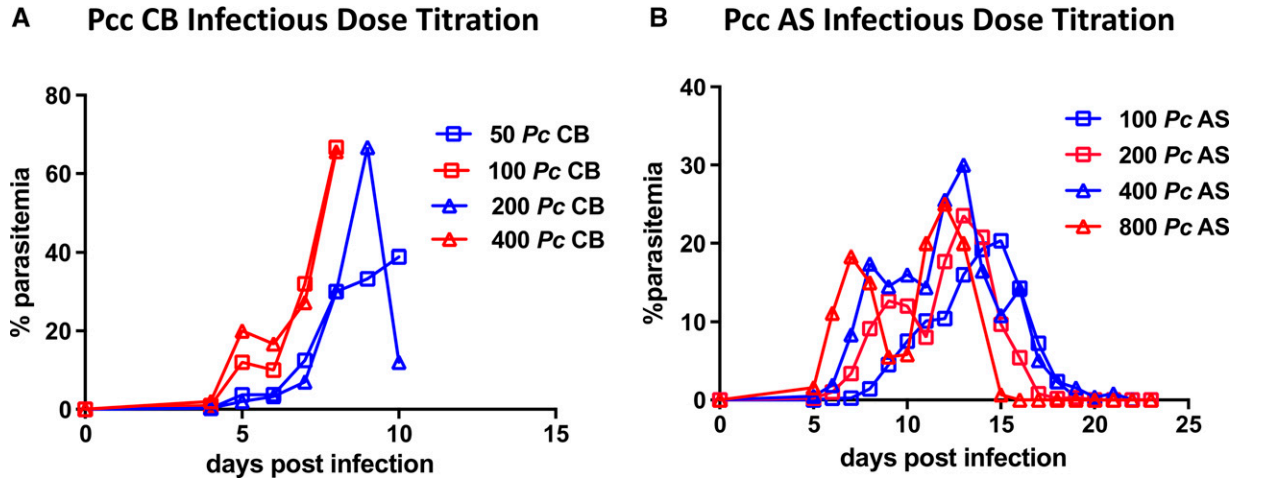


FIGURE 4. Thicket rats (TRs) are susceptible to infection with *Plasmodium chabaudi* CB and AS. (**A**) Parasitemia in four TRs inoculated with 50, 100, 200, or 400 *Plasmodium chabaudi chabaudi* (*Pcc*) CB sporozoites (SPZ). (**B**) Parasitemia in four TRs inoculated with 100, 200, 400, or 800 *Pcc* AS SPZ. Each line represents one animal.

TABLE 3

TR infection with *Plasmodium chabaudi chabaudi* CB by mosquito bite

| TR | Fed mosquitoes | Fed mosquitoes with sporozoites | Parasitemia |
|---|---|---|---|
| 1 | 3 | 2 | Negative |
| 2 | 3 | 2 | Positive |
| 3 | 6 | 2 | Negative |
| 4 | 5 | 3 | Positive |

TR = thicket rat.

TABLE 4

*Plasmodium yoelii* 17XNL pyrimethamine chemoprophylaxis vaccination against 200 *P. yoelii* SPZ challenge

| | $3 \times 10^3$ SPZ × 3 | |
|---|---|---|
| | No. protected/no. challenged | % Protected |
| TR | 0/4 | 0 |
| BALB/c | 3/4 | 75 |

TR = thicket rat; SPZ = sporozoite.

these models have been good tools to screen out ineffective vaccines, they have not been as successful in selecting effective vaccines.[16] A more rigorous rodent model that is not easily protected via immunization would be useful to qualify or prioritize potential vaccine candidates.

A new phylogenetic analysis from the Sackler Institute for Comparative Genomics at the American Museum of Natural History shows that malarial parasites found in tree-dwelling rats share a sister relationship with *P. falciparum* and *Plasmodium reichenowi*.[17] Although *Plasmodium* parasites in rodents differ from *Plasmodium* parasites in humans (e.g., the existence of the PfEMP1 antigen family in *P. falciparum* but not in rodent parasites[18,19]), individual gene analysis, regardless of methods, consistently shows that *P. falciparum* and *P. reichenowi* are sister taxa and the three species that infect rodents were always recovered as a monophyletic group.[20,21] Many of the analyses placed *P. falciparum* and *P. reichenowi* as sisters to *P. chabaudi*, *P. berghei*, and *P. yoelii* as described previously by Perkins.[17] Since the malarial parasites found in rodents share a sister relationship with *P. falciparum*, developing a TR model to study malaria may specifically accelerate progress toward the goal of a human malaria vaccine against *P. falciparum*.

Early studies by Yoeli in the 1960s and 1970s investigated the use of "tree rats" (*Thamnomys surdaster*) as experimental models for *Pb* infection, characterizing the course of infection in wild rats as chronically mild with low parasitemia, in contrast to the severe, fulminant disease observed in laboratory-bred species.[22,23] Yoeli's studies also provided early insight to the histologic pathology in liver tissue induced by *Pb* sporozoite inoculation in *T. surdaster*.[24] Subsequent to those early studies, rodents formerly known as *T. surdaster* are now referred to as *G. surdaster*.[25]

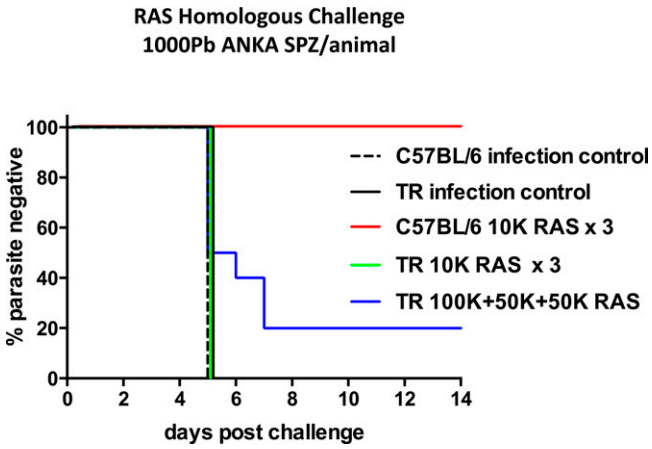


FIGURE 5. Radiation-attenuated sporozoites (RAS) immunization fully protects C57BL/6 mice, but elicits partial protection in thicket rat (TR) against high-dose homologous *Plasmodium berghei* (*Pb*) challenge. Percent parasite negative in C57BL/6 mice ($N = 10$) and TR ($N = 10$) immunized three times with $10^4$ RAS 3–4 weeks apart, TR ($N = 10$) immunized three times with $10^5$, $5 \times 10^4$, and $5 \times 10^4$ RAS 3–4 weeks apart, and naïve controls ($N = 5$ per group) challenged with 1,000 *Pb* ANKA SPZ.

The TRs transferred from Kinshasa for these studies were identified by a team of experts from the Laboratory of Zoonotic Pathogens, Rocky Mountain Laboratories, and the NMNH Smithsonian Institute to be *G. dolichurus*, which is currently regarded as a synonym for *G. surdaster*. The name likely represents a species composite in need of taxonomic revision. Such a qualifier would simply acknowledge the appreciable increase in recognized rodent species that is occurring concurrent with the increased application of molecular tools to taxonomic problems.[26] In the future, TRs in the northern part of the Democratic Republic of Congo may be separated out and referred to as *G. surdaster*, whereas the TRs in the south of Democratic Republic of Congo (LMIV TR) might continue to be referred to as *G. dolichurus*. The area where these TRs were captured is the same area where *Pb* was originally isolated,[9,10] hence the evidence favors that this is the natural host of *Pb*.

Our results support the findings of Chatterjee and others,[7] which suggested that TR (*G. surdaster*), the natural host for *Pb*, would be a more stringent rodent model to extrapolate to human malaria. TRs are highly susceptible to infection with doses as low as 25 *Pb* SPZ, and develop parasitemia after as few as one infected mosquito bite. We expand on previous findings by establishing TR susceptibility to other rodent forms of malaria including *Py* and *Pcc*, which resulted in patent parasitemia in at least 50% of animals.

Confirming previous findings, the natural host was more difficult to protect by RAS immunization in contrast to artificial hosts like inbred mice.[7] Similar to humans who require immunization with > 1,000 mosquitoes carrying *Pf*RAS to achieve sterile immunity,[27] TRs required higher doses of RAS to achieve only partial protection against homologous challenge. We expanded on previous findings by testing CVac methods of immunization, and observed similar trends: higher doses of CVac using PYR were required for protection in some TRs versus all inbred mice. Similar SPZ doses were used in both the RAS and CVac studies, whereas RAS experiments were conducted using *Pb* and CVac studies were conducted using *Py*. Our results confirm that TRs are a more stringent rodent model for evaluating whole-organism vaccines for malaria and, as seen in humans, vaccine efficacy can be enhanced by increasing vaccine dose.[28]

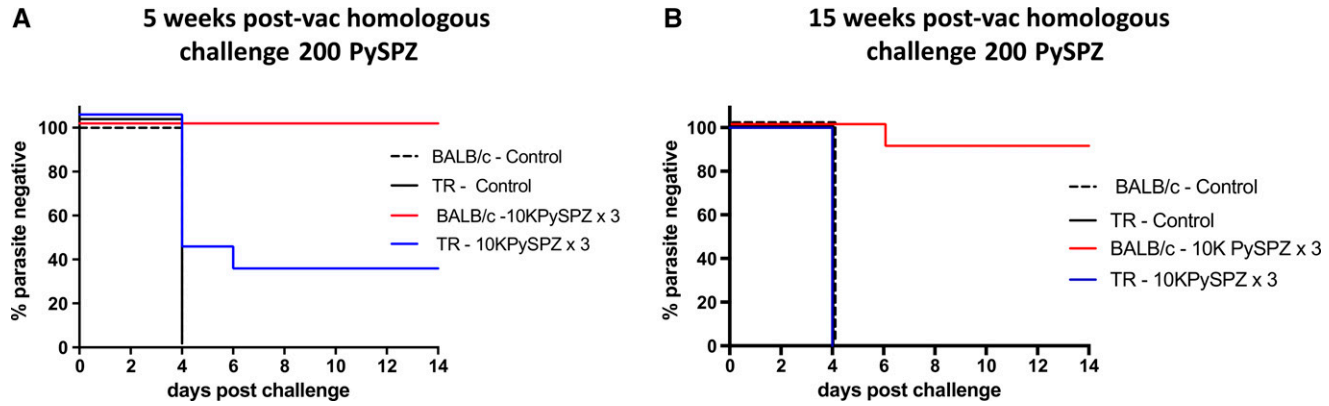


FIGURE 6. High-dose chemoprophylaxis vaccination fully protects BALB/c mice but only partially protects thicket rat (TR) against homologous *Plasmodium yoelii* (*Py*) challenge. (**A**) Percent parasite negative in BALB/c and TR immunized three times via $10^4$ *Py* sporozoites (SPZ) inoculation coupled with pyrimethamine (PYR) treatment and controls against challenge with 200 *Py*SPZ 5 weeks postimmunization. (**B**) Percent parasite negative in BALB/c and TR immunized three times via $10^4$ *Py*SPZ inoculation coupled with PYR treatment and controls against challenge with 200 *Py*SPZ 15 weeks postimmunization.

The different requirements to achieve protection in TRs and artificial models may be due to different mechanisms of protection; for example, innate immunity characterized by the infiltration of mononuclear cells, neutrophils, and eosinophils is prevalent in artificial hosts, whereas it is absent in TRs.[3,7] In addition, there may be differences in antigenic targets between the two models; for example, in laboratory mice, both immunizing and challenge SPZ were halted at the uninucleate, trophozoite stage, whereas development to submature schizonts was observed in *Pb* in TRs.[3,7] Perhaps "the 'adaptation' of a parasite to an artificial host actually translates in immunological terms into defense mechanisms that in most circumstances are more effective than those seen in the natural host."[3]

Received September 12, 2016. Accepted for publication November 29, 2016.

Published online January 23, 2017.

Note: Supplemental figures appear at www.ajtmh.org.

Acknowledgments: We would like to thank Andre Laughinghouse and Kevin Lee of Laboratory of Malaria and Vector Research for providing all of the mosquitoes used in this project; Jean Langhorne for providing the *Plasmodium chabaudi chabaudi* parasites; Robert Morrison for helping with the thicket rat (TR) sequence analysis; Tom G. Schwan from the Laboratory of Zoonotic Pathogens in Montana, and Darrin Lunde and Michael Carleton from the Smithsonian for their work to identify TR; and J. Patrick Gorres for help with writing and submitting the manuscript.

Financial support: This research was supported and funded by the Intramural Research Program of the National Institute for Allergy and Infectious Disease of the National Institutes of Health.

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