A Comparison of Kenyan Biomphalaria pfeifferi and B. Sudanica as Vectors for Schistosoma mansoni, Including a Discussion of the Need to Better Understand the Effects of Snail Breeding Systems on Transmission

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A COMPARISON OF KENYAN BIOMPHALARIA PFEIFFERI AND B. SUDANICA AS VECTORS FOR SCHISTOSOMA MANSONI, INCLUDING A DISCUSSION OF THE NEED TO BETTER UNDERSTAND THE EFFECTS OF SNAIL BREEDING SYSTEMS ON TRANSMISSION

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ABSTRACT: In Kenya, schistosomes infect an estimated 6 million people with >30 million people at risk of infection. We compared compatibility with, and ability to support and perpetuate, Schistosoma mansoni of Biomphalaria pfeifferi and Biomphalaria sudanica, 2 prominent freshwater snail species involved in schistosomiasis transmission in Kenya. Field-derived B. pfeifferi (from a stream in Mwea, central Kenya) and B. sudanica (from Nawa, Lake Victoria, in western Kenya) were exposed to S. mansoni miracidia isolated from fecal samples of naturally infected humans from Mwea or Nawa. Juvenile (<6 mm shell diameter), young adult (6–9 mm), and adult snails (>9 mm) were each exposed to a single miracidium. Schistosoma mansoni developed faster and consistently had higher infection rates (39.6–80.7%) in B. pfeifferi than in B. sudanica (2.4–21.5%), regardless of the source of S. mansoni or the size of the snails used. Schistosoma mansoni from Nawa produced higher infection rates in both B. pfeifferi and B. sudanica than did S. mansoni from Mwea. Mean daily cercariae production was greater for B. pfeifferi exposed to sympatric than allopatric S. mansoni (583–1,686 vs. 392–1,232), and mean daily cercariae production among B. sudanica were consistently low (50–590) with no significant differences between sympatric or allopatric combinations. Both non-miracidia-exposed and miracidia-exposed B. pfeifferi had higher mortality rates than for B. sudanica, but mean survival time of shedding snails (9.3–13.7 wk) did not differ significantly between the 2 species. A small proportion (1.5%) of the cercariae shedding B. pfeifferi survived up to 40 wk post-exposure. Biomphalaria pfeifferi was more likely to become infected and to shed more cercariae than B. sudanica, suggesting that the risk per individual snail of perpetuating transmission in Kenyan streams or lacustrine habitats may differ considerably. High infection rates exhibited by the preferential self-fertilizing B. pfeifferi relative to the out-crossing B. sudanica point to the need to investigate further the role of host breeding systems in influencing transmission of schistosomiasis by snail hosts.

Vector-borne diseases including malaria, dengue, Zika virus, and trypanosomiasis continue to pose major challenges to public health (Smith et al., 1998; Greenwood and Mutabingwa, 2002; San Martin et al., 2010). Similarly, snail-transmitted infections also remain problematic in the developing world, and although snails are not vectors in a more conventional sense in that they do not bite their hosts to perpetuate transmission, they play an indispensable role in transmission and are considered to be vectors by the WHO (2016).

With a few exceptions, digeneans (digenetic trematodes or flukes) use snails as first intermediate hosts, enjoying a remarkably productive period of asexual reproduction within snails that culminates with the production of cercariae that may continue for months and in some cases over a year (Mutuku et al., 2014). The prolonged production and release of numerous cercariae into the environment gives the life cycles of human-infecting schistosomes considerable stability, thereby challenging control efforts. Given the vast populations of snails that occupy many natural transmission sites, control of snail-transmitted diseases is a formidable challenge. When schistosomiasis control has been most successful is when snail control has been implemented (Lelo et al., 2014; Njenga et al., 2014; Sokolow et al., 2016), highlighting the importance of knowing more about the biology of the snail hosts and their interactions with snail-transmitted parasites of human and veterinary concern.

The competence of snails to serve as hosts for schistosomes is influenced by several different factors including, but not limited to, infection prevalence as measured by the proportion of schistosome-exposed snails that actually produce and release (shed) cercariae, the length of time required to complete sporocyst development for the first release of cercariae following exposure to infection (the pre-patent period), the longevity of infected snails, duration of actual shedding of the schistosome-exposed snails, and daily output of cercariae from infected snails (Ibikounlé et al., 2012). It is also important to appreciate that schistosome snail hosts exist in complex environmental settings that can influence their capacity to support transmission. They must simultaneously cope with exposure to potential infection with several other species of digenetic trematodes, which may even be more common than schistosomes (Loker et al., 1981; Mohammed et al., 2016) and that also have the potential to cause castration, thereby strongly affecting fitness of the snails. Moreover, infection with other trematode species may alter susceptibility to infection with schistosomes (Spatz et al., 2012). Finally, the suitability of snail environments often varies dramatically with season (Charbonnel et al., 2005), which is
anticipated to influence the snail’s breeding system (for instance, selfing vs. out-crossing), which in turn might influence their competence in resisting infection by parasites (Howard and Lively, 1994; Gibson et al., 2016).

In Africa, transmission of intestinal schistosomiasis caused by Schistosoma mansoni is enabled by 12 species of Biomphalaria with Biomphalaria pfeifferi and Biomphalaria sudanica being the most prominent intermediate hosts in Kenya (Loker et al., 1993; Brown, 1994). Biomphalaria pfeifferi is widely distributed in tributaries feeding Lake Victoria, canals in the Mwea irrigation scheme in central Kenya, small impoundments, and both seasonal and perennial streams throughout the country, except in the tropical lowland belt along coastal Kenya. Biomphalaria sudanica is mainly found along the shores of Lake Victoria and other larger water bodies like Lake Jipe (Loker et al., 1993; Brown, 1994).

As part of a series of studies exploring the role of Kenyan Biomphalaria snails in the transmission of S. mansoni, we first examined the compatibility of field-derived B. pfeifferi to S. mansoni miracidia obtained from infected school children (Mutuku et al., 2014). We were particularly interested in learning if S. mansoni exhibits a greater degree of compatibility with its local B. pfeifferi snail populations than it does with other B. pfeifferi populations further removed geographically. Both sympatric and allopatric combinations of parasites and snails exhibited high compatibility (approximately 50% at a dose of 1 miracidium per snail), with an increase in infection and mortality rates as the miracidial dose was increased. Approximately 3% of B. pfeifferi from Asao, western Kenya, exposed to a low dose of sympatric miracidia (1 or 5) continued to shed cercariae for as long as 58 wk post-exposure (PE). We were also interested in comparing B. pfeifferi and B. sudanica with respect to their role in transmission of S. mansoni. Using a polymerase chain reaction (PCR) assay to detect S. mansoni in snails, we established that during 24 days of pre-patent development following exposure to a single S. mansoni miracidium, 48.3% of B. pfeifferi harbored successfully developing parasites as compared to only 23.5% for B. sudanica. At 40 days PE, by which time it was expected that any successful infection should have culminated in cercariae production, only 14.7% of B. sudanica had either shed cercariae or harbored viable parasites, whereas the comparable figure for B. pfeifferi was 47.6% (Lu et al., 2016). These results are suggestive that B. pfeifferi offers a more conducive environment for schistosome development than B. sudanica during the pre-patent period.

In contrast to the study of Mutuku et al. (2014), which dealt exclusively with B. pfeifferi, one aim of the current study was to examine the relative compatibility of field-derived Kenyan B. pfeifferi and B. sudanica to S. mansoni. Additionally, we were interested in learning if B. pfeifferi was more susceptible to infection with S. mansoni derived from a sympatric locality, one in which its transmission likely depended exclusively on B. pfeifferi, and if this snail species would be equally susceptible to S. mansoni taken from an allopatric area where B. sudanica routinely transmitted the parasite. Conversely, is B. sudanica more compatible with S. mansoni derived from the same location where it is routinely transmitted by B. sudanica? We were also interested in documenting other parameters associated with vectorial competence including length of pre-patent period, the number of cercariae produced daily, and the duration of shedding of cercariae by infected snails.

**MATERIALS AND METHODS**

**Parasite and snail sources**

Schistosoma mansoni eggs were obtained from pooled fecal samples from 5 school children aged 6–12 yr from Mukuo village, Mwea, Kirinyaga County, central Kenya (GPS coordinates 0°40'54"S, 03°20'36"E, altitude 1,098 m), or from Nawa village, Kisumu County, western Kenya (GPS coordinates 0°06’0"S, 03°42’75"E, altitude 1,272 m). Schistosome eggs were concentrated and hatched, and miracidia used to infect snails as described by Mutuku et al. (2014). Snails were collected from the field from Mukou stream, in Mwea, central Kenya, and B. sudanica were collected from Nawa beach, on the shores of Lake Victoria, in Nawa village, Kisumu, and identifications were confirmed as B. sudanica or B. pfeifferi based on known geographical and habitat preferences (Loker et al., 1993; Brown, 1994; Dejong et al., 2001, 2003; Steinauer et al., 2009) and conchological characters (Brown, 1994). The 2 species are distinct genetically and were not found coexisting in the habitats we examined. Biomphalaria snails collected were isolated and screened for digenean infection, and any snail found to be shedding cercariae of any type was discarded. Prior to exposure to S. mansoni, all non-shedders were maintained for an additional 4 wk in aquaria, both to adapt the snails to laboratory conditions and to permit re-screening to determine if they were still negative for digenean infections (Mutuku et al., 2014).

**Experimental design**

A reciprocal cross-infection experiment was conducted whereby B. pfeifferi snails from Mwea and B. sudanica from Nawa were exposed to S. mansoni miracidia from either Mwea or Nawa. Snails to be exposed to S. mansoni were categorized into 3 groups depending on size (shell diameter)/age: (1) juveniles < 6 mm shell diameter; (2) young adults 6–9 mm; and (3) adults > 9 mm (a total of 6 sympatric and 6 allopatric combinations). For each of the 12 possible combinations, 100 pre-screened snails found not to be shedding any digenetic trematodes were exposed to 1 S. mansoni miracidium each. For each of the 3 snail size categories from the 2 locations, a group of 100 snails was not exposed to the parasite and served as unexposed controls. A total of 1,800 snails and 1,200 miracidia were used for this experiment. Starting at 1 wk PE, the snails were examined once a week, for any snails shedding S. mansoni cercariae, for over a period of at least 24 wk, or until the snails died using the procedure described below. Snails were counted and screened individually for evidence of shedding schistosome or any other cercariae, and the number of surviving snails recorded. For snails that were found to be shedding, the total number of cercariae they produced for 2 hr between 1000 hr and 1200 hr was determined as described below.

**Determination of cercariae output from infected snails**

Each snail was placed in an individual well of a 24-well plastic culture plate, each well containing 1 ml of aged dechlorinated water. The plate was placed in indirect sunlight for 2 hr between 1000 hr and 1200 hr. Individual wells were then examined under a dissecting microscope for presence of cercariae. For the snails that had shed cercariae, the contents of the well were mixed gently using a micropipette, and an aliquot of 50 µl was then obtained and placed in a gridded Petri dish. Two drops of Lugol’s iodine
were then added to stain and immobilize the cercariae, which were then counted with the aid of a dissecting microscope and a tally counter. The number of cercariae counted was multiplied by 20 to obtain the total number of cercariae that were produced by the snail during the 2 h period. This procedure was used for all the shedding snails at 6, 10, and 14 wk PE.

**Ethical considerations**

Approval for this study was obtained from the KEMRI Scientific and Ethics Review Unit (SERU) and was referenced SERU SSC No. 2373 and from the University of New Mexico (UNM) Institutional Review Board and referenced 18115. Children were selected for enrollment into the study because they are the most vulnerable to schistosomiasis, contribute significantly to environment contamination and parasite transmission, are easily accessible from their schools, and are regularly offered treatment. Recruitment of human study subjects and their participation and care was done as described previously (Mutuku et al., 2014). Consent to participate in the study was obtained from parents or guardians. The information and data obtained from the study participants were stored securely within KEMRI on password-protected computers. This study was conducted with the approvals of the National Commission for Science, Technology and Innovation (NACOSTI), Permit NACOSTI/P/16/9609/12754, and the National Environment Management Authority (NEMA), Permit NEMA/AGR/46/2014.

**Statistical analyses**

Data analysis was conducted using IBM SPSS version 21.0 statistical software and Microsoft Excel. Descriptive statistics such as proportions were used to summarize categorical variables, while measures of central tendency such as mean, standard error, and range were used to summarize continuous variables. Odds ratio (OR) and 95% confidence interval (CI) were used to estimate the strength of association between outcome and exposure variables. A P value less than 0.05 was considered statistically significant.

**RESULTS**

**Duration of pre-patent period for *S. mansoni***

in snails

The pre-patent period for *S. mansoni* was found to be shorter in *B. pfeifferi* than in *B. sudanica* (Fig. 1). For all *S. mansoni* and *B. pfeifferi* combinations, some snails were shedding cercariae by 4 wk PE, with sympatric combinations having higher proportions of early shedders (3–5.8%) compared to allopatric combinations (1.4–1.5% shedders). Allopatric combinations showed higher proportions of snails beginning shedding at 6 wk PE. For *B. sudanica*, except for young adults exposed to sympatric *S. mansoni*, none of the other groups had shed by 4 wk PE, and only a small percentage (1.2–3.4%) shed by 5 wk PE. Even for juvenile *B. sudanica*, it took up to 6 wk for shedding to commence. There was no obvious overall tendency for younger snails to shed earlier than older snails.

**Prevalence of *S. mansoni***

Infection in snails

With respect to infection prevalence as measured by shedding of cercariae, snails in each exposure combination attained their peak prevalence of infection at 6 wk PE, with an overall 30.6% prevalence among the 755 surviving exposed snails. *Biomphalaria sudanica* were significantly less likely to develop cercariae-producing infections (48 of 414 surviving snails or 8.6%) than *B. pfeifferi* (183 of 341 surviving snails or 53.7%), regardless of the size of snails used or the source of *S. mansoni* (Fig. 1; Suppl. Tables S1, S2). For *B. sudanica* of all 3 size/age groups, the sympatric *B. sudanica*-transmitted Nawa *S. mansoni* isolate produced higher prevalence of infection than the allopatric *B. pfeifferi*-transmitted *S. mansoni* isolate from Mwea, but the differences were not significant. The opposite was true for *B. pfeifferi* where the allopatric Nawa isolate of *S. mansoni* achieved higher prevalence levels than the sympatric Mwea isolate (significant for juvenile snails). In other words, for both snail species, and for all 3 size/age classes, the Nawa-derived *S. mansoni* isolate always produced more patent infections than the Mwea-derived *S. mansoni* isolate. The overall percentage of infection achieved among all snails exposed to Nawa *S. mansoni* (145 of 369 or 39.3%) was significantly higher (*P* = 0.0078) than for *S. mansoni* derived from Mwea (86 of 386 snails or 22.3%) (Fig. 1; Table S2). Overall, relative to the juvenile snail prevalence level of 32.7%, infection prevalence of young adult and adult snails were not significantly different (27.7%, OR = 0.79 [95% CI = 0.55–1.13]; *P* = 0.1938), and (31.8%, OR = 0.96 [95% CI = 0.64–1.42]; *P* = 0.8213), respectively (Table S1).

**Snail mortality by 10 wk post-exposure to *S. mansoni***

Except for the adult snails, mortality was higher for *B. pfeifferi* than *B. sudanica*, regardless of infection status (Fig. 2; Table II). Overall, relative to juvenile exposed snails, morality among the unexposed control snails for both *B. sudanica* and *B. pfeifferi* was significantly greater than for species exposed to *S. mansoni* (*P* = 0.0372 and 0.0422, respectively).
significantly lower at 20.0%, (OR = 0.16 [95% CI = 0.12–0.23]; \(P < 0.0001\)) and 36.0%, (OR = 0.37 [95% CI = 0.27–0.51]; \(P < 0.0001\)), respectively. For the miracidium-exposed snails, relative to juvenile snails, young adult snails were 41% less likely to die; however, there was no significant increase in mortality for the adult snails when all the snails were considered together 62.20% (OR = 1.11 [95% CI = 0.84–1.48]; \(P < 0.4686\)).

Survival of snails with *S. mansoni* cercariae infections

For snails shedding *S. mansoni* cercariae (Table I), except for the adult snails, *B. sudanica* had higher mean survival time compared with *B. pfeifferi*, with *B. sudanica* exposed to sympatric *S. mansoni* achieving slightly longer mean survival times (9.5–12.2 wk) than with allopatric *S. mansoni* (9.5–11.0 wk). However, whereas no infected *B. sudanica* survived past 29 wk PE, a small percentage (1.5%) of young and adult *B. pfeifferi* exposed to sympatric *S. mansoni* survived up to 40 wk PE. By comparison, the longest recorded survival of unexposed *B. sudanica* and *B. pfeifferi* was 43 and 49 wk, respectively.

Cercariae production

Overall, infected *B. pfeifferi* produced more cercariae than *B. sudanica*, with almost all the mean counts for the former being higher than the latter species (Fig. 3; Table II). For *B. pfeifferi*, sympatric combinations usually had a higher mean cercariae production (583 [95% CI 404–762] to 1,686 [95% CI 886–2,486]) than allopatric combinations (392 [95% CI 255–529] to 1,232 [95% CI 936–1,528]). There was no obvious trend for smaller snails to produce fewer cercariae than bigger/older snails, but the highest counts did come from young adult or adult snails. There was no obvious overall tendency for cercariae production to either increase or decrease over 3 successive periods of observation spread over an interval of 56 days. The highest number of cercariae produced at a single observation time was 4,460 by an adult *B. pfeifferi* exposed to sympatric *S. mansoni*.

For *B. sudanica*, generally more cercariae were produced by snails with sympatric than allopatric *S. mansoni* infections (161 [95% CI 103–218] to 360 [95% CI 279–441]), but again this was not always the case. In these monomiracidial infections, no *B. sudanica* snails produced more than 1,000 cercariae during the observation period, though this was common with infected *B. pfeifferi* with sympatric *S. mansoni*.

**DISCUSSION**

Following exposure to *S. mansoni*, for *B. pfeifferi* relative to *B. sudanica*, we found the pre-patent period tended to be shorter, the prevalence of infection as measured by shedding significantly higher, the mortality at 10 wk higher, average survivorship of infected snails marginally shorter, and the average daily production of cercariae significantly higher. In general, the size/age of the snails exposed (<6, 6–9, and >9 mm shell diameter) did not strongly affect most parameters studied for either snail species.

In our hands, following exposure to a single miracidium, Kenyan field isolates of *B. pfeifferi* consistently attained a prevalence of infection of more than 40%. We have now noted
this to be the case whether *S. mansoni* is derived from locations sympatric to the *B. pfeifferi* isolate (both for the Asao stream in west Kenya and now observed twice for *B. pfeifferi* from canals in the Mwea rice scheme in central Kenya) or from allopatric locations (Mutuku et al., 2014). High susceptibility of *B. pfeifferi* to allopatric *S. mansoni* isolates has been shown to be the case regardless of whether the *S. mansoni* isolates come from regions in which *B. pfeifferi* is the usual host or from regions where *B. sudanica* is the normal host. For instance, 80% of juvenile *B. pfeifferi* from Mwea in central Kenya became infected following exposure to a single miracidium of *S. mansoni* from Nawa, Lake Victoria, approximately 300 km to the west. Our results are in agreement with most but not all previous studies (Frandsen, 1979; Victoria, approximately 300 km to the west. Our results are in agreement with most but not all previous studies (Frandsen, 1979; Southgate et al., 2000; Ibikounlé et al., 2012; Adriko et al., 2013; Lu et al., 2016) in documenting high levels of compatibility of *B. pfeifferi* using a combination of an *S. mansoni*-specific PCR-based detection assay, dissection, and shedding methods (Lu et al., 2016), it was noted that more *B. pfeifferi* (54.5%) than *B. sudanica* (38.9%) were positive for *S. mansoni* at 1–4 days PE. This suggested that penetration of miracidia was somewhat higher for *S. mansoni* in *B. pfeifferi*. By 8–24 days PE, the proportion of dissection-positive/PCR positive snails was over 2 times higher in *B. pfeifferi* than in *B. sudanica*. By 40 days PE, the proportion of all snails that was shedding was over 12 times higher, and the proportion of all snails that was shedding was over 25% of sympatric *B. sudanica*, even though this same isolate proved to be very compatible with allopatric *B. pfeifferi* (80% prevalence). Comparing the responses to *S. mansoni* of lab-reared *B. sudanica* and field-derived *B. pfeifferi* using a combination of an *S. mansoni*-specific PCR-based detection assay, dissection, and shedding methods (Lu et al., 2016), it was noted that more *B. pfeifferi* (54.5%) than *B. sudanica* (38.9%) were positive for *S. mansoni* at 1–4 days PE. This suggested that penetration of miracidia was somewhat higher for *S. mansoni* in *B. pfeifferi*. By 8–24 days PE, the proportion of dissection-positive/PCR positive snails was over 2 times higher in *B. pfeifferi* than in *B. sudanica*. By 40 days PE, the proportion of all snails that was shedding was over 12 times higher, and the proportion of all snails that was shedding was over 12 times higher than for *B. sudanica*. *Schistosoma mansoni* also developed faster in *B. pfeifferi* than in *B. sudanica* (Lu et al., 2016).

In contrast to Adriko et al. (2013) and in agreement with Frandsen (1979), our results consistently show lower levels of success for *S. mansoni* in *B. sudanica*, in either sympatric or allopatric combinations. The Nawa isolate of *S. mansoni*, which was recovered from individuals from the shores of Lake Victoria, where their infections most probably originated from *B. sudanica*, never infected more than 25% of sympatric *B. sudanica*, even though this same isolate proved to be very compatible with allopatric *B. pfeifferi* (80% prevalence). Comparing the responses to *S. mansoni* of lab-reared *B. sudanica* and field-derived *B. pfeifferi* using a combination of an *S. mansoni*-specific PCR-based detection assay, dissection, and shedding methods (Lu et al., 2016), it was noted that more *B. pfeifferi* (54.5%) than *B. sudanica* (38.9%) were positive for *S. mansoni* at 1–4 days PE. This suggested that penetration of miracidia was somewhat higher for *S. mansoni* in *B. pfeifferi*. By 8–24 days PE, the proportion of dissection-positive/PCR positive snails was over 2 times higher in *B. pfeifferi* than in *B. sudanica*. By 40 days PE, the proportion of all snails that was shedding was over 12 times higher, and the proportion of all snails that was shedding was over 12 times higher than for *B. sudanica*. *Schistosoma mansoni* also developed faster in *B. pfeifferi* than in *B. sudanica* (Lu et al., 2016).

Also of considerable relevance to understanding the capacity of snails to transmit schistosomiasis is their longevity, especially their duration of shedding. We observed that *B. pfeifferi* of all age

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**Table II. Analysis of mean cercariae production within each snail-parasite combination at 6, 10, and 14 wk post-exposure (PE).**

<table>
<thead>
<tr>
<th>Snail size</th>
<th>Snail species</th>
<th>Snail source</th>
<th>Parasite source</th>
<th>Number of shedding snails</th>
<th>Mean cercariae output for 2 hr (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6 wk PE</td>
<td>10 wk PE</td>
<td>14 wk PE</td>
</tr>
<tr>
<td>Juveniles</td>
<td><em>B. sudanica</em></td>
<td>Nawa</td>
<td><em>B. pfeifferi</em></td>
<td>17 7 4</td>
<td>258 (146-370)</td>
<td>451 (274-628)</td>
</tr>
<tr>
<td></td>
<td><em>B. sudanica</em></td>
<td>Nawa</td>
<td><em>B. pfeifferi</em></td>
<td>2 1 0</td>
<td>150 (51-249)</td>
<td>250</td>
</tr>
<tr>
<td></td>
<td><em>B. sudanica</em></td>
<td>Mwea</td>
<td><em>B. pfeifferi</em></td>
<td>25 10 5</td>
<td>984 (789-1,179)</td>
<td>583 (404-762)</td>
</tr>
<tr>
<td></td>
<td><em>B. pfeifferi</em></td>
<td>Mwea</td>
<td><em>B. pfeifferi</em></td>
<td>46 10 2</td>
<td>1,032 (862-1,201)</td>
<td>392 (255-529)</td>
</tr>
<tr>
<td>Young adults</td>
<td><em>B. sudanica</em></td>
<td>Nawa</td>
<td><em>B. pfeifferi</em></td>
<td>17 11 1</td>
<td>161 (103-218)</td>
<td>250 (173-328)</td>
</tr>
<tr>
<td></td>
<td><em>B. sudanica</em></td>
<td>Mwea</td>
<td><em>B. pfeifferi</em></td>
<td>5 3 0</td>
<td>310 (48-572)</td>
<td>590 (243-937)</td>
</tr>
<tr>
<td></td>
<td><em>B. pfeifferi</em></td>
<td>Mwea</td>
<td><em>B. pfeifferi</em></td>
<td>28 14 5</td>
<td>1,051 (814-1,288)</td>
<td>760 (489-1,031)</td>
</tr>
<tr>
<td></td>
<td><em>B. pfeifferi</em></td>
<td>Mwea</td>
<td><em>B. pfeifferi</em></td>
<td>29 9 8</td>
<td>607 (442-772)</td>
<td>513 (288-739)</td>
</tr>
<tr>
<td>Adults</td>
<td><em>B. sudanica</em></td>
<td>Nawa</td>
<td><em>B. pfeifferi</em></td>
<td>23 15 10</td>
<td>851 (628-1,073)</td>
<td>859 (613-1,104)</td>
</tr>
<tr>
<td></td>
<td><em>B. sudanica</em></td>
<td>Mwea</td>
<td><em>B. pfeifferi</em></td>
<td>23 15 1</td>
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<td>Mwea</td>
<td><em>B. pfeifferi</em></td>
<td>23 15 1</td>
<td>851 (628-1,073)</td>
<td>859 (613-1,104)</td>
</tr>
</tbody>
</table>

FIGURE 3. Mean cercariae produced by *Biomphalaria pfeifferi* and *Biomphalaria sudanica* at 6, 10, and 14 wk post-exposure. Sympatric combinations are represented in red and allopatric combinations in blue. Color version available online.
groups, both unexposed controls and exposed snails, suffered high mortality by 10 wk PE, generally higher than seen for \( B. \) sudanica. Exposure combinations with high prevalence of infection had particularly high mortality by 10 wk PE, so this provides some explanation for the high mortality observed. Another factor we cannot exclude is that the \( B. \) pfeifferi used were transported from central to western Kenya and may have suffered both from transportation and different environmental conditions at Kisian. We have also noted that some \( B. \) pfeifferi isolates adapt better to laboratory conditions than others. In spite of the high mortality at 10 wk PE, some \( B. \) pfeifferi nonetheless survived and continued to shed for up to 40 wk PE. In contrast, none of the exposed \( B. \) sudanica survived past 29 wk PE. As previously noted (Mutuku et al., 2014), some individual field-derived \( B. \) pfeifferi when experimentally exposed to \( S. \) mansoni as young adults can support production of \( S. \) mansoni cercariae for over a year.

With respect to the number of cercariae released per day, on average \( B. \) pfeifferi produced more cercariae than \( B. \) sudanica. For individual \( B. \) pfeifferi, >2,000 cercariae were often recovered from a 2-hr shedding period. By contrast, none of the experimentally exposed \( B. \) sudanica were observed to shed more than 900 cercariae in a comparable interval, in agreement with results by Frandsen (1979). Low infection and cercariae production levels help to explain the difficulties we have experienced in the past in trying to maintain the \( S. \) mansoni life cycle in the laboratory in \( B. \) sudanica. In contrast to our results, a study utilizing Ugandan \( B. \) pfeifferi snail isolates demonstrated that \( B. \) sudanica produced more cercariae than \( B. \) pfeifferi though the difference was not significant (Adriko et al., 2013).

The results of our study are also of relevance to evolutionary biologists interested in the Red Queen hypothesis, particularly the topic of how sexual reproduction in host populations influences susceptibility to coevolving parasites. Studies of other digenean-snail combinations have shown that high prevalence of digenean infection favors higher proportions of sexually reproducing snails. This is because the parasites are able to adapt readily to clonal asexual hosts and achieve high levels of sterilizing infection among them, whereas host individuals produced by sexual crosses may express rare traits conferring higher resistance to infection (Howard and Lively, 1994; Vergara et al., 2014; Gibson et al., 2016). With respect to the present study, we note as have several others that \( B. \) pfeifferi is highly susceptible to \( S. \) mansoni and in nature is often infected with several digenetic trematodes (Loker et al., 1981; Mohammed et al., 2016). A number of studies have documented that although \( B. \) pfeifferi is capable of cross-fertilization, it is a strong preferential self-fertilizer (Jarne and Theron, 2001; Charbonnel et al., 2005; Campbell et al., 2010). The latter mode of reproduction generally leads to an excess of homozygosity and a loss of genetic diversity, and \( B. \) pfeifferi populations consist of a series of relatively demarcated lineages separated from one another by strong preferential selfing (Jarne and Theron, 2001). These are all characteristics that might be expected to favor high levels of parasitism. We lack a general understanding in \( S. \) mansoni transmission foci for how many such \( B. \) pfeifferi lineages exist, how spatially distinct or temporally stable they are, and how much they may vary in rate with other \( B. \) pfeifferi lineages with which they must contend. These other digenetic species can be as common or more so than \( S. \) mansoni and also impose fitness costs as they also can cause castration (Esch and Fernandez, 1994; Lafferty and Kuris, 2009). It will be interesting to learn if under heavy pressure from parasitism including \( S. \) mansoni and other abundant digeneans like amphisomes (Laidemitt et al., 2016) whether \( B. \) pfeifferi populations increase out-crossing rates such that they might then fare better in a co-evolutionary arms race with outcrossing parasites (King et al., 2011; Koskella et al., 2011; Singh et al., 2015). Alternatively, perhaps some genotypes of \( B. \) pfeifferi are more resistant to digeneans than others, and selective pressure from digenean parasitism may favor increased frequency of resistant genotypes. Although the impermanent nature of the stream habitats often colonized by \( B. \) pfeifferi may prevent stable parasite populations from building such that the presumed advantages in rapid colonizing ability favored by self-fertilization might predominate over advantages in resistance to parasites resulting from sex, our studies suggest high levels of parasitism can persist for years in streams that do not necessarily flood or dry on an annual basis (M. R. Laidemitt, pers. comm.).

Further investigation of the interactions between digenean parasitism (including \( S. \) mansoni) and both outcrossing rates and population composition studies for \( B. \) pfeifferi are clearly warranted, including efforts to determine if out-crossed progeny enjoy greater resistance to digenean infection.

\( B. \) sudanica and \( B. \) choanomphala also deserve consideration in a broader evolutionary context. \( B. \) sudanica has been characterized as an out-crosser (Standley et al., 2014). In the context used by the authors, this designation was meant to apply to both \( B. \) choanomphala and \( B. \) sudanica given that other studies suggested the 2 regularly undergo genetic exchange (the 2 taxa represent distinctive ecophenotypes) and \( B. \) choanomphala is the name with taxonomic priority (Standley et al., 2011). Here we use the name \( B. \) sudanica rather than \( B. \) choanomphala to apply to the shore-inhabiting form, because the latter is generally considered to be a deep-water snail. In any case, the exact nature of the genetic exchange between the 2 named taxa deserves further study for additional populations. Also, assuming that the lakeshore-inhabiting form \( B. \) sudanica is an out-crooser, it is of interest and consistent with theory that its experimental susceptibility to infection with either sympatric or allopatric \( S. \) mansoni is lower than seen with \( B. \) pfeifferi. Furthermore, overall prevalence levels with \( S. \) mansoni and other digeneans appear to be lower in \( B. \) sudanica than in \( B. \) pfeifferi in natural habitats monitored for over 2 yr (M. R. Laidemitt, pers. comm.) though extraneous environmental factors might play an important role in dictating such infection levels as well. Any gain in resistance achieved by \( S. \) mansoni by out-crossing might be expected to affect not just \( S. \) mansoni, but as many as 16 additional digenean species, many transmitted by shoreline-inhabiting birds, that commonly infect this snail in nature as well. Although experimentation with additional isolates is needed, the low infection levels we retrieve with \( B. \) sudanica following experimental exposure to \( S. \) mansoni are suggestive of the presence in \( B. \) sudanica of resistance traits that may prove useful with respect to developing new control efforts based on introductions of resistant snails into natural populations of schistosome-susceptible snails. Last, the relationships between \( B. \) choanomphala and digenean infection deserve much more scrutiny. This taxon is typically but not always recovered from deeper lake water. Deepwater habitats have been considered as coevolutionary “cold spots” as compared to shallower shoreline habitats frequented by avian definitive.
hosts (Howard and Lively, 1994; King et al., 2009). For B. choanomphala, does its preferred habitat provide a refugium from digenean infection? Although it is clear that B. choanomphala can be infected by S. mansoni in deeper water, the extent to which it is exposed to this and other digenean species may be considerably diminished relative to B. sudanica. If so, then does this taxon when in deep water revert to more frequent self-fertilization, possibly abandoning the costs of maintaining resistance to digenean infection given their lower exposure rates (Sheldon and Verhulst, 1996)?

Another observation of interest from our data is that S. mansoni from Nawa, where it is transmitted by B. sudanica, produced higher infection levels in both snail species than the B. pfeifferi-transmitted isolate of S. mansoni isolate from Mwea. This is consistent with the idea that ongoing coevolutionary interactions of S. mansoni with a sexually reproducing host confers on it properties of infectivity that guarantee it a higher likelihood of success when confronted with a selling species like B. pfeifferi. Study of further reciprocal exposure experiments involving the same 2 snail species and isolates of S. mansoni derived from each would be of interest to further document this possibility. Also of interest would be to learn if and how the interactions between sexual vs. selling snails might also influence trade-offs that might occur with respect to virulence in the definitive host (Davies et al., 2001).

In conclusion, we were interested in determining which of the two most prominent intermediate host snails for S. mansoni in Kenya is more efficient in transmission of the intestinal schistosomiasis parasite by measuring traits that affect transmission. At least some B. sudanica and B. pfeifferi could support full development of either allopatrically or sympatrically derived S. mansoni regardless of snail size/age, but B. pfeifferi were significantly more likely to become infected and had higher daily rates of cercariae production than B. sudanica. Even though B. sudanica seems less efficient in transmission of the parasite on a per snail basis, this species occurs in vast numbers in its natural habitat. Abundance may thus compensate for low compatibility such that B. sudanica can readily sustain transmission in communities living around the shores of the lake. The persistence of a proportion of long-term B. pfeifferi sheds, though it may seem insignificant, could nonetheless play a significant role in initiating reinfections in the face of sustained mass drug administration. Because of differences in the breeding systems of B. pfeifferi and B. sudanica, the interactions of these 2 host species with S. mansoni and other digeneans may prove to be instructive in understanding the importance of sex in resistance to parasites.

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