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Controlling *Taenia solium* and soil transmitted helminths in a northern Lao PDR village: Impact of a triple dose albendazole regime

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ABSTRACT

Taenia solium taeniasis-cysticercosis and soil-transmitted helminths (STHs) are parasitic Neglected Tropical Diseases endemic throughout Southeast Asia. Within Lao PDR, a remote northern hill tribe village had previously been identified as a hyper endemic focus for *T. solium*. To reduce this observed prevalence, a One Health intervention covering both pigs and humans was implemented, which included two Mass drug administrations (MDA1 and MDA2) for village residents using a triple dose albendazole 400 mg treatment regime. In addition to the effect on *T. solium* levels, the dual impact of this anthelmintic regime on STHs within the community was also monitored.

Faecal samples were collected pre and post MDA1 and MDA2 and analysed for the presence of *Taenia* species and the STHs *Ascaris lumbricoides*, *Trichuris trichiura* and hookworm species. The McMaster technique was used to measure the changes in both prevalence and intensity of infection. Molecular characterisation of *Taenia* and hookworm species was conducted to detect zoonotic species.

The level of taeniasis within the sampled population decreased by 79.4% after MDA1, remained steady during the five month inter-treatment interval and decreased again by 100% after MDA2. The prevalence of STHs decreased by 65.5% and 62.8% after MDA1 and MDA2 respectively; however an increase to 62.1% of pre MDA1 levels was detected during the inter-treatment interval. Individually, hookworm prevalence decreased by 83.4% (MDA1) and 84.5% (MDA2), *A. lumbricoides* by 95.6% and 93.5% and *T. trichiura* by 69.2% and 61%. The intensity of infection within the sampled population also decreased, with egg reduction rates of 94.4% and 97.8% for hookworm, 99.4% and 99.3% for *A. lumbricoides* and 77.2% and 88.5% for *T. trichiura*. Molecular characterisation identified a *T. solium* tapeworm carrier from 21.6% (13/60) of households in the village. *T. saginata* was identified in 5% (3/60) of households. The zoonotic hookworm *A. ceylanicum* was detected in the resident dog population.

These results suggest that the triple dose albendazole 400 mg treatment regime achieved a significant reduction in the level of taeniasis whilst simultaneously reducing the STH burden within the village. The increased STH prevalence detected between MDAs reflects the need for behavioural changes and a sustained chemotherapy programme, which may also need to include the resident dog population.

1. Introduction

Taenia solium taeniasis-cysticercosis and soil-transmitted helminths (STHs) are parasitic Neglected Tropical Diseases (NTDs) endemic throughout Southeast Asia (World Health Organisation, 2012). The conditions for their transmission include sub-optimal hygiene and sanitation and close contact with animal reservoirs, all of which are common to developing country contexts worldwide.

T. solium has been reported in China, Nepal, India, Philippines, Indonesia, Thailand, Cambodia, Vietnam and Lao PDR (Conlan et al., 2008; Willingham et al., 2010). Programmes targeting *T. solium* have not been successful in eliminating the parasite from any region within Southeast Asia, despite the necessary control tools being

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available (Willingham et al., 2010). One of the main reasons cited for this failure is the lack of sufficient integrated surveillance in both the human and animal populations (Willingham et al., 2010). Pigs are the natural intermediate host, harbouring encysted *T. solium* larvae in the musculature (cysticercosis); therefore surveillance of pig carcasses in slaughterhouses is vital to disease control. While humans harbour the adult tapeworm, determining the infection as *T. solium* (as opposed to the non-pathogenic *T. saginata* and *T. asiatica* human tapeworm species) requires morphological identification of expelled adult worms or molecular tools, both of which are not regularly available in ongoing, large-scale surveillance programmes. A significant human health risk is posed when people accidently ingest *T. solium* eggs, either through auto-infection or from contaminated environments as a result of poor hygiene and sanitation. In this case, the larval form of the parasite may develop, often with a predilection for the brain. This results in neurocysticercosis, a major cause of acquired epilepsy in developing countries (García et al., 2003). A patient with a single adult *T. solium* tapeworm can release thousands of eggs into the environment, a potential source of neurocysticercosis to both themselves and others in close contact. Consequently, identification and treatment of taeniasis patients is of great importance, and subsequent intervention programmes which attempt to break the transmission cycle will require a One Health approach targeting both humans and pigs within those environments.

Similarly, STH infections are endemic throughout Asia; this region holds 67% of the global infection, currently estimated to occur in around 1 billion people worldwide (De Silva et al., 2003; Hotez et al., 2008). The most common human STHs include roundworms (*Ascaris lumbricoides*), whipworms (*Trichuris trichiura*) and the hookworms (*Necator americanus* and *Ancylostoma duodenale*). Individually, infection with any of these STHs can have a detrimental effect on human health; however the impact of infection increases where multiple species are present; a commonly occurring syndrome called polyparasitism (Conlan et al., 2012; Lymbery and Thompson, 2012). The use of chemotherapy is the cornerstone to controlling parasitic infections within human populations; however evidence of sustained control in endemic regions is still limited. A combination of factors such as sociocultural issues around MDA compliance and ongoing environmental contamination from human and animal reservoirs can significantly reduce the efficacy of these programmes (Parker and Allen, 2014; Heukelbach et al., 2002). For instance within Southeast Asia, dogs have been found to harbour the zoonotic hookworm *Ancylostoma ceylanicum*; (Traub et al., 2008; Inpankaew et al., 2014) dogs therefore remain a potentially continuous source of STH infection that is not addressed through an MDA intervention in the human population alone.

Over the last few decades several studies have investigated human parasitism within Lao PDR, with reported prevalences of *Taenia* spp. ranging from 0.6% to 3.7% and STHs from 62% to 86% (Conlan et al., 2012; Rim et al., 2003; Sayasone et al., 2009; Chai and Hongvanthong, 1998). The study by Rim et al. (2003) instigated a biannual national MDA programme targeting STHs within school children, commencing in 2006. However, subsequent studies have continued to report both high prevalences of STHs and the continued presence of *T. solium* in Lao PDR (Conlan et al., 2012; Sayasone et al., 2009; Soukhathammavong et al., 2012). More recently, a study investigating pig-associated zoonoses conducted across four provinces in Lao PDR identified several areas where *T. solium* is suspected to exist at elevated levels (Inthavong et al., 2012).

Subsequent investigation into one of these suspected hyperendemic regions revealed a taeniasis prevalence of 26.1%, confirmed as *T. solium* via genetic sequencing of a subset of samples (Okello et al., 2014). Given the serious human health implications posed by *T. solium*, a two year One Health intervention was planned for this village, aiming to break *T. solium* transmission at several points of the life cycle. This consisted of a three pronged approach that combined interventions in both the pig and human reservoirs with a communication and advocacy campaign that brought in various social and economic assessments. Given the human MDA intervention was assumed to concurrently impact on the level of STHs within the village, the burdens of these parasites were incorporated into the monitoring framework. It was expected this would improve the overall cost-benefit of the intervention, simultaneously providing data to measure the effect of anthelmintic treatment against multiple parasites. This research paper examines the impact of two albendazole triple dose regimes, undertaken five months apart, on the prevalences of *T. solium* and STHs within the target community, as determined by microscopy and PCR. The concurrent pig intervention is still ongoing and therefore not the focus of this paper, with the findings from the sociocultural work reported elsewhere (Bardosh et al., 2014).

2. Materials and methods

2.1. Study site

The study village is located in the province of Phongsaly approximately 700 km north of Vientiane in a mountainous area bordering Vietnam, with limited vehicle access during the wet season (May–October). The village is homogenous for the Tai Dam ethnic group, an animistic culture which practises the consumption of raw pork associated with ancestral sacrificial ceremonies throughout the year (Bardosh et al., 2014). Approximately 300 people (298 and 295 at the times of MDA) are dispersed amongst 60 households.

2.2. Ethics

This study was approved by Australia's Commonwealth Scientific and Industrial Research Organisation (CSIRO) Animal, Food and Health Sciences Human Research Ethics Committee (CAFHS HREC), approval number 13/10. The study also has ethical approval from the Lao PDR Ministry of Health's Council of Medical Science National Ethics Committee for Health Research (NECHR), approval number 013/NEHCR. The study is registered with the Australia New Zealand Clinical Trials Registry (ANZCTR), trial number ACTRN12614001067662. Consent to undertake the intervention was sought from the village chief, and all MDA participants undertook a consultation process and provided written consent via a signature or thumb print.

2.3. Human mass drug administration

Following an initial baseline study in January 2013 (Okello et al., 2014), two MDAs were conducted in October 2013 and March 2014. Treatment consisted of triple dose albendazole 400 mg (Eskazole®, GlaxoSmithKline) delivered over three consecutive days and offered to all eligible residents of the village. Eligibility required participants to be over six years of age, not pregnant or breastfeeding and not suffering acute illness. Treatment was administered by the Lao PDR Ministry of Health Department of Communicable Disease Control (DCDC) and Department of Prevention and Hygiene, who were also available for the post treatment monitoring of adverse reactions. Each village household was assigned a number (1–60), which was then used to co-ordinate the three day anthelmintic administration and monitoring process. Treatment was distributed to individual households on a daily basis to avoid the potential cysticidal effect of high dose albendazole if multiple tablets were ingested simultaneously (Product Information Eskazole® Tablets, 2015).

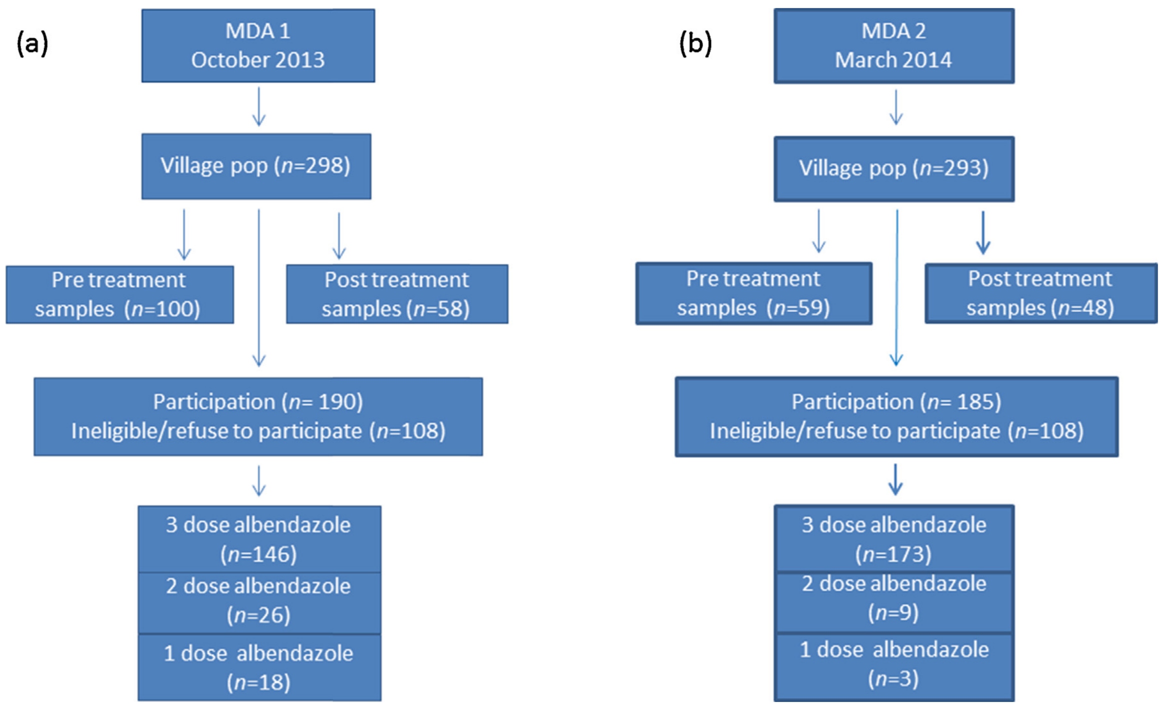

Fig. 1. Schematic presentation of the number of participants for (a) MDA1 and (b) MDA2; the number of faecal samples volunteered and collected pre/post each MDA and compliance from participants for the triple-dose protocol.

2.4. Sample collection

All village participants were asked to volunteer faecal samples, collected on four occasions throughout the monitoring period according to the following timeline; October 2013 (pre-MDA 1 monitoring), November 2013 (post-MDA 1 monitoring), March 2014 (Pre-MDA 2 monitoring) and April 2014 (post-MDA 2 monitoring) (Fig. 1). Only those people who obtained treatment were asked to volunteer post MDA samples. Villagers were given household numbered plastic bags for faecal collection, toilet paper, soap, and educational posters outlining the lifecycle of *T. solium* and associated hygiene and sanitation messages. Faecal samples were collected 24 h prior to the administration of anthelmintics, and again at 7–21 days post treatment. In addition a small number of village dog faecal samples ($n = 13$) were opportunistically collected and subjected to microscopy and molecular analysis. All collected samples were preserved on the day of collection (2 parts faeces, 8 parts preservative) in 10% formalin (for microscopy analysis) and 70% ethanol (for molecular characterisation) and analysed at Murdoch University in Perth, Australia.

2.5. Sample analysis

2.5.1. Microscopy

The formalin fixed samples were washed twice with distilled H_2O before flotation techniques were implemented. Quantitative data was obtained for all sampling events during the two MDA programmes through the McMaster method as described by Levecke et al. (2011). Two grams of washed faeces was weighed and used for this technique. The eggs per gram of faeces (EPGs) detected were then used to measure parasite intensity within the sampled population pre and post anthelmintic treatment.

2.5.2. Statistical analysis

Parasite prevalences were expressed as a percentage of positive samples found by microscopy, with 95% confidence intervals calculated assuming a binomial distribution using the software Quantitative Parasitology 3.0 (Rozsa et al., 2000). Egg reduction rates (ERR) were calculated for all STHs monitored using the following formula; $\mathrm{ERR}(\%) = 100 \times \left(1 - \frac{\text{arithretic mean egg counts at follow up}}{\text{arithretic mean egg count at follow base line}}\right)$ and used as a measure for the reduction in intensity of infection (worm burden). ERR's were not calculated for *Taenia* spp. as the intermittent nature of expelling of proglottids does not allow for a correlation between egg output and worm burden. The increase in prevalence of STHs between the two anthelmintic treatment periods was determined using the following formula;

$100 \times \left(\frac{\%\text{prevalencebeforeMDA2}}{\%\text{prevalencebeforeMDA1}}\right)$. Differences in prevalences observed between sampling events was tested using the Fisher's exact test.

2.5.3. Molecular characterisation

Molecular characterisation of *Taenia* spp. and hookworm spp. was conducted on human and dog faecal samples to determine the risk of zoonotic species present within the village.

DNA extraction: A 2 ml aliquot of each ethanol-preserved sample was centrifuged; ethanol eluted and used for DNA extraction using the Maxwell® 16 Instrument (Promega, Madison, USA) as per manufacturer's instruction.

Amplification of *Taenia* species: A multiplex PCR amplifying the *rrnS* locus was used for differentiation of *Taenia* species from human and dog faecal samples, as per Trachsel et al., (2007). The 267 bp amplicons representing *Taenia* spp. were sequenced using the internal sequencing primer Cest 5_{seq} (Trachsel et al., 2007).

Amplification of hookworm species: A PCR amplifying the ITS1-5.8S-ITS2 region was used for differentiation of hookworm species from human and dog faecal samples, with slight modifications from Traub et al. (2008). Using primers RTHW1F and RTHW1R a 380 bp product was amplified. The PCR reaction was performed in 25 μl volumes consisting of 1 μl of extracted DNA, 2.5 mM $MgCl_2$, 1 × reaction buffer (20 mM Tris–HCL, pH 8.5 at 25 °C, 50 mM KCl), 200 μM of each dNTP, 10 ρmol of each primer, 1 unit of Taq DNA polymerase (Fisher Biotec, Perth Australia), and dH_2O. Conditions for amplification were initiated with a denaturing step of 94 °C for 5 min, then 40 cycles of 94 °C for 30 s, 60 °C for 30s and 72 °C for 30 s, followed by a final extension of 72 °C for 7 min. Amplicons were sequenced in both directions.

Purification of genetic material: PCR products were purified using an Agencourt AMPure XP system (Beckman Coulter Inc., Brea USA) and sequence reactions were performed using the Big Dye Terminator Version 3.1 cycle sequencing kit (Applied Biosystems) according to the manufacturer's instructions. Reactions were electrophoresed on an ABI 3730 48 capillary machine.

Identification: Resultant nucleotide sequences were compared with published sequences on NCBI using the basic alignment search tool (BLAST). Further sequence analysis was conducted using the sequence alignment program Sequencer™ 4.8 (Gene Codes Corporation, Ann Arbor, USA).

3. Results

3.1. Coverage of the human MDA intervention

The MDA regimes conducted in October 2013 and March 2014 administered treatment to approximately 64% of the village residents on both occasions (Fig. 1). Those that did not participate (108) were either ineligible (pregnant, breastfeeding or under the age of 6), absent from the village, or did not consent to treatment. Of those treated, 76.8% and 93.5% (MDA 1 and 2 respectively) received the recommended protocol of 3 doses of albendazole 400 mg over three consecutive days, while 13.7% and 4.9% of participants received 2 doses and 9.5% and 1.6% received 1 dose (Fig. 1).

3.2. Mass drug administration 1: October 2013

Pre-intervention faecal samples for MDA1 were obtained from 33.5% (100/298) of the total village population across 75% (45/60) households. Microscopy analysis revealed an overall parasite prevalence of 90% (95% CI = 83–95). The greatest individual species prevalence was *T. trichiura* (60%) followed by hookworm (56%), *A. lumbricoides* (43%), and *Taenia* spp. (18%) (Table 1).

Post MDA1 faecal samples were obtained from 30.5% (58/190) of the treated population across 55.7% (34/60) of households. Overall parasite prevalence was found to be 31% (95% CI = 20–44), reflecting a reduction in prevalence of 66% (Fisher's exact test, $P < 0.0001$). The greatest prevalence reduction for individual parasite species was detected for *A. lumbricoides* (95.6%; Fisher's exact test, $P < 0.0001$), followed by hookworm (83.4%; Fisher's exact test, $P < 0.0001$), *Taenia* spp. (79.4%; Fisher's exact test, $P = 0.012$) and lastly *T. trichiura* (69.2%; Fisher's exact test, $P < 0.0001$). Egg reduction rates (ERR) for each STH monitored found that the average EPG within the sampled population was reduced for *A. lumbricoides* by 99.4%, followed by hookworm 94.4% and *T. trichiura* 77.2% (Table 1).

3.3. Mass drug administration 2: March 2014

Pre-intervention faecal samples for MDA2 were obtained from 20% (59/295) of the total village population across 58.3% (35/60) households. The overall parasite prevalence obtained through microscopy techniques was found to be 55.9% (95% CI = 43–68), which represents an increase from the post-MDA1 prevalence of 31% (Fig. 2). The greatest individual species prevalence was detected for *T. trichiura* (42.4%) followed by *A. lumbricoides* (32.2%), hookworm (27.1%), and *Taenia* spp. (3.4%) (Table 1).

Post-MDA2 faecal samples were obtained from 25.9% (48/185) of treated individuals across 53.3% (32/60) households. Overall parasite prevalence was found to be 20.8%, reflecting a reduction of 62.8% (Fisher's exact test, $P < 0.0001$). The greatest reduction in individual parasite species was detected for *Taenia* spp. (100%) followed by *A. lumbricoides* (93.5%; Fisher's exact test, $P < 0.0001$), hookworm (84.5%; Fisher's exact test, $P = 0.002$) and lastly *T. trichiura* (61%; Fisher's exact test, $P = 0.006$). Egg reduction rates (ERR) found that the average EPG within the sampled population was reduced for *A. lumbricoides* by 99.3%, followed by hookworm (97.8%) and *T. trichiura* (88.5%) (Table 1). During the 5 month inter-treatment interval between MDA1 and MDA2, an increase in STH prevalence was detected, with the overall prevalence reaching 62.1% of pre-MDA1 levels (Fig. 2). Among the individual parasite species detected *A. lumbricoides* had the greatest increase in prevalence, reaching 74.8% of pre-MDA1 levels followed by 70.6% for *T. trichiura* and 48.4% for hookworm. Prevalences for *Taenia* sp. consistently declined over the monitoring period (Fig. 2).

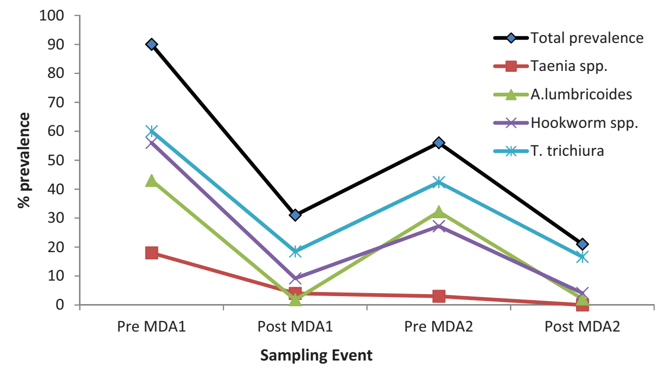

Fig. 2. Overall parasite prevalence (%) and individual parasite species prevalences (%) detected pre and post MDA1 and MDA2.

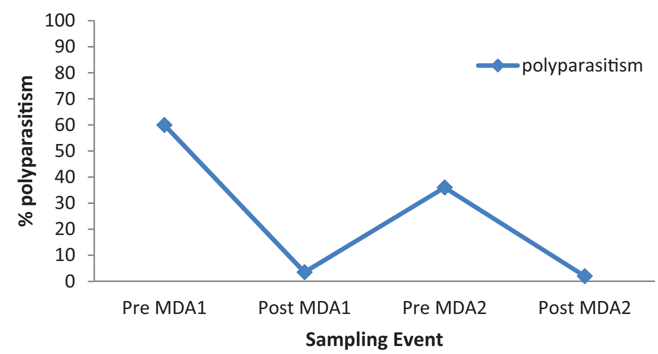

Fig. 3. The level of polyparasitism (%) detected pre and post MDA1 and MDA2.

3.4. Polyparasitism

The number of samples for which polyparasitism (co-infection with more than one parasite) was detected displayed a similar pattern to the observations of singular parasite levels during the intervention. At the pre-MDA1 sampling of October 2013, polyparasitism was detected in 60% of samples. This decreased post-MDA1 to 3.5%, increased during the inter-MDA interval to 36%, before decreasing again post-MDA2 to 2% (Fig. 3).

3.5. Molecular characterisation of *Taenia* spp. and hookworm spp.

Taeniid eggs were detected by microscopy in a total of 21 human samples over the 4 sampling events, of which 19 were molecularly characterised and species identification achieved (Table 2). Of these, 15 were identified as *T. solium*, with the remaining 4 matching published sequences for *T. saginata*; *T. asiatica* was not detected. At the household level, 26.6% (16/60) of village households were found to have a tapeworm carrier. Of these households,

Table 1
The prevalences obtained for *Taenia* spp. *Ascaris lumbricoides*, Hookworm spp. *and Trichuris trichiura* using microscopy techniques during the MDA monitoring period. Reduction in parasite prevalence and egg reduction rates (ERR) are calculated for hookworm, *A. lumbricoides* and *T. trichiura* and presented as a percentage (95% confidence intervals in parentheses).

Sample date	*Taenia sp.*	Hookworm		*A. lumbricoides*		*T. trichiura*		Total prevalence
	Prevalence	Prevalence	Avg EPG	Prevalence	Avg EPG	Prevalence	Avg EPG	
Pre MDA1 October 2013 (n = 100)	18% (n = 18) (11.3–27)	56% (n = 56) (46–65.6)	233	43% (n = 43) (33.5– 53)	2818	60% (n = 60) (50–69.5)	166.5	90% (=90) (82.6–94.7)
Post MDA1 November 2013 (n = 58)	3.7% (n = 2) (0.6–12.7)	9.3% (n = 5) (3.7–20.1)	13	1.9% (n = 1) (0.01–9.9)	17.6	18.5% (n = 10) (9.9–31.3)	38	31% (n = 18) (20.3–43.9)
Prevalence reduction	79.4%	83.4%		95.6%		69.2%		65.5%
ERR MDA1			94.4%		99.4%		77.2%	
Pre MDA2March 2014 (n = 59)	3.4% (n = 2) (0.6–11.6)	27.1% (n = 16) (16.7–39.8)	247	32.2% (n = 19) (21–45)	2358	42.4% (n = 25) (30.4–55.2)	127	55.9% (n = 33) (43.2–68.2)
Post MDA2 April 2014 (n = 48)	0 0	4.2% (n = 2) (0.7–14.3)	5.3	2.1% (n = 1) 5(0.1–11)	16.6	16.7% (n = 8) (7.8–30)	14.6	20.8% (n = 10) (11.1–34.3)
Prevalence reduction	100%	84.5%		93.5%		61%		62.8%
ERR MDA2			97.8%		99.3%		88.5%	

81.3% (13/16) were identified as *T. solium* and 18.7% (3/16) were identified as *T. saginata.* None of the dog samples were positive for taeniids either by microscopy or molecular techniques.

A subset of hookworm positive human samples detected by microscopy were molecularly characterised and species identification achieved. Genotypic information was obtained for 31 samples over the 4 sampling events. Of these, 22 (70.9%) were identified as *Necator americanus,* 2 (6.4%) as *Ancylostoma duodenale,* 2 (6.4%) as *A. ceylanicum* and unusually 6 (19.4%) were identified as *A. braziliense.* One of these samples contained a mixed infection with *N. americanus* and *A. ceylanicum*. Genotypic information was obtained for 9 dog samples and of these 4 (44.4%) were identified as *A. ceylanicum*, 4 as *A. caninum* (44.4%) and 2 as *N. americanus* (22.2%). One of these samples contained a mixed infection with *A. ceylanicum* and *A. caninum*.

4. Discussion

The primary objective of this study was to monitor the impact of a targeted drug administration on the high levels of *T. solium* within the village, utilising an albendazole 400 mg triple dose regime. Secondary to this but of substantial value was the ability to simultaneously monitor the impact this treatment had on the levels of STH infections also existing within the community. While a recognised limitation of this study was that a varying proportion of unidentified faecal samples were obtained throughout the monitoring period, the statistically significant reduction in both *T. solium* and STH prevalence indicate that the sampling frame was sufficient to detect an effect from chemotherapy treatment. The results from this sample set should therefore be considered as representative for the treated village population.

4.1. Impact of triple dose albendazole treatment against taeniasis

The use of albendazole to target *T. solium* was chosen over other possibilities such as niclosamide and praziquantel due to its reported broad-spectrum efficacy against both *Taenia* spp. and STHs, high compliance, acceptability to the Lao PDR Ministry of Health and ease of administration (Horton, 2000; Steinmann et al., 2011). In addition there have been reports of serious adverse reactions in asymptomatic neurocysticercosis patients when given praziquantel, of which there were suspected cases present in this village (Okello et al., 2014; Inthavong et al., 2013; Wandra et al., 2011). The triple dose 400 mg regime was chosen due to its reported success in similar studies treating taeniasis. (Steinmann et al., 2011; de Kaminsky, 1991). These studies found that a single dose of albendazole 400 mg had a cure rate of approximately 50% against *Taenia* spp. infections, while a 3-dose treatment can achieve a cure rate of up to 100%. Indeed, the 3-dose treatment regime appeared to have a significant impact on the level of taeniasis within this population. Prevalences were observed to decrease by 79.4% after the first intervention (18–3.7%), remaining steady during the inter-treatment interval and decreased further by 100% after MDA2. For a number of reasons treatment compliance with the MDA1 in October 2013 was relatively low, with 77% of treated individuals receiving 3 doses and the remaining 23% receiving 1 or 2 doses, hence the requirement for MDA2. Compliance for MDA2 was substantially greater, with 93.5% of treated individuals receiving 3 doses and subsequent sampling detected 0% prevalence for taeniasis by microscopy. Whilst it is recognised that sensitivity for the detection of *Taenia* spp. eggs with microscopy is low, this is the methodology used for many human taeniasis studies to date. (Conlan et al., 2008; Steinmann et al., 2011; de Kaminsky, 1991). In addition, detection was com-

Table 2
Molecular characterisation of *Taenia* spp. with reference to samples positive for taeniid ova on microscopy, the number of households with a member positive for taeniasis and the *Taenia* species identified.

Sampling event	PCR/micro positive	Individual households	Genotyping results of *Taenia* sp.	
			T. solium	*T. saginata*
October 2013 (pre MDA1)	16/18	14	14	3
November 2013 (post MDA1)	2/2	2	2	0
March 2014 (pre MDA2)	1/1	1	0	1
April 2014 (post MDA2)	0	0	0	0
Total	19/21	[a]17	16	4[a]

[a] Number includes two samples from the same household.

plemented by PCR which can often detect taeniid DNA missed on microscopy, although in this study microscopy and molecular techniques were consistent.

The high level of taeniasis detected within the village gained significance with the molecular characterisation of *T solium* in 15/19 taeniasis positive samples. This provided confirmatory evidence that this village was indeed a hotspot for *T. solium* infections as previously suggested (Okello et al., 2014). At the household level, genotyping revealed a *T. solium* tapeworm carrier in at least one occupant from 21.6% (13/60) of households in the village dispersed across a wide area. *T. saginata* was identified in only 5% (3/60) of households. Given the widespread and dominant prevalence of *T. solium,* coupled with the relatively low levels of hygiene and sanitation as described by Bardosh et al. (2014), the potential for cysticercosis in this close living community is considered to be extremely high, further validating the suspicion that a large number of residents currently suffer from cysticercosis (Okello et al., 2014).

A major explanation for this hyper endemic focus is the high consumption of raw and/or undercooked pork, practised regularly throughout the year for various animistic ceremonies at both the household and village level (Bardosh et al., 2014). Whilst buffalo are kept by a small proportion of the village, they are rarely consumed given their high economic value; reflected by the low prevalence of *T. saginata* detected. Lack of adequate latrine provision perpetuates the parasite life cycle via the common practice of promiscuous defecation and the free ranging smallholder pig production system (Bardosh et al., 2014). The village is also quite isolated from the wider community due to its geographical position and inaccessibility during the wet season. This isolation, and the village practice of raising pigs predominantly for personal consumption rather than trade, reduces any dilution effect which may have been achieved through the regular sale and purchase of pigs from outside the village. This then has the potential for a saturation of cysticercosis positive pigs in this isolated and close living community.

4.2. Impact of triple dose albendazole treatment against STHs

The triple dose albendazole treatment protocol used in this study had previously been reported to be efficacious against STHs, particularly hookworm, which can pose significant health risks to at-risk members of the community such as pregnant women and children (Bethony et al., 2006; Hotez et al., 2004). A recent review (Keiser and Utzinger, 2008) found that single dose albendazole (400 mg) was more efficacious than single dose mebendazole (500 mg) against hookworm. Further to this a study involving school-age children in Lao PDR found that while single dose albendazole performed better than mebendazole against hookworm infections both single dose regimes displayed low efficacy (36% vs 17%) (Soukhathammavong et al., 2012). Steinman et al. (2011) conducted a comparative study using single dose and triple dose albendazole regimes and found that a significantly greater efficacy against hookworm was attained with the triple dose treatment, reporting cure rates against individual STHS of 92% for hookworm, 96% for *A. lumbricoides* and 56% against *T. trichiura.*

Similarly, the triple dose treatment regime in this study displayed a significant post-treatment impact in STH prevalence each time. Overall levels of parasitism were found to decrease by 65.5% and 62.8% for MDA1 and MDA2 respectively. Individually, hookworm prevalence decreased by 83.4% (MDA1) and 84.5% (MDA2), *A. lumbricoides* by 95.6% and 93.5% and *T. trichiura* by 69.2% and 61%. In addition, the intensity of infection within the sampled group also greatly decreased, with ERRs found for hookworm of 94.4% and 97.8%, *A. lumbricoides* of 99.4% and 99.3% and *T. trichiura* of 77.2% and 88.5%. The intensity of infection is often linked with the level of host morbidity, given a high number of parasite ova suggest a high worm burden (Bethony et al., 2006; Anderson and May, 1991). Therefore the significant reduction in parasite ova observed here suggests a marked decrease in worm burden and subsequently an improved level of health and well-being for many of the treated individuals within the village, as reflected in concurrent socioeconomic research throughout this intervention (Okello WO et al., in preparation). Importantly, the level of polyparasitism, another detrimental factor for host health, was also substantially reduced from 60% in October 2013 to 2% in April 2014. The significance of this is emphasised statistically with reports that globally, *A. lumbricoides, T. trichiura* and hookworm species are responsible for 1.32, 0.64 and 3.23 million Disability Adjusted Life Years (DALYs) respectively (Murray et al., 2012). Grouped together, the STHs have the highest DALY calculation of all Neglected Tropical Diseases according to the most recent Global Burden of Disease (GBD) study (Hotez et al., 2014).

The pre-treatment parasitism level of 90% detected in October 2013 was found to be comparatively high, particularly when compared to the recent large scale study in northern Lao PDR where the overall STH prevalences presented here were found to be greater than any provincial (70.6%) or high risk ethnic group (85.2%) detected by Conlan et al. (2012). These results support the suggestion that hot spots for parasitism exist within Lao PDR which are not being adequately addressed by the school-based worming programme, consistent with suggestions by others (Conlan et al., 2012; Soukhathammavong et al., 2012). Reasons for the hyper endemic status of this community are most likely linked to their relative isolation, in conjunction with high levels of poverty and sub-optimal sanitary conditions resulting in significant environmental contamination and a constant cycle of re-infection.

4.3. Challenges of mass drug administration

This study experienced many of the challenges recognised by others in achieving significant and sustainable cure rates with MDAs, specifically, eligibility and compliance of community members, and the level of re-infection which is attained post treatment (Keiser and Utzinger, 2008; Parker and Allen, 2011; Jia et al., 2012).

4.3.1. Eligibility and compliance

Obtaining full coverage within marginalised rural settings can be affected by eligibility and compliance of the targeted population. This is especially significant in populations with high numbers of children or those not accustomed to taking drugs, and were identified as a significant factor in the coverage achieved by the anthelmintic treatment regime within this village. For both treatment programmes, approximately one third of the village residents were either not eligible (pregnant, breastfeeding, less than 6 years or age) or not willing to participate; for those that did, full treatment compliance ranged from 76.8% in MDA1 to 93.5% in MDA2. Whilst compliance was undoubtedly improved through the use of Eskazole® (albendazole; Zentel) – a chewable tablet with a sweet taste that does not result in excessive mouth foaming or require a purgative –researchers still could not achieve 100% compliance (Bardosh et al., 2014). Surprisingly, the greatest number of non-compliant participants was school-age children who had participated in the bi-annual school deworming programme. These individuals either did not want to participate due to the stigma and fear of large worms being purged during the process, or their parents would not consent to their participation, fearful of them having too many drugs (Bardosh et al., 2014).

4.3.2. Reinfection and zoonoses

Overlaying the issues around eligibility and compliance are the environmental and behavioural factors associated with poor communities such as promiscuous defecation and lack of sanitary conditions, which support a high frequency of parasite transmission and therefore re-infection rate. This study observed substantial reinfection with STHs in the five month interval between the two treatment periods, with hookworm reaching 48% of the October 2013 pre-treatment levels, *A. lumbricoides* 75% and *T. trichiura* 71%. This is a common problem; a recent meta – analysis reviewing the reinfection rate of STHs post-treatment found that reinfections can achieve 100% of pre-treatment levels within 12 months if chemotherapy is not sustained (Jia et al., 2012). The reinfection levels observed in this study certainly reflect this rapid reinfection rate. In addition to reinfection from environmental contamination, consideration needs to be given to the sympatric species operating as reservoir hosts within these communities. Pigs are the obvious reservoir host for *T. solium*, and as such porcine chemotherapy remains a significant component of this One Health intervention. The dog is another important animal reservoir within these communities, hosting a number of zoonotic gastrointestinal parasites such as hookworm. Evidence for dog–human transmission was detected within this study with the identification of the zoonotic hookworm *A. ceylanicum* in both dog and human samples, and the dog hookworm *A. braziliense* in human samples. *A. ceylanicum* is prevalent throughout SE Asia and has been implicated as a zoonosis transmitted by dogs on several occasions (Conlan et al., 2012; Traub et al., 2008; Inpankaew et al., 2014, 2007; Areekul et al., 1970). The finding of *A. braziliense* however, was unusual as this species of hookworm has not been found to cause patent infection in humans, as distinct from its role as a cause of cutaneous larva migrans. Whilst the finding of *A. braziliense* DNA in human faecal samples may suggest a ‘new’ infection, it is perhaps more likely the result of accidental ingestion of parasite ova by humans and therefore this observation represents a spurious infection. Similarly, what appeared to be dog whipworm eggs, *Trichuris vulpis,* were observed in several human samples. This observation has been previously reported and until substantial proof can be provided is assumed to also be spurious in nature (Steinmann et al., 2015). In addition to zoonotic transmission dogs have been implicated in the mechanical transmission of human parasites such as *A. lumbricoides*, whereby parasite ova are passed though the dog gastrointestinal tract and back into the environment for possible transmission to humans (Traub et al., 2002). Evidence for this was observed in the village dog samples collected with parasite ova of *Ascaris* sp. and *Trichuris* sp. of human (or pig) origin and DNA for the human hookworm *N. americanus* being detected. Therefore, despite the small dog sample size, these findings suggest dogs in this village do play an important role in both the zoonotic and mechanical transmission of human parasites and add an additional element to the rate of infection achieved post anthelmintic treatments. As such the authors recommend this species be considered in any large scale One Health parasite intervention.

5. Conclusions

Despite the recognised challenges of implementing the MDA, the results presented here suggest that treatment with triple dose albendazole has achieved a significant reduction in the level of taeniasis, whilst also dramatically reducing the current level of STHs within the village. Whilst the detected increase in the STH prevalence between the first and second treatment interventions reflects the need for broader environmental and behavioural changes and sustained chemotherapy, the level of taeniasis at this point has remained reduced since the first treatment. Ongoing monitoring will ascertain if the *T. solium* lifecycle has been sufficiently restricted by the concurrent interventions in the village pig population. This treatment regime may prove beneficial for other suspected hot spots where a targeted approach is warranted. Finally, the authors strongly recommend the consideration of *T. solium* in broader STH and sanitation programmes currently being rolled out under various development frameworks. Conversely, those implementing *T. solium* control programmes should be encouraged to monitor the broader impacts of their interventions on STHs and/or other coexisting parasites in their target communities, further promoting the economic and policy rationales for integrated approaches to NTD control.

Competing interests

The authors declare that they have no competing interests.

Authors’ contributions

AO, AA, BK, PI, JA and AT were involved in the study design. AA, AO, BK and PI undertook the collection of samples and monitoring of the treatment regime. AA conducted the laboratory analysis and drafted the manuscript with subsequent input from AO, AT and JA.

All authors read and approved the final manuscript.

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