

Susceptibility of 7 freshwater gastropod species in Zimbabwe to infection with *Gastrodiscus aegyptiacus* (Cobbold, 1876) Looss, 1896

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ABSTRACT

Gastrodiscosis outbreaks due to *Gastrodiscus aegyptiacus* were recorded in horses in the vicinity of Harare, Zimbabwe, in the absence of *Bulinus forskalii*, *B. senegalensis* and *Cleopatra* sp. which are considered to be the only intermediate host snails. This suggested the possibility of other snail species acting as intermediate hosts in the life cycle of the trematode. A study was carried out to determine the susceptibility of 7 freshwater snail species to infection with *G. aegyptiacus*. First generation (F-1) of 5 freshwater pulmonate snail species, *Bulinus tropicus*, *Bulinus globosus*, *Biomphalaria pfeifferi*, *Helisoma dyuri* and *Physa acuta* that were bred in the laboratory, and 2 prosobranch snail species, *Melanoides tuberculata* and *Cleopatra* sp. that were collected from the field were used in this study. Data pertaining to mortalities and cercariae shedding were recorded throughout the experimental period. The prosobranch snails, *M. tuberculata* and *Cleopatra* sp. were susceptible to *G. aegyptiacus* with a minimum prepatent period of 45 days and 54 days, respectively. *Bulinus tropicus*, *P. acuta* and *H. dyuri* were susceptible as evidenced by the presence of different generations of rediae and mature cercariae on dissection at 59 days post-infection although attempts to induce the snails to shed from 28 days post-infection did not produce cercariae. *Bulinus globosus* and *Bio. pfeifferi* were refractory to infection. The results revealed the ability of *G. aegyptiacus* to infect *M. tuberculata*, *Cleopatra* sp., *B. tropicus*, *P. acuta* and *H. dyuri* under experimental conditions and this may explain the recorded outbreaks of gastrodiscosis in equine populations in Zimbabwe in the absence of the known intermediate hosts. *Bulinus tropicus* is considered as the most likely major intermediate host of *G. aegyptiacus* because of its wide distribution in Zimbabwe and is well adapted to a wide variety of environments.

Key words: *Gastrodiscus aegyptiacus*, gastropods, survival rates, susceptibility.

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INTRODUCTION

Gastrodiscus aegyptiacus is a common intestinal fluke of equines and pigs⁵. Although gastrodiscosis is considered to be of little economic importance, adverse clinical signs and fatalities have been attributed to the trematode^{1–3}.

Bulinus forskalii^{8,10}, *B. senegalensis*⁸, and snails of the genus *Cleopatra*⁸ have been reported as the intermediate hosts of *G. aegyptiacus*. Outbreaks of gastrodiscosis have been reported in Zimbabwe but none of these snail species were observed within the affected areas. The most recent outbreak occurred at Inkomo Barracks in 2001 and only *Bulinus tropicus* was recovered from a natural water source around the grazing area (S Mukaratirwa, Faculty

of Veterinary Science, unpubl. data, 2002). *Bulinus forskalii* is not widely distributed in Zimbabwe and *B. senegalensis* has not been reported to occur in Zimbabwe⁹. This may indicate the existence of other intermediate host(s), especially among the more prevalent species of gastropods.

The aim of this study was to determine the susceptibility of common freshwater gastropod species to *G. aegyptiacus* infection.

MATERIALS AND METHODS

First-generation (F-1) populations of freshwater pulmonate snails *B. globosus*, *B. tropicus*, *Biomphalaria pfeifferi*, *Physa acuta* and *Helisoma dyuri* were obtained from Blair Research Laboratory, Harare. These freshwater pulmonate snails are characterised by lack of an operculum, have no true gill and the mantle cavity serves as an air breathing organ⁵.

Two freshwater prosobranch snail species

namely *Melanoides tuberculata* and *Cleopatra* sp. were collected from a perennial river on a farm about 20 km northeast of Chinhoyi and from Lake Kariba, respectively. These freshwater prosobranchs are characterised by having a comb-like gill, a ctenidium situated within the mantle cavity and in front of the heart and all have an operculum attached to the foot and closing the aperture⁵. The snails were identified according to Brown and Kristensen⁴. Snails with a shell height of ≥ 5 mm were selected for *M. tuberculata* and ≥ 3 mm for *Cleopatra* sp. The selected snails were screened for patent trematode infection and only the negative ones were used in the experiment.

Fresh faecal samples destined for collection of *G. aegyptiacus* eggs were collected from the rectums of horses at Inkomo Barracks, Harare. The faeces were mixed with water and crushed using a pestle and mortar. The suspension obtained was passed through a sieve with pore size of 250 μ m to remove large debris. The eggs were isolated by passing the suspension through a sieve with a pore size of 63 μ m. The eggs were deposited in glass Petri dishes with distilled water and incubated in the dark at 27 °C for a minimum of 12 days. The water in the Petri dishes was changed every 4 days to minimise fungal contamination.

Embryonated eggs were induced to hatch by taking the Petri dishes out of the dark, changing the water and exposing the contents to artificial illumination using a 100-watt bulb for 1 hour, after which miracidia was released.

F-1 snails with a shell height range of 8–10 mm together with field-collected *M. tuberculata* and *Cleopatra* sp. were individually exposed to 2 miracidia of *G. aegyptiacus* in tissue culture plates as described by Chingwena *et al.*⁶ An uninfected *B. tropicus* F-1 generation group was used as control.

Frequent checks were made to ensure that all snails being exposed remained under water in the tissue culture plates. The snails were left overnight before being transferred to 2-litre plastic aquaria at a density of 5 snails per aquarium. They were fed dried lettuce and commercial

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Table 1: Experimental infection of 7 gastropod species with *Gastrodiscus aegyptiacus*.

Species	Number of snails exposed	Number surviving up to Days 59 and 73 post-exposure	Mortality (%)	Snails harbouring larvae of <i>G. aegyptiacus</i>		Minimum prepatent period in days	Snails refractory (%)	Total infected (%)
				Shedding (%)	Dissection (%)			
<i>C. ferruginea</i> *	59	43	27	14	58	53	42	58
<i>M. tuberculata</i> *	80	64	20	6	46	45	54	46
<i>B. tropicus</i> †	95	18	81.1	0	88.9	—	10.1	88.9
<i>B. globosus</i> ‡	9	2	77.8	0	0	—	100	0
<i>Bio. pfeifferi</i> †	11	3	72.7	0	0	—	100	0
<i>P. acuta</i> ‡	45	30	33.3	0	96.6	—	3.4	96.6
<i>H. duryi</i> †	9	6	33.3	0	100	—	0	100

*Prosobranch snails, †pulmonate snails.

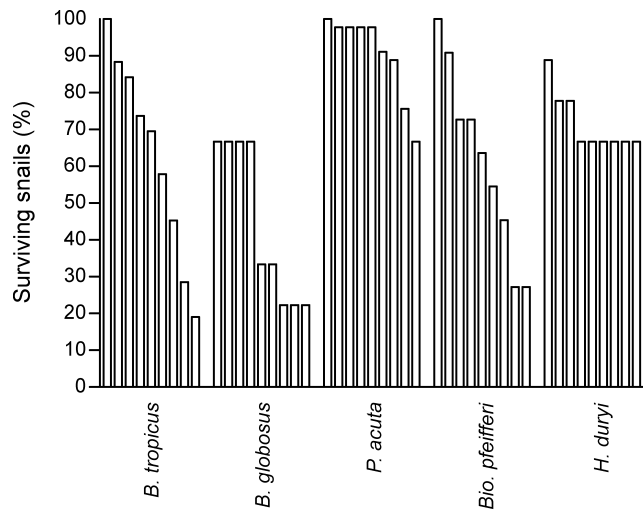


Fig. 1: Weekly survival rates (%) of pulmonate species after infection with *Gastrodiscus aegyptiacus*.

trout pellets and monitored daily for mortalities. From 28 days post exposure to miracidia, snails were examined every 3rd day for shedding of cercariae using standard methods as described by Frandsen and Christensen⁷.

At Day 59 post-exposure, all surviving *B. globosus*, *B. tropicus*, *B. pfeifferi*, *P. acuta* and *H. duryi* snails were dissected and examined under a dissecting microscope to determine the presence of larval stages of *G. aegyptiacus*. *Melanoides tuberculata* and *Cleopatra* sp. were dissected on Day 73 post-exposure.

The snails were designated 'susceptible' if, on shedding, they released pigmented cercariae with an unforked tail, distinct eyespots and a large ventral sucker at the posterior end of the body^{7,10} or on dissection the snails harboured rediae or cercariae with morphological features as described by Malek¹⁰.

RESULTS

The incubation period of *G. aegyptiacus* eggs ranged from 12 to 14 days. The findings in the study showed that *Gastrodiscus aegyptiacus* are susceptible to *B. tropicus*, *P. acuta*, *H. duryi*, *Cleopatra* sp. and *M. tuberculata* but with varying degrees of susceptibility (Table 1).

The prosobranchs *Melanoides tuberculata* (6%) and *Cleopatra* sp. (14%) released cercariae of *G. aegyptiacus* during the experimental period. *Melanoides tuberculata* shed more than 70 cercariae/snail/day while *Cleopatra* sp. shed up to 5 cercariae/snail/day. *Gastrodiscus aegyptiacus* had a shorter prepatent period in *M. tuberculata* snails (45 days) than *Cleopatra* sp. (53 days). The mortality rate of *M. tuberculata* post-infection was 20% compared to 28% of *Cleopatra* sp.

On dissection of both the pulmonate and the prosobranch snails, daughter and mature rediae and cercariae were found

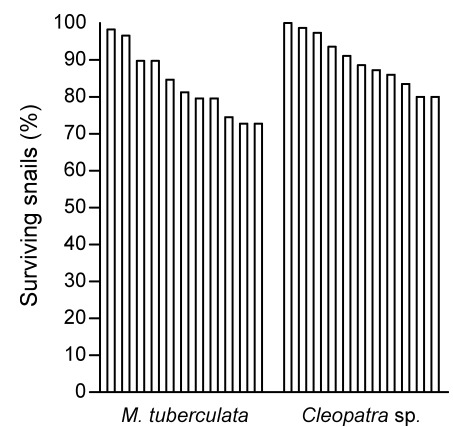


Fig. 2: Weekly survival rates (%) of prosobranch species after infection with *Gastrodiscus aegyptiacus*.

in *H. duryi* with the highest prevalence of infection (100%), followed by *P. acuta* (96.6%), *B. tropicus* (88.9%), *Cleopatra* sp. (58%) and *M. tuberculata* (46%).

Weekly survival rates for both pulmonates and prosobranchs are shown in Figs 1 and 2. Of the pulmonates, *B. tropicus* had the highest mortality rate post-exposure (81.1%) and *P. acuta* and *H. duryi* had the lowest mortalities (both 33.3%). Mortality rates were higher in the laboratory-bred snails than in *M. tuberculata* (20%) and *Cleopatra* sp. (27%), which were collected from the field. *Bulinus globosus* and *Bio. pfeifferi* were refractory to infection and had mortality rates of 77.8% and 72.7%, respectively (Table 1). The mortality rate of the uninfected *B. tropicus* was 5%.

DISCUSSION

The incubation period of *G. aegyptiacus* eggs in this study ranged between 12 and 14 days which is similar to that reported by Malek¹⁰.

This experiment indicated that *M. tuberculata*, *Cleopatra* sp., *B. tropicus*, *P. acuta* and *H. duryi* may act as intermediate hosts of

G. aegyptiacus. *Bulinus forskalii* is not widely distributed in Zimbabwe⁹, and the fact that it was not found in the stream around the grazing areas, further excludes it as the major intermediate host of *G. aegyptiacus* in Zimbabwe.

Bulinus tropicus is reported to be the most widely distributed freshwater snail in Zimbabwe⁹ and is well adapted to a wide variety of environments⁵. The above factors combined with a high infection rate recorded in this study indicate that *B. tropicus* is most likely the major intermediate host for *G. aegyptiacus* in Zimbabwe. *Physa acuta* and *H. duryi* are least likely to be the main intermediate hosts of *G. aegyptiacus* in Zimbabwe as these are exotic species that were introduced to Africa in the last 30 years. *Helisoma duryi* was introduced deliberately as a competitor against intermediate hosts for schistosomes⁵ and is not widespread, probably due to its inability to self-fertilise, although the risk of it taking over as the main intermediate hosts cannot be ruled out. *Melanoides tuberculata* is viviparous and reproduces parthenogenetically and hence colonises new areas rapidly and the extent to which the snail species contrib-

utes to the transmission of *G. aegyptiacus* in Zimbabwe needs to be verified.

Bulinus tropicus, *P. acuta* and *H. duryi* did not shed cercariae despite harbouring mature cercariae on dissection. This could have been due to the fact that the experimental conditions in our study were not able to simulate the field conditions for shedding of cercariae.

Mortalities in pulmonate species were very high compared with uninfected controls. This could have been caused by poor adaptation of the infected snails to the parasite. Trematode infections have been known to cause mortalities in their snail hosts, especially if the infectious doses are high. This is unlikely in this experiment, however, as only 2 miracidia per snail were used.

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