Australian Government
Australian Centre for
International Agricultural Research

Final report

project	**Management of pig-associated zoonoses in Lao PDR**
project number	AH/2006/161
date published	May 2012
prepared by	James Conlan
co-authors/ contributors/ collaborators	Prof. Andrew Thompson Dr Stuart Blacksell Prof. Stan Fenwick Dr Khamphouth Vongxay Dr Boualam Khamlome
approved by	Dr Doug Gray, Research Program Manager Animal Health, ACIAR
final report number	FR2012-14
ISBN	978 1 921962 72 1
published by	ACIAR GPO Box 1571 Canberra ACT 2601 Australia

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1 Acknowledgments

We would like to thank the many women, men and children in northern Laos who very generously gave their time and patience and whose cooperation greatly contributed to the success of this project. We would also like to thank the many Lao researchers in the National Animal Health Centre and government partners in the provincial agriculture and forestry office (PAFO) and district agriculture and forestry office (DAFO). We are greatly indebted to the Department of Hygiene and Prevention, Ministry of Health, for their strong support and coordination of the human health studies and the respective provincial and district health offices. We are also greatly appreciative of the diagnostic and technical support provided by our many research partners, the Mahidol-Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand; the Armed Forces Research Institute for Medical Sciences (AFRIMS), Bangkok, Thailand; the Tropical Disease Research Laboratory, Khon Kaen University, Khon Kaen, Thailand; the Department of Infectious, Parasitic and Immunomediated Diseases, Istituto Superiore di Sanita, Rome, Italy; the Institute of Tropical Medicine, Antwerp, Belgium; the National Centre for Laboratory and Epidemiology (NCLE), Vientiane, Lao PDR; the Institut Francophone pour la Médecine Tropicale (IFMT), Vientiane, Lao PDR; the CSIRO Australian Animal Health Laboratory, Geelong, Australia; and the Australian Centre for International and Tropical Health (ACITH), University of Queensland, Brisbane, Australia. We would also like to acknowledge the support of the Lao and Australian governments that supported this research.

2 Executive summary

Improving livestock production systems has been assigned a high priority in the Lao government's rural development strategy for food security and poverty reduction. Pig production is becoming increasingly important for food security in Laos. The vast majority of pigs are produced in the smallholder sector using low-input traditional methods and these farming practices present opportunities for the transmission of a broad range of medically important zoonotic infectious diseases. Smallholder farmers can be exposed through occupational, environmental and food-borne contact with infected animals. Impacts include ill health and associated disabilities, decreased access to pork markets and decreases in pig production. Moreover, control of zoonotic diseases is a high priority for the Lao Ministry of Health and several initiatives have recently been undertaken in collaboration with the Ministry of Agriculture and Forestry to address zoonotic diseases. The "Management of pig associated zoonoses in Lao PDR" project was initiated to establish the evidence base regarding the presence and socioeconomic impact of pig associated zoonoses in Laos ran from January 2008 to December 2010.

The project took a 'one-health' approach to investigate multiple zoonotic diseases to determine the prevalence and risk factors associated with infection in Luangprabang, Oudomxay, Xiengkhuang and Huaphan provinces in northern Laos. Furthermore, the costs associated with pig associated parasitic zoonoses were estimated to underpin the science and provide policy makers further evidence to support the implementation of control measures for these diseases. The project was managed by the School of Veterinary and Biomedical Science, Murdoch University in partnership with the Lao Department of Livestock and Fisheries. Through the DLF the project worked with provincial and district agriculture and forestry offices to conduct slaughter-house and village based animal studies. Human health research was conducted by the Department of Hygiene and Prevention (DHP), Ministry of Health, and through the DHP, the project linked with provincial and district health offices to conduct village based research activities. The project's research activities were also supported by the Wellcome Trust-Mahosot Hospital-Oxford University Tropical Medicine Research Collaboration, Microbiology Laboratory, Mahosot Hospital, Vientiane and the Mahidol-Oxford Tropical Medicine Research Unit (MORU), Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand.

The project found that the human, pig and dog populations studied had a very high prevalence of parasitism, in particular polyparasitism, and zoonotic transmission between humans and animals was apparent for multiple species including taeniaisis/cysticercosis, trichinellosis, *Giardia* and soil transmitted helminths. Cysticercosis in the human population was relatively rare with detected prevalence less than 2%, although we observed a focal distribution with high prevalence detected in some villages. *Taenia saginata* was the dominant *Taenia* species infecting people and *T. hydatigena* was the dominant species infecting pigs. *Trichinella spiralis* was the only species detected in pigs and we found serological evidence that human exposure to *Trichinella* larvae was common. The project also found that the zoonotic viruses, Japanese encephalitis virus (JEV) and hepatitis E virus (HEV), are an unmanaged threat to human health with evidence that JEV is hyper-epizootic with peak transmission in the wet season.

The socioeconomic impact of selected pig-associated zoonoses was found to have a significant impact on the economy of northern Laos and warrants policy attention. A substantial proportion of the economic burden was borne by the agricultural sector due to carcass condemnations and downgrading and losses in productivity. The project was very successful at establishing the evidence base for the occurrence and impact of pig associated zoonoses and as a direct consequence, the results have directly resulted in multiple research partners developing new research for development initiatives in the area zoonotic infectious diseases.

3 Background

Greater than 80% of all households in Lao PDR are agricultural holdings, of which greater than 90% are smallholder producers using predominantly traditional practices. One of the important ways rural families maintain livelihoods is to generate income through the production of a small number of livestock. Pig production plays a very important role in this income generation and approximately 70% of households in the northern provinces raise pigs (MAF, 2000). Poorer households have a heavy reliance on small livestock (pigs and poultry) for income generation, often contributing greater than 50% to household income, especially in remote areas. Many of the poorest districts are found in the southern provinces bordering Vietnam and in the northern provinces bordering China and Vietnam (WFP, 2001), where agricultural production is impeded by a rugged mountainous environment and unexploded ordinance. These same regions have the greatest density of pigs per square kilometre of agricultural land (FAO, 2005) and per capita pig density is highest in the northern provinces (WFP, 2001). A survey (Phengsavanh and Stür, 2006) of northern Lao PDR describes three main pig production systems: (a) seasonal or year round free-range scavenging, (b) confinement in an enclosure with the provision of feed and basic housing and (c) confinement in a pen with a raised slatted floor and the provision of feed. A combination of these management systems may exist in any one village but in general Hmong people living in mountainous upland areas raise sows for piglet production in a free range scavenging environment. The *Lao loum* people who tend to live in the valleys and lowland areas predominantly buy weaned piglets to fatten before sale in pens (Phengsavanh and Stür, 2006). So there is generally a movement of piglets from mountainous upland areas to lowland fattening production systems. Exotic or cross-breed pigs produced in semi-intensive and intensive production systems generally supply large urban markets due to a demand for low fat pork. Smallholder producers generally have access to markets but are highly reliant on 'middlemen' traders to purchase pigs, the distance pigs travel is largely dependent on where traders originate. Indigenous breed pigs produced in the smallholder sector generally supply localised markets (district and provincial centres) and there is generally a lack of detailed information regarding the supply chain for indigenous breed pork; including point of slaughter before sale in markets, meat inspection and registration of pork traders. There is no doubt pig production is important to the livelihood of farmers and to the food security of the population in general, however there is a general lack of data on zoonotic diseases associated with pigs in Laos. Data on pig associated zoonoses is limited to several case studies of trichinellosis outbreaks, cysticercosis related condemnations of pig carcasses in Luangprabang and evidence in humans that Japanese encephalitis is endemic. There is no data describing the epidemiology and specific risk factors for exposure of pigs and humans to zoonotic diseases in Laos. Moreover, there is no data describing the potential economic impacts of pig-associated zoonoses and no specific programs to control these diseases in the pig and human populations.

The tapeworm, *Taenia solium*, is an important zoonotic agent with a lifecycle that involves pigs and humans. In humans, who can be the definitive and intermediate host, *T. solium* infection can present in two forms, intestinal tapeworm infestation (taeniasis) and larval cysts in muscle tissue and the brain (cysticercosis); the later possibly resulting in severe neurological damage or death. Humans acquire taeniasis when eating raw or partially cooked pork contaminated with *T. solium* cysticerci (larval cysts). After ingestion, the larval stage establishes in the intestine and develops into the adult tapeworm. Adult worms shed thousands of eggs in the faeces of infected individuals that can cause infection in pigs and humans. Ingestion of eggs results in larval worms migrating to different parts of the human and pig body where they form cysts. The infection cycle is maintained in production systems with extensive pig farming practices where pigs are exposed to human effluent through poor sanitation. The disease is closely associated with the poor who do not have access to improved pig management practices, clean water and/or adequate sanitation. Pork consumers are exposed along the supply chain and risks increase in an environment where pork is not subject to thorough inspection at slaughter. Taeniasis/cysticercosis is considered one of the most important zoonoses in Southeast Asia (Willingham, 2002) and evidence from Cambodia suggests that the prevalence of *T. solium* infection in locally bred pigs raised in traditional farms is 25% and as high as 36% in some provinces (Sovyra, 2005), indicating that the disease could be widespread and a serious economic issue for Laos.

Trichinellosis, a disease caused by ingesting the larval stage of the nematode *Trichinella* in raw or undercooked pork, is also a recognised problem in Laos with two recent outbreaks in 2005 receiving considerable attention. The first outbreak occurred in Bolikhamxay province (Sayasone et al. 2006) and the other in Oudomxay province (Barennes et al., 2006), with the latter resulting in approximately 600 human infections and in complete cessation of pork and pig sales in the Oudomxay market for three months. No reliable prevalence data for pigs is available for *Trichinella* infections in Laos; however, it is expected to be endemic as it is in southern China and Thailand. In a southern province of China near the border with Laos, pig trichinellosis has been closely associated with upland-free range farming (Cui et al. 2006). Traditionally, Lao people eat uncooked or partially cooked meat (Rim et al. 2003; Sayasone et al. 2006) which places them at considerable risk of contracting these and many other food-borne zoonotic pathogens. In addition, sale of infected pork through markets places the wider community at risk considering the consumption of uncooked pork is common in Laos (Rim et al. 2003). Trichinellosis results in high morbidity and in severe cases death, and is rated a priority zoonosis in the Southeast Asian region (Willingham, 2002).

There are a number of other zoonotic diseases that are emerging or re-emerging in Asia that pose substantial risks for smallholder farmers and pig production, including Hepatitis E virus (HEV) (Blacksell et al 2006) and Japanese encephalitis virus (JEV), *Streptococcus suis* (Yu et al, 2006), leptospirosis (Boqvist, et al 2005), salmonellosis, cryptosporidiosis and toxoplasmosis. The importance of these diseases in the Lao context is unclear; in Vietnam however, *S. suis* is a major cause of meningitis (pers. comm.. Dr. Paul Newton) and HEV and JEV are listed by the Lao Ministry of Health and the WHO as priority zoonotic diseases.

There is sufficient evidence to indicate that pig associated zoonotic disease is an important issue although there is limited information that details the extent and distribution of the problems and the associated social and economic costs. A recent economic and consumption survey (LECS 3, 2003) clearly demonstrates that ill health has a major impact on work force participation and significantly contributes to the cycle of poverty. The role that livestock play in causing ill health in humans in Laos is poorly understood. *Taenia solium* cysticercosis is an internationally recognised high priority disease and is listed by the World Health Organisation (WHO) as one of the ‘Neglected Tropical Diseases’. The international burden of neglected tropical diseases are currently underestimated (Engels and Savioli, 2006) and scientific evidence is required to inform decision making processes regarding control and health and agricultural policy. The original concept for this project arose after Australian project staff involved in ACIAR project AH/2003/001 had discussions with tropical infectious disease specialists working at Mahasot Hospital, Vientiane and Lao veterinarians working at the National Animal Health Centre. Clearly, cysticercosis, trichinellosis and other zoonoses were seen as a serious threat to public health and to livestock production and marketing, yet little was known of these diseases in Lao PDR.

4 Objectives

The aim of the project was to establish the evidence base and define the socioeconomic impact of pig associated zoonoses in Lao PDR and to identify and test appropriate interventions. The specific project objectives as set out in the project proposal are:

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1) Establish the prevalence, distribution and socioeconomic impacts of pig associated zoonoses in the Lao PDR.

- Introduce surveillance and diagnostic capacity for the food-borne parasitic diseases, cysticercosis/taeniasis and trichinellosis and strengthen capacity for the identification of faecal pathogens such as *Cryptosporidium* and *Salmonella*.
- Adapt established survey methodologies to conduct cross-sectional investigations of priority zoonotic diseases in four target provinces
- Knowledge survey and education needs assessment of 12 target villages
- Develop, distribute and test the effectiveness of education/public awareness material in 12 target villages
- Conduct socioeconomic impact assessment surveys

2) Determine the risks associated with human and pig transmission at the village level and identify appropriate and sustainable disease control strategies.

- Conduct quantitative epidemiological studies on human and pig disease in at least 12 target villages
- Conduct qualitative and observational epidemiological studies in 12 target villages
- Participatory problem diagnosis and development of a basket-of-options for disease control in 6 villages

3) Implement and evaluate control measures to minimise disease impacts on smallholder producers and the wider community.

- Implement disease control interventions and monitor uptake, understanding and sustainability in 6 villages
- Evaluate control interventions through continued surveillance
- Monitor and evaluate project activities and conduct follow-up socio-economic impact assessment surveys
- Assisting the Lao-EU project in developing relevant meat inspector training/course material

4) To develop appropriate regional and national communication strategies for the wider uptake of project outcomes to manage pig associated zoonoses.

- Workshops and training courses engaging international, national, provincial and district stakeholders
- Communication of project outcomes regionally and internationally through networks, conferences and workshops.
- Publication of project outcomes in peer reviewed scientific publications and workshop proceedings.

5 Methodology

The pig zoonoses project was conducted in Laos and managed by the School of Veterinary and Biomedical Sciences, Murdoch University, in partnership with the Lao Department of Livestock and Fisheries (DLF), Ministry of Agriculture and Forestry. Murdoch University provided the international project leader who was based in Vientiane and the DLF provided the Lao project leader, a project scientist and supporting laboratory staff. Through the DLF, the project linked with provincial and district agriculture and forestry offices (PAFO and DAFO) to conduct slaughter-house and village based research activities in one district in each of four provinces: Xiengngeun district in Luangprabang province, Xay district in Oudomxay province, Viengxay district in Huaphan province and Pek district in Xiengkhuang province. The project also linked with the Department of Hygiene and Prevention (DHP), Ministry of Health through the the Francophone Institute of Tropical Medicine (IMFT). The DHP provided an infectious diseases doctor to coordinate research and through the DHP, the project linked with provincial and district health offices (PHO and DHO) to conduct village based research activities. The project's research activities were also supported by the Wellcome Trust-Mahosot Hospital-Oxford University Tropical Medicine Research Collaboration, Microbiology Laboratory, Mahosot Hospital, Vientiane and the Mahidol-Oxford Tropical Medicine Research Unit (MORU), Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand.

The pig zoonoses project was heavily reliant on laboratory testing to achieve the objectives and not all of the testing could be done at the National Animal Health Centre (NAHC, livestock laboratory) and the National Centre for Laboratory and Epidemiology (NCLE, human health laboratory) due to capacity constraints that could not be addressed within the budget and timeframe of the project. The genetic typing of Hepatitis E virus (HEV) together with the human and pig HEV and Japanese encephalitis virus (JEV) serology was conducted at the Armed Forces Research Institute for Medical Sciences (AFRIMS) in Bangkok and this was facilitated and coordinated by MORU. The Nipah virus (NiV) and swine influenza virus (SIV) serology was conducted by the CSIRO Australian Animal Health Laboratory (AAHL) in Geelong.

The human faecal samples were tested at Khon Kaen University, Khon Kaen, Thailand. Egg detection from faecal samples of dogs and pigs was conducted at the NAHC and also at the School of Veterinary and Biomedical Sciences, Murdoch University. All molecular characterisation of parasite eggs (human, dog and pig faeces) were done at Murdoch University.

Cysticercosis antigen detection ELISA was conducted at the NAHC for pig serum and the NCLE for human serum. *Trichinella* muscle digestion from pigs was performed at the NAHC and *Trichinella* antibody ELISA was performed at the NCLE for human serum. Molecular characterisation of *Trichinella* larvae recovered from pigs was carried out at the European Union Reference Laboratory for Parasites, Istituto Superiore di Sanità, Rome, Italy. Molecular characterisation of tapeworms collected was conducted at the NAHC.

Socio-economic modelling of the costs associated with cysticercosis and trichnellosis were out sourced to the School of Population Health, University of Queensland.

5.1 Objective 1: Establish the prevalence, distribution and socioeconomic impacts of pig associated zoonoses in the Lao PDR

Laboratory training was conducted at the National Animal Health Centre in relevant serological and parasitological procedures for trichinellosis, cysticercosis and enteric parasite egg detection.

A through chain assessment of the pork supply chain was conducted at the start of the project in the first quarter of 2008. The study was designed to describe the pig production systems supplying the pigs for slaughter in the selected districts and to understand the different actors in the supply chain from production to consumption. The initial survey was also designed to inform the design of the slaughter-house surveys with regards to slaughter-house locations, animal numbers, slaughter-practices, meat inspection capacity and animal traceability. The survey consisted of international, national, provincial and district animal health workers and was conducted in 8 villages in each district. Key informant interviews were conducted with village pig producers, pig traders, pork sellers and district and provincial agriculture staff using a combination of check-list questionnaires and interactive techniques.

A second survey was conducted in 2008-2009 in the slaughter house identified in the first survey. Pig surveys were conducted at three slaughter-points in Xiengkhuang and Oudomxay provinces from May to September 2008 and at two collection points in Huaphan and Luangprabang provinces from October 2008 to January 2009. The survey team consisted of trained district and provincial agricultural and forestry government staff who visited the slaughter-points approximately every two weeks. All pigs brought for slaughter on the nights the survey team visited were examined post-mortem and a blood sample was collected. The tongue and diaphragm pillar muscles were excised and examined for cysts and sent to the laboratory in Vientiane. Pork traders prevented muscle slicing; as such, the heart, liver, mesentery and omentum and other viscera were examined for the presence of *Taenia* cysts, as were all exposed muscle surfaces. Presence of cysts and data on location, age, breed, sex and production system at last point of sale were recorded on a data collection sheet and sent to Vientiane Capital with the blood sample and any cysts found. Tonsil and diaphragm muscle were disgeted to detect *Trichinella* larvae and blood and faecal samples were tested by serology and for the presence of enteric parasites respectively.

5.2 Objective 2: Determine the risks associated with human and pig transmission at the village level and identify appropriate and sustainable disease control strategies.

Follow-up surveys were conducted in six villages in each district in the human, pig and dog populations in the dry season from January to March 2009. For the human study, fourteen households were randomly selected in each village and all household members ≥6 years old were asked to participate. A household questionnaire was administered to the head of each household with his/her family present to assess the house characteristics, assets owned, ownership of animals, ethnicity, education levels. An individual questionnaire was administered to all study participants, with younger participants (<15 years) interviewed in the presence of a parent or guardian. Data on raw meat consumption and practices related to disease transmission were collected. Blood and faecal samples were collected and stored at 4 °C for serological and detection of enteric parasites, respectively.

Faecal samples were collected from 181 pigs ≤6 months old with the permission of owners in the same villages as the human survey described above from January to March

2009. Samples were stored in RNA preservation media for molecular diagnostic testing and in formalin and alcohol for the detection of enteric parasites.

Dog faecal samples were collected with the permission of owners in the same villages as the human survey described above from January to March 2009. Samples were collected digitally and preserved in 10% formalin. Demographic data and the age and sex were recorded with the sample.

The human and animal studies were approved by the Murdoch University ethics committees and the human study was approved by the Lao Ministry of Health's research ethics committee.

5.3 Objective 3: Implement and evaluate control measures to minimise disease impacts on smallholder producers and the wider community.

Within the timeframe of the project, only one component of this objective was completed. Meat and carcass inspection training was conducted for the project staff involved in the survey and district staff working with the "Lao – EU Farmer Support Project" were also trained in meat inspection techniques.

5.4 Objective 4: To develop international, regional and national communication strategies for the wider uptake of project outcomes for the management of pig associated zoonoses.

Project results were communicated regionally and internationally through the publication of peer reviewed journals and the presentation of results at international conferences and regional workshops. Research was also reported on at the 2010 annual meeting and the closing meeting held in December 2010 in Vientiane. During the course of the project regular consultations were held with the World health Organisation (WHO) and the Lao Ministry of Health and other stakeholders in human and animal health development, including NGO's and international development organisations.

6 Achievements against activities and outputs/milestones

Objective 1: Establish the prevalence, distribution and socioeconomic impacts of pig associated zoonoses in the Lao PDR

no.	activity	outputs/ milestones	completion date	comments
1.1	Introduce surveillance and diagnostic capacity for the food-borne parasitic diseases, cysticercosis/taeniasis and trichinellosis and strengthen capacity for the identification of faecal pathogens such as *Cryptosporidium* and *Salmonella*	Transfer of new diagnostic technologies.	Aug 2008	Laboratory equipment procured and SOPs established. Monoclonal and polyclonal antibodies for ELISAs were sourced from the Institute of Tropical Medicine in Belgium and from the reference laboratory for *Trichnella*, Rome, Italy.
		National and provincial staff trained in new diagnostic methods and established diagnostic test methods enhanced.	December 2008 May 2010	NAHC staff received training in Trichinelosis muscle digestion and cysticercosis and trichinellosis antibody detection by ELISA. Expert training on faecal egg floatation and microscopy was provided to detect Helminth eggs in pig and dog faeces. Ministry of Health laboratory staff were trained in cysticercosis and trichinellosis antibody ELISA.
		Diagnostic sample submission network implemented	May 2008	The submission network was established for the pig surveys and was initiated in six districts of the four survey provinces. Samples collected included blood (serum), diaphragm, tongue, faeces and cysts
1.2	Adapt established survey methodologies to conduct investigations of zoonotic disease occurrence in four target provinces.	A quantitative description of the epidemiology of pig associated zoonotic disease in four provinces.	Dec 2009 Dec 2010	Pig surveys were completed in March 2009 and diagnostic tests and analysis were complete by Dec 2009 Human serology for parasitic diseases were completed in May 2010 and viral zoonoses in Dec 2010.
		Detailed understanding of the extent of the disease threat facing smallholder farmers	Dec 2010	The full results of pig and human diagnostic tests and analysis were completed in the final months of the project. Delays were experienced for all human tests as our samples were tested in multiple laboratories and had to fit with work schedules in those labs.
1.3	Knowledge survey and communications needs assessment of target villages	Knowledge gaps at the district and village level identified and documented.	Jul 2009	The life cycle of *Trichinella* and Taeniasis/cysticercosis are complex and substantial knowledge gaps exist at all levels of government and at the village level. Most villagers were unaware of the range of infectious diseases people could contract from pigs.

		The preferred and most appropriate method of delivering resource material identified.	Dec 2009	Owing to the general lack of knowledge of transmission pathways and life cycles, the communication focused on providing good quality information in a poster and flip chart format for awareness raising at villager and district levels for the parasitic zoonosis cysticercosis.
		2 MSc students trained at IFMT	Dec 2008	One Master's student from IFMT was co-supervised by the Australian project leader and his thesis examined the risks associated with hepatitis E virus between rural and urban women.
1.4	Develop, distribute and test the effectiveness of education/public awareness material	Information and educational packages for villagers and district extension staff	Dec 2010	Communication material was field tested in the third quarter of 2010 and the general role out of the package was not done in this project. The package consisted of a taeniasis/cysticercosis life cycle poster and accompanying flip chart detailing risk factors and interventions to prevent infection/transmission.
		Survey results of knowledge uptake and understanding	Not completed	The education package was not completed in time to role it out. The material was provided to the follow up ACIAR funded pig health project and to the NAHC and DHP.
1.5	Conduct socioeconomic impact assessment surveys	A qualitative description of the social impacts of zoonotic diseases.	Dec 2010	In retrospect, the original project design should not have included socio-economic analysis without detailed understanding of the disease prevalence, impacts on market access and health determinants, such as access to health services, health costs and disabilities associated with chronic disease. Health sociologists and economists from the University Of Queensland were brought in to assess the economic impact of cysticercosis and trichinellosis after we had sufficient data to proceed.
		A qualitative and quantitative description of the economic impact of disease.	Dec 2010	
		Baseline data on disease impacts.	Dec 2010	

PC = partner country, A = Australia

Objective 2: Determine the risks associated with human and pig transmission at the village level and identify appropriate and sustainable disease control strategies

no.	activity	outputs/ milestones	completion date	comments
2.1	Conduct quantitative epidemiological studies on human and pig disease in 12 target villages	A quantitative description of the epidemiology at the village level.	Dec 2010	Pig surveys were not conducted in village as we did not have suitable diagnostic capacity to gain meaningful results and as such could not be justified on an animal ethics basis. Pig surveys were conducted in abattoirs only. Six villages were selected in each province, 24 in total, to strengthen the statistical power of the surveys. The risk factors and survey results were communicated to government officials at national, provincial and district level through annual project meetings when results were available. Communication of survey results to villagers was undertaken only in instances where *Taenia solium* taeniasis was detected. Results for viral zoonoses and Helminth serology were not available in time for an effective communication strategy back to villages to be initiated. This was unfortunate and may have negative implications for further surveillance work in these communities.
		Risk factors for disease transmission identified and communicated to villagers, government and others	Dec 2010	
2.2	Conduct qualitative and observational epidemiological studies in target villages	A qualitative description of the epidemiology at the village level	Dec 2008 August 2010	Supply chain analysis was conducted for pigs and pork in Oudomxay and Xiengkhuang March 2008 and in Luangprabang and Huaphan in October 2008. District government officers, traders, slaughterhouses, markets and farmers were involved in the survey. Qualitative studies were conducted in conjunction with the MoH in Luangprabang and Oudomxay to pilot methods of identifying high risk communities for cysticercosis (spikes in epilepsy, carcass rejections at slaughter, trader exclusions etc).
		Risk factors for disease transmission identified and communicated to villagers, government and others	Dec 2010	The risk factors and survey results were communicated to government officials at national, provincial and district level through annual project meetings when results were available. Communication of survey results to villagers was as described in activity 2.1, above.
2.3	Participatory problem diagnosis and development of a basket-of-options for disease control	Identification of the problems causing increased risk of disease transmission.	Not undertaken	For *Trichinella* and cysticercosis, the problems causing increased risk of disease transmission are well known, poor pig management, access to human faeces, scavenging, open defecation, poverty, low education levels. We did not specifically undertake participatory problem diagnosis, but our results (discussed below) provide insights into specific
		Appropriate interventions to introduce in villages identified and documented.	Not undertaken	

		Basket-of-options designed in consultation with farmers	Not undertaken	problems facing the different ethnic groups in northern Laos, the Mon-Khmer ethnic family (mainly Khmu) were at increased risk of cysticercosis and the Lao-Tai (Lao loum) were at increased risk of trichinellosis. Other risk factors are described in results section below.

PC = partner country, A = Australia

Objective 3: Implement and evaluate control measures to minimise disease impacts on smallholder producers and the wider community

no.	activity	outputs/ milestones	completion date	comments
3.1	Implement disease control interventions and monitor uptake, understanding and sustainability in 6 villages	Disease control technologies implemented at village level.	Not undertaken	Within the time frame of the project, this objective was overly optimistic and was always going to be difficult to achieve.
		Limitations to uptake, understanding and sustainability identified.	Not undertaken	Within the time frame of the project, this objective was overly optimistic and was always going to be difficult to achieve.
3.2	Evaluate control interventions through continued surveillance	Disease control options quantitatively assessed.	Not undertaken	Within the time frame of the project, this objective was overly optimistic and was always going to be difficult to achieve.
		Results communicated to villagers, government and others.	Not undertaken	Within the time frame of the project, this objective was overly optimistic and was always going to be difficult to achieve.
3.3	Monitor and evaluate project activities and conduct follow-up socio-economic impact assessment surveys	Relative success of the basket-of-options to control disease measured and documented.	Not undertaken	Within the time frame of the project, this objective was overly optimistic and was always going to be difficult to achieve.
		Socio-economic impact of disease control assessed in comparison to baseline.	Not undertaken	Within the time frame of the project, this objective was overly optimistic and was always going to be difficult to achieve.
3.4	Assisting Lao-EU project in developing relevant meat inspector training material/course	Qualified meat inspectors trained.	April 2008	6 Lao-EU project staff participated in a PZP training course in March 2008.
		Possible accreditation system put in place (Lao-EU project are driving this intervention).	Not undertaken	PZP were not invited to participate in further work in meat inspector training and no accreditation system is currently in place.
		Strengthened meat inspection capacity of district and provincial abattoirs.	Dec 2008	More than 20 DAFO and PAFO received advanced meat inspector training from the 6 districts participating in the slaughterhouse surveys.

PC = partner country, A = Australia

Objective 4: To develop appropriate regional and national communication strategies for the wider uptake of project outcomes to manage pig associated zoonoses.

no.	activity	outputs/ milestones	completion date	comments
4.1	Workshops and training courses engaging international, national, provincial and district stakeholders	Communication of project results to interested parties.	Dec 2010	
		Information exchange with other interested parties.	Dec 2010	
4.2	Communication of project outcomes regionally and internationally through networks, conferences and workshops.	• Project staff attending and presenting information at international and regional conferences.	Dec 2010	Project leader (A)
4.3	Communication of project outcomes regionally and internationally	Publication of results in peer-reviewed scientific journals.	Dec 2010	At the time of report writing, five review articles related to zoonoses in SE Asia and Laos arising from project activities have been published as well as two research articles, Hepatitis E genotyping study and soil-borne helminthiasis. A further seven research articles are currently in various stages of publication/preparation, (i) *Taenia* ecology, (ii) trichinellosis epidemiology, (iii) viral zoonoses in pigs, (iv) JEV and HEV in humans, (v) health economic modelling study, (vi) giardiasis and trichomoniasis molecular epidemiology and (vii) parasite ecology in humans, pigs and dogs in northern Laos. A further study on bovine zoonoses that was funded by the WHO as a direct result of the PZP activities is currently under review for publication.
		Communication of material to FAO/WHO Campaign for the control of parasitic zoonoses.	Dec 2010	The diagnostic training activities in the NCLE directly resulted in the PZP supporting a MoH and WHO investigation of trichinellosis in Xayabouly province in 2009/2010. The results of the JEV survey work in humans and pigs were used by the WHO in deliberations to shape JEV vaccination policy in conjunction with the MoH in Dec 2010. The WHO staff regularly attended workshops and annual meetings in 2008 and 2010 where results were relayed. FAO staff were invited but did not attend any meetings for the PZP. Regular communication with the WHO over the course of the project lead to the funding of a bovine zoonoses project to better understand the role of cattle and buffalo in *Taenia* ecology amongst other bovine zoonoses.

PC = partner country, A = Australia

7 Key results and discussion

7.1 Establishing the evidence base for zoonotic infectious diseases

7.1.1 Through-chain assessment of pork supply from production to plate

In Xiengkhuang province, piglets are produced predominantly by farmers of the Hmong-Mien ethnic family in remote villages and supply farmers in lowland areas (predominantly farmers of Lao-Tai ethnic family). Piglets are supplied either by direct farmer-to-farmer trade, through live animal markets in Phonsavan or via middlemen traders. On occasion, farmers in lowland areas have breeding stock but this was not a common observation. The live animal markets that supply piglets to urban and peri-urban pig producers are supplied by all districts of Xiengkhuang and a significant proportion of piglets come from Huaphan province. Pigs slaughtered in the abattoir in Phonsavan are predominantly sourced locally from urban and peri-urban pig farmers but occasionally from semi-remote villages, other districts or other provinces, depending on demand. The small slaughter-points in Nongphet and Khangkai townships almost exclusively sourced pigs for slaughter from surrounding villages. The preferred breed for consumption was *Moo laat* (indigenous breed pig) and traders observed that cysticercosis in pigs was rarely seen (<1%). Official meat inspectors from the district agricultural office reportedly inspected every carcass at slaughter.

In Oudomxay province, the live trade of piglets is predominantly by the direct sale of pigs from farmer-to-farmer whereby management and production practices improved down the supply chain. We observed multiple steps in the supply: small piglets up to 10kg were sold to a fattener farmer and kept to 15-20 kg, subsequent sale to a farmer who would keep them to 20-30kg and the final stage farmer would buy a 25-30kg grower pig and keep and sell for slaughter at 50-60kg. Most pigs slaughtered in Oudomxay were sourced from Xay district and a small number were sourced from other districts. There was little evidence that pigs were sourced from other provinces and at the time of the survey in 2008 the sale of pigs across the border with China was banned. The slaughter point at Km32 village sourced pigs mostly from surrounding villages and all traders rarely observed cysticecosis in pigs (<1%). Official meat inspectors from the district agricultural office reportedly inspected every carcass at slaughter.

Luangprabang was the largest market for pigs in the survey. Local breed pigs were sourced from all districts of Luangprabang and other provinces by middleman traders and directly sourced by slaughter traders. At the time of the survey in 2008, there was no official slaughterhouse in Luangprabang province and slaughter-traders killed pigs in backyard operations and meat inspections were carried out at the market rather than at the point of slaughter. The supply of live piglets was mostly via direct farmer-to-farmer but trade production practices generally improved down the supply chain. Like Xiengkhuang, Hmong farmers were important suppliers of piglets. The small slaughter-point operators in Xiengnguen mostly sourced slaughter pigs from surrounding villages in Xiengnguen district. Traders in Luangprabang indicated that cysticercosis was not common and approximately 1% of carcasses were condemned due to infestation.

In Huaphan province, the supply of live piglets for fattening was via direct farmer-o-farmer trade and some middlemen operators supplied piglets to pig producers in the urban and peri-urban villagers of Xamneua. Xamneua slaughter traders mostly sourced pigs from Xamneua district and other districts of Huaphan province but we were informed that in 2008 shortages in supply resulted in many pigs being sourced from Viengkham district in Luangprabang province. The traders indicated that Viengkham district pigs were a high risk for cysticercosis but the need for a regular supply outweighed the risk. Traders in Huaphan indicate that between 3 and 5% of pigs were infected with cysticercosis.

In general, pork supply was through officially recognised meat traders and we found little evidence of itinerant meat trade in northern Laos. Figure 7.1 summarises the supply of live animals and pigs through a diverse and variable supply chain.

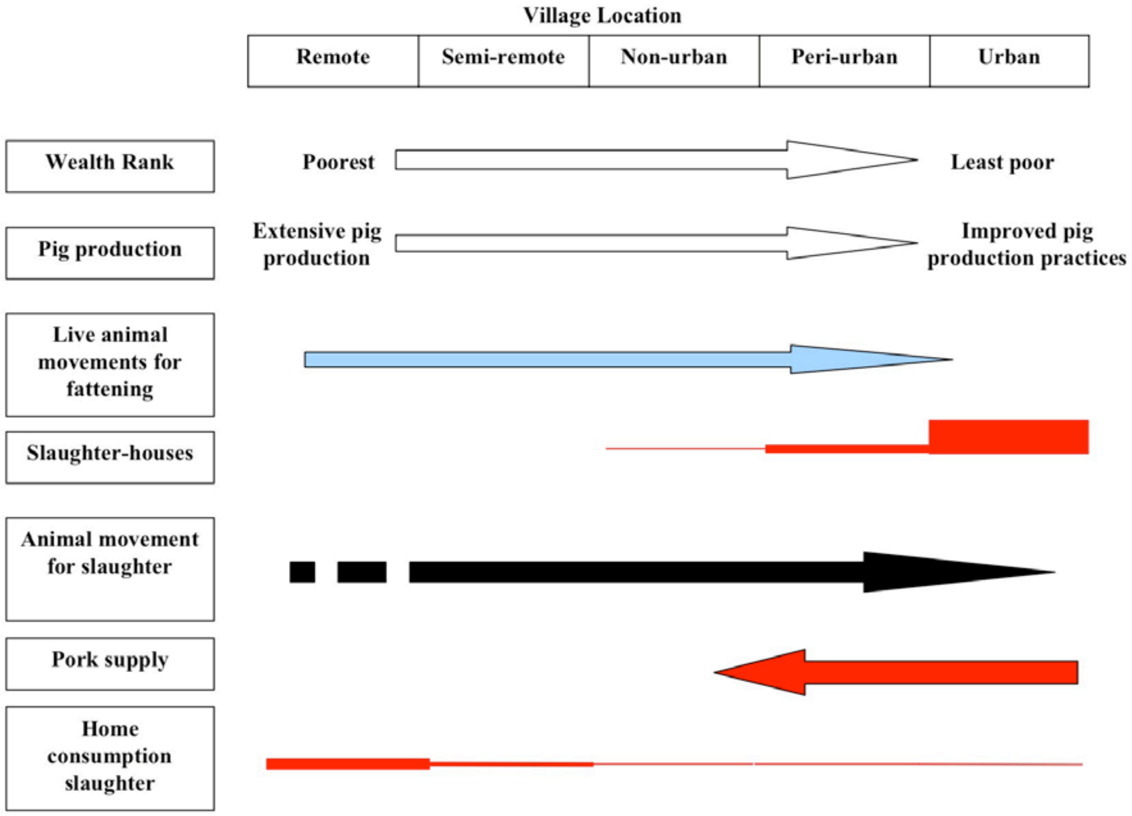

Figure 7.1 Schematic model of pig and pig meat supply along the supply chain in northern Laos.

7.1.2 Cysticercosis and taeniasis ecology and epidemiology in northern Laos

Pig study: The inspection data results from Oudomxay province were dropped from the analysis due to the submission of incorrectly completed forms. A total of 590 pig carcasses, with a matching serum sample, were inspected in three provinces, 209 in Luangprabang, 190 in Huaphan and 191 in Xiengkhuang province. Data on the variables 'breed', 'age', 'sex' and 'production system at last point of sale' were collected for 538, 528, 540 and 518 pigs, respectively (Table 7.1). Carcass inspection detected five pigs (0.8%) with cysts consistent with the morphology of *T. solium*; 1.0-1.5 cm fluid filled muscle cysts with a single white scolex. All infected pigs were heavily infected (without counting) and had viable and degenerated cysts and increasing age was significantly associated with *T. solium* detection (Table 7.1). One hundred and thirty two (22.4%) carcasses were detected with cysts consistent with the morphology of *T. hydatigena*; large, visceral, fluid filled cysts with a single white scolex. The prevalence of *T. hydatigena* detection was significantly greater in free-range pigs (Table 7.1). Two pigs, one each from Luangprabang and Huaphan, had a dual infection with *T. solium* and *T. hydatigena*. One pig in Huaphan province was detected with cysts consistent with *T. asiatica*; very small fluid filled cysts present in the liver, spleen and lung. This pig was a 15-month-old female purchased from a penned production system. Non-specific liver lesions consistent with parasitemia were detected in 16 (2.7%) pigs; these were considered inspection negative in the absence of histopathology to definitively detect *Taenia*.

The sera of 404 (68.5%) pigs were reactive in the AgELISA and no significant association was observed for province, breed, age, sex and production system (Table 7.1). All five *T. solium* and one *T. asiatica* inspection positive pigs had serum samples reactive in the AgELISA. Of the 132 *T. hydatigena* inspection positive pigs, 129 were reactive and three were non-reactive. Fourteen of the 16 pigs with non-specific liver lesions were serum reactive in the AgELISA and two were non-reactive. Table 7.2 describes the estimates of true prevalence of *T. solium*, *T. hydatigena* and *T. asiatica* cysticercosis in pigs using the Maximum Likelihood Estimator (MLE) for carcass inspection with sensitivity ranging from 0.1 to 1.0 and assuming specificity was equal to 1.0.

The evidence suggests that *T. hydatigena* was the dominant species infecting pigs in Laos, with a strong infection pressure being exerted by a relatively large, unmanaged free ranging dog population (*Canis lupus familiaris*). No other definitive hosts for *T. hydatigena* are found in Laos. The pig study was limited by the diagnostic protocols employed. In the pig study, the Ag-ELISA was not able to differentiate the three *Taenia* species and the inspection data could only account for one third of the serologically positive animals. The sensitivity of detecting *T. solium* cysts at slaughter can be variable and estimates range from 20-60% but specificity has been estimated to be 100%. We used a standard maximum likelihood estimator to calculate true prevalence, which was only valid if both specificity and sensitivity were known. We made the assumption that inspection was 100% specific and calculated true prevalence through a range of sensitivities of detecting cysts at slaughter; meaning prevalence was estimated from one degree of freedom in the observed data at each increment of sensitivity. Since inspection was constrained by traders restricting muscle slicing, we assumed the sensitivity of detecting *T. solium* cysts was at the lower end of the range. No data was available that allowed us to estimate the sensitivity of detecting *T. hydatigena* in pigs. Since *T. hydatigena* metacestodes mature in the fat of the mesentery and omentum and the size can vary from 1-7 cm, these cysts could be easily missed, particularly in Lao indigenous breed pigs that typically have a very high fat content. The adjusted inspection prevalence data for *T. solium* (2.8-4.2%), *T. hydatigena* (44.7-55.9%) and *T. asiatica* (0.6-0.8%) accounted for the majority of the serologically positive animals (Table 7.2). It is evident that future *Taenia* research in Southeast Asia will require the use of more robust diagnostic protocols for the animal and human studies. The 'gold standard' for cysticercosis in pigs, dissection taking 0.5 cm slices through half a carcass, is both expensive and time consuming and not conducive to large and geographically diverse studies.

Table 7.1. Prevalence of pig cysticercosis (*Taenia solium*, *T. hydatigena* and *T. asiatica*) by carcass inspection and seroprevalence of pig cysticercosis by the AgELISA by location, sex, breed, age and production system at last point of sale.

Risk factor	**N (%)**	**Carcass inspection prevalence of pig cysticercosis (95% CI)**		**Cysticercosis AgELISA pos**
		***T. solium* (95% CI)**	***T. hydatigena* (95% CI)**	**Prevalence (95% CI)**
Total	590	0.8 (0.1, 1.6)	22.4 (19.0, 25.7)	68.5 (64.7, 72.2)
Province (N=590)				
Luangprabang	209 (35.4)	1.4 (0.0, 3.1)	22.5 (16.8, 28.2)	71.3 (65.1, 77.5)
Huaphan	190 (32.2)	1.1 (0.0, 2.5)	26.3 (20.0, 32.6)	70.5 (64.0, 77.1)
Xiengkhuang	191 (32.4)	0.0 (0.0, 0.0)	18.3 (12.7, 23.8)	63.3 (56.5, 70.2)
Age (months) (N=528)				
≤6	70 (13.3)	0	27.1 (16.6, 37.7)	68.6 (57.6, 79.6)
7-12	250 (47.4)	0	22.8 (17.6, 28.0)	73.2 (67.7, 78.7)
13-18	128 (24.2)	1.6 (0.0, 3.7)	19.5 (12.6, 26.4)	68.0 (59.8, 76.1)
≥18	80 (15.2)	3.8 (0.0, 8.0)	25.0 (15.4, 34.6)	63.8 (53.1, 74.4)
Breed (N=538)				
Lao indigenous	473 (87.9)	1.1 (0.1, 2.0)	24.7 (20.8, 28.6)	72.1 (68.0, 76.1)
Exotic	37 (6.9)	0	10.8 (0.6, 21.0)	56.8 (40.5, 73.0)
Cross-breed	28 (5.2)	0	14.3 (1.1, 27.5)	64.3 (46.2, 82.4)
Sex (N=540)				
Female	275 (50.9)	0.7 (0.0, 1.7)	22.5 (17.6, 27.5)	69.5 (64.0, 74.9)
Male	130 (24.1)	2.3 (0.0, 4.9)	20.0 (13.1, 26.9)	67.7 (59.6, 75.8)
Castrated male	135 (25.0)	0	26.7 (19.2, 34.2)	72.6 (65.0, 80.2)
Production system at last point of sale (N=518)				
Penned/Corralled	345 (66.6)	1.2 (0.0, 2.3)	19.4 (15.2, 23.6)	67.2 (62.3, 72.2)
Free-roaming	167 (32.2)	0.6 (0.0, 1.8)	29.3 (22.4, 36.3)	74.3 (67.6, 80.9)
Mixed	6 (1.2)	0	33.3 (0.0, 74.7)	66.7 (25.3, 100.0)

Table 7.2. Estimated true prevalence of pig cysticercosis (*Taenia solium*, *T. hydatigena* and *T. asiatica*) and dog taeniasis (*T. hydatigena*) adjusted by the Maximum-Likelihood-Estimation model for increments of test sensitivity, assuming specificity of 100%.

Test sensitivity (%)	**Estimated true prevalence of pig cysticercosis, % (95% CI)**		
	T. solium	***T. hydatigena***	***T. asiatica***
100	0.8 (0.1, 1.6)	22.4 (19.0, 25.7)	0.2 (0.0, 0.5)
90	0.9 (0.1,1.8)	24.9 (21.1, 28.6)	0.2 (0.0, 0.6)
80	1.1 (0.1, 2.0)	28.0 (23.8, 32.2)	0.2 (0.0, 0.6)
70	1.2 (0.2, 2.3)	32.0 (27.2, 36.8)	0.2 (0.0, 0.7)
60	1.4 (0.2, 2.6)	37.3 (31.7, 43.0)	0.3 (0.0, 0.8)
50	1.7 (0.2, 3.2)	44.7 (38.0, 51.5)	0.3 (0.0, 1.0)
40	2.1 (0.3, 4.0)	55.9 (47.5, 64.3)	0.4 (0.0, 1.3)
30	2.8 (0.4, 5.3)	74.6 (63.4, 85.8)	0.6 (0.0, 1.7)
20	4.2 (0.5, 7.9)	-	0.8 (0.0, 2.5)
10	8.5 (1.1, 15.9)	-	1.7 (0.0, 5.0)

-, >100% prevalence calculated; Shaded cells indicates biologically plausible estimates of the true prevalence of respective *Taenia* species.

Human study: A total of 1582 people in 332 households were eligible to participate in this survey. Of these, 1306 (82.7%) individuals from 321 households aged 6-91 years provided blood and faecal samples, a completed questionnaire and had valid laboratory test results. Overall, the Mon-Khmer and Lao-Tai ethnic families had the highest compliance, 88.9 and 88.6% of eligible persons, respectively. The Hmong-Mien ethnic family had the lowest compliance with only 62.5% of eligible persons providing a stool and blood sample and a completed questionnaire. Non-compliance due to mental illness was negligible with two persons, one each from the Lao-Tai and Hmong-Mien ethnic groups, prevented from participating by their respective families. The final survey population consisted of 553 Lao-Tai (42.3%), 523 Mon-Khmer (40.1%) and 230 (17.6%) Hmong-Mien. No Sino-Tibetan people were recruited into this study. The majority of Lao-Tai people were from Huaphan province (49.0%), no Mon-Khmer people were selected in Huaphan and Xiengkhuang province and the majority of Hmong-Mien people were from Xiengkhuang province (70.4%). The highest proportion of impoverished participants was from the Mon-Khmer ethnic family and the highest proportion of least and less poor participants were from the Lao-Tai ethnic family. The Mon-Khmer and Hmong-Mien ethnic families had the highest proportion of participants defecating in the open and the highest proportion of people living in a household with an illiterate female head of household.

The prevalence of cysticercosis and taeniasis stratified by ethnicity for population and individual variables are summarised in Table 7.3. The prevalence of cysticercosis AgELISA positivity was 2.2% (95% CI: 1.4-3.0%), ranging at the village level from 0.0-11.3%; 14 villages had no detectable cysticercosis cases and 10 villages had at least one seropositive case. Greater than half the cases (15/29) were detected in three villages in Oudomxay province. On univariate analysis, only province was significantly associated with cysticercosis AgELISA positivity. After controlling for clustering at the household level by random effects logistic regression, only Luangprabang province (Adjusted OR=0.26, 95% CI=0.07, 0.98) was significantly associated with reduced risk of cysticercosis AgELISA positivity.

The prevalence of *Taenia* egg positivity was 2.9% (95% CI: 2.0-3.8%) and the estimated taeniasis prevalence, egg positive plus self-reporting, was 8.4% (95% CI: 6.9-9.9%), ranging at the village level from 0.0-6.9% and 0.0-17.0%, respectively. The proportion of people reporting a history of taeniasis was 27.0% (95% CI: 24.5-29.4%). For individuals with current taeniasis, 90.0% (95% CI: 84.3-95.7%) reported having a history of taeniasis compared to 21.2% (95% CI: 18.8-23.5%) for uninfected individuals. Only egg positive and/or self-reporting cases were considered in the risk factor analysis. On univariate analysis, a history of taeniasis was very strongly associated with increased risk of having a current taeniasis infection. Other factors significantly associated with taeniasis were gender, province, age, ethnicity and consumption of raw meat. Two multivariate analyses of risk factors associated with taeniasis were carried out, including and excluding the variable “history of taeniasis” (Table 7.4). History of taeniasis was strongly correlated with increasing age (χ^2=121.9, P<0.001) and inclusion in the model gave the perception that increasing age was protective (Table 7.4). The exclusion of “history of taeniasis” from the analysis resulted in gender, province of origin, age, ethnicity, and infrequent consumption of uncooked beef being significantly associated with a current taeniasis infection (Table 7.4). The consumption of uncooked pork and uncooked fermented pork were not significantly associated with taeniasis after controlling for other risk factors.

The proportion of people reporting consumption of any uncooked meat, uncooked pork, fermented pork sausage and uncooked beef was 50.2% (95% CI: 47.5-52.9%), 13.9% (12.1-15.8%), 28.8% (26.3-31.2%) and 36.7% (34.1-39.4%), respectively. The prevalence of eating any uncooked meat increased significantly with age (χ^2=145.8, P<0.001), similar results were observed for eating uncooked beef (χ^2=214.2, P<0.001), uncooked pork (χ^2=48.7, P<0.001) and fermented pork sausage (χ^2=41.3, P<0.001). Uncooked beef consumption had the highest peak prevalence of 56.9% (95% CI: 50.4-63.4%) in the 40-54 year age group; the peak prevalence of eating uncooked pork and uncooked fermented pork sausage was 20.9% (95% CI: 15.5-26.2%) and 37.8% (95% CI: 31.3-44.2%), respectively, also in the 40-54 year age group.

Of the 110-taeniasis positive individuals who were treated with niclosamide, proglottids were expelled from 35 people and PCR revealed 33 tapeworms were *T. saginata* and two were *T. solium*. The *T. solium* worms were recovered from a 7-year-old male from Oudomxay province who was AgELISA negative and from a 34-year-old male from Xiengkhuang province who was AgELISA positive. Both cases reported not eating uncooked pork or fermented pork sausage. The *T. saginata* worms were recovered from 27 males and six females (age range: 19-78) from all provinces. Thirty-two of the *T. saginata* cases were AgELISA negative and 32 reported eating uncooked beef. Both *T. solium* cases were egg positive by FECT and one was self-reported. Sixteen of the 33 *T. saginata* cases (48.5%) were egg positive by FECT and 29/33 (87.9%) were self-reported.

Our *Taenia* study had a number of important limitations and most notable was the relatively small sample size; this was evident in a lack of statistical power to detect significant risk factors associated with cysticercosis even though the majority of cases occurred in three Mon-Khmer villages in Oudomxay province. Secondly, we sought to recruit all eligible household members ≥6 years old and compliance varied for the different ethnic groups. The majority of the low compliance in the Hmong-Mien ethnic family was evident in Huaphan province and could be explained by an aversion to venipuncture and embarrassment in giving a faecal sample, both closely linked to cultural beliefs and customs. This could be corrected in future studies by using a finger-prick and blood spot sampling method, the use of trained Hmong-Mien women to administer the surveys and strengthening of the consultation process.

We used a circulating antigen ELISA rather than an antibody ELISA because the latter method tends to overestimate prevalence in endemic areas. Human cysticercosis was relatively rare in northern Laos at the community level (2.2%) but there was strong evidence of a focal distribution with just over half of the seropositive cases residing in three villages in Oudomxay province. In Asia, a focal distribution of human cysticercosis has also been observed in northern Vietnam, Indonesian Papua and China, so our results support evidence that cases tend to cluster in geographically restricted localities. The relatively high prevalence of cysticercosis in young children was unexpected and corresponded with a relatively high prevalence of taeniasis in the same age group, even though the 7-year old boy with *T. solium* taeniasis was cysticercosis Ag-ELISA negative. The specific exposures leading to increased prevalence in this age group warrants further investigation.

We observed high taeniasis prevalence (8.4%) with spatial variation based on self-reporting and detection of *Taenia* eggs in a single stool. Self-reporting methods improve detection of tapeworm carriers but may lack specificty. Our results show conclusively that *Taenia* egg detection was a gross underestimate of true prevalence and we obtained 18 additional tapeworm specimens after treating egg negative individuals who self-reported infection, however we cannot estimate the specificity of self-reporting from these results.

The strongest risk factor for having a current taeniasis infection was having a history of taeniasis. Ninety percent of taeniasis cases reported having a history of taeniasis indicative of a high prevalence of taeniasis re-infection, which was not surprising considering the very high prevalence of eating uncooked meat; particularly beef. Infrequent consumption of beef was strongly associated with taeniasis in both multivariate models and indicates a possible link with raw meat consumption at festivals. We did not undertake studies of cysticercosis in cattle and buffalo in this study, however a recent slaughterhouse based study in five northern provinces detected cysticercosis antigen in 52% of cattle and 21% of buffalo (Vongxay et al, manuscript in preparation). The evidence therefore suggests that taeniasis re-infection was predominantly due to eating uncooked beef and infection with *T. saginata*. The two *T. solium* cases were not associated with knowingly eating uncooked pork and may have arisen simply from inadvertent undercooking.

Table 7.3. Prevalence of human cysticercosis and taeniasis for selected population characteristics

Population characteristic	N (%)	Proportion Cysticercosis AgELISA positive (95% CI)	Proportion taeniasis positive [a] (95% CI)
Total	1306	2.2 (1.4, 3.0)	8.4 (6.9, 9.9)
Gender			
Female	656 (50.2)	2.1 (1.0, 3.2)	4.7 (3.1, 6.4)
Male	650 (49.8)	2.3 (1.2, 3.5)	12.2 (9.6, 14.7)
Province			
Oudomxay	383 (29.3)	3.9 (2.0, 5.9)	13.1 (9.7, 16.4)
Luangprabang	348 (26.6)	1.2 (0.0, 2.3)	9.2 (6.1, 12.2)
Huaphan	280 (21.4)	1.1 (0.0, 2.3)	5.4 (2.7, 8.0)
Xiengkhuang	295 (22.6)	2.4 (0.6, 4.1)	4.4 (2.1, 6.8)
Ethnicity			
Lao-Tai	553 (42.3)	1.6 (0.6, 2.7)	7.8 (5.5, 10.0)
Mon-Khmer	523 (40.0)	3.3 (1.7, 4.8)	11.7 (8.9, 14.4)
Hmong-Mien	230 (17.6)	1.3 (0.0, 2.8)	2.6 (0.5, 4.7)
Age (years)			
6-10	217 (16.6)	3.2 (0.9, 5.6)	7.3 (3.8, 10.9)
11-14	183 (14.0)	1.1 (0.0, 2.6)	2.2 (0.0, 4.3)
15-24	258 (19.8)	1.2 (0.0, 2.5)	4.3 (1.8, 6.7)
25-39	282 (21.6)	1.8 (0.2, 3.3)	13.5 (9.5, 17.5)
40-54	225 (17.2)	3.1 (0.8, 5.4)	11.6 (7.3, 15.8)
≥55	141 (10.8)	3.5 (0.5, 6.6)	10.6 (5.5, 15.8)
Household wealth status			
Most poor	225 (17.2)	3.1 (0.8, 5.4)	10.2 (6.2, 14.2)
Very poor	246 (18.8)	4.1 (1.6, 6.6)	11.0 (7.0,14.9)
Poor	283 (21.7)	1.1 (0.0, 2.3)	6.7 (3.8, 9.6)
Less poor	288 (22.1)	2.1 (0.4, 3.7)	7.3 (4.3, 10.3)
Least poor	264 (20.2)	1.1 (0.0, 2.4)	7.6 (4.4, 10.8)
Open defecation	445 (34.1)	2.2 (0.8, 3.6)	8.8 (6.9, 10.7)
History of taeniasis	352 (27.0)	2.8 (1.1, 4.6)	28.1 (23.4, 32.8)
Raw pork consumption			
Does not eat	1124 (86.1)	2.3 (1.4, 3.2)	6.9 (5.4, 8.3)
Weekly	18 (1.4)	5.6 (0.0,17.3)	5.6 (0.0, 17.3)
Monthly	61 (4.7)	1.6 (0.0, 4.9)	19.7 (9.4, 29.9)
Every few months	65 (5.0)	1.5 (0.0, 4.6)	16.9 (7.6, 26.3)
Once or twice per year (or less)	38 (2.9)	0.0 (0.0, 0.0)	23.7 (9.5, 37.8)
Fermented pork consumption			
Does not eat	930 (71.2)	2.5 (1.5, 3.6)	7.0 (5.3, 8.6)
Weekly	93 (7.1)	1.1 (0.0, 3.2)	5.4 (0.7, 10.0)
Monthly	139 (10.6)	1.4 (0.0, 3.4)	17.3 (10.9, 23.6)
Every few months	99 (7.6)	2.0 (0.0, 4.8)	10.1 (4.1, 16.1)
Once or twice per year (or less)	45 (3.5)	0.0 (0.0, 0.0)	13.3 (3.0, 23.7)
Raw beef consumption			
Does not eat	826 (63.3)	2.4 (1.3, 3.5)	4.1 (2.8, 5.5)
Weekly	49 (3.8)	2.0 (0.0, 6.1)	16.3 (5.6, 27.0)
Monthly	199 (15.3)	1.5 (0.0, 3.2)	17.1 (11.8, 22.4)
Every few months	160 (12.3)	1.9 (0.0, 4.0)	12.5 (7.3, 17.7)
Once or twice per year (or less)	72 (5.5)	2.8 (0.0, 6.7)	19.4 (10.1, 28.8)
Male head literate	1078 (82.5)	2.4 (1.5, 3.3)	8.2 (6.5, 9.8)
Female head literate	802 (61.4)	1.9 (0.9, 2.8)	8.2 (6.3, 10.1)

[a] Taeniasis refers to *Taenia* egg positive individuals plus people self-reporting tapeworm segments in stool

Table 7.4. Risk factors significantly (P<0.050) associated with taeniasis, as determined by multiple logistic regression modelling controlling for household clustering

Model[a]	Risk factor	Adjusted OR[b]	95% CI[c]
Model 1	Oudomxay	Ref.	
	Luangprabang	0.71	0.40, 1.29
	Huaphan province	0.32	0.12, 0.84
	Xiengkhuang province	0.38	0.15, 0.93
	6-10 years old	Ref.	
	11-14 years old	0.17	0.05, 0.62
	15-24 years old	0.24	0.09, 0.67
	25-39 years old	0.42	0.17, 1.04
	40-54 years old	0.29	0.11, 0.75
	≥55 years old	0.22	0.08, 0.60
	Lao-Tai ethnicity	Ref.	
	Mon-Khmer ethnicity	0.90	0.45, 1.83
	Hmong ethnicity	0.34	0.12, 0.96
	No previous taeniasis	Ref.	
	History of taeniasis	32.98	15.63, 69.56
	Raw beef (doesn't eat)	Ref.	
	Raw beef (weekly)	0.81	0.27, 2.37
	Raw beef (monthly)	1.48	0.71, 3.09
	Raw beef (once very few months)	1.07	0.49, 2.35
	Raw beef (Once or twice per year)	4.13	1.50, 11.36
Model 2	Female	Ref.	
	Male	2.20	1.34, 3.63
	Oudomxay province	Ref.	
	Luangprabang province	0.65	0.38, 1.13
	Huaphan province	0.26	0.11, 0.63
	Xienkhuang province	0.36	0.15, 0.86
	6-10 years old	Ref.	
	11-14 years old	0.24	0.07, 0.75
	15-24 years old	0.42	0.17, 1.00
	25-39 years old	1.11	0.53, 2.34
	40-54 years old	0.86	0.39, 1.90
	≥55 years old	0.82	0.34, 1.94
	Lao-Tai ethnicity	Ref.	
	Mon-Khmer ethnicity	0.86	0.45, 1.65
	Hmong ethnicity	0.26	0.10, 0.70
	Raw beef (doesn't eat)	Ref.	
	Raw beef (weekly)	1.68	0.61, 4.65
	Raw beef (monthly)	2.43	1.27, 4.65
	Raw beef (once very few months)	1.88	0.91, 3.87
	Raw beef (once or twice per year)	5.99	2.51, 14.25

[a] Model 1, Taeniasis OR adjusted for gender, province, age, history of taeniasis, ethnicity and frequency of raw fermented pork sausage, raw pork and raw beef consumption

Model 2, Taeniasis OR adjusted for gender, province, age, ethnicity, raw fermented pork sausage consumption, raw pork consumption, raw beef consumption

[b] OR, odds ratio

[c] CI, confidence interval

7.1.3 Trichinellosis ecology and epidemiology in northern Laos

Pig study: Eight-hundred and thirty-nine samples, diaphragm and tonsil tissue, were submitted to the central laboratory in Vientiane for testing by the artificial digestion method. Tissue samples were pooled into 10x 10g lots (total weight 100g), digested, filtered and washed and the larvae visualised under a low power light microscope. Individual samples from *Trichinella* larvae positive pools were tested by the digestion method using the remaining diaphragm and tonsil tissue. *Trichinella* larvae were recovered from 15 pigs (1.8%) and the highest prevalence was detected in Xiengkhuang province (4.1%) and Oudomxay province (2.3%)(Table 7.5). The majority of positive pigs had low intensity infections with less than one larvae per gram of tissue (66.7%) and only two pigs (13%) had *Trichinella* detected at greater than 10 larvae per gram of tissue (Table 7.6). *Trichinella* larvae were recovered from indigenous breed pigs only and no exotic or cross breed pigs were positive for *Trichinella* larvae.

Larvae that were recovered from sixteen digestion pools were sent to the *Trichinella* reference laboratory in Rome, Italy, and tested by PCR to determine the species. Thirteen isolates were *T. spiralis* and the DNA from the remaining three isolates could not be amplified. All 15 isolates recovered from individual samples were also sent to Rome for speciation; unfortunately the DNA was degraded during the digestion process and could not be amplified by PCR. The evidence from the pooled isolates however, indicates that *T. spiralis* is endemic in the pig population of Laos and consumption of uncooked, under-cooked or fermented pork poses a substantial risk of acquiring trichinellosis.

Table 7.5. Prevalence of pig trichinellosis detected by tongue and diaphragm muscle digestion

Province	No. tested	*Trichinella* positive	Proportion
Oudomxay	175	4	2.3
Luangprabang	218	0	0.0
Huaphan	224	2	0.9
Xiengkhuang	222	9	4.1
Total	839	15	1.8

Table 7.6. Intensity of trichinella infection and location animals infected

Intensity of infection (lpg*)	No. of infections	Location
0.1 – 0.9	10	Oudomxay (4); Huaphan (1); Xiengkhuang (5)
1 – 10	3	Xiengkhuang (3)
>10	2	Huaphan (1); Xiengkhuang (1)
Total	15	

*lpg, larvae per gram.

Human study: As described above in section 7.1.2, 1582 participants were recruited to the human study and 1419 individuals had serum samples taken and questionnaires administered that could be analysed for human trichinellosis exposure and risk assessment. Table 7.7 provides details of trichinellosis ES ELISA prevalence for different population characteristics for all positives where the signal to noise ratio (S/N) was greater than 1 and strong positives designated as S/N>1.5. Prevalence for ES ELISA positivity (S/N>1 and S/N>1.5) was highest in the male population, in Oudomxay province and in the Lao-Tai ethnic family. Prevalence increased with increasing age, increasing wealth status and with consumption of raw pork or fermented pork sausage (*Som moo*). Prevalence of ES ELISA positivity was also highest in the population who predominantly purchased pork from a market as opposed to those who predominantly consumed pork slaughtered at home or harvested from the wild.

Controlling for multiple risk factors and household clustering (Table 7.8), trichinellosis ES ELISA positivity (Model 1, S/N>1) was significantly associated with being male, residing in Oudomxay province, increasing age, being of Lao-Tai ethnicity and with purchase of pork from a market. When the odds ratios were calculated for strong positive ES ELISA results, the results were similar to model 1 but frequent consumption of fermented pork sausage was also significantly associated with trichinellosis test positivity. Consumption of raw pork was not significantly associated with trichinellosis ES ELISA positivity in either model and possibly reflects the small sample size and lack of statistical power in the analysis.

We used a crude ES ELISA to test for *Trichinella* positivity and this test lacks specificity in populations where multiple intestinal parasites circulate in the survey population. This was the case in the population of northern Lao PDR (see section 7.1.6 below) where multiple parasite infections were commonly detected although, we did not observe an association with ES ELISA positivity and other intestinal helminthiasis. The reverse was actually seen, whereby *Trichinella* ES ELISA prevalence was highest in older males belonging to the Lao-Tai ethnic group and this group had the lowest prevalence of intestinal helminthiasis. This gives a measure of confidence that we were detecting anti-*Trichinella* antibodies but this could not be confirmed. We sent a small sample of human sera to the reference laboratory in Rome for western blot analysis and there was poor agreement between the ES ELISA results and western blot positivity. This was not unexpected however, since the western blot test is validated for acute infection and the very specific banding pattern is observed in people with acute infection with clinical disease. No tests are currently validated for population studies where people may have been exposed to a small number of larvae resulting in subclinical infection, similarly no serological tests have been validated for population studies where people may have been exposed to larvae many months or years before the blood sample was taken. The antibodies raised against *Trichinella* larvae infestation may be influenced by the number of larvae ingested and the time period after exposure and the western blot test is an imperfect test for cross-sectional population surveys. We therefore do not have a gold standard test to measure our survey results against. The results we have presented may have over estimated prevalence and are indicative of the population exposure to *Trichinella* larvae and suggest that consumption of fermented pork sausage is an important risk factor for acquiring infection.

The evidence from Laos indicates that trichinellosis may be endemically stable. The minimum number of larvae required to cause clinical disease has been estimated to be between 70 and 150 larvae and in this Lao study, the volume of fermented sausage consumed in a sitting was most often less than 50 grams. The prevalence of *T. spiralis* larvae in backyard and free-range pigs was relatively low and the majority harboured a low worm burden (<1 lpg) suggesting that in a community where uncooked pork is consumed, most infections will be subclinical. Severe clinical cases predominantly occur as sporadic point source outbreaks or sporadic isolated cases as was the case in the 2005 trichinellosis outbreak in Oudomxay province and the 2004 outbreak in Bolikhamxay province. Trichinellosis endemic stability requires verification by well-designed and comprehensive epidemiological studies of pigs and people but it could provide important insights for the implementation of disease control initiatives.

Table 7.7. Prevalence of human trichinellosis tested by the excretory-secretory (ES)-ELISA, stratified by gender, province, wealth status, age, ethnicity and frequency of *Som moo* (fermented pork sausage) and un-cooked pork consumption. Results are presented for all positives (signal to noise (S/N) ratio >1.0) and strong positives (S/N ratio >1.5).

Population characteristic	Population size		Proportion ES-ELISA positive (95% CI)	
	N	%	All positives	Strong positive
Survey population	1419	100	19.1 (17.1, 21.1)	11.2 (9.6, 12.9)
Gender				
Female	719	50.7	15.4 (12.8, 18.1)	9.3 (7.2, 11.4)
Male	700	49.3	22.8 (19.7, 26.0)	13.3 (10.8, 15.8)
Province				
Oudomxay	412	29.0	22.8 (18.7, 26.9)	15.8 (12.2, 19.3)
Luangprabang	373	26.3	17.7 (13.8, 21.6)	9.9 (6.8, 13.0)
Huaphan	294	20.7	11.2 (7.6, 14.9)	6.5 (3.6, 9.3)
Xiengkhuang	340	24.0	22.9 (18.4, 27.4)	11.5 (8.1, 14.9)
Wealth Status				
Most poor	253	17.8	9.9 (6.2, 13.6)	4.3 (1.8, 6.9)
Very poor	270	19.0	17.4 (12.9, 22.0)	11.1 (7.3, 14.9)
Poor	297	20.9	14.8 (10.8, 18.9)	9.7 (6.3, 13.1)
Less poor	313	22.1	24.0 (19.2, 28.7)	15.3 (11.3, 19.3)
Least poor	286	20.2	28.0 (22.7, 33.2)	14.6 (10.6, 18.8)
Age (years)				
6-11	296	20.9	6.8 (3.9, 9.6)	3.4 (1.3, 5.4)
12-19	329	23.2	12.2 (8.6, 15.7)	6.4 (3.7, 9.0)
20-34	297	20.9	22.6 (17.8, 27.3)	13.1 (9.3, 17.0)
35-49	277	19.5	30.7 (25.2, 36.2)	19.5 (14.8, 24.1)
≥50	220	15.5	26.8 (20.9, 32.7)	16.4 (11.4, 21.2)
Ethnicity				
Lao-Tai	583	41.1	26.1 (22.5, 29.6)	17.2 (14.1, 20.2)
Mon-Khmer	564	39.8	13.1 (10.3, 15.9)	6.6 (4.5, 8.6)
Hmong-Mien	272	19.2	16.5 12.1, 21.0)	8.5 (5.1, 11.8)
Som moo consumption				
Does not eat	1023	72.1	15.0 (12.8, 17.1)	8.2 (6.5, 9.9)
Once per week	96	6.8	25.0 (16.2, 33.8)	18.8 (10.8, 26.7)
Once per month	149	10.5	33.6 (25.9, 41.2)	18.8 (12.4, 25.1)
Once every few months	104	7.3	33.7 (24.4, 42.9)	24.0 (15.7, 32.4)
Once or twice per year or less	47	3.3	19.1 (7.5, 30.8)	10.6 (1.5, 19.8)
Uncooked pork consumption				
Does not eat	1224	86.3	18.0 (15.8, 20.1)	10.5 (8.7, 12.2)
Once per week	20	1.4	15.0 (0.0, 32.1)	5.0 (0.0, 15.5)
Once per month	66	4.7	18.2 (8.6, 27.7)	10.6 (2.9, 18.2)
Once every few months	71	5.0	31.0 (20.0, 42.0)	21.1 (11.4, 30.9)
Once or twice per year or less	38	2.7	36.8 (20.8, 52.9)	23.7 (9.5, 37.8)
Main source of pork supply				
Other (home slaughter, wild…)	431	30.4	9.2 (6.5, 12.0)	3.7 (1.9, 5.5)
Market	988	69.6	23.4 (20.7, 26.0)	14.6 (12.4, 16.8)

Table 7.8. Risk factors significantly (P<0.050) associated with trichinellosis ES-ELSA positivity, as determined by multiple logistic regression modelling controlling for household clustering

Model[a]	Risk factor	Adjusted OR[b]	95% CI[c]
Model 1	Female	Ref.	
	Male	2.10	1.46, 3.00
	Oudomxay province	Ref.	
	Luangprabang province	0.28	0.15, 0.55
	Huaphan province	0.05	0.02, 0.13
	Xiengkhuang province	0.25	0.11, 0.55
	6-11 years old	Ref.	
	12-19 years old	2.33	1.18, 4.60
	20-34 years old	6.64	3.38, 13.07
	35-49 years old	10.65	5.37, 21.14
	≥50 years old	6.60	3.29, 13.24
	Lao-Tai ethnicity	Ref.	
	Mon-Khmer ethnicity	0.14	0.07, 0.30
	Hmong ethnicity	0.36	0.18, 0.74
	Other (home slaughter, wild…)	Ref.	
	Market pork supply	2.13	1.14, 3.97
Model 2	Female	Ref.	
	Male	2.11	1.32, 3.36
	Oudomxay province	Ref.	
	Luangprabang province	0.18	0.08, 0.44
	Huaphan province	0.02	0.01, 0.07
	Xiengkhuang province	0.09	0.03, 0.24
	6-11 years old	Ref.	
	12-19 years old	2.99	1.14, 7.87
	20-34 years old	9.32	3.60, 24.16
	35-49 years old	14.26	5.52, 36.84
	≥50 years old	8.75	3.34, 22.91
	Lao-Tai ethnicity	Ref.	
	Mon-Khmer ethnicity	0.06	0.02, 0.17
	Hmong ethnicity	0.29	0.12, 0.73
	Som moo (doesn't eat)	Ref.	
	Som moo (weekly)	2.75	1.10, 6.90
	Som moo (monthly)	1.11	0.54, 2.31
	Som moo (once very few months)	1.84	0.86, 3.97
	Som moo (Once or twice per year)	0.99	0.30, 3.26
	Other (home slaughter, wild…)	Re.	
	Market pork supply	4.18	1.72, 10.16

[a] Model 1, Odds ratio of ES ELISA reactive serum (S/N>1.00) adjusted for gender, province, age, history of taeniasis, ethnicity and frequency of raw fermented pork sausage, raw pork and raw beef consumption

Model 2, Odds ratio of ES ELISA reactive serum (S/N>1.50) adjusted for gender, province, age, ethnicity, raw fermented pork sausage consumption, raw pork consumption, raw beef consumption

[b] OR, odds ratio

[c] CI, confidence interval

7.1.4 Hepatitis E genetic typing study in young pigs

One hundred and eighty-one faecal samples were collected from pigs ≤6 months old (median: 3 months). Samples were stored in RNA preservation media and were tested for HEV and genotyped at AFRIMS, Bangkok. Twenty-one faecal samples (11.6%) had detectable HEV RNA. Samples were collected from pigs from 95 households in 23 villages (Table 7.9). There was a significant difference in observed prevalence between the 4 provinces (P=0.012) since no pigs sampled in Huaphan province had detectable HEV RNA (Table 7.9).

Table 7.9. Detection of HEV RNA in faeces of pigs in northern Lao PDR

Province[1]	No. of villages	No. of pigs	Median pig age (months)	Age range (months)	Proportion pigs HEV positive (95%CI)	HEV positive villages (%)
XK	6	39	3	1.5 - 4.5	12.8 (1.8 - 23.8)	2 (33.3)
HUA	5	45	3	2.0 - 5.0	0.0 (0.0 - 0.0)	0 (0.0)
OUD	6	46	4	1.5 - 6.0	17.4 (6.0 - 28.8)	5 (83.3)
LP	6	51	4	2.0 - 6.0	15.7 (5.4 - 26.0)	3 (50.0)
Total	23	181	3	1.5 - 6.0	11.6 (6.9 - 16.3)	10 (43.5)

[1] XK, Xiengkhuang; HUA, Huaphan; OUD, Oudomxay; LP, Luangprabang

Sequence analysis of a 240-bp fragment of the HEV RNA indicated that all 21 Lao isolates belonged to genotype 4 and were 89.6-100% identical to each other. When Lao sequences were compared to known human and pig HEV isolates obtained from GenBank, they had 88.3-97.1% sequence homology with genotype 4 strains. Genetic variability was observed within the 21 Lao HEV isolates and we tentatively assigned them into four apparent groups and a lone genetically distinct isolate.

This was the first time HEV virus had been characterised in the pig population of northern Laos and the first report of genotype 4 HEV in any host species in Laos. Moreover, almost half of the village pig herds were infected. This finding has epidemiological significance since the growing body of evidence indicates possible geographical partitioning of genotypes 3 and 4 in the Mekong sub-region of Southeast Asia. Evidence suggests that genotype 3 has been isolated from pigs in Cambodia and Thailand (Caron et al., 2006; Cooper et al., 2005) and genotype 4 from humans in Vietnam (Hijikata et al., 2002; Koizumi et al., 2004) and pigs in northern Laos. The Lao and Vietnamese HEV isolates originate from northern regions and to date no data on the genotypes of HEV circulating in the southern regions of these countries are available. Genotype 4 HEV has been associated with the most severe form of HEV infection (fulminant hepatitis with severe morbidity) as compared to genotype 3 HEV. This has important implications for the wellbeing of pig farmers and slaughterhouse workers who are exposed to genotype 4 HEV in northern Laos.

Age-stratified prevalence peaked at 15.8% (3/19) in 1-2 month old pigs (Figure 7.2) but no significant difference (P=0.514) in HEV infection was observed for piglet age due to the small sample size. The age-stratified HEV RNA positivity data suggests that pigs were infected at a young age with peak prevalence observed in 1-2 month old piglets. This finding is consistent with experimental studies showing that piglets start to shed virus from 1 month of age (Kanai et al., 2010) after the waning of IgA maternal antibodies by 3 weeks of age (de Deus et al., 2008). Piglets can shed virus for up to 4 months post infection (Kanai et al., 2010) providing a continued source of environmental contamination and enabling HEV to remain endemic over the dry season months, November to March. As a consequence, any control measures will need to take into account the quality of housing and management of faecal material from young piglets.

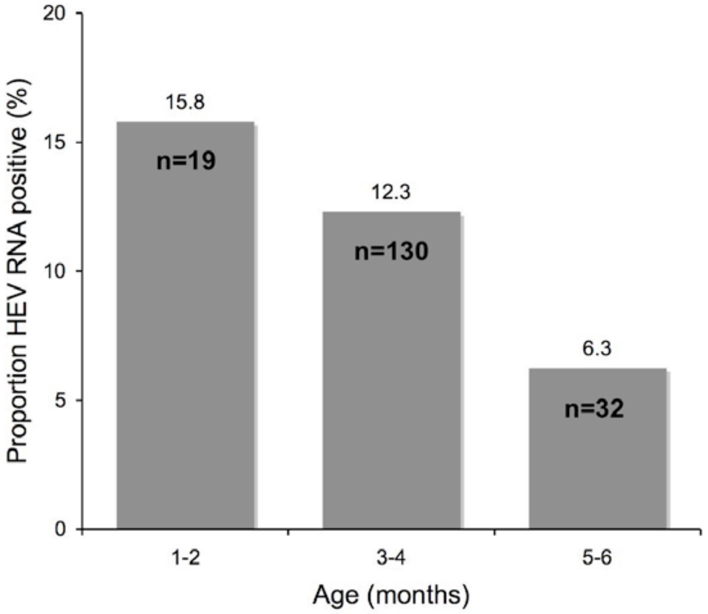

Figure 7.2 Proportion of pigs HEV RNA positive by age-category

This study provides the first data set from Laos that can be used to examine the source of human HEV disease. The pig isolates we detected had sequence homology with human isolates from southern China and pose a threat for human health in northern Laos; however, from this limited study we cannot form a strong sense of the impact on the human population. A collaborative research approach with public health specialists will be required to uncover the impacts on communities in northern Laos. In addition, further work will be required to determine if the dry season prevalence we observed was baseline and a source of epidemic transmission in the monsoonal wet season.

7.1.5 Prevalence and distribution of viral zoonoses in pigs in northern Laos

Seven hundred and twenty nine serum samples were tested at AFRIMS for antibodies to Japanese encephalitis virus (JEV) and 724 samples were tested by ELISA for Hepatitis virus (HEV). In total 727 and 722 samples were subjected to analysis for JEV and HEV, respectively, after omission of test results for samples with labelling errors. Seven hundred and twenty six serum samples were tested at CSIRO AAHL for SIV and NiV. Seven samples had labelling errors and 719 samples were included in the final analysis. Complete data was collected for location of slaughterhouse and the collection date and only partial data was available for pig age, breed and production system at last point of sale. Data on age was available for 656 animals and the median age was 12 months (25-75 percentile range: 8-16 months). The majority of slaughtered animals were indigenous breed swayback black pigs (83.9%) and the majority were purchased by slaughter-traders from a penned production system (72.4%). The last point of sale provided no indication of the production systems encountered during the life of the animals.

Antibodies to JEV were detected by HI in 543 of 727 pigs (74.7%; 95% CI: 71.5-77.9%), of which, inhibition titres of 20, 40 and ≥ 80 were observed in 26 (3.6%), 55 (7.6%) and 462 (63.6%) pigs, respectively. In Oudomxay and Xiengkhuang provinces, where samples were collected in the wet season months, there was a significantly lower seroprevalence of anti-JEV antibodies detected by HI in pigs in Xiengkhuang province and pigs in the 4-6 month old age range. In contrast, 4-6 month old pigs had the highest prevalence of anti-JEV IgM antibodies (Table 7.10). In Luangprabang and Huaphan provinces, where samples were collected in the dry season months, there was a significantly lower seroprevalence of anti-JEV antibodies detected by HI in pigs in Luangprabang province and pigs raised in a free range production system (Table 2). Anti-JEV IgM antibodies were not detected in samples collected from Luangprabang and Huaphan provinces.

IgM antibody was detected in 17 of 329 pigs (5.2%; 95% CI: 2.7-7.6%) in Oudomxay and Xiengkhuang provinces, and comprised 11 pigs sampled from Oudomxay province and six from Xiengkhouang province. Fifteen of the 17 IgM positive sera had HI titres ≥ 80 and the remaining two samples had HI titres of 40. Age data was available for 265/329 pigs and peak seroprevalence (11.6%) was observed in the youngest age class of animal (Table 7.10) and was 20.7% (6/29) and 5.0% (2/40) of 4-6 month-old pigs in Oudomxay and Xiengkhuang provinces, respectively.

This study demonstrates unequivocally that JEV was widespread in the pig population of northern Lao PDR and a seroprevalence of 74.7% was indicative of a hyper-epizootic state. There is no JEV vaccination of pigs in northern Lao PDR and these results represent natural transmission. The youngest pigs in our survey population were 4 months old and maternal antidodies wane after 2 months, the detected antibodies were therefore indicative of active JEV infections rather than passive immunity.

Antibodies to JEV were detected in all provinces and significant differences in prevalence was observed between the provinces in each temporal sampling frame. It was unlikely that the observed seroprevalence between the two temporal sampling frames was influenced by the timing of sample collection since Hi can detect antibodies to JEV for three years. The differences we encountered were likely due to factors such as pig density, rice paddy production and Culex mosquito abundance. This was further supported by the finding that pigs purchased for slaughter from free-range production systems had lower seroprevalence compared to penned pigs, with the former production systems being encountered predominantly in upland rice growing areas with limited paddy. However, the observed prevalence in all four provinces was very high.

Prevalence of IgM antibodies against JEV peaked in June and July, corresponding with the start of the wet season and water filling of rice paddies providing suitable breeding conditions for Culex mosquitoes. In pigs, IgM antibodies are detected within 2-3 days post-infection and can be detected in serum for up to 3 weeks, indicating that the IgM positive pigs we detected were recently infected and that peak transmission and greatest risk for human infection corresponds with the first half of the wet season. This peak in Lao pigs was consistent with a peak transmission to Thai people in June and July. Since we do not present a single sampling frame over a complete year, caution should be exercised in interpreting seasonal transmission patterns. We would not expect, however, highly active transmission in the dry season months due to a lack of mosquito breeding sites but the impact of irrigated rice production on Culex mosquito abundance in the dry season of northern Lao PDR remains to be determined.

The IgM ELISA results in pigs provides limited evidence that JEV is not maintained in the pig population throughout the year, being consistent with an epizootic pattern of transmission. This could be due to a combination of relatively low animal densities, a short duration of viremia, ranging from 1-3 days, and a decrease in mosquito vector abundance in the dry season winter months. The migration patterns of ardeid birds could therefore have a strong influence on JEV transmission patterns and several ardeid bird species breed in Lao PDR during the wet season months and other species over-winter during the dry season months. The role of these migratory birds in maintaining JEV in an epizootic state in Lao PDR warrants further investigation.

In pigs, the most clinically significant manifestation of a JEV infection is reproductive failure in sows due to abortion and abnormal farrowing. The very high seroprevalence of JEV in young pigs ≤6 months indicates that JEV would have little or no impact on the reproductive potential of local indigenous breed sows. Southeast Asian indigenous breed sows sexually mature from 6-8 months and the majority of sows in Laos would have protective immunity by the age of first oestrus. The impact on the reproductive potential of indigenous breed boars may however be more significant. Southeast Asian indigenous breed boars can reach sexual maturity from 2-3 months of age and infection of sexually mature boars can cause infertility. Since the smallholder pig sector in Laos has low productivity, we believe the effect of JEV on this pig producing sector warrants greater scrutiny, with particular reference to boar infertility.

Seven hundred and twenty two serum samples were tested by the HEV ELISA and 152 (21.1%) tested positive. One hundred and sixty three (22.6%) samples had an inconclusive borderline test result; the remaining 407 (56.4%) samples were negative for antibodies to HEV. In Oudomxay and Xiengkhuang provinces, where samples were collected in the wet season months, there was no observed spatial difference in seroprevalence. Seroprevalence was significantly higher in the early stages of the wet season, May and June, and significantly higher in indigenous and cross-breeds compared to exotic breed pigs (Table 1). In Luangprabang and Huaphan provinces, where samples were collected in the dry season, there was no observed spatial difference in seroprevalence and no observed difference for pig breeds. There was, however, a significant decrease in seroprevalence for December 2008 (Table 7.11). In the wet season collection sites, seroprevalence peaked in 4-6 month old pigs (41.2%; Table 7.11), however, the reverse was observed for the dry season collection sites where 4-6 month old pigs had the lowest seroprevalence (3.6%; Table 2). Age-related seroprevalence in the current study peaked in 4-6 month old pigs sampled in the wet season and 7-12 month old pigs sampled in the dry season. The combined temporal and age prevalence data indicates that young animals are an important reservoir of HEV at the beginning of the wet season. We have demonstrated that young pigs are an important reservoir of HEV and work should now be undertaken to determine the impact of human HEV disease and the source of infection, particularly for farmers who engage in piglet production (e.g. Hmong farmers in Xiengkhuang).

Seven hundred and nineteen serum samples were tested by the NiV comparative ELISA of which 716 (99.6%) were negative and three (0.4%) returned an inconclusive test result. All three ELISA inconclusive sera were tested by the NiV neutralisation assay of which two samples were negative and the third was toxic to the cell line. We found no serological evidence of NiV infection of pigs in Lao PDR, but by no means does this confirm absence of NiV in this country. Pteropid fruit bats are present in Laos and to better understand the epidemiology and risks of NiV, surveys of these competent reservoir host species will be required.

Twenty three out of 719 (3.2%) pig serum samples were reactive in the SIV ELISA, of which 13 (1.8%) were considered positive and 10 samples had an inconclusive result. Twenty ELISA reactive sera were tested by HI for H3N2 (Nakorn Pathom), two samples were HI positive with titres 160 and 640 the remaining 18 sera were negative by HI for H3N2. Twenty-one and 23 sera were tested by HI for H1N1 (Ratchaburi) and H1N1 (2009-Pandemic), respectively, and all were negative. Furthermore, 14 ELISA negative sera were tested by HI for H3N2 (Nakorn Pathom) and two samples were HI positive. No ELISA negative sera were tested by HI for H1N1 due to insufficient sera. Serological data for SIV indicated low levels of virus circulation in the Lao pig population for the period May 2008 to January 2009, however the SIV ELISA has not been fully validated for pig serum and a limited number of subtypes were screened by HI due to the small amount of esera tested. These factors may have contributed to the low frequency of detection, but this low frequency of antibody to SIV may also derive from a low-density pig population acting as a natural barrier to maintenance of virus endemicity.

Table 7.10 Seroprevalence of anti-Japanese encephalitis virus (JEV) and anti-Hepatitis E virus (HEV) antibodies in Oudomxay and Xiengkhuang provinces, samples were collected from May to September 2008 (wet season months).

Population characteristic	Japanese encephalitis virus (JEV) serology							Hepatitis E virus (HEV) serology			
	Number tested	JEV HI assay			JEV IgM ELISA			Number tested	HEV IgG ELISA		
		% Pos[a]	95% CI[b]	*P*	% Pos[a]	95% CI[b]	*P*		% Pos[a]	95% CI[b]	*P*
Province											
Oudomxay	143	90.2	85.3, 95.1	<0.001	7.7	3.3, 12.1	0.082[c]	141	29.8	22.1, 37.4	0.958
Xiengkhuang	186	70.4	63.8, 77.0		3.2	0.7, 5.8		183	30.1	23.3, 36.8	
Collection date											
May 2008	40	77.5	64.0, 91.0	0.148	2.5	0.0, 7.6	0.694[c]	39	35.9	20.1, 51.7	0.008
June 2008	144	76.4	69.4, 83.4		6.3	2.2, 10.3		141	39.0	30.9, 47.2	
July 2008	84	78.6	69.6, 87.5		7.1	1.5, 12.8		83	18.1	9.6, 26.5	
August 2008	38	78.9	65.4, 92.5		2.6	0.0, 8.0		38	21.1	7.5, 34.6	
September 2008	23	100	-		0.0	-		23	21.7	3.5, 40.0	
Breed											
Indigenous	191	80.6	0.75, 0.86	0.898	6.3	2.8, 9.8	0.499[c]	190	35.8	28.9, 42.7	0.003
Exotic	68	82.4	73.1, 91.6		2.9	0.0, 7.1		66	13.6	5.1, 22.1	
Cross-breed	18	77.8	56.5, 99.1		0.0	-		16	31.25	5.7, 56.8	
Age (months)											
4-6	69	69.6	58.4, 80.7	0.017	11.6	3.8, 19.3	0.022[c]	68	41.2	29.2, 53.2	0.099
7-12	173	85.0	79.6, 90.3		2.9	0.4, 5.4		170	27.1	20.3, 33.8	
>12	23	87.0	72.1, 100.0		4.3	0.0, 13.4		23	34.8	13.7, 55.8	
Production type											
Penned	257	81.7	77.0, 86.5	0.161[c]	5.4	2.7, 8.2	1.000[c]	253	30.0	24.4, 35.7	0.32[c]
Free range	4	50.0	0.0, 100.0		0.0	-		4	0.0	-	
Mixed	0	-	-		-	-		-	-		

[a] Pos, Positive; [b]CI, confidence interval; [c] Fishers Exact Test (all other *P*-values calculated using χ^2-test)

Table 7.11 Seroprevalence of anti-Japanese encephalitis virus (JEV) and anti-Hepatitis E virus (HEV) antibodies in Luangprabang and Huaphan provinces, samples were collected from October 2008 to January 2009 (dry season months).

Population characteristic	Japanese encephalitis virus (JEV) serology[c]				Hepatitis E virus (HEV) serology			
	Number tested	JEV HI assay			Number tested	HEV IgG ELISA		
		% Pos.	95% CI[b]	*P*		% Pos.	95% CI[b]	*P*
Province								
Luangprabang	209	59.8	53.1, 66.5	<0.001	209	12.4	7.9, 17.0	0.402
Huaphan	189	83.6	78.3, 88.9		189	15.3	10.2, 20.5	
Collection date								
October 2008	14	78.6	54.0, 100.0	0.275	14	14.3	0.0, 35.3	0.001
November 2008	113	70.8	62.3, 79.3		113	21.2	13.6, 28.9	
December 2008	107	64.5	55.3, 73.7		107	2.8	0.0, 6.0	
January 2009	164	75.0	68.3, 81.7		164	15.9	10.2, 21.5	
Breed								
Indigenous	367	70.6	65.9, 75.2	0.987	367	14.4	10.8, 18.1	0.370
Exotic	10	70.0	35.4, 100.0		10	10.0	0.0, 32.6	
Cross-breed	11	72.7	41.3, 100.0		11	0.0	-	
Age (months)								
4-6	28	78.6	62.4, 94.8	0.637	28	3.6	0.0, 10.9	0.161
7-12	169	71.6	64.7, 78.5		169	16.6	10.9, 22.2	
>12	193	69.9	63.4, 76.5		193	13.0	8.2, 17.7	
Production type								
Penned	206	77.2	71.4, 83.0	0.001	206	13.1	8.5, 17.8	0.919
Free range	167	60.5	53.0, 68.0		167	14.4	9.0, 19.7	
Mixed	6	100.0	-		6	16.7	0.0, 59.9	

[a] Pos, Positive; [b] CI, confidence interval; [c] All serum samples collected in Luangprabang and Huaphan provinces were not reactive in the JEV IgM ELISA

7.1.6 Epidemiology of Japanese encephalitis and Hepatitis E virus in human populations of northern Laos

There are inherent difficulties in analysing flavivirus HI results from an environment where JEV co-circulates with all four Dengue viruses, as is the case in Laos, due to cross-reactivity in the HAI assay. We tested 1136 human serum samples and 765 (67.3%) had evidence of anti-flavivirus antibodies in the HAI assay. Of these samples, 620/1136 (54.6%) had evidence of anti-JEV antibodies and 217/1136 (19.1%) had exclusively anti-JEV antibodies in the HAI assay. In the flavivirus HAI assay, samples were considered positive if they produced an HAI reactive titer > 1:10. All samples were tested for JEV and the four dengue virus (DEN1 – DEN4) serotypes. Samples were scored as either JEV or DEN1 – DEN4 if they produced a two-fold higher titer to the homologous viruses or scored as flavivirus (FLAVI) if samples were positive for DEN and JEV without a two-fold higher titre. The results are summarised in table 7.12b below. JEV positivity was markedly lower in Xiengkhuang province (7.1%) compared to the other three provinces (43.1% - 64.1%) and prevalence increased with increasing age. Prevalence of JEV positivity was highest in the Lao-Tai ethnic group and prevalence was higher in people who lived in villages that produced rice (both upland and lowland rice). Although the villages that produced upland rice almost always also produced lowland paddy rice. Dengue virus positivity was low for serotypes 1-3 but prevalence of DEN4 was 42.5% in Xiengkhuang province and disproportionately affected Hmong people (33.6%). The reason for this finding is not immediately clear and the finding that DEN4 was the predominant flavivirus circulating in Xiengkhuang province warrants further investigation. The prevalence of DEN4 positivity was highest in people who lived in villages not producing upland rice and this may indicate that the lowland Hmong villages on the Xiengkhuang plateau provide favourable conditions for competent mosquito vectors to proliferate.

The results of the Japanese encephalitis HI testing are difficult to interpret in the absence of virus neutralisation assays to differentiate past JEV infection from past exposure to Dengue virus, but we have been able to clearly show that JEV is hyperendemic in the human population of northern Laos and that there exists spatial, age and ethnic group heterogeneity. The results of the pig survey indicate that the peak period of exposure occurs in the wet season and the combined human and pig results provide important weight to the argument in favour of widespread vaccination of the human population to protect against JEV infection.

Hepatitis E virus antibody results are presented in Table 7.12a below. There was an increase in sero-prevalence with an increase in age and wealth score and male subjects had a higher sero-prevalence than females. Sero-prevalence was highest in Xiengkhuang and Huaphan provinces and Mon-Khmer people had lowest sero-prevalence. Interestingly, ser-prevalence increased with increasing wealth, with lowest prevalence observed in the poorest households. It is not immediately clear why this observation was made and further work is required to examine specific exposures that predispose the least poor households to exposure. The Hepatitis E test results are unable to inform on the genotype causing infection and we are unable to comment on the source of infection, whether it be waterborne or zoonotic transmission from pigs. However the results contribute to the growing body of evidence that HEV is prevalent in the Lao population and further work is required to determine the source of human infections. As described above, genotype 4 HEV is prevalent in the pig population and may be a source of infection for humans. Furthermore, almost one third of all females surveyed had evidence of past exposure to HEV virus and prevalence increased with increasing age, indicating that women of reproductive age are at risk of exposure to this virus and severe clinical hepatitis during pregnancy may occur.

Table 7.12a Prevalence of anti-HEV antibodies in the human population stratified by gender, province, wealth score, age and ethnicity.

Population characteristic	Population size		Proportion HEV antibody ELISA positive
	N	%	
Survey population	1136	100	37.9
Gender			
Female	591	52.0	31.3
Male	545	48.0	45.1
Province			
Oudomxay	357	31.4	33.1
Luangprabang	237	20.9	32.5
Huaphan	262	23.1	49.6
Xiengkhuang	280	24.6	37.9
Wealth Status			
Most poor	221	19.5	10.9
Very poor	233	20.5	25.8
Poor	249	21.9	38.6
Less poor	245	21.6	57.1
Least poor	188	16.5	59.0
Age (years)			
6-11	245	21.6	12.2
12-19	273	24.0	24.9
20-34	235	20.7	48.1
35-49	215	18.9	56.7
≥50	168	14.8	58.3
Ethnicity			
Lao-Tai	490	43.1	40.0
Mon-Khmer	432	38.0	32.6
Hmong-Mien	214	18.8	43.9

Table 7.12b Prevalence of anti-JEV, dengue virus (DEN) and anti-flavivirus (FLAVI) antibodies in the human population.

Population characteristic	N	JEV	DEN1	DEN2	DEN3	DEN4	FLAVI
Total survey population	1136	39.4 (36.6, 42.3)	2.2 (1.3, 3.1)	0.8 (0.2, 1.3)	0.8 (0.8, 1.3)	13.6 (11.6, 15.6)	10.6 (8.8, 12.4)
Province							
Oudomxay	357	43.1 (38.0, 48.3)	2.5 (0.9, 4.2)	0.8 (0.0, 1.8)	0.3 (0.0, 0.8)	0.0 (0.0, 0.0)	3.4 (1.5, 5.2)
Luangprabang	237	44.7 (38.4, 51.1)	3.8 (1.4, 6.2)	2.1 (0.3, 3.9)	0.8 (0.0, 2.0)	0.4 (0.0, 1.2)	20.7 (15.5, 25.8)
Huaphan	262	64.1 (58.3, 69.9)	2.3 (0.5, 4.1)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	12.9 (8.9, 17.1)	11.5 (7.6, 15.3)
Xiengkhuang	280	7.1 (4.1, 10.2)	0.4 (0.0, 1.1)	0.4 (0.0, 1.1)	2.1 (0.4, 3.8)	42.5 (36.7, 48.3)	10.4 (6.8, 13.9)
Age							
6-11	245	20.8 (15.7, 25.9)	0.8 (0.0, 1.9)	0.8 (0.0, 1.9)	0.0 (0.0, 0.0)	20.4 (15.3, 25.5)	2.9 (0.8, 4.9)
12-19	273	39.6 (33.7, 45.4)	1.8 (0.2, 3.4)	0.0 (0.0, 0.0)	0.4 (0.0, 1.1)	12.8 (8.8, 16.8)	2.9 (0.9, 4.9)
20-34	235	46.8 (40.4, 53.2)	2.6 (0.5, 4.6)	0.4 (0.0, 1.3)	04 (0.0, 1.3)	16.2 (11.4, 20.9)	6.8 (13.8, 24.3)
35-49	215	47.0 (40.3, 53.7)	4.2 (1.5, 6.9)	1.4 (0.0, 2.9)	1.4 (0.0, 3.0)	9.3 (5.4, 13.2)	19.1 (13.8, 24.3)
>=50	168	46.4 (38.9, 54.0)	1.8 (0.0, 3.8)	1.8 (0.0, 3.8)	2.4 (.0, 4.7)	6.5 (2.8, 10.3)	28.6 (21.7, 35.4)
Ethnicity							
Lao-Tai	490	48.6 (44.1, 53.0)	1.4 (0.03, 2.5)	1.0 (0.1, 1.9)	1.4 (0.4, 2.5)	16.5 (13.2, 19.8)	13.9 (10.8, 16.9)
Mon-Khmer	432	36.8 (32.2, 41.4)	3.0 (1.4, 4.6)	0.9 (0.0, 1.8)	0.5 (0.0, 1.1)	0.2 (0.0, 0.7)	8.3 (5.7, 10.9)
Hmong-Mien	214	23.8 (18.1, 29.6)	2.3 (0.3, 4.4)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	33.6 (27.3, 40.0)	7.5 (3.9, 11.0)
Wealth status							
Most poor	221	27.6 (21.7, 33.5)	1.8 (0.0, 3.6)	0.9 (0.0, 2.2)	0.5 (0.0, 1.3)	8.5 (4.9, 12.3)	5.9 (2.8, 9.0)
Very poor	233	37.3 (31.1, 43.6)	1.7 (0.0, 3.4)	0.4 (0.0, 1.3)	0.4 (0.0, 1.3)	12.9 (8.6, 17.2)	6.4 (3.3, 9.6)
Poor	249	48.2 (42.0, 54.4)	3.6 (1.3, 5.9)	0.4 (0.0, 1.2)	0.8 (0.0, 1.9)	16.9 (12.2, 21.5)	9.6 (6.0, 13.3)
Less poor	245	41.6 (35.4, 47.8)	1.6 (0.0, 3.2)	0.4 (0.0, 1.2)	1.6 (0.0, 3.2)	21.2 (16.1, 26.4)	13.5 (9.2, 17.8)
Least poor	188	41.5 (34.4, 48.6)	2.1 (0.1, 4.2)	2.1 (0.1, 4.2)	0.5 (0.0, 1.6)	5.9 (2.5, 9.2)	18.6 (13.0, 24.2)
Water supply							
Treated water supply	131	38.2 (29.8, 46.5)	5.3 (1.5, 9.2)	1.5 (0.0, 3.6)	1.5 (0.0, 3.6)	4.6 (1.0, 8.2)	19.1 (12.3, 25.8)
Other	1005	39.6 (36.6, 42.6)	1.8 (1.0, 2.6)	0.7 (0.2, 1.2)	0.7 (0.2, 1.2)	14.7 (12.5, 16.9)	9.5 (7.6, 11.3)
Low land paddy rice							
No	149	19.4 (13.1, 25.8)	4.7 (1.3, 8.1)	1.3 (0.0, 3.2)	0.7 (0.0, 2.0)	6.7 (2.7, 10.7)	14.1 (8.5, 19.7)
Yes	987	42.4 (39.4, 45.5)	1.8 (1.0, 2.7)	0.7 (0.2, 1.2)	0.8 (0.3, 1.4)	14.6 (12.4, 16.8)	10.0 (8.2, 11.9)
Upland rice cultivation							
No	246	26.8 (21.3, 32.4)	1.2 (0.0, 2.6)	0.4 (0.0, 1.2)	2.4 (0.5, 4.4)	28.5 (22.8, 34.1)	14.2 (9.8, 18.6)
Yes	890	42.9 (39.7, 46.2)	2.5 (1.4, 3.5)	0.9 (0.3, 1.5)	0.3 (0.0, 0.7)	9.4 (7.5, 11.4)	9.6 (7.6, 11.5)

7.1.7 A 'one health' approach to understanding the role of dogs and other parasite species that influence the health and well-being of vulnerable populations in northern Laos

While this project was primarily concerned with the occurrence and impact of pig associated zoonotic infectious diseases, the people and pigs of northern Laos are but two host species in a dynamic environment where pathogen-pathogen, host-host and host-pathogen interactions influence the transmission of infectious diseases.

7.1.7.1 Taenia hydatigena and village dogs

For the *Taenia* study, a crucial component of research that was undertaken was the prevalence survey of dogs to determine the prevalence of adult *Taenia hydatigena* in this host species. We used egg detection for *T. hydatigena* taeniasis in dogs instead of copro-antigen ELISA as we were unable to source suitable diagnostic reagents during the course of this study. Faecal samples were collected from 105 dogs from 21 villages, 32 (30.5%), 30 (28.6%), 11 (10.5%) and 32 (30.5%) from Oudomxay, Luangprabang, Huaphan and Xiengkhuang provinces, respectively. All dogs were raised in an unrestrained manner, the median age was 12 months (range: 2-108) and 63 (60%) were female and 42 (40%) were male. Two dogs (1.9% (95% CI: 0.0-4.6)) were *Taenia* egg positive; one a 12-month-old male dog from Xiengkhuang province and the other a 3-year-old male dog from in Luangprabang province. We used a maximum likelihood estimator to approximate prevalence; in the plausible range of diagnostic sensitivity the prevalence was estimated to be 4.8-9.5% of dogs (Table 7.13). No published data provide a reliable estimate of the diagnostic sensitivity and specificity of *T. hydatigena* egg detection in dog faeces. *Taenia hydatigena* proglottids are predominantly shed without defecation indicating that the sensitivity of detecting eggs in stool samples would be lower than the estimated 62.5% observed for *T. solium* taeniasis. Our prevalence estimates of *T. hydatigena* were in the order of 5-10% of village dogs and this corresponded to prevalence in pigs of 50-60%.

Under a strong infection pressure, immunity to *Taenia* infection in the intermediate host can be acquired within two weeks of exposure to eggs and embryos entering muscles after one week will not survive, meaning there exists a short window of opportunity for infection throughout the life of the intermediate host. Immune-mediated *Taenia* competition in the intermediate host has been well documented for the ovine tapeworms, *T. ovis* and *T. hydatigena*, where *T. ovis* cyst development can be inhibited by pre-exposure to *T. hydatigena* eggs. There are no plausible reasons why these same immune-mediated competitive interactions do not occur in pigs, meaning that *T. solium* cyst development would be inhibited by ingestion of *T. hydatigena* eggs. The evidence presented here indicates that pigs may be exposed to *T. hydatigena* eggs at a young age through coprophagia, feed, water and/or soil contamination and provide pigs with protective immunity due to genus conserved immunogenic antigens.

Table 7.13 Estimated true prevalence of *Taenia hydatigena* taeniasis in village dogs adjusted by the Maximum-Likelihood-Estimation model for increments of test sensitivity, assuming specificity of 100%.

Test sensitivity	Estimated taeniasis prevalence (95% CI)
70	2.7 (0.0 – 6.4)
60	3.2 (0.0 – 7.5)
50	3.8 (0.0 – 9.0)
40	4.8 (0.0 – 11.3) **
30	6.3 (0.0 – 15.1) **
20	9.5 (0.0 – 22.6)**
10	19.0 (0.0 – 45.2)

** Biological plausible range of test sensitivity

7.1.7.2 Polyparasitism in the human population

The overall prevalence of infection with any soil transmitted helminth (STH) tested by the formalin-ether concentration technique was 70.6%; the prevalence of *Ascaris lumbricoides*, *Trichuris trichiura*, hookworm and *Strongyloides stercoralis* were 26.1, 41.5, 46.3 and 8.9%, respectively (Table 7.14). The species-specific prevalence of STH by population characteristic are described in detail in Table 7.14. There was no significant difference (P<0.05) in prevalence between male and females for all parasite species with the exception of *S. stercoralis*, where the prevalence in males was more than double that of females. There was a significant difference in province specific prevalence for all parasite species; Xiengkhuang province had the lowest prevalence for all STH and Luangprabang province had the highest prevalence for all species except hookworm. Prevalence of *A. lumbricoides*, *T. trichiura* and hookworm decreased significantly with increasing household wealth and *S. stercoralis* increased significantly with increasing wealth. There was a significant difference between age groups for *A. lumbricoides* with peak prevalence observed in 20-34 year-olds, *T. trichiura* prevalence decreased significantly with increasing age up to 49 years old with a slight rise in the oldest cohort, whereas *S. stercoralis* increased significantly with increasing age up to 49 years old. The prevalence of helminthiasis was significantly greatest in the Mon-Khmer ethno-linguistic family for all STH with *A. lumbricoides* and *T. trichiura* infection significantly greater in the cohort who defecated in the open. Infection with *S. stercoralis* was not associated with the literacy of the male or female head-of-household, whereas *A. lumbricoides* and *T. trichiura* infections were both significantly greater in households with an illiterate male or female head-of-household. Hookworm infection was significantly greater in households with an illiterate female head-of-household but not the male head-of-household.

The significant risk factors associated with STH infection a 231 after controlling for household clustering at the community level and in high-risk sub-populations are summarised in Table 7.15. At the community level, there was strong evidence of spatial, ethnicity and wealth variation in having a STH infection. Study participants living in Luangprabang and Huaphan provinces had an increased risk of ascariasis, trichuriasis or having any STH infection as compared to Oudomxay province residents. Study participants from Xiengkhouang province had a reduced risk of ascariasis or strongyloidiasis and people from Huaphan province had an increased risk of hookworm infection. People in the wealthier quintiles had a reduced risk of having an STH infection with the exception of strongyloidiasis. People of Mon-Khmer ethnicity had increased risk of ascariasis, trichuriasis or any STH infection compared to Lao-Tai people, whereas people of Hmong-Mien ethnicity had a moderately increased risk of hookworm infection but a marked reduction in risk of trichuriasis or strongyloidiasis. At the community level, open defecation was a significant risk for trichuriasis and overall, men had a slightly increased risk of having any STH infection.

People of Mon-Khmer ethnicity are a highly vulnerable group with respect to STH infection in northern Laos. This group of people were more likely to defecate in the open, were more likely to be poor and more likely to have an illiterate male or female head of household. These findings were reflected in the high prevalence of STH infection and the increased risk in comparison with other ethnic groups. Concurrent infections with STH (polyparasitic infections) were common for the Mon-Khmer ethno-linguistic family and for the poorest households, indicating that health and development consequences were potentially greater in these groups. Recent studies in the Philippines demonstrated that low to moderate intensity multiple infections were associated with anaemia. Less clear however is the impact of multiple infections on cognitive function. Multiple studies indicate that STH infections have a detrimental effect on cognitive performance and educational outcomes in school children, of particular note are recent data from geographically diverse regions indicating that light and moderate polyparasitic infections have a detrimental effect. In the Lao context, polyparasitic STH infections are highly prevalent in high-risk groups and may have a strong role in perpetuating the cycle of poverty.

These same poor Mon-Khmer families also harboured the highest prevalence of taeniasis and cysticercosis, even though the results were not statistically significant for cysticercosis due to small sample size for a rare disease. The results indicate that poverty and health are closely related and have an impact on the educational outcomes, work performance, productivity and food security. The potential to have an impact in these highly vulnerable Mon-Khmer communities, with the inherent challenges or low education levels, language barriers and cultural differences will require a sensitive but systematic approach to education, sanitation and health interventions over an extended period of time. Our results indicate that the mass administration of mebendazole in northern Laos for the treatment of STH is having limited impact for the poorest households and Mon-Khmer communities. Furthermore, the majority of the Mon-Khmer households in our survey population were in Oudomxay province and in this province 90% of pigs had evidence of previous JEV infection, placing the human population at substantial risk of becoming infected with this potentially deadly virus.

Table 7.14 Prevalence of soil-transmitted helminths by population characteristics.

Population characteristic	*A. lumbricoides*	*T. trichiura*	Hookworm	*S. stercoralis*	Any STH[b]
Survey population	26.1	41.5	46.3	8.9	70.6
Gender					
Female	28.0	40.8	43.7	5.4	68.1
Male	24.1	42.1	49.0	12.5	73.2
Province					
Oudomxay	24.5	45.6	50.8	7.6	75.8
Luangprabang	40.6	60.8	41.8	18.8	81.5
Huaphan	30.0	52.2	51.6	6.6	77.8
Xiengkhouang	7.2	2.9	40.5	1.6	44.1
HH wealth status					
Most poor	37.6	53.6	59.2	7.6	86.4
Very poor	31.8	49.8	57.2	6.7	82.0
Poor	30.6	41.2	43.0	5.2	68.0
Less poor	15.6	29.8	41.4	10.8	60.0
Least poor	16.5	35.2	33.0	14.2	59.6
Age (years)					
6-11	25.9	48.3	43.8	4.5	70.3
12-19	29.2	44.4	41.7	6.9	71.2
20-34	30.4	37.2	48.6	10.5	71.3
35-49	23.3	35.9	49.6	12.2	72.2
≥50	19.6	41.1	48.6	11.2	67.3
Ethnicity					
Lao-Tai	17.7	36.2	39.7	9.9	61.9
Mon-Khmer	39.4	60.6	50.6	11.2	85.2
Hmong-Mien	17.5	14.9	51.9	2.2	60.4
Defecation site					
Latrine	21.3	35.4	44.8	9.2	65.8
Open	35.1	53.0	49.1	8.2	79.8
Male head of HH[a]					
Literate	23.9	37.9	45.8	8.7	68.5
Illiterate	36.6	58.6	48.7	9.5	81.0
Female head of HH[a]					
Literate	22.9	36.3	40.9	9.2	64.5
Illiterate	30.8	49.2	54.5	8.5	79.7

[a] HH, household; [b] Any STH, soil-transmitted helminth, including all four STH infections.

Table 7.15 Risk factors significantly (*P*<0.050) associated with soil-transmitted helminth infections at the community level and in vulnerable sub-populations (6-11 year-old children and women of childbearing age, 15-49 years-old), as determined by multiple logistic regression modelling controlling for household clustering.

Parasite	Risk factor [h]	Adjusted OR [a]	95% CI [b]	*P*
A. lumbricoides [c]	Luangprabang province	5.08	2.95, 8.72	<0.001
	Huaphan province	5.55	2.62, 11.76	<0.001
	Less poor	0.36	0.19, 0.69	0.002
	Least poor	0.27	0.13, 0.54	<0.001
	Mon-Khmer ethnicity	4.11	2.16, 7,82	<0.001
T. trichiura [d]	Luangprabang province	4.11	2.29, 7.39	<0.001
	Huaphan province	5.46	2.58, 11.56	<0.001
	Xiengkhuang province	0.12	0.04, 0.32	<0.001
	Least poor	0.46	0.21, 0.99	0.048
	20 – 34 year-olds	0.46	0.29, 0.73	0.001
	35 – 49 year-olds	0.42	0.27, 0.67	<0.001
	Mon-Khmer ethnicity	2.40	1.27, 4.55	0.007
	Hmong-Mien ethnicity	0.36	0.17, 0.76	0.007
	Open defecation	1.62	1.05, 2.51	0.030
	Mon-Khmer ethnicity	4.13	1.71, 9.95	0.002
	Open defecation	3.06	1.63, 5.76	0.001
Hookworm [e]	Huaphan province	1.89	1.01, 3.53	0.045
	Least poor	0.42	0.24, 0.76	0.004
	Hmong-Mien ethnicity	1.74	1.04, 2.90	0.033
S. stercoralis [f]	Male	2.87	1.85, 4.45	<0.001
	Luangprabang province	2.56	1.44, 4.53	0.001
	Xiengkhuang province	0.17	0.06, 0.51	0.002
	20 – 34 year-olds	2.66	1.30, 5.47	0.008
	35 – 49 year-olds	2.51	1.23, 5.11	0.011
	≥ 50 years-old	2.19	1.03, 4.67	0.042
	Hmong-Mien ethnicity	0.26	0.09, 0.69	0.007
Any STH [g]	Male	1.42	1.06, 1.90	0.020
	Luangprabang province	3.07	1.67, 5.63	<0.001
	Huaphan province	3.81	1.80, 8.06	<0.001
	Poor	0.36	0.17, 0.73	0.005
	Less poor	0.33	0.16, 0.68	0.003
	Least poor	0.22	0.10, 0.49	<0.001
	Mon-Khmer ethnicity	2.90	1.54, 5.50	0.001

[a] OR, odds ratio
[b] CI, confidence interval
[c] OR adjusted for province, wealth status, ethnicity, age group, defecation site and male and female head-of-household literacy
[d] OR adjusted for province, wealth status, ethnicity, age group, defecation site and male and female head-of-household literacy
[e] OR adjusted for gender, province, wealth status, ethnicity and female head-of-household literacy
[f] OR adjusted for gender, province, wealth status, ethnicity and age group
[g] OR adjusted for gender, province, wealth status, ethnicity, defecation site and male and female head-of-household literacy of-household literacy
[h] Reference comparators: Province, '*Oudomxay province'*; Wealth status, '*most poor'*; Ethnicity, *'Lao-Tai'*; Age group, *'6-11 years-old'*.

7.1.7.3 Other zoonotic parasites associated with dogs

Two hookworm species, *Necator americanus* and *Ancylostoma ceylanicum*, that have been associated with human disease in Laos were detected in the village dog populations. In total, 94 of 105 dogs (89.5%) had hookworm eggs detected by microscopy. Twenty-three of 94 (24.5%) faecal samples positive for hookworm were analysed by PCR and sequencing to identify the species. The PCR-sequencing protocol was able to successfully amplify and characterise 18 (78.3%) of these samples. Single species amplification of DNA from *A. ceylanicum*, *A. caninum*, *A. braziliense* and *N. americanus* were detected in 7 (38.9%), 2 (11.1%), 1 (5.6%) and 1 (5.6%) dogs, respectively. Dual species amplification of DNA from *A. ceylanicum* and *A. caninum* were detected in 4 (22.2%) dogs and dual species amplification of DNA from *A. ceylanicum* and *N. americanus* were detected in 3 (16.7%) dogs. Overall, *A. ceylanicum* was the most prevalent hookworm species detected in village dogs, 14/18 (77.8%). Dogs infected with *A. ceylanicum* or *A. caninum* were detected in all four provinces, *A. braziliense* was detected only in Luangprabang province and *N. americanus* was detected in two villages in Oudomxay province and two villages in Luangprabang province. In Oudomxay province where *N. americanus* was detected in dogs, all residents of one village reported open defecation and all residents of the other village reported latrine use. These findings have important implications for human hookworm disease since *N. americanus* has not been previously associated with zoonotic transmission, further studies to confirm patency in dogs will therefore be required. Furthermore, the control of hookworm disease in dogs will require special consideration since the treatment of dogs may alter the distribution and occurrence of *T. hydatigena* in the dogs and pigs and by extension the immune-mediated suppression of *T. solium* in the Lao pig population.

Other enteric zoonotic parasites infecting dogs included *Giardia duodenalis*, *Trichomonas* spp., liver fluke (*Opisthorchis viverrini* or *Clonorchis sinensis*) and ascaris, the results are summarised in Table 7.16. Molecular and phylogenetic analysis of dog and human *Giardia* isolates indicated that zoonotic transmission between these hosts species occurs with human and dog isolates in Assemblage B and D clustering together.

Table 7.16 Enteric parasitic infections of village dogs (n=105)

Parasite	**Proportion of dogs infected (%)**
Taenia spp.*	1.9
Hookworm*	89.5
Cryptosporidium spp.	2.9
*Giardia duodenalis**	28.6
Toxocara canis	21.0
Ascaris*	24.8
Coccidiosis	3.8
Trichuris spp.	19.0
Opisthorchis viverrini or *Clonorchis sinensis**	1.0
Spirometra spp.*	13.3
Spirocerca spp.	10.5
Entamoeba spp.	1.9
Trichomonas spp.*	4.8
Any parasite	**98.1**

* Parasite with zoonotic potential

7.1.7.4 Enteric parasitic infections of young pigs

In total, 181 faecal samples were collected from young pigs ≤6months of age as described for the HEV genotypying above. One hundred and two formalin preserved samples were sent to Murdoch University for analysis by Sodium nitrate flotation to detect helminth eggs and protozoan oocysts. The results are summarised in Table 7.17.

Table 7.17 Enteric parasites infecting young pigs ≤6months of age

Parasite	**Proportion of pigs infected (%)**
*Balantidium coli**	68.6
Giardia spp.*	2.9
Taenia spp.*	2.0
Paragonimus spp.*	1.0
Trichomonas spp.*	36.3
Entamoeba spp.	37.3
Trichuris suis	20.6
Strongyle	61.8
Strongyloides spp.*	20.6
Coccidiosis	33.3
Any parasite	**99.0**

* Parasite with zoonotic potential

The majority of pigs were infected with the ciliate protozoan *Balantidium coli* and this parasite has been recognised as a potential zoonoses, particularly for immuno-compromised individuals. The human enteric parasite study did not detect any cases of balantidiosis and this parasite may not be clinically significant at a general population level but should be considered important for highly vulnerable people with weakened immune systems, including the elderly, people infected with human immunodeficiency virus (HIV) and other conditions. We did not determine the species of *Strongyloides* infecting pigs, but *S. stercoralis* is infectious to pigs and it will be important to consider pigs as a reservoir host for programs seeking to control soil transmitted Helminths. Similarly, we did not determine the species of *Paragonimus*, *Giardia* or *Trichomonas*, but all have the potential to be transmitted to people and pigs should be considered a potential source of human illness.

Interestingly, coccidiosis was detected in a third of the young pigs tested and could be a cause of piglet diarrhoea and piglet mortality. While this is not a zoonotic threat, significant looses to the pig producing sector occur as a consequence of piglet mortality and research to understand the role of coccidiosis in piglet losses is warranted, as is the exploration of management and husbandry practices to reduce the impact of this enteric parasite.

We were able to determine that polyparasitism in young pigs was highly prevalent with greater than 90% of animals tested having two or more enteric parasites and two thirds having three or more (Figure 7.3). This finding is significant in that the potential for zoonotic transmission is high and piglet growth and feed conversion may be severely constrained.

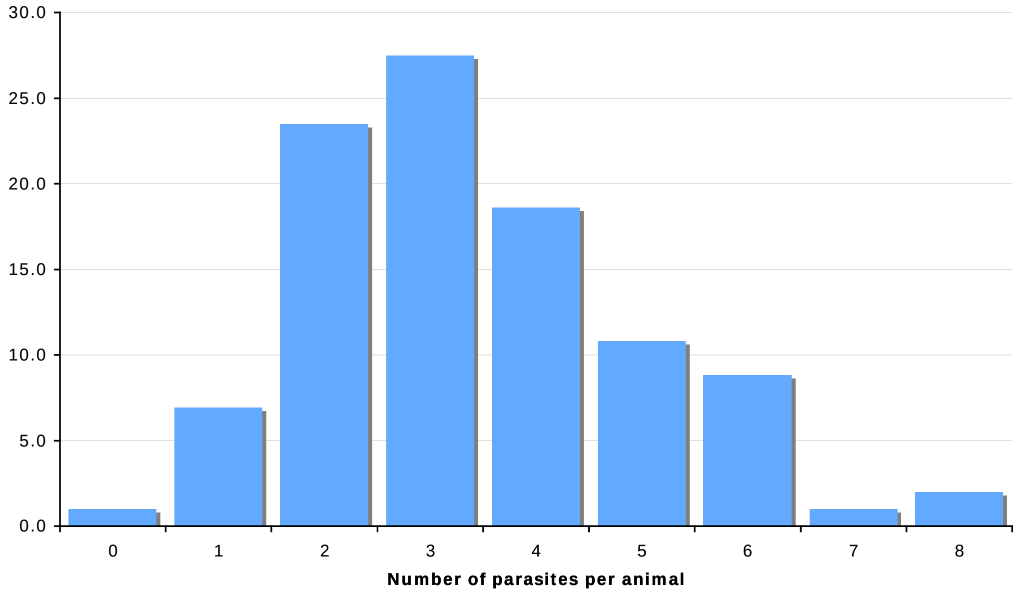

Figure 7.3 Polyparasitism in young pigs ≤6 months of age (n=102).

7.2 Socio-economic impact assessment

During the project planning, it was considered necessary to conduct socioeconomic evaluation of the impact of pig-associated zoonoses in Laos PDR to assist in resource allocation decisions especially in the healthcare sector as diseases that seemingly have a low or tolerable health impact may actually result in significant economic losses for the community. Expertise in this area was sought through Dr Stuart Blacksell's adjunct appointment at the Australian Centre for International Tropical Health (ACITH) at the University of Queensland (UQ) via a recognised expert in the area, Prof Maxine Whittaker and Adnan Choudhury (Research Assistant).

Original data from Oudomxay, Luangprabang, Huaphan and Xiengkhuang provinces were captured through the human health survey and a pig-abattoir study. The human health survey covered 333 households (1515 individuals) from 24 randomly selected villages in the 4 study regions. Prevalence rates for cysticercosis, taeniasis and trichinellosis in humans and pigs were derived from the surveillance studies performed earlier in the project.

The total costs of pig-associated zoonotic diseases as estimated by the model are displayed in Table 7.2.1. The total costs directly incurred by human beings through healthcare expenditure are extremely low. This is likely due to a mixture of the low prevalence and low impact (e.g. relatively cheap treatment) of taeniasis, trichinellosis and cysticercosis. Hospitalisation costs formed the bulk of human health costs. As an individual had on average a likelihood of 1.96-2.62% probability to acquire cysticercosis, this implied the total likelihood of being hospitalised by NCC was approximately between 0.04% and 0.06%. This is reflected in the low per person/year costs in Table 7.2.2. Luangprabang carries by far the greatest cost to these illnesses. This is likely due to a relatively higher proportion of uncooked or partially cooked pork consumers in Luangprabang compared to other provinces. Oudomxay also had a relatively high proportion of uncooked or partially cooked pork consumers compared to Xiengkhuang and Huaphan; however its cost of illness was on par with these provinces. This is likely due to Oudomxay having the second lowest population in the study regions. This hypothesis is supported by Oudomxay having a comparable cost of illness to Huaphan while having twice the human cost estimation (reflecting the higher pork consumption rates). The confidence intervals for all of the provinces are extremely wide and likely due to the large amount of unknowns and assumptions made in the model.

Table 7.2.1. Annual Costs due to Pig-Associated Zoonotic Diseases

	Mean	**LCI 95%**	**HCI 95%**
Huaphan (non HCM)	$89,909	$27,395	$198,090
Animal Costs Only	$79,002	$20,305	$183,335
Human Costs Only (non HCM)	$10,907	$547	$46,189
Annual Human Costs (HCM)	$297,612	$237,974	$360,620
Total Annual Cost (HCM)	**$376,614**	**$287,557**	**$495,776**
	Mean	**LCI 95%**	**HCI 95%**
Luangprabang (non HCM)	$135,051	$43,679	$286,178
Animal Costs Only	$114,523	$30,414	$261,775
Human Costs Only (non HCM)	$20,528	$2,974	$72,225
Annual Human Costs (HCM)	$1,096,640	$882,713	$1,313,586
Total Annual Cost (HCM)	**$1,211,163**	**$975,573**	**$1,465,837**
	Mean	**LCI 95%**	**HCI 95%**
Oudomxay (non HCM)	$89,560	$28,201	$192,293
Animal Costs Only	$74,990	$19,625	$172,863
Human Costs Only (non HCM)	$14,571	$1,570	$55,365
Annual Human Costs (HCM)	$427,611	$341,976	$515,141
Total Annual Cost (HCM)	**$502,601**	**$396,094**	**$629,750**
	Mean	**LCI 95%**	**HCI 95%**
Xiengkhuang (non HCM)	$73,831	$22,781	$161,015
Animal Costs Only	$64,773	$17,046	$149,515
Human Costs Only (non HCM)	$9,058	$572	$37,785
Annual Human Costs (HCM)	$397,401	$319,939	$477,266
Total Annual Cost (HCM)	**$462,175**	**$364,796**	**$575,622**

HCM=Human Capital Method to estimate costs associated with loss of productivity and disability

Provinces with larger populations generally have larger costs due to higher numbers of sufferers. As such, per person estimates may provide a better guide of the intensity of the illness in a given province. As the results in Table 7.2.2 show, Huaphan has the lowest cost per person. This is most likely due to Huaphan having the lowest proportion of uncooked or partially cooked pork consumers of the four study regions.

Table 7.2.2. Annual cost of pig-associated disease per-person, by province

	Mean	LCI 95%	HCI 95%
Huaphanh	$1.34	$1.02	$1.76
Luang Prabang	$2.98	$2.40	$3.60
Oudomxay	$1.90	$1.49	$2.37
Xieng Khouang	$2.01	$1.59	$2.51

Due to variable food consumption habits in Lao, southern parts of the country may have different rates of pork consumption and as such cost per-person estimates cannot readily be applied Lao-wide. The outputs of this model contain noticeably wide confidence intervals which were expected due to the number of assumptions and grey data sources used. The difference in methodologies and quality of inputs of this Lao model and other more rigorous studies are readily seen in its output.

The results of this model provide insight into the impact of cysticercosis, taeniasis and trichinellosis in four provinces of northern Laos. These results show that pig-associated zoonotic diseases have a significant impact on the economy of the selected provinces and deserve policy attention. The results are not definitive but allow decision makers and donors to understand the potential extent of a disease in order to make resource allocation decisions. Further research could refine the estimates calculated by this study. Additionally this study demonstrates that economic evaluations of neglected, under measured diseases can be undertaken as the first step to address the policy neglect. Owing to the spatial clustering of cysticercosis, additional studies may also be able to provide greater insight into social impacts such as labour shortages, dependency, stigma and school participation.

7.3 Pilot study to identify high risk areas for cysticercosis and taeniasis transmission using key informant interviews

The project was able to determine that there was a low prevalence of *Taenia solium* tapeworm carriers in northern Laos and these cases are difficult to detect. In our study the estimated prevalence of *T. solium* taeniasis based on total taeniasis prevalence in combination with the molecular diagnostic testing was 0.5% and this corresponded with approximately 2.2% prevalence of human cysticercosis and 3-4% prevalence of pig *T. solium* cysticercosis. Our results also indicated that human cysticercosis was focally distributed meaning that hot-spots for transmission will be difficult to detect using cross-sectional surveys. Surveys would have to be very large and expensive to identify hot spots. Since disease control requires interruption of the human-pig-human transmission cycle, we need to first devise methods of detecting hot-spots using non-invasive risk assessment of existing data followed by more robust assessment of disease status in pigs and people (Figure 7.5) and validating the system through the assessment process.

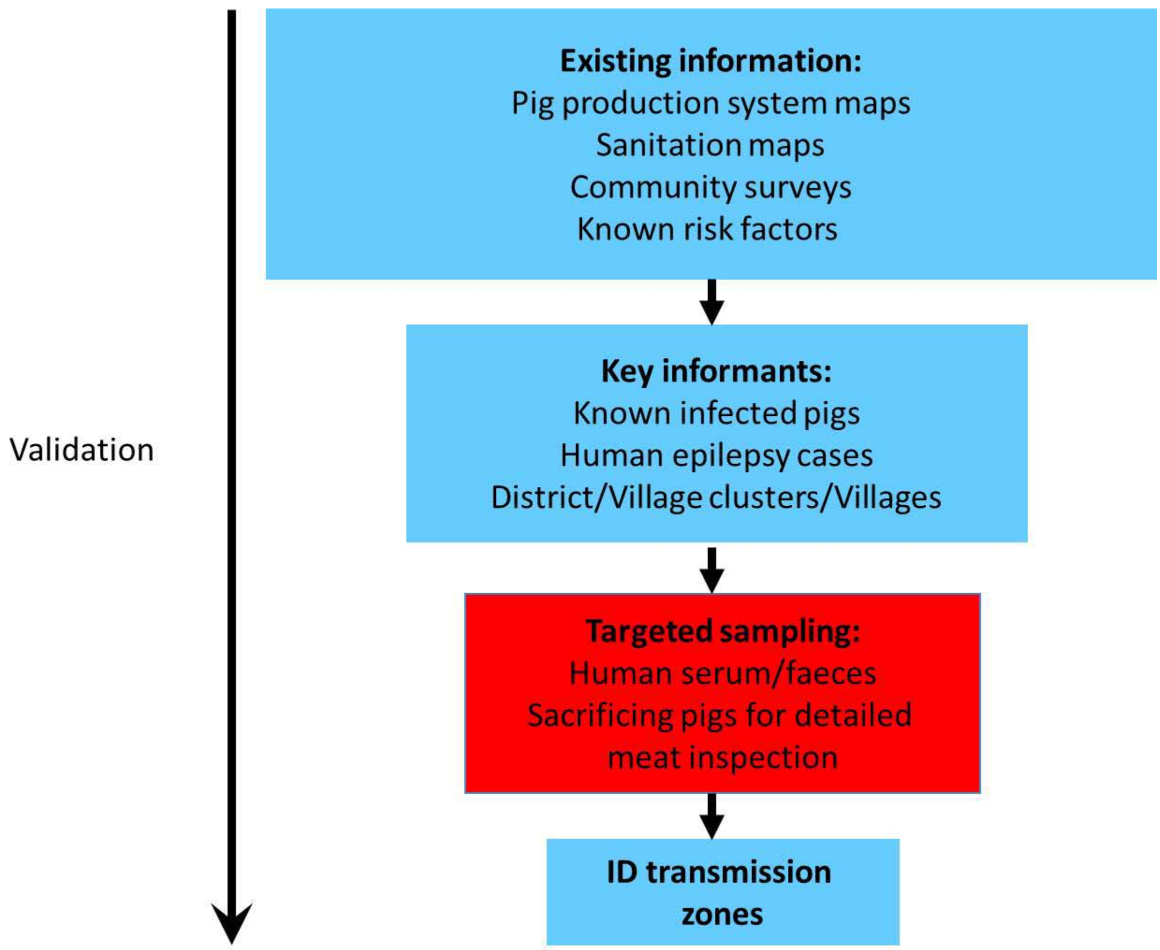

Figure 7.5 Schematic flow diagram of process to identify *Taenia solium* transmission hot-spots.

Important characteristics of the assessment process are using non-invasive detection methods in the initial stages, sensitive and specific in detecting human and pig cases, cost effective, rapid and reproducible, and utilising key informants to provide reliable information. For the purpose of this survey we identified pig traders, slaughterhouse workers, meat sellers and government meat inspectors as key informants to provide information on pig cysticercosis. For human *T. solium* taeniasis detctetion we assumed epilepsy cases as a proxy for human cysticercosis and by extension taeniasis. The key informants identified were medical clinics, medical practitioners and hospital records for mature onset unexplained epilepsy. We conducted pilot studies in Luangprabang and Oudomxay provinces to determine if the key informants listed above could provide suitable information. For pig cysticercosis, the data collected in both provinces was of limited use due to the complexity of the supply chain and detection of cysticercosis at the final slaughter step. Slaughter-traders, meat sellers and inspectors were not able to identify the origin of pigs that were infected with cysticercosis. The lack of traceability along the supply chain will need to be addressed before these key informants can be used to identify hot-spots, or alternatively other key informants need to be identified. For human epilepsy cases, the data was limited in that hospital and clinic records were heavily biased towards patients within a short distance of the hospital or clinic and there was no way of determining the cause of the epilepsy. A previous study in Laos indicates that head trauma associated with motor vehicle accidents is the leading cause of epilepsy and we could distinguish causation in our pilot study.

The pilot study demonstrated that the methodology will need to be adaptable and key informants will need to be carefully identified and may differ between different provinces. Human neurocysticercosis cases present with general neurological symptoms that are difficult for district and provincial hospital staff to differentiate so this will be a major limitation. Identification of pig cysticercosis hot-spots may provide the best available data as traders generally have a good idea about high risk areas. Traders in Huaphan province identified Viengkham in Lunagprabang province as a hot spot and this knowledge should be gathered by developing a structured and validated questionnaire for meat and slaughter traders. Once hot-spots are identified, structured, invasive surveys using robust diagnostic methods should then be conducted to determine if there is evidence of human and pig *T. solium* transmission.

7.4 Communications and educational material for cysticercosis

The PZP Information Education and Communication component consists of a small package of public health materials, comprising a set of print materials that explain the transmission cycle of the pork tapeworm (*Taenia solium*) and the steps people need to make in order to break the cycle and avoid contracting the parasite. These steps, if adopted, would also have a significant benefit in preventing other infectious diseases associated with food and poor hygiene and sanitation.

At the outset of the IEC development process a brief was written with the PZP management team, representatives of the MOH and DLF. The main requirement was that the media explain the transmission cycle of the pork tapeworm and the steps people need to make in order to avoid contracting it. An existing poster developed in Africa was provided as a starting point for possible adaptation for the Lao context. Taking into consideration various factors the team agreed to produce a large poster outlining the cycle of transmission and a flip chart focusing on 5 ways of stopping the transmission. These would incorporate images and a small amount of text with simplified readily understandable messages.

Various factors were considered during the initial design process in line with standard practices and reflected in the final design:

• Proposed target audiences for the media were initially defined as family groups and in particular people who handle pigs or raw pork for the family to eat.

• Ethnicities targeted were primarily Lao Loum although PZP has worked with other groups including Khmu and Hmong.

• Expected literacy levels of target audiences were also a major consideration

• It was envisaged that this IEC package would eventually be employed by the MOH and other agencies working in pig/human public health.

• While PZP covered costs of design and production, budgetary constraints mean that the financing of large-scale reproduction will have to be met by the agencies who will use the materials.

• Preliminary designs were reviewed by PZP team members and pre-tested with 15 groups in 3 villages in Vientiane Province. The results of that test were evaluated and some changes were incorporated into the final designs.

• The final consultation with a broad audience (final project meeting for PZP) highlighted a few important points: reproduction of the material in other Lao ethnic minority group languages where a written language exists (e.g. Khmu); testing the material and adaptation for illiterate people; incorporate the material into broader health promotion strategies; possibly include a preserved tapeworm in the delivery

• The material is not intended to be left in the village as it requires explanation from trained district health and livestock staff; perhaps flyers outlining the 5 steps can be left in the village

• The staff who will deliver this material will require training and should be incorporated with MOH departments specializing in IEC delivery

The poster and flip chart outlining the five steps to stopping transmission can be accessed on the ACIAR webpage at www.aciar.gov.au.

8 Impacts

8.1 Scientific impacts – now and in 5 years

Diagnostic tests and scientific methods used during the project are already in the public domain so we expect no scientific impact regarding diagnostics development.

The project has however been able to identify a novel and potentially important aspect of *Taenia* ecology in pigs and people. A paper was accepted to the international journal *Trends in Parasitology* discussing the role of inter-specific competition in moderating *Taenia* transmission dynamics. If this hypothesis is eventually demonstrated there will likely be substantial regional and international scientific impact. The project has been able to show that *Taenia saginata* is the dominant species infecting humans and that *Taenia hydatigena* is the dominant species in pigs. These two findings support the competition model stated above. Of significant scientific impact was the finding of a focal distribution of *Taenia solium* cysticercosis in the human population, more than half of the cases detected were from just three Khmu villages in Oudomxay province, indicating that surveillance and future research will need to take a systematic and targeted approach to village selection rather than randomised selection of villages, similarly, control measures will need to be tailored to specific production systems and ethnicities. Impacts in fives years could be development of a fully validated risk based approach to the selection of areas to target for disease control with the implementation of a suite of diagnostic tools to confirm the transmission of *Taenia solium* and measure control activities. It is unlikely that a traditional cross-sectional or prevalence survey will result in successful identification of *Taenia solium* hot-spots due to the cost and vast geographical scope of such surveys. It will be critical to future control initiatives for novel risk based tools to be developed and validated.

We have also been able to show that risk for human disease can occur at all stages in the pork supply chain but pig and human infection with *Taenia solium* and subsequent cysticercosis is most likely (greatest risk) at the upstream end of the supply chain and particularly in poor remote villages producing piglets in a free range environment with poor sanitation. In contrast, the evidence indicates that greatest risk of human trichinellosis is at the downstream end, affecting predominantly more wealthy people from the Lao-Tai ethnic population. This reflects the food-borne transmission of *Trichinella* and the faecal-oral transmission of *Taenia solium* cysticercosis. We have provided important insights into the risk of pig infections at the upstream end and these pig producers should be a target of interventions. However, improvements to pig production through the entire supply chain will be critical to controlling food-borne parasites.

The work that we conducted on Japanese encephalitis virus in pigs was the first sero-epidemiological data for Laos. The evidence indicates that JEV is in a hyper-epizootic state and peak transmission occurs in the wet season. These results are an important body of work for the Ministry of Health and the World Health Organisation (WHO) in developing a plan for the introduction of vaccination for JEV. The results have been shared with public health specialists at the WHO prior to a meeting in October 2010 to discuss vaccination against JEV. Similarly our work on Hepatitis E virus has had immediate scientific impact with the finding that there appears to be geographic partitioning of genotype 3 and 4 in SE Asia. In five years time, these findings will likely contribute to further investigations to better understand the distribution of HEV in SE Asia and to determine if JEV is reintroduced in to the Lao pig population from ardeid birds or if the virus over-winters in mosquito larvae.

The results of this project have directly influenced the development of other research initatives aimed at controlling zoonotic infectious diseases, these include the new ACIAR Project AH/2009/001 *Increased productivity and reduced risk in pig production and market*, the International Livestock Research Institute (ILRI) EcoZEID Project and the

Bovine Zoonoses Project funded by the World health Organisations (WHO) Small Grants Program.

8.2 Capacity impacts – now and in 5 years

The parasitology diagnostic capacity of the National Animal Health Centre (NAHC) has been substantially improved. The NAHC now has world standard diagnostic capacity for Trichinella detection from muscle tissue from pigs and other susceptible animals.

Laboratory based parasite egg detection capacity has also been improved through the introduction of simple and sensitive egg flotation techniques not previously used.

Laboratory testing capacity at the National Centre for laboratory and Epidemiology has also benefited from project activities with staff now proficient in serological detection of human trichinellosis and cysticercosis.

Improvements have also been made to meat inspection protocols and an additional 18 inspectors have been trained in improved detection methods for cysticercosis.

The Lao Project Leader undertook a John Dillion Fellowship study tour in Australia and received project management training and invaluable insight into the management and operations of world class Australian research institutes. This will have continued positive impacts on project management capacity within the National Animal Health Centre.

8.3 Community impacts – now and in 5 years

8.3.1 Economic impacts

Economic impacts were not measurable over the course of the three year project. Economic impacts are likely to arise from improvements to pig management and from improvements to health and increased work force participation, and these could not be achieved in the short time frame of this project.

8.3.2 Social impacts

Pigs have particular significance for food security and poverty reduction in Laos and the outcomes of this project provided valuable information on the occurrence and impact of pig associated zoonoses. While we had no immediately measurable social impacts, there remains a potential for significant social impact within 5 – 10 years if our findings are acted upon. We found that Mon-Khmer people are a highly vulnerable population and disproportionately affected by zoonoses and other diseases that negatively impact on their social advancement. The elimination or reduction in open defecation and improved management of pigs could have major impact on diarrhoeal diseases, cysticercosis and soil-borne helminthiasis such as hookworm infection, trichuriasis, ascariasis and strongyloidiasis.

8.3.3 Environmental impacts

We did not observe negative or positive environmental impacts and during the course of this study and do not envisage impacts in the future.

8.4 Communication and dissemination activities

The main mechanisms for communicating and disseminating research results from the project to the scientific community and organisations working in the area of health development were through publication in the international scientific literature, presentation at regional and international scientific meetings and conferences and the presentation of results at meetings in Vientiane.

Communication of project results back through the Lao community was not completed within the three years of the project; however we developed a communications package in Lao and English for use by the Ministry of Health and Ministry of Agriculture and Forestry and international development partners. The package was specific for taeniasis and cysticercosis but the messages presented will also likely have positive impacts on other food-borne and hygiene related infectious diseases.

9 Conclusions and recommendations

9.1 Conclusions

The key findings of the project were:

1. Taenia solium taeniasis and cysticercosis are focally distributed and prevalence was high in populations where pig production practices, meat consumption habits and poor sanitation supports transmission. The overall population prevalence for human cysticercosis was relatively low but this should not be misconstrued as cysticercosis not being of public health importance.

2. *Taenia saginata* was the dominant tapeworm infecting people and this was strongly influenced by the very high prevalence of consuming uncooked beef. *Taenia hydatigena* was the dominant *Taeniid* infecting pigs and this may have a role in suppressing *T. solium* infection through immune mediated interactions in the intermediate host.

3. Exposure to *Trichinella*, as detected by the E/S-ELISA, was widespread in the human survey population of northern Laos and the Lao-Tai ethnic group were the most likely to have had antibodies detected. Seropositivity was associated with consumption of fermented pork sausage. *Trichinella spiralis* larvae were recovered from pigs at slaughter and the majority (66.7%) of infected pigs had worm burdens less than 1 larvae per gram. Sporadic outbreaks of trichnellosis will continue to occur in Laos while consumption of uncooked pork is common. The majority of pigs are produced by smallholder farmers and management and husbandry in this farming sector are not sufficient to break the *T. spiralis* lifecycle.

5. Almost all pigs and dogs and three quarters of the human population showed evidence of parasitism, and importantly polyparasitism was common.

6. Besides cysticercosis and trichinellosis, multiple parasitic zoonoses were detected in pigs, including *Balantidium coli*, *Paragonimus*, *Giardia* or *Trichomonas* and *Strongyloides*.

7. The Mon-Khmer ethnic group (which predominantly comprised Khmu people) were a highly vulnerable group, accounting for the most cases of cysticercosis and soil transmitted helminthiasis. These latter infections have been known to negatively influence growth performance, educational outcomes and productivity, all of which strongly influence the entrenchment of poverty.

8. Japanese encephalitis virus is an unmanaged threat to human health; with strong evidence that JEV is hyper-epizootic in the pig population of northern Laos and the peak period of transmission occurs in the wet season. In the human population, serological evidence indicates that the majority of the population have been exposed by the time they reach adulthood.

9. Hepatitis E virus is prevalent in the pig population and genotype 4 is the predominant species infecting pigs. The peak period of transmission in pigs occurs in the wet season and poses the greatest risk of human infection.

10. Other non-zoonotic parasitic infections in young pigs may be constraining production; there was a high prevalence of strongyles, coccidiosis and *Endamoeba*.

11. Pig-associated zoonotic diseases have a significant impact on the economy of northern Laos and deserve policy attention.

9.2 Recommendations

Table 9.1

Research questions: **What we didn't know at the beginning of the projects**	**Conclusions:** **What we know now as result of the project *and* from other sources**	**Implications:** **What does this increase in knowledge mean for next users and final beneficiaries of the project?**	**Recommendations:** **On this basis, what recommendations is the project making for continued research on zoonotic diseases?**
Is the estimate of 10% prevalence of human cysticercosis accurate?	No, the evidence indicates that human cysticercosis is relatively rare in the human population, but there was strong evidence of focal distribution and high prevalence in some villages.	Population level cross-sectional prevalence studies will provide little benefit for disease control initiatives. Investigative methods specifically designed to identify hot-spots of transmission will be required.	Develop and validate survey methods to detect transmission hot-spots, including the use of key informants, overlaying existing data on agricultural production, hospital data, meat consumption habits and sanitation maps, in combination with well-designed epidemiological surveys of humans and pigs using good quality diagnostic testing
Are there specific factors that may increase the risk of zoonotic disease?	Yes, the evidence indicates that spatial, socio-economic and ethno-linguistic factors influence risk of becoming infected with a zoonotic infectious disease.	Knowledge of specific risk factors allows public and veterinary health practitioners to devise and target control programs at those groups most likely to become infected.	Socio-cultural factors have long been known to have a strong controlling influence on transmission of neglected tropical diseases but social research has also been neglected. Future research should fully utilise health sociologists with SE Asian experience to better understand things such as cultural understanding of risk and decision making processes based on those understandings.
Are there other related parasites that may interact with *Taenia solium* in the intermediate host?	Yes, our evidence indicates that *T. hydatigena* from dogs is the dominant *Taeniid* infecting pigs.	This dog tapeworm may act to suppress *T. solium* infection and transmission through immune mediated interactions.	Controlled animal studies to characterise the interreactions between *T. solium* and *T. hydatigena* in pigs.
Is *Trichinella* widespread in the pig population and does this result in human infection?	Yes and no, we observed almost 2% prevalence in pigs with spatial variation and variable worm burdens. This translated to a high seroprevalence in humans, particularly in the Lao-Tai ethnic group.	Our results can not inform on prevalence of disease in humans, rather it indicates exposure only. The results indicate endemic stability in pigs and there will be sporadic outbreaks of human trichinellosis while raw pork consumption is commonly practiced. The high seroprevalence of trichinellosis in Lao-Tai people and low worm burdens in pigs indicates that there could be a high level of	This needs to be supported with improved diagnostic tests that are appropriate for community surveys with improved specificity.

		exposure without widespread clinical disease.	
Is Japanese encephalitis virus enzootic or epizootic in the pig population	The available evidence indicates that the virus is most likely epizootic, i.e. transmission does not occur through-out the entire year and the virus is therefore reintroduced into the pig population.	For animal health, this information has practical implications for herd and breeding management. Boar infertility may be a problem and pregnant sows may abort if susceptible to infection during peak periods of transmission. For human health, there are implications for vaccination program design and management of pigs during the peak period of transmission.	Two recommendations are apparent: Surveys need to be conducted across the entire country (or in selected provinces in southern, central and northern Laos). Abattoirs provide ready access to animals but the age class means most animals will already have been exposed to virus. Surveys targeting piglets across a whole year should be initiated to detect IgM antibody or virus by PCR. Secondly, surveys of mosquitoes and birds will provide information on the source of virus.
Is there a peak period of viral zoonoses transmission?	Yes, our evidence indicates the start of the wet season is the peak period of transmission for JEV and HEV in pigs.	This period of peak transmission in pigs would also likely result in increased human infection and implementation of control measures need to account for this peak.	Survey pigs in southern, central and northern Laos over a complete season to confirm our results and select young animals to detect current or recent infection to identify peak in transmission.
What genotypes of HEV are circulating in the Lao pig population?	In northern Laos, our evidence indicates that genotype 4 is the dominant genotype and no other genotypes were detected.	Genotype 4 viruses have been shown to result in more severe clinical disease in humans compared to the other zoonotic genotype 3 viruses. Genotyping of human HEV infections will provide insight into severity and source of infection.	Studies now need to be conducted in the pig populations of southern and central Laos to determine if genotype 3 is also circulating in the country. Greater collaboration with hospitals and clinics will be required to determine the link between pigs and human HEV disease.
Do pig associated zoonotic parasites have a substantial cost that warrants policy attention?	Yes, the costs associated with *T. solium* and trichinella are disproportionately borne by the agricultural sector (pig producers and traders) due to carcass downgrading or disposal.	There is a substantial cost to pig producers, the nature of human disease indicates that most infected people do not seek medical care and costs associated with human disease may have been underestimated in this model.	Research is required to understand the social impacts such as labour shortages, dependency, stigma and school participation on people infected with cysticercosis and trichinellosis. In addition, the model was limited by gaps in knowledge and these could be addressed in future work.

- A critical limitation in *Taenia* research in pigs is the lack of good quality, sensitive, fully validated and readily available diagnostic tests that are capable of discriminating between the three *Taenia* species that can infect pigs in Asia, *T. solium*, *T. hydatigena* and *T. asiatica*. Without these tests becoming available, the detection of high risk areas will be dependent on slaughter and dissection of pigs which is neither an economically or logistically viable option. Detection of cysticercosis at slaughter is not reliable and results from inspection should not be the only data used to formulate policy. Furthermore, use of inspection data at slaughter is limited by the almost complete lack of traceability back to farm. Investment in developing new diagnostic technologies or validating existing tests in Asia should be a priority.

- Our data indicates that human taeniasis and cysticercosis (*T. solium*) occurs in hotspots and cross-sectional or prevalence surveys may not be the best option to identify where these hotspots exist, and by extension determine where interventions need to be targeted. We strongly recommend that non-traditional epidemiological approaches be undertaken (or developed) to identify vulnerable communities, including the use of key informants, overlaying existing data on agricultural production, hospital data, meat consumption habits and sanitation maps, in combination with well-designed epidemiological surveys of humans and pigs using good quality diagnostic testing.
- Undertake controlled *in vivo* studies to investigate the immune mediated interactions between *T. solium* and *T. hydatigena* in the pig intermediate host. The nature and extent of this interaction will inform decisions on controlling parasites in dogs and by extension the transmission of *T. hydatigena* in pigs, it may also inform on the use of natural *T. hydatigena* infection in dogs and pigs to control or limit the transmission of *T. solium* in pigs.
- Japanese encephalitis virus epidemiology is not fully understood. Our results indicate that JEV is hyper-epizootic but our results do represent a complete year. Further studies to determine the spatial and temporal patterns of JEV transmission in pigs, birds and humans will be required to inform public health officials seeking to control JE by vaccination.

10 References

10.1 References cited in report

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10.2 List of publications produced by project

1. Conlan, J.V., Vongxay, K., Khamlome, B., Dorny, P., Sripa, B., Elliot, A., Blacksell, S.D., Fenwick, S. and Thompson, R.C.A A cross-sectional study of *Taenia solium* in a region where four *Taenia* species are co-endemic reveals competition may be protective. *American Journal of Tropical Medicine and Hygiene* (In press)
2. Conlan, J.V., Vongxay, K., Jarman, R.G., Fenwick, S., Thompson, R.C.A., and Blacksell, S.D. Serologic study on pig associated viral zoonoses in Laos. *American Journal of Tropical Medicine & Hygiene* (In press)
3. Vongxay, K., Conlan, J.V., Khounsy, S., Dorny, P., Fenwick, S., Thompson, R.C.A. and Blacksell, S.D. Sero-prevalence of major bovine-associated zoonotic infectious diseases in the Laos. *Vector-borne and zoonotic diseases* (In press)
4. Conlan, J.V., Khamlome, B., Vongxay, K., Elliot, A., Pallant, L., Sripa, B., Blacksell, S.D., Fenwick, S. and Thompson, R.C.A. (2012) Soil-transmitted helminthiasis in Laos: a community-wide cross-sectional study of humans and dogs in a mass drug administration environment. *American Journal of Tropical Medicine & Hygiene* 86(4):624–634
5. Conlan, J.V., Jarman, R.G., Vongxay, K., Chinnawirotpisan, P., Melendrez, M.C., Fenwick, S., Thompson, R.C.A., and Blacksell, S.D. (2011) Hepatitis E virus is prevalent in the pig population of Lao People's Democratic Republic and evidence exists for homogeneity with Chinese Genotype 4 human isolates. *Infection, genetics and evolution* 11(6):1306-11
6. Conlan, J.V., Sripa, B., Attwood, S.W. and Newton, P.N. (2011) A review of parasitic zoonoses in a changing Southeast Asia. *Veterinary Parasitology* 182(1):22-40
7. Thompson, R.C.A. and Conlan J.V. (2011) Emerging issues and parasite zoonoses in the SE Asian and Australasian region. *Veterinary Parasitology* 181(1):69-73
8. Willingham, A.L., Wu, H-W., Conlan, J. and Satrija, F. (2010) Combating *Taenia solium* Cysticercosis in Southeast Asia: An Opportunity for Improving Human Health and Livestock Production. *Advances in Parasitology* 72: 235-266

9. Conlan, J.V., Vongxay, K., Fenwick, S., Blacksell, S.D. and Thompson, R.C.A. (2009) Does interspecific competition have a moderating effect on *Taenia solium* transmission dynamics in Southeast Asia? *Trends in Parasitology* 25(9): 398-403

10. Conlan, J.V., Khounsy, S., Inthavong, P., Fenwick, S., Blacksell, S.D. and Thompson, R.C.A. (2008) A review of taeniasis and cysticercosis in the Lao People's Democratic Republic. *Parasitology International* 57(3): 252-255

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11. Choudhury, A., Conlan, J.V., Blacksell, S.D., Thompson, R.C.A., Fenwick, S., Vongxay, K., Khamlome, B. and Whittaker, M. (2012) Conducting Socioeconomic Evaluations in Information Constrained Settings: The Impacts of Pig-associated Zoonoses in Lao PDR. *EcoHealth*

10.3 List of planned publications

In addition to the research publications listed above, the project team are currently writing manuscripts intended for publication. The listed publications outlined below are indicative only and represent work outstanding. The target journals for submission are veterinary parasitology and tropical medicine publications.

12. Conlan, J.V., Pozio, E., Vongxay, K., Khamlome, B., Blacksell, S.D., Fenwick, S. and Thompson, R.C.A (2012) Patterns and risks of trichinellosis in pigs and humans in the Lao People's Democratic Republic.
13. Blacksell, S.D., Conlan, J.V., Khamlome, B., Vongxay, K., Fenwick, S., Thompson, R.C.A., and Jarman, R.G. (2012) Japanese encephalitis and dengue virus seroprevalence and risk factor analysis in communities of northern Laos.
14. Blacksell, S.D., Conlan, J.V., Khamlome, B., Vongxay, K., Fenwick, S., Thompson, R.C.A., and Jarman, R.G. (2012) Hepatitis E virus seroprevalence and risk factor analysis in communities of northern Laos.
15. Pallant, L., Conlan, J.V., Khamlome, B., Vongxay, K., Elliot, A., Sripa, B., Blacksell, S.D., Fenwick, S. and Thompson, R.C.A. (2012) Giardiasis in the human and dog populations of northern Laos: implications for zoonotic transmission.
16. Conlan, J.V., Khamlome, B., Vongxay, K., Elliot, A., Pallant, L., Sripa, B., Blacksell, S.D., Fenwick, S. and Thompson, R.C.A. (2013) Patterns of polyparasitism in the human, dog and pig populations of Laos: spatial and socio-economic determinants.
17. Conlan, J.V., Blacksell, S.D., Fenwick, S., Vongxay, K., Khamlome, B. and Thompson, R.C.A. (2013) Semi-quantitative risk assessment for taeniasis and cysticercosis in Laos: implications for the control and management of human cysticercosis.
18. Conlan, J.V., Blacksell, S.D., Fenwick, S., Vongxay, K., Khamlome, B. and Thompson, R.C.A. (2013) The neglected tropical diseases of southeast Asia: a focus on Laos and the role of livestock and companion animals in shaping human health.

Other publications, that have not yet been identified, may emerge from the data gathered during this project and this list is not necessarily exhaustive.

10.4 International workshop and conference proceedings

1. Conlan, J.V., Inthavong, P., Khounsy, S., Blacksell, S.D., Fenwick, S. and Thompson, R.C.A. (2007) Cysticercosis in the Lao People's Democratic Republic : Country Report. *Proceedings of the 7th meeting for the Regional Network on Asian Schistosomiasis and other Helminthic Zoonoses (RNAS+), 5 - 7 September 2007, Lijiang, China.*

2. Conlan, J.V., Khamlome, B., Vongxay, K., Sripa, B., Blacksell, S.D., Fenwick, S. and Thompson, R.C.A. (2009) Heminth infections and risk factor analysis among communities in Northern Lao PDR. *6th Seminar on Food- and Water-borne Parasitic Zoonoses, 2-4 December 2009, Bangkok, Thailand.* (Oral presentation)

3. Thompson, R.C.A., Conlan, J.V., Khamlome, B., Vongxay, K., Blacksell, S.D., Fenwick, S. (2009) Taenia ecology and competition in people, pigs and dogs in Southeast Asia. *22rd International Conference of the World Association for the Advancement of Veterinary Parasitology, 9-13 August 2009*, Calgary, Canada. (Oral presentation)

4. Conlan, J.V., Inthavong, P., Khounsy, S., Elliot, A., Pallant, L.J., Blacksell, S.D., Fenwick, S. and Thompson, R.C.A. (2010) Dogs shaping the ecology of parasitic zoonoses in Southeast Asia. *XIIth International Congress of Parasitology (ICOPA 2010), 15-20 August 2010, Melbourne, Australia (Oral presentation)*

5. Conlan, J.V., Inthavong, P., Khounsy, S., Elliot, A., Pallant, L.J., Blacksell, S.D., Fenwick, S. and Thompson, R.C.A. (2010) A study on human taeniasis and cysticercosis in the Lao PDR: a risk factor analysis and discussion of interspecific tapeworm competition. *XIIth International Congress of Parasitology (ICOPA 2010), 15-20 August 2010, Melbourne, Australia* (Poster presentation)

6. Conlan, J.V., Inthavong, P., Khounsy, S., Elliot, A., Pallant, L.J., Blacksell, S.D., Fenwick, S. and Thompson, R.C.A. (2010) Dogs and pigs as potential reservoirs of zoonotic parasites in the Lao PDR. *XIIth International Congress of Parasitology (ICOPA 2010), 15-20 August 2010, Melbourne, Australia* (Poster presentation)

7. Thompson, R.C.A., Conlan, J.V., Khamlome, B., Vongxay, K., Blacksell, S.D., Fenwick, S. (2010) Zoonotic infections of pigs and dogs in Southeast Asia – update and future threats. *Joint International Tropical Medicine Meeting 2010 (JITMM2010) 1-3 December 2010, Bangkok, Thailand.* (Oral presentation)

8. Conlan, J.V., Khamlome, B., Vongxay, K., Blacksell, S.D., Fenwick, S. and Thompson, R.C.A. (2010) Sympatric co-existence of Taenia spp. in pigs – implications for human transmission? *Joint International Tropical Medicine Meeting 2010 (JITMM2010) 1-3 December 2010, Bangkok, Thailand.* (Oral presentation)

9. Blacksell, S.D., Conlan, J.V., Khamlome, B., Vongxay, K., Fenwick, S. and Thompson, R.C.A. (2010) Hepatitis E virus infection in the larger Mekong region. *Joint International Tropical Medicine Meeting 2010 (JITMM2010) 1-3 December 2010, Bangkok, Thailand.* (Oral presentation)

10. Conlan, J.V., Khamlome, B., Vongxay, K., Elliot, A., Pallant, L.J., Blacksell, S.D., Fenwick, S. and Thompson, R.C.A. (2011) Zoonotic hookworm transmission in a highly endemic region of Southeast Asia. *23rd International Conference of the World Association for the Advancement of Veterinary Parasitology, 21-25 August 2011, Buenos Aires, Argentina.* (Oral presentation)

11 Appendixes

11.1 Appendix 1: Information Education and Communication component

The communications package is accessible in a separate file.