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Multi-strain compatibility polymorphism between a parasite and its snail host, a neglected vector of schistosomiasis in Africa

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ABSTRACT

Interactions between *Schistosoma mansoni* and its snail host are understood primarily through experimental work with one South American vector species, *Biomphalaria glabrata*. However, 90% of schistosomiasis transmission occurs in Africa, where a diversity of *Biomphalaria* species may serve as vectors. With the long-term goal of determining the genetic and ecological determinants of infection in African snail hosts, we developed genetic models of *Biomphalaria sudanica*, a principal vector in the African Great Lakes. We determined laboratory infection dynamics of two *S. mansoni* lines in four *B. sudanica* lines. We measured the effects of the following variables on infection success and the number of cercariae produced (infection intensity): (i) the combination of parasite and snail line; (ii) the dose of parasites; and (iii) the size of snail at time of exposure. We found one snail line to be almost completely incompatible with both parasite lines, while other snail lines showed a polymorphism in compatibility: compatible with one parasite line while incompatible with another. Interestingly, these patterns were opposite in some of the snail lines. The parasite-snail combination had no significant effect on the number of cercariae produced in a successful infection. Miracidia dose had a strong effect on infection status, in that higher doses led to a greater proportion of infected snails, but had no effect on infection intensity. In one of the snail-schistosome combinations, snail size at the time of exposure affected both infection status and cercarial production in that the smallest size class of snails (1.5–2.9 mm) had the highest infection rates, and produced the greatest number of cercariae, suggesting that immunity increases with age and development. The strongest predictor of the infection intensity was the size of snail at the time of shedding: 1 mm of snail growth equated to a 19% increase in cercarial production. These results strongly suggest that infection status is determined in part by the interaction between snail and schistosome genetic lines, consistent with a gene-for-gene or matching allele model. This foundational work provides rationale for determining the genetic interactions between African snails and schistosomes, which may be applied to control strategies.

1. Introduction

Schistosomiasis is a neglected tropical disease that afflicts between an estimated 189–229 million people worldwide, primarily those living in poverty (GBD 2016 Disease and Injury Incidence and Prevalence Collaborators, 2017) While several species of *Schistosoma* can cause schistosomiasis, *Schistosoma mansoni* is among the most prevalent in human infections. Trapped eggs produced by paired adult worms living in the mesenteric veins of the large intestine cause the pathology. Eggs that are successful at penetrating the venules and the intestinal wall, are released into the lumen and excreted into the environment with feces. When eggs contact freshwater, the free-swimming larvae, miracidia, hatch, and seek and penetrate freshwater snails. Following development, multiplication of the schistosome within the snail results in the release of hundreds to thousands of free-swimming infectious larvae, cercariae, into the freshwater environment daily. The cercariae seek a vertebrate host to

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penetrate and establish within, amassing to tens of thousands of cercariae in the lifetime of a single snail (Tavalire et al., 2016; Mutuku et al., 2021). Thus, infected snails serve as a persistent source of infection. Even when people are successfully treated with praziquantel to clear schistosome infection, they rapidly become reinfected through the water sources upon which they rely. The recent World Health Organization Road Map for Neglected Tropical Diseases recommends as a critical action that snail control be implemented as well as new snail control intervention strategies in order to reach schistosomiasis elimination goals (WHO, 2021).

Currently, snail control typically consists of applying molluscicides such as niclosamide which are indiscriminately toxic and not very tractable in large water bodies as snails rapidly recolonize from nearby areas (Yang et al., 2020; Zhu et al., 2022). Additionally, recent studies suggest that unless molluscicide treatment is extensive and continuous, a schistosome boom is predicted to occur as snails recolonize with little competition for resources (Malishev & Civitello, 2019; Civitello et al., 2022). To discover novel interventions, researchers have been working to understand the immune defense of snails against schistosomes. Although much has been learned regarding the snail-schistosome system, this work has almost exclusively focused on one South American vector, *Biomphalaria glabrata*. While this research has uncovered valuable insight on snail-schistosome dynamics, African vectors of schistosomiasis have been neglected, despite the fact that 90% of schistosomiasis transmission occurs in sub-Saharan Africa, and not South America where *B. glabrata* occurs (Hotez & Kamath, 2009). There is a critical need to understand the host-parasite relationships of African snails and to develop laboratory models for these important systems.

In Africa, there are 11 described species of *Biomphalaria* (Brown, 1994). While some African *Biomphalaria* are highly susceptible to infection with *S. mansoni*, such as *B. pfeifferi* (Tchuem Tchuenté et al., 1999; Southgate et al., 2000; Mutuku et al., 2014, 2017), *B. sudanica* appears to be relatively resistant, even when genetically diverse snail populations are exposed to diverse schistosome populations (Mutuku et al., 2021). This pulmonate snail was originally described from Southern Sudan from the marshy tributaries of the River Bhar el Gazal and has been reported from both marsh and lacustrine habitats in Kenya, Ethiopia, Sudan, Uganda, and Tanzania (von Martens, 1870; Williams & Hunter, 1968; Magendantz, 1972; Loker et al., 1981; Brown, 1994; Erko et al., 2006; Kazibwe et al., 2006); however, most research has focused on populations residing in Lake Victoria, where *B. sudanica* is the primary vector of *S. mansoni* (Gouvras et al., 2017). Genetic work has indicated that *B. sudanica* of Lake Victoria is very similar to its sister taxon, *B. choanomphala* of Lake Victoria, also a vector species for *S. mansoni*; and it has been suggested that these two species be synonymized (Bandoni et al., 2000; Standley et al., 2011, 2014). Because the two forms of this snail differ not only in their morphology, but also their susceptibility to *S. mansoni* (*B. sudanica* is more resistant while *B. choanomphala* is more susceptible), we will use the name *B. sudanica* for simplicity and to indicate that our work focuses on this species/ecomorph from Lake Victoria in Kenya.

A primary goal of the present study was to assess the relative compatibility of four laboratory lines of *B. sudanica* originally collected from Lake Victoria, with two lines of *S. mansoni*, one originating from Kenya and the other from Puerto Rico. We use the term "compatibility" rather than resistance or susceptibility to indicate that the infection status (i.e. shedding cercariae positive or negative) is determined by the interaction between the snail immune system and the schistosome immune evasion strategies (Basch, 1975; Richards & Shade, 1987; Webster & Davies, 2001; Théron & Coustau, 2005; Mitta et al., 2012). A second goal was comparing the intensity, number of cercariae produced by infected snails, relative to snail size, during a single day of infection. We additionally investigated the effects of the snail size at time of exposure and the dose of miracidia each snail was exposed to on the infection status and intensity of infection. Overall, we document a compatibility polymorphism between lines of *S. mansoni* and *B. sudanica*, signifying a likelihood of a genetic interaction that can be investigated further in these medically important species.

2. Materials and methods

2.1. Snails and parasites

This study included four lines of *B. sudanica* that originated from the Kisumu region of Lake Victoria, Kenya. Three lines were purposely inbred after collection from the wild with three generations of selfing (110, 111 and 163). The fourth line (KEMRIwu) was not purposely inbred but had been maintained in captivity since 2010. The snail lines have a genome observed heterozygosity of 0.21% (KEMRIwu), 0.13% (Line 110), and 0.11% (Line 163) (data not shown). Prior to experiments, snails were maintained in 10 l plastic shoe boxes with 8 l of aerated and filtered artificial spring water. They were supplemented with calcium carbonate and most lines were fed green leaf lettuce twice a week and supplemented with Aquatic Freshwater Snail Food Mix #2 (Aquaticblend edfoods.com) during breeding. Line 163 did not thrive on lettuce and instead primarily was fed snail food mix and supplemented with lettuce. Dietary effects on infection status and intensity are currently assessed in another study in our laboratory. Room temperatures were maintained at around 24–25 ^{o}C and lights were kept on a 12:12 light-dark cycle.

We used two lines of *S. mansoni*. The NMRI (Naval Medical Research Institute) line was obtained through the BEI Resources Schistosomiasis Resource Center (Lewis et al., 2008), and shipped overnight in mouse livers. This parasite line was originally collected in the early 1940's from infected humans in Puerto Rico. The second *S. mansoni* line, UNMKenya, originated from Kenya and kept in the laboratory since 2017. This parasite strain was maintained in hamsters at the University of New Mexico and eggs in saline were shipped overnight to Western University of Health Sciences for snail infections. The line was originally imported from Mwea, Kenya (central) in 2013 and maintained in *B. pfeifferi*. To introduce more genetic variation to the UNMKenya line, fresh material was added in 2017, representing parasites collected from Asao, Kenya (western) and maintained in *B. choanomphala* and hamster definitive hosts.

2.2. Snail exposures and assessment of infection

Due to the large number of snails exposed, infections were carried out over the course of multiple trials. During the week prior to exposures, snails were collected from breeding tanks, and sorted into size classes of approximately 1 mm ranging from 1.5 mm to 9.5 mm in diameter of the shell at the widest width. On the day of exposures, eggs were freed from liver tissue by massaging the liver through a tissue sieve with 0.85% saline solution. The egg mixture was rinsed once or twice with 0.85% saline solution in an Erlenmeyer flask (500 ml) using a decanting method. Artificial spring water was added to the flask, and the flask was exposed to light for 15–20 min to stimulate miracidial hatching. To concentrate the miracidia, most of the flask, except the neck, was covered with a dark cloth, causing the photophilic miracidia to move to the lighted part of the flask so that they could be collected easily. Twenty-four-well tissue culture plates were used for exposing snails to miracidia. To achieve the correct dose, miracidia were pipetted into wells and the number confirmed visually with a stereomicroscope. Wells were topped off with 2 ml of artificial spring water before adding a single snail to each well. Snails remained in the infection plates overnight before being moved to aquaria at a density of about 36 snails per aquarium. Husbandry was the same as for breeding except that diet was strictly green leaf lettuce twice a week except for Line 163 which received snail food mix twice a week. Cloth covers were placed over the aquarium racks (chrome shelving units of approximately 152 × 61 × 137 cm) to dim the light and reduce early release of cercariae. The cloth does not block all light but creates a dim environment. Room lights were kept on a 12:12 light-dark cycle.

Infection status was determined visually, using a stereomicroscope.

To assess, snails were isolated in 2 ml of artificial spring water in 24-well tissue culture plates and wells were examined for swimming cercariae after 2 h. The number of cercariae (intensity) produced by infected snails was estimated by counting the number of cercariae in a 200 µl subsample, taken after homogenizing with a pipette. Cercariae were killed and stained with iodine, and the number counted multiplied by 10, except where snails shed less than 10 cercariae, in which case, a total cercarial count was recorded. Shell diameters were measured in mm at their widest width using digital calipers. Snails were assessed at 3 time intervals: 8, 10 and 14 weeks post-exposure. Only snails that did not shed cercariae were retained and reassessed at the next assessment period. This was done to be sure to detect infections that may have required a longer than expected prepatent period.

2.3. Descriptive statistics and analysis

Because snails were assessed for infection multiple times, prevalence was calculated as the total number of infected snails summed across the three assessment intervals (8, 10 and 14 weeks) divided by the total number of snails screened at the first screening interval (8 weeks), multiplied by 100. For statistical analyses, the software R was used (R Core Team, 2019), including packages *car* (Fox & Weisberg, 2019), *ggplot2* (Wickham, 2009), *MASS* (Venables & Ripley, 2002). Line 163 was highly resistant to both parasite lines, so only descriptive statistics are reported with no statistical analysis. We used presence of infection (cercarial shedding positive or negative) as a measure of infection success, and also compared cercarial production (intensity) among snail schistosome combinations. For intensity analyses, we used shell diameter size at the time of shedding as a covariate as our past work has shown that this measurement is significantly associated with cercarial production (Tavalire et al., 2016; Spaan et al., 2022), as well as others (e.g. Graham, 2003; Civitello et al., 2020).

All analyses used a dose of 5 miracidia except for the analysis of dose effects on infection status and intensity. The size classes included in each analysis vary depending on available data. Such as, where analysis compared the effect of schistosome and snail line, the following exposure size classes were included: 1.5–4.9 mm snails for KEMRIwu and Line 110, and 3.0–4.9 mm snails for Line 111. For analysis that included compatible snail-schistosome combinations (e.g. KEMRIwu-UNMKenya, 110-NMRI and 111-UNMKenya) all exposure sizes were used.

2.3.1. Effect of schistosome and snail line on infection status and intensity

Infection status. A generalized linear model (GLM) with binomial family and logit link function and ANOVA with type III sum of squares was used to determine the effect of snail line (KEMRIwu or 110) and parasite line (UNMKenya or NMRI) on infection status while accounting for size classes (1.5–2.9; 3.0–3.9; and 4.0–4.9 mm). All two-way interactions were included in the model. No violation of model assumptions occurred. Snail Line 111 was not included in this GLM because fewer size classes were tested, and fewer replicates were included. To understand the only significant interaction from this model (between snail and parasite line), models were run separately on each snail line; KEMRIwu and 110. Thus, a GLM with binomial family and logit link function was used to determine the effect of parasite line on infection status for each snail line separately. Here we included snail Line 111 with fewer size classes (3.0–3.9 and 4.0–4.9 mm).

Intensity. To determine whether one of the snail-schistosome combinations (KEMRIwu-UNMKenya, 110-NMRI and 111-UNMKenya) produced more cercariae, while accounting for the size of snail at time of shedding, a GLM with negative binomial family distribution was used to account for the response variable being count data and overdispersion. No further violation of model assumptions occurred. Scaling of the continuous variable, the size of snail at time of shedding, was done by standardizing the coefficient estimates (i.e. centering by subtracting the mean of each variable and dividing it by its standard deviation) to ensure that all variables contribute evenly to the scale.

2.3.2. Effect of snail size at time of exposure on infection status and intensity

To determine the effect of snails exposed to parasites at different size classes on infection status and intensity, an ANOVA with type III sum of squares and a GLM with either binomial family and logit link function or negative binomial family distribution was used for infection status and intensity, respectively. Only the most compatible snail-parasite combinations were analyzed: KEMRIwu-UNMKenya, 110-NMRI and 111-UNMKenya. Models were run separately for each snail-parasite combination. Again, the continuous variable, size of snail at time of shedding, was scaled and accounted for in the intensity models.

2.3.3. Effect of dose (5 vs 10 miracidia) on infection status and intensity

To determine the effect of parasite dose on infection status, an ANOVA with type III sum of squares and a GLM with either binomial family and logit link function or negative binomial family distribution was used for infection status and intensity, respectively. Only two of the most compatible snail-parasite combinations were compared KEMRIwu-UNMKenya and 110-NMRI, due to small sample sizes with other combinations. Only the 4.0–4.9 mm size class was included as dose data were not available for the other size classes. Again, the variable, size of snail at time of shedding, was scaled and accounted for in the intensity models.

2.3.4. Effect of parasite line exposure on survival

A GLM with binomial family and logit link function was used to determine the effect of parasite line (UNMKenya and NMRI) on survival (8 weeks post-exposure) for each snail line separately (KEMRIwu, 110 and 111). No violation of model assumptions occurred.

3. Results

A total of 3638 snails were exposed across all snail lines, parasite lines, miracidia doses, and exposure sizes (Table 1). However, the number of snails in each analysis varies depending on available data for each question of interest.

3.1. Effect of schistosome and snail line on infection status and intensity

Opposite patterns of compatibility with hosts and parasite strains were found in this study. There was a significant interaction between snail line (KEMRIwu and 110) and parasite line (UNMKenya and NMRI) in the odds of infection at an exposure dose of 5 miracidia, after accounting for exposure size (ANOVA, $\chi^2_{(1)} = 100.4$, $P < 0.0001$; Supplementary Table S1).

For KEMRIwu snails, exposure to 5 miracidia from UNMKenya parasites significantly increased the odds of infection by 3157% compared to exposure with the NMRI line of parasites (GLM, $\beta_0 = -4.34$, $\beta = 3.48$, $z = 6.8$, $P < 0.0001$; Fig. 1, Table 2). On the contrary, for snails of Line 110, exposure to 5 miracidia from UNMKenya parasites significantly decreased the odds of infection by 83% compared to exposure with the NMRI parasites (GLM, $\beta_0 = -2.67$, β = -1.75, $z = -2.9$, $P = 0.0038$; Fig. 1, Table 2). For snails of Line 111, exposure to 5 miracidia from UNMKenya parasites significantly increased the odds of infection by 452% compared to NMRI line of *S. mansoni* (GLM, $\beta_0 = -3.72$, $\beta = 1.71$, $z = 3.6$, $P = 0.0004$; Fig. 1, Table 2). Line 163 was highly resistant to both parasite lines with no NMRI infections and 0–1% UNMKenya infection (Fig. 1, Table 1).

We compared cercarial production between compatible snail combinations. Although the number of cercariae produced by KEMRIwu snails infected with UNMKenya parasites was 32% lower than the Line 110 snails infected with NMRI parasites after accounting for snail size at shedding, there was no significant difference (GLM, $\beta_0 = 5.62$, $\beta = -0.39$, $z = -1.8$, $P = 0.0669$; Fig. 2, Table 3). Similarly, there was no significant difference in the number of cercariae produced by snails of Line 111 infected with UNMKenya parasites and snails of Line 110 infected with NMRI parasites after accounting for snail size at shedding

Table 1
Summary of snail experimental details including, snail lines, size classes exposed, total number of snails exposed per replicate and total, total number of snails infected and total number of snails surviving to the first assessment period (8 weeks post-exposure).

Exposure size (mm)				1.5–2.9			3.0–3.9			4.0–4.9			5.0–5.9			6.0–6.9			8.0–9.5		
Dose	Snail line	Parasite line	Trial	Exp.	Surv.	Inf.	Exp.	Surv.	Inf.	Exp.	Surv.	Inf.	Exp.	Surv.	Inf.	Exp.	Surv.	Inf.	Exp.	Surv.	Inf.
5	KEMRI-wu	UNM-Kenya	1	72	30	13	72	42	15	72	46	10	88	82	25	103	91	26	34	32	11
			2	120	59	21	48	43	17	95	83	27	–	–	–	–	–	–	–	–	–
			3	–	–	–	–	–	–	88	81	30	–	–	–	–	–	–	–	–	–
			Total	192	89	34	120	85	32	255	210	67	88	82	25	103	91	26	34	32	11
		NMRI	1	96	83	3	110	70	1	48	44	0	–	–	–	–	–	–	–	–	–
			2	–	–	–	–	–	–	72	65	0	–	–	–	–	–	–	–	–	–
			3	–	–	–	–	–	–	96	74	0	–	–	–	–	–	–	–	–	–
			Total	96	83	3	110	70	1	216	183	0	–	–	–	–	–	–	–	–	–
	110	UNM-Kenya	1	120	111	3	72	59	0	72	60	0	–	–	–	–	–	–	–	–	–
			2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
			Total	120	111	3	72	59	0	72	60	0	–	–	–	–	–	–	–	–	–
		NMRI	1	72	48	9	110	106	13	60	53	11	96	82	1	72	61	3	48	35	0
			2	–	–	–	96	66	1	120	110	1	–	–	–	96	89	1	–	–	–
			Total	72	48	9	206	172	14	180	163	12	96	82	1	168	150	4	48	35	0
	111	UNM-Kenya	1	67	48	8	72	67	4	94	92	16	–	–	–	–	–	–	–	–	–
			2	–	–	–	72	67	16	–	–	–	–	–	–	–	–	–	–	–	–
			Total	67	48	8	144	134	20	94	92	16	–	–	–	–	–	–	–	–	–
		NMRI	1	–	–	–	60	44	4	67	66	0	–	–	–	–	–	–	–	–	–
			2	–	–	–	–	–	–	102	100	2	–	–	–	–	–	–	–	–	–
			Total	–	–	–	60	44	4	169	166	2	–	–	–	–	–	–	–	–	–
	163	UNM-Kenya	1	26	1	1	34	20	0	24	7	0	–	–	–	–	–	–	–	–	–
			2	–	–	–	–	69	1	–	–	–	–	–	–	–	–	–	–	–	–
			Total	26	1	1	–	89	1	24	7	0	–	–	–	–	–	–	–	–	–
		NMRI	1	–	–	–	91	67	0	–	–	–	–	–	–	–	–	–	–	–	–
			Total	–	–	–	91	67	0	–	–	–	–	–	–	–	–	–	–	–	–
10	KEMRI-wu	UNM-Kenya	1	–	–	–	–	–	–	88	74	37	–	–	–	–	–	–	–	–	–
			2	–	–	–	–	–	–	48	32	11	–	–	–	–	–	–	–	–	–
			3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
			Total	–	–	–	–	–	–	136	106	48	–	–	–	–	–	–	–	–	–
		NMRI	1	–	–	–	–	–	–	72	70	0	–	–	–	–	–	–	–	–	–
			2	–	–	–	–	–	–	24	21	1	–	–	–	–	–	–	–	–	–
			3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
			Total	–	–	–	–	–	–	96	91	1	–	–	–	–	–	–	–	–	–
	110	UNM-Kenya	1	–	–	–	72	57	0	72	57	0	–	–	–	–	–	–	–	–	–
			2	–	–	–	–	–	–	24	24	9	–	–	–	–	–	–	–	–	–
			Total	–	–	–	72	57	0	96	81	9	–	–	–	–	–	–	–	–	–
		NMRI	1	–	–	–	–	–	–	72	64	16	–	–	–	–	–	–	–	–	–
			2	–	–	–	–	–	–	42	39	1	–	–	–	–	–	–	–	–	–
			Total	–	–	–	–	–	–	114	103	17	–	–	–	–	–	–	–	–	–
	111	UNM-Kenya	1	–	–	–	72	65	11	72	66	4	–	–	–	–	–	–	–	–	–
			2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
			Total	–	–	–	72	65	11	72	66	4	–	–	–	–	–	–	–	–	–
		NMRI	1	–	–	–	–	–	–	102	92	1	–	–	–	–	–	–	–	–	–
			2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
			Total	–	–	–	–	–	–	102	92	1	–	–	–	–	–	–	–	–	–
	163	UNM-Kenya	1	–	–	–	–	–	–	72	65	1	–	–	–	–	–	–	–	–	–
			2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
			Total	–	–	–	–	–	–	72	65	1	–	–	–	–	–	–	–	–	–
		NMRI	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
			Total	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Abbreviations: Exp., exposed; Surv., survived; Inf., infected.
Note: A total of 3638 snails were exposed to 5 or 10 miracidia from either *S. mansoni* NMRI or UNMKenya line. Each snail line included up till three replicate trials across six exposure size classes.

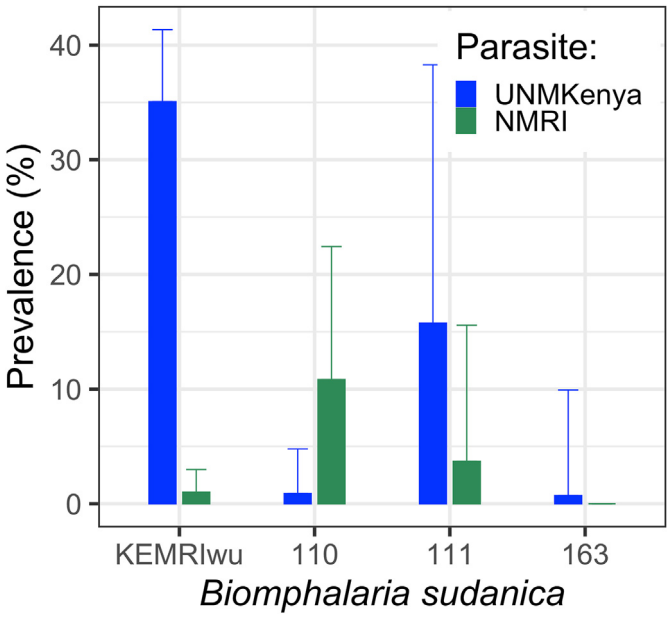

Fig. 1. Prevalence of infection of four lines of *Biomphalaria sudanica* after exposure to one of two lines of *Schistosoma mansoni* with a dose of five miracidia. Exposure snail sizes included 1.5–4.9 mm snails for KEMRIwu line and Line 110, 3.0–4.9 mm snails of Line 111, and 3.0–3.9 mm snails of Line 163. Error bars represent 95% confidence intervals.

(GLM, $\beta_0 = 5.62$, $\beta = -0.15$, $z = -0.6$, $P = 0.5565$; Fig. 2, Table 3). The number of cercariae produced increased by 19% for every 1 mm increase in snail size at shedding, after accounting for compatible snail-schistosome combinations (GLM, $\beta_0 = 5.62$, $\beta = 0.17$, $z = 2.3$, $P = 0.0194$; Table 3).

3.2. Effect of snail size at time of exposure on infection status and intensity

KEMRIwu snails in the 4.0–6.9 mm size classes exposed to UNMKenya line had 56–60% lower odds of infection compared to the 1.5–2.9 size class (Table 4, Fig. 3). Similarly, snails of Line 110 in the 3.0–6.9 mm size classes, exposed to the NMRI line had a 75–96% lower odds of infection than the smallest size class (1.5–2.9 mm) (Table 4, Fig. 3). There was no significant difference in the odds of infection between any of the exposure size classes for snails of Line 111 that were exposed to 5 miracidia from the UNMKenya line (Table 4, Fig. 3).

When considering the UNMKenya line of *S. mansoni*, there was no association between snail exposure size and the number of cercariae produced while accounting for size of snail at time of shedding for any infections for either the KEMRIwu or 111 snail lines (ANOVA, KEMRI-UNMKenya: $\chi^2_{(5)} = 8.5$, $P = 0.1314$, or 111-UNMKenya: $\chi^2_{(2)} = 5.4$, $P =$

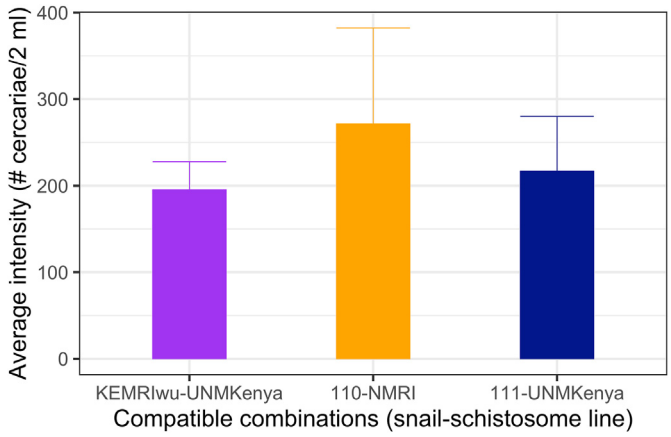

Fig. 2. Mean number of cercariae produced by compatible snail-schistosome combinations, when exposed to 5 miracidia. All exposure snail sizes are combined. No significant differences were found. Error bars represent 95% confidence intervals.

0.0672; Fig. 3, Supplementary Table S2). The number of cercariae produced by snails of Line 110 infected with NMRI parasites differed, depending on the size of snail at exposure (ANOVA, $\chi^2_{(4)} = 49.1$, $P < 0.0001$; Supplementary Table S2). The number of cercariae produced by this combination exposed at larger sizes (3.0–3.9 mm, 4.0–4.9 mm, 5.0–5.9 mm, and 6.0–6.9 mm) was significantly lower (70%, 68%, 99%, and 93%, respectively) compared to those exposed at the smallest size (1.5–2.9 mm), after accounting for size at shedding (Table 5, Fig. 3).

3.3. Effect of dose (5 vs 10 miracidia) on infection status and intensity

KEMRIwu snails exposed to 10 miracidia of UNMKenya line had 99% higher odds of getting infected compared to snails exposed to a lower dose of 5 miracidia (GLM, $\beta_0 = -0.94$, $\beta = 0.63$, $z = 2.8$, $P = 0.0046$, Table 6). However, after accounting for size at time of shedding, there was no significant difference in the expected number of cercariae produced by KEMRIwu snails exposed to 10 *vs* 5 miracidia (Dose 5: $\overline{\mathscr{X}} \pm$ 95% CI = 230 ± 67 cercariae/2 ml; Dose 10: $\overline{\mathscr{X}} \pm$ 95% CI = 212 ± 65 cercariae/2 ml; ANOVA, $\chi^2_{(1)} = 0.14$, $P = 0.7103$). Similarly, snails of Line 110 exposed to 10 miracidia of NMRI line had 319% higher odds of getting infected compared to snails exposed to a lower dose of 5 miracidia (GLM, $\beta_0 = -2.68$, $\beta = 1.43$, $z = 3.4$, $P = 0.0006$; Table 6). Also, after accounting for size at time of shedding, there was no significant difference in the expected number of cercariae produced by snails of Line 110 exposed to 10 *vs* 5 NMRI miracidia (Dose 5: $\overline{\mathscr{X}} \pm$ 95% CI = 210 ± 135 cercariae/2 ml; Dose 10: $\overline{\mathscr{X}} \pm$ 95% CI = 193 ± 93 cercariae/2 ml; ANOVA, $\chi^2_{(1)} = 0.04$, $P = 0.8484$).

Table 2
Summary of the generalized linear model output with a binomial family, logit link function to determine the effect of parasite line on infection status (shedding positive or negative) for each snail line separately, *B. sudanica* KEMRIwu, 110 and 111. Odds ratios represent the back-transformed estimates (β). Reference level for parasite line is NMRI.

	Estimate (β) ± SE	Odds ratio (CI)	*z*-value	*P*-value
B. sudanica Line KEMRIwu				
Intercept (β_0)	−4.34 ± 0.50	0.01 (0.004–0.03)	−8.6	< 0.0001
Parasite: UNMKenya	3.48 ± 0.51	32.57 (13.61–106.61)	6.8	**< 0.0001**
B. sudanica Line 110				
Intercept (β_0)	−2.67 ± 0.17	0.07 (0.05–0.10)	−15.3	< 0.0001
Parasite: UNMKenya	−1.75 ± 0.61	0.17 (0.04–0.49)	−2.9	**0.0038**
B. sudanica Line 111				
Intercept (β_0)	−3.72 ± 0.45	0.02 (0.01–0.05)	−8.2	< 0.0001
Parasite: UNMKenya	1.71 ± 0.48	5.52 (2.36–16.16)	3.6	**0.0004**

Abbreviations: CI, confidence interval; SE, standard error.

Table 3
The effect of compatible snail-schistosome combinations on intensity (number of cercariae produced) for the generalized linear model output with a negative binomial family. Odds ratios represent the back-transformed estimates (β). Reference level for snail-schistosome combination is *B. sudanica* 110-NMRI.

	Estimate (β) ± SE	Odds ratio (CI)	*z*-value	*P*-value
Intercept (β_0)	5.62 ± 0.19	275.50 (193.22–411.69)	29.1	< 0.0001
B. sudanica KEMRIwu-UNMKenya	−0.39 ± 0.21	0.68 (0.44–1.01)	−1.8	0.0669
B. sudanica 111-UNMKenya	−0.15 ± 0.25	0.86 (0.52–1.41)	−0.6	0.5565
Snail size at shedding (mm)[a]	0.17 ± 0.07	1.19 (1.02–1.39)	2.3	**0.0194**

Abbreviations: CI, confidence interval; SE, standard error.
[a] Continuous variable, snail size at time of shedding was scaled.

Table 4
The effect of snail size at time of exposure on infection status (shedding positive or negative) for the generalized linear model output with a binomial family, logit link function, for each snail line, *B. sudanica* KEMRIwu, 110, and 111 with their compatible parasite. Odds ratios represent the back-transformed estimates (β). Reference level for exposure size is 1.5–2.9 mm.

Variable	Estimate (β) ± SE	Odds ratio (CI)	*z*-value	*P*-value
B. sudanica KEMRIwu-UNMKenya				
Intercept (β_0)	−0.12 ± 0.34	0.89 (0.45–1.75)	−0.3	0.7317
Exposure size: 3.0–3.9 mm	−0.49 ± 0.41	0.61 (0.27–1.37)	−1.2	0.2305
Exposure size: 4.0–4.9 mm	−0.83 ± 0.37	0.44 (0.21–0.92)	−2.2	**0.0272**
Exposure size: 5.0–5.9 mm	−0.87 ± 0.42	0.42 (0.18–0.95)	−2.1	**0.0369**
Exposure size: 6.0–6.9 mm	−0.93 ± 0.41	0.40 (0.18–0.89)	−2.3	**0.0244**
Exposure size: 8.0–9.5 mm	−0.74 ± 0.50	0.48 (0.18–1.25)	−1.5	0.1356
B. sudanica 110-NMRI				
Intercept (β_0)	−1.14 ± 0.38	0.32 (0.14–0.65)	−3.0	0.0031
Exposure size: 3.0–3.9 mm	−1.38 ± 0.50	0.25 (0.09–0.69)	−2.7	**0.0062**
Exposure size: 4.0–4.9 mm	−1.54 ± 0.49	0.21 (0.08–0.57)	−3.1	**0.0018**
Exposure size: 5.0–5.9 mm	−3.27 ± 1.08	0.04 (0.002–0.21)	−3.0	**0.0024**
Exposure size: 6.0–6.9 mm	−2.08 ± 0.64	0.12 (0.03–0.41)	−3.3	**0.0011**
Exposure size: 8.0–9.5 mm	−16.43 ± 1097.25	<0.01 (<0.01–> 2064)	−0.02	0.9881
B. sudanica 111-UNMKenya				
Intercept (β_0)	−1.68 ± 0.39	0.19 (0.08–0.37)	−4.4	< 0.0001
Exposure size: 3.0–4.0 mm	−0.53 ± 0.45	0.59 (0.25–1.50)	−1.2	0.2390
Exposure size: 4.0–5.0 mm	−0.16 ± 0.47	0.85 (0.34–2.25)	−0.3	0.7300

Abbreviations: CI, confidence interval; SE, standard error.

3.4. Effect of parasite line exposure on survival

For KEMRIwu snails, exposure to 5 miracidia from UNMKenya parasites significantly decreased the odds of survival by 46% compared to exposure with the NMRI line of parasites (GLM, $\beta_0 = 1.36$, $\beta = -0.62$, $z = -4.1$, $P < 0.0001$, Table 7). However, there was no significant effect of parasite line on the odds of survival for either snails of Line 110 (GLM, $\beta_0 = 1.63$, $\beta = 0.28$, $z = 1.3$, $P = 0.2070$; Table 7) or snails of Line 111 (GLM, $\beta_0 = 2.40$, $\beta = -0.22$, $z = -0.7$, $P = 0.4640$; Table 7).

4. Discussion

The results presented in this study indicate variation in the compatibility between lines of an African snail vector of schistosomiasis, *B. sudanica*, and the parasite, *S. mansoni*. Findings demonstrate that infection status is dependent on the combination of snail and parasite genotypes, dose, and the size of snail at time of exposure. Furthermore, another infection outcome, the intensity of infection, varies among different size classes of snails. Our large sample sizes and the repeatability of these outcomes strongly suggests that the result is based on genetic interactions between the host and parasite, consistent with matching allele or gene-for-gene models of host-parasite compatibility (Flor, 1956; Frank, 1993). The relationship between the South American snail *B. glabrata* and *S. mansoni* also has been described as a compatibility polymorphism (e.g. Basch, 1975; Richards & Shade, 1987; Webster & Woolhouse, 1998; Mitta et al., 2012; Theron et al., 2014) with the combination of snails and parasites determining the outcome of infection.

Another important finding from this study is that the size of a shedding snail greatly impacts the number of cercariae produced so that an increase in a single millimeter of snail diameter results in a 19% increase in cercariae. In the *B. glabrata-S. mansoni* system, a similar relationship has been observed, with a millimeter increase in snail diameter resulting in an 11% increase in cercariae (Tavalire et al., 2016). The finding that larger snails produce more parasites has been reported in other snail-trematode studies and supports the idea that energetics and nutrient availability is a key regulator of cercarial production. Energy diverted from reproduction after parasitic castration is converted to enhanced snail growth and thus parasite growth (Baudoin, 1975; Sorensen & Minchella, 2001; Lafferty & Kuris, 2009; Faro et al., 2013). Additionally, high quality nutrient diets, or increased availability of nutrients, such as through reduced competition, results in larger snails that produce more cercariae (Sandland & Minchella, 2003; Civitello et al., 2020; Civitello & Hartman, 2021; Desautels et al., 2022), which in turn increases transmission likelihood of schistosomes to definitive host populations.

Under the controlled experimental conditions, we found no significant differences in cercarial production between the compatible snail and schistosome combinations. However, we suspect that testing a broader array of genotypes could show such differences. For instance, in comparing *B. sudanica* with its sister species/ecophenotype, *B. choanomphala*, *B. sudanica* produces about half as many cercariae as *B. choanomphala*, despite *B. sudanica* snails' much larger size (Mutuku et al., 2021). Also, work with the *B. glabrata-S. mansoni* system has indicated genetic components to cercarial production for both the parasite (Le Clec'h et al., 2021) and snail (Tavalire et al., 2016).

In the present study, dose-dependent effects on infection status, but not on cercarial production when comparing 5 *vs* 10 miracidia were observed. Results from previous studies of schistosomes in *B. glabrata* have been mixed with some papers reporting a positive association (Gérard et al., 1993; Théron et al., 1997) of dose and cercarial production, others reporting no effect (Blair & Webster, 2007), or a negative

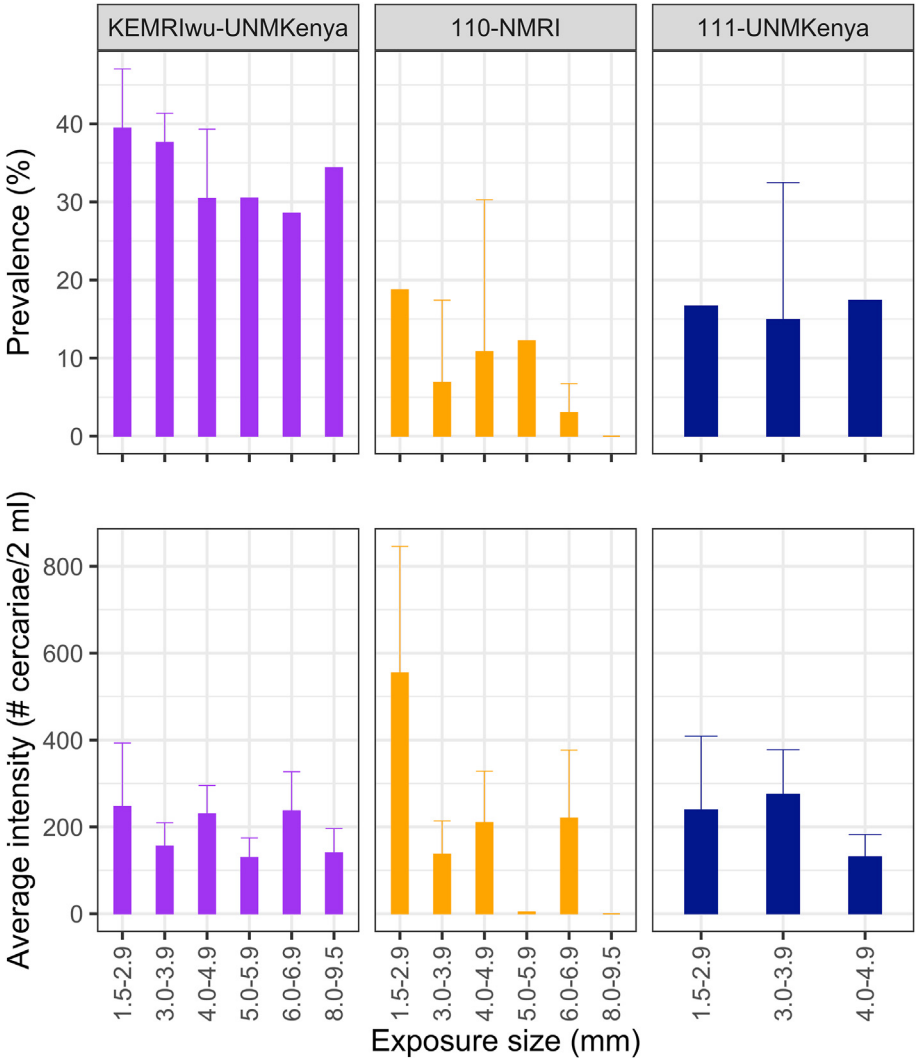

Fig. 3. Prevalence of infection and mean cercarial production for three snail lines after exposure to their more compatible parasite line at various sizes. All exposures were performed with a dose of 5 miracidia. Measurements indicate snail shell diameter at time of exposure and error bars represent 95% confidence intervals. KEMRIwu snails in the 4.0–6.9 mm size classes and snails of Line 110 in the 3.0–6.9 mm size classes had lower odds of infection compared to the smallest (1.5–2.9 mm) size class (Table 4). The number of cercariae produced across exposure sizes was not significantly different except that the mean number produced by snails of Line 110 at 1.5–2.9 mm was greater than those exposed at larger size classes (ANOVA, $\chi^2_{(4)} = 49.1$, $P < 0.0001$).

Table 5
The effect of exposure size on intensity (number of cercariae produced) for the generalized linear model output with a negative binomial family, for *B. sudanica* Line 110 with their compatible parasite (NMRI). Odds ratios represent the back-transformed estimates (β). Reference level for exposure size is 1.5–2.9 mm.

Variable	Estimate (β) ± SE	Odds ratio (CI)	*z*-value	*P*-value
Intercept (β_0)	6.35 ± 0.23	573.85 (377.21–932.16)	27.3	< 0.0001
Exposure size: 3.0–3.9 mm	−1.21 ± 0.32	0.30 (0.15–0.58)	−3.7	**0.0002**
Exposure size: 4.0–4.9 mm	−1.13 ± 0.30	0.32 (0.18–0.57)	−3.8	**0.0002**
Exposure size: 5.0–5.9 mm	−5.77 ± 0.84	0.003 (0.001–0.02)	−6.9	**< 0.0001**
Exposure size: 6.0–6.9 mm	−2.56 ± 0.61	0.07 (0.02–0.27)	−4.2	**< 0.0001**
Snail size at shedding (mm)[a]	0.78 ± 0.16	2.19 (1.57–2.97)	4.8	**< 0.0001**

Abbreviations: CI, confidence interval; SE, standard error.
[a] Continuous variable, snail size at time of shedding was scaled.

Table 6
Summary of the generalized linear model output with a binomial family, logit link function to determine the effect of dose on infection status (shedding positive or negative) for each snail line (*B. sudanica* KEMRIwu and 110) with their compatible parasite at exposure size 4.0–4.9 mm separately. Odds ratios represent the back-transformed estimates (β). Reference level for dose is 5 miracidia.

Variable	Estimate (β) ± SE	Odds ratio (CI)	*z*-value	*P*-value
B. sudanica KEMRIwu-UNMKenya				
Intercept (β_0)	−0.94 ± 0.15	0.39 (0.29–0.51)	−6.4	< 0.0001
Dose: 10	0.69 ± 0.24	1.99 (1.24–3.21)	2.8	**0.0046**
B. sudanica 110-NMRI				
Intercept (β_0)	−2.68 ± 0.31	0.07 (0.04–0.12)	−8.6	< 0.0001
Dose: 10	1.43 ± 0.42	4.19 (1.88–9.72)	3.4	**0.0006**

Abbreviations: CI, confidence interval; SE, standard error.

Table 7
Summary of the generalized linear model output with a binomial family, logit link function to determine the effect of parasite line on survival at 8 weeks post-exposure for each snail line separately, *B. sudanica* KEMRIwu, 110, and 111. Odds ratios represent the back-transformed estimates (β). Reference level for parasite line is NMRI.

Variable	Estimate (β) ± SE	Odds ratio (CI)	*z*-value	*P*-value
B. sudanica KEMRIwu				
Intercept (β_0)	1.36 ± 0.12	3.91 (3.10–4.98)	11.3	< 0.0001
Parasite: UNMKenya	−0.62 ± 0.15	0.54 (0.40–0.72)	−4.1	**< 0.0001**
B. sudanica 110				
Intercept (β_0)	1.63 ± 0.13	5.11 (4.01–6.59)	12.9	< 0.0001
Parasite: UNMKenya	0.28 ± 0.22	1.32 (0.86–2.07)	1.3	0.2070
B. sudanica 111				
Intercept (β_0)	2.40 ± 0.24	11.05 (7.11–18.28)	10.0	< 0.0001
Parasite: UNMKenya	−0.22 ± 0.31	0.80 (0.43–1.44)	−0.7	0.4640

Abbreviations: CI, confidence interval; SE, standard error.

relationship due to reduced growth rates of snails exposed to high doses (Tavalire et al., 2016). One interesting finding is that when very small (1.5–2.9 mm) snails of Line 110 were exposed to NMRI parasites, they produced many more cercariae than snails exposed at larger sizes, even though there were no difference in snail size at time of shedding across different exposure sizes (data not shown). Therefore, indicating that this result is not simply the effect of snails being exposed at a small size staying small and still producing more cercariae per unit snail.

The size of snail at the time of exposure had an impact on infection rate in that the smallest size class (1.5–2.9 mm) had the highest prevalence; however, differences were statistically significant in comparison with only some of the larger size classes. Increasing resistance with size has been reported previously for some strains of *B. glabrata* and *B. alexandrina* (a vector of schistosomiasis in Egypt) after exposure to schistosomes, with the general conclusion that juvenile snails are more susceptible than adult snails (Théron et al., 1998; Niemann & Lewis, 1990; Abou-El-Naga et al., 2015; Ben-Ami, 2019). *Biomphalaria sudanica* in our laboratory become sexually mature and begin to lay eggs between 5 and 6 mm and rarely get larger than 10 mm (M.L. Steinauer pers. obs.), thus we tested a nearly full range of sizes for these snails. The results for the snails of Line 110 exposed to NMRI parasites across size classes were consistent with the pattern of declining susceptibility with development in that prevalence declined generally with size. Larger snails were almost completely resistant to infection. However, KEMRIwu snails exposed to UNMKenya parasites did not follow this pattern and larger snails were still quite susceptible to infection. Variation in this pattern is intriguing because it suggests a potential mechanism whereby some snails resistance to parasites are influenced by maturation, while others are constant throughout the snail's life span.

We also measured survivorship of snails after exposure, comparing survivorship after exposure of the more compatible parasite to that of the least compatible parasite. There was a large survivorship difference in the KEMRIwu snails. Those exposed to the NMRI line (incompatible) had a significantly larger odds of survivorship than those exposed to the UNMKenya parasite (compatible), suggesting that the successful infection, rather than simply exposure and penetration, reduces snail survivorship. Although, other studies also found reduced survivorship with *S. mansoni* infection across *B. alexandria* (Mangal et al., 2010), *B. pheifferi* (Mutuku et al., 2014), and various *B. glabrata* lines (Tavalire et al., 2016), *B. pheifferi* exposed to *S. mansoni* from either a sympatric or allopatric source did not differ in survival rates as one would expect the sympatric combination between snail and schistosome to have higher survival rates (Mutuku et al., 2014). Interestingly, Tavalire et al. (2016) found that the *B. glabrata* line with the lowest survival rate had the highest cercarial production, hypothesizing that the parasite was able to acquire more resources to enhance its growth or that the immune defense of that snail line was more energetically costly. The latter being a possible explanation for the reduced survivorship with successful infection.

5. Conclusions

In summary, we provide the first report of infection dynamics of multiple genetic lines of *S. mansoni* in *B. sudanica*, a vector host in the Great Lakes region of Africa that remains understudied despite its importance in transmission of schistosomes to humans. Our results indicate heterogeneity in several infection traits based on the snail-schistosome combination and documents an experimental system that may be used to further elucidate mechanisms underpinning of these traits in endemic regions of sub-Saharan Africa. Understanding the interactions between snails and schistosomes will provide important perspectives for disease modelling and prediction and will also inform snail-based control measures.

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Ethical approval

Project approval received under the relevant bodies, including KEMRI Scientific Review Unit (permit # KEMRI/RES/7/3/1), Kenya's National Commission for Science, Technology, and Innovation (permit # NACOSTI/P/22/148/39 and NACOSTI/P/15/9609/4270), Kenya Wildlife Services (permit # WRTI-0136-02-22 and # 0004754), and National Environment, Management Authority (permit # NEMA/AGR/159/2022 and # NEMA/AGR/46/2014) (Registration # 0178). The UNMKenya parasite strain was maintained in hamsters at the Western University of Health Sciences under approval of the IACUC (ACUP# R20IACUC039) or at the University of New Mexico (IACUC# 22-201290-MC), in which case eggs in saline were shipped overnight to Western University of Health Sciences for snail infections.

CRediT authorship contribution statement

Johannie M. Spaan: conceptualization, methodology, formal analysis, investigation, data curation, writing - original draft, writing - review & editing, visualization, project administration. Tom Pennance: conceptualization, methodology, investigation, data curation, writing - original draft, writing - review & editing, visualization, project administration. Nicole Sims, Jewell Roth, and Yvonne Lam: investigation, writing - review & editing. Martina R. Laidemitt, Eric S. Loker, Fredrick Rawago, George Ogara, Maurice R. Odiere: resources, writing - review & editing. Michelle L. Steinauer: conceptualization, methodology, investigation, resources, data curation, writing - original draft, writing - review & editing, project administration, supervision, funding acquisition. All authors read and approved the final manuscript.

Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

he data generated or analyzed during this study are available via the Figshare data repository (*B. sudanica* compatibility master data: https://doi.org/10.6084/m9.figshare.22312819.v1).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crpvbd.2023.100120.

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