Dexrazoxane as a viable microsporidia control agent in *Anopheles gambiae*

Tiago G. Zeferino [1 ¶]
Luís M. Silva [1,2 * ¶]

[1] Institute of Biology, University of Neuchâtel, Rue Emile-Argand 11, 2000 Neuchâtel, Switzerland
[2] Department of Zoology, University of British Columbia, 6270 University Boulevard, Vancouver, BC V6T 1Z4, Canada

¶ Both authors contributed equally

* Corresponding author
E-mail: luis.silva@ubc.ca (LMS)

ORCID
TGZ: 0009-0004-0767-5200
LMS: 0000-0002-8203-4006

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Abstract

Microsporidia have long been proposed as biological agents for controlling disease vectors and the parasites they transmit. However, their study in vector biology has been constrained due to challenges in manipulating microsporidia within hosts. In this study, we investigated the effect of Dexrazoxane, a candidate drug against microsporidiosis, on the establishment and development of *Vavraia culicis* infection in its natural host, the mosquito *Anopheles gambiae*, the main malaria vector. Our findings show that Dexrazoxane significantly reduces spore load, particularly in mosquitoes reared individually, without affecting the overall infection success of the parasite. This result aligns with studies in *Caenorhabditis elegans*, where Dexrazoxane inhibited new spore production without hindering initial spore integration into the host gut cells. Dexrazoxane's DNA topoisomerase II inhibitor mechanism likely explains its impact on mosquito development, as larvae exposed to the drug failed to emerge as adults. These findings highlight Dexrazoxane's potential as a viable tool for controlling microsporidia in adult mosquitoes and hope to enhance the study of mosquito-microsporidia interactions. Further research is required to explore its broader application in vector-borne disease control, including malaria.

Introduction

Microsporidia are common obligate parasites of many animals, and their prevalence has been increasingly reported over the past decades across a wide range of taxa, sparking a surge in microsporidia studies [1–7]. While research in human-infecting microsporidia mainly focuses on clearing them (i.e., microsporidiosis) [8], in invertebrates such as insects, their study is more nuanced, as evidence suggests microsporidia may serve as biological control agents for certain pests [9] and vector species, and the diseases they transmit [10–12].

Nearly half of the microsporidia species that infect insects have specialised in targeting dipterans, such as flies and mosquitoes, which are vectors for several human diseases. Among those infecting mosquitoes, *Vavraia culicis* [13] is one of the most well-studied microsporidia species [14–23]. This generalist intracellular parasite is known to infect several mosquito genera, including *Anopheles gambiae*, the major vector of malaria [18]. Infection typically occurs during the larval stage when mosquito larvae ingest spores along with food in water bodies. Once the spores penetrate the host's gut wall, they develop and produce new spores. The limited immune response of *An. gambiae* to this particular microsporidian species [24] allows for a steady progression of the infection, characterised by continuous consumption of host resources and accumulation of spores in the body cavity, leading to costs in host longevity [18, 25] and fecundity [14].

Although *An. gambiae* incurs significant costs from *V. culicis* infection, it comes with a benefit in protection against co-infection with *Plasmodium spp.* by negatively affecting oocyst development [26,

27]. These findings led the community to hypothesise that microsporidian parasites, such as the one in this study, could be used as biological control agents for mosquitoes and their parasites by reducing the mosquito lifespan and parasite transmission to mammal hosts, respectively [11, 26, 27]. However, the mechanisms underlying this interaction remain unclear, partly due to the technical challenges of manipulating microsporidian infections within mosquito hosts.

Hence, to facilitate the control and study of microsporidians within the mosquito host, a candidate chemical, Dexrazoxane, recently shown to have anti-microsporidian activity in *Caenorhabditis elegans* [29] was tested. Due to its antimitotic and redox activity, Dexrazoxane has been extensively used as a protector agent against the cardiotoxicity of chemotherapy in humans [30]. Its mechanism of action is similar to ethylenediaminetetraacetic acid (EDTA) but diffuses into cells more readily [31], where it binds to iron and lowers oxidative stress formation [32]. However, its role against microsporidia has only recently been studied. For instance, in *C. elegans*, Dexrazoxane is known to inhibit *Nematocida parisii* development after it has established itself in the gut cells [29]. While it does not prevent initial infection, it suppresses the production of new spores, thereby hindering disease progression and transmission. Given its promising effects on microsporidia infection in *C. elegans*, we aimed to test whether these effects extend to microsporidian infections in mosquitoes, using *An. gambiae* and *V. culicis* as the experimental system. Our findings suggest that Dexrazoxane's effect might be more general than previously thought, extending to mosquito-microsporidia interactions. This result may enhance our understanding of microsporidia interactions with their host and other parasites and could potentially inform and fuel future vector-borne disease control strategies.

Materials and Methods

Experimental system

We used the *An. gambiae s.s* Kisumu strain [33], which had been maintained at our standard laboratory conditions (about 600 individuals per cage, constant access to 6% sucrose solution, 26 ± 1ºC, 70 ± 5% relative humidity and 12 h light/dark) for many years before the experiments.

The microsporidian *V. culicis floridensis* was provided by J.J Becnel (USDA, Gainesville, FL, USA). Originally discovered in *Ae. albopictus,* this parasite was later found to parasitise several mosquito genera, including *Aedes*, *Culex*, *Anopheles*, *Culiseta*, *Ochlerotatus* and *Orthopodomyia* [20, 34, 35]. We maintained it by alternately infecting *Aedes aegypti* and *An. gambiae* to ensure its status as a generalist parasite, as described previously [18].

V. culicis is an obligatory, intracellular parasite. Mosquitoes are infected when, as larvae, they ingest the spores along with their food. After several rounds of replication, the parasite produces new

93 infectious spores that spread to gut and fat body cells. The spores are released to larval sites when
94 infected larvae die, when adults die on the surface of water, or when eggs covered with spores are laid
95 onto the water's surface.

96 *Rearing and maintenance of experimental mosquitoes*

97 Freshly hatched (0-3 hours old) *An. gambiae* larvae were individually placed into 12-well culture plates,
98 each containing 3 ml of deionized water. The larvae received Tetramin Baby® fish food every day
99 according to their age (0.04, 0.06, 0.08, 0.16, 0.32 and 0.6 mg/larva respectively on days 0, 1, 2, 3, 4
100 and 5 or older [36]).

101 In a first experiment, we assessed the impact of Dexrazoxane on larval development and spore growth.
102 Two-day-old larvae were exposed to either 0 or 10,000 spores of *V. culicis*, and received one of our
103 three Dexrazoxane doses (0 µM, 30 µM and 60 µM) at one of three administration times (four, five, or
104 six days after egg hatching), in a fully combinatorial design. Survival and pupation of the larvae were
105 tracked daily.

106 In a second experiment, we assessed the impact of Dexrazoxane on spore growth at the adult stage and
107 if being reared individually as opposed to collectively could influence this growth. Two-day-old larvae
108 were exposed to either 0 or 10,000 spores of *V. culicis*. Pupae were transferred to individual 50 ml
109 falcon tubes with approximately 10 ml of deionised water [37]. Female adults were moved to 150 ml
110 cups (5.5 cm Ø x 10 cm) covered with a net or cages, depending on their treatment. The cups contained
111 a 50 mm Ø petri dish (to prevent mosquitoes from drowning) floating on the surface of 50 ml deionised
112 water and a 10 x 7 cm rectangular filter paper to maintain humidity. The mosquitoes were provided
113 with cotton balls soaked in a 6% sucrose solution or a 6% sucrose solution supplemented with 60 µM
114 of Dexrazoxane, which was replaced every other day.

115 On the 8th day, alive individuals were collected in a 2 ml Eppendorf tube containing 0.1 ml of deionised
116 water and stored at -20º C for spore load quantification. A stainless-steel bead (Ø 5 mm) was added to
117 each tube and the samples were homogenised using a Qiagen TissueLyser LT at a frequency of 30 Hz
118 for two minutes. The number of spores in each sample was then counted using a haemocytometer with
119 0.1 µl of the sample under a phase-contrast microscope (400x magnification).

120 *Statistical analysis*

121 All analyses were conducted with the R software [38] version 4.4.1, using the packages DHARMa [39],
122 car [40], glmmTMB [41], emmeans [42] and multcomp [43]. Significance was assessed with the
123 "Anova" function of the "car" package [40]. We used a type III ANOVA in the case of a significant

interaction and a type II ANOVA otherwise. When relevant, we performed post-hoc multiple comparisons with the package "emmeans", using the default Tukey adjustment.

The presence of spores was coded as a binary response (presence or absence of detectable spores) and analysed with a generalised linear model with a binomial distribution of errors, where the explanatory variables were diet (with or without Dexrazoxane), type or rearing (individual or collective) and their interaction. Because we counted the number of spores in a haemocytometer containing 0.1 µl of the sample (i.e. 1/1000 of the total volume), the detection threshold was estimated to be 1000 spores.

Spore load of mosquitoes with positive spore counts was analysed with a generalised linear model with a negative binomial distribution of errors. The explanatory variables were diet (with or without Dexrazoxane), type or rearing (individual or collective), and their interaction.

Results

Larval exposure to Dexrazoxane delayed larval development and blocked pupation, independently of the dose, with none of the treated larvae surviving past pupation (as opposed to Dexrazoxane-unexposed larvae). That led us to discard the use of this chemical during the larval stage.

The proportion of individuals harbouring spores was lower for individuals reared in cups than for individuals reared in cages (0.65 vs 0.85, $\chi^2 = 12.25$, df = 1, $p < 0.001$, **Fig. 1a**) but was not affected by the consumption of Dexrazoxane (0.75 vs 0.75, $\chi^2 = 0.00$, df = 1, $p = 0.945$) or their interaction ($\chi^2 = 0.28$, df = 1, $p = 0.595$). In contrast, the spore load of mosquitoes with positive counts was reduced by half in individuals consuming Dexrazoxane, but only when were reared in cups (4.9×10^4 vs 1.2×10^5 in cups, and 9.5×10^4 vs 6.7×10^4 in cages, interaction type of rearing * diet: $\chi^2 = 10.35$, df = 1, $p = 0.001$, **Fig. 1b**). For further details on the statistical analysis see **Table 1**.

152 **Table 1. Effect of exposure to Dexrazoxane on infection.** Statistical models carried on to test for
153 effect of exposure to Dexrazoxane, rearing condition (individual in cup, or collective in cage) and their
154 interaction on the presence of spores (Model 1a) and number of spores (Model 1b). See Materials and
155 methods for more details on the experimental design and statistical tests. Values in bold are statistically
156 significant.

Tested effect			
Model 1a – Presence of spores	**df**	χ^2	***p***
Exposure to Dexrazoxane	1	0.00	0.945
Rearing (Cup/Cage)	1	12.25	**<0.001**
Exposure to Dexrazoxane: Rearing (Cup/Cage)	1	0.28	0.595
Model 1b – Spore load	**df**	χ^2	***p***
Exposure to Dexrazoxane	1	2.13	0.144
Rearing (Cup/Cage)	1	4.68	**0.030**
Exposure to Dexrazoxane: Rearing (Cup/Cage)	1	10.35	**0.001**

157

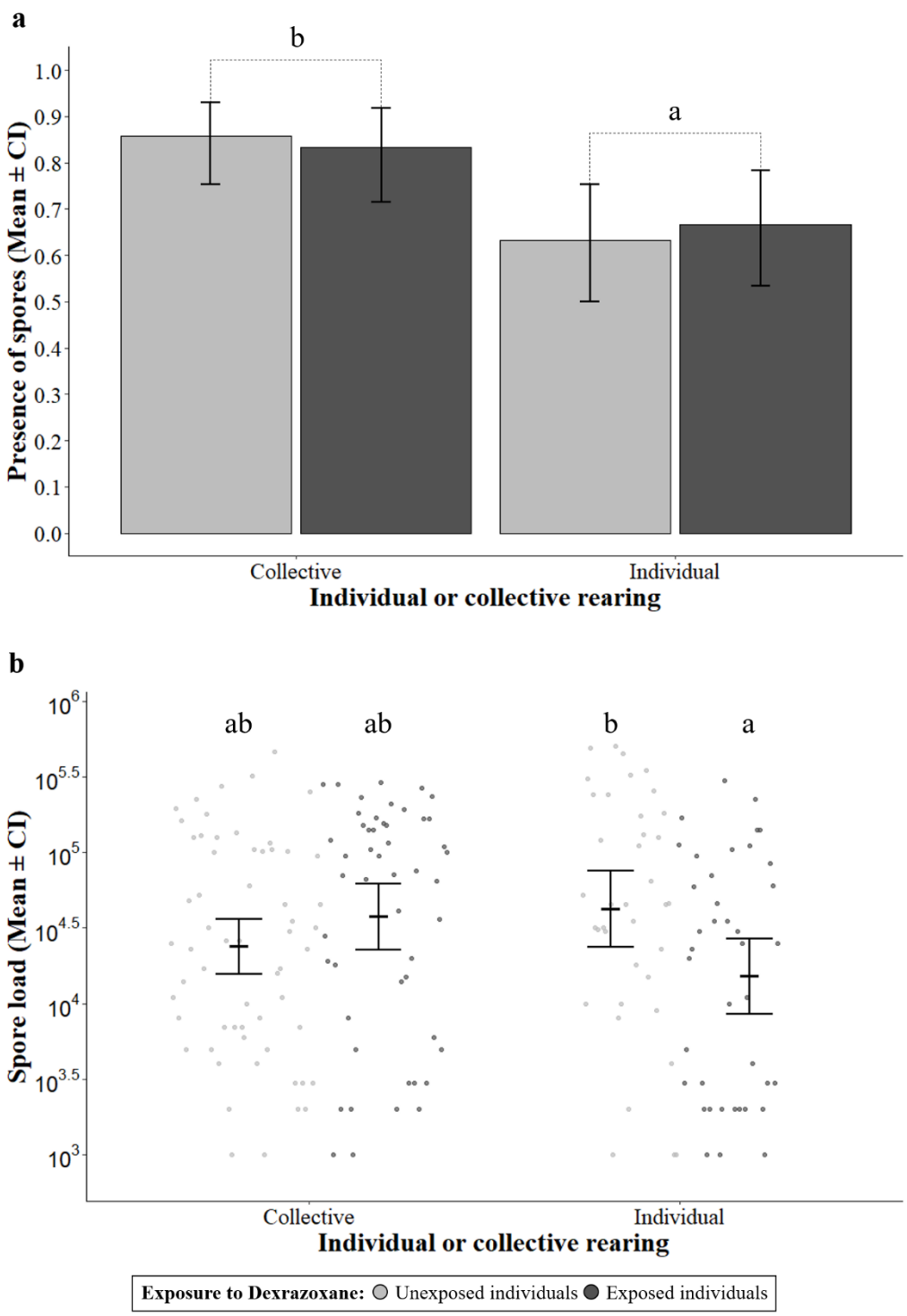

Figure 1. Parasite load of infected mosquitoes after consuming a specific diet for seven days. The proportion of mosquitoes reared individually or collectively that had detectable spores for **(a)** each Dexrazoxane diet and their spore load **(b)**. The sample sizes were 70, 60, 60 and 60 for **(a)**, and 60, 50, 38 and 40 for **(b)**. Error bars show the 95% confidence intervals of the means. Letters indicate statistically significant differences from multiple comparisons. See Table 1 for further details on the statistical analyses.

165 # Discussion

166 Our study investigates the effect of Dexrazoxane on the infection by the microsporidian *V. culicis* in
167 the mosquito host *An. gambiae*. The findings extend the current knowledge of microsporidia-mosquito
168 interactions and suggest potential strategies for controlling microsporidia infections and mosquito-
169 borne diseases like malaria.

170 The primary finding from this study is that Dexrazoxane significantly reduces the spore load of infected
171 mosquitoes but only under certain rearing conditions, mainly when mosquitoes are raised individually
172 in cups **(Fig. 1b)**. Moreover, although the number of spores produced drops, the number of successful
173 infections did not differ, compared to unexposed **(Fig. 1a)**. This result aligns with previous research on
174 its inhibitory effects on microsporidian development in *C. elegans* [29], which demonstrated
175 Dexrazoxane primarily reduces new spore production, rather than affecting the initial spore integration
176 into the host gut wall. This reduction in spore numbers came at no cost to host survival.

177 The reduced spore load in mosquitoes raised individually in cups highlights a potential indirect effect
178 of Dexrazoxane on the mosquito immune response or microsporidian metabolism. While any
179 hypothesis cannot be ruled out, this inhibition is likely due to the drug's mechanism of action. For human
180 purposes, Dexrazoxane primarily functions through iron chelation, where it blocks circulating iron from
181 being used to fuel oxidative stress and tissue damage. Although this mechanism has been shown to
182 affect *Plasmodium falciparium* development *in vitro* [44], the same cannot be said for microsporidia.
183 In fact, *C. elegans* infection with the microsporidia *N. parisii* can be blocked using Dexrazoxane but
184 cannot be reverted through iron supplementation [29]. This result suggests inhibition in microsporidian
185 infections might not happen via iron chelation but instead through another mechanism of action.
186 Interestingly, Dexrazoxane also acts as a DNA topoisomerase II inhibitor, which likely explains why
187 larvae exposed to the drug failed to emerge as adults (see Materials and Methods). In a study on *Ae.*
188 *aegypti*, larvae treated with Dexrazoxane exhibited morphological defects and low survival during both
189 larval and pupal stages [45]. Therefore, it is plausible that *V. culicis* relies on the same protein or a
190 structurally similar one and inhibiting this protein during the parasite growth phase might block spore
191 production and infection progression.

192 It is important to note that the context of mosquito hosts introduces additional complexity compared to
193 previous studies performed *in vitro* or with *C. elegans* [29, 44]. Dexrazoxane's reduction in spore load,
194 especially in isolated conditions, suggests that other factors, such as social interactions and spore
195 transmission, may influence its efficacy. These factors should be carefully controlled and studied in
196 future research, along with determining the optimal dosage. Despite these complexities, the results of
197 this study open the door for further investigation into microsporidia as potential biological control
198 agents, particularly for *V. culicis.* Moreover, the strategic timing of the suppression of the infection

progression, without completely clearing it, offers several advantages for studying interactions with vector-borne parasites, such as *Plasmodium.* We believe this approach will allow researchers to investigate the mechanisms behind microsporidia-driven inhibition, for instance, whether it is driven by collateral immunity or resource competition. A further understanding of these mechanisms will allow us to better prepare and design vector control strategies, which are essential today.

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302

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306 Author contributions

307 LMS and TGZ designed and conducted the experiments, analysed the data and wrote the manuscript.

308 Data availability

309 All data generated or analysed during this study are included as Supplementary Information files.

310 Additional Information

311 The authors declare no competing interests.

312 Figure legends

313 **Figure 1. Parasite load of infected mosquitoes after consuming a specific diet for 7 days.** The
314 proportion of mosquitoes reared individually or collectively that had detectable spores for **(a)** each
315 Dexrazoxane diet, and their spore load **(b)**. The sample sizes were 70, 60, 60 and 60 for **(a)**, and 60, 50,
316 38 and 40 for **(b)**. Error bars show the 95% confidence intervals of the means. Letters indicate

statistically significant differences from multiple comparisons. See Table 1 for further details on the statistical analyses.

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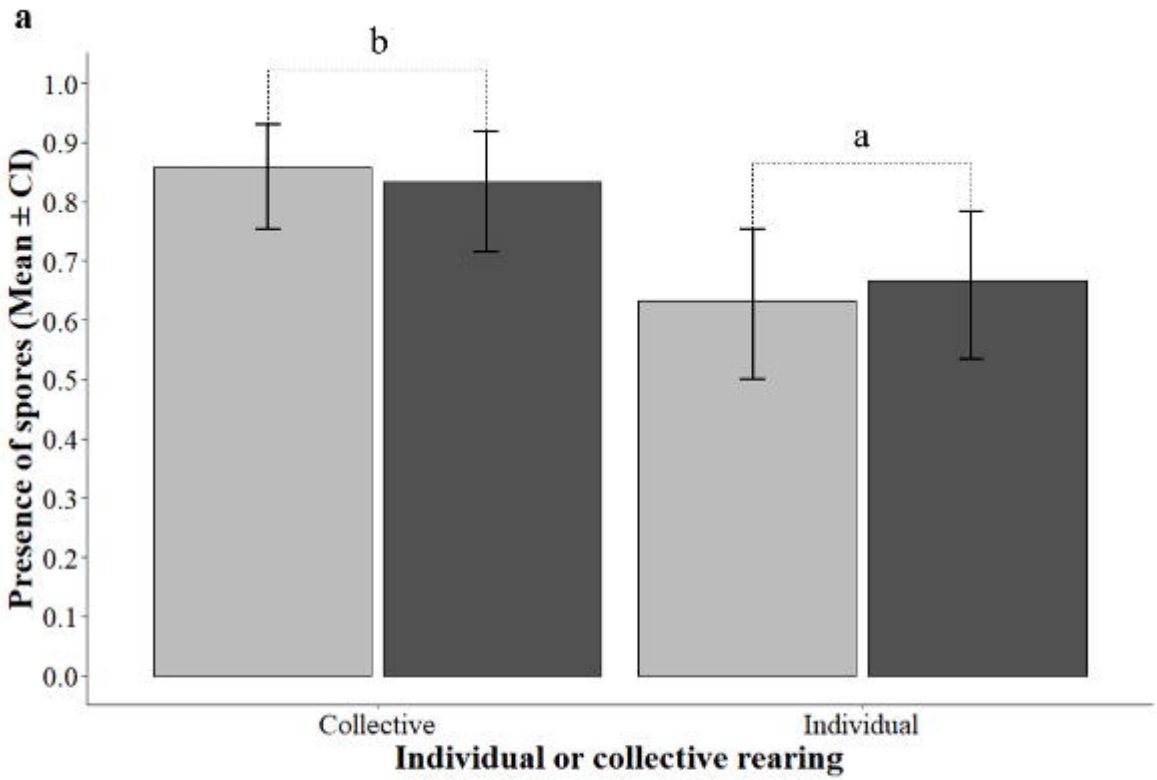
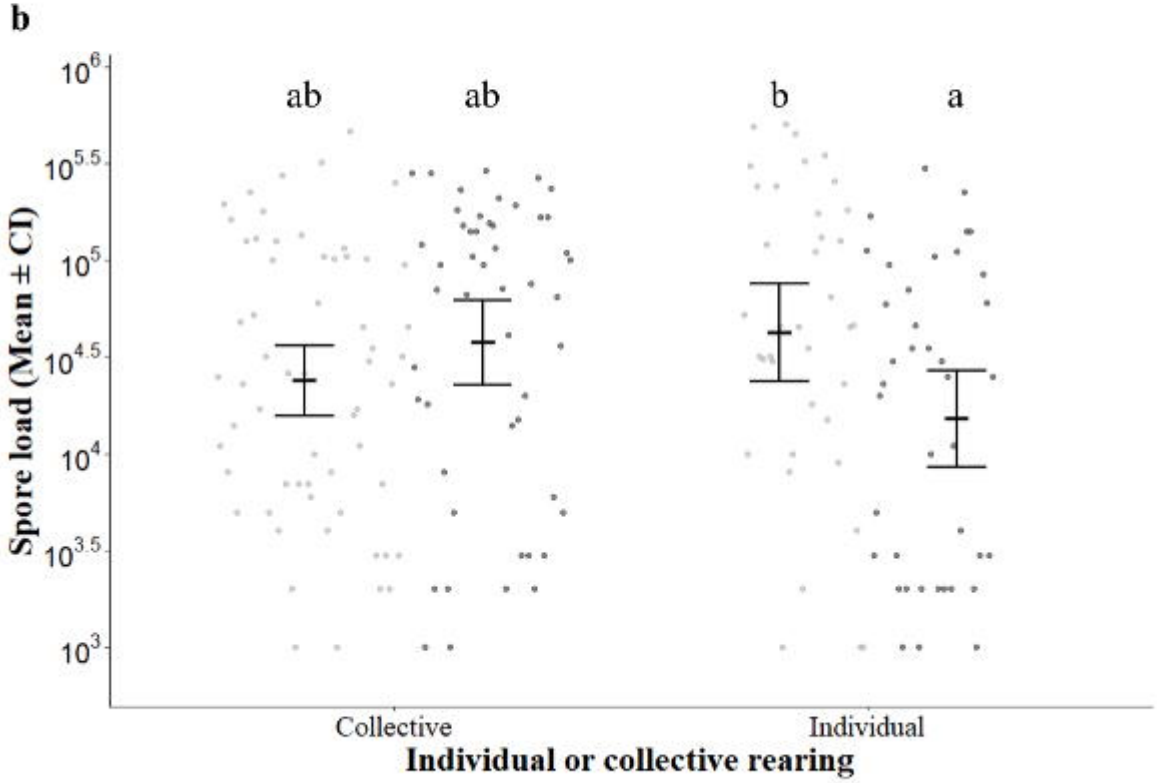

a
b
a
Presence of spores (Mean ± CI)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Collective
Individual
Individual or collective rearing


b
ab
ab
b
a
Spore load (Mean ± CI)
10^{6}
$10^{5.5}$
10^{5}
$10^{4.5}$
10^{4}
$10^{3.5}$
10^{3}
Collective
Individual
Individual or collective rearing
Exposure to Dexrazoxane: Unexposed individuals Exposed individuals