Human infections and co-infections with helminths in a rural population in Guichi, Anhui Province, China

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Abstract

Helminth infections are believed to be common in tropical and subtropical countries. A cross-sectional study was carried out in two villages located in Guichi District in Anhui Province, the People's Republic of China, where multiparasitism was investigated using parasitological tests. The data collected were fitted to Bayesian multi-level models to profile risk factors for helminth infections. The prevalence of *Schistosoma (S.) japonicum*, *Ascaris (A.) lumbricoides* and *Trichuris (T.) trichiura* were 0.43% (range: 0-0.87% at the village level), 2.28% (range: 1.69-2.88%), and 0.21% (range: 0-0.42%), respectively. No hookworm infection was found. With regard to multiparasitism, only a 33-year-old female was found to be co-infected with *S. japonicum* and *A. lumbricoides*. Multiparasitism was unexpectedly rare in the study area, which contrasts with results from other studies carried out elsewhere in the country. The long-term usage of albendazole for individuals serologically positive for schistosomiasis may be the main reason, but this needs to be confirmed by future studies.

Introduction

Schistosomiasis japonica, a water-associated disease with a long history in China, remains a public health problem (Gray *et al.*, 2010). Although great progress has been achieved with regard to control and/or local elimination of the disease through implementation of a multitude of control measures for more than six decades, approximately 65 million individuals are still at risk (Utzinger *et al.*, 2005; Zhou *et al.*, 2005) and the infection often overlaps with other parasitic worm infections, such as the soil-transmitted helminths (STHs), in particular *Ascaris (A.) lumbricoides*, hookworm and *Trichuris (T.) trichiura* (Yapi *et al.*, 2014). Soil-transmitted helminths are among the most common globally endemic geo-helminths, which are also widely spread in China (Hotez *et al.*, 1997). According to the Global Burden of Disease (GBD) study 2010, schistosomiasis and STHs together account for about 32.6% of the total burden of tropical parasites (Hotez *et al.*, 2014).

Multiparasitism is believed to be common among deprived populations, especially in rural areas where sanitation conditions are poor

(Bhutta *et al.*, 2014). It has been documented that even low-intensity infections with several helminths can cause significant morbidity (de Silva, 2003; Ezeamama *et al.*, 2005). So far, the available, published literature on multiparasitism comes mostly from Africa, while data from Southeast Asia (Mazigo *et al.*, 2013, 2014; Singer, 2013) and China are scarce (Steinmann *et al.*, 2008).

A main intervention to reduce the morbidity due to schistosomiasis is the periodic treatment of at-risk populations with the drug praziquantel, while transmission control of the infection as well as eventual elimination of the disease altogether focuses on interrupting the parasite's life cycle, which depends on a snail intermediate host and a large number of definitive, mammalian hosts (Yang *et al.*, 2014). Although there is no systematic STH control in China, preventive chemotherapy is routinely used when these infections are discovered (Ellis *et al.*, 2007). An exploration of risk factors associated with multiple diseases (*e.g.*, schistosomiasis and ascariasis) would contribute to developing and implementing cost-effective control strategies (Yapi *et al.*, 2014). Schistosome and STHs infections are likely to proliferate under favourable climatic and environmental conditions, especially in remote and poor areas where essential facilities, such as clean water and sanitation, are not available (Ziegelbauer *et al.*, 2012). Physical environmental factors related to these diseases have been well investigated in previous studies, but social and behavioural determinants are still poorly understood (Mofid *et al.*, 2011; Yang *et al.*, 2014).

In this study, we investigated multiparasitism in an area historically known as strongly endemic for schistosomiasis, focusing on two villages belonging to Guichi District Anhui Province in the mid-eastern part of China. The impact of social factors on both single parasitic infections and multiple simultaneous ones was also investigated. We used parasitological data obtained from a cross-sectional survey and discuss the results in the context of potential impacts of social and behavioural factors and associated interventions. A Bayesian multilevel modelling approach was employed to account for spatial pattern of diseases and local conditions.

Materials and Methods

Study area and study population

The residents of Heping and Niutoushan, two neighbouring villages located at the lake and marshland area in Guichi District, Chizhou, Anhui Province, made up the study population. The Yangtze River passes through the northern part of the study area (Figure 1). Individuals aged 5 to 65 years were invited to participate and the location of each household that accepted to be part of the study was recorded using a hand-held global positioning system (GPS) instrument (Thales MobileMapper; https://www.crunchbase.com/organization/thales-navigation-inc). The survey was conducted in October and November 2012.

Questionnaire survey

All participants were interviewed by questionnaire requesting information on age, sex, education and occupation. The following personal behavioural information was also included: i) source of drinking water; ii) consumption of uncooked vegetables; iii) sanitation, *i.e.* hand wash after defecation; iv) way of fertilising the fields; v) role of footwear when undertaking agricultural activities; vi) awareness of the parasite's life-cycle. Participants under 15 years of age were assisted by their parents or legal guardians when answering the questions. Household-level information, such as income and type of toilet, was obtained from the head of each household. Income data was subjectively classified into three categories: very poor [<10,000 renminbi (RMB)/year], poor (10,000-20,000 RMB/year) and least poor (>20,000 RMB/year).

Parasitological examination

The indirect haemagglutination assay (IHA) test (Hirst, 1942) was used to examine the participants serologically, while Kato-Katz (Katz *et al.*, 1972) was used for faecal examination (3 thick-smear slides of from one stool specimen). Each slide was read blindly by an experienced laboratory technicians for the presence of eggs from *Schistosoma (S.) japonicum* and STHs (*A. lumbricoides*, *Ancylostoma duodenale*, *Necator americanus* and *T. trichiura*). In addition, 10% of slides were re-read by a senior laboratory technician. All the individuals found to be either IHA-positive or egg-positive by stool examination were diagnosed again, both serologically and parasitologically.

Statistical analysis

A Bayesian modelling analysis framework was developed with data that could be classified into different hierarchies. Considering the hierarchical nature of the data in our study, for example, individual-level data was assigned to level 1 and household-level data were assigned to level 2.

Let Y_{ij} denote the infection status (*i.e.* negative=0 or positive=1) of individual i within household j, which was assumed to follow a Bernoulli distribution [$Y_{ij} \sim Be(p_{ij})$] where p_{ij} is the probability of being serological/parasitological infection. To fit the data, we first used a spatial, multi-level logistic regression model as follows:

$$\operatorname{logit}(p_{ij}) = \alpha + \sum_n \beta_n X_n + u_j + v_j \qquad \text{eq. 1}$$

where the covariates X_n are the risk factors at each individual/household level, and u_j and v_j the household-level random effects that account for the (non-spatial) clustering of individuals and the spatial effects between households, respectively. The non-spatial random effect u_j was assumed to have a normal distribution, namely $u_j \sim N(0, \sigma_1^2)$, where σ_1^2 is the between-household variance. The spatial random effect was assumed to have a normal distribution that has a conditional autoregressive (CAR) structure:

$$v_j \sim N(0, \sigma_2^2) \qquad \text{eq. 2}$$

and

$$[v_j \mid v_k, j \neq k, \sigma_2^2] \sim N\left(\frac{\sum_k v_k w_{jk}}{\sum_k w_{jk}}, \frac{\sigma_2^2}{\sum_k w_{jk}}\right) \qquad \text{eq. 3}$$

where σ_2^2 is the spatial variance and w_{jk} the weight of the neighbour k for household j (j and k are IDs for different household); if i, j are neighbours, w_{jk}=1, otherwise w_{jk}=0. The neighbours of a household j were defined as those within a buffer region of 1 km centred at j. A non-spatial multi-level logistic model was also fitted for comparative purpose, in which the spatial random effect v_j was removed from model (1), namely:

$$\log it(p_{ij}) = \alpha + \sum_n \beta_n X_n + u_j \quad \text{eq. 4}$$

Bayesian methods were employed to fit the two models using WinBUGS 1.1.4 software (MRC Biostatistics Unit; Cambridge, UK). Priors for α and β_n were set using the vague normal distribution N(0,0.0001), and prior distributions for the inverse of σ_1^2 and σ_2^2 were specified using the vague gamma distribution *Gamma*(0.5,0.0005). To estimate the model parameters, we used a Markov Chain Monte Carlo (MCMC) simulation (Gelfand and Smith, 1990). Specifically, two separate chains starting from different initial values were run for 30,000 iterations: the first 10,000 iterations were discarded as burn-ins and the remaining 20,000 iterations from each chain were used for parameter estimation. Convergence was checked by visual examination of the time series plots of samples from each chain. The Bayesian information criterion (BIC) (Spiegelhalter *et al.*, 2002) was used to compare the goodness of fit for the models. A model with a smaller BIC is considered to fit the data better.

Table 1. Parasitological and serological tests at the village level.

Village	*A. lumbricoides*	*T. trichiura*	*S. japonicum* Parasitology°	*S. japonicum* Serology#
Heping	12 (1.7%)	3 (0.4%)	0	109 (15.4%)
Niutoushan	20 (2.9%)	0	6 (0.9%)	83 (12.0%)

A. lumbricoides, Ascaris lumbricoides; T. trichiura, Trichuris trichiura; S. japonicum, Schistosoma japonicum. The number of people found positive is provided with the prevalence within parentheses. °Use of Kato-Katz test; #use of indirect haemagglutination assay test.

Table 2. Estimates of parameters by multi-level models for schistosomiasis japonica prevalence in two villages in Guichi District, Anhui Province.

Level	Risk factor		*S. japonicum* infection Non-spatial model OR	Non-spatial model 95% CI	Spatial model OR	Spatial model 95% CI
Individual	Age		1.04*	1.02, 1.06	1.05*	1.01, 1.11
	Sex	Male	1	1		
		Female	1.02	0.73, 1.45	0.86	0.57, 1.33
	Education	Illiteracy	1	1		
		Primary school	1.34	0.85, 2.13	1.28	0.70, 2.42
		High school	0.10	0.01, 12.23	0.59	0.37, 1.02
	Occupation	Student	1	1		
		Fisherman	47.68*	9.21, 85.90	9.59	0.30, 112.36
		Peasant	4.36*	1.07, 24.30	2.59	0.06, 196.44
	Source of drinking water	Well	1	1		
		Pond, lake, river	0.08	0.01, 13.21	0.19	0.03, 2.08
		Tap	1.99	0.97, 4.31	2.02	0.91, 4.79
	Consumption of uncooked food	Yes	1		1	
		No	1.08	0.67, 1.78	1.04	0.62, 1.77
	Hand wash after defecation	Yes	1	1		
		No	0.97	0.53, 1.70	1.01	0.53, 1.86
	Fertilising with night soil in the fields	Yes	1	1		
		No	1.16	0.72,1.84	1.26	0.74, 2.15
	Barefoot when undertaking agricultural activities	Yes	1	1		
		No	0.61	0.36, 1.05	0.90	0.32, 2.47
		Never undertake	0.33*	0.15, 0.71	0.58	0.12, 3.22
	Aware of how the disease is transmitted	Yes	1	1		
		No	1.01	0.66, 1.56	0.88	0.39, 1.61
Household	Income	Most poor	1	1		
		Poor	0.37	0.12, 1.17	0.27*	0.08, 0.99
		Least poor	0.75	0.28, 2.19	0.60	0.18, 2.07
	Type of toilet	Sanitary	1			
		Outdoor	1.48	0.50, 4.34	1.54	0.43, 5.28
		Indoor	1.18	0.60, 2.48	0.89	0.22, 2.60
Variation	σ1		0.05e-03	0.01e-03, 0.79e-03	0.75e-03	0.22 e-03, 13.29e-03
	σ2		-	-	5.54	(1.56, 24.04)
	BIC		1350.25		2453.27	

S. japonicum, Schistosoma japonicum; OR, odds ratio; CI, confidence interval; BIC, Bayesian information criterion. *P≤0.05.

Results

Figure 2 shows locations of households from the two villages in Guichi District. Overall, 1403 individuals from 671 households were included in the study that included slightly more females (n=714 or 50.9%) than males (n=689 or 49.1%). With regard to occupation, most were peasants (n=952 or 67.8%), followed by students (n=258 or 18.5%) and fishermen (n=193 or 13.7%). Table 1 shows the status of infection for each parasite species.

Infections detected

The overall prevalence of *S. japonicum* infection was 0.4%. At the village level, the schistosomiasis prevalence was 0 and 0.9% in Heping and Niutoushan village, respectively, while the schistosomiasis seroprevalence at the village level ranged from 12.0 to 15.4%. As the number of parasitological positive cases (n=6) was too low to be fitted, the number of serological positive cases (n=192) was considered for further analysis.

The overall prevalence of *A. lumbricoides* infection was 2.3% whilst at the village level the prevalence was 1.7 and 2.9% in Heping and Niutoushan, respectively. For *T. trichiura* infection, the overall prevalence was 0.2% with all the cases (n=3) in Heping. No cases of hookworm infection were found in either village. The *T. trichiura* and hookworm infections were not further analysed due to very small number of cases found.

There was only one individual (a 33-year-old female) in Niutoushan village co-infected with *S. japonicum* and *A. lumbricoides*. However, when we included the *S. japonicum* sero-positive cases, 5 individuals (3 males and 2 females with ages ranging between 43 and 61) were simultaneously infected with *A. lumbricoides* in this village. The co-infection prevalence was too low to be considered for risk analysis.

Model comparison and risk profiles

Tables 2 and 3 summarise the goodness-of-fit of our models and risk factors for *S. japonicum* and *A. lumbricoides* infections, respectively. In each table, the BIC value for model 2 (*i.e.*, the non-spatial model) was smaller than that obtained for model 1, indicating that model 2 fitted the data better.

As shown in Table 2, older individuals were at a significantly higher risk of infection with *S. japonicum* compared with younger individuals [odds ratio (OR)=1.04; 95% confidence interval (CI)=1.02-1.06]. Occupation was a highly significant risk factor for *S. japonicum* infection, with fisherman at the highest risk (OR=47.68; 95% CI=6.21-85.90). In addition, individuals who had never undertaken agricultural activities had a significantly lower risk (OR=0.33; 95% CI=0.15-0.71) of being infected than those who were barefoot in the fields.

Neither age nor gender was associated with *A. lumbricoides* infection (Table 3); however, income and being barefoot in the fields were significantly associated with the infection. Of note, the peasant group was at a significantly higher risk (OR=6.84; 95% CI=1.41-37.34) of infection with *A. lumbricoides* compared to the student group.

Discussion

We investigated the status of multiparasitism in a population from Guichi District, Anhui Province in eastern China as it was once one of the most endemic areas of schistosomiasis japonica in the country (Zhang *et al.*, 2008; Zhao *et al.*, 2005) and schistosomiasis usually overlaps with STHs (Yapi *et al.*, 2014). A previous study (Ellis *et al.*, 2007) indicated that one third of the population harboured at least two parasite species concurrently in an environmentally similar area in the same river basin. Both blood and stool samples were collected in our study to determine the infection history of schistosomiasis and the current status of parasite infection. The survey was conducted during the dry season (October to November) to minimise potential seasonal effect.

Our data suggest that infection by multiparasitism is rare in this population. *A. lumbricoides* infection was mosty prevalent in the vil-

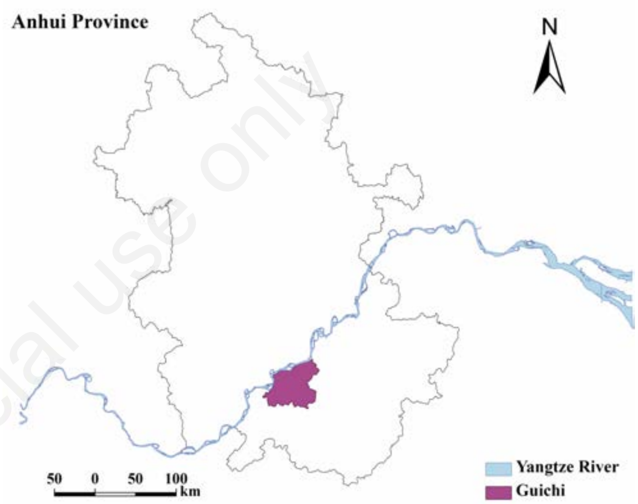

Figure 1. Map of Anhui Province with Guichi District.

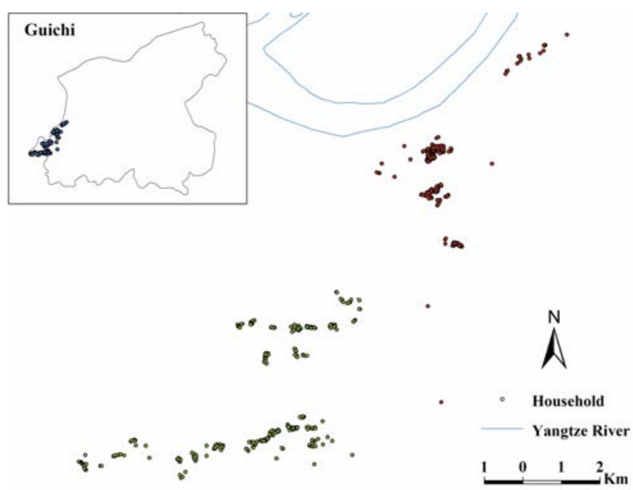

Figure 2. Location of households belonging to the two study villages in Guichi District, Anhui Province. Circles in red are households from Niutoushan and those in green are households from Heping; the map was created using the AcrGIS software (version 10.0, ESRI Inc., Redlands, CA, USA).

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lages ranging from 1.7 to 2.9%, followed by *S. japonicum* (0 to 0.9%) and *T. trichiura* (0 to 0.4%); no infection with hookworm was found. For multiparasitism, only a 33-year-old female was found to harbour two parasite species (*S. japonicum* and *A. lumbricoides*) concurrently. Our observations are not consistent with the results from some other reports in China. The survey carried out in the Poyang Lake region in 2007 found that helminth infections were common (Ellis *et al.*, 2007), and another study conducted in a village of a south-western mountainous area in 2006 suggested that multiparasitism was hyper-endemic (Steinmann *et al.*, 2008). This low burden of multiparasitism is probably, according to the discussion with the head of the local anti-schistosomiasis station, due to periodic mass administration of anthelmintics, *i.e*. albendazole. As an area historically known for high prevalence rates of schistosomiasis japonica (Zhang *et al.*, 2008; Zhao *et al.*, 2005), routine examinations of *S. japonicum* infection are carried out throughout Guichi District. During these examinations for schistosomiasis, individuals, who were stool-positive for schistosome eggs were given praziquantel. This control policy has been implemented for several decades (Wang *et al.*, 2009), which is probably the main reason for rare multiparasitism as albendazole and similar drugs used have a broad-spectrum effect on intestinal parasites. This intervention ended in 2013, and a follow-up study will be able to test our speculation in the near future.

To better understand the roles of social and behavioural determinants in the transmission of helminth infections, we employed a Bayesian multi-level modelling approach considering the spatial and hierarchical attributes of our data, and some previous studies used similar modelling approaches (Barreto *et al.*, 2011; Cohen *et al.*, 2007; Mossong *et al.*, 2008). According to the BIC value, the non-spatial model, which excluded the spatial correlation between households, had a better fit than the spatial model and was therefore employed to investigate the risk factors. This indicates that the household-level random effect can explain the residuals variation after adjusting for current risk factors, and that spatial correlation at such a small scale as the household level can be neglected when modelling helminth infections.

The results from the non-spatial model (Table 2) identified, as expected, age and occupation as significant determinants for schistosomiasis. For example, individuals who had never undertaken agricul-

Table 3. Estimates of parameters by multi-level models for Ascaris prevalence in two villages in Guichi District, Anhui Province.

Level	Risk factor		*A. lumbricoides infection*			
			Non-spatial model		Spatial model	
			OR	95% CI	OR	95% CI
Individual	Age		0.97	0.94, 1.04	0.98	0.96, 1.08
	Sex	Male	1		1	
		Female	0.86	0.40, 1.85	0.88	0.57, 1.36
	Education	Illiteracy	1		1	
		Primary school	0.62	0.23, 1.82	0.69	0.43, 1.11
		High school	0.08	0.01, 13.12	0.29	0.01, 8.39
	Occupation	Student	1		1	
		Fisherman	0.02	0.01, 87.79	0.54	0.02, 4.44
		Peasant	6.84*	1.41, 37.34	1.03	0.58, 1.81
	Source of drinking water	Well	1		1	
		Pond, lake, river	0.03	0.01, 37.52	0.38	0.01, 11.64
		Tap	2.00	0.53, 8.80	0.80	0.47, 1.37
	Consumption of uncooked food	Yes	1		1	
		No	0.61	0.23, 1.72	0.73	0.46, 1.19
	Hand wash after defecation	Yes	1		1	
		No	0.47	0.09, 1.84	0.64	0.27, 1.42
	Fertilising with night soil in the fields	Yes	1		1	
		No	1.74	0.57, 4.93	0.99	0.55, 1.76
	Barefoot when undertaking agricultural activities	Yes	1		1	
		No	0.20*	0.07, 0.58	0.57	0.34, 0.98
		Never undertake	0.21*	0.04, 0.97	0.58	0.28, 1.20
	Aware of how the disease is transmitted	Yes	1		1	
		No	0.60	0.26, 1.48	0.70	0.44, 1.11
Household	Income	Most poor	1		1	
		Poor	0.49*	0.34, 0.69	0.57	0.23, 1.36
		Least poor	0.48*	0.29, 0.58	0.74	0.40, 1.35
	Type of toilet	Sanitary	1		1	
		Outdoor	3.36	0.56, 25.28	0.95	0.35, 2.40
		Indoor	0.99	0.26, 5.27	0.70	0.41, 1.23
Variation	σ1		0.05e-03	0.01e-03, 0.79e-03	1.89e-03	1.77e-03, 2.00e-03
	σ2		-	-	2.30	2.18, 2.46
	BIC		642.05		1785.09	

A. lumbricoides, Ascaris lumbricoides; OR, odds ratio; CI, confidence interval; BIC, Bayesian information criterion. *P≤0.05.

tural activities were found to have a significantly lower risk for infection. These patterns might be explained by the fact that these individuals, due to their occupation and activities, would be more likely to have had contact with water and infected host snails. The higher prevalence among older individuals also supports this hypothesis. Nevertheless, socioeconomic status (quantified by income) was not a significant factor for *S. japonicum* infection in our study, which is not consistent with the results from previous studies (Agbo *et al.*, 1999; Steinmann *et al.*, 2007; Xu *et al.*, 2011). We observed that family members with higher household income were mainly fisherman, which might at least in part, explain the inconsistency. The non-spatial model in Table 3 shows that income and being barefoot in the fields were variables significantly associated with *A. lumbricoides* infection. A previous study indicated that poorer households were less likely to use safe drinking water and appropriate sanitation facilities (Schmidlin *et al.*, 2013). Surprisingly, we found that, for both diseases, awareness of how the disease is transmitted did not reduce infection rates.

Although mass chemotherapy is fundamental in the control of helminths, a locally adapted, feasible and sustainable control strategy must also consider the social-behavioural context (Wang *et al.*, 2009). In fact, in many parts of the world where periodic, anthelminthic treatment of millions of people is integrated with improved socioeconomic conditions, the mean infection intensity diminished (Brooker *et al.*, 2006; Fenwick, 2006; Utzinger and de Savigny, 2006). Therefore, a strategy to interrupt the helminth transmission must be based on the integration of key factors at each level. At the individual level, according to our study, fishermen and peasants should be the targeted population for such interventions encouraging self-protection, *e.g.*, wearing shoes in the field and avoid unsafe water. At the household level, improved socioeconomic status combined with improved sanitation and clean water facilities, is a crucial for the prevention of infection.

Some shortcomings of our study should be mentioned. First, when modelling *S. japonicum* risks, we used the sero-positive cases of schistosomiasis due to the very small number of stool-positive cases. Although sero-positivity does not necessarily indicate the current status of infection, it provides an estimate of the infection pressure irrespective of recent control activities (Steinmann *et al.*, 2007) and the burden of schistosomiasis in the study area. Screening methods have been frequently used in areas of low infection intensity after repeated rounds of chemotherapy (Hillyer and Soler, 1999; Hoshino-Shimizu *et al.*, 1992; Steinmann *et al.*, 2007). Second, even the parasitological test is not perfect. We used only the Kato-Katz method when screening stool samples although utilisation of 2-3 methods is recommended (Steinmann *et al.*, 2008).

Conclusions

The Bayesian multi-level model is a useful approach to capture the hierarchical nature of our epidemiological data and identify the social and behavioural determinants of infection at the local scale. Our risk analyses on single-species infections should contribute to designing effective control and mitigation strategies to fight helminthiases at the local scale. Although results from previous reports from other parts of China (Ellis *et al*., 2007; Steinmann *et al*., 2008) show that multiparasitism is common, it is currently rare in the study area.

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