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A Cross-Sectional Study of *Taenia solium* in a Multiple Taeniid-Endemic Region Reveals Competition May be Protective

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Abstract. We conducted cross-sectional surveys for taeniasis and cysticercosis in humans, pigs, and dogs in four northern provinces of Laos. Human cysticercosis and taeniasis prevalence was 2.2% (95% confidence interval [CI] = 1.4–3.0%) and 8.4% (95% CI = 6.9–9.9%), respectively. Eating uncooked beef, being male, province of residence, age, and ethnicity were significant risk factors for taeniasis and only province of residence was a significant risk factor for cystiercosis. Thirty-five human tapeworms were recovered during the survey and 33 (94.3%) and 2 (5.7%) were identified as *Taenia saginata* and *T. solium*, respectively. Maximum-likelihood adjusted prevalence of *T. solium* and *T. hydatigena* in pigs was 4.2% (95% CI = 0.5–7.9%) and 55.9% (95% CI = 47.5–64.3%), respectively, and *T. hydatigena* taeniasis in dogs was 4.8% (95% CI = 0.0–11.3%). *Taenia hydatigena* and *T. saginata* were the most prevalent taeniids in the respective pig and human populations and together may suppress *T. solium* transmission.

INTRODUCTION

Taenia solium is a zoonotic tapeworm that has a life cycle involving humans as the definitive adult-stage host (taeniasis) and pigs as the intermediate larval-stage host (cysticercosis). In humans, who can also be inadvertently infected with larval-stage cysticerci after ingesting eggs, the most severe clinical manifestation of infection is neurocysticercosis when cysticerci establish in the central nervous system, causing serious neurological sequelae such as epilepsy and in severe cases, death. In Southeast Asia, the epidemiology of *T. solium* is complicated by the co-endemicity of other *Taenia* species, where three species cause taeniasis in humans (*T. solium*, *T. saginata*, and *T. asiatica*) and three species cause cysticercosis in pigs (*T. solium*, *T. asiatica*, and *T. hydatigena*).[1–5]

Taenia solium infection disproportionately affects the poorest communities worldwide where conditions are suitable for the completion of the tapeworm life cycle, including free-roaming pig production, inadequate sanitation, poor hygiene, and low levels of education. Such conditions exist in many rural communities in Laos. However, to date no studies have been undertaken to investigate *T. solium* in a multi-species context. In Laos, evidence of human cysticercosis is limited to a small study that found a seroprevalence of antibody against *T. solium* cysticercosis of 4.8%[6] and an ill-defined case of neurocysticercosis in northern Laos.[7] The only data from pigs is based on carcass inspection and indicated a prevalence of 1–2%.[8]

Human taeniasis is also poorly understood. Many studies have reported taeniasis prevalence without determining or reporting the species causing infection,[6,9–17] and prevalence estimates range from 0% to 14%, with a high degree of spatial variation.[8] Only *T. saginata* has been reported in southern Laos[18,19] and *T. solium* and *T. saginata* have been reported in northern Laos.[7]

The principal objective of the present study was to investigate *Taenia* spp. infection in humans, pigs, and dogs in four provinces in northern Laos by 1) conducting studies to estimate the prevalence and risk of taeniasis and cysticercosis in humans, 2) identifying the *Taenia* species causing taeniasis in humans, 3) estimating the prevalence of cysticercosis in pigs and of *T. hydatigena* taeniasis in dogs, and 4) combining results of different studies to draw conclusions on the ecologic factors controlling human and pig infections.

MATERIALS AND METHODS

Ethics statement. Informed consent was obtained from all human adult participants and from the parents or legal guardians of minors (children < 15 years of age). The study protocol was reviewed and approved by the Murdoch University Human Ethics Committee (Project no. 2008/266) and the Lao Ministry of Health National Ethics Committee for Health Research (no. 239/NECHR) before commencing this study.

For the studies involving dogs and pigs, the protocols were reviewed and approved by the Murdoch University Animal Ethics Committee (Project no. R2108/07), which adheres to the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes. The Lao Department of Livestock and Fisheries does not, at this time, have a committee to review and approve scientific research protocols involving animals.

Human survey. *Study Site.* Laos is an ethnically diverse country that has 49 distinct ethnic groups classified into four ethno-linguistic families, Lao-Tai, Mon-Khmer, Hmong-Mien, and Sino-Tibetan, making up 67%, 24%, 8%, and 1% of the population, respectively.[20] The study was conducted in four provinces in northern Laos: Oudomxay, Luangprabang, Huaphan, and Xiengkhuang (Figure 1), where all four ethno-linguistic families are represented. Provinces were selected in consultation with the Lao government, and the guiding principles of selection were accessibility from Vientiane and priority areas for poverty alleviation, rural development, and improving pig production.

Survey design. In each province, one district was randomly selected for inclusion in this study (Figure 1). The survey was

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FIGURE 1. Study sites in northern Laos. 1, Xay District, Oudomxay Province; 2, Xiengngeun District, Luangprabang Province; 3, Pek District, Xiengkhuang Province; 4, Viengxay District, Huaphan Province.

conducted in six randomly selected villages in the dry season during January–March 2009 to maximize study participation and minimize negative impacts on seasonal labor demands. The number of villages selected was constrained by the human resources available at the three levels of government administration: national, provincial, and district. Villages were selected from official listings provided by provincial government offices, and villages were excluded if a four-wheel drive vehicle could not access them.

For the sample size calculation, we conservatively estimated that 4% of households would have at least one cysticercosis or taeniasis case on the basis of prevalence data for northern Vietnam[21] and prevalence estimates for Laos.[22] At a precision of 10% and 95% confidence level (Equation 1) and correcting for a finite population of 150 households per village on the basis of averaged data supplied by district agriculture and forestry offices (Equation 2), 14 households were randomly selected from each village by using a random number table. The two equations used were

$$N_0 = \frac{Z^2 \times P(1P)}{a^2} \quad (1)$$

$$N_1 = \frac{nN_0}{n + (N_0 - 1)} \quad (2)$$

where N_0 was the sample size required for simple random sampling, Z was the z-score for the required confidence level (1.96), P was the estimated proportion of affected households, and α was the required precision expressed as a proportion (0.1). N_1 was the corrected sample size and n was the finite population size.

All household members ≥ 6 years of age were asked to participate. Village chiefs were given advance notice of the survey timing and meetings were conducted in villages the day before sampling to select households. In cases where a household refused to participate, the village chief selected a household with similar characteristics. A household questionnaire was administered to the head of each household with his or her family present to assess the house characteristics, assets owned, ownership of animals, ethnicity, education levels and literacy of the male and female heads of household, the person with greatest responsibility for preparing food, and person with the greatest responsibility for primary care. An individual questionnaire was administered to all study participants, with younger participants (< 15 years of age) interviewed in the presence of a parent or guardian who may have provided assistance in answering questions.

Data on frequency of raw meat consumption, latrine use, tapeworm segments seen in feces, and a history of taeniasis were collected. For those who consumed raw meat, we asked them to estimate the frequency of raw meat consumption: weekly, monthly, every few months, and infrequent (once or twice per year or less often). Questionnaires were administered in the Lao language and were pre-tested with persons who did not otherwise participate in the survey. In the circumstances where a person could not understand the Lao language, a household member, relative, or village chief provided verbal translation of questions to study participants with their consent.

A venous blood sample of 2–3 mL was collected and the serum fraction was stored at –20°C. Labeled plastic bags for a single fecal sample were handed out. Fecal samples were collected from the participants the next day and stored in two preservation solutions, 10% formalin and 80% ethanol, for microscopy and molecular analysis, respectively.

Persons who were *Taenia* egg positive or self-reported seeing fecal segments were treated with niclosamide and a purgative (bisacodyl) according to manufacturer's instructions (Vechaphant Baesaj, Visonic, Thailand) during November–December 2009. All participants were provided with detailed information on the risks associated with *T. solium* taeniasis and the need to safely dispose of all stools and adhere to strict hand hygiene measures. Buckets and soap were provided to all participants.

Adults were treated with 2 g of niclosamide and 15 mg of bisacodyl two hours post-treatment, children > 34 kg were treated with 1.5 g of niclosamide and 10 mg of bisacodyl, and children 11–34 kg were treated with 1 g of niclosamide and 5 mg of bisacodyl. All fecal samples were examined for scoleces and proglottids for two days after treatment. Expelled worm segments were preserved in 80% ethanol and transported back to Vientiane at ambient temperature where they were stored at 4°C until testing.

Animal surveys. Opportunistic pig surveys were conducted at three slaughter-points in Xiengkhuang and Oudomxay Provinces from May–September 2008 and at two collection points in Huaphan and Luangprabang Provinces from October 2008–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. Pork traders prevented muscle slicing; as such, the heart, liver, mesentery, omentum, and other viscera were examined for *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 with the blood sample and any cysts found.

Dog fecal samples were collected in the same villages as the human survey described above during January–March 2009 using a semi-structured approach. Dogs were selected if they belonged to the same household as those randomly selected for the human survey. If no dogs were present in a household, then dogs were opportunistically identified in the village. The permission of owner's was granted before sampling was undertaken. Fecal samples were collected by using manual digital extraction and preserved in two preservation solutions, 10% formalin and 80% ethanol. Demographic data and the age and sex were recorded with the sample. The samples were sent to Vientiane at ambient temperature and subsequently stored at 4°C before processing.

Laboratory analysis. Formalin-preserved human feces was transported at room temperature to Khon Kaen University (Khon Kaen, Thailand) where samples were analyzed by the formalin-ether-concentration technique and microscopy. Formalin-preserved dog feces was transported to Murdoch University (Perth, Western Australia, Australia) at ambient temperature and examined for taeniid eggs by the saturated sodium nitrate flotation technique and microscopy.

Preserved human and pig serum samples were tested by an enzyme linked immunosorbent assay (ELISA) for *Taenia* metacestode circulating antigens[23–25] using modifications introduced by Dorny and others.[26] The optical density (OD) of the human samples were read at 490 nm with a reference at 650 nm, and the OD of the pig samples were read at 490 nm. The cut-off value was calculated as described by Dorny and others[24] by using a panel of eight negative serum samples from human and pig populations in Laos. A ratio for each test was calculated by dividing the OD of the test sample by the cut-off value, and a ratio > 1 was considered positive. Samples were retested if the coefficient of variation was > 50% or if the OD of the test sample was close to the cut-off value.

DNA was isolated from proglottids expelled post-niclosamide treatment by using the DNeasy Blood and Tissue Extraction Kit (QIAGEN, Hilden, Germany). A multiplex polymerase chain reaction (PCR) for cytochrome c oxidase subunit 1 gene (*cox1*) was performed for species identification of *T. saginata*, *T. solium*, and *T. asiatica*. Primers and PCR protocols were as described[27] with modifications. A PCR cocktail contained 0.4 μM of each primer (Sigma-Aldrich, St. Louis, MO, 1.25 units of GoTaq DNA polymerase in GoTaq reaction buffer supplemented with 2 mM $MgCl_2$ (Promega, Madison, WI), and 0.2 mM of each dNTP (Promega) in a final 50-μL reaction volume. The amplification protocol consisted of 3 minutes at 94°C; followed by of 35 cycles of 30 seconds at 94°C, 30 seconds at 56°C, and 90 seconds at 72°C; plus one cycle of 5 minutes at 72°C. Subsequently, PCR-amplified products were subjected to electrophoresis on 1.5% agarose gels with a DNA ladder (Hyperladder II; Bioline, London, United Kingdom). Positive control DNA for the three *Taenia* species were extracted from proglottids (kindly supplied by the Institute of Tropical Medicine, Antwerp, Belgium).

Data analysis. The questionnaire and laboratory test data were entered into a spreadsheet (Excel; Microsoft, Redmond, WA) and subsequent analysis was carried out in STATA/IC version 10 (Stata Corp LP, College Station, TX). The socioeconomic status of each household was calculated by use of principal component analysis of household assets[28,29] after replacement of missing values with the mean of the respective asset for that ethnic group. All assets were dichotomous. The households were ranked into wealth quintiles according to their cumulative standardized asset scores.

Prevalence of cysticercosis seropositivity in the human and pig populations were calculated as the proportion of positive antigen ELISA results in the sampled population. Taeniasis prevalence in humans and dogs was calculated as the proportion of fecal samples with taeniid eggs in the sampled population. In addition, human taeniasis prevalence was calculated for those persons who had eggs detected and/or self-reported tapeworm segments in their feces. In the pig study, the Pearson's chi-square test and Fisher's exact test were used to explore associations between infection status (carcass inspection and antigen ELISA seropositivity) and age, breed, sex, and production system at last point of sale.

For risk factor analysis in the human study, taeniasis was defined as taeniid egg–positive and/or self-reporting segments. Univariate logistic regression without adjustment was used to test associations between infection status (cysticercosis or taeniasis) and sex, location, ethno-linguistic family, age, wealth status, defecation site, taeniasis, history of taeniasis, uncooked meat consumption habits, and literacy of selected household members. Risk factors significant or borderline significant (cut-off $P \leq 0.10$) in the univariate analyses were included in a multivariate random effects logistic regression model adjusting for the effect of household clustering. The results are reported as adjusted odds ratios and 95% confidence intervals (CIs). The final analysis only considered persons with complete parasitologic and serologic data. Missing data on literacy of household members because of death, divorce, or other factors were replaced with the mean for that village and rounded to 0 or 1 (illiterate or literate).

A maximum-likelihood estimator (MLE) (Equation 3) with 95% CI (Equation 4)[30] was used to calculate adjusted prevalence for dog *T. hydatigena* taeniasis and pig cysticercosis detected by inspection at slaughter, for *T. solium*, *T. hydatigena* and *T. asiatica* according to the equations

$$\text{MLE} = \frac{p(1-\text{sp})}{\text{se}+\text{sp}-1} \tag{3}$$

$$95\%\text{CI} = \frac{p+\text{sp}-1-1.96\sqrt{\{p(1p)/N\}}}{\text{se}+\text{sp}-1}, \quad \frac{p+\text{sp}-1+1.96\sqrt{\{p(1-p)/N\}}}{\text{se}+\text{sp}-1} \tag{4}$$

where p was the observed prevalence, sp was the test specificity, se was the test sensitivity, and N was the sample size. Calculations were made through 10% increments of test sensitivity assuming specificity was 100%. For dog *T. hydatigena* taeniasis, 100% specificity was assumed because *Echinococcus* spp. are not endemic to Southeast Asia. Cystic echinococcosis has been rarely reported from mainland Southeast Asia and nothing is known of its epidemiology.[31] For pig cysticercosis, carcass inspection specificity has been estimated to be at or very close to 100%.[26]

RESULTS

Human study. A total of 1,582 persons in 332 households were eligible to participate in this survey. Of these

TABLE 1
Demographic differences between survey participants compliant (C) and non-compliant (NC), stratified by ethnicity, Laos*

Characteristic	Lao-Tai			Mon-Khmer			Hmong-Mien		
	C, no. (%)	NC, no. (%)	*P*	C, no. (%)	NC, no. (%)	*P*	C, no. (%)	NC, no. (%)	*P*
Sex									
F	277 (89.1)	34 (10.9)	0.168†	270 (91.5)	25 (8.5)	0.826†	109 (67.3)	53 (32.7)	0.267†
M	276 (92.3)	23 (7.7)		253 (91.0)	25 (9.0%)		121 (72.9)	45 (27.1)	
Province									
Oudomxay	58 (96.7)	2 (3.3)	0.029‡	297 (90.8)	30 (9.2)	0.779†	28 (96.6)	1 (3.4)	< 0.001‡
Luangprabang	91 (84.3)	17 (15.7)		226 (91.5)	21 (8.5)		31 (96.9)	1 (3.1)	
Huaphan	271 (92.5)	22 (7.5)		0	0		9 (17.6)	42 (82.4)	
Xiengkhuang	133 (89.3)	16 (10.7)		0	0		162 (75.0)	54 (25.0)	
Household wealth status									
Most poor	14 (93.3)	1 (6.7)	0.381†	162 (93.1)	12 (6.9)	0.635‡	49 (51.6)	46 (48.4)	< 0.001‡
Very poor	59 (89.4)	7 (10.6)		152 (88.4)	20 (11.6)		35 (67.3)	17 (32.7)	
Poor	176 (91.2)	17 (8.8)		61 (91.0)	6 (9.0)		46 (78.0)	13 (22.0)	
Less poor	178 (93.2)	13 (6.8)		54 (93.1)	4 (6.9)		56 (73.7)	20 (26.3)	
Least poor	126 (86.9)	19 (13.1)		94 (91.3)	9 (8.7)		44 (95.7)	2 (4.3)	
Age (years)									
6–10	69 (85.2)	12 (14.8)	0.035‡	99 (90.8)	10 (9.2)	0.001‡	49 (65.3)	26 (34.7)	0.203‡
11–14	75 (90.4)	8 (9.6)		70 (81.4)	16 (18.6)		38 (67.9)	18 (32.1)	
15–24	105 (86.8)	16 (13.2)		102 (88.7)	13 (11.3)		51 (68.0)	24 (32.0)	
25–39	135 (93.8)	9 (6.2)		109 (97.3)	3 (2.7)		38 (67.9)	18 (32.1)	
40–54	93 (96.9)	3 (3.1)		98 (97.0)	3 (3.0)		34 (77.3)	10 (22.7)	
≥ 55	76 (89.4)	9 (10.6)		45 (90.0)	5 (10.0)		20 (90.9)	2 (9.1)	

* C = compliant; NC = non-compliant. Missing data for non-compliant: Lao-Tai =14; Mon-Khmer = 16; Hmong-Mien = 41.
† For chi-square test for difference between C and NC.
‡ For Fisher's exact test for difference between C and NC.

persons, 1,306 (82.7%) individuals from 321 households aged 6–91 years provided blood and fecal 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; only 62.5% of eligible persons provided fecal and blood samples and a completed questionnaire. Non-compliance caused by mental illness was negligible; two persons, one each from the Lao-Tai and Hmong-Mien ethnic groups, were protected by their respective

TABLE 2
Survey population characteristics stratified by ethnicity, Laos*

Characteristic	Total		Lao-Tai		Mon-Khmer		Hmong-Mien			
	No.	%	No.	%	No.	%	No.	%	$\chi^{2\dagger}$	*P*
Sex										
F	656	50.2	277	50.1	270	51.6	109	47.4	1.2	0.562
M	650	49.8	276	49.9	253	48.4	121	52.6		
Province										
Oudomxay	383	29.3	58	10.5	297	56.8	28	12.2	1,000.0	< 0.001
Luangprabang	348	26.7	91	16.5	226	43.1	31	13.5		
Huaphan	280	21.4	271	49.0	0	0.0	9	3.9		
Xiengkhuang	295	22.6	133	24.1	0	0.0	162	70.4		
Wealth status										
Most poor	225	17.2	14	2.5	162	31.0	49	21.3	292.1	< 0.001
Very poor	246	18.8	59	10.7	152	29.1	35	15.2		
Poor	283	21.7	176	31.8	61	11.7	46	20.0		
Less poor	288	22.1	178	32.2	54	10.3	56	24.4		
Least poor	264	20.2	126	22.8	94	18.0	44	19.1		
Age (years)										
6–10	217	16.6	69	12.5	99	18.9	49	21.3	26.7	0.003
11–14	183	14.0	75	13.6	70	13.4	38	16.5		
15–24	258	19.8	105	19.0	102	19.5	51	22.2		
25–39	282	21.6	135	24.4	109	20.8	38	16.5		
40–54	225	17.2	93	16.8	98	18.7	34	14.8		
≥ 55	141	10.8	76	13.7	45	8.6	20	8.7		
Defecation site										
Latrine	861	65.9	475	85.9	275	52.6	111	48.3	171.6	< 0.001
Open	445	34.1	78	14.1	248	47.4	119	51.7		
Male head of HH										
Illiterate	228	17.5	25	4.5	164	31.4	39	17.0	134.4	< 0.001
Literate	1,078	82.5	528	95.5	359	68.6	191	83.0		
Female head of HH										
Illiterate	504	38.6	83	15.0	296	56.6	125	54.4	225.4	< 0.001
Literate	802	61.4	470	85.0	227	43.4	105	45.6		

* HH = household.
† Calculated across all groups and ethnicities.

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 persons were recruited into this study. Differences between the compliant and non-compliant persons who were eligible to participate in the study stratified by ethnic family are shown in Table 1.

Survey population structures stratified by ethnicity are shown in Table 2. Significant differences were observed for all characteristics with the exception of sex. Most (49.0%) Lao-Tai persons were from Huaphan Province, no Mon-Khmer persons were selected in Huaphan and Xiengkhuang Provinces, and most (70.4%) Hmong-Mien persons were from Xiengkhuang Province. The highest proportion of impoverished participants were 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 persons living in a household with an illiterate female head of household (Table 1).

The prevalence of cysticercosis and taeniasis stratified by ethnicity for population and individual variables are shown in Table 3. The prevalence of cysticercosis antigen ELISA positivity was 2.2% (95% CI = 1.4–3.0%), ranging at the village level from 0.0% to 11.3%; 14 villages had no detectable cysticercosis cases and 10 villages had at least one seropositive case. Greater than half (15 of 29) of the cases were detected in three villages in Oudomxay Province. Univariate analysis showed that only province was significantly associated with cysticercosis antigen ELISA positivity. After controlling for clustering at the household level by random effects logistic regression, only Luangprabang Province (adjusted odds ratio = 0.26, 95% CI = 0.07–0.98) was significantly associated with reduced risk of cysticercosis antigen ELISA 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-reported, was 8.4% (95% CI = 6.9–9.9%), ranging at the village level from 0.0% to 6.9% and 0.0% to 17.0%, respectively. The proportion of persons reporting a history of taeniasis was 27.0% (95% CI = 24.5–29.4%). For persons with current taeniasis, 90.0% (95% CI = 84.3–95.7%) reported having a history of taeniasis compared with 21.2% (95% CI = 18.8–23.5%) for uninfected persons. Only egg positive and/or self-reporting cases were considered in the risk factor analysis. Univariate analysis showed that a history of taeniasis was strongly associated with increased risk of having a current taeniasis infection. Other factors significantly associated with taeniasis were sex, 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 4). History of taeniasis was strongly correlated with age ($\chi^2 = 121.9$, $P < 0.001$), and inclusion in the model gave the perception that age was protective (Table 4). The exclusion of history of taeniasis from the analysis resulted in sex, province of origin, age, ethnicity, and infrequent consumption of uncooked beef being significantly associated with a current taeniasis infection (Table 4). The consumption of uncooked pork and uncooked fermented pork were not significantly associated with taeniasis after controlling for other risk factors.

TABLE 3

Prevalence of cysticercosis (antigen-capture ELISA) and taeniasis (egg detection plus self-reported) by population level and individual level characteristics, stratified by ethnicity, Laos*

Characteristic	Proportion cysticercosis antigen ELISA positive (95% CI)				Proportion egg positive or self-reported taeniasis (95% CI)			
	Total	LT	MK	HM	Total	LT	MK	HM
Total	2.2 (1.4–3.0)	1.6 (0.6–2.7)	3.3 (1.7–4.8)	1.3 (0.0–2.8)	8.4 (6.9–9.9)	7.8 (5.5–10.0)	11.7 (8.9–14.4)	2.6 (0.5–4.7)
Sex								
F	2.1 (1.0–3.2)	1.1 (0.0–2.3)	3.7 (1.4–6.0)	0.9 (0.0–2.7)	4.7 (3.1– 6.4)	3.9 (1.7–6.3)	7.0 (4.0–10.1)	0.9 (0.0–2.7)
M	2.3 (1.2–3.5)	2.2 (0.4–3.9)	2.7 (0.7–4.8)	1.7 (0.0–4.0)	12.2 (9.6–14.7)	11.6 (7.8–15.4)	16.6 (12.0–21.2)	4.1 (0.5–7.7)
Province								
Oudomxay	3.9 (2.0–5.9)	0.0	5.1 (2.5–7.6)	0.0	13.1 (9.7–16.4)	12.1 (3.4–20.7)	13.8 (9.9–17.8	7.1 (0.0–17.3)
Luangprabang	1.1 (0.0–2.3)	2.2 (0.0–5.3)	0.9 (0.0–2.1)	0.0	9.2 (6.1–12.2)	9.9 (3.6–16.1)	8.8 (5.1–12.6)	9.7 (0.0–20.7)
Huaphan	1.1 (0.0–2.3)	1.1 (0.0–2.4)	–	0.0	5.4 (2.7–8.0)	5.5 (2.8–8.3)	–	0.0
Xiengkhuang	2.4 (0.6–4.1)	3.0 (0.0–5.9)	–	1.9 (0.0–4.0)	4.4 (2.1–6.8)	9.0 (4.1–14.0)	–	0.6 (0.0–1.8)
Wealth status								
Most poor	3.1 (0.8–5.4)	0.0	3.7 (0.8–6.6)	2.0 (0.0–6.1)	10.2 (6.2–14.2)	0.0	13.6 (8.2–18.9)	2.0 (0.0–6.1)
Very poor	4.1 (1.6–6.6)	0.0	5.9 (2.1–9.7)	2.9 (0.0–8.7)	11.0 (7.0–14.9)	11.9 (3.4–20.4)	13.2 (7.7–18.6)	0.0
Poor	1.1 (0.0–2.3)	1.1 (0.0–2.7)	0.0	2.2 (0.0–6.6)	6.7 (3.7–9.6)	7.4 (3.5–11.3)	9.8 (2.1–17.5)	0.0
Less poor	2.1 (0.4–3.7)	2.8 (0.4–5.3)	1.9 (0.0–5.6)	0.0	7.3 (4.3–10.3)	8.4 (4.3–12.5)	7.4 (0.2–14.6)	3.6 (0.0–8.6)
Least poor	1.1 (0.0–2.4)	1.6 (0.0–3.8)	1.1 (0.0–3.2)	0.0	7.6 (4.4–10.8)	6.3 (2.0–10.7)	9.6 (3.5–15.6)	6.8 (0.0–14.6)
Age (years)								
6–10	3.2 (0.9–5.6)	4.3 (0.0–9.3)	4.0 (0.1–8.0)	0.0	7.3 (3.9–10.9)	2.9 (0.0–7.0)	14.1 (7.2–21.1)	0.0
11–14	1.1 (0.0–2.6)	0.0	1.4 (0.0–4.3)	2.6 (0.0–8.0)	2.2 (0.0–4.3)	2.7 (0.0–6.4)	2.9 (0.0–6.9)	0.0
15–24	1.2 (0.0–2.5)	1.0 (0.0–2.8)	2.0 (0.0–4.7)	0.0	4.3 (1.8–6.7)	2.9 (0.0–6.1)	6.9 (1.9–11.9)	2.0 (0.0–5.9)
25–39	1.8 (0.2–3.3)	0.7 (0.0–2.2)	2.8 (0.0–5.9)	2.6 (0.0–8.0)	13.5 (9.5–17.5)	13.3 (7.5–19.1)	14.7 (7.9–21.4)	10.5 (0.3–20.7)
40–54	3.1 (0.8–5.4)	1.1 (0.0–3.2)	5.1 (0.7–9.5)	2.9 (0.0–8.9)	11.6 (7.3–15.8)	12.9 (6.0–19.8)	13.3 (6.4–20.1)	2.9 (0.0–8.9)
≥ 55	3.5 (0.5–6.6)	3.9 (0.0–8.4)	4.4 (0.0–10.7)	0.0	10.6 (5.5–15.8)	7.9 (1.7–14.1)	20.0 (7.8–32.2)	0.0
Defecation site								
Latrine	2.2 (1.2–3.2)	1.9 (0.7–3.1)	3.2 (1.2–5.4)	0.9 (0.0–2.7)	8.8 (6.9–10.7)	7.6 (5.2–10.0)	13.5 (9.4–17.5)	2.7 (0.0–5.8)
Open	2.3 (0.9–3.6)	0.0	3.2 (1.0–5.4)	1.7 (0.0–4.0)	7.6 (5.2–10.1)	9.0 (2.5–15.5)	9.7 (6.0–13.4)	2.5 (0.0–5.4)
Male head of HH								
Illiterate	1.3 (0.0–2.8)	0.0	1.8 (0.0–3.9)	0.0	9.6 (5.8–13.5)	4.0 (0.0–12.3)	11.6 (6.6–16.5)	5.1 (0.0–12.4)
Literate	2.4 (1.5–3.3)	1.7 (0.6–2.8)	3.9 (1.9–5.9)	1.6 (0.0–3.3)	8.2 (6.5–9.8)	8.0 (5.6–10.3)	11.7 (8.4–15.0)	2.1 (0.0–4.1)
Female head of HH								
Illiterate	2.8 (1.3–4.2)	0.0	3.7 (1.5–5.9)	2.4 (0.0–5.1)	8.7 (6.3–11.2)	7.2 (1.5–12.9)	11.8 (8.1–15.5)	2.4 (0.0–5.1)
Literate	1.9 (0.9–2.8)	1.9 (0.7–3.2)	2.6 (0.5–4.7)	0.0	8.2 (6.3–10.1)	7.9 (5.4–10.3)	11.4 (7.3–15.6)	2.9 (0.0–6.1)

* ELISA = enzyme-linked immunosorbent assay; CI = confidence interval; LT = Lao-Tai ethnicity; MK = Mon-Khmer ethnicity; HM = Hmong-Mien; HH = household.

TABLE 4

Risk factors significantly ($P < 0.050$) associated with taeniasis, as determined by multiple logistic regression modeling controlling for household clustering, Laos*

Model†	Population characteristic	Risk factor	Adjusted OR	95% CI
1	Province	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
	Age (years)	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
	Ethnicity	Lao-Tai ethnicity	Ref.	
		Mon-Khmer ethnicity	0.90	0.45–1.83
		Hmong ethnicity	0.34	0.12–0.96
	Previous taeniasis	No previous taeniasis	Ref.	
		History of taeniasis	32.98	15.63–69.56
	Raw beef consumption	Doesn't eat	Ref.	
		Weekly	0.81	0.27–2.37
		Monthly	1.48	0.71–3.09
		Every few months	1.07	0.49–2.35
		Infrequent	4.13	1.50–11.36
2	Sex	Female	Ref.	
		Male	2.20	1.34–3.63
	Province	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
	Age (years)	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
	Ethnicity	Lao-Tai ethnicity	Ref.	
		Mon-Khmer ethnicity	0.86	0.45–1.65
		Hmong ethnicity	0.26	0.10–0.70
	Raw beef consumption	Doesn't eat	Ref.	
		Weekly	1.68	0.61–4.65
		Monthly	2.43	1.27–4.65
		Every few months	1.88	0.91–3.87
		Infrequent	5.99	2.51–14.25

* OR = odds ratio; CI = confidence interval; Ref. = referent; Infrequent = consumes once or twice per year or less often.
† Model 1 = taeniasis OR adjusted for sex, 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 sex, province, age, ethnicity, and raw fermented pork sausage consumption, raw pork consumption, and raw beef consumption.

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 ($\chi^2 = 145.8$, $P < 0.001$), and similar results were observed for eating uncooked beef ($\chi^2 = 214.2$, $P < 0.001$), uncooked pork ($\chi^2 = 48.7$, $P < 0.001$), and fermented pork sausage ($\chi^2 = 41.3$, $P < 0.001$) (Figure 2). 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 (Figure 2).

Of the 110-taeniasis positive persons who were treated with niclosamide, proglottids were expelled from 35 persons and PCR showed that 33 tapeworms were *T. saginata* and 2 were *T. solium*. The *T. solium* worms were recovered from a seven-year-old boy from Oudomxay Province who was antigen ELISA negative and from a 34-year-old man from Xiengkhuang Province who was antigen ELISA positive. Both persons 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 years) from all provinces. Thirty-two of the *T. saginata* cases were antigen ELISA negative, and 32 reported eating uncooked beef. Both *T. solium* cases were egg positive by formol ethyl acetate concentration and one was self-reported. Sixteen (48.5%) of the 33 *T. saginata* cases were egg positive by formol ethyl acetate concentration and 29 (87.9%) of 33 were self-reported.

Pig study. Inspection data results from Oudomxay Province were not used in the analysis because of 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. 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 5). 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

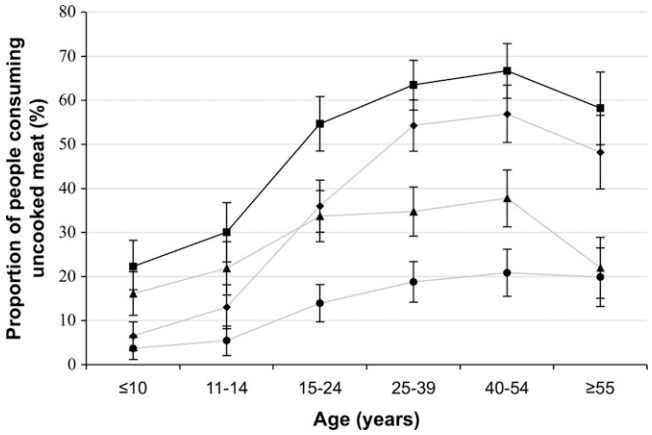

FIGURE 2. Proportion of study population consuming uncooked meat by age category. Solid black line indicates any uncooked meat; gray line with circles indicates uncooked pork; gray line with triangles indicates uncooked fermented pork sausage; gray line with diamonds indicates uncooked beef.

degenerated cysts and age was significantly associated with *T. solium* detection (Table 5). One hundred 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 5). Two pigs, one each from Luangprabang and Huaphan Provinces, had a dual infection with *T. solium* and *T. hydatigena*. One pig in Huaphan Province was detected with cysts consistent with *T. asiatica*; small fluid-filled cysts present in the liver, spleen, and lung. This pig was a 15-month-old female obtained from a penned production system. Non-specific liver lesions consistent with parasitemia were detected in 16 (2.7%) pigs; these pigs were considered inspection negative in the absence of histopathologic test results to definitively identify *Taenia*.

Serum samples from 404 (68.5%) pigs were reactive in the antigen ELISA, and no significant association was observed for province, breed, age, sex, and production system (Table 5). All five *T. solium* and one *T. asiatica* inspection-positive pigs had serum samples reactive in the antigen ELISA. Of 132 *T. hydatigena* inspection-positive pigs, 129 were reactive and three were non-reactive. Fourteen of the pigs with non-specific liver lesions were serum reactive in the antigen ELISA and two were non-reactive.

Estimates of true prevalence of *T. solium*, *T. hydatigena*, *T. asiatica* cysticercosis in pigs and *T. hydatigen*a taeniasis in dogs using the maximum-likelihood estimator for carcass inspection with a sensitivity ranging from 10 to 100% and assuming specificity was 100% are shown in Table 6.

Dog study. Fecal 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 (Table 1). All dogs were raised in an unrestrained manner, the median age was 12 months (range = 2–108 months), 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 village 5 in Xiengkhuang Province and the other a 3-year-old male dog from village 15 in Luangprabang Province. Estimates of true prevalence in the plausible range of diagnostic sensitivity ranged from 4.8% to 6.3% (Table 6).

DISCUSSION

This study investigated *T. solium* in the context of multiple *Taenia* species interacting in a host assemblage that includes

TABLE 5

Prevalence of pig cysticercosis (*Taenia solium*, *T. hydatigena*, and *T. asiatica*) by carcass inspection and seroprevalence of pig cysticercosis (antigen-capture ELISA) by location, sex, breed, age, and production system at last point of sale, Laos*

		Carcass inspection prevalence of pig cysticercosis (95% CI)				Cysticercosis antigen-capture ELISA positivity	
Risk factor	No. (%)	*T. solium* (95% CI)	*P*	*T. hydatigena* (95% CI)	*P*	Prevalence (95% CI)	*P*
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)	0.335†	22.5 (16.8–28.2)	0.173‡	71.3 (65.1–77.5)	0.177‡
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		18.3 (12.7–23.8)		63.3 (56.5–70.2)	
Age (months) (n = 528)							
≤ 6	70 (13.3)	0	0.010†	27.1 (16.6–37.7)	0.629‡	68.6 (57.6–79.6)	0.389‡
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)	0.077†	72.1 (68.0–76.1)	0.107‡
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)							
F	275 (50.9)	0.7 (0.0–1.7)	0.151†	22.5 (17.6–27.5)	0.423‡	69.5 (64.0–74.9)	0.674‡
M	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)	1.000†	19.4 (15.2–23.6)	0.024†	67.2 (62.3–72.2)	0.253†
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)	

* ELISA = enzyme-linked immunosorbent assay; CI = confidence interval; Mixed = sometimes penned and sometimes free-roaming.
† By Fisher's exact test.
‡ By Pearson's chi-square test.

TABLE 6

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%, Laos*

Test sensitivity (%)	Estimated true prevalence of pig cysticercosis, % (95% CI)			Estimated true prevalence of *T. hydatigena* taeniasis in village dogs, % (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)	1.9 (0.0–4.5)
90	0.9 (0.1–1.8)	24.9 (21.1–28.6)	0.2 (0.0–0.6)	2.1 (0.0–5.0)
80	1.1 (0.1–2.0)	28.0 (23.8–32.2)	0.2 (0.0–0.6)	2.4 (0.0–5.6)
70	1.2 (0.2–2.3)	32.0 (27.2–36.8)	0.2 (0.0–0.7)	2.7 (0.0–6.4)
60	1.4 (0.2–2.6)	37.3 (31.7–43.0)	0.3 (0.0–0.8)	3.2 (0.0–7.5)
50	1.7 (0.2–3.2)	44.7 (38.0–51.5)†	0.3 (0.0–1.0)	3.8 (0.0–9.0)
40	2.1 (0.3–4.0)	55.9 (47.5–64.3)†	0.4 (0.0–1.3)	4.8 (0.0–11.3)†
30	2.8 (0.4–5.3)†	74.6 (63.4–85.8)	0.6 (0.0–1.7)†	6.3 (0.0–15.1)†
20	4.2 (0.5–7.9)†	–	0.8 (0.0–2.5)†	9.5 (0.0–22.6)
10	8.5 (1.1–15.9)	–	1.7 (0.0–5.0)	19.0 (0.0–45.2)

* CI = confidence interval; – = > 100% prevalence calculated.
† Biologically plausible estimates of the true prevalence of respective *Taenia* species.

humans, pigs, dogs, and bovines. We have documented the sympatric occurrence of four *Taenia* species in northern Laos where conditions suit *T. solium* transmission and hyperendmicity. We observed a substantial proportion of the population practicing open defecation, uncooked pork consumption was relatively common, and pig production systems were rudimentary. However, we observed a low prevalence of human cysticercosis in the survey population. The following discussion draws on the human and animal studies to hypothesize that *T. hydatigena* hyperendemicity in pigs and a high prevalence of persons in Laos eating uncooked beef were the strongest factors controlling *Taenia* ecology in this country.

This study had a number of important limitations and most notable was the relatively small sample size. There was a lack of statistical power to detect significant risk factors associated with cysticercosis even though most cases occurred in three Mon-Khmer villages in Oudomxay Province. Second, we sought to recruit all eligible household members ≥ 6 years of age and compliance varied for the different ethnic groups. Most 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 fecal sample, both closely linked to cultural beliefs and customs. This limitation could be corrected in future studies by using a fingerprick and blood spot sampling method, the use of trained Hmong-Mien women to administer the surveys, and strengthening the consultation process. This study limitation possibly led to an underestimation of cysticercosis prevalence in this ethnic group because poor household members were most likely to have refused participation and the three cysticercosis cases were identified in the three poorest quintiles. However, taeniasis was more common in the wealthier quintiles of the Hmong-Mien ethnic group, and our data may have represented an overestimate. Families also tended to exclude household members who were mentally ill or frail, which might have had a limited effect on the estimate of taeniasis and cysticercosis prevalence within the survey population. Because the number of refusals due to frailty or mental illness was small, this effect was assumed to be negligible.

We required an accurate estimate of current human cysticercosis infection and used a circulating antigen ELISA rather than an antibody ELISA, which tends to overestimate prevalence in disease-endemic areas.[21,32,33] One preliminary study in Vietnam using computed tomography and cutaneous nodule biopsy as a gold standard found a high sensitivity (94.4%) and specificity (100%) of detecting active human cysticercosis with the antigen ELISA,[34] indicating the results in our study were reliable. Human cysticercosis was relatively rare in northern Laos at the community level (2.2%), but there was strong evidence of a focal distribution because just over half of the seropositive cases came from three villages in Oudomxay Province. In Asia, a focal distribution of human cysticercosis has also been observed in northern Vietnam,[21] Indonesian Papua,[35] and China,[36] our results provide further 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, although the seven-year old boy with *T. solium* taeniasis was cysticercosis antigen ELISA negative. The specific exposures leading to increased prevalence in this age group warrants further investigation.

We observed a high taeniasis prevalence (8.4%) with spatial variation on the basis of self-reporting and detection of *Taenia* eggs in a single fecal sample, and these results were comparable with those of other studies in southern and central Laos.[6,8–17,19] Somers and others[21] estimated that self-reporting in Vietnam grossly overestimates true prevalence, but in our study almost half (17 of 35) of treated persons who expelled proglottids were initially detected by self-reporting alone. This finding was consistent with results of Flisser and others,[37] who reported improved detection of tapeworm carriers by using self-reporting methods. Somers and others[21] also found *Taenia* egg prevalence almost identical to copro-antigen prevalence, which was inconsistent with studies conducted elsewhere.[38,39] 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 persons who self-reported infection. Unfortunately, no scoleces were recovered after treatment, and proglottids were not recovered from more than half (20 of 38) of the egg-positive cases. These results are comparable with other studies using niclosamide and magnesium sulfate[40] but poor in comparison to the use of pre- and post-niclosamide purging with electrolyte-polyethyleneglycol salt.[41] The later purging salt was not suitable for use in the field in Laos. Thirty-three (94.3%) of the tapeworm specimens were identified by PCR as *T. saginata*, and because the efficacy of niclosamide in treating *T. solium* and *T. saginata* are assumed to be similar,[42] the data indicates that *T. saginata* was the dominant species infecting persons in Laos.

The strongest risk factor for having a current taeniasis infection was a history of taeniasis. Ninety percent of persons with taeniasis reported a history of taeniasis, although this finding could refer to a single infection with sporadic shedding of segments. Despite this potential bias, results indicated a high prevalence of taeniasis re-infection, which was not surprising considering the high prevalence of eating uncooked meat, particularly beef. Infrequent consumption of beef, which is typically prepared as *laap* (a traditional meat salad prepared with lime juice, mint, chili, and pounded rice) was strongly associated with taeniasis in both multivariate models and indicates a possible link with raw meat consumption at festivals. More detailed investigations will be required to understand specific cultural practices and food preparation. 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.[43] The evidence therefore suggests that taeniasis re-infection was predominantly caused by 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.

The animal studies provide important insights into the ecology of *Taenia* in Laos and mainland Southeast Asia in general. Evidence suggests that *T. hydatigena* was the dominant species infecting pigs in Laos, and a strong infection pressure was exerted by a relatively large, unmanaged dog population (*Canis lupus familiaris*). No other definitive hosts for *T. hydatigena* are found in Laos. Under a strong infection pressure, immunity in the intermediate host can be acquired within two weeks of exposure to eggs and embryos entering muscles after one week will not survive,[44,45] indicating that there is 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*, in which *T. ovis* cyst development can be inhibited by pre-exposure to *T. hydatigena* eggs.[45] There are no plausible reasons why these same immune-mediated competitive interactions do not occur in pigs, indicating 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 because of genus-conserved immunogenic antigens. However, controlled studies will be required to elucidate the immune-mediated interactions in pigs, if they exist, and determine the impact on transmission dynamics.

The pig and dog studies were limited by the diagnostic protocols used. We used egg detection for *T. hydatigena* taeniasis in dogs instead of copro-antigen ELISA because we were unable to source suitable diagnostic reagents during the course of this study. No published data provide a reliable estimate of the diagnostic sensitivity and specificity of *T. hydatigena* egg detection in dog feces. Because proglottids are predominantly shed without defecation,[46] we believe that sensitivity would be much lower than the estimated 62.5% observed for *T. solium* taeniasis.[38,47] Our prevalence estimates of *T. hydatigena* were in the order of 5–6% of village dogs and this corresponded to prevalence in pigs of 50–60%.

In the pig study, the antigen ELISA was not able to differentiate the three *Taenia* species,[33,48] and 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% to 60%,[26,49,50] but specificity has been estimated to be 100%.[26] Bayesian approaches have been applied to model true prevalence of *T. solium* cysticercosis in the face of imperfect tests.[26] However, in this present study, *T. hydatigena* and *T. asiatica* co-endemicity excluded the antigen ELISA results and the Bayesian model could not be applied. Instead of a Bayesian approach, we used a standard maximum-likelihood estimator to calculate true prevalence, which was only valid if specificity and sensitivity were known.[30] 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. Because 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 were available that enabled us to estimate the sensitivity of detecting *T. hydatigena* in pigs. Because *T. hydatigena* metacestodes mature in the fat of the mesentery and omentum and the size can vary from 1 to 7 cm,[50] these cysts could be easily missed, particularly in indigenous breed pigs in Laos that typically have a high fat content.[51] The adjusted inspection prevalence data for *T. solium*, *T. hydatigena*, and *T. asiatica* accounted for most serologically positive animals.

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 is dissection. However, dissection, which involves taking 0.5-cm slices through half a carcass,[52] is expensive and time-consuming, and not conducive to large a geographically diverse studies. There is a genuine need for a robust, cheap, sensitive, specific, validated, and readily available test that can differentiate *Taenia* species in pigs.[1,49] Similarly, improved tests for sensitive and specific detection of human *T. solium* taeniasis cases are required, and these tests are currently being developed and validated,[53] but validation in multiple populations around the world should be a priority.

We have documented the occurrence of four *Taenia* species in Laos. The study indicated a low prevalence of *T. solium* cysticercosis and taeniasis in the human population and a focal distribution in northern Laos. The evidence also suggests that natural parasite competition and local food customs have an influence on *T. solium* transmission, presenting real opportunities for control and possible elimination. With a concerted effort to identify, treat, and follow-to-cure *T. solium* tapeworm carriers, thereby reducing the infection pressure on pigs, continued exposure of pigs to *T. hydatigena* eggs may assist in further reducing *T. solium* transmission. With time, and continued improvements to sanitation and pig husbandry in Laos, we might expect significant reductions in human cysticercosis prevalence.

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