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Multiparasitism and intensity of helminth infections in relation to symptoms and nutritional status among children: A cross-sectional study in southern Lao People's Democratic Republic

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A B S T R A C T

The occurrence and spatial distribution of intestinal helminth infection in children is fairly well understood. However, knowledge on how helminth infections govern intestinal morbidity is scarce. We conducted a cross-sectional study to assess and quantify the relationship between single and multiple species helminth infection with clinical and self-reported morbidity indicators and nutritional status in Champasack province, southern Lao People's Democratic Republic (Lao PDR). A random sample of 1313 children, aged 6 months to 12 years, from villages in nine rural districts were enrolled and examined for helminth infection using duplicate Kato-Katz thick smears. Morbidity was assessed by self-reported symptoms, coupled with clinical examination and appraisal of nutritional status and anaemia. Bivariate and multivariate logistic regression was employed to study associations between helminth infection and morbidity indicators and anaemia. We found considerable morbidity among the surveyed children, including hepatomegaly (13.7%), pale conjunctiva (13.2%) and abdominal pain (10.4%). Anaemia was recorded in 60.4% of the children, whilst signs of stunting and low body mass index (BMI) were observed in 49.8% and 33.3% of the surveyed children, respectively. Hookworm and *Opisthorchis viverrini* were the predominant helminth species with prevalences of 51.0% and 43.3%, respectively. The prevalence of *Schistosoma mekongi* in the surveyed children was 5.6%. Multiple species helminth infections were recorded in 40.4% of the study cohort. Morbidity was associated with specific helminth species infection (e.g. *S. mekongi* with hepatomegaly; adjusted odds ratio (aOR): 9.49, 95% confidence interval (CI): 2.07–43.51) and multiparasitism (e.g. two or more helminth species with abdominal pain; aOR: 2.40, 95% CI: 1.46–3.93). Anaemia was associated with hookworm infection (aOR: 1.64, 95% CI: 1.16–2.34) and multiparasitism (aOR: 1.64, 95% CI: 1.18–2.29). Low BMI was associated with *O. viverrini* infection (aOR: 1.68, 95% CI: 1.14–2.49) and multiparasitism (aOR: 1.42, 95% CI: 1.01–2.00). The multiple strong associations reported here between helminth infections (single or multiple species) and intestinal morbidity among children in rural parts of southern Lao PDR call for concerted efforts to control helminth infections, which in turn might improve children's health and development.

1. Introduction

Parasitic worm infections, including soil-transmitted helminths (STHs), food-borne trematodes (FBTs) and *Schistosoma* spp., remain important public health issues in low- and middle-income countries in the tropics and subtropics (Colley et al., 2014; Fürst et al., 2012b; Murray et al., 2012; Pullan et al., 2014). More than 5 billion people are at risk of helminthiases, an estimated 2 billion are infected and hundreds of millions suffer from some kind of morbidity (Hotez et al., 2008; Pullan and Brooker, 2012; Utzinger et al., 2012). The occurrence of morbidity varies from individual to individual and is related to the type, number and intensity of helminth species and host factors (e.g. school-aged children and pregnant women are of particular risk of morbidity) (Hall et al., 2008; Quinnell, 2003). In children, chronic helminth infections may cause a number of negative health outcomes, such as impaired physical and cognitive development (Al-Mekhlafi et al., 2005; Bundy et al., 2009; Fürst et al., 2012a,b; Oninla et al., 2010). Additionally,

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helminth infections are aetiological factors of anaemia and protein-energy malnutrition, which account for half of child mortality in developing countries (Al-Mekhlafi et al., 2005; Oninla et al., 2010).

In Lao People's Democratic Republic (Lao PDR) helminthiasis are highly endemic with distinct spatial patterns depending on the helminth species. For example, STHs (i.e. *Ascaris lumbricoides*, hookworm and *Trichuris trichiura*) show highest prevalence rates in the northern provinces (Rim et al., 2003), *Opisthorchis viverrini* and other FBTs are particularly prevalent in the lowlands in the Mekong River basin (Forrer et al., 2012; Phongluxa et al., 2013; Sayasone et al., 2007, 2011) and *Schistosoma mekongi* is restricted to the lower Mekong River basin (Lao-Cambodian border) (Muth et al., 2010; Sayasone et al., 2011). In many provinces, the distributions of various helminth species are geographically overlapping, and hence, multiple species helminth infections are a common phenomenon (Phongluxa et al., 2013; Sayasone et al., 2011). Multiparasitism is of concern, as multiple species helminth infections can exacerbate morbidity (Ezeamama et al., 2005; Steinmann et al., 2010). However, data pertaining to morbidity associated with single and multiple species helminth infections and intensity are scarce in resource-constrained settings, including Lao PDR.

We conducted a cross-sectional parasitological, clinical and questionnaire survey focussing on children aged between 6 months and 12 years in nine rural districts of Champasack province in the southern part of Lao PDR where STHs, FBTs and *S. mekongi* co-exist. Our aim was to assess and quantify the relationship between single and multiple species helminth infections with clinical and self-reported morbidity indicators and nutritional status.

2. Materials and methods

2.1. Ethics statement

Ethical approval for the current study was obtained from the Ministry of Health (MOH) of Lao PDR (reference no. 027/NECHR) and the ethics committee of Basel, Switzerland (EKBB; reference no. 255/06). Permission for field and laboratory work was obtained from the MOH, the Provincial Health Office (PHO) and the District Health Office (DHO). Village meetings were held and local authorities and inhabitants were given detailed explanations about the objectives, procedures and potential risks and benefit of the study. An information sheet in the local language was read aloud to parents in the presence of their children and sufficient time was given to pose any kind of questions related to the study. Parents were asked for written informed consent and a local witness observing this procedure also signed the consent form. Participation was voluntary, and hence, parents could withdraw their children from the study anytime without further obligations. Children found positive for any intestinal helminth infection were treated according to national guidelines (MOH, 2004).

2.2. Study area

The study was carried out in Champasack, the largest province of southern Lao PDR, covering an area of 15,415 km^2, stretching from 13°55′ to 15°29′ N latitude and from 105°11′ to 106°46′ E longitude. According to the 2005 census, the total population of Champasack province was 603,370 inhabitants (Census 2005, Lao Statistics Bureau, Vientiane; www.nsc.gov.la). The study was carried out in all nine rural districts of Champasack province (i.e. districts of Bachieng, Champasack, Khong, Mounlapamok, Paksong, Pathoumphone, Phonthong, Sanasomboun and Soukhouma). Of note, helminth infections, including multiparasitism (Chai et al., 2005; Eom et al., 2014; Sayasone et al., 2009) and the prevalence and extent of malnutrition in Champasack province are similar to other rural parts of Lao PDR (Kamiya, 2011).

2.3. Study design and population

Epidemiological data were collected during a cross-sectional, community-based survey carried out between January and May 2007 in Champasack province (Forrer et al., 2012). We employed a two-stage sampling method. In a first step, 54 villages were randomly selected. Second, in each village 15–20 households were selected at random. All children of the selected household aged between 6 months and 12 years were invited to participate.

2.4. Field and laboratory procedures

Data on behaviour (e.g. food consumption and hygiene) and medical history were obtained from each child with assistance of parents or legal guardians using a pre-tested questionnaire. For children aged <6 years, only parents or legal guardians were interviewed. Older children (up to 12 years) participated during the interviews with their parents or caregivers. A household questionnaire was administered to heads of household to obtain socioeconomic data, such as access to clean water and sanitation, house construction material, and ownership of household assets and livestock.

Anthropometric data were collected from all enrolled children. Exact child age was obtained from birth records and other official documents (e.g. vaccination card, vaccination book registry and household registry book). Children's weight was measured using a digital scale (Tanita Corporation; Tokyo, Japan). A child was weighed bare-foot wearing light clothes to the nearest 0.1 kg. Height was measured using a measuring board to the nearest 0.1 cm. Children aged below 2 years were measured in supine position.

Study participants provided a single stool sample. Within 2 h of stool collection, duplicate Kato-Katz thick smears, using 41.7 mg templates, were prepared, adhering to standard protocols (Katz et al., 1972; Yap et al., 2012). In brief, Kato-Katz thick smears were allowed to clear for 30 min before examination under a light microscope by experienced laboratory technicians. Helminth eggs were counted and recorded for each species separately. For quality control, 10% of the Kato-Katz thick smears were re-examined by a senior technician. Helminth species-specific egg counts were compared with the original readings and, whenever discrepancies were observed (i.e. negative versus positive result; helminth egg counts differing by more than 10%), the slides were read a third time and results discussed until agreement was reached.

A fingerpick blood sample was taken from each child and assessed for haemoglobin (Hb), using a B-haemoglobin photometer (Hemocue AB; Angelholm, Sweden). Morbidity data were obtained from each participant by recall of symptoms within 2 weeks prior to the survey (e.g. abdominal bloating, abdominal pain, bloody diarrhoea and watery diarrhoea) and by clinical examination (e.g. big belly, pale sub-conjunctiva, tenderness of abdomen, hepatomegaly and splenomegaly). Clinical examinations were conducted by an experienced physician. Hepatomegaly was determined by palpating the left liver lobe along the xiphoid-umbilicus line in the supine position. It was classified as absent or present when the left liver lobe was not palpable or palpable, accordingly (Biays et al., 1999). Splenomegaly was classified using the Hackett score (Hackett, 1944).

2.5. Statistical analysis

Survey data were double-entered and cross-checked using EpiData 3.1 (EpiData Association; Odense, Denmark). Only those

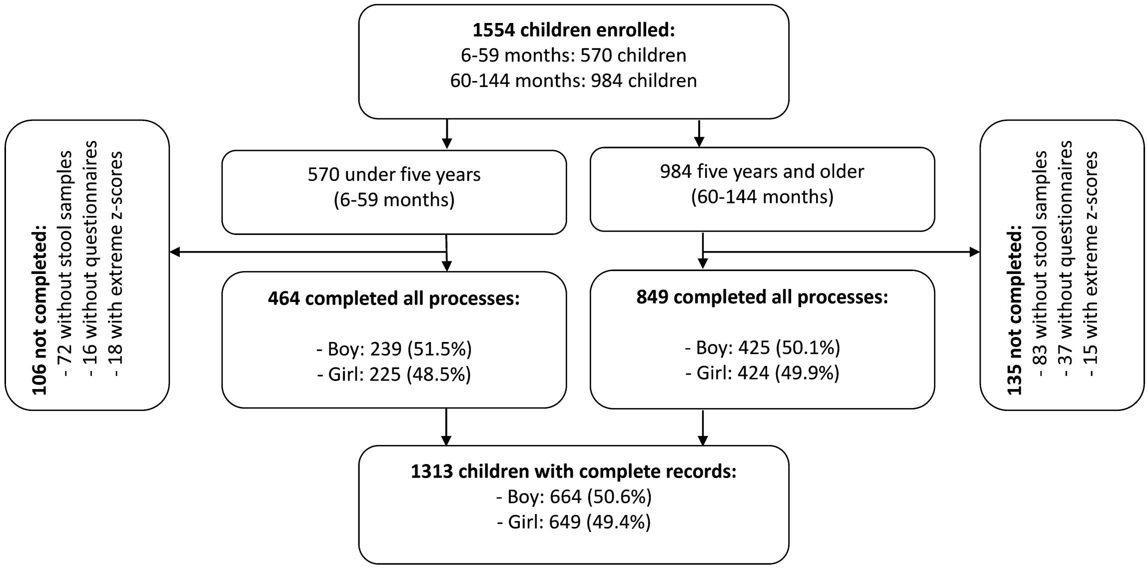

Fig. 1. Children's compliance of survey in nine districts of Champasack province, southern Lao PDR, 2007.

children with complete data records were included in the final analysis. For summary statistics, children were classified into two age groups (i.e. 6 months to 5 years and >5 years to 12 years).

Anaemia was determined according to age-specific Hb levels using World Health Organization (WHO) cut-offs (WHO, 2014). Children aged <5 years were considered anaemic if their Hb levels were below 11.0 g/dl, whilst for older children up to 12 years of age, the cut-off was 11.5 g/dl.

Helminth infection intensities were classified into light, moderate and heavy, according to eggs per 1 g of stool (EPG) and thresholds put forward by Maleewong et al. (1992) and WHO guidelines (WHO, 2005). In brief, light, moderate and heavy hookworm infections were 1–1999 EPG, 2000–3999 EPG and ≥4000 EPG; whilst the respective cut-offs for *A. lumbricoides* were 1–4999 EPG, 5000–49,999 EPG and ≥50,000 EPG, those of *T. trichiura* and *O. viverrini* were 1–999 EPG, 1000–9999 EPG and ≥10,000 EPG, and *S. mekongi* intensity classes were 1–100 EPG, 101–399 EPG and ≥400 EPG. Additionally, children were grouped into five helminth infection/intensity classes, as follows: (i) no infection; (ii) any single helminth species infection with light intensity; (iii) any single helminth species infection with moderate or heavy intensity; (iv) any multiparasitism with light intensity; and (v) any multiparasitism with moderate or heavy intensity.

Anthropometric data were calculated in STATA version 10.1 (Stata Corporation; College Station, TX, USA) using a readily available macro-statistical package developed by a technical group of WHO (2007). The WHO child growth standards 2007 for under-five children and children aged 5–19 years (merged data NCHS/WHO) were used as references (de Onis et al., 2007). The following four anthropometric indicators were considered (i) weight-for-height (wasting); (ii) height-for-age (stunting); (iii) weight-for-age (underweight); and (iv) body mass index (BMI) for age. A child was defined as being undernourished when z-score measurement of these indicators were below −2 standard deviations (SDs) of the population reference (Alom et al., 2012; de Onis et al., 2007).

House construction material and ownership of household assets and livestock were used to build an asset-based socioeconomic index, using principal component analysis (PCA). This approach has been widely and successfully used to construct household socioeconomic status (see, for example, Forrer et al., 2012). Households were classified in five wealth quintiles, the first quintile corresponding to the poorest and the fifth to the least poor population group.

We used χ^2 statistics to compare proportions. Associations between morbidity indicators (outcome) and helminth infection status (predictor) were assessed by using (i) a bivariate logistic regressions and (ii) a multivariable logistic regression analysis (stepwise backwards elimination), adjusted for sex, age-group, socioeconomic status, nutritional status and personal hygiene. Crude odds ratio (cOR) and adjusted odds ratio (aOR) and corresponding 95% confidence intervals (CIs) were calculated. Variables exhibiting an association at a significance level of 0.15 were included in multivariate logistic regression models. Multiple linear regression models were used to associate helminth infections with mean Hb levels as previously performed (Degarege et al., 2014). Statistical significance was defined as *P* value <0.05.

3. Results

3.1. Compliance and population characteristics

A total of 1554 children aged between 6 months and 12 years were enrolled (Fig. 1). Among them, 241 (14.6%) failed to submit a stool sample, were absent during the clinical examination and questionnaire survey, or had extreme z-score values (too low or high according to WHO definitions). The remaining 1313 children had completed all study procedures, and hence were retained for the final analysis. There were 649 (49.4%) girls and 664 (50.6%) boys. In terms of age, 464 (35.3%) of the children were below 5 years, whilst the remaining 849 children were older, up to 12 years.

3.2. Helminth infections

Table 1 summarises children's helminth infection status, stratified by sex and two age groups. Hookworm was the predominant species (51.0%), followed by *O. viverrini* (43.3%), *A. lumbricoides* (16.8%), *T. trichiura* (10.7%), *S. mekongi* (5.6%) and *Taenia* spp. (1.2%). 307 children were free of any helminth infection (23.4%), 476 children (36.2%) harboured a single helminth species and the remaining 530 children (40.4%) were infected with at least two and up to five helminth species concurrently. The following helminth infection intensities profiles were observed: single infection with light infection intensities (27.5%), single infection with moderate and heavy infection intensities (8.8%), multiparasitism with light

Table 1
Situation of helminth infections among 1313 children of Champasack province, southern Lao PDR in 2007, stratified by sex and age group.

Infections	Overall	Sex				Age group			
		Females	Males	χ^2	*P*-value	6 months–5 years	5–12 years	χ^2	*P*-value
All infections									
Opisthorchis viverrini	569 (43.3)	277 (42.7)	292 (44.0)	0.22	0.636	117 (25.2)	452 (53.2)	95.95	<0.001
Schistosoma mekongi	74 (5.6)	41 (6.3)	33 (5.0)	1.12	0.290	21 (4.5)	53 (6.2)	1.66	0.197
Hookworm	670 (51.0)	323 (49.8)	347 (52.3)	0.81	0.367	194 (41.8)	476 (56.1)	24.40	<0.001
Ascaris lumbricoides	221 (16.8)	109 (16.8)	112 (16.8)	0.01	0.970	90 (19.4)	131 (15.4)	3.37	0.066
Trichuris trichiura	140 (10.7)	71 (10.9)	69 (10.4)	0.10	0.748	37 (8.0)	103 (12.1)	5.44	0.020
Taenia spp.	16 (1.2)	9 (1.4)	7 (1.1)	0.30	0.583	2 (0.4)	14 (1.7)	3.70	0.054
Individual infection									
Negative	307 (23.4)	155 (23.9)	152 (22.9)			173 (37.3)	134 (15.8)		
Only *Opisthorchis viverrini*	209 (15.9)	106 (16.4)	103 (15.5)			54 (11.6)	154 (18.3)		
Only *Schistosoma mekongi*	9 (0.7)	6 (0.9)	3 (0.5)			5 (1.1)	4 (0.5)		
Only hookworm	193 (14.7)	103 (15.9)	90 (13.6)			79 (17.0)	114 (13.4)		
Only *Ascaris lumbricoides*	58 (4.5)	25 (3.9)	33 (5.0)			24 (5.2)	34 (4.0)		
Only *Trichuris trichiura*	7 (0.5)	2 (0.3)	5 (0.8)			3 (0.7)	4 (0.5)		
Any multiparasitism	530 (40.4)	252 (41.9)	278 (38.8)	5.75	0.452	126 (27.2)	404 (47.6)	103.5	<0.001
Number of helminth species									
Single species	476 (36.3)	242 (37.3)	234 (35.2)			165 (35.6)	311 (36.6)		
Two species	393 (29.9)	179 (27.6)	213 (32.1)			87 (18.8)	305 (35.9)		
Three or more species	138 (10.5)	73 (11.3)	65 (9.8)	3.61	0.307	39 (8.4)	99 (11.7)	92.40	<0.001
Intensity of infections									
Any single helminth species with light	331 (25.2)	176 (27.1)	155 (23.3)			108 (23.3)	223 (26.3)		
Any single helminth species with moderate+	145 (11.0)	66 (10.2)	79 (11.9)			57 (12.3)	88 (10.4)		
Any multiparasitism with light	219 (16.7)	98 (15.1)	121 (18.2)			26 (5.6)	193 (22.7)		
Any multiparasitism with moderate+	311 (23.7)	154 (23.7)	157 (23.6)	4.99	0.288	100 (21.6)	211 (24.9)	113.61	<0.001

P-value calculated from χ^2–test

infection intensities (16.6%) and multiparasitism with moderate and heavy infection intensities (23.7%).

3.3. Clinical assessments

Table 2 shows the frequency of reported symptoms, stratified by sex and age group. The most frequently reported symptom was abdominal pain (10.4%). Abdominal bloating and watery diarrhoea were reported by 4.0% of the children each, whilst bloody diarrhoea was reported by 1.2% of the children. There was no significant sex difference in self-reported morbidity indicators. Abdominal bloating and watery diarrhoea were reported significantly more often in children aged 6 months to 5 years compared to their older counterparts (both $P < 0.001$). Hepatomegaly (13.7%) was the most frequent clinical sign diagnosed in the physical examination, followed by pale sub-conjunctiva (13.2%), "big belly" (6.9%), splenomegaly (4.3%) and tenderness of abdomen upon palpation (1.0%). "Big bellies" were significantly more often diagnosed in the younger age group compared to children aged above 5 years.

3.4. Anaemia and nutritional status

Overall, 793 children (60.4%) were found anaemic with no significant difference by sex (males: 62.1% versus females: 58.7%, $P = 0.216$) and age group (6 month to 5 years: 60.3% versus 5–12 years: 60.4%, $P = 0.978$). Table 3 summarises the nutritional status of children examined, stratified by sex and age group. Overall, high prevalences of stunting (49.4%), underweight (47.9%) and low BMI for age (33.3%) were observed. Wasting was determined in 10.7% of children surveyed. We did not observe any significant difference between boys and girls. However, children aged 5–12 years had a significantly lower nutritional status than their younger counterparts (e.g. stunting: 51.1% versus 46.3%, $P = 0.048$; underweight: 54.8% versus 35.3%, $P = 0.001$; low BMI for age: 44.8% versus 12.3%, $P < 0.001$). The opposite trend was observed for wasting (9.5% versus 13.6%, $P = 0.025$).

3.5. Predictors of clinical outcomes

Stepwise multiple logistic regression analysis revealed that helminth infections (e.g. single and multiple species infection and different infection intensity profiles) were significant predictors for different morbidity indicators. Table 4 summarises identified associations between helminth infection and morbidity indicators. Of particular note is that *S. mekongi* infection showed significant associations with palpable hepatomegaly (aOR: 9.49, 95% CI: 2.07–43.51), splenomegaly (aOR: 53.7, 95% CI: 6.44–448.79), self-reported bloody diarrhoea (aOR: 23.33, 95% CI: 4.04–134.66) and pale sub-conjunctiva (aOR: 6.13, 95% CI: 1.44–26.19). Similarly, multiple species helminth infection was associated with hepatomegaly and splenomegaly with a trend for higher aORs the higher the intensity of multiple species helminth infections.

None of the other single species helminth infection was significantly associated with morbidity indicators, except for a positive association between hookworm infection and pale sub-conjunctiva (aOR: 1.91, 95% CI: 1.21–3.01). Multiple species helminth infection showed strong associations with morbidity (e.g. any multiple species helminth infection with moderate and high infection intensity was significantly associated with reported watery diarrhoea (aOR: 2.35, 95% CI: 1.26–4.39), abdominal bloating (aOR: 2.00, 95% CI: 1.09–3.67) and abdominal pain (aOR: 1.84, 95% CI: 1.26–2.67).

The presence of palpable hepatomegaly in children showed a strong association with any multiple species helminth infection (aOR: 3.67, 95% CI: 2.49–5.40). The association was particularly strong between any multiple species helminth infection with moderate and heavy infection intensity (aOR: 7.91, 95% CI: 5.40–11.60). The presence of palpable splenomegaly was strongly associated

Table 2
Frequency of clinical outcomes obtained from physical examination of study children in Champasack province, southern Lao PDR in 2007, stratified by sex and age group ($n = 1313$).

Clinical outcomes	Overall	Sex				Age group (years)			
	n (%)	Girls, *n* (%)	Boys, *n* (%)	χ^2	*P*-value	<5, *n* (%)	5–12, *n* (%)	χ^2	*P*-value
Symptoms in past two weeks									
Abdominal pain	137 (10.4)	69 (10.6)	68 (10.2)	0.05	0.817	57 (12.3)	80 (9.4)	2.63	0.105
Abdominal bloating	52 (4.0)	26 (4.1)	26 (3.9)	0.01	0.933	30 (6.5)	22 (2.6)	11.84	0.001
Watery diarrhoea	52 (4.0)	22 (3.5)	30 (4.5)	1.10	0.295	31 (6.7)	21 (2.5)	13.96	<0.001
Bloody diarrhoea	16 (1.2)	9 (1.4)	7 (1.1)	0.30	0.583	8 (1.7)	8 (0.9)	1.52	0.217
Physical examinations									
Hepatomegaly	180 (13.7)	88 (13.6)	92 (13.9)	0.02	0.876	64 (13.8)	116 (13.7)	0.01	0.948
Pale sub-conjunctiva	173 (13.2)	74 (11.4)	99 (14.9)	3.55	0.060	72 (15.5)	101 (11.9)	3.44	0.064
Big belly	90 (6.9)	50 (7.5)	40 (6.2)	0.96	0.327	56 (12.1)	34 (4.00)	30.56	<0.001
Splenomegaly*	57 (4.3)	31 (4.3)	26 (3.9)	0.59	0.444	17 (3.7)	40 (4.7)	0.79	0.373
Tenderness of abdomen	13 (1.0)	6 (0.9)	7 (1.1)	0.06	0.812	2 (0.4)	11 (1.3)	2.29	0.130

P-value obtained from Chi-square test.
* Splenomegaly: Hackett score ≥2.

with multiple species helminth infection (aOR: 14.13, 95% CI: 3.39–58.86) and any multiple species helminth infection with moderate or heavy infection intensity (aOR: 10.41, 95% CI: 4.73–22.92).

3.6. *Predictors of nutritional status and anaemia*

Table 5 summarises the main associations between helminth infection and nutritional indicators, readily adjusted for potential predictors, such as sex, age group, personal hygiene, socioeconomic status and recent history of illness, as obtained from stepwise multivariable logistic regression analysis. The presence of anaemia showed a strong association with helminth infection. For example, *S. mekongi* infection (aOR: 1.52, 95% CI: 1.08–2.15), hookworm infection (aOR: 1.64, 95% CI: 1.16–2.34) and multiple species helminth infection (two helminth species infection, aOR: 1.65, 95% CI: 1.24–2.20; three or more helminth species infections, aOR: 1.53, 95% CI: 1.15–2.04) were significantly associated with anaemia. With regard to malnutrition, low BMI for age was significantly associated with multiple species helminth infection (two helminth species infection, aOR: 1.40, 95% CI: 1.01–1.94; three or more helminth species, aOR: 1.45, 95% CI, 1.03–2.04) and helminth infection intensity (any multiparasitism with light infections, aOR: 1.42, 95% CI: 1.01–2.00).

Other nutritional indicators such as stunting and wasting were not associated with any of the helminth infection intensity profiles. The presence of stunting in a child was significantly associated with low socioeconomic status and wasting was associated with self-reported watery diarrhoea (aOR: 2.58, 95% CI: 1.07–5.84) and bloody diarrhoea (aOR: 3.99, 95% CI: 1.10–14.50).

Linear regression analysis, adjusted for age group, sex, weight and height, showed that the mean Hb level was significantly lower in children who harboured a single helminth species compared to non-infected children (i.e. for *S. mekongi*, coefficient: −1.42, 95% CI: −2.35 to −0.48; for hookworm, coefficient: −0.33, 95% CI: −0.58 to −0.07; Fig. 2a). Regarding helminth infection intensity profiles, the linear regression model revealed a lower mean Hb level in children who had positive stool examination compared to non-infected children (Fig. 2b). Single infections with moderate or heavy infection intensities were significantly associated with a lower mean Hb level (coefficient: −0.32, 95% CI: −0.60 to −0.04; Fig. 2b).

4. Discussion

In Lao PDR, helminthiases remain issues of considerable public health relevance, at local, provincial and national level. Single and multiple species helminth infections and associated morbidity patterns are governed by social-ecological systems. Over the past 15 years, data have been accumulated regarding people's infection status with individual helminth species and their spatial distribution at different scales (Eom et al., 2014; Forrer et al., 2012; Rim et al., 2003; Sayasone et al., 2011). However, the current knowledge on morbidities due to specific helminth infections, and particularly concurrent infections with multiple helminth species in the same host, is scarce. In a previous study, we have reported on hepatobiliary excess morbidity associated with concurrent *S. mekongi*–*O. viverrini* infection in adults in southern Lao PDR (Sayasone et al., 2012). Here, we further this line of scientific inquiry, as we report on the association of single and multiple species helminth infection with intestinal morbidity among children aged 6 months to 12 years.

We carried out a large cross-sectional survey in all nine rural districts of Champasack province, situated in the southern part of Lao PDR. Our aim was to deepen the understanding of how helminth infections, multiparasitism and different infection intensity profiles influence children's health and nutritional status. We employed the widely used Kato-Katz technique for the diagnosis of helminth infections (Bergquist et al., 2009; Speich et al., 2010). Additionally, children were interviewed for a recent history of illness and

Table 3
Frequency of nutritional status among 1313 children from Champasack province, southern Lao PDR in 2007, stratified by sex and age groups.

Nutritional indicators	Overall	Sex				Age group (years)			
		Girls	Boys	χ^2	*P*-value	<5	5–12	χ^2	*P*-value
Stunting (*z*-score ≤ 2)	649 (49.4)	312 (48.1)	337 (50.8)	0.94	0.332	215 (46.3)	434 (51.1)	3.71	0.048
Underweight (*z*-score ≤ 2)	629 (47.9)	310 (47.8)	319 (48.0)	0.01	0.920	164 (35.3)	465 (54.8)	45.37	0.001
Wasting (*z*-score ≤ 2)	144 (10.7)	71 (10.9)	73 (11.0)	0.01	0.975	63 (13.6)	81 (9.5)	5.01	0.025
Low BMI for age (*z*-score ≤ 2)	437 (33.3)	202 (31.1)	235 (35.4)	2.69	0.101	57 (12.3)	380 (44.8)	142.50	<0.001

P-value calculated from chi-square test.

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Table 4
Association between helminth infections and outcomes of clinical examination among 1313 study from Champasack province, southern Lao PDR in 2007, using stepwise multiple logistic regression (backwards elimination).

Morbidity indicators	Significant associations	cOR (95% CI)	aOR (95% CI)
Symptoms in past two weeks			
Watery diarrhoea	Multiple helminth species infections		
	No infection	1.00	1.00
	Any multiparasitism	1.17 (0.59–2.31)	2.35 (1.26–4.39)
	Helminth infection intensity		
	No infection	1.00	1.00
	Any multiparasitism with moderate+	1.64 (0.80–3.33)	2.40 (1.34–4.32)
Bloody diarrhoea	Single helminth species		
	No infection	1.00	1.00
	Only *S. mekongi*	14.33 (2.45–83.90)	23.33 (4.04–134.66)
Abdominal bloating	Helminth Infection intensity		
	No infection	1.00	1.00
	Any multiparasitism with moderate+	1.18 (0.60–2.35)	2.00 (1.09–3.67)
Abdominal pain	Multiple helminth species infections		
	No infection	1.00	1.00
	Two helminth species	1.34 (0.81–2.22)	1.62 (1.13–2.32)
	Three or more helminth species	2.52 (1.42–4.49)	2.40 (1.46–3.93)
	Helminth infection intensity		
	No infection	1.00	1.00
	Any multiparasitism with moderate+	1.99 (1.21–3.27)	1.84 (1.26–2.67)
Physical examinations			
Pale sub-conjunctiva	Single helminth species infection		
	No infection	1.00	1.00
	Only hookworm	1.84 (1.10–3.08)	1.91 (1.21–3.01)
	Only *S. mekongi*	4.15 (0.99–17.39)	6.13 (1.44–26.19)
	Helminth infection intensity		
	No infection	1.00	1.00
	Any multiparasitism with light	1.74 (1.05–2.88)	1.62 (1.07–2.46)
Tenderness of abdomen	Multiple helminth species infections		
	No infection	1.00	1.00
	Three or more helminth species	3.02 (0.67–13.70)	3.64 (1.10–12.12)
Hepatomegaly	Single helminth species infection		
	No infection	1.00	1.00
	Only *S. mekongi*	6.81 (1.59–29.18)	9.49 (2.07–43.51)
	Multiparasitism		
	No infection	1.00	1.00
	Two helminth species	3.06 (1.84–5.11)	2.49 (1.72–3.61)
	Three or more helminth species	6.37 (3.61–11.27)	5.36 (3.37–8.52)
	Any multiparasitism	3.82 (2.34–6.22)	3.67 (2.49–5.40)
	Helminth infection intensity		
	No infection	1.00	1.00
	Any single helminth species with moderate+	2.84 (1.53–5.26)	3.02 (1.78–5.11)
	Any multiparasitism with moderate+	6.65 (4.02–10.98)	7.91 (5.40–11.60)
Splenomegaly	Single helminth species infection		
	No infection	1.00	1.00
	Only *S. mekongi*	43.57 (5.34–355.20)	53.75 (6.44–448.79)
	Multiparasitism		
	No infection	1.00	1.00
	Two helminth species	10.39 (2.44–44.21)	20.70 (2.52–45.75)
	Three or more helminth species	22.88 (5.23–100.09)	25.12 (5.71–110.45)
	Any multiparasitism	13.47 (3.24–55.98)	14.13 (3.39–58.86)
	Helminth infection intensity		
	No infection	1.00	1.00
	Any single helminth species moderate+	6.58 (1.31–33.03)	3.41 (1.16–10.02)
	Any multiparasitism with light	6.53 (1.40–30.55)	3.46 (1.32–9.12)
	Any multiparasitism with moderate+	18.72 (4.46–78.63)	10.41 (4.73–22.92)

cOR: crude odds ratio; aOR: adjusted odds ratio.
aOR obtained from stepwise multiple logistic regression (backwards elimination), adjusted for sex, age group, socioeconomic status, nutritional status and personal hygiene.

underwent a clinical examination for appraisal of current diseases. Anthropometric data (i.e. height and weight) were determined and anaemia was assessed by means of a fingerprick sample tested for Hb level.

Our data confirm that helminth infections are highly prevalent in Champasack province. Indeed, microscopic examination of duplicate Kato-Katz thick smears showed that over three-quarter of the children were infected with at least one helminth species. Hookworm was the predominant species with slightly more than half of the children infected (51.0%). Given the high marginal prevalence of various helminth species, multiparasitism was common; two out of five children with complete data records harboured two or more helminth species concurrently. Interestingly, most of the children diagnosed with single helminth species were characterised by light infection intensity profiles (25.2%), whilst children with multiple species helminth infections showed moderate and heavy infection intensity profiles (23.7%). Helminth infections (either single or multiple species and particularly those of moderate and heavy intensities) were significantly associated with clinical morbidity, anaemia and malnutrition. Hence, findings reported here are of relevance to further clarify whether and to what extent helminth infections impact on children's health, nutritional status and overall development (Hall et al., 2008; Taylor-Robinson et al., 2012).

Table 5
Predictors of nutritional status of 1313 study children, Champasack province, southern Lao PDR in 2007, using stepwise multiple logistic regression (backwards elimination).

Morbidity indicators	Significant associations	cOR (95%, CI)	aOR (95%, CI)
Anaemia	Single helminth species infection		
	No infection	1.00	1.00
	Only *S. mekongi*	1.33 (0.97–1.82)	1.52 (1.08–2.15)
	Only hookworm	1.51 (1.09–2.09)	1.64 (1.16–2.34)
	Multiple helminth species infections		
	Two helminth species	1.48 (1.09–2.02)	1.65 (1.24–2.20)
	Three or more helminth species	1.48 (1.09–2.02)	1.53 (1.15–2.04)
	Helminth infection intensity		
	No infection	1.00	1.00
	Any multiparasitism with light	1.60 (1.13–2.38)	1.64 (1.18–2.29)
Stunting	Household socioeconomic status		
	Richest	1.00	1.00
	Poor	1.17 (0.82–1.67)	1.63 (1.09–2.44)
	Very poor	1.65 (1.17–2.32)	2.42 (1.62–3.62)
	Poorest	1.38 (0.98–1.94)	1.93 (1.29–2.87)
Wasting	Watery diarrhoea in past two weeks	1.05 (1.69–6.07)	2.58 (1.07–5.84)
	Bloody diarrhoea in past two weeks	3.79 (1.30–11.06)	3.99 (1.10–14.50)
Low BMI for age (z-score ≤ 2)	Single helminth species		
	No infection	1.00	1.00
	Only *O. viverrini*	1.68 (1.16–2.45)	1.68 (1.14–2.49)
	Multiple helminth species infections		
	No infection	1.00	1.00
	Two helminth species	1.40 (1.02–1.91)	1.40 (1.01–1.94)
	Three or more helminth species	1.41 (1.04–1.91)	1.45 (1.03–2.04)
	Intensity of infections		
	No infection	1.00	1.00
	Any multiparasitism with light	1.59 (1.10–2.31)	1.42 (1.01–2.00)
	Household socioeconomic status		
	Richest	1.00	1.00
	Poorest	1.49 (1.04–2.14)	1.58 (1.12–2.21)
	Raw vegetables	1.36 (1.05–1.76)	1.38 (1.02–1.86)

SN: small number; cOR: crude odds ratio; aOR: adjusted odds ratio.
aOR obtained from stepwise multiple logistic regression (backwards elimination), adjusted for sex, age group, personal hygiene, household socioeconomic status and recent history of illness.

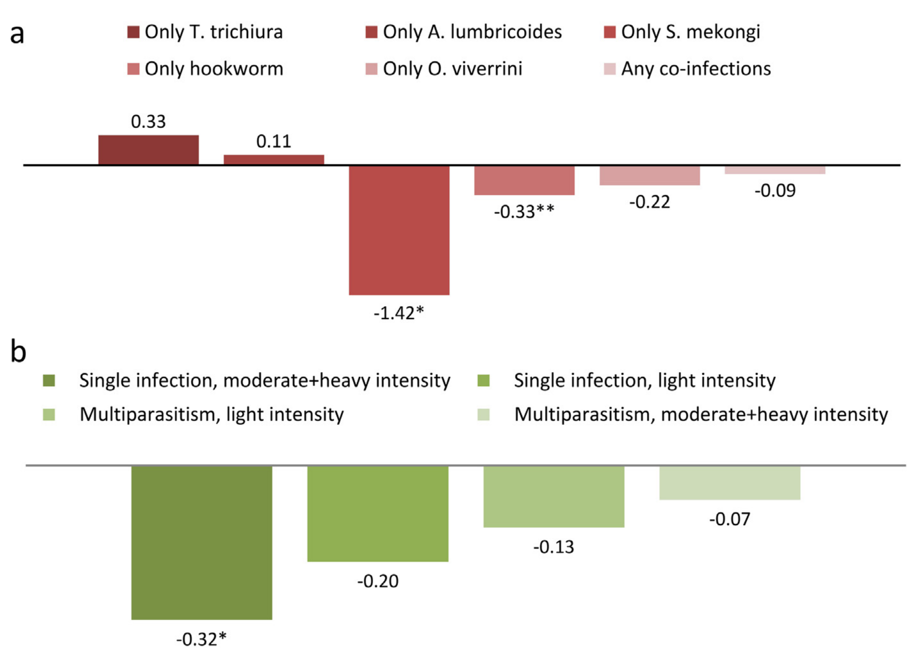

Fig. 2. (a) Differences in mean haemoglobin levels among infected compared to non-infected children ($n = 1313$), adjusted for age group, sex, weight and height. * $P = 0.003$ (95% CI: −2.35 to −0.48); ** $P = 0.012$ (95% CI: −0.58 to −0.07). (b) Coefficients of association between infection intensity and haemoglobin levels among infected compared to non-infected children ($n = 1313$), adjusted for age group, sex, weight and height. * $P = 0.027$ (95% CI: −0.60 to −0.04).

4.1. Strengths and limitations

Our study has several strengths and limitations that are offered for discussion. A major strength is the rigorous appraisal of morbidity, including self-reported morbidities (e.g. abdominal bloating, abdominal pain, bloody diarrhoea and watery diarrhoea), clinical morbidities (e.g. big belly, pale sub-conjunctiva, tenderness of abdomen, hepatomegaly and splenomegaly), quantification of Hb levels for assessment of anaemia and examination of malnutrition. An additional strength is the analytical approach taken (i.e. multi-variate analysis), which allowed for adjustment of potential confounders, such as demographic, socioeconomic and personal behavioural data.

In terms of limitations, the following issues must be emphasised. First, we employed a cross-sectional study design, and hence we cannot infer causality. Second, our diagnostic approach consisted of the collection of a single stool sample per child that was subjected to duplicate Kato-Katz thick smear examination. Clearly, collection of multiple consecutive stool samples (instead of single specimens), examination of triplicate or quadruplicate Kato-Katz thick smears per sample (instead of duplicate Kato-Katz thick smears) and use of multiple diagnostic methods (e.g. Kato-Katz plus ether-concentration techniques instead of Kato-Katz only) would have resulted in higher diagnostic accuracy (Knopp et al., 2008; Lovis et al., 2012; Steinmann et al., 2008). Third, we did not assess children's daily food consumption patterns. However, such patterns play an important role in children's malnutrition, because consumption of a diverse diet including animal meat products are associated with lower odds of stunting and underweight, as shown in neighbouring Cambodia (Darapheak et al., 2013). Fourth, anaemia is the consequence of multiple causes, which interact with each other in a complex manner. Among these factors are the inherited haemoglobinopathies of primary importance in Lao settings (Savongsy et al., 2008; Tritipsombut et al., 2012). It must be noted that using a haemoglobin photometer for Hb measurement, the identification of the exact type of anaemia and the fraction of iron-deficiency anaemia is not possible. Only the latter is associated with helminth infections (Brooker et al., 2008; Ngui et al., 2012). Fifth, we made no attempts to diagnose for other concurrent infections, such as *Plasmodium* spp. (the causative agent for malaria), bacteria and viruses. Yet, infections with these pathogens and co-infection with helminths could have affected children's nutritional status, self-reported morbidity and general health (Becker et al., 2013; Manning et al., 2012; Mondal et al., 2012). However, given the large number of children examined and limited human and financial resources for the current study, it was operationally not feasible to diagnose all these infections.

4.2. Policy implications

Our findings have important bearings on policy. First, the high rates of specific helminth infections (i.e. hookworm and *O. viverrini*) and the extent of multiparasitism at the time this study was carried out in the southern part of Lao PDR is alarming. Our findings are in line with previous reports (Chai et al., 2007; Eom et al., 2014; Sayasone et al., 2009, 2011), which call for urgent public health measures to remedy this situation. Our observations and results reported by other investigators before provide a strong rationale for scaling-up helminthiases control measures in Lao PDR and Champasack province in particular. In fact, the deworming programmes targeting school-aged children and more recently preschool-aged children were initiated in 2005, but Champasack province was only included in the late 2007, hence, just after the launch of the current study (Phommasack et al., 2008). In the meantime, the deworming programme has gone to scale (Lao PDR, 2014; Phommasack et al., 2008). It will be interesting to reassess the helminthiasis situation nowadays. Repeated rounds of deworming might have improved children's health status, particularly in children under the age of 5 years, who are at highest risk of morbidity (Bryce et al., 2005). In addition, most recently, several pilot interventions focussing on multiple parasitic infections and using ecohealth approaches were implemented. The results of their evaluation will contribute to the wider discussion how multi-parasitic infection is best addressed.

Second, our findings showed that a single helminth species infection negatively affects the health of children. *S. mekongi* infection was significantly associated with reported bloody diarrhoeal episodes, pale sub-conjunctiva, hepatomegaly and splenomegaly obtained from clinical examination. Furthermore, our models revealed the significantly lower mean of Hb among children, who had hookworm, *O. viverrini* and *S. mekongi* infection, compared to non-infected children. These findings are in agreement with previous reports published elsewhere (Degarege et al., 2014; Righetti et al., 2013). Indeed, hookworm infection and schistosomiasis cause chronic intestine inflammation with continued intestinal blood loss (Gyorkos et al., 2011; Larocque et al., 2005; Ngui et al., 2012; Righetti et al., 2013) and are thus drivers of anaemia (Chen et al., 2012; Ngui et al., 2012; Pasricha et al., 2008).

Third, multiparasitism has previously been shown to be a potential cause for child anaemia and malnutrition. Even with light infection intensity, the impact on health is considerable (Bustinduy et al., 2013; Degarege et al., 2014). In this study, the results confirm that multiple helminth infections concurrently were significant predictors for occurrence of clinical morbidities such as watery diarrhoeal episodes, abdominal discomforts, hepatomegaly and splenomegaly. Moreover, they were also significantly associated with anaemia and a low BMI for age among the study children. However, no statistically positive association between helminth infections and stunting, underweight as well as wasting has been found in this study. Similar findings of not statistically association between helminth infections and malnutrition have been previously observed elsewhere in Nigeria (Oninla et al., 2010) and in Kenya (Bustinduy et al., 2013). It is widely acknowledged that in developing countries, malnutrition is multi-factorial and it is difficult to establish its causal link (Bustinduy et al., 2013; Oninla et al., 2010). In the present study, we found that the socioeconomic status of family predicted significantly a child's stunting and the occurrence of watery and bloody diarrhoeal episodes were predictors of wasting.

5. Conclusion

We conclude that helminth infections and malnutrition are highly prevalent among children living in the rural districts of Champasack province, and most likely elsewhere in Lao PDR. Helminth infections and multiparasitism are significantly associated with observed morbidities. We believe that these morbidities can be resolved if adequate control measures are in place such as health education, coupled with periodic de-worming, micronutrient supplementation, improving access to clean water and sanitation.

Author contributions

SS and PO conceived and developed the study; SS analysed the data and interpreted results together with PO; SS and PO drafted the manuscript; JU and KA assisted with manuscript revision; all authors read and approved the final submitted manuscript; SS and PO are guarantors of the paper.

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