Am. J. Trop. Med. Hyg., 103(5), 2020, pp. 1893–1901
doi:10.4269/ajtmh.20-0183

Dynamics and Outcomes of *Plasmodium* Infections in *Grammomys surdaster* (*Grammomys dolichurus*) Thicket Rats versus Inbred Mice

Solomon Conteh,[1] Jacqueline Kolasny,[1] Yvette L. Robbins,[1] Pati Pyana,[2] Philippe Büscher,[3] Javonn Musgrove,[1] Brandi Butler,[1] Lynn Lambert,[1] John Patrick Gorres,[1] and Patrick E. Duffy[1]*

[1]*Laboratory of Malaria Immunology and Vaccinology (LMIV), National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), Bethesda, Maryland;* [2]*Department of Parasitology, Institut National de Recherche Biomedicale (INRB), Kinshasa, Democratic Republic of the Congo;* [3]*Department of Biomedical Sciences, Unit of Parasite Diagnostics, Institute of Tropical Medicine, Antwerp, Belgium*

Abstract. Investigations of malaria infection are often conducted by studying rodent *Plasmodium* species in inbred laboratory mice, but the efficacy of vaccines or adjunctive therapies observed in these models often does not translate to protection in humans. This raises concerns that mouse malaria models do not recapitulate important features of human malaria infections. African woodland thicket rats (*Grammomys surdaster*) are the natural host for the rodent malaria parasite *Plasmodium berghei* and the suspected natural host for *Plasmodium vinckei vinckei*. Previously, we reported that thicket rats are highly susceptible to diverse rodent parasite species, including *P. berghei*, *Plasmodium yoelii*, and *Plasmodium chabaudi chabaudi*, and are a more stringent model to assess the efficacy of whole-sporozoite vaccines than laboratory mice. Here, we compare the course of infection and virulence with additional rodent *Plasmodium* species, including various strains of *P. berghei*, *P. yoelii*, *P. chabaudi*, and *P. vinckei*, in thicket rats versus laboratory mice. We present evidence that rodent malaria parasite growth typically differs between the natural versus nonnatural host; *G. surdaster* limit infection by multiple rodent malaria strains, delaying and reducing peak parasitemia compared with laboratory mice. The course of malaria infection in thicket rats varied depending on parasite species and strain, resulting in self-cure, chronic parasitemia, or rapidly lethal infection, thus offering a variety of rodent malaria models to study different clinical outcomes in the natural host.

INTRODUCTION

Malaria is the leading cause of death in Africa. The WHO estimated 219 million cases of malaria worldwide in 2017, resulting in 435,000 deaths, largely attributable to *Plasmodium falciparum*, the most prevalent and deadly malaria parasite in Africa.[1] Despite widespread use of preventative measures including insecticide-treated bed nets, indoor residual spraying, and antimalarial drugs, the reduction in malaria burden globally has stalled over the last 3 years.[1] Evidence of increasing resistance in parasites and vectors raises concerns that recent progress in malaria control will be reversed,[2,3] and, thus, vaccines and other novel interventions are urgently needed. However, vaccine development has been hampered by the lack of suitable animal models that can reliably qualify interventions that translate to protection in humans.[4] Most animal models used to study malaria infection use nonnatural hosts such as inbred laboratory mice rather than natural hosts.

The African woodland thicket rat *Grammomys surdaster* (henceforth referred to as "thicket rat") is the natural host for *Plasmodium berghei* (*Pb*) and suspected to be the natural host for *Plasmodium vinckei vinckei* (*Pvv*) parasites.[5] In a previous study, we demonstrated that thicket rats are susceptible to infection with various parasite species, including *Pb*, *Plasmodium yoelii* (*Py*), and *Plasmodium chabaudi chabaudi* (*Pcc*).[6] Consistent with previous studies,[7] we also showed that whole sporozoite (SPZ) vaccine regimens sufficient to protect laboratory mice against malaria challenge were insufficient to protect thicket rats, which required higher SPZ dosages to achieve even short-term sterile immunity after radiation-attenuated sporozoite vaccination or chemoprophylaxis vaccination.[6] This suggests that thicket rats may offer a more stringent model to identify whole SPZ vaccines that should be considered for assessment in human trials.

Here, we further investigate the thicket rat model by assessing the course of infection and the lethality caused by additional *Plasmodium* strains and species in both natural (*G. surdaster* thicket rat) and nonnatural hosts (inbred mice) without treatment. We examined hematological parameters during infection with parasites that naturally infect this thicket rat (*Pb* strains ANKA and K173; *Pvv* v67) to determine any differences between natural or nonnatural models or between blood stage (BS)–induced versus SPZ-induced infection. We hypothesized that the natural host would be more susceptible to infection but would limit *Plasmodium* parasitemia moreso than nonnatural hosts.

MATERIALS AND METHODS

Animals (hosts). All experiments adhered to the protocol of the Laboratory of Malaria Immunology and Vaccinology (LMIV) 1E animal study protocol that was approved by the Institutional Animal Care and Use Committees of the National Institute of Allergy and Infectious Diseases (NIAID), NIH.

Thicket rats. We previously speciated the thicket rats used in this study as *G. surdaster* or *Grammomys dolichurus*, known to be the natural host for *P. berghei* and suspected to be that of *P. vinckei vinckei*.[5] Thicket rats were captured in the Katanga Province in the southern region of the Democratic Republic of Congo, at Fungurume and Lumata, which are located 200 km and 50 km from Lubumbashi, respectively. Thicket rats were reared at the LMIV, NIAID, NIH.

BALB/c, C57BL/6, and CD1 mice. BALB/c and C57BL/6 mice were used as nonnatural hosts and were obtained from NIH-approved vendors. CD1 mice were used to maintain the parasite life cycle to support SPZ production in mosquitoes. Donor-infected BALB/c and C57Bl/6 mice were used to

* Address correspondence to Patrick E. Duffy, Laboratory of Malaria Immunology and Vaccinology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, 29 Lincoln Dr., Bldg. 29B, Rm. 4NN06, Bethesda, MD 20892-2903. E-mail: patrick.duffy@nih.gov

TABLE 1

Summary of *Plasmodium* species studied in mice and thicket rats: prior publication vs. current study

	Plasmodium species/strains used	
	Conteh, January 2017	Current study
Mice	*Pb* ANKA	*Pb* K173
	Pb NK65	*Pvv* Katanga
	Py 17XNL	*Pvv v67*
		Pv
		Pv Cameroon 8EL8
		Pv brucechwatti 1/69
		Pv petteri 197CR27
		Pcc ER
		Py YM
		Py XNL
Thicket rats	*Pb* ANKA	*Pb* K173
	Pb NK65	*Pvv* Katanga
	Py 17XNL	*Pvv v67*
	Pcc CB	*Pv*
	Pcc AS	*Pv* Cameroon 8EL8
		Pv brucechwatti 1/69
		Pv petteri *197CR27*
		Pcc ER
		Py YM
		Py XNL

Pb = Plasmodium berghei; *Pcc = Plasmodium chabaudi chabaudi*; *Pv = Plasmodium vinckei*; *Pvv = Plasmodium vinckei vinckei*; *Py = Plasmodium yoelii*.

prepare BS parasite inocula to initiate infection in their respective species.

Mosquitoes. *Anopheles stephensi* mosquitoes were originally obtained from Nijmegen, The Netherlands, and reared at the Laboratory of Malaria and Vector Research (LMVR), NIH. Three- to 7-day-old mosquitoes were fed on infected mice. To allow for SPZ development after blood meals, mosquitoes were maintained as follows: for *Py* and *Pv* strains, mosquitoes were maintained for 14–21 days in an environmental chamber (EC) set at 24°C temperature; for *Pb* and *Pvv* strains, 18–25 days in an EC set at 19–21°C temperature. Relative humidity was set at 80% for all parasite strains. The light cycle for *Pb* and *Pvv* was set for 12 hours of light and 12 hours of darkness; for *Py* and *Pv* strains, the light cycle was set for 10 hours of light (two lights on), 2 hours of dusk (one light), 10 hours of darkness (no light), and 2 hours of dawn (one light).

Parasites. The parasite species and strains used in these infection studies are summarized in Table 1, with additional information on natural hosts, vectors, and origins provided in Tables 2–5. The *Pb* ANKA strain was obtained from the LMVR; the *Pb* K173 strain from the University of Glasgow, United Kingdom; the *Pcc* strain from the National Institute for Medical Research in London, United Kingdom; the *Py* strain from the Center for Infectious Disease Research, Seattle, Washington; and *Pv* and *Pvv* strains from BEI Resources (Fairfax, VA; formerly known as MR4) in frozen cryopreserved blood samples (stabilates). These parasites were expanded in BALB/c, C57BL/6, and CD1 mice, and in thicket rats, at the LMIV, NIH, and BS stabilates were stored in the liquid nitrogen vapor phase.

Sporozoite-induced infections. Infected mosquitoes (14–21 days after mosquito infection for *Py* strains and 18–27 days after infections for *Pb* and *Pvv* v67) were dissected to remove the salivary glands, which were placed in 0.1-mL medium E 199 (Quality Biologicals, Gaithersburg, MD, Catalog Number 112-022-101) containing 0.2% bovine serum albumin (BSA) in a 1.5-mL protein Lo-Binding Eppendorf tube. The salivary glands were triturated 20 times using a 1-mL syringe and a 26G needle to release SPZs from the glands. Sporozoites were counted using a disposable hemocytometer, and the SPZ/mL was determined. The challenge (or infection) dose was chosen for each strain to ensure that all mice were reliably infected. Animals were inoculated intravenously (IV) using a syringe and 27G–29G needle or 29G 0.3-mL insulin syringe, with 400 SPZs for *Py* XNL, 500 SPZs for *Py* YM, and 1,000 SPZs for *Pvv* v67 in 200 µL of E199 containing 0.2% BSA. Sporozoite infections were not performed for *Pb* K173 nor for *P. vinckei* strains, except *Pvv* v67; SPZs could not be generated from these parasite strains possibly because of a loss of transmission capacity after numerous serial passages of BS parasites.

Blood stage-induced infections. Donor BALB/c mice were inoculated intraperitoneally (i.p.) with each parasite stabilite, and then 5–8 days after inoculation, parasitemia levels were monitored daily by Giemsa-stained blood smears. On reaching 3% parasitemia, infected blood was collected and used to infect subject animals by intravenous inoculation with 100,000 parasitized erythrocytes (PEs) in 100 µL of phosphate buffered saline.

Parasitemia calculation. To calculate parasitemia in both donor mice and subject rodents, thin blood smears were

TABLE 2

Parasites tested for which the natural host is *T. rutilans*

(Sub) species	Natural vector[15]	Laboratory vector	Origin[5]
Py 17XNL	*Unknown*	*A. stephensi*	Country: CAR Isolated from *T. rutilans* captured in CAR in 1965
Pcc AS isolate	Unknown	*A. stephensi*	Country: CAR History: *T. rutilans*. Captured: Boulard (CAR)
Pcc CB isolate	Unknown	*A. stephensi*	Country: CAR History: *T. rutilans*. Captured: Boulard (CAR)
Pcc ER isolate	Unknown	*A. stephensi*	Country: CAR History: Originally isolated from *T. rutilans*, La Maboké field station, CAR.
Pv	*A. cinctus* *A. dureni*	NA	This species is found in Central Africa, including Congo and Nigeria
Pv brucechwatti, strain 1/69	*A. cinctus* *A. dureni*	NA	Country: Nigeria History: Isolate collected in 1969 by Killick-Kendrick from mosquito
Pv petteri strain 197CR27	*A. cinctus* *A. dureni*	NA	No mosquito transmission Subclone of isolate CR; country: CAR History: *T. rutilans*. Captured: Boulard (CAR)
Pv Cameroon strain 8EL8	*A. cinctus* *A. dureni*	NA	Original isolate Esekam inoculated intravenously obtained from *Thamnomys* species, collected in Esekam, Cameroon, in 1974

A. cinctus = Anopheles cinctus; *A. dureni = Anopheles dureni*; *A. stephensi = Anopheles stephensi*; CAR = Central African Republic; *Pcc = Plasmodium chabaudi chabaudi*; *Pv = Plasmodium vinckei*; *Py = Plasmodium yoelii*; *T. rutilans = Thamnomys rutilans*.

TABLE 3
Lethality of malaria parasites that naturally infect thicket rats to their natural or laboratory hosts

Parasite strain	Infectious inoculum	Thicket rats (natural host)	C57BL/6 (laboratory host)	BALB/c (laboratory host)
Pb ANKA	SPZ	Lethal	Lethal	–
Pb ANKA	BS	Lethal	Lethal	–
Pb NK65	SPZ	Lethal	Lethal	–
Pb NK65	BS	Lethal	Lethal	–
Pb K173	BS	Nonlethal/varies	Lethal	Lethal
Pvv v67	SPZ	Lethal	Nonlethal	Nonlethal
Pvv v67	BS	Lethal	Lethal	Lethal
Pvv Katanga	BS	Nonlethal	–	Lethal

BS = blood stage; *Pb* = *Plasmodium berghei*; *Pvv* = *Plasmodium vinckei vinckei*; SPZ = sporozoite.

collected on a slide and stained with 10% Giemsa diluted in deionized water. Parasitemia level was calculated by counting the number of PEs in 2,000–3,000 erythrocytes (Es), determining the percentage of PEs or parasitemia. If parasitemia was very high, then parasitemia levels were calculated by determining how many high-powered microscopic fields (200–300 E/field) were examined to reach a count up to 200 PEs. Parasitemia was calculated as (200 PE/number of Es counted) × 100.

Hematology assessment. Thicket rats, and C57BL/6 and BALB/c mice were infected IV with either SPZs or BS of *Pb* ANKA, *Pb* K173, and *Pvv* v67 (i.e., species that naturally infect thicket rats), and samples were collected at three different time points: preinfection, middle of infection (4–6 dpi), and study endpoint (time of euthanasia or self-cure). Complete blood count was performed by the NIH Clinical Center Department of Laboratory Medicine/Hematology using a Siemens Advia 120 hematology analyzer (Siemens Health Care Incorporated, Deerfield, IL).

End of study criteria for individual animals. Infected animals were euthanized when they became moribund, parasitemia exceeded 50%, or %hematocrit fell below 20% (severe anemia). Based on our prior experience, malaria infections in thicket rats that cause a drop in hematocrit less than 20% or parasitemia > 50% are uniformly fatal. A parasite was considered "lethal" if the infected host was euthanized according to these criteria.

RESULTS

We previously examined the susceptibility of thicket rats and C57BL/6 mice to SPZ-induced infection with *Pb* ANKA, *Pb* NK65, *Py* 17XNL, *Pcc* AS, or *Pcc* CB.[6] We compared courses of parasitemia in thicket rats and laboratory mice initiated by BS parasite and/or SPZ inoculations of these species and several additional *Plasmodium* species and strains (Table 1 summarizes the *Plasmodium* species tested in this study and in our previous 2017 study). The geographic origin and the natural mosquito vector vary among these parasites originally isolated from *G. surdaster* (Table 6), from the thicket rat species *Thamnomys rutilans* (Table 2), or from their corresponding mosquito vectors.

Peak parasitemia is often delayed in thicket rats versus mice during BS-induced infections. Mice and thicket rats were inoculated with rodent malaria parasites including those that naturally infect *G. surdaster* (*Pvv* Katanga, *Pb* K173, and *Pvv* v67). In general, thicket rats reduced average peak parasitemia and delayed the average time to peak parasitemia compared with laboratory mice (Figure 1).

Among parasite strains that naturally infect thicket rats, thicket rats completely controlled BS-induced infection with *Pvv* Katanga (self-cure) (Figure 2A) and partially controlled BS-induced infection with *Pb* K173 (Supplemental Figure S1A), whereas mice experienced rapid parasite growth and succumbed to BS-induced infection with both species (Figure 1, red asterisks). *Plasmodium vinckei vinckei* v67 was lethal to both thicket rats and mice after BS parasite inoculation (Figure 2D). Among these three parasite strains that naturally infect thicket rats, only *Pvv* v67 yielded infected mosquitoes; after SPZ inoculation, *Pvv* v67 SPZ-induced infection was lethal to thicket rats but not to mice (Supplemenatal Figure S1B).

Among parasites not known to naturally infect *G. surdaster*, *P. vinckei* infections initiated by BS parasite inoculation

TABLE 4
Lethality of other rodent malaria parasites to thicket rats or laboratory hosts

Parasite strains	Infectious inoculum	Thicket rats	C57BL/6	BALB/c mice
Py XNL	SPZ	Lethal/varies	Nonlethal	Nonlethal
Py XNL	BS	Lethal/varies	Nonlethal	Nonlethal
Py YM	SPZ	Lethal	Lethal	Lethal
Py YM	BS	Lethal	Lethal	Lethal
Pcc ER	SPZ	Nonlethal	Nonlethal	–
Pcc ER	BS	Nonlethal	Nonlethal	–
Pcc AS	SPZ	Nonlethal	–	Nonlethal
Pcc AS	BS	Nonlethal	–	Nonlethal
Pcc CB	SPZ	Nonlethal/varies	–	Nonlethal
Pcc CB	BS	Nonlethal	–	Nonlethal
Pv	BS	Nonlethal	–	Lethal
Pv brucechwatti 1/69	BS	Nonlethal	–	Lethal
Pv petteri	BS	Nonlethal	–	Nonlethal
Pv Cameroon 8EL8	BS	Lethal	–	Nonlethal

BS = blood stage; *Pcc* = *Plasmodium chabaudi chabaudi*; *Pv* = *Plasmodium vinckei*; *Py* = *Plasmodium yoelii*; SPZ = sporozoite.

TABLE 5
Peak parasitemia (level and day) in thicket rats and mice for various parasites

Parasite *species*/strain	Infectious inoculum	Host	Average peak parasitemia (range %)	Day(s) to peak parasitemia
Pb K173	BS	Thicket rats	"High": 23.8 (8.7–38.0)	9–27
			"Low": 0.2 (0.1–0.4)	4–12
Pb K173	BS	C57BL/6	14.8 (11.3–18.1)	6–7
Pb K173	BS	BALB/c	18.2 (15.0–23.6)	6
Py YM	SPZ	Thicket rats	47.6 (21.4–70.7)	7–9
Py YM	SPZ	C57BL/6	43.6 (30.7–50.0)	8–13
Py YM	SPZ	BALB/c	79.4 (71.7–87.5)	7
Py YM	BS	Thicket rats	42.3 (11.9–64.9)	6–8
Py YM	BS	C57BL/6	78.1 (64.6–93.2)	6
Py YM	BS	BALB/c	74.5 (60.8–78.1)	6
Py XNL	SPZ	Thicket rats	23.7 (20.3–25.7)	13–21
Py XNL	SPZ	C57BL/6	24.0 (19.3–29.7)	18–23
Py XNL	SPZ	BALB/c	12.4 (10.0–13.1)	10–11
Py XNL	BS	Thicket rats	24.6 (15.0–40.8)	9–15
Py XNL	BS	C57BL/6	25.7 (20.7–32.4)	15–18
Py XNL	BS	BALB/c	7.9 (5.5–10.2)	8–9
Pcc ER	SPZ	Thicket rats	12.0 (10.2–14.0)	7–9
Pcc ER	SPZ	C57BL/6	7.3 (5.0–11.3)	8–9
Pcc ER	BS	Thicket rats	26.4 (24.1–28.8)	8–13
Pcc ER	BS	C57BL/6	23.9 (23.4–24.5)	7–8
Pv	BS	Thicket rats	7.5 (0.2–14.9)	8–9
Pv	BS	BALB/c	81.6 (78.0–90.0)	5
Pv brucechwatti 1/69	BS	Thicket rats	6.2 (0.0–13.5)	10–11
Pv brucechwatti 1/69	BS	BALB/c	36.4 (31.3–38.7)	6–8
Pv petteri	BS	Thicket rats	15.2 (6.7–29.9)	9–12
Pv petteri	BS	BALB/c	35.0 (30.0–40.0)	8
Pv Cameroon 8EL8	BS	Thicket rats	34.0 (29.6–38.5)	8
Pv Cameroon 8EL8	BS	BALB/c	36.2 (32.1–44.4)	6–8
Pvv v67	SPZ	Thicket rats	39.2 (28.3–46.8)	8
Pvv v67	SPZ	C57BL/6	16.8 (7.0–33.7)	12–13
Pvv v67	SPZ	BALB/c	34.4 (25.4–55.3)	12–13
Pvv v67	BS	Thicket rats	54.8 (8.4–86.3)	7–13
Pvv v67	BS	C57BL/6	34.6 (24.2–40.8)	6
Pvv v67	BS	BALB/c	65.4 (38.0–85.0)	6–7
Pvv Katanga	BS	Thicket rats	19.9 (14.5–22.0)	11–19
Pvv Katanga	BS	BALB/c	41.4 (11.4–60.3)	5

BS = blood stage; *Pb* = *Plasmodium berghei*; *Pcc* = *Plasmodium chabaudi chabaudi*; *Pv* = *Plasmodium vinckei*; *Pvv* = *Plasmodium vinckei vinckei*; *Py* = *Plasmodium yoelii*; SPZ = sporozoite.

revealed two patterns in thicket rats versus mice: 1) thicket rats controlled BS parasitemia, whereas BALB/c mice experienced rapid parasite expansion starting 4–5 days after inoculation with *P. vinckei* (Figure 2B) and *Pv* brucechwatti 1/69 (Figure 2C); 2) mice and thicket rats experienced similar moderate parasite growth with *Pv* petteri (Figure 2E) and *Pv* Cameroon 8EL8 (Figure 2F), albeit thicket rats succumbed to the latter but not the former, whereas mice self-cured with both strains.

Plasmodium yoelii (both YM and XNL strains) growth was generally similar between thicket rats and mice whether initiated by BS parasite or by SPZ inoculation (Figure 3), albeit *Py* XNL was lethal by 9–15 days after BS-induced infection and 13–21 days after SPZ-induced infection in thicket rats but nonlethal in mice (Figure 3B and D). *Plasmodium chabaudi chabaudi* ER growth was also similar in thicket rats and mice (Figure 4).

Sporozoite-induced infections yielded similar or lower parasitemia than BS-induced infections. For the four strains (*Pcc* ER, *Py* YM, *Py* XNL, and *Pvv* v67) that yielded SPZs in mosquitoes, both BS-induced and SPZ-induced infections were studied to assess their impact on parasite growth in the three rodent models (*Pcc* ER was not tested in BALB/c). BS parasite inoculation yielded higher peak parasitemia than SPZ inoculation for six of 11 different host/parasite combinations (*Pvv* v67 in thicket rats and both BALB/c and C57BL/6 mice, *Pcc* ER in thicket rats and C57BL/6 mice, and *Py* YM in C57BL/6 mice), whereas the other five host/parasite combinations resulted in similar peak parasitemias after the two routes of inoculation (Figure 5). Peak parasitemia was sometimes markedly higher in mice versus thicket rats (e.g., *Py* YM [Figure 5]) and sometimes markedly lower (e.g., *Pvv* v67 in C57BL/6 [Figure 5]), but in many cases, peak parasitemia was similar in mice and thicket rats.

Lethality and hematological dynamics in thicket rats and mice. More often than not, the lethality or nonlethality of different parasites was consistent between thicket rats and laboratory mice; notably, *Pvv* Katanga (which naturally infects thicket rats) was nonlethal in thicket rats but lethal in mice (Tables 3–5). We compared hematological changes in thicket rats and mice infected with *Pb* ANKA, *Pb* K173, and *Pvv* v67, which are species known to naturally infect thicket rats. All infections in mice (other than SPZ-induced *Pvv* v67) were lethal, and all resulted in hematocrit decreases. All infections in thicket rats resulted in decreased hematocrits except *Pb* K173 BS-induced infection, wherein hematocrit was stable through day 6; three of six thicket rats succumbed to infection (days 8 and 10), at which time their hematocrits had dropped (Figure 6B). Thicket rats and laboratory mice generally exhibited similar decreases in hematocrit. An exception was SPZ-induced *Pvv* v67

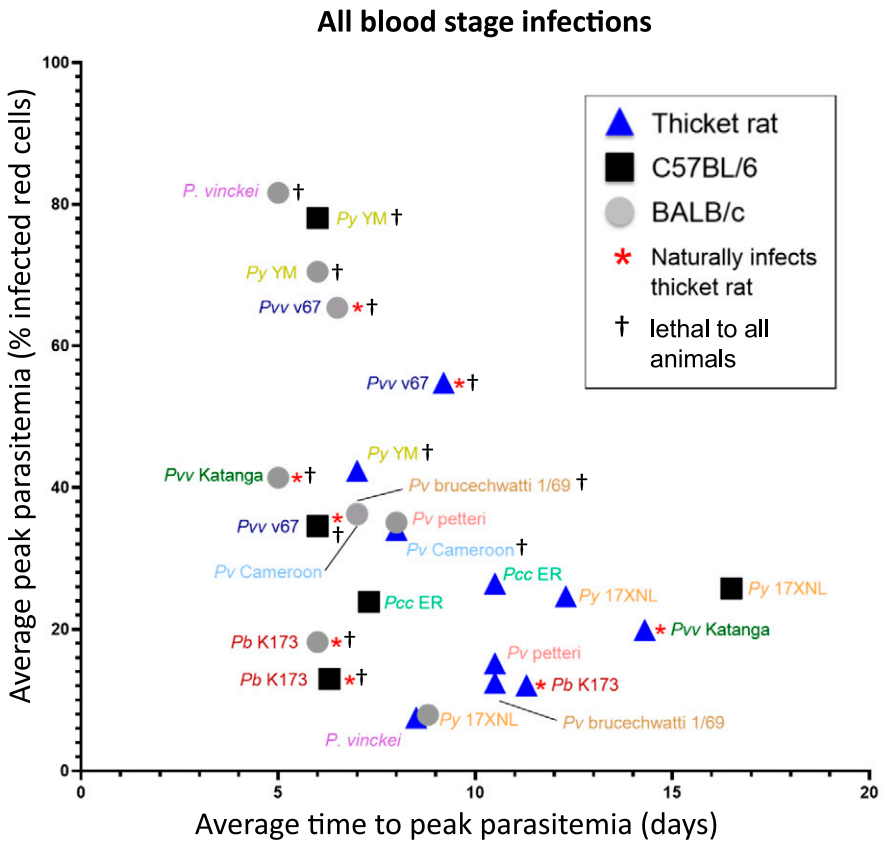

FIGURE 1. Dynamics of parasitemia during BS–induced infections with various rodent malaria species. Each host–parasite combination is represented by a colored symbol (rodent) and text label (parasite). Placement on scattergram indicates the average peak parasitemia of all animals in that group and their average time to peak parasitemia. *Indicates that this parasite species/subspecies is known or thought to naturally infect *Grammomys surdaster* (*dolichurus*). †Indicates that animals in this model met the criteria for euthanasia, including parasitemia > 50%, severe anemia (%hematocrit < 20%), or moribundity. BS = blood stage; *Pb* = *Plasmodium berghei*; *Pcc* = *Plasmodium chabaudi chabaudi*; *Pv* = *Plasmodium vinckei*; *Pvv* = *Plasmodium vinckei vinckei*; *Py* = *Plasmodium yoelii*. This figure appears in color at www.ajtmh.org.

infections, wherein thicket rats experienced sharp hematocrit declines and succumbed to infection, whereas mice experienced gradual declines and controlled the infection (Figure 6C and E). Changes in red blood cell counts were consistent with hematocrit changes (Supplemental Figure S2).

Most commonly, absolute lymphocyte (Supplemental Figure S3) and total white blood cell (WBC) counts (Supplemental Figure S4) decreased over the initial 4–6 days of infection and increased thereafter, with the exception of *Pvv* v67 SPZ-induced infections which did not show a consistent pattern (Supplemental Figures S3E and S4E). The decrease in WBC counts from baseline to day 4 generally corresponded to a decrease in hematocrit in mice but not thicket rats over the course of the infection. The correlation between average drop in WBC by day 4 and average decrease in hematocrit by the end of the study in different models was significant by Spearman's rank test in in C57BL/6 mice ($P = 0.017$), but not in BALB/c mice ($P = 0.175$) (Supplemental Figure S5).

DISCUSSION

Most animal models used in malaria infection studies use nonnatural hosts such as inbred laboratory mice rather than natural hosts. However, the use of these models has hindered vaccine development, as protective efficacy in mice has often failed to translate to efficacy in humans. *Grammomys surdaster* (*dolichurus*) is the natural host to several rodent malaria species, including *Pb* ANKA, *Pb* NK65, and *Pb* K173, and the suspected host of *Pvv* v67 and *Pvv* Katanga (Table 6). We previously demonstrated that thicket rats are relatively difficult to protect by whole-SPZ immunization in contrast to inbred mice. Similar to humans, thicket rats required higher doses of vaccine to achieve only partial protection against homologous challenge, suggesting that thicket rats provide a more stringent animal model for evaluating whole-organism vaccines.[6] Here, we compared the course of rodent *Plasmodium* infection in natural hosts (thicket rats) versus nonnatural hosts (laboratory mice) to explore whether host–parasite interactions may differ between these models.

The course of parasitemia differed between thicket rats and mice to lesser or greater degrees, depending on the strain of rodent *Plasmodium* species, and the difference was particularly marked for some strains of *P. vinckei* (Figure 2A–C). Thicket rats controlled BS-induced infections with *Pvv* Katanga, *P. vinckei*, and *Pv* brucechwatti 1/69, whereas laboratory mice experienced rapid parasite

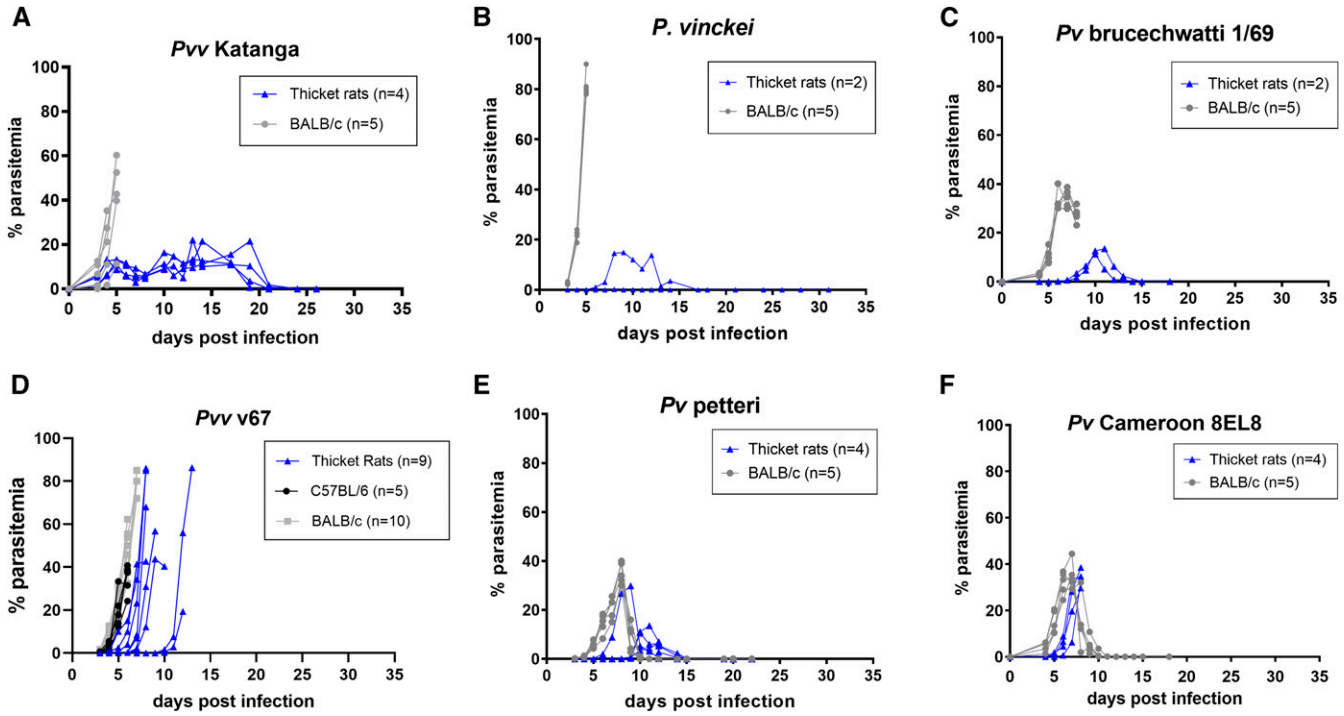

FIGURE 2. Blood stage–induced infections with various strains of *Plasmodium vinckei*. Each line represents a single animal. (**A**) *Plasmodium vinckei* infections were lethal in BALB/c and nonlethal in thicket rats; two thicket rats inoculated with *P. vinckei* BS did not develop patent parasitemia. (**B**) *Plasmodium vinckei* brucechwatti 1/69 infections were lethal in BALB/c and nonlethal in thicket rats; two thicket rats inoculated with *P. vinckei* brucechwatti 1/69 BS did not develop patent parasitemia. (**C**) Both thicket rats and BALB/c mice controlled *P. vinckei* petteri infection. (**D**) *Plasmodium vinckei* Cameroon 8EL8 infection was lethal in thicket rats by day 9 postinfection, whereas BALB/c mice controlled the infection by day 11 postinfection. (**E**) *Plasmodium vinckei vinckei* v67 was lethal to both thicket rats and mice; data presented represent a combination of two separate studies. (**F**) *Plasmodium vinckei vinckei* Katanga parasitemia was controlled by thicket rats, but the infection was lethal in BALB/c. BS = blood stage; *Pv* = *Plasmodium vinckei*; *Pvv* = *Plasmodium vinckei vinckei*. This figure appears in color at www.ajtmh.org.

growth and succumbed to these infections by 5–7 days after inoculation (Figure 2). Conversely, mice controlled *Pvv* v67 parasitemia after SPZ- but not BS-induced infection, succumbing to the latter, whereas thicket rats succumbed after parasite growth spurts following both SPZ- and BS-induced infections (Figure 2D, Supplemental Figure S1B). Laboratory mice commonly progressed to peak parasitemia within a short period of time, whereas thicket rats developed peak parasitemia later and often self-cured (Figure 1). This pattern generally supported our hypothesis that thicket rats would control parasitemia moreso than laboratory mice. Parasite lines that achieved > 50% parasitemia typically did so after explosive growth (within 5–9 days, e.g., Figure 1) in both thicket rats and mice (Tables 3–5). Other *Plasmodium* infections not known to naturally infect thicket rats resulted in chronic parasitemia (e.g., *Pcc* ER [Figure 4]) or rapidly lethal infection (e.g., *Py* YM [Figure 3A and C]). Overall, thicket rats offer a variety of rodent malaria models to study different clinical outcomes in the natural parasite host, including hyperparasitemia, chronic parasitemia, severe anemia, moribundity, and self-cure.

We hypothesized that as natural hosts, thicket rats would be permissive to initial infection (reflecting parasite evolution) but would tolerate infection and/or sustain chronic parasitemia without succumbing (reflecting host or parasite evolution). The unexpected lethality of *Pb* ANKA and *Pvv* v67 that are naturally infecting species in thicket rats could result from increased virulence of parasites that have been passaged in nonnatural hosts (laboratory mice) for decades.[8–10] Continued passage in the natural host may attenuate virulence of these strains and should be evaluated in future. The method used to inoculate SPZs for infection may also have contributed to lethality, as intravenous inoculation is more efficient in causing infection than mosquito bite.[11]

Earlier studies showed that passage through mosquitoes ameliorated virulence of subsequent *Plasmodium chabaudi chabaudi* infections of C57BL/6 mice.[10] In all infected animals, peak parasitemia levels appeared similar or higher in BS-induced versus SPZ-induced infections (Figure 5). Nevertheless, lethality was just as likely with SPZ-induced as BS-induced infections in the same host, with few exceptions (Tables 3–5). Overall, these results support the hypothesis that parasite growth can be attenuated by transmission through the mosquito vector[10]; however, this does not appear to avert lethality.

In humans, malaria infection often causes anemia and leukopenia,[12] and severe malarial anemia is a common cause of childhood death in endemic countries. In both laboratory mice and thicket rats, anemia less than 20% hematocrit and parasitemia greater than 50% are related to fatal outcomes; we used these as well as moribundity as the criteria for euthanasia. Among the parasite species that naturally infect thicket rats, rodents that received *Pb* ANKA, *Pb* K173, and *Pvv* v67 uniformly met these criteria

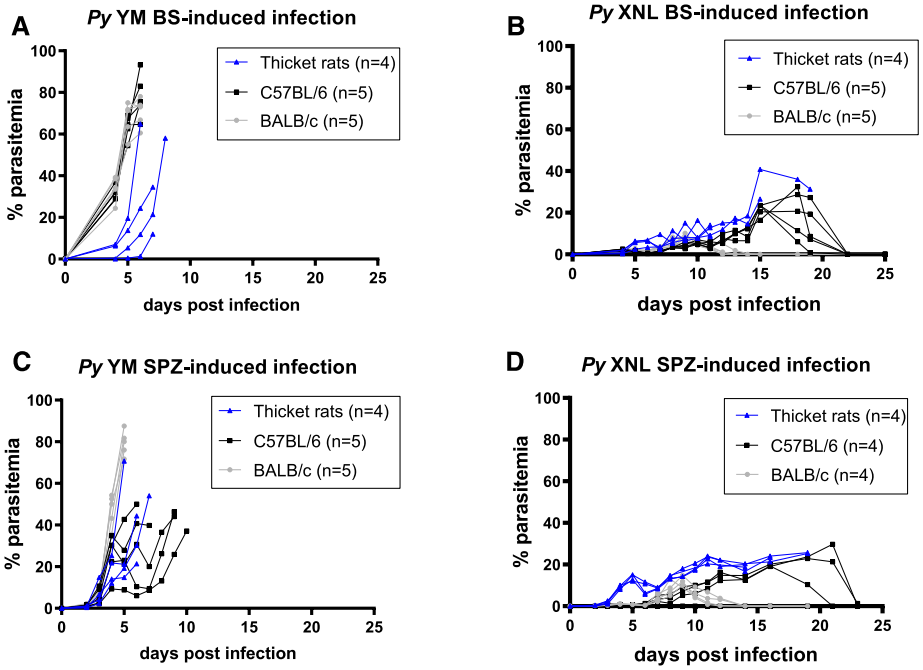

FIGURE 3. Blood stage–induced and SPZ-induced infections with two strains of *Plasmodium yoelii*. Each line represents one animal. (**A**) *Plasmodium yoelii* YM BS-induced infection was lethal in all mice and in all thicket rats by days 6 and 8, respectively. (**C**) *Plasmodium yoelii* YM SPZ was also lethal in BALB/c mice by day 7, thicket rats by day 8, and C57BL/6 mice by day 10 postinfection. (**B** and **D**) *Plasmodium yoelii* XNL BS-induced and SPZ-induced infections were controlled by mice but lethal in thicket rats. One C57BL/6 mouse did not develop patent parasitemia during SPZ-induced infection. BS = blood stage; *Py* = *Plasmodium yoelii*; SPZ = sporozoite. This figure appears in color at www.ajtmh.org.

for euthanasia, with the exception of five of 10 thicket rats that received *Pb* K173 BS-induced infection, and all mice that received *Pvv* v67 SPZ-induced infection (Figure 6, Table 3). Absolute lymphocyte and WBC counts generally decreased during the early stages of infection, with a nadir on days 4–6, except for *Pvv* v67 SPZ-induced infection which resulted in modest changes throughout follow-up (Supplemental Figures S3 and S4). A consistent observation in mice but not thicket rats was that a decrease in WBC by day 4 corresponded to a decrease in hematocrit by study end (Supplemental Figure S5); the correlation was significant by Spearman's rank test in C57BL/6 mice ($P = 0.017$), but not in BALB/c mice ($P = 0.175$). Previous studies have suggested that malaria-induced anemia in nonnatural rodent models may be immune-mediated, involving phagocytic cells and CD4 T cells in semi-immune BALB/c mice and involving CD8 T cells in aged nonimmune Wistar rats with low *P. berghei* parasitemias.[13,14] Future studies should examine the relative contributions of immune cell subsets to the anemia that develops in thicket rats.

In summary, rodent malaria parasite growth characteristics typically differ between the natural versus nonnatural host, suggesting that host–parasite interactions in the latter alter the natural course of infection. Such differences could account for the difficulty in translating protection observed in preclinical studies using nonnatural animal hosts to efficacy in clinical trials. Therefore, thicket rats should be evaluated for their utility to assess interventions such as adjunctive therapies or BS vaccines that previously showed efficacy in mice but not humans. The thicket rat genome has been sequenced, and reagents are being generated to investigate

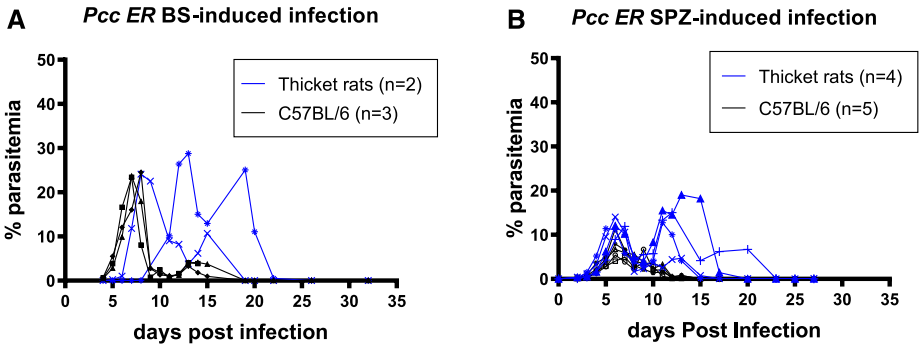

FIGURE 4. Blood stage–induced and SPZ-induced infections with *Pcc* ER. Each line represents one animal. (**A** and **B**) Both C57BL/6 mice and thicket rats controlled parasitemia, although thicket rats showed a more variable course with delayed peak parasitemias during recrudescences in a subset of animals. BS = blood stage; *Pcc* = *Plasmodium chabaudi chabaudi*; SPZ = sporozoite. This figure appears in color at www.ajtmh.org.

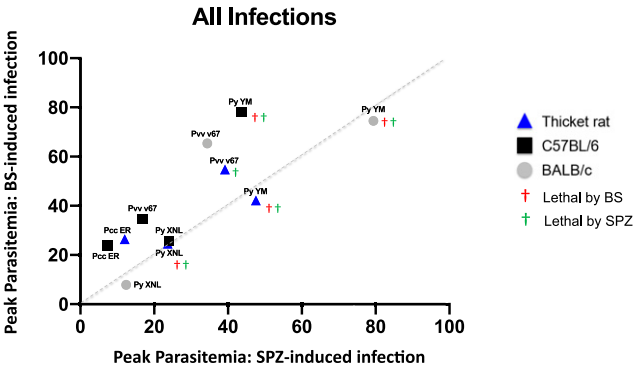

FIGURE 5. Summary of all BS–induced and SPZ-induced infections. Each shape and color represent a host–parasite combination. In general, BS-induced infection induced a higher average peak parasitemia than SPZ-induced infection for five of 10 different host/parasite combinations (*Pcc* ER, *Pvv* v67, and *Py* YM in C57BL/6 mice), whereas the other five host/parasite combinations showed similar average peak parasitemia. BS = blood stage; *Pcc* = *Plasmodium chabaudi chabaudi*; *Pvv* = *Plasmodium vinckei vinckei*; *Py* = *Plasmodium yoelii*; SPZ = sporozoite. This figure appears in color at www.ajtmh.org.

the thicket rat immune response during infections and vaccinations. Wild thicket rats should be captured to determine which additional parasites they naturally harbor, using newly available tools.

Received March 10, 2020. Accepted for publication June 25, 2020.

Published online August 17, 2020.

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

Acknowledgments: We would like to thank Sachy Orr-Gonzales, Kirk Simmons-Butler, and Alaysies Queen for providing help in collecting blood smears; Andre Laughinghouse and Kevin Lee of the Laboratory of Malaria and Vector Research for providing the mosquitoes; Jennifer Kwan for help with statistical analysis; Jean Langhorne for providing *Plasmodium chabaudi chabaudi* parasites and data visualization, and BEI Resources (MR4) for providing *P. vinckei* parasites used for this project.

Financial support: This work was supported by the Intramural Research Program of the National Institute of Allergy and Infectious Diseases, National Institutes of Health.

Disclosure: All animal experiments were conducted under protocol LMIV 1E ASP approved by the Institutional Animal Care and Use Committees (IACUC). All data generated or analyzed during this study are included in this published article.

Authors' addresses: Solomon Conteh, Jacqueline Kolasny, Yvette L. Robbins, Javonn Musgrove, Brandi Butler, Lynn Lambert, John Patrick Gorres, and Patrick E. Duffy, Laboratory of Malaria Immunology and Vaccinology (LMIV), National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), Bethesda, MD, E-mails: solomon.conteh@nih.gov, jacqueline.kolasny@nih.gov, yvette.robbins@nih.gov, javonn.musgrove2@chp.edu, brandi.butler@nih.gov, lelambert@niaid.nih.gov, john.gorres@nih.gov, and patrick.duffy@nih.gov. Pati Pyana, Department of Parasitology, Institut National de Recherche Biomedicale (INRB), Kinshasa, Democratic Republic of Congo, E-mail: ppyana@yahoo.fr. Philippe Büscher, Department of Biomedical Sciences, Unit of

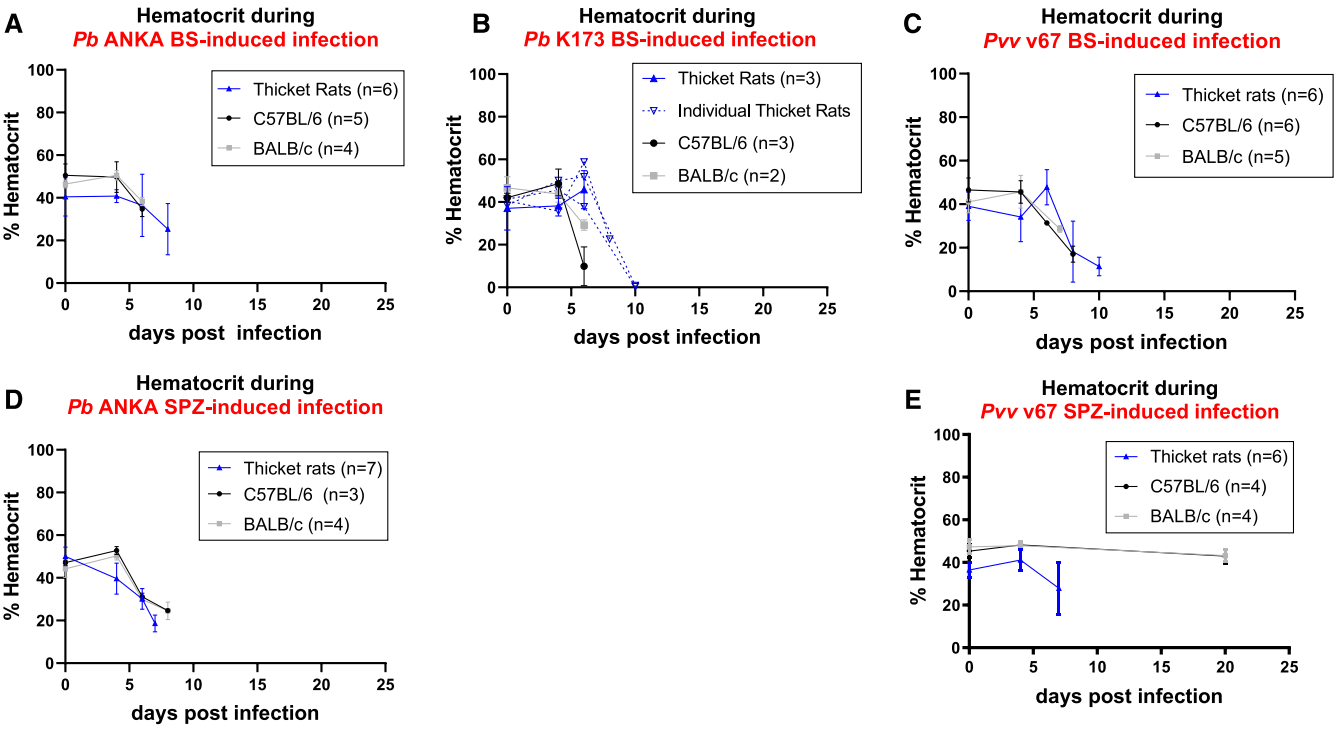

FIGURE 6. Hematocrit changes in rodents during BS–induced or SPZ-induced infection with *Pb* ANKA, *Pb* K173, and *Pvv* v67. For animals inoculated with SPZs to induce infection, 2 days were subtracted to calculate days postinfection to account for liver stage development. *Plasmodium berghei* K173 failed to produce gametocytes, and, hence, SPZ infections were not possible. (**A** and **D**) *Plasmodium berghei* ANKA infections caused a drop in hematocrit that was consistently greater after SPZ- vs. BS-induced infection. (**B**) *Plasmodium berghei* K173 BS-induced infections caused moderate (BALB/c) to severe (C57BL/6) hematocrit drops in mice but not in thicket rats by day 6; three thicket rats that succumbed to infection on days 8 and 10 showed moderate or severe hematocrit drops (dashed lines), whereas the other three infected thicket rats did not demonstrate illness and hematocrits were not assessed after day 6. (**C**) *Plasmodium vinckei vinckei* v67 BS-induced infections reduced hematocrits in all animals, whereas (**E**) *Pvv* v67 SPZ-induced infections reduced hematocrit in thicket rats but not mice. BS = blood stage; *Pb* = *Plasmodium berghei*; *Pvv* = *Plasmodium vinckei vinckei*; SPZ = sporozoite. This figure appears in color at www.ajtmh.org.

TABLE 6
Parasites tested for which the natural host is thicket rat *G. surdaster*

(Sub) species	Natural vector[15]	Laboratory vector	Origin[5]
Pb ANKA isolate	*A. dureni*	*A. stephensi*	Forest gallery on the River Kisanga, near Lubumbashi Katanga Province, DRC
Pb K173 isolate	Unknown	NA	Isolated from *G. surdaster* caught in forest gallery River Kisanga, near Lubumbashi
Pb NK65 isolate	*A. dureni*	*A. stephensi*	Isolated from *A. dureni* millecampsi, caught in forest gallery, River Kisanga, near Lubumbashi
Pvv v-67 isolate	*A. dureni*	*A. stephensi*	Isolated on the Kinga River near Kamena, Katanga, from *A. dureni*, DRC
Pvv Katanga isolate	*A. dureni*	*A. stephensi* (very low sporozoite [SPZ] yield)	Original Mulata isolate from *A. dureni* in Kamena (River Kanga), Katanga, DRC

A. dureni = *Anopheles dureni*; *A. stephensi* = *Anopheles stephensi*; DRC = Republic of Zaire; *G. surdaster* = *Grammomys surdaster*; NA = not applicable; *Pb* = *Plasmodium berghei*; *Pvv* = *Plasmodium vinckei vinckei*.

Parasite Diagnostics, Institute of Tropical Medicine, Antwerp, Belgium, E-mail: pbuscher@itg.be.

REFERENCES

1. WHO, 2018. *World Malaria Report 2018*. Geneva, Switzerland: World Health Organization.
2. malERA Refresh Consultative Panel on Insecticide and Drug Resistance, 2017. malERA: an updated research agenda for insecticide and drug resistance in malaria elimination and eradication. *PLoS Med 14:* e1002450.
3. Menard D, Dondorp A, 2017. Antimalarial drug resistance: a threat to malaria elimination. *Cold Spring Harb Perspect Med 7:* a025619.
4. Waters H, 2011. Better animal models needed for malaria vaccine development, experts say. *Nat Med* 21. Available at: http://blogs.nature.com/spoonful/2011/10/better_animal_models_needed_fo_1.html.
5. LUMC, ■. Plasmodium berghei*: Life Histories and Stabilates (Deep-Frozen Samples) of Isolates, Lines, and Clones Maintained at the University of Edinburgh*. Available at: https://www.lumc.nl/sub/1040/att/811071156182556/811130228382556/908051142222557.pdf. Accessed January 23, 2018.
6. Conteh S et al., 2017. *Grammomys surdaster*, the natural host for *Plasmodium berghei* parasites, as a model to study whole-organism vaccines against malaria. *Am J Trop Med Hyg 96:* 835–841.
7. Chatterjee S, Ngonseu E, Van Overmeir C, Correwyn A, Druilhe P, Wery M, 2001. Rodent malaria in the natural host–irradiated sporozoites of *Plasmodium berghei* induce liver-stage specific immune responses in the natural host *Grammomys surdaster* and protect immunized *Grammomys* against *P. berghei* sporozoite challenge. *Afr J Med Med Sci 30:* 25–33.
8. Ebert D, 1998. Experimental evolution of parasites. *Science 282:* 1432–1435.
9. Mackinnon MJ, Read AF, 2004. Immunity promotes virulence evolution in a malaria model. *PLoS Biol 2:* E230.
10. Spence PJ, Jarra W, Levy P, Reid AJ, Chappell L, Brugat T, Sanders M, Berriman M, Langhorne J, 2013. Vector transmission regulates immune control of *Plasmodium* virulence. *Nature 498:* 228–231.
11. Conteh S, Chattopadhyay R, Anderson C, Hoffman SL, 2010. *Plasmodium yoelii*-infected *A. stephensi* inefficiently transmit malaria compared to intravenous route. *PLoS One 5:* e8947.
12. Akinosoglou KS, Solomou EE, Gogos CA, 2012. Malaria: a haematological disease. *Hematology 17:* 106–114.
13. Evans KJ, Hansen DS, van Rooijen N, Buckingham LA, Schofield L, 2006. Severe malarial anemia of low parasite burden in rodent models results from accelerated clearance of uninfected erythrocytes. *Blood 107:* 1192–1199.
14. Safeukui I et al., 2015. Malaria induces anemia through CD8+ T cell-dependent parasite clearance and erythrocyte removal in the spleen. *mBio 6:* e02493-14.
15. Janse C, 2019. *Introduction to* Plasmodium berghei*: Malaria Parasites that Infect Murine Rodents from Central Africa*. Available at: https://www.lumc.nl/org/parasitologie/research/malaria/berghei-model/introduction-berghei/. Accessed April 11, 2019.