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SUMMARY

Understanding of the larval trematode infections in snail intermediate hosts is essential in designing appropriate control measures. The current study was designed to determine larval trematode infection and seasonal variations in freshwater intermediate host snails Iringa and Arumeru Districts where trematode infections in cattle are endemic. A repeated cross-sectional study was adopted whereby Snails were collected three times a year (dry, early wet and mid wet). The selection of water bodies for sampling was based on random sampling and snails were collected by scooping method. The collected snails were identified based on the published morphological keys. A total of 2,016 snails were collected and 134 (6.6%) were found to be infected with trematode larvae. Six species of snails were identified that were *Radix natalensis*, *Bulinus africanus* group snails, *Bulinus tropicus* group snails, *Bulinus forskalli* group snails, *Biomphalaria pfeifferi* and *Ceratophallous natalensis*. Five morphologically different types of cercariae were identified that included gymnocephalous, amphistomes, mammalian *Schistosoma*, avian *Schistosoma* and Xiphidiocercariae. The findings on overall seasonal snail infection rates indicated low rate during the wet season while peak of infections were in the dry and early wet seasons. It is concluded that domesticated ruminants in the study areas are at high risk of getting infected with the trematodes (*Fasciola* and amphistomes) during dry and early wet seasons. Therefore, deworming of domesticated ruminants with an effective flukicide is recommended at the end of the wet season and followed by a treatment in mid dry season and the last treatment in early wet season.

Keywords: Trematode, Cercariae, Freshwater snails, Tanzania, Sub Saharan Africa

INTRODUCTION

Digenetic trematodes have a complicated life cycle which requires freshwater snails as intermediate hosts where the trematode larval stages sporocysts, rediae and cercaria develop (Soulsby, 1982). Prevalence and intensity of cercariae infections in freshwater snails are the most important risk factors in determining the rate of transmission of digenetic trematodes from the snail-intermediate host to the next definitive host (Margolis *et al.*, 1982; Anderson and May, 1991). Knowledge of the larval trematode infections in snail intermediate hosts is important because high levels of trematode infections may in some cases reflect high risks for the definitive hosts to get infected with trematode parasites. Furthermore, understanding of larval trematode infections in endemic areas may reveal the existence of certain species that could be manipulated to attain biological control of snail-transmitted diseases (Loker *et al.*, 1981). However, in Tanzania, little information is available on the abundance, diversity and the larval trematode infection dynamics in freshwater snails in areas where trematode infections of domesticated ruminants are endemic. The present study was conducted to determine larval trematode infection rates in freshwater intermediate host snails in Iringa and Arumeru Districts where trematode infections in cattle are endemic (Keyyu *et al.,* 2005; Nzalawahe *et al.,* 2014). The information is useful for designing appropriate control measures of trematode infections in cattle.

MATERIALS AND METHODS

Descriptions of the study area

This study was conducted at Iringa and Arumeru Districts of Iringa and Arusha regions respectively. The location of Iringa District Council is 7° 0′ 8.30″ S, 34° 37′ 0″ E and the villages involved were from the highland area (altitude >1500m) where communal grazing is practiced and are characterized by numerous hills and valleys with many permanent rivers, streams, pools and ponds.

The location of Arumeru District is 3° 8′ 0″ S, 36° 52′ 0″ E and the villages involved were those practicing communal grazing with irrigation activities during the dry season while during the wet season some villages practice tethering and zero grazing and some practiced communal grazing.

The selected villages in Iringa District Council included Ilalasimba, Ndiwili, Kiponzero, Lupembelwasenga and Ihomasa while for those in Arumeru District were Makiba, Lekitatu, USA-River, Olkung'wado and Uwiro. These selected villages were reported to have high *Fasciola gigantica* and paramphistome infections in cattle in Arumeru (Nzalawahe *et al.,* 2014) and Iringa (Nzalawahe *et al.,* 2015).

Study design and snails sampling

Sampling of snails at selected water bodies was conducted three times in mid dry, early wet and mid wet seasons. Water bodies that were infested with snail intermediate hosts and were also frequently accessed by cattle were selected using simple random sampling In Iringa District Council the snail collections were conducted in October 2013 (dry), December 2013 (early wet) and April 2014 (mid wet) whilst in Arumeru District collection was conducted in November 2014 (dry), January 2015 (early wet) and May 2015 (mid wet). Collection of snails was achieved by the scooping method as described by Coulibaly and Madsen (1990) undertaken for 20 – 30 minutes at each site visited. Collected snails were identified using morphological keys (Mandahl-Barth, 1962**;** Frandsen *et al.,* 1980; Brown, 1994).

Cercarial shedding was induced by placing each snail in a 10 ml beaker filled with 6 ml of distilled water and exposed to artificial light overnight. The following morning water in each beaker was poured into petri dishes, and examined for the presence of cercariae using a dissecting microscope.

Snails that were not shedding cercariae were further exposed to sunlight for 1 – 4 hours and re-examined for cercariae shedding. The harvested cercariae were identified morphologically using the guide to identification of cercariae from African fresh water snails (Frandsen and Christensen, 1984).

Data analysis

Data were entered into Microsoft Excel window 10 and imported into R version 2.15.0 software for statistical analysis (R Development Core Team, 2012). The overall snail infection rates were determined by computing descriptive statistics. Seasonal variations in cercariae infections in snails were determined by Chi square test and were considered significant at $p<0.05$ at 95% confidence interval.

RESULTS

A total of 2016 snail intermediate hosts of digenetic trematodes were collected at selected water bodies in Iringa District Council and Arumeru District. The snails included *R. natalensis, B. africanus, B. tropicus, B. forskalli, B. pfeifferi* and *C. natalensis.* Of the collected snails, 134 (6.6%) were infected with five different cercariae of digenetic trematodes (Table 1 and Figure. 1). However, there was no snail observed to be infected with more than one cercarial type (mixed infection).

The highest snails infection rate was observed in *B. forskalli* (10.7%) followed by *B. pfeifferi* (9.1%), *B. africanus* (8.3%), *R. natalensis* (6.3%), *C. natalensis* (6.1%) and *B. tropicus* (0.6%) (Table1).
Gymnocephalous cercariae (*Fasciola* type) constituted 1.7% of all positive cases followed by amphistomes cercariae (1.6%) and xiphidiocercariae (1.4%) (Table1).

Amphistomes cercariae were detected in *B. pfeifferi* (Iringa District Council) and *C. natalensis* (Arumeru). Gymnocephalous cercariae were detected in *R. natalensis* (Iringa Rural and Arumeru), *B. africanus* (Iringa District Council) and *B. pfeifferi* (Arumeru) (Table 1-3).

Mammalian *Schistosoma* cercariae were detected in *B. africanus, B. forskalii and B. tropicus groups snails* (Iringa) and *S. mansoni* cercariae from Arumeru District in *B. pfeifferi* (Arumeru).

*A*vian *Schistosoma* cercariae were detected in *R. natalensis*, *B. forskalli* and *B. pfeifferi* whereas Xiphidiocercariae (cercariae of trematodes infecting amphibians) were found in *B. pfeifferi*, *B. forskalli*, *R. natalensis* and *C. natalensis* (Table 1).

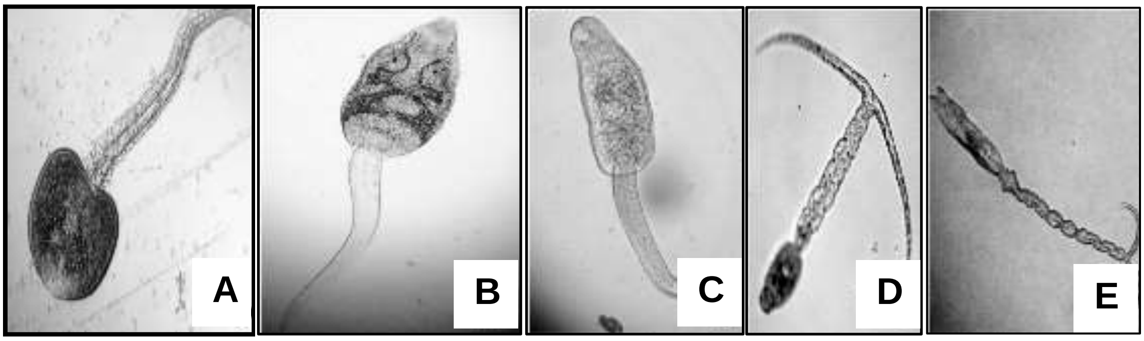

Figure 1: Type of the cercariae that were found to infect snail intermediate hosts: (A) Gymnocephalous cercariae (B) Amphistome cercariae (C) Xiphidiocercariae (D) Avian Schistosoma cercariae (E) Mammalian Schistosoma cercariae.

In Iringa District Council, gymnocephalous cercariae were detected during the dry (4.2%) and early wet (5.9%) seasons whilst amphistome cercariae were recovered in early wet (11.9%) and mid wet (0.2%) seasons respectively. In addition, mammalian and avian *Schistosoma* cercariae were observed during the dry (3.2% and 1.9%) and mid wet seasons (0.4% and 3.1%) respectively (Table 2) but Xiphidiocercariae were observed during early wet (1.7%) and mid wet (4%) seasons (Table 2). In Arumeru District, gymnocephalous and amphistomes cercariae were observed in dry (1.3% and 0.5%) and early wet (1.3% and 0.9%) seasons respectively (Table 3). Mammalian (0.4%) and avian *Schistosoma* cercariae (0.4%) were observed in early wet season whilst xiphidiocercaria (0.9%) was observed during the mid wet season (Table 3). In Iringa District Council the proportions of snails (*R. natalensis* and *B. africanus*) shedding gymnocephalous cercariae were significantly higher in dry (7.83%) and early wet (14.28%) seasons compared to mid wet season (0.00%) ($P < 0.000$), whilst proportion of *B. pfeifferi* shedding amphistomes cercariae was significantly higher in early wet (26.4%) season compared to mid wet (0.80%) and dry (0.00%) seasons ($p < 0.000$).

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Table 1: Overall cercariae infections rate in snails collected in Iringa and Arumeru Districts, Tanzania

Snails species	Number of infected snails with each trematode cercariae						Infection rate (%)
	n	GC	AC	MSC	ASC	Xiph	
R. natalensis	573	21	-	-	9	6	6.3
B. africanus	181	7	-	8	-	-	8.3
B. tropicus	363	-	-	2	-	-	0.6
B. forskalli	149	-	-	2	8	6	10.7
B. pfeifferi	652	7	29	1	7	15	9.0
C. natalensis	98	-	4	-	-	2	6.1
Total snails	2016	35	33	13	24	29	
Infection rate of each type of cercariae		1.7%	1.6%	0.6%	1.2%	1.4%	
Overall infection rate							6.6

GC = Gymnocephalous, AC= Amphistome cercariae, MSC= Mammalian *Schistosoma*, ASC= Avian *Schistosoma*, XC= Xiphidiocercaria

Table 2: Overall season-wise snail infection rates in Iringa District Council, southern highlands, Tanzania

Season	Type of trematode cercariae							Infection rate
	Snails species	n	GC(%)	AC (%)	MSC (%)	ASC (%)	XC (%)	
Dry	*R. natalensis*	83	11 (13.3)	-	-	4 (4.8)	-	18.1
	Bu. africanus	83	2 (2.4)	-	8 (9.6)	-	-	12.1
	B. tropicus	65	-	-	2 (3.1)	-	-	3.1
	B.forskalli	-	-	-	-	-	-	-
	B. pfeifferi	78	-	-	-	2 (2.6)	-	2.6
	Total	309	13	-	10	6	-	
	Infection (%)		4.2	-	3.2	1.9	-	9.4
Early wet	*R. natalensis*	78	9 (11.5)	-	-	-	-	11.5
	B. africanus	20	5 (25.0)	-	-	-	-	25.0
	B. tropicus	30	-	-	-	-	-	-
	B.forskalli	0	-	-	-	-	-	-
	B. pfeifferi	106	-	28 (26.4)	-	-	4 (3.8)	30.2
	Total	234	14	28	-	-	4	
	Infection (%)		5.9	11.9	-	-	1.7	19.7
Mid wet	*R. natalensis*	150	-	-	-	5 (3.3)	2 (1.3)	4.8
	B. africanus	78	-	-	-	-	-	-
	B. tropicus	43	-	-	-	-	-	-
	B.forskalli	149	-	-	2 (1.3)	8 (5.4)	10 (6.7)	13.4
	B. pfeifferi	124	-	1 (0.8)		4 (3.2)	10 (8.1)	12.1
	Total	544	-	1	2	17	22	
	Infection (%)			0.2	0.4	3.1	4.0	7.7

GC = Gymnocephalous, AC= Amphistome cercariae, MSC= Mammalian *Schistosoma*, ASC= Avian *Schistosoma*, XC= Xiphidiocercariae

Moreover, proportion of *Bulinus* species shedding mammalian *Schistosoma* cercariae was significantly higher in dry season (6.76%) compared to early wet (0.74%) season (p<0.00) whilst avian *Schistosoma* cercariae were significantly higher during the mid wet season compared to early wet season (p<0.014). The proportions of snails shedding xiphidiocercariae were significantly higher during the mid wet season compared to the dry season (p<0.000). The differences in snails infection rates between seasons were not statistically significant in Arumeru District. The overall seasonal snails infection rates in Iringa District Council were significantly higher than in Arumeru District (p=0.000).

Table 3: Overall season-wise snail infection rates in Arumeru District, northern Tanzania

Season			**Type of trematode cercariae**					**Infection rate (%)**
	Snails species	n	GC (%)	AC (%)	MSC (%)	ASC (%)	XC (%)	
Dry	*R. natalensis*	151	1 (0.7)	-	-	-	-	0.7
	B. tropicus	19	-	-	-	-	-	-
	B. pfeifferi	169	4 (2.4)	-	-	-	-	2.4
	C. natalensis	60	-	2 (3.3)	-	-	-	3.3
	Total	399	5	2	-	-	-	
	Infection (%)		1.3	0.5	-	-	-	1.8
Early wet	*R. natalensis*	61	-	-	-	-	-	-
	B. tropicus	26	-	-	-	-	-	-
	B. pfeifferi	121	3 (2.5)	-	1 (0.8)	1 (0.8)	-	4.1
	C. natalensis	27	-	2 (7.4)	-	-	2 (7.4)	14.8
	Total	235	3	2	1	1	2	
	Infection (%)		1.3	0.9	0.4	0.4	0.9	3.8
Mid wet	*R. natalensis*	50	-	-	-	-	4	8.0
	B. tropicus	80	-	-	-	-	-	-
	B. pfeifferi	54	-	-	-	-	1	1.9
	C. natalensis	11	-	-	-	-	0	-
	Total	295	-	-	-	-	5 (1.7)	
	Infection (%)		-	-	-	-		1.7

GC = Gymnocephalous, AC= Amphistome cercariae, MSC= Mammalian *Schistosoma*, ASC= Avian *Schistosoma*, XC= Xiphidiocercariae

DISCUSSION

The current work recorded five morphologically different types of cercariae infecting freshwater snail intermediate hosts in the study area. The cercarial types recovered were among those reported in the previous studies in Mwanza region, Tanzania (Loker *et al.*, 1981) and elsewhere in Africa (Chingwena *et al.*, 2002a).

The overall snail infection rate (6.6%) was low which correspond with the findings from other studies (Chingwena *et al.*, 2002a; Nkwengulila and Kigadye, 2005). However, other authors reported higher snail infection rates (Loker *et al.*, 1981; Phiri *et al.*, 2007). The low snail infection rates could be attributed to low parasite pressure, with contact between miracidia and snails a rare event (Chingwena *et al.*, 2002a).

The high rates of snail mortality caused by parasites (Wright, 1966; Sousa, 1992) and acquired resistance to infection by the host resulting from host–parasite co-evolution (Begon *et al.*, 1990) may also be responsible for this result. Mixed infections were not observed in individual snails and this concurs with findings reported in the previous studies (Nkwengulila and Kigadye, 2005; Phiri *et al.*, 2007).

Mixed infections are rare (Kendall, 1964), this could be explained by double infections being more pathogenic compared to single species infections and consequently snails with mixed infections have higher mortality compared to those with single infections (Sousa, 1992), and may therefore be under-represented in snail collections (Chingwena *et al.,* 2002a).
However, other authors (Sousa 1993; Lafferty *et al.,* 1994) have reported the inter-species antagonism as an explanation for the rare mixed infections. In the present results high infection rate of cercariae was observed in *B. forskalli* and this is in agreement with the previous studies (Nkwengulila and Kigadye, 2005) but in contrast with Loker *et al.,* (1981) who reported highest infection rate in *R. natalensis.*

Gymnocephalous (*Fasciola* type) cercariae were recorded in *R. natalensis*, *B. africanus* and *B. pfeifferi*. *Radix natalensis* is reported to be the only intermediate host for *F. gigantica* and hence the observed gymnocephalous infections in *R. natalensis* were of *F. gigantica*. The gymnocephalous like cercariae observed in *B. africanus* and *B. pfeifferi* raise a concern on whether these snails may be involved in the transmission of *F. gigantica*.

However, this invites further investigations based on the experimental infections and the use of molecular tools to characterize the Gymnocephalous cercariae. Comparable findings have been reported in Mwanza region, Tanzania (Loker *et al.,* 1981). Natural infections of *R. natalensis* with gymnocephalous (*Fasciola* type) cercariae have been reported in Uganda (Ogambo-ongoma, 1971), Zimbabwe (Chingwena *et al.,* 2002b), Zambia (Phiri *et al.,* 2007) and in Nigeria (Schillhorn, 1980; Duwa and Oyeyi, 2009), whilst gymnocephalous cercariae infections in *B. africanus* have been reported in Nigeria (Duwa and Oyeyi, 2009).

Amphistome cercariae were detected in *B. pfeifferi* (Iringa and Arumeru) and *Ceratophalous natalensis* (Arumeru) and hence these snail species seem to play a major role in transmission of amphistomes in the two Districts. Comparable results in the country have been reported by Loker *et al.* (1981). In contrast to the natural infections of amphistomes in *Bulinus natalensis,* a close relative of *B. tropicus* (Makundi *et al.,* 2006), and *B. forskalli* (Loker *et al.,* 1981) have previously been reported in Tanzania, however, no infections in these snail species were found in this study. Although natural infections of amphistomes in *B. tropicus* were not recorded in the present study, the observed mammalian *Schistosoma* infection in *B. tropicus* in Iringa District Council indicates that it plays a role in the transmission of Amphistomes, since *B. tropicus* becomes susceptible to *S. bovis* when it has previously been exposed to amphistomes (Southgate *et al.,* 1985, 1989).

The study in Arumeru (Nzalawahe *et al.,* 2014) recorded *S. bovis* infections in cattle and *B. tropicus* was the only *Bulinus* species collected in the study area so this may suggest that *B. tropicus* may be susceptible to amphistomes in the District. Natural infections of *B. tropicus*, *B. pfeifferi*, *B. forskalli* and *C. natalensis* with amphistomes have been reported elsewhere in Africa (Dinnik and Dinnik, 1954; Dinnik, 1961; Dinnik, 1965; Graber and Daynes, 1974; Southgate *et al.,* 1989; Mukaratirwa *et al.,* 1998; Chingwena *et al.,* 2002b).

Previous studies in Tanzania have reported *B. africanus* (Kassuku *et al.,* 1986), *B. forskalli* (Mutani et al., 1983; Mwambungu, 1988), *B. globosus* (Mwambungu, 1988) and *B. natalensis* (Makundi *et al.,* 2006) serving as the intermediate host snails for *S. bovis*. This study also reported the mammalian *Schistosoma* infections in *B. africanus*, *B. tropicus*, *B. forskalli* and *B. pfeifferi*.

Since the recovered mammalian *Schistosoma* cercariae were not characterized to the species level, the observed mammalian *Schistosoma* infections in *Bulinus africanus* snails group, could be that of *S. haematobium* or *S. bovis* as they serves as an intermediate host for both species, (Brown, 1994; Chingwena *et al.,* 2002b; Stothard *et al.,* 2013). The *Schistosoma* infections in *B. pfeifferi* was observed in Arumeru District only which signify the presence of *Schistosoma mansoni* infections in human.

The seasonal variations in the infection of *R. natalensis* with gymnocephalous (*Fasciola* type) cercariae and infection of *B. pfefferi* with amphistomes cercariae observed in Iringa District Council is consistent with the previous epidemiological study in cattle in the same District that recorded cattle picking more infection of *Fasciola* and paramphistomes from the early dry season than picking up at the end of the dry season and early wet season. The higher prevalence of gymnocephalous and amphistome ceracarie infection during the dry and early wet seasons could be attributed to reduced water volumes in the habitats during the dry season, accompanied by increased contact with livestock due to the scarcity of pasture, and increased grazing around water bodies, thereby favouring the accumulation of *Fasciola* and amphistome eggs in close proximity to potential snail habitats which leads to increased frequency of contact between miracidia and snail intermediate hosts hence escalating the prevalence of infection in snails (Chingwena *et al.,* 2002a).

The high infection rates of the xiphidiocercariae and avian *Schistosoma* cercariae during the mid wet season compared to the observed low infections of other trematodes in Iringa District Council, suggests they might exert some influence on the prevalence of other trematode infections. Speculations that xiphidiocercariae may be playing a modifying role in lowering the prevalence of the trematodes of veterinary or medical importance has been reported in previous studies (Wright, 1966; Loker *et al.,* 1981) and they recommended further investigations to elucidate the biocontrol potential of these trematode species.

The observed high overall infection rates of snails between seasons in Iringa District Council compared to Arumeru District could be due to less direct control (chemotherapy) of trematode infections in cattle in Iringa than Arumeru District hence more environmental contamination with trematode eggs in Iringa Rural than Arumeru District, on top of the possible difference in geo-climatic conditions (Karimi *et al.,* 2004). The current work reveals the diversity of larval trematode parasites in snails in Iringa District Council and Arumeru District of Tanzania, and thus necessitates further investigations to expound the bio-control potential of these trematode species.

This study used the technique of induced shedding of cercariae to determine the snails infection which is less sensitive and specific than other detection methods such as PCR (Hamburger *et al.,* 2004), since it cannot detect prepatent infections. It is concluded that overall seasonal snail infection rates were low rate during the wet season while peak of infections were in the dry and early wet seasons and thus domesticated ruminants in the study areas could be at high risk of getting infected with the trematodes (*Fasciola* and amphistomes) during dry and early wet seasons.

Therefore, deworming of domesticated ruminants with an effective flukicide is recommended at the end of the wet season and followed by a treatment in mid dry season and the last treatment in early wet season. Also, future studies should be carried out using advanced molecular tools for speciation of larval trematode infections and snails which are highly sensitive and specific and should further our understanding of the epidemiology of trematode infections (Mostafa *et al.,* 2003; Hamburger *et al.,* 2004; Velusamy *et al.,* 2004). However, these have not been fully employed for the detection of infections in snails under field conditions (Chingwena *et al.,* 2002a). Further studies on snail populations and infection dynamics based on the monthly sampling are recommended for designing cost effective snail control programs.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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