Associations between trematode infections in cattle and freshwater snails in highland and lowland areas of Iringa Rural District, Tanzania

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SUMMARY

The epidemiology of trematode infections in cattle was investigated within highland and lowland areas of Iringa Rural District, in southern Tanzania. Fecal samples were collected from 450 cattle in 15 villages at altitudes ranging from 696 to 1800 m above the sea level. Freshwater snails were collected from selected water bodies and screened for emergence of cercariae. The infection rates in cattle were *Fasciola gigantica* 28·2%, paramphistomes 62·8% and *Schistosoma bovis* 4·8%. Notably, prevalence of trematode infections in cattle was much higher in highland (altitude > 1500 m) as compared with lowland (altitude < 1500 m) areas and was statistically significant (*P*-value = 0·000) for *F. gigantica* and paramphistomes but not for *S. bovis*. The snails collected included *Lymnaea natalensis, Bulinus africanus, Bulinus tropicus, Bulinus forskali, Biomphalaria pfeifferi, Melanoides tuberculata* and *Bellamya constricta* with a greater proportion of highland (75%) than lowland (36%) water bodies harbouring snails. Altitude is a major factor shaping the epidemiology of *F. gigantica* and paramphistomes infections in cattle in Iringa Rural District with greater emphasis upon control needed in highland areas.

Key words: *Fasciola gigantica*, paramphistome, *Schistosoma bovis*, altitude, Tanzania, cattle, sub-Saharan Africa.

INTRODUCTION

Trematode infections in domestic ruminants caused by *Fasciola gigantica, Fasciola hepatica*, paramphistomes, *Schistosoma bovis* and *Dicrocoelium hospes* are known to be present in Tanzania (Mahlau, 1970; Hyera, 1984; Kassuku *et al*. 1986; Keyyu *et al*. 2005; Walker *et al*. 2008) and are considered to be major constraints to cattle production (Mwabonimana *et al*. 2009; Swai and Ulicky, 2009; Mellau *et al*. 2010; Komba *et al*. 2012; Nzalawahe and Komba, 2013; Nzalawahe *et al*. 2014). In Kenya, deliberate infection of Boran cattle with *F. gigantica* metacercariae caused production losses from the combination of liver condemnations and reduction in liveweight gain to the value of US $23.41 (10·34%) per head (Wamae *et al*. 1998). In northern Tanzania, estimated annual losses have been reported of US$18 000 due to liver condemnation in Arusha abattoir (Mwabonimana *et al*. 2009), and US$1780 due to liver condemnation and US $5943 due to weight loss caused by chronic fasciolosis at Hai town abattoir (Swai and Ulicky, 2009). In Iringa region, Ministry of Livestock Development and Fisheries staff describe acute intestinal paramphistomosis as a cause of death of weaner calves (personal communication).

The occurrence of trematode infections typically depends on the presence and distribution of freshwater snail intermediate hosts (Schillhorn van Veen, 1980; Brown, 1994) which in turn varies with the climatic conditions (Mungube *et al*. 2006). The highest rate of trematode infections in cattle in Africa has been reported to occur in areas of extended annual rainfall associated with wetlands and marshy areas (Yilma and Malone, 1998; Pfukenyi *et al*. 2006) and irrigation activities (Traore, 1989; Nzalawahe *et al*. 2014) with risk decreasing in areas with shorter wet season (Yilma and Malone, 1998; Pfukenyi *et al*. 2006). The other areas that have been reported to favour the occurrence of trematode infections includes wet and humid areas (Majok *et al*. 1993) and areas with high stocking rate of animals (Cheruiyot, 1983). Previous epidemiological studies of trematode infections in cattle (Kassuku *et al*. 1986; Makundi *et al*. 1998; Keyyu *et al*. 2005) in Iringa Rural District were primarily conducted in the highlands (altitude ~1500 m) which are characterized with high annual rainfall, and wet grazing lands (Makundi *et al*. 1998).

Since the study of 2005 rainfall has declined and in the lowland area there have been extensive droughts. Thus further studies were required to compare the highland and lowland areas and to have a scientific

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basis for recommending what control regimes should be instated for bovine trematode infections in Iringa District.

MATERIALS AND METHODS

Description of the study area

This study was conducted in Iringa Rural District, Iringa Region, in the southern highlands of Tanzania. The eastern part of the District is a highland area comprising numerous hills and valleys with many permanent rivers, streams and ponds, while the western side is semi-arid flat lowland, characterized by dry grazing land with thickets and scattered bushes (Makundi *et al*. 1998). The level of annual rainfall ranges between 500 and 2700 mm, with the lowland areas receiving less than 600 mm (Iringa District Council, 2013) and climatic conditions for the year of the study (2013) were typical of long term trends for the District. Village cattle keepers practice communal grazing under a traditional management system which is not restrictive and grazing land and water are freely available to all cattle. Highland and lowland villages are generally far enough apart that there is little movement of cattle between the two. The highlands are rich in permanent water bodies including rivers, streams, irrigation canals, pools and swampy areas, and cattle generally graze in their home villages where pasture and water are ample throughout the year. In the lowlands there are only a few permanent water bodies (the Ruaha river, the Mtera dam and artificially constructed pools found in a few villages). Here, cattle keepers are mainly pastoralists (e.g. Maasai, Manga'ti) and agro-pastoralists (Sukuma) with large herds which they tend to move to other parts of Tanzania (e.g. Mbeya, Dodoma and Coastal region) during the dry season in search of more abundant pastures and water supplies. Tanzanian shorthorn zebu is the predominant cattle breed, with the 'Iringa Red' type being commonest in highland villages while the Maasai zebu type is common in lowland villages. Products containing the fasciolicides albendazole, nitroxynil and oxyclozanide are available in this area, although doubts are often expressed about their quality and financial constraints mean that farmers rarely treat animals unless they show severe clinical signs of disease.

Study design, sample size and selection of the study villages

A cross-sectional study was carried out from February to March 2013 to determine the prevalence of trematode infections in cattle, while visits for identification of the potential snail intermediate hosts were conducted at two inspection time-points in March and September 2013. The cross-sectional study was conducted using a cluster-sample design, with villages as clusters. Study villages were selected using probability proportional to size, based on their cattle populations reported by the local District Veterinary Office. Sample size was estimated using Bennett's formula for cluster sample surveys (Bennett *et al*. 1991). A total of 15 study villages were selected and for purposes of subsequent analyses further divided into two groups according to altitude, namely highland villages located at altitudes 1500 m above the sea level or greater and lowland villages located at altitudes below 1500 m.

Coprological examination

A total of 450 cattle were selected and faecal samples were collected per rectum, and processed using the Flukefinder® (Richard Dixon, ID, USA), a double sieving concentration method, in accordance with the manufacturer's instructions. Briefly, fecal samples were fixed with 70% ethanol to prevent hatching of *S. bovis eggs* on exposure to freshwater during sample processing. Faeces (2 g) were mixed with 30 mL of water, poured into the Flukefinder® unit and flushed well with water. Larger fecal debris were retained by the larger diameter sieve (125 nm) and discarded, while fecal material including trematode eggs retained in the smaller diameter sieve (30 nm) was back washed into a 100 mL plastic cup. This was allowed to settle for 5 min, then the supernatant was poured off and the sediment poured into a petri dish. Water was added to fill the petri dish, and then the contents were allowed to settle again for 30 sec before pouring off the supernatant. Three drops of methylene blue were added to the remaining sediment which was examined for the presence of trematode eggs using a dissecting microscope. Eggs were identified morphologically using standard keys (Soulsby, 1982; Valero *et al*. 2009).

Sampling of snails and cercarial harvesting

At each village area liaison took place with a local authority representative and local guide who knew the area was recruited to identify water bodies frequented by cattle. Snails were collected in March (mid-wet season) and September (mid-dry season) to identify water bodies that could serve as potential transmission sites for trematodes throughout the year; some are mainly frequented by cattle herds during the wet season and then up until the mid-dry season when they tend to dry up, whereas others serve mainly as water sources in the late dry season. The scooping method as described by Coulibaly and Madsen (1990) was undertaken for 20–30 min at each site visited. Collected snails were identified using morphological keys (Mandahl-Barth, 1962; Frandsen *et al*. 1980; Brown, 1994). Collection of snails was conducted between 11:00 and 14:00,

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Table 1. Egg-patent trematode infections in cattle in study villages in Iringa Rural District, Tanzania

Village	Cattle population	Altitude (m)	*F. gigantica*		Paramphistome		*S. bovis*	
			n +ve[a]	Prev.[b] (%)	n +ve	Prev. (%)	n +ve	Prev. (%)
Lupembel-wasenga	567	1800	30	100	30	100	4	13·3
Ihomasa	712	1787	23	76·7	26	86·7	0	0·0
Kiponzero	759	1750	28	93·3	28	93·3	1	3·3
Ndiwili	234	1736	16	53·3	27	90	3	10
Ilalasimba	913	1525	14	46·7	16	53·3	0	0·0
Igingilanyi	555	1376	0	0·0	22	73·3	5	16·7
Chamdindi	1106	1280	0	0·0	12	40	0	0·0
Iguluba	1334	1101	0	0·0	10	33·3	0	0·0
Kitisi	996	922	5	16·7	3	10	0	0·0
Malizanga	719	891	2	6·7	23	76·7	1	3·3
Luganga	2241	791	5	16·7	19	63·3	0	0·0
Mkombilenga	729	761	2	6·7	8	26·7	0	0·0
Mbuyuni	3100	750	0	0·0	14	46·7	0	0·0
Izazi	1096	704	1	3·3	20	66·7	4	13·3
Migoli	1962	703	1	3·3	25	83·3	0	0·0
Overall	17023		127	28·2	283	62·9	18	4·0

30 cattle sampled at random per village, $n = 450$ overall.

[a] n +ve: number of cattle positive by coprological examination.

[b] Prev.: prevalence.

following which counting, identification and placing in beakers lasted until 16:00. Cercarial shedding was achieved by placing each snail in a 10 mL beaker filled with 6 mL of distilled water and exposing to light overnight. The following morning water in each beaker was poured into the Petri dish, and examined at 40× magnification for the presence of cercariae. If no cercarial shedding was observed, snails were further exposed to the light till afternoon, then re-examined. The harvested cercariae were identified morphologically using published keys (Frandsen and Christensen, 1984).

Statistical analysis

Data were collated in Microsoft Excel 2007 and imported into R version 2.15.0 software for statistical analysis (R Development Core Team, 2012). Occurrence of potential trematode vectors was analysed by logistic regression, using altitude as the explanatory variable. Trematode infections of cattle were analysed similarly, using observation of intermediate host snails and age and sex of cattle as additional explanatory variables. Model comparisons were conducted by analysis of deviance using Akaike's information criterion to ascertain the minimum adequate model in each case. Differences in prevalence of trematode infections among cattle of varying age, sex and location were determined by χ^2 tests.

RESULTS

Cattle population

The age distribution of the 450 cattle in the study was 31 (6·9%), 97 (21·6%) and 322 (71·6%) calves, weaners and adults, respectively. Among the 150 cattle sampled in highland villages, the age distribution was 6 (4·0%), 27 (18·0%) and 117 (78%) and among the 300 cattle sampled in lowland villages 25 (8·3%), 70 (23·3%) and 205 (68·3%), respectively, for the same age groups. Although there were relatively fewer adult cattle in the highland village sample and more calves and weaners in the lowland village sample, this difference was not significant (χ^2, 2 D.F. = 5·35, $P = 0{\cdot}069$). Ten of the cattle sampled were identified as being of the 'Iringa Red' type, all located in two highland villages (Ilalasimba $n = 9$, Ndiwili $n = 1$), the remainder all being of the Maasai zebu type.

Trematode infections in cattle

Eggs of *F. gigantica*, paramphistomes and *S. bovis* were identified by coprological examination of cattle. These infections were detected in 127 (28·2%), 283 (62·8%) and 18 (4·0%) of the 450 cattle examined, and in 11 (73·3%), 15 (100%) and 6 (40%) of the 15 villages, respectively (Table 1). Adult cattle had the highest prevalence of trematode infections (Table 2). The differences in prevalence between the age groups was statistically significant for *F. gigantica* ($P < 0{\cdot}05$) and paramphistomes ($P < 0{\cdot}001$) but not for *S. bovis*. The highest prevalences of *F. gigantica*, paramphistomes and *S. bovis* were observed above 1500 m (Table 2).

The differences in prevalence between the highlands and lowlands were highly significant for *F. gigantica* ($P < 0{\cdot}001$) and paramphistomes ($P < 0{\cdot}05$) but not for *S. bovis* ($P > 0{\cdot}1$). The prevalence of trematode infections among study villages

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Table 2. Egg-patent trematode infections in different age-groups of cattle in Iringa Rural District, Tanzania

Age-group	Parasite	*N* infected	Prevalence (%)
Adult (⩾2 years old) *N* = 322	*F. gigantica*	102	31·7
	Paramphistome	225	69·9
	S. bovis	17	5·3
Weaner (<2 years old) *N* = 128	*F. gigantica*	25	19·5
	Paramphistome	58	45·3
	S. bovis	1	0·8

showed extensive spatial variation (Table 3 and Fig. 1) and ranged from 0 to 100% for *F. gigantica* and paramphistomes and 0–16·7% for *S. bovis*. The highest individual village prevalences of *F. gigantica* and paramphistomes, both 100%, were observed at Lupembelwasenga, the highland village at greatest altitude (1800 m), whereas for *S. bovis* it was at Igingilanyi (1376 m) in the lowlands (Table 1). In the two highland villages (Ilalasimba and Ndiwili) where both breed types were observed, 5 of 10 (50%) Iringa Red cattle and 25 of 50 (50%) Maasai zebu cattle had egg-patent infections with *F. gigantica*, whereas 4 of 10 (40%) Iringa Red and 39 (78·0%) of 50 Maasai Zebu had egg-patent paramphistome infections, the difference being significant ($P = 0·024$, Fisher's exact test). None of the 10 Iringa Red cattle were found to be excreting *S. bovis* eggs; indeed *S. bovis* was not observed in any cattle in Ilalasimba, where 9 of these 10 cattle were located, and nor were *S. bovis* eggs observed in feces the single Iringa Red animal examined in Ndiwili, although they were present in 3 of 29 Maasai zebu cattle examined at that site.

Concurrent infections of all 3 trematode parasites were observed in 7 (1·6%) of the cattle examined, while 112 (24·9)% had mixed infection with *F. gigantica* and paramphistomes, significantly more often than expected by chance ($\chi^2 = 48·5$, $P < 0·001$) if the 2 were independent; these mixed infections were seen in greater than expected numbers in the highlands ($n = 102$ [68%], $\chi^2 = 17·2$, $P < 0·001$), but not the lowlands ($n = 10$ [3·3%], $\chi^2 = 0·75$, $P = 0·39$). Fecal egg counts for *F. gigantica* ranged from 0 to 298, for paramphistomes from 0 to 625 and for *S. bovis* from 0 to 2 eggs g^{-1}, respectively, with the highest numbers from cattle in the highlands.

In cattle in which both *F. gigantica* and paramphistome eggs were detected in feces ($n = 112$), there was a significant positive correlation between the log-transformed egg counts ($r^2 = 0·244$, $P < 0·001$). No correlation was observed between egg counts in cattle with mixed infections with *F. gigantica* and *S. bovis* ($r^2 = 0·0733$, $P = 0·557$), or with paramphistomes and *S. bovis* ($r^2 = 0·00585$, $P = 0·795$).

Distribution of snails

During March 2013 (wet season), a total of 431 trematode intermediate host snails were collected at selected water bodies in highland areas, including 140 *Bulinus forskalli*, 111 *Biomphalaria pfeifferi*, 95 *Lymnaea natalensis*, 55 *Bu. africanus* and 30 *Bulinus tropicus*. Avian schistosome cercariae were shed by some of these snails (20 *Bi. pfeifferi*, 11 *L. natalensis*, 10 *Bu. forskalli*, 8 *Bu. africanus* and 2 *Bu. tropicus*), but none shed cercariae of mammalian trematodes. During the same period, 80 intermediate host snails were collected at lowlands sites, including 39 *Bi. pfeifferi*, 19 *Bu. forskalli*, 10 *Bu. africanus* and 12 *Bu. tropicus*. Again, avian schistosome cercariae, but not mammalian trematode cercariae were shed by some of these snails (4 *Bu. forskalli*, 4 *Bi. pfeifferi* and 2 *Bu. africanus*). *Melanoides tuberculata* and *Bellamya constricta* were also collected at some lowland sites, but not examined for cercarial shedding as they were are not well recognized as intermediate hosts for trematodes.

During September 2013 (dry season), 309 intermediate host snails were collected at selected water bodies in highland villages, including 100 *Bu. africanus*, 83 *L. natalensis*, 78 *Bi. Pfeifferi* and 48 *Bu. tropicus*. *Gymnocephalous* cercariae were detected in 13 *L. natalensis* and 2 *Bu. africanus*. Amphistome cercariae were found in 1 *Bi. pfeifferi*. Mammalian *Schistosoma* cercariae were found in 7 *Bu. africanus* and 1 *Bu. tropicus*. Avian *Schistosoma* cercariae were found in 4 *L. natalensis* and 2 *Bi. pfeifferi*. During the same period, 33 intermediate host snails were collected at selected water bodies in lowland villages, including 23 *Bi. pfeifferi* and 10 *L. natalensis*. None of these snail shed cercariae. *Melanoides tuberculata* and *Be. constricta* were also collected at these lowland sites.

Of the water bodies visited (Table 4 and Fig. 2), 8/10 (80·0%) in the highlands and 12/19 (63·2%) in the lowlands areas, were found to harbour intermediate host snail species. *Lymnaea natalensis* and *Bi. pfeifferi* were found at 9 and 8 sites at altitude ranges of 801–1814 m and 955–1790 m, respectively, whereas *Bulinus* species were found at 11 sites at altitudes ranging from 1268 to 1814 m. *Melanoides tuberculata* and *Be. constricta* were restricted to the lowlands at altitudes ranging 696–895 m. The number of sites harbouring snail intermediate hosts differed significantly between the highlands (>1500 m) and lowlands (<1500 m) for *L. natalensis* (Fisher's exact test: $P < 0·01$) and *Bi. pfeifferi* (Fisher's exact test: $P < 0·05$) but not for *Bulinus*

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Table 3. Egg-patent trematode infections in cattle in highland and lowland areas of Iringa Rural District, Tanzania

Area	*N*	Parasite	*N* infected	Prevalence (%)
Highland (⩾1500 m)	150	*F. gigantica*	111	74·0
		Paramphistome	127	84·7
		S. bovis	8	5·3
Lowland (<1500 m)	300	*F. gigantica*	16	5·3
		Paramphistome	156	52·0
		S. bovis	10	3·3

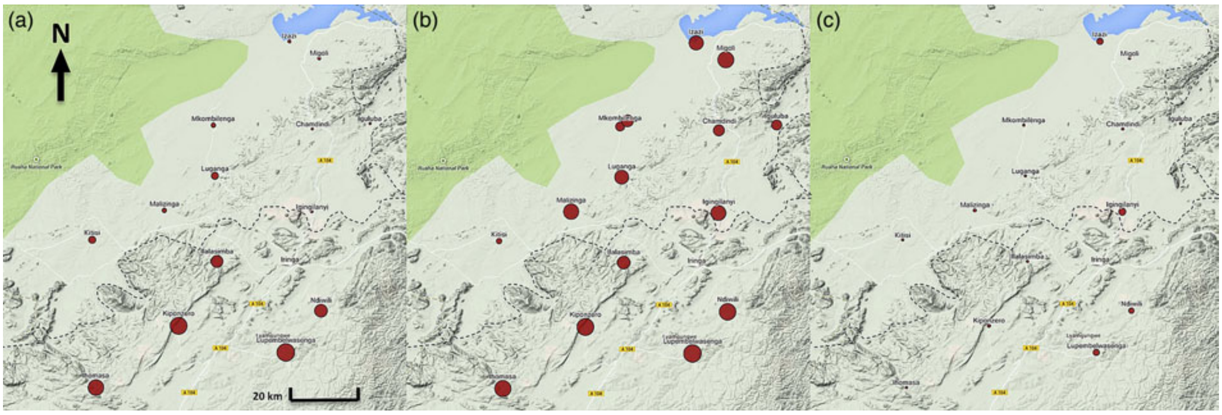

Fig. 1. Distribution of trematode infections in cattle in villages in Iringa Rural District. Circles proportional to prevalence. (a) *Fasciola gigantica*: smallest circles (Chamdindi, Igingilanyi, Iguluba and Mbuyuni) 0% prevalence; largest circle (Lupembelwasenga) 100% prevalence; (b) Paramphistomes: smallest circle (Kitisi) 3% prevalence; largest circle (Lupembelwasenga) 100% prevalence; (c) *Schistosoma bovis*: smallest circles (Ihomasa, Ilalasimba, Chamdindi, Iguluba, Kitisi, Luganga, Mkombilenga, Mbuyuni, Migoli) 0% prevalence; largest circle (Igingilanyi) 16·7% prevalence. Dashed lines show demarcation between lowland (top left) and highland (bottom right) areas.

species (Fisher's exact test: $P = 0{\cdot}22$). Logistic regressions showed a significant effect of altitude on the incidence of *L. natalensis* ($P < 0{\cdot}01$), *Bulinus* sp. or *Bi. pfeifferi* ($P < 0{\cdot}001$), all of which were more likely to be found at higher altitude, and *M. tuberculata* ($P < 0{\cdot}001$), more likely at lower altitude (Fig. 3).

Linear modelling of trematode prevalence

Fasciola gigantica, paramphistome and *S. bovis* prevalences in cattle were modelled using altitude, observation of intermediate host snails and age and sex of cattle as explanatory variables (Fig. 4). Altitude, observation of *L. natalensis* and age of cattle were retained in the minimum adequate model for *F. gigantica* prevalence, while altitude and observation of any (potential) intermediate host snail and cattle age were retained for paramphistome prevalence. Simplified models using altitude as the only explanatory variable were used to calculate altitudes at which 50% prevalence would be expected using −intercept/coefficient. Prevalences increased with altitude, such that the model for *F. gigantica* (coefficient 0·004687, s.e. 0·0004359; intercept −7·276, s.e. 0·6633) predicted 50% prevalence at 1552 m and that for paramphistomes (coefficient 0·001533, s.e. 0·0002562; intercept −1·240, s.e. 0·3028) predicted 50% prevalence at 809 m. Prevalence of *S. bovis* was too low for an adequate model.

DISCUSSION

The present findings and previous studies (Keyyu *et al*. 2005: Nzalawahe *et al*. 2014) in Tanzania and elsewhere in Africa (Pfukenyi *et al*. 2005) revealed that with *F. gigantica* and paramphistomes are the predominant egg-patent trematode infections of cattle. While *S. bovis* was less prevalent in cattle investigated in this study, it is clearly an important parasite in Iringa District and the wider region (De Bont *et al*. 1994; Makundi *et al*. 1998; Pfukenyi *et al*. 2005; Yabe *et al*. 2008). Keyyu *et al*. (2005) recorded prevalences of 44·9% for *F. gigantica* and 82·7% for amphistomes in cattle above 1200 m altitude, consistent with the prevalences of 111/210 (52·9%) for *F. gigantica* and 161/210 (76·7%) for paramphistomes in villages in our study above this altitude (Table 1). Makundi *et al*. (1998) found 8 (3·9%) of 205 cattle in villages in Iringa District to be infected with *S. bovis*, a prevalence similar to the 4% in the present study.

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Table 4. Occurrence of snails at water bodies in Iringa Rural District, Tanzania

Village	Habitat description	Altitude (m)	Latitude	Longitude	*Lymnaea* sp.	Other snail species
Ihomasa	Stream	1814	S08°06·919	E35°09·739	*L. natalensis*	*Bu. africanus*, *Bu. tropicus*
Lupembel-wasenga	Swamp	1790	S08°01·241	E35°40·770	*L. natalensis*	*Bu. africanus*, *Bu. forskali*, *Bu. tropicus*, *Bi. pfeifferi*
Makongati	Stream	1742	S07°53·472	E35°26·024	–	–
Kiponzero	Stream, Pool	1727	S07°56·945	E35°23·248	*L. natalensis*	*Bu. africanus*, *Bu. forskali*, *Bi. pfeifferi*
Negabihi	Pool	1715	S07°56·021	E35°45·703	–	*Bu. forskali*
Ihami	Stream	1713	S08°00·894	E35°20·936	–	–
Ndiwili	Swamp	1705	S07°54·496	E35°46·517	*L. natalensis*	*Bu. africanus*, *Bu. tropicus*, *Bi. pfeifferi*
Ilalasimba	Stream	1512	S07°46·524	E35°29·526	*L. natalensis*	*Bi. pfeifferi*
Igingilanyi	Ditch	1376	S07°38·475	E35°45·019	–	*Bu. africanus*, *Bu. tropicus*, *Bu. forskali*
Kising'a	Ditch	1370	S07°31·196	E35°46·055	–	*Bu. forskali*, *Bu. africanus*
Mkungugu	Reservoir	1287	S07°32·241	E35°47·559	*L. natalensis*	*Bu. africanus*, *Bi. pfeifferi*
Chamdindi	Ditch, Reservoir	1271	S07°25·093	E35°45·097	–	*Bu. forskali*
Iguluba	Stream, Reservoir	1105	S07°24·212	E35°54·553	–	–
Idodi	Stream	955	S07°46·891	E35°11·397	–	*Bi. pfeifferi*
Malizanga	River, Reservoir	886	S07°38·262	E35°20·915	–	*M. tuberculata*
Kitisi	River	895	S07°43·028	E35°09·126	–	*M. tuberculata*
Luganga	Trough	801	S07°32·670	E35°29·182	*L. natalensis*	*M. tuberculata*
Mbuyuni	Trough	750	S07°23·543	E35°30·139	–	–
Mkombilenga	River	749	S07°24·467	E35°28·931	–	–
Izazi	River	707	S07°10·903	E35°41·377	–	*M. tuberculata*
Migoli	Dam	701	S07°08·642	E35°46·223	–	*M. tuberculata*, *Be. constricta*

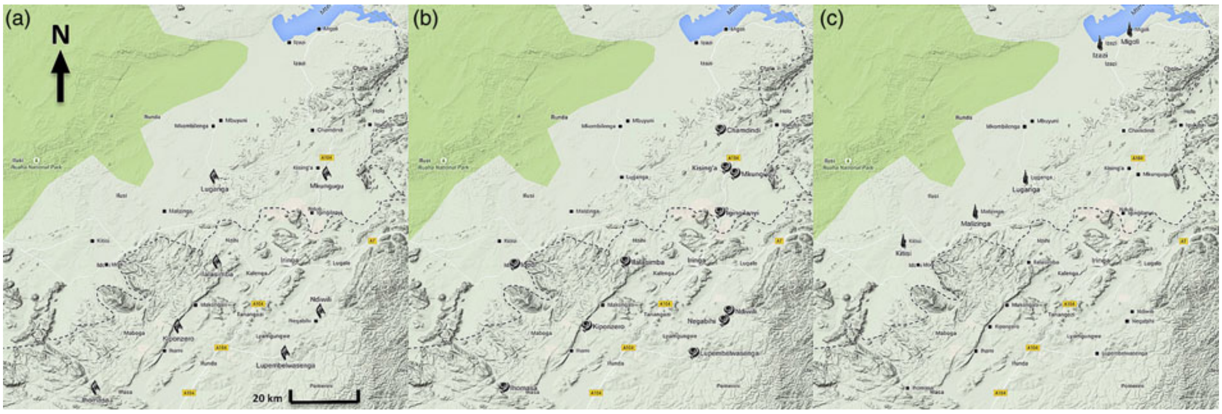

Fig. 2. Distribution of intermediate host snails in water bodies in Iringa Rural District. Snail symbols: intermediate host snails observed; solid squares snails not observed. (a) *Lymnaea natalensis*; (b) *Bulinus* sp. or *Biomphalaria* sp.; (c) *Melanoides tuberculata*.

Fasciola gigantica and paramphistome infections were more prevalent in cattle of the highlands (74·0 and 84·7%, respectively) compared with the lowlands (5·3 and 52·0%, respectively). *Fasciola gigantica* and paramphistome infection prevalences were both significantly higher in adult cattle than in weaners ($P < 0·05$), reflecting their greater length of exposure to infection (Waruiru *et al*. 2000; Keyyu *et al*. 2005; Pfukenyi *et al*. 2006). Despite there being only 10 cattle of the Iringa Red type in the study sample, there was significantly a lower rate of egg-patent paramphistome infections

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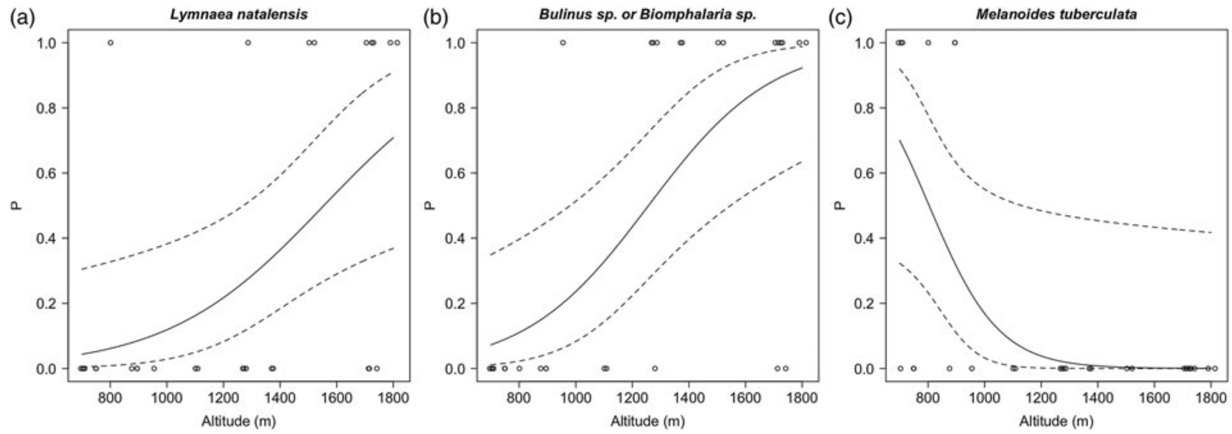

Fig. 3. Altitude and occurrence of intermediate host snails in Iringa Rural District. Solid lines: fitted logistic regression model; dotted lines: 95% confidence interval. (a) *Lymnaea natalensis*. Regression co-efficients: intercept −5·618 (S.E. 2·127); altitude = 0·003614 (S.E. 0·001458); $P_{occurrence}$ 0·5 at altitude 1554 m; (b) *Bulinus* sp or *Biomphalaria* sp. Regression co-efficients: intercept −5·745 (S.E. 1·989); altitude = 0·00457 (S.E. 0·001542); $P_{occurrence}$ 0·5 at altitude 1257 m; (c) *Melanoides tuberculata* sp., Regression co-efficients: intercept 6·546 (S.E. 3·416943); altitude −0·008142 (S.E. 0·004045); $P_{occurrence}$ 0·5 at altitude 804 m.

(40%) in these cattle than in Maasai zebu-type cattle (78%) in the same villages. The small number of animals involved means these results should be interpreted with caution, and no evidence of breed association with susceptibility was found for either *F. gigantica* or *S. bovis*.

Distribution of intermediate host snails was broadly consistent with that of trematode infection in cattle, in keeping with understanding of their vectorial competence. Linear modelling of the data showed that villages above approximately 1550 m had over 50% prevalence of *F. gigantica* and greater than 50% likelihood of presence of its principal intermediate host *L. natalensis*. The detection of *F. gigantica* infections in cattle in some lowland villages where *L. natalensis* intermediate hosts were not found, might be due to either migration of cattle herds to wetter areas during the dry season, or to the presence of temporary water bodies acting as focal points for trematode transmission during the rainy season and early dry season, and possibly harbouring snails washed from the highlands by flood water.

Villages above 1260 m had greater than 50% likelihood of *Bulinus* or *Biomphalaria* sp. being present. *Bulinus africanus* and *Bu. forskalli* act as primary intermediate hosts for *S. bovis* (Kumar, 1999), while *Bu. tropicus* also act as intermediate hosts for *S. bovis* after previous infection with paramphistomes (Southgate *et al.* 1985, 1989). Although too few *S. bovis* infections were detected in cattle to allow modelling of its relationship to altitude, its observed distribution in cattle roughly corresponded presence of these intermediate hosts in the more easterly part of the study area (Figs 1c and 2b).

Paramphistomes use a wide range of aquatic snails as their intermediate hosts including *Lymnaea* sp., *Bulinus* sp. and planorbid species (e.g. *Biomphalaria* sp.). Although it was not possible to identify paramphistomes to species level on the basis of egg morphology, previous studies have identified *Calicophoron microbothrium* and *Cotylophoron jacksoni* in cattle slaughtered in Iringa (Keyyu *et al.* 2006). *Bulinus tropicus* is recognized as an intermediate host for *C. microbothrium*, while intermediate hosts for *C. jacksoni* are poorly described (Kumar, 1999). With the sole exception of *L. natalensis* present at 801 m in Luganga village, *Lymnaea, Bulinus* and *Biomphalaria* sp. were not found at any sampling site below 900 m in altitude (Table 4). Nevertheless, paramphistomes occurred in cattle at all sampling sites below this altitude, and at high prevalence in 4 locations (Migoli: 703 m, 83·3% prevalence; Izazi: 704 m, 66·7%; Mbuyuni: 750 m, 46·7%; and Malizanga: 891 m, 76·7%). *Melanoides tuberculata* were encountered in many of these low altitude sites (Table 4) consistent with the possibility that this snail species is involved in transmission of paramphistomes as reported in an experimental study in Zimbabwe (Chingwena *et al.* 2002).

The highest risk of trematode infection in cattle in East and southern Africa has been reported to occur in areas of high annual rainfall, with risk decreasing in areas of shorter wet season (Malone *et al.* 1998; Pfukenyi *et al.* 2006). High rainfall favours the development and survival of the developmental stages in intermediate host snails, whereas arid areas are generally unsuitable for occurrence of trematode infections in cattle. In addition to high rainfall and suitable temperatures for the survival of the snails, the Iringa highlands provide a number of wet/swampy grazing areas, streams, rivers and reservoirs which allows snail activity throughout the year. In contrast, the lowlands are characterized by dry grazing lands with low rainfall and high temperatures, especially during the dry season. Cattle have access to few natural water bodies and reservoirs

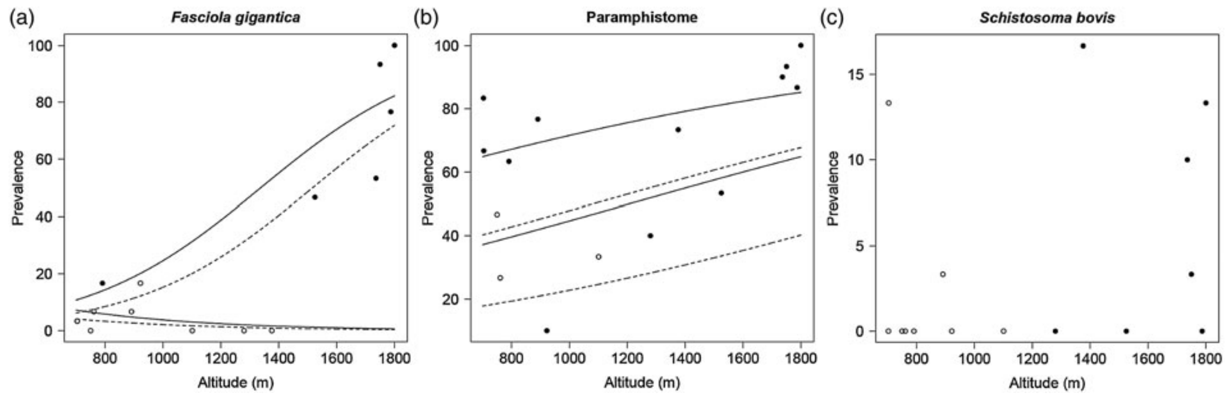

Fig 4. Altitude and trematode infections in cattle in Iringa Rural District: fitted logistic regression models. Symbols: village prevalences in cattle; solid lines: fitted models for adult cattle (age ⩾24 months); dotted lines: fitted model for juvenile cattle (age <24 months). (a) *Fasciola gigantica*. Open symbols, lower solid and dotted lines: villages where *Lymnaea natalensis* not observed; closed symbols, upper solid and dotted lines: villages where *L. natalensis* observed. Regression co-efficients: intercept −1·569 (S.E. 1·420); *L. natalensis* present −3·469 (S.E. 1·726); age >24 months 0·5957 (S.E. 0·3591); altitude −0·002266 (S.E. 0·001610); *L. natalensis* present:altitude 0·005586 (S.E. 0·001723); (b) Paramphistomes. Open symbols, lower solid and dotted lines: villages where no intermediate host snails observed. Closed symbols, upper solid and dotted lines: villages where (potential) intermediate host snails (*L. natalensis, Bulinus* sp., *Biomphalaria* sp. or *Melanoides tuberculata*) observed. Regression co-efficients: intercept −2·254 (S.E. 0·3711); (potential) intermediate host snails present 1·136 (S.E. 0·2736); age > 24 months 1·007 (S.E. 0·2306); altitude 0·001032 (S.E. 0·0002802); (c) *Schistosoma bovis*. Open symbols: no intermediate host snails observed. Closed symbols: intermediate host snails (*Bulinus* sp., *Biomphalaria* sp.) observed. (Prevalences too low for reliable model fitting).

hence snail habitats have a highly focal distribution. Moreover, the majority of ditches and reservoirs that harbour *Bulinus* and *Biomphalaria* species during the rainy season dry up by the middle of the dry season, resulting in a seasonal transmission pattern. The longer period of transmission explains why trematode burdens are higher in cattle in the highlands. Similar findings have been reported in Zimbabwe (Pfukenyi *et al*. 2005, 2006). In Uganda, Ogambo-Ongoma (1972) and Howell *et al*. (2012) found high prevalences of trematode parasites (*F. gigantica* and paramphistomes) at low altitudes (<1500 m), but this might be due to the different eco-climatic conditions in their study areas (Malone *et al*. 1998).

Gymnocephalous cercariae were shed by *L. natalensis* and *Bu. africanus* while mammalian *Schistosoma* cercariae and amphistome cercariae were found in *Bu. africanus, Bulinus forskali* and *Bi. Pfeifferi*, respectively. The shedding of gymnocephalous, amphistome and mammalian *Schistosoma* cercariae suggested the presence of *Fasciola*, paramphistomes and S. *bovis*/*Schistosoma haematobium* infections in the snails. Avian *Schistosoma* cercariae and Xiphidiocercariae which are not of veterinary importance were found in all 3 genera of snails.

Intermediate host snails were found to shed cercariae of veterinary/medical importance during the dry season (September 2013) and but not the wet season (March 2013) and only in highland areas but not the lowlands. This is consistent with the higher prevalence of *F. gigantica* and paramphistome infection in cattle in the highlands compared with the lowlands, and with earlier results (Keyyu *et al*. 2005) that showed the proportion of cattle with egg patent infections increased during the course of the dry season, peaking at the end of the dry season and the early part of the wet season.

The observation of greater than expected numbers of mixed infections with *F. gigantica* and paramphistomes, especially in highland areas ($P < 0{\cdot}001$), suggests that in these areas either they may share intermediate host species, e.g. *L. natalensis*, or that their respective intermediate hosts have similar environmental distributions. The significant positive correlation ($r^2 = 0{\cdot}244$, $P < 0{\cdot}001$) between the log-transformed egg counts in cattle in which both *F. gigantica* and paramphistome eggs were detected in feces was consistent with the assertion that interactive infections between these two trematode types are mutually inclusive (Yabe *et al*. 2008). As in previous studies (Yabe *et al*. 2008), no such correlation was observed between infections with *F. gigantica* and *S. bovis* infections or between infections with paramphistome and *S. bovis*.

This study has established that trematode parasites (*F. gigantica* and paramphistomes) are prevalent in cattle of Iringa Rural District, with those in the highlands worse affected than those in the lowlands. These observations suggest that interventions for the control of *F. gigantica* and paramphistomes are currently needed in the highlands (Keyyu *et al*. 2003). Intervention could be based on anthelmintic treatment of cattle, which would require assessment of the effectiveness of the locally available flukicides, and control of intermediate hosts, which likewise

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would require detailed investigation of seasonal snail population dynamics and infection rates. Moreover further studies are needed at lowlands area to confirm the involvement of *M. tuberculata* in the transmission of paramphistomes.

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