Australian Government

Australian Centre for International Agricultural Research

Final report

Small research and development activity

project	**Impact assessment of Taenia solium control in Phongsali province, Lao PDR and development of future opportunities for the control of zoonotic parasitic infections**
project number	LS/2018/201
date published	25 September 2020
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final report number	FR2021-005
ISBN	978-1-922635-00-6
published by	ACIAR GPO Box 1571 Canberra ACT 2601 Australia

Contents

List of Abbreviations

ACIAR	Australian Centre for International Agricultural Research
ASF	African Swine Fever
DLF	Department of Livestock and Fisheries (Lao PDR)
DCDC	Department of Communicable Disease Centre (CDC)
CMPE	Centre for Malaria and Parasite Epidemiology (Lao PDR)
FAO	Food and Agriculture Organisation
FBPD	Food Borne Parasitic Disease
KAP	Knowledge, Attitude Practice
LMIC	Low Middle Income Countries
MU	Murdoch University
MOH	Ministry of Health (Lao PDR)
NAHL	National Animal Health Laboratory (Lao PDR)
NUOL	National University of Lao (Lao PDR)
NTD	Neglected Tropical Disease
OIE	World Organisation for Animal Health
SHF	Small holder farmers
STH	Soil Transmitted Helminths
TPHI	Tropical Public Health Institute (Lao PDR)
WHO	World Health Organisation

1 Executive summary

Taenia solium is a food borne parasitic disease transmitted between people and pigs and is endemic throughout Southeast Asia. Human infections with the larval stage of this parasite may result in brain cysts, a major cause of acquired epilepsy in endemic regions (Ndimubanzi et al., 2010). Transmission is maintained in areas where human sanitation is poor, free ranging pig systems are employed and ingesting raw/undercooked pork is common, as seen in rural regions of Lao PDR.

A previous ACIAR project (AH2009/001) conducted a two-year human/pig intervention aimed at eliminating *T. solium* from a village in northern Lao PDR. This protocol provided some of the first empirical evidence from Southeast Asia of how positive outcomes for both human and animal health can be achieved under a One Health strategy (Okello et al., 2015). The objective of this SRA was to revisit the intervention village and conduct a five-year impact assessment by determining current human parasitic infections and community practices around animal husbandry, food preparation and village sanitation

The parasitological testing ascertained that sustained control of *Taenia solium* had not occurred and parasite prevalence had returned to similar levels seen prior to the commencment of intervention strategies. However, the prevalence of soil transmitted helminths (STHs) was markedly decreased and importantly the intensity of infection (number of parasites per person) had decreased significantly. This variation in results was clarified through village surveys and interviews which revealed what environmental and behaviour change had occurred since 2014.

The village experienced a dramatic increase in household toilets since the 2014 intervention (6 to 50) aided by UNDP. The village members did not indicate that anti-parasitic drugs had been administered to the community in the previous 5 years, therefore this sustained reduction in STH levels appeared to be achieved through change in both environment (toilet facilities) and behaviour (preference to use toilets and associated hygiene practices). However free-ranging pig farming systems still predominated, human defecation in the field occurred out of habit and necessity and raw pork consumption was still considered essential for many cultural ceremonies, the sum of which contribute to the continual transmission of *T. solium*.

Overall, the main recommendations from this impact study are:

- Control programs for zoonotic diseases such as *T. solium* should include a social science arm aimed at understanding how best to affect behaviour change associated to risk factors for disease transmission. This impact assessment discovered that while therapeutic intervention was initially effective against eliminating *T. solium,* cultural and behavioural practices related to pig husbandry, hygiene and food safety remained unchanged allowing for the disease to perpetuate.
- Sustained control of STHs within the community is more likely to be achieved if therapeutic treatment is combined with environmental changes, (increase in toilets) and behavioural changes (use of toilets/handwashing). Future STH intervention programs should therefore co-ordinate with other WASH-focused programs.
- Intervention strategies which target multiple pathogens can provide opportunities for synergies with other government/NGO programs. In this case, post 2014 a UNDP program provided materials for building toilets, and district health officials provided

education on toilet use and handwashing, which combined with community level MDA has led to a sustained control of STHs within the community.

2 Introduction

Taenia solium is a zoonotic food borne parasitic disease endemic throughout Southeast Asia (WHO 2012). Pigs are the natural intermediate host, harbouring encysted *T. solium* larvae in the musculature (cysticercosis); while humans harbour the adult tapeworm (taeniasis). 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 (NCC), a major cause of acquired epilepsy in developing countries (Garcia et al., 2003). Transmission is maintained in areas where human sanitation is poor, free ranging pig systems are employed and ingesting raw/undercooked pork is common, as seen in rural regions of Lao PDR.

Most farmers in the rural areas of Lao PDR maintain subsistence farming with a small number of native 'mulat' pigs per household. In many cases pigs receive little health and husbandry inputs, while the common practice of self-slaughter of animals within villages does little to combat health risks from zoonotic disease such as *T. solium*. Improving the quality and health of pigs for market (both domestic and international) is a development priority for the Lao government and economic trade. Research around food safety is therefore key to the future expansion of any livestock trade. Likewise, the impact of foodborne parasites is an important concern for the Lao Ministry of Health and the control of *T. solium* is a specific priority within the national health program (Lao MoH, 2015).

Programmes targeting *T. solium* have not been successful in eliminating the parasite from any region within Southeast Asia, despite the necessary control tools being available (Willingham et al., 2010). In 2014 an ACIAR funded project (AH2009/001) completed a human/pig intervention aimed at eliminating *T. solium* from the village of Om Phalong in northern Lao PDR (Ash et al., 2015; Okello et al., 2015). This involved treating both pigs and people with anti-parasitic drugs, along with community education around food safety and hygiene practices. Whilst *T. solium* was the targeted pathogen in this intervention the antiparasitic treatments administered to both people and pigs were effective against a wide range of soil transmitted helminths (STHs) providing additional health benefits for both (Ash et al 2015). This provided some of the first empirical evidence from SE Asia of how positive outcomes for both human and animal health can be achieved under a One Health strategy and was the first project to mitigate this disease in SE Asia (Okello et al., 2016). Whilst initially successful, the sustainable control of taeniasis within the human population after such a programme was unknown and warranted investigation.

Therefore, the main objective of this SRA was to revisit the intervention village and conduct an impact assessment of the 2014 intervention, including both biological analysis (level of taeniasis and STHs) and socio-economic analysis (pig production, village infrastructure). The level of success identified, combined with examining social/cultural influences, is valuable information in developing a methodology to potentially identify further at-risk villages for *Taenia solium* transmission.

In addition to the impact assessment, we aimed to develop links with key human and animal health stakeholders and discuss the main research priorities surrounding foodborne parasitic disease within Lao PDR. Ultimately this would be used to develop opportunities and confirm research questions for the follow-on project (approved in 2020; LS-2014-055) investigating transmission risks of zoonotic parasitic infections and the best

methods for the detection and control of identified pathogens within the region from both an epidemiological and socio-economic perspective.

The key questions this project intended to address included:

- Did a One Health therapeutic intervention succeed in reducing the level of taeniasis in the targeted village in the long term?
- Has there been any major social/cultural changes within the village since the intervention, and if so, what impact may this have had on the risks of zoonotic transmission?
- How can findings from this project feed into future control programmes and policies for foodborne parasitic disease in the Asia region?
- What are the key research questions identified from the Lao government and international organisations concerned with zoonotic disease?

3 Objectives

There were four main objectives:

1) Conduct an impact assessment of the 2014 therapeutic intervention of people and pigs in the target project site in Phongsali province.

2) Develop links with key stakeholders and discuss the priorities for the control of food-borne parasitic disease with Lao PDR.

   Key stakeholders include members within the Lao Ministry of Health, Dept. of Livestock & Fisheries, National University of Lao and National Institute of Public Health along with international agencies WHO; FAO and OIE, all of whom are concerned with food-borne parasitic disease in the region. Together we can identify what are the key research questions and what are the next steps favourable and feasible for all parties going forward.

3) Investigate cultural/social behaviours which may correlate with increased transmission of zoonotic parasites.

   A key issue for the control of *T. solium* is the lack of an easy diagnostic tool for the detection of taeniasis/cysticercosis in people/pigs, and at this point there is no indication of when one may be available. Until this is achieved another strategy to narrow the target range is to understand what cultural/social characteristics are associated with higher risk factors. We will visit 2-3 villages in the region that have previously been identified (through serology) as apparently high or low for taeniasis and/or cysticercosis and gather cultural/social data to see if any patterns emerge for the for presence/absence of this zoonotic parasite. This initial data will then be used to springboard into a larger project which will incorporate a wider collection of villages in the northern regions of Lao.

4) Develop the full proposal for LS/2014/055 to reflect current research opportunities and stakeholder interests for a larger project investigating foodborne parasitic diseases

   Throughout the duration of this SRA we will aim to identify the key priorities and research questions as determined by stakeholders and the result of this SRA. The results from the impact assessment combined with preliminary social/cultural data obtained and in-country priorities will be used to develop and begin implementation of a large project investigating zoonotic parasitic infections within the region.

4 Methodology

4.1 Study Site

The project study site was a hill tribe village located in Mai district in the northern province of Phongsali, near the Vietnam border (Figure 1). The village is largely isolated, being located in a remote mountainous area with one road connecting the village to the closest market town of Mai (40min drive). Whilst the road to the village has been greatly improved since 2014, it is a steep dirt road still not accessible during the wet season (May-October). Field visits to the village required a 2 days drive from Vientiane where project staff were located.

This village had been the focus of a human/animal therapeutic intervention designed to mitigate the transmission of the zoonotic tapeworm *Taenia solium,* as described in Okello et al (2015).

Figure 1. Map of Laos indicating the intervention area (red dot).

The village consisted of 248 people living in 45 households and was comprised of almost 100% Tai Dam ethnic group, an animist culture which incorporates raw meat eating for many religious ceremonies (Kashinaga 2009). Houses are situated around a small river which borders threes sides of the village, with a central area containing the majority of houses (Figure 2).

Photo 1: Central region of the village containing the highest density of housing

Subsistence farming predominates, with free ranging pig systems routinely practiced, however, this village had been significantly affected by African Swine Fever (ASF) in 2019 and the few remaining pigs were being housed in pens in an effort to avoid contracting the disease (Figure 3-4).

Photos 2-3: Pig pens housing two pigs which survived the recent ASF epidemic

4.2 Impact Assessment

The impact assessment incorporated two visits to Om Phalong village in March and December 2019. Ethical approval to undertake human research was obtained from both Murdoch University Human Ethics Committee (2019/02) and the Lao Ministry of Health National Ethics committee for Health Research (2019.10.Pho). The first visit in March included faecal sampling of the human population to determine parasite levels, and a survey to determine changes to the village since the previous intervention in 2014.

Faecal samples were obtained from community members voluntarily and were collected each morning over 3 consecutive days. All samples were preserved on the day of collection (2 parts faeces, 8 parts preservative) in 10% formalin for microscopy analysis, 5% formalin for copro-antigen testing and 70% ethanol for molecular characterisation (Figure 5-6). Samples were sent to Murdoch University, Australia for both qualitative and quantitative microscopy analysis, and for subsequent molecular characterisation of positive *Taenia* species. Samples were also sent to Ghent University, Belgium to undergo coproantigen ELISA testing.

All testing methods employed in this project replicated those that had been conducted in the previous intervention (AH/2009/001) which was to ensure results could be confidently compared between time points. Specifically, this included general quantitative microscopy techniques to ascertain both presence and intensity of infection with *Taenia* sp. and STHs, a *Taenia* specific copro-antigen ELISA test as per Allan and Craig (2006) and molecular characterisation to confirm the species of *Taenia* present as per Traschel et al., (2007).

In addition to replicating these methods, samples which had been sent to Murdoch University were also used to validate published testing methods advocating sensitivity and/or specificity for *Taenia solium* and STHs. The aim was to identify potential methods which could be employed under field conditions and/or low resource laboratories. This was ongoing throughout the project and included techniques such as *Taenia* species specific conventional PCR and qPCR, high resolution melt qPCR for detection of STHs and loop-mediated isothermal amplification (LAMP) for *Taenia* detection.

Photos 4-5: Field staff (Murdoch and Lao MoH) preserving faecal samples for later analysis

The village surveys were developed based on previous research instruments used in 2013 and review of the literature (Appendix 1). These were conducted by government MoH personnel with assistance from provincial MoH staff. The first interview was conducted with the village chief and then MoH staff visited each household to conduct separate interviews. The survey was aimed at detecting key changes since the 2014 intervention and consisted of six main sections:

1. Village/respondent demographics
2. *T. solium* intervention 2014
3. Pig and dog ownership and husbandry
4. Water and sanitation
5. Food preparation and diet
6. Health and well being

The second visit in December 2019 sought to further understand the factors influencing ongoing transmission of the disease. During this visit, small group interviews (SGIs) were held with key informants. The SGIs were conducted using a semi-structured interview schedule, developed based on the March 2019 survey findings to focus on areas that required further information (see Appendix 2 and 3). Accordingly, SGIs were held on three main topics:

1. Cultural ceremonies and practices
2. Pig husbandry and trade
3. Health and hygiene

A targeted sampling strategy was developed for each topic to obtain a range of views including from authorities on a subject area (e.g., spiritual and community leaders), different age groups and people with more/less experience selling/trading pigs (Table 1). Discussions were held with men and women separately for most topics (Photos 6-7). In addition, interviews were held with village officials at the start and at the end of the visit. The latter was particularly important for clarifying findings and addressing any inconsistencies. Note, that despite the intention to keep the interviews small this was not always possible, although the targeted group tended to dominate the discussion at times it was useful to ask other community members questions to see whether they agreed with what was being said.

Table 1: Small group interview topics and participants

Topic	**Males**	**Females**
Cultural ceremonies and practice	19	23
Pig husbandry and trade	5+	-
Health and hygiene	4	12
Village chief / vice village chief	2	-
Total Interviewees	30	35

+ The group discussing pig husbandry and trade started off with mostly men, but women joined he group as it progressed

Photos 6-7: Small group interviews being held with men and women of the village.

4.3 Project team and Development of future opportunities

A new project team was assembled for this project, as personnel who had worked on AH/2009/001 were no longer available. Personnel from the National University of Laos (NUoL) were significant members of the new project team and included Dr Malavanh Chittavong, who assisted in all field activities throughout the duration of the project. In addition, NUoL staff from the social science department were employed to assist in the March SGI interview activities.

Personnel from the MoH were instrumental in the March impact assessment field activities and included Dr Bounnaloth Insisiengmay (Dept of Communicable Disease Control) and staff from the Centre for Malaria and Parasitology Epidemiology (CMPE).

Murdoch University staff included Dr Amanda Ash, project leader, who assisted in all field activities with support from Sarah Keatley (March impact assessment) and Dr Davina Boyd (December small group interview work). The parasitological analysis was conducted at Murdoch University Parasitology Laboratory. Validation of published parasitology testing methods was also conducted here, with the view that these could be implemented in future projects.

Throughout the duration of the project stakeholder meetings and discussions were held with key members involved in food borne parasitic disease in Lao to identify future opportunities for research. Dr Chittavong and Dr Insisiengmay participated in all of these actives and included other key members from:

- MOH : DCDC and CMPE
- NUOL – Faculties of Agriculture Science, Veterinary Science and Social Science.
- NAHL - Parasitology department
- OIE – Lao office
- TPHI – Parasitology research department and Epilepsy research group
- WHO – Lao office

5 Achievements against activities and outputs/milestones

5.1 Activity 1: OIE-FAO-WHO Meeting on Foodborne Parasite Control in Asia (October 2018)

Activity: Attend the OIE-FAO-WHO workshop on control of foodborne and zoonotic parasitic diseases (FBPD) in Asia scheduled for October 2018 in Luang Prabang. This meeting will provide the perfect opportunity to meet with all relevant stakeholders for foodborne parasitic diseases in Lao PDR. There will also be opportunity to meet interested parties in Vientiane who were unable to attend this meeting and will be instrumental to the project.

Intended Output(s) – *Collate key research questions identified by workshop attendees to input into full project proposal. Formulate the logistics with in-country partners for the scheduled January impact assessment.*

Achievements – The project leader (PL) attended the WHO Meeting to Accelerate Prevention and Control of Neglected Foodborne Parasitic Zoonoses in Selected Asian Countries 16-18 October 2018. This workshop was a joint initiative organised by WHO, OIE and FAO. Representatives from all countries within the Asia region were invited to present data on the occurrence of foodborne parasitic diseases within their respective countries. The information shared and conversations held with meeting delegates provided a comprehensive background on the key research questions surrounding FBPD in the region and in Laos specifically. This provided a baseline for development of the full project proposal. PL also presented a brief overview of the *Taenia solium* project from AH/2009/001, which provided delegates background information and context for further discussion surrounding future research on *T. solium* within Lao. The official report of the meeting can be found here.

The opportunity to develop collaborations with Lao delegates was productive, with many of these contacts instrumental in achieving subsequent activities and objectives of this SRA. Specifically, key contacts were made with Lao Ministry of Health personnel in the Department of Communicable Disease Control (DCDC) and the Centre for Malaria, Parasitology and Epidemiology (CMPE), World Health Organisation Lao staff and also OIE staff currently in Lao PDR.

5.2 Activity 2: Field data collection

Activity: Revisit the AH/2009/001 intervention village in Phongsali province and assess the level of taeniasis within the village community. This would be 4 years post intervention and would provide an indication of the success/longevity treatment has had on the community. If the number of tapeworm carriers (taeniasis) has remained low this would then provide proof of concept for potential large-scale interventions within endemic areas.

Output: Ascertain the level of success from the previous medical/veterinary intervention.

Achievements:

A short window existed to conduct the impact assessment due to wet season weather constraints, therefore a new team had to be quickly assembled and appropriate approvals obtained in a short period of time. This involved:

December 2018: Meeting with personnel from the CDCD and CMPE and the PL in Vientiane to engage support and recruit field personnel

January 2019: Meetings with potential collaborators at the MoH, Tropical Public Health Institute (TPHI), World Health Organisation (WHO) and OIE. Introductions were also made with ACIAR in-country representatives. During this time Dr Malavanh Chittavong was a significant recruitment and provided great assistance for the project and is considered suitable for the larger project.

January 2019: Human ethics proposals were written and submitted in English for Murdoch University ethics committee approval and subsequently translated for submission to the Lao Human Ethics Committee. Approval was received from both Murdoch and Lao Human ethics committees (Murdoch = 2019/002; Lao = 041/NECHR).

February 2019: Data collection was a success in that village members were willing to participate immediately, and objectives were achieved in record time. This high level of engagement was likely possible due to AH/2009/001, which community members remembered as a positive experience and were happy to participate again. This highlighted the importance of building good relationships with project participants in achieving good project outcomes. Sufficient numbers of faecal samples (n=144) were obtained in the allocated time for parasitological testing and these were analysed by project staff at Murdoch University.

March – June 2019: Analysis of faecal samples and survey data obtained during the impact assessment was performed. To ensure that results could be compared with the 2013-2014 intervention, the same diagnostic techniques were employed which required samples to be sent to the Institute of Tropical Medicine in Belgium for copro-antigen testing and to Murdoch University for microscopy and PCR testing. Full results are presented in section 7.

5.3 Activity 3: Socio-cultural Studies

Activity: Investigate the cultural/social characteristics of 2-3 villages within the region which have previously been identified as having a high or low incidence of taeniasis and /or cysticercosis. This will be in the form of a survey designed to uncover risk-factors associated with the transmission of *T. solium*. The survey will include risk factors identified in previous projects (free-ranging pigs, environments contaminated with human faeces; eating raw/undercooked pork) and will also include other potential factors uncovered in previous ACIAR projects but not yet researched. These include but are not limited to:

- Ethnic make-up of each village - multi vs single ethnicities
- Number of dogs in a village infected with *Taenia hydatigena* – a potential protective element
- Number of pigs owned and kept for consumption vs those that are for trade
- Age of pigs when slaughtered for consumption
- Frequency of ceremonies involving consumption of raw pork.

Output: Identification of potential risk patterns associated with high-risk villages which can be used for large scale surveys

Achievements: Investigation of the cultural/social characteristics which many influence transmission of *T. solium* was conducted in only one village, the original intervention site. This occurred in two stages 1) during the impact assessment of February 2019 and 2) in December 2019.

February 2019 - The village chief and village householders undertook a questionnaire designed to identify changes in village practices since the human/animal therapeutic intervention 4 years ago. There were six main sections of the survey 1) village demographics; 2) knowledge retained from 2014 *T. solium* intervention 3) pig and dog ownership; 4) water and sanitation; 5) food preparation and diet; and 6) health and wellbeing (see appendix 1-2). These surveys were conducted by MoH and NuOL staff with assistance from provincial health staff.

March – May 2019 - Survey data was compiled and analysed to identify any significant changes related to the previous intervention and further transmission of *Taenia solium* (objective 2-3) Full results of the survey are detailed in section 7.

December 2019 – After the wet season project staff returned to the site to conduct rapid ethnographical activities, as described by Bardosh et al (2014). The program included three main activities:

- Key informant interviews to explore some of the unexpected or unexplained findings from the earlier survey
- Small group interviews to better understand community KAP relating to health and their attitudes towards consumption of raw pork particularly for ceremonial purposes
- Guided observations of animal populations, husbandry practices and WASH facilities

Small group interviews were designed to understand community knowledge, attitudes and practices (KAP) relating to human health, pig production and ceremonial consumption of raw pork.

5.4 Activity 4 – Full Proposal Development

Activity: Throughout the project researchers will be assessing the key research areas and priorities identified by all stakeholders regarding foodborne parasites within the region with the view to develop the follow-on full project. A particular need already identified is to extend the research of foodborne parasites in the pork value chain entering bordering countries such as Vietnam.

Output: Draft full proposal for large project.

Achievements: Continual conversations throughout the project held with key stakeholders in Lao provided significant justification and support for the development of

future opportunities for the control of zoonotic parasitic infections. Significant meetings/workshops are listed below.

October 2018 – Activity 1 resulted in key contacts with Lao Ministry of Health personnel in the Department of Communicable Disease Control (DCDC) and the Centre for Malaria, Parasitology and Epidemiology (CMPE), World Health Organisation Lao staff and also OIE staff currently in Lao.

February 2019 – Meeting with the Director General of the MoH (DCDC dept) identified common goals in achieving the mitigation of zoonotic parasitic disease, indicating support from the Lao government for future work

March 2019 – A stakeholder meeting was held in Vientiane gathering all interested parties for a larger project investigating FBPD in northern Lao. This involved persons from the Lao MoH, DLF, WHO, ACIAR and Murdoch university. It was agreed that FBPD was a concern in Laos and little activity has been undertaken in the northern provinces. Many opportunities and potential collaborations were identified for future research into this area that could complement existing donor and government activities in the south.

December 2019 – Meeting with project personnel discuss results from the impact assessment and further planning for future research opportunities

Overall, meetings with stakeholders confirmed that FBPD such as *T. solium* is a priority for the Lao government, with significant interest and engagement evident from all parties for developing a larger project investigating ways to mitigate future transmission of *T. solium* in pigs. Of note the emergence of African Swine Fever in the region has resulted in a closure of international livestock trade, therefore research opportunities in this area are currently negligible. However, opportunities exist for re-developing in-country trade and improving current biosecurity and food safety as the Lao pig supply is slowly restocked.

6 Key results and discussion

6.1 Parasitological results

6.1.1 Community-level parasite prevalences

Prevalence levels obtained here represent a cross-section of the village population and therefore presented at a community level. This aligns with the analysis conducted in the 2014 intervention study.

In March 2019, 144 faecal samples (58% of target population) were voluntarily collected. Microscopy analysis revealed an overall parasite prevalence of 63.2%. The greatest individual species prevalence was hookworm (39%) followed by *T. trichiura* (17%), *A. lumbricoides* (13%) and *Taenia* spp. (9%). (Table 2).

Table 2 The prevalences obtained for *Taenia* spp. *Ascaris lumbricoides*, Hookworm spp. *and Trichuris trichiura* using microscopy techniques prior to the MDA in 2013, immediately after the MDA, 1 year post and 5 years post MDA. Reduction in intensity of infection is reflected by eggs per gram of faeces (EPG) calculated for hookworm, *A. lumbricoides* and *T. trichiura*.

Sample date (number collected)	***Taenia sp.***	**Hookworm**		***A. lumbricoides***		***T. trichiura***		**Total prevalence**
	Prevalence	Prevalence	Avg EPG	Prevalence	Avg EPG	Prevalence	Avg EPG	
Pre Intervention Oct 2013 (n=100)	18% (n=18)	56% (n=56)	233	43% (n=43)	2818	60% (n=60)	166.5	90% (n=90)
Post Intervention (Immediate) Apr 2014 (n=48)	0	4.1% (n=2)	5.3	2.1% (n=1)	16.6	16.5%(n=8)	14.6	21% (n=10)
Post Intervention (1 year) Jan 2015 (n=139)	0.7% (n=1)	19.5% (n=27)	52	32% (n=45)	1723	32% (n=45)	64	57% (n=79)
Post Intervention (5 Years) Mar 2020 (n=144)	**9% (n=13)**	**39% (n=56)**	**23**	**13% (n=19)**	**184**	**17% (n=24)**	**19**	**63.2% (n=86)**

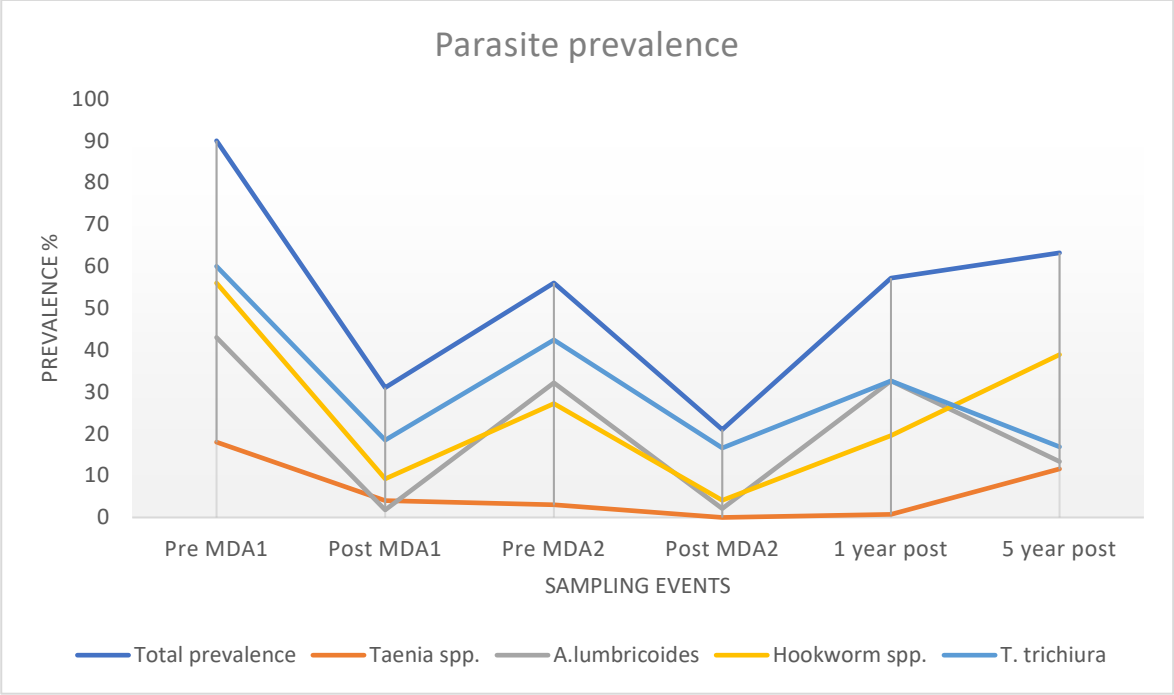

Figure 9. Overall parasite prevalence (%) and individual parasite species prevalences detected at all time points from October 2013 (pre MDA1) through to March 2019 (5 year post).

The overall prevalence, detected in March 2019 was slightly increased from the results obtained in January 2015,1 year post intervention (63.2% vs 57.2%), however when individual parasite genera were considered only *Taenia* (0.7% vs 9%) and hookworm (19.5% vs 39%) were detected at higher levels. The detected prevalences for both *Ascaris* and *Trichuris* were greatly reduced (32% vs 13% and 32% vs 17% respectively) (Table 2, Figure 9).Importantly the quantitative analysis which measured intensity of infection from Eggs Per Gram of faeces (EPG) values, had decreased for all STHs (Table 2).

Co-infection with more than one parasite (polyparasitism) was also found to be lower during this study than the January 2015 (18% vs 32%) (Figure 10). This indicated that while STH infections remained in the community, infection levels had decreased in i) the number of different parasite genera infecting people, and ii) the intensity of infection with those specific parasites was decreased.

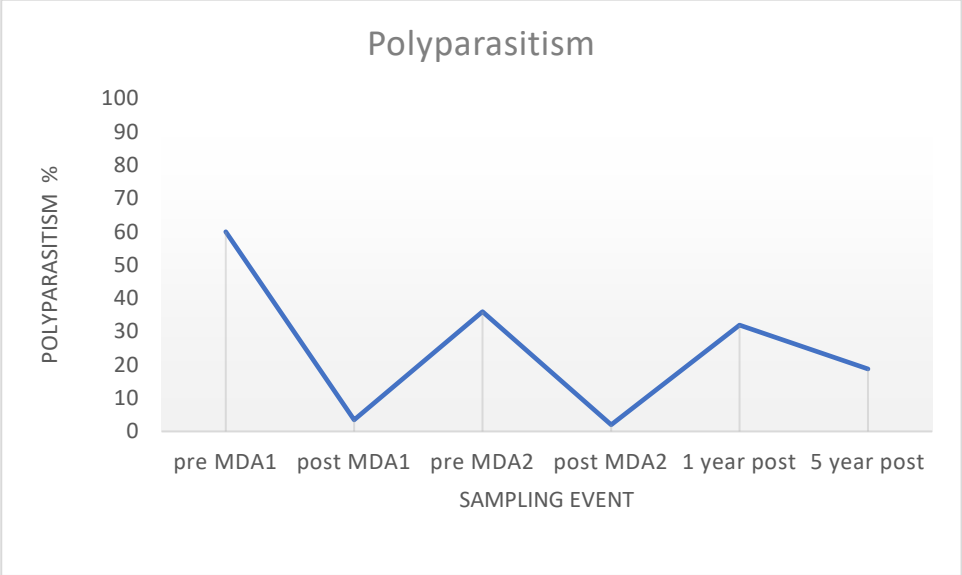

Figure 10. The level of polyparasitism (%) detected from October 2013 (pre MDA1) through to March 2019 (5 year post).

6.1.2 *Taenia* species

Microscopy testing detected *Taenia* sp. in 13 samples (9%) which was increased from January 2015 (0.7%). (Figure 11).

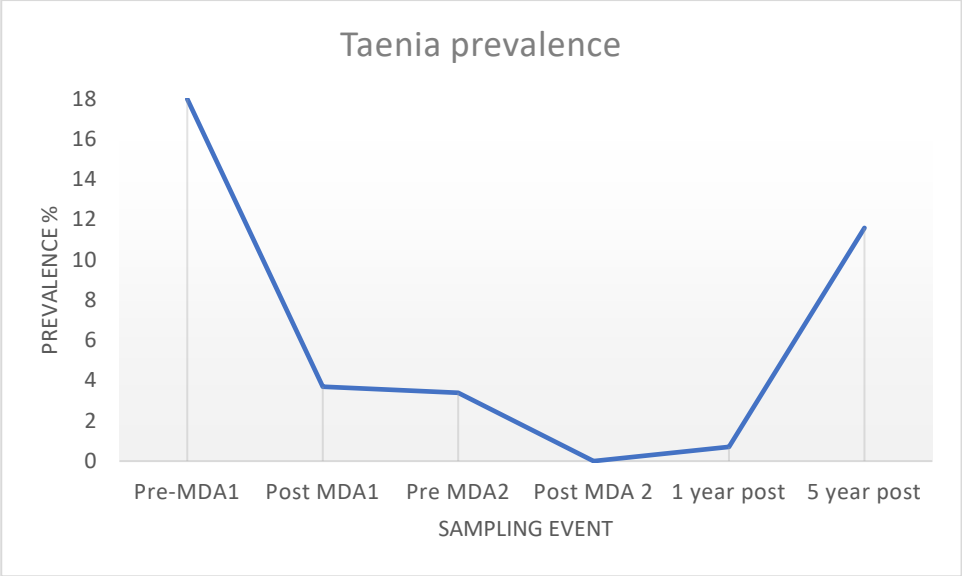

Figure 11. Prevalence of *Taenia* sp. detected during at all time from October 2013 (pre MDA1) through to March 2019 (5-year post).

A total of 140 samples were tested using the coproantigen ELISA test per Allan and Craig (2006) for the presence of *Taenia* spp., of which 41 tested positive (29.3%). This test has been shown to be more sensitive than microsocpy (Allan et al 1996) and similar higher detection rates were observed in both the October 2013 (28.7%) and January 2015 (6.5%) sampling events. The copro-antigen test is currently for research use only (ie is not commerically available) and has limitations with obtaining appropriate controls to enable confident cut-off level. However ultimately, these results support the findings that the prevalence of *Taenia* has returned to similar levels seen prior

to the commencment of intervention strategies. Microscopy and the coproantigen ELISA are unable to determine which species of *Taenia* are present (Allan and Craig 2006; Schantz et al., 1989) therefore molecular characterisation of all positive samples was conducted, consistent with Ash et al (2015).

Of the 13 microscopy positives, 12 2samples were identified as *T. solium* at two different gene loci (rrnS and COX1). No samples were positive for *T.saginata* or *T.asiatica.* The remaining sample (1/13) was confirmed as *Taeniaformis hydatigera. Taeniaformis hydatigera* is a tapeworm commonly found in cats and other felids but has also been detected in dogs. Infection with this tapeworm has rarely been detected in humans and in this instance may be a reflection of diet rather than a parasitic infection. Village members are known to source their protien from a range of animals including dogs, cats and wildlife, therefore tapeworm DNA from these sources could be passed through. Confirmation of whether this was a product of diet or an infection would require adult worms to be passed in human stools.

6.1.3 Validation of diagnostic tools

A range of published techniques were trialled using the positive samples obtained during the impact assessment. These were assessed for sensitivity and specificity and for their potential use either under field conditions or in low resource laboratories. The preliminary results of the benefits and challenges identified for each assay trialled are listed in Table 3 .

Table 3. The projected benefits and challenges identified from diagnostic assays trialled during this project.

Diagnostic Assay	Benefits	Challenges
High resolution Melt qPCR for soil transmitted helminths (Cunningham et al., 2018)	Highly sensitive and detects mixed infections of STHS. Can also detect new genotypes. If required can sequence the qPCR product to confirm species and/or genotype Quick method for high throughput testing	Need to sequence products to confirm genotypes without confirmed positive control samples. Need to maintain good positive controls, either commercial or cloned. Requires a quantstudio thermocycler which is expensive and requires continual calibration and servicing.
Summary: Has potential as a general assay and also for a first screen of large field sample sets		
Multiplex PCR differentiating between *Taenia solium*, *Taenia saginata* and *T. asiatica*.	Species specific for all three human *Taenia* species Ability to determine multiple species simultaneously	Requires a PCR thermal cycler and subsequent maintenance/servicing to be sustained.

Diagnostic Assay	Benefits	Challenges
(Yamasaki et al., 2004)	Can use generic reagents if using this as a singleplex PCR, which are easier to obtain and cheaper than commercial mastermix reagents. Uses one gene region (COX1)	Commercial mastermix is required if using this as a multiplex PCR. These reagents are often expensive Works well differentiating between *T. saginata* and *T. solium,* but problems associated with confidently detecting *T. asiatica.*
Summary: This assay has potential but will need further validation to ensure it is specific for all three Taenia species.		
Multiplex qPCR differentiating between *Taenia solium*, *Taenia saginata* and *T. asiatica*. (Nguyen et al., 2018)	Species specific for all three human *Taenia* species therefore no need to sequence Ability to determine multiple species simultaneously Can use a portable qPCR machine, Magnetic Induction cycler, potential for field samples.	Need to maintain good positive controls, either commercial or cloned. Specificity for *T. asciatica* was not able to be validated as need appropriate controls Reagents can be expensive Magnetic Induction Cycler machines are limited to 48 samples
Summary: This assay has potential as a field based qPCR but will need further validation to ensure it is specific for all three Taenia species.		
Loop-mediated isothermal Ampliciation (LAMP) with colorimetric assay (Nkouawa et al., 2016)	Field applicable technique which only requires reagents and a heat block. Can determine presence of *Taenia* sp. Technique is quick Trialed with colourmetric reagents for a field easy result species simultaneously	Need further testing to confirm *Taenia* species Sensitivity of this technique is currently poor as published primers are not looping primers. Development of suitable primers needed Colourmetric assay not very sensitivity to visualize LAMP

Diagnostic Assay	Benefits	Challenges
	Can use a portable qPCR machine, Magnetic Induction cycler, potential for field samples.	result. Other methods need to be trialed e.g. turbidity or DOT ELISA.
Summary: This assay has the most potential as a cheap easy field applicable assay, however it needs extensive validation on both reagents and visualizing techniques		

Overall, these diagnostic assays have potential for laboratories which possess a thermocycler (PCR machine) and are able to source appropriate reagents and controls. Potential for field-based testing was also identified but not conclusive on these preliminary results. Further validation is required to ensure they are sensitive, specific and robust.

6.2 Village surveys and small group interview results

The project visited the target village on two occasions, the first time (Mar 2019) to conduct quantitative survey with individual households and the second time (Dec 2019) to conduct small group interviews (SGI) with selected members of the community. Over 100 people participated in the study.

6.2.1 VILLAGE BACKGROUND

Demographics

As of December 2019, the target village had a population of 248 people (122 females and 126 males) from 60 families, living in 45 houses. Over the past 6 years the population has declined, with more than 10 households moving away for work (Table 2); no new families have moved to the village. There are 42 children that attend school in the village, although not all school age children attend school.

Table 4: Target village population 2013 – 2019

	2013	**March 2019**	**December 2019**
Village population	330	276	248
Number of females	-	143	122
Number of males	-	133	126
Number of families		70	60
Number of households/houses	40*	-	45

* According to the village chief there were only 40 households at this time, but Bardosh et al (2014) reported that there were 62

Migration and mobility

In terms of movement of people (including villagers, traders, others) to and from the village; since the intervention in 2014 the road has been improved considerably. The village chief explained that this means: access to the district is better, that transport is easier; that cars can now get to the village during the rainy season (although there are sometimes problems); that the road through to Vietnam is much better so there's more traffic; and, that more Vietnamese are able to come through by this road. However, while the road enables more people to come into the village, the survey suggests that many villagers do not leave the village frequently or for long periods. Only 30% (12) of the respondents said that household members leave the village weekly or monthly and 13 % (5) reported that a household member left the villager once or twice per year. That said, there does appear to be some individuals that routinely travel from the village, for instance there is a school teacher that teaches in a village 60 km away; the village school is staffed by three teachers from Mai District. In addition, 21% (8) od the survey respondents reported that family members travel to their agricultural lands to work for a period of time in the sanaam. The amount of time varied and included daily, a few days, the entire rainy season, two months and the whole year. There was also some indication that people had interactions with neighbouring villages, but details of this were not explored. There are four neighbouring villages, the nearest is 4 km away and furthest more than 7 km, the ethnic groups living in these villages include Khamu, Tai Dam, Lao Sung Kor and Arkha.

Interventions

Over the period 2014 – 2019 there have been project interventions in the target village on almost a yearly basis. In 2017/2018 the Provincial Agriculture and Forestry Office (PAFO) ran a vaccine programme for haemorrhagic septicaemia in cattle and buffalo. In 2018/2019 the district hospital has been to the village to teach people how to use the toilets and to wash their hands after using the toilets. Currently (2019/2020), FAO and CARE International have nutrition programs and construction of a Nutrition and Agricultural Extension Office targeting child malnutrition by producing food (vegetables and poultry) is underway. In addition, at various times there have been health programs (e.g., vaccinations for Hepatitis B and doctor visits for eye disease). It was not confirmed by the district office, but the level of intervention suggests this village has been selected as a focal village by the government which means that it will continue to be targeted for government and externally funded projects.

6.2.2 PIG OWNERSHIP & HUSBANDRY

Ownership

There are three main reasons people kept pigs; "ceremonies, eating and selling." At the time of the first visit in 2019, 70% (26) of the survey respondents owned pigs, most owned 1 or 2 pigs whereas five people owned 4-7 pigs. At that time, the village chief reported that the number of pigs had declined in the village but did not give any indication of the extent of this decline. In the 2013 survey, slightly more people said they owned pigs (78%), certainly the number of pigs owned per person appears to have been higher (an average of 4.5 pigs per person in 2013 compared with 2.1 in March 2019) although this could be due to the time that the survey took place (i.e., in the ceremonial and/or reproduction cycle). However, during the second visit in 2019 a significant decline in pig numbers was reported due to African Swine Fever (ASF). In August alone 150-200 pigs had died and at the time of the second visit only 20 pigs remained in the village.

Regardless of the decline prior or post ASF, pigs continue to be important to people. Aside from their importance as a religious offering, source of protein and income, some people indicated that pigs "are part of their family" and that they "love the pigs." Understandably, respondents were devastated by the recent losses and reported that it was their intention to continue with pig

production the following year; their plan was to obtain “piglets from a neighbouring village, if they had any.” Some suggested that they would make improvements to their pig husbandry practices as a result of the disease outbreak, as outlined below.

Husbandry

Taenia solium transmission depends on pigs having access to human faeces; one strategy to reduce access is to ensure that pigs are securely penned at all times (Lekule and Kyvsgaard, 2003). Bardosh et al (2014) found there was a seasonal variation to pig management, with pigs free ranging in the dry season and penned in the wet season. However, small pigs were often free anytime throughout the year as they were able to escape from the pens. The 2019 study found little change to these practices; some people interviewed (27%) reported that they were now penning their pigs in the wet and dry seasons, but, according to the village chief and vice village chief, most pigs are still free range in the dry season and penned outside the village (in or near the rice fields) in the wet/cropping season. The latter is a ‘village law’ for all livestock, with consequences if the livestock damage a crop. In the case of pigs, if a pig damages a cassava crop then “the person who owns the crop is entitled to kill the pig and ask the owner to take it away.” Given that the system has not changed much in the last six years, scavenging pigs can still potentially consume contaminated human faeces, if not all year at least for part of the year. Given the recent ASF outbreak, the vice village chief thought that it could be important in the future to “keep pigs penned at all times so that they could not all scavenge together.” One farmer advised that they had penned their remaining pigs because they did not want to become infected. However, while the local pigs that are owned by people might be better managed in the future, as outlined below, wild pigs that are entirely free range are also being consumed, perhaps more so than local pigs. As village members mentioned they defecate in the open when they visit their fields or go hunting (see below) it is possible that wild boar have had access to contaminated human faeces and are another source of transmission for *T. solium*.

In the SGI, farmers described what looking after the pigs involved. One farmer described giving their pigs “a bath” each morning, another described what they were fed which included “cassava root and taro that have been boiled then mixed with rice bran as well as banana stem.” Another person remembered a technician from Vientiane capital coming to tell them how to build a good pig pen, one where the “floor is dry and clean and the floor is washed if it is dirty.” However, the pig pens that were observed were largely a simple construction of bamboo and natural fibres.

The village chief and SGI participants reported that no deworming/vaccination of pigs had taken place since the intervention in 2014. However, the former village chief reported that he had gone to PAFO himself to buy vaccine (which was assumed to be for CSF) and had administered it himself. Regardless, it seems that very few animals had been treated since 2014. The group we spoke to report no obstacle to paying for a vaccine and the village animal health worker who participated in the discussion said he had experience giving vaccines to “cattle and chickens, but never tried with pigs.” He felt that people would accept vaccines/deworming in the future. The other participants also said they accepted this, and given the recent deaths had intentions to do so in the future. However, there does seem to be some pervasive misconceptions about vaccinated pigs, in particular when and whether they are safe to eat with some people believing “that the vaccine is still in the body of the pig.” This was also reported in Bardosh et al (2014) indicating this is an important belief. Post the 2014 intervention villagers kept their vaccinated pigs penned, whereas unvaccinated pigs were allowed to scavenge. The village chief also reported that most people elected not to eat the vaccinated pigs at that time, instead eating unvaccinated or wild pigs.

6.2.3 PIGS FOR CEREMONIES, CONSUMPTION & SALE

Ceremonies

Tai Dam people have a number of cultural ceremonies, that involve offering and eating raw meat and liquor; these include for New Year's, village spirit ceremony, household ancestral ceremony, weddings, new houses and deaths, as reported by Bardosh et al (2014). It was explained by the spiritual leaders that, women are the spiritual leaders for all ceremonies except New Year's, although a man can lead other ceremonies if they have been taught. The spiritual leader's role is to chant from the ritual manuscript and make offerings to the gods, ancestors and talisman; the kind of offering depending on the ceremony. The respondents reported that nowadays raw pork is offered as part of the new year, wedding, new house and village ceremonies. As part of these ceremonies it is also common for villagers to consume pork.

The pigs offered at the ceremonies can be local or wild, or meat can be purchased from the market. The pigs killed for ceremonies are slaughtered in the village "outside the house, wherever there is space." The women explained that "the men kill the pigs because they are big and they cannot control them, but also because Tai Dam believe that women should not help the men kill the pigs, or touch the pig, especially at New Year's." It is also an "old cultural belief that men should prepare the food for the gods"; typically, raw larb and blood, whereas the food prepared for the guests is prepared by the women. Respondents explained that raw pork is given to the gods because they "follow the tradition and ways of their parents, and that it is important for them to follow the culture and not break it." There is a strong belief that "the gods don't like cooked food, they like medium cooked and raw food, but not fully cooked; the gods prefer to eat raw food." The respondents were asked if they thought there was a reason for this but did not have an explanation beyond that "it is tradition".

For guests attending the ceremonies, respondents indicated that cooked pork is served; all the people we spoke to including the village chief and three female spiritual leaders who preside over many village ceremonies were adamant that this was the case. It was reported that this change came about following the project intervention in 2014. Survey and small group interview data suggests that this is the case, for the majority of villagers, but that others still eat raw pork. In the survey, 21% of the respondents said that they eat raw larb and/or blood monthly, every few months or once or twice per year and in the small group interviews respondents reported that "70-80% of the people stopped eating raw pork, even raw wild pork after the project came." Consumption of raw pork is discussed further in the section on health and hygiene (7 2.4).

However, while pigs are central to many cultural ceremonies, on a yearly basis very few pigs are killed for this purpose. For example, in 2019 respondents reported that only "1-2 local pigs were consumed; about 60-100 kg of meat." The village chief confirmed this saying that the only local pigs killed last year were for Tai Dam New Year's where "two families killed pigs and had a big ceremony." He and the villagers also reported that in general very few animals are killed per year or per ceremony, "for the previous year (2018) there were no additional ceremonies and typically there were zero to two additional ceremonies in a year."

General consumption

Outside of cultural ceremonies, it seems that pork and indeed meat is not a major part of the villager's diet. Survey respondents described a diet mostly of fish, vegetables, bamboo shoots and eggs, and for some rodents, birds and buffalo. However, some people consume pork meat at times during the year and there are three main sources of meat:

1. *Wild pork:* if a person kills a wild pig then there is a rule that dictates that they can keep half, but that the other half must be distributed to other villagers. Although some villagers reported that this would be shared with every household, further probing suggests that priority is given to the village chief, village authority and immediate neighbours. How many people receive meat also depends on the size of the animal; the larger the animal the more meat there was to go around, but most wild pigs caught were small. Some of the poorer women reported that they "only eat a very little when a wild pig is caught" because they were mostly small and when they were not small the person that killed the pig was allowed to sell some of their share. In these circumstances "only people with a lot of money can afford to buy it." The village chief reported that in a year a minimum of three and maximum of six wild pigs were distributed, but the small group interview respondents reported it was much more than this saying 30-40 wild pigs were killed and eaten last year. Wild pigs are shot or captured and then slaughtered in the village although there is a preference to capture because "if the pig is dead they cannot get the blood."
2. *Meat (wild or local) purchased from the Mai District market:* villager's own pigs seem to be kept largely for ceremonies or sale whereas pork for general consumption (and some ceremonies) was purchased from the market. Some of the men explained that "every time they go to the town/market they purchase meat, but they do not go very often" whereas some of the women explained that they "don't have the money" to purchase pork very often."
3. *Meat sold in the village:* a less common source of pork meat was from within the village, it was explained that "on some occasions we kill a growing pig and sell to our neighbours, before killing we ask our neighbours how many kilos they want and record it."

Of these three sources, it seems that wild pork is more readily available and likely to be consumed throughout the year, and it seems that wild pork in particular is used to prepare raw larb. As explained by respondents "we stopped eating raw pork since the project came, except when we catch a wild pig, we eat the wild pig raw because wild raw larb is more delicious than local raw larb; people still eat raw wild pig and other animals."

Several factors seem to determine whether a person consumes more or less pork throughout the year: 1) whether they are influential and therefore are more frequently gifted wild pork; 2) if they (or their family) have reason to and the means to travel to Mai District; 3) whether they have the money to purchase pork from the market (and at times locally).

Sale

The sale of pigs is a common source of cash income, although only 50% of survey respondents reported that they sell their pigs. For those that do sell their pigs, 88% were sold when the pigs were less than one year old (see Table 4). This is consistent with findings from the earlier study where people said they preferred to sell their pigs when they were smaller because there were fewer feed inputs, they fetched a higher price and they tasted better (Bardosh et al 2014).

Table 5: Age and price of pig at sale

Age of pigs	% people selling	Average price (Kip)
3 months	36	400,000 (10 kg) – 500,000 (12 kg)
6 – 7 months	28	-
6 – 12 months	16	-
> 1 year	4	-

Age of pigs	% people selling	Average price (Kip)
3 – 3.5 years	8	1,000,000

In the interview people explained that they keep the piglets with the sow for three months and sell them when they weigh 10-12 kg, for any pigs that they keep for longer they keep for wedding parties. The piglets are sold to traders that come to the village, who sells on to their customers and also keep some of the weaning pigs for fattening. Most of the piglets are sold at celebration for the New Year, but the middleman comes anytime to buy. Some pigs are also sold to neighbours for cultural ceremonies.

In terms of people that had a more commercial approach to pig production, only four of the interviewed households keep pigs that they fatten and sell. The fattened pigs are sold to the slaughter-house in Mai District. The reason provided for fattening pigs was that "they have a large area of land away from the village, that they use to grow cassava and bananas which they can also feed their pigs." However, based on the prices provided for piglets versus older pigs, it seems that selling piglets is quite lucrative. It was interesting to note that the price of piglets per kg has increased from 25,000 Kip/kg in 2013 to 40,000 Kip/kg in 2019, which may have been related to significant reduction in pig numbers due to ASF. Unfortunately, little was learned about the market for pigs and how this has/has not changed as people were focused on the recent loss of their pigs.

6.2.4 HEALTH & HYGIENE

Staying healthy was recognised as important by all the people we spoke to, it was associated with being, being happy, comfortable and having a long life. It was also associated with being rich as staying healthy reduced out of pocket healthcare costs. The majority (97%) of survey respondents felt that their family's health was good and individuals in the SGIs reported few health problems. Colds and the flu seemed to be the main health problems in the village, especially "when the season changes" along with joint pain, high blood pressure, kidney stones (a small number of people). A shortness of breath identified as issues for older people and eye infections and childhood diseases for younger people. Diarrhoea was reported to be an issue especially in the dry season when the weather is hot, and they are in the rice fields where they tend to drink water from the stream. Eating sour fruits was linked to episodes of stomach aches among children and women.

Explanations regarding how diseases are transmitted or what caused them to get sick included:

- "*maybe its [*flu*] transmitted in the air*"
- "*I think they breath the [*sickness*] from the air, for example for flu, people normally get it in the cold season*"
- "*Cold weather, it makes us weak and makes it easy to get sick*"
- "*Eating raw larb/blood you can get an infection; the tapeworm egg will get into my blood and kill me*"
- "*for diarrhoea, drinking un-boiled water from the stream, the water in the stream is not clean, there's a lot of falling leaves and branches that make the water quality bad and the water is not clean because of the birds and wild animals urine and faeces*"

- "*eating a lot of sour fruit causes stomach aches and diarrhoea*" [the amount eaten was given as the reason, but follow up identified that the fruits being eaten are sour because they are unripe]

The steps that people took to stay healthy included:

- Good nutrition
- Washing hands before preparing food
- Washing the food before preparing it
- Eating well cooked food
- Washing dishes
- Drinking boiled water – unless in the fields/forest
- Washing hands after using the toilet
- Washing clothes
- Good personal hygiene
- Clean house

The survey results and interviews, highlighted that most people associated being healthy with being clean, including "eating clean" and "staying clean" and "having a cleaner place" and many of these practices were well now well established. Even so, there are some exceptions including those relating to the use of toilets, handwashing, drinking clean water and consumption of raw larb.

Access to and use of toilets

Prior to the *T. solium* intervention in 2013, a water and sanitation project supported the installation of toilets and a town water supply. Bardosh et al (2014) reported that this intervention took place in 2004 and at that time materials were provided for the construction of toilets, although not everyone constructed a toilet at that time citing a lack of education, lack of water, limited space and repurposing of the materials as reasons for this. It is not clear whether there was a follow up intervention, but the village officials described a UNDP project that provided materials for the construction of toilets. The village chief explained that people had to transport the materials from Mai District by themselves, this was an 8-12 hour walk and it took until around 2015 until all the toilets were complete. Today, according to the village chief all houses except for three have a toilet, the households that do not have a toilet share with others. The toilets are now all enclosed (unlike in 2013), but the materials the walls are made of vary (Figure 12). In the 2019 interviews, everyone we spoke to said they used a toilet "for number twos," but in the survey a small number of people reported that they defecate in the open (14%). Some individuals also mentioned they defecate in the open when they visit their fields or go hunting

Figure 12. Example of a new brick toilet facility recently added to this house.

Potable water for drinking/cooking

A water system was installed at the same time as the toilets, this system includes two large tanks for storing water, one upstream and one downstream in *nor namvarn stream*. These tanks are concrete blocks which are fully enclosed (figure 13). Water is piped from the stream to the tanks and then to the village. There are six taps in the village and water is also piped to people's houses. As with the toilets, the materials were provided by the project, but the villagers provided the labour. Many people we spoke to in the survey (67%) and interviews said that they accessed water from the government tap, but others said they accessed drinking water directly from the stream. It seems that when people are in the village they can more easily access this water supply and they boil their drinking water. However, when they village the field/forest they are unable to do so. As mentioned above, people are aware that un-boiled water can make them sick.

Figure 13. One of the water storage tanks

Handwashing

Most of the people surveyed (84%) said they washed their hand after going to the toilet, but the remainder (all males) said they never did or only sometimes. This was confirmed by interview participants (men and women) who conceded that men in particular were not always diligent about washing their hands, comments included:

- “I don’t think all men wash their hands and use soap after using the toilet… some men only use water, but only a few”
- “some men are lazy to wash their hands [after going to the toilet or before cooking], I have to force my husband to wash his hands”

As for whether people washed their hands before cooking, the survey responses were similar, most people said that they always did (86%) and the rest sometimes or never. In the interviews this seemed to be especially important to some, as one woman remarked “we don’t know how to cook if we don’t wash our hands” but not practiced by others. A young man laughed at himself because he tended to wash his hands after cooking and not before, “because he’s in a hurry all the time.”

Soap was used by a third (33%) of survey respondents all of the time and about half (48%) some of the time; the remainder (19%) never used it. It seems that hand washing in general has increased since 2013 where 70% of the respondents said they washed their hands after using the toilet compared to 88% in 2019. The use of soap has increased somewhat; in 2013 it was observed only on a few occasions in wealthier households. These changes may have been brought about by the district hospital intervention that teaches people how to use the toilets and wash their hands, although it was reported that while this intervention “targeted everyone, most of the participants were women.”

For the people that do not wash their hands. to some extent this could be due to lack of awareness of the important and/or not having formed the habit of hand washing, but limited understanding of germ theory of disease also seems to be a factor. Although cleanliness was considered an

important strategy for staying healthy, people we spoke to did not think that diseases could be transferred by touching something, like another person's hand, or that the disease was a material thing resulting from say a cough.

Consumption of raw larb

Some people we spoke to felt very strongly that eating raw larb (type of meat salad) was bad for their health, as one young man said "for me, eating raw is like eating disease, but others believe raw larb makes them healthier." This was reiterated by the village chief "people don't care about health, even if people die they still prefer to eat raw larb" and echoed an earlier statement reported in Bardosh et al (2014) where one respondent claimed that even though he knows eating raw pork may be bad from him, "it doesn't matter – today I am having fun. If I get a worm or even die I don't care. I will still eat this meat!"

Age and gender seem to influence how likely it is for someone to eat raw larb. Older people (like the village chief) seemed to prefer to eat raw pork, whereas the youth union members we spoke to understood the risks associated with eating raw pork and other young adults indicated that they had "never tried raw larb before" and that they "prefer cooked larb." Children were not served raw pork, although Bardosh et al., (2014) reported some instances where younger people would eat it anyway, and this was also reflected in some positive taeniasis cases in children (Okello et al 2016). The consumption of raw pork also seems to be more common among men, many women we spoke to said they were "afraid to eat raw pork." In general, there was perception that men are stronger and healthier than women (and perhaps invincible and therefore able to eat raw pork). This was reported by older men we spoke to – "men don't get sick compared to women, they work hard and drink rice wine, this is why they don't get sick" and younger men – "men are stronger than women, men work hard and eat a lot, women are weaker compared to men because they produce a lot of babies and they are more selective about what they eat." The women also agreed saying that "women have poor health because they give birth" but they also felt that this is because women work hard (perhaps harder than the men) and "do all the tasks for men."

For future control strategies, these findings highlight the importance of a gender strategy. It was also evident that endorsement of the village chief would be critical for garnering community wide support; he himself has not yet been convinced to change this behaviour.

As for what people do when they get sick, most said they go to the hospital (57%) or the doctor (27%) which could be at the hospital. Historically, it seems that medicinal herbs were used, but while the village chief still said this was common for people to "heal themselves" the female spiritual leaders reported that it was now more common to take people to hospital. Some of the younger people we spoke to also reported going to the pharmacy to purchase medication especially "if we feel unwell in our intestines" when this happens they said they would buy "de-worming tablets" and that they would do this every 3-4 years, but that they have not done this since the project "because we feel we don't have tapeworm." Perhaps an unintended consequence of the project is that people felt that they had been 'healed' permanently.

6.3 Discussion

6.3.1 General discussion

Parasitological testing has ascertained that while a sustained reduction in STHs had occurred, similar results had not been obtained for *T. solium.* Transmission of STHs is linked with open defecation and lack of hygiene practices and is more likely to occur in population dense areas such as the village environment where people and contaminated environments are in close proximity

(Ruberanziza et al., 2019). Focus group discussions (section 7.2) revealed that the number of latrines had greatly increased, with all but two households now in possession of a latrine, and government run education programs on appropriate use of toilets and handwashing had been recently conducted. The uptake in the use of toilets as reported in this project was noted in previous FGDs (Bardosh et al., 2014) whereby women especially valued the benefit of toilets in place of open defecation. Therefore, it likely that when toilets were obtained and people used them and employed good hygiene practices, a sustained reduction in STHs has been achieved.

However, while latrine access is likely to have positively influenced the prevalence of STHs in the community, cultural practices related to raw pork and pig husbandry remained largely unchanged since the 2014 intervention. Pigs were mostly free-ranging and open defecation still occurred from a minority of the village population, particularly in the field. Focus groups advised that the community had reduced their consumption of raw pork (attributed to education from AH/2009/001) but the ceremonial practices involving raw pork were still on-going and raw wild pork was a preference when caught. Understanding how behaviour change can be affected towards long held cultural practices is an important area of research when considering zoonotic disease and requires further investigation (Burniston et al., 2015, Goodwin et al 2012). For instance, in this project a gender and age bias towards the consumption of raw pork was reported with women and younger men less likely to eat raw pork to avoid disease. While village elders such as the chief are important influencers, focussing on women and younger men may provide an opportunity for instigating future behaviour change.

6.3.2 Lessons learned from village surveys and small group interviews

The surveys and small group interviews found that there have been some changes in behaviour since the 2014 intervention, but the changes are not community wide:

- The seasonal variation in pig management (free ranging in dry season/penned in wet season) was still commonplace, however after losing 80% of pigs due to ASF farmers commented they would consider full-time penning and vaccination to prevent this happening again.
- The number of households with toilets has dramatically increased (16% to 93%) with 100% of people surveyed reported using them, however 14% responded that they also practice open defecation.
- The majority of people (84%) stated they washed their hands after using the toilet and before cooking, but soap is still not routinely used.
- There was a reported decline in eating raw pork from survey respondents (70-80%) especially at ceremonies, but conversely an increase in the consumption of wild pork and pork from the market was reported. Some people (especially men and the older generation) still have a preference for eating wild raw larb and will not consider modifications such as freezing for food safety.

Lessons learned for future control strategies include:

- The ASF outbreak is a potential platform for encouraging improvements to animal husbandry (penning) and biosecurity (vaccination and anthelmintics).
- Education regarding vaccines and when it is safe to consume the meat, as well as timing of vaccines so as not to coincide with key ceremonies (consumption) is likely to be important.

- Mechanisms for scheduling and implementing ongoing vaccination is also likely to be important; the village animal health worker (where available) could be trained to manage and administer vaccines.
- There is a need to consider other sources of raw meat which could be contaminated with *T. solium* cysts, e.g. wild boar, general wildlife, dogs.
- Differences in the behaviour of men/women in terms of consumption of raw pork and hygiene practices highlight the importance of a gender strategy.
- Education regarding germ theory and ongoing health management could be valuable.
- Endorsement of the village chief is critical for garnering community wide support; and involving all village officials is likely to improve outcomes.

6.3.3 Future opportunities for control of zoonotic parasitic disease

Discussion with Lao stakeholders combined with the results obtained in this project have confirmed that there is a need for further research investigating *T. solium*, particularly in northern Laos. In particular, the need for deeper social science research around affecting cultural and behavioural change required for interventions to be sustainable. It appears that while antiparasitic drugs are needed in the first instance to eliminate parasitic infections, changing entrenched cultural behaviours is essential for sustained control and for unravelling where and how *T. solium* hotspots occur and are maintained (Bardosh et al 2016, Gabriel et al., 2016, Pearson et al 2016, Sripa et al 2016).

The emergence of ASF within the region has raised challenges for livestock farmers but may provide opportunities in the control of *T. solium* and other pig-borne zoonoses such as Japanese encephalitis and Hepatitis E which are endemic in Lao PDR. The study village had lost approximately 80% of their pigs, which was extremely distressing to the community as while pigs are valued as both a source of food and income, they are highly valued for cultural ceremonies mentioned above. They intend to restock when possible and some SHFs indicated they would change animal husbandry practices, specifically penning so they don't get sick from other pigs and vaccination if available. Providing additional education around these husbandry changes may be more readily accepted given the current climate of contagions. Therefore, opportunities may include:

i. a reduction in the animal reservoir for *T solium* will assist in eliminating the disease in hotspot communities
ii. behaviour change around animal husbandry, may be more readily adopted to ensure the survival of newly acquired pigs,
iii. the project will be well placed to assist with any NGO activities around re-establishing SHF pig production.

6.3.4 Validation of diagnostic assays

Many new diagnostic assays are published with the view that they improve on the current techniques however most are not 'road tested' for their viability outside the well-equipped research laboratory. We trialled a range of molecular based assays detecting human *Taenia* species (Nguyen et al., 2018; Nkouawa et al., 2016; Yamasaki et al., 2004) and STHs (Cunningham et al., 2018) with varying results. Preliminary results for the conventional and real-time multiplex PCR for *Taenia* spp. (Nguyen et al, 2018; Yamasaki et al., 2004) found the assays to be sensitive and have potential for high-throughput testing, however specificity needs to be further tested to ensure all

human *Taenia* spp. are routinely detected. Likewise, the real-time PCR for the simultaneous decection of several STH parasites (Cunningham et al., 2018) was specific for multiple parasite species and has the potential to replace traditional microscopy, provided appropriate controls can be sourced and maintained. Limitations with these molecular based assays include the need for specific laboratory equipment, (thermocyclers), complex (non-standard) reagents and appropriate controls, all of which can be difficult to source locally. In the Lao context National laboratories often have appropriate laboratory equipment but reagents can be difficult to source. This limitation may be alleviated if standard reagents, more readily sourced, can be optimised to work in these assays and will be the subject of further investigations and trials.

Provincial laboratories and field conditions are not suitable for assays requiring PCR thermocyclers, however assays using LAMP, which combines the specificity of PCR in detecting parasite species, without the use of expensive equipment have great potential for point of care diagnostics. Preliminary validation of a published LAMP assay (Nkouawa et al., 2016) indicated specificity and visualization was poor however avenues for improving these limitations have been identified and will be the subject of further investigation and trials. Overall, these assays show potential for but need to be further investigated for sensitivity, specificity and feasibility for low resource laboratories. Assays using the LAMP technique are of most interest due to their potential use in provincial and field settings.

7 Impacts

7.1 Scientific Impacts – now and in 5 years

Eliminating *T. solium* from endemic regions is extremely challenging and currently rarely achieved. The results from this project have provided evidence on what impact/s a One Health control program may have on disease transmission within a community and in doing so guide future research strategies.

The simultaneous administration of anti-parasitic drugs to people and pigs to eliminate infections of *T. solium* was effective in the short term however was not sustained and the reasons for this have become clearer from this project. Attitudes towards food safety and hygiene have largely remained the same between the 2014 intervention and this impact assessment, in that open defecation still occurred (although reduced), pigs are still free-ranging and raw pork is still consumed. In 2014 it was reported a passive strategy had been used to affect behaviour change in this village, involving pamphlets and posters showing biomedical images that while valued were perhaps not effective communication materials for long-term change (Bardosh et al., 2014). The ability to affect behaviour change is now recognised as a vital component of intervention programs and requires knowledge of the local environment in order to tailor appropriate strategies (Bieri et al., 2013; Briscoe and Aboud, 2012; Carabin et al., 2018). Future strategies should therefore heed these findings and include a strong social science focus aimed at understanding the social/behavioural/cultural/environmental conditions of a community involved in zoonotic disease control programs in order to have a significant and sustained impact.

The initial burden of STHs within the community was reduced in 2014, however evidence for an increase in parasite prevalence was detected throughout the monitoring period (Ash et al., 2017). Sustained control of STHs within the community is more likely to be achieved if therapeutic treatment is combined with environmental changes, (increase in household toilets) and behavioural changes (use of toilets and handwashing). Future STH control strategies should therefore attempt to co-ordinate with organisations such as UNDP or UNICEFs Water Sanitation and Hygiene (WASH) program. Overall, the results obtained from this impact study should influence the scientific design of future control programs attempting to mitigate the transmission of zoonotic disease.

7.2 Capacity impacts – now and in 5 years

This project required a new team to be assembled and new partnerships made with the National University of Lao and the Ministry of Health. Through these new partnership staff at NUoL and MOH have gained capacity to:

- conduct ethnographical research,
- understand challenges in control of zoonotic disease
- design one health projects for zoonotic disease
- work in a co-ordinated way with different government agencies

This increased capacity and knowledge base should allow further research activities to be conducted both independently and collegially.

7.3 Community impacts – now and in 5 years

This SRA was assessing the impact of a previous project (AH/2009/001) rather than changing economic, social or environmental conditions within the community at this point. However, there is likely to be impacts in 5 years' time as this impact assessment will be used to inform a future project (building on AH/2009.001) aimed at investigating and developing interventions to mitigate food borne disease in northern Lao PDR.

7.3.1 Economic impacts

Not applicable

7.3.2 Social impacts

Not applicable

7.3.3 Environmental impacts

Not applicable

7.4 Communication and dissemination activities

Activities undertaken in the project to disseminate project results was largely through project meetings and conference presentations.

Project meetings were attended by staff from MoH (CDCD and CMPE); NUoL (Faculty of Agriculture and Veterinary Science); NAHL (parasitology department) and Murdoch University. Meetings were:

- An initial project meeting was held in Vientiane to update the newly formed team with an overview of the project, including past results, and to plan field activities for the upcoming impact assessment.
- A post impact assessment project meeting was held to disseminate the results obtained and to propose future work.

Conference papers were presented at:

- The Regional symposium on research into smallholder pig production, health, and pork safety", Hanoi, Vietnam
- The annual conference of the Australian Society for Parasitology in Adelaide (Australia).

Both conference papers presented the preliminary results of the project which were available at that time.

Further conference papers were planned which would have presented the final results (lessons learned) but these were cancelled due to COVID related travel restrictions.

A guest lecture was delivered to the Tropical Public Health Institute postgrad students (Masters in Public Health). The lecture provided a background on zoonotic parasitic disease and described the research currently being conducted on *T. solium* in northern Lao.

8 Conclusions and recommendations

8.1 Conclusions

This impact assessment has determined that mitigating the continual transmission of *Taenia solium* between pigs and people will require a strong social science/communication program centred around activities which can affect sustainable change in community behaviour and practices.

As demonstrated by Okello et al (2015), the simultaneous administration of anti-parasitic drugs to eliminate infections of *T. solium* in both the human and pig population was effective in reducing the level of infection within a community in the short term. However, this project has demonstrated that long-term control is not likely to occur unless associated disease transmission risk factors are reduced. These risk factors include free roaming pigs having access to human faeces, people eating raw pork and poor hygiene, the sum of which contribute to the continual transmission of *T. solium.* Eliminating one or more of these risk factors would greatly increase the chance of long-term control. Identifying methods which will be accepted by communities, based on risk factors present in the community, will be key to sustainable control.

Within the study village it was found that some behaviour/environmental change had occurred, specifically the use of toilet facilities when they become available and the associated hygiene practices such as washing hands. The village experienced a dramatic increase in household toilets since the 2014 intervention (6 to 50) and the preferred use of these has likely influenced a marked decrease in the level of environmental contamination with STHs. This effect was reflected in the parasitology results which detected a significant reduction in STHs within the community, and importantly a significant reduction in the intensity of infection. The village members did not indicate that anti-parasitic drugs had been administered to the community in the previous 5 years and therefore this sustained reduction in STH levels appeared to be achieved through change in both environment (toilet facilities) and behaviour (preference to use toilets associated hygiene practices).

A similar sustained reduction in the prevalence of *T. solium* within the community was not observed and had returned to pre-2014 levels. The village surveys and small group interview work revealed that free-ranging pig farming systems still predominated, human defecation in the field occurred out of habit and necessity and raw pork consumption was still considered essential for many cultural ceremonies, all of which combined contribute to the continual transmission of *T. solium.* Under the current village system, a therapeutic approach appears to be the most effective tool to manage infection in the short term, but likely unsustainable outside donor-funded programs. This highlights the need to further identify - and link with - programs which can affect behaviour change around the known key risk factors.

While the emergence of ASF has been devastating to SHFs in the region it may provide a potential platform for encouraging improvements to animal husbandry which will assist in the control of *T. solium.* Specifically, the apparent willingness to pen pigs in future to avoid disease and implementing vaccination programs, would significantly reduce the chances of pigs becoming infected and therefore cultural preferences for eating raw pork would no longer be a risk factor.

Overall, the results from this study confirm that deeper social science research around culture and affecting behaviour change is required for developing appropriate tools and intervention strategies which can bring about long-term control for complex diseases such as *T. solium*.

8.2 Recommendations

Overall, the main recommendations from this impact study are:

- Control programs for zoonotic diseases such as *T. solium* should include a social science arm aimed at understanding how best to affect behaviour change associated to risk factors for disease transmission. This impact assessment discovered that while therapeutic intervention was initially effective against eliminating *T. solium,* cultural and behavioural practices related to pig husbandry, hygiene and food safety remained unchanged, the sum of which have most likely contributed to the continual transmission of *T. solium*

- Sustained control of STHs within the community is more likely to be achieved if therapeutic treatment is combined with environmental changes, (increase in household toilets) and behavioural changes (use of toilets and handwashing). Future STH intervention programs should therefore co-ordinate with programs such as WASH.

- Intervention strategies which target multiple pathogens can provide opportunities for synergies with other government/NGO programs. In this case, post the 2014 intervention a UNDP program provided materials for building toilets, and district health officials provided education on toilet use and handwashing, which combined has led to a sustained control of STHs within the community.

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9.1 List of publications produced by project

Conference Papers

Ash A et al., September 2019. *Taenia solium control – is it achievable? Results of a pilot study in northern Lao PDR*. Australian Society for Parasitology Annual Conference, Adelaide.

Ash A et al., March 2019. *Taenia solium control – is it achievable? Results of a pilot study in northern Lao PDR*. Regional symposium on research into smallholder pig production, health, and pork safety, Hanoi, Vietnam.