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***Toxocara vitulorum & Fasciola gigantica***  
**in Cattle and Buffalo**  
**in Northern Laos**

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A thesis submitted in fulfilment of the requirements for  
the degree of Doctor of Philosophy

Faculty of Veterinary Science

The University of Sydney



2014



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## STATEMENT OF SOURCES & CONTRIBUTION OF OTHERS

I declare that this thesis is my own original work and has not been submitted either in whole or in part for another degree at this or any other university or other institution for tertiary education. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given.

This thesis contains two peer reviewed publications, two papers that have been submitted to journals for review and one draft paper ready for submission. I am the primary author of these papers and they are presented in the layout format required by the journal to which they were submitted. Each paper is presented for reading as a separate journal paper and for this reason there is some repetition of data and information amongst chapters in this thesis.

Financial support for the PhD research was provided through the Australian Centre for International Agricultural Research (ACIAR) funded project 'Best Practice Health and Husbandry in Cattle and Buffalo, Lao PDR' (AH 2006/159) and the W Richards Trust, University of Sydney.

Field work was conducted with assistance of Lao Department of Livestock and Fisheries extension personnel, appointed to work for the ACIAR AH 2006/159 project and an Asian Development Bank funded livestock development project entitled 'Northern Region Sustainable Livelihood through Livestock Development Project'. A number of final year veterinary students from the University of Sydney and a Charles Sturt University veterinary science graduate were involved with the ACIAR research project as part of their rural practice rotation and also assisted in fieldwork.

All contributors have been acknowledged or where appropriate are co-authors of papers, which are part of this thesis.

The project had the approval of the animal and human ethics committees of the University of Sydney (permit numbers N00/6-2009/3/5105 and 05-2009/11382 respectively).

Luzia Rast,



Date: 14<sup>th</sup> October 2013

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Rast, L, Lee, S, Nampanya, S, Toribio, J-ALML, Khounsy, S & Windsor, PA 2013, 'Prevalence and clinical impact of *Toxocara vitulorum* in cattle and buffalo calves in northern Lao PDR', *Tropical Animal Health and Production*, vol. 40, no. 2, pp. 1224-1241.

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## LIST OF ABBREVIATIONS

ACIAR	Australian Centre for International Agricultural Research
ADB	Asian Development Bank
APTA	Asia Pacific Trade Agreement
ASEAN	Association of South East Asian Nations
BPHH	Best Practice Health and Husbandry in cattle and buffalo, Lao PDR
CIAT	International Centre for Tropical Agriculture
DAFO	District Agriculture and Forestry Office
DLF	Department of Livestock and Fisheries, Lao PDR
ELISA	Enzyme-linked immunosorbent assay
EPG	Eggs per gram
FAO	Food and Agriculture Organisation (of the United Nations)
FEC	Faecal egg count
FMD	Foot and Mouth Disease
GMS	Greater Mekong Subregion
HS	Haemorrhagic Septicaemia
ILRI	International Livestock Research Institute
LAO PDR	Lao People's Democratic Republic (or Laos)
LDP	Northern Region Sustainable Livelihood through Livestock Development Project (or NRSLLDP)
MAFF	Ministry for Agriculture, Forestry and Fisheries
NAFC	Northern Agriculture and Forestry College
NAFES	National Agriculture and Forestry Extension Services
NAFRI	National Agriculture and Forestry Research Institute
NAHC	National Animal Health Centre Laos
NGO	Non-Government Organisation
NRSLLDP	Northern Region Sustainable Livelihood through Livestock Development Project (or LDP)
OIE	Office Internationale des Épizootics (World Organisation for Animal Health)
PAFO	Provincial Agriculture and Forestry Office
PCR	Polymerase Chain Reaction
PB	Partial Budget
RFLP	Restriction Fragment Length Polymorphism
UoS	University of Sydney
VVW	Village Veterinary Worker
WTO	World Trade Organisation

## ABSTRACT

In South-East Asia agricultural production including livestock is predominately produced within mixed smallholder farming systems. These mostly operate at subsistence levels. Cattle and buffalo are typically kept as assets rather than for optimal production purposes. Economic growth and urbanisation in the region continues to lead to increased demand for red meat products. This provides opportunities and pressures for smallholder farmers to increase their livestock outputs and supply this market with consistent and high quality products. Laos is well placed to supply this increasing regional demand but constraints inhibiting optimal production outputs from smallholder farming systems need to be addressed. These constraints include low capacity animal health systems, lack of infrastructure, traditional low input/low output farming methods and endemic livestock diseases. There is limited documentation about disease prevalence in large ruminants and basic production benchmarks such as reproductive, morbidity and mortality rates. Anecdotal reports indicate that *Fasciola gigantica* and *Toxocara vitulorum* are endemic in Laos and contribute to substantial production losses. This is despite the availability of relative cheap and simple treatment technology for *T.vitulorum* and substantial past research investment in the region on both parasites.

The research presented in this thesis contributes to knowledge on the prevalence of *T.vitulorum* and *F.gigantica* in cattle and buffalo and the impact of these parasitic infections on production in mixed smallholder farming systems in northern Laos, which are typical for many other parts of South-East Asia. It further contributes to defining and documenting basic large ruminant production parameters within these production systems and quantifies the financial impact of *T.vitulorum* treatment of calves. Importantly this research identified a large gap in knowledge and in the sustained adoption of effective control practices for large ruminant internal parasites and explored pathways and gives recommendations to address this.

The thesis presents data from seven separate field studies completed in northern Laos between 2009 and 2012. Two cross-sectional field surveys were conducted and results showed that both *T.vitulorum* in cattle and buffalo calves and *F.gigantica* in adult cattle and buffalo had high apparent prevalence (22.6% and

17.2% respectively) and were geographically widespread throughout northern Laos. Results further indicated that both parasites affected cattle and buffalo at similar levels and that no specific clinical signs were associated with either parasite. This was in line with past research on *F.gigantica* infection but not for *T.vitulorum* with limited past research and anecdotal reports indicating that toxocariasis causes diarrhoea and rough coats in calves and especially so in buffalo calves.

Slaughterhouse surveys were conducted in five main provincial slaughterhouses in northern Laos. Results showed a prevalence of faecal eggs for *F.gigantica* (34.1%) plus liver lesions consistent with *F.gigantica* infection in 71.0% of slaughtered cattle and buffalo providing further evidence of the endemic nature of this parasite and its potential clinical impact across northern Laos. Additional findings of the slaughterhouse surveys were that a large proportion of slaughtered female animals were pregnant (44% cattle, 47% buffalo), 9.8% of slaughtered animals had FMD lesions and meat inspectors were rarely present for the entire slaughter process with no condemnation of any products.

Two separate farmer surveys on a sample of farmers that had their large ruminants tested for either *T.vitulorum* or *F.gigantica* were conducted using face-to-face semi-structured interviews. Results of the *T.vitulorum* farmer survey (n=273) showed that there was a relative high rate of awareness (62.3%) about this parasite amongst farmers and that specific knowledge about its epidemiology and potential clinical impact was lacking. Only 2.5% of farmers used pyrantel treatment of calves at the recommended age and dose rate. Results of the farmer survey (n=326) for *F.gigantica* showed smallholders had very limited knowledge about fasciolosis in large ruminants despite 20.6% reporting having seen leaf shaped parasites in livers of slaughtered cattle or buffalo in the past. None of the interviewed farmers treated larger ruminants to control liver fluke.

Analyses of large ruminant production data found annual calf morbidity and mortality rates of 42.6% (CI 0.38-0.47) and 37.3% (CI 0.33-0.42) respectively; and adult morbidity and mortality rates of 7.4% (CI 0.06-0.09) and 2.8% (CI 0.003-0.05) respectively. Further, results showed low reproductive performance

of 0.6 and 0.4 calf per year for cattle and buffalo respectively with first calving ages of 36 months reported for both species.

Two separate field treatment trials were conducted. For *T.vitulorum* calves were treated when they were <50 days old and results showed that locally produced and available pyrantel was effective, with a  $\geq 95\%$  reduction of *T.vitulorum* eggs in faeces four, eight and twelve weeks post treatment in cattle and buffalo calves compared to untreated calves of the same age. Results for imported triclabendazole oral drench and locally available triclabendazole/albendazole tablets manufactured in Vietnam, showed both produced >90% reduction of faecal egg counts in adult cattle and buffalo four, eight and twelve weeks post treatment compared to untreated animals. In addition there was a trend of increased weight gain in treated buffalo compared to the untreated control group indicating that treatment of fasciolosis may result in heavier buffalo.

Financial analysis using partial budgeting and data from our surveys showed that there was a large net benefit of USD 3.69-14.86 per calf for treatment with pyrantel (12.5 mg/kg) once only between 14-21 days of age compared to no treatment.

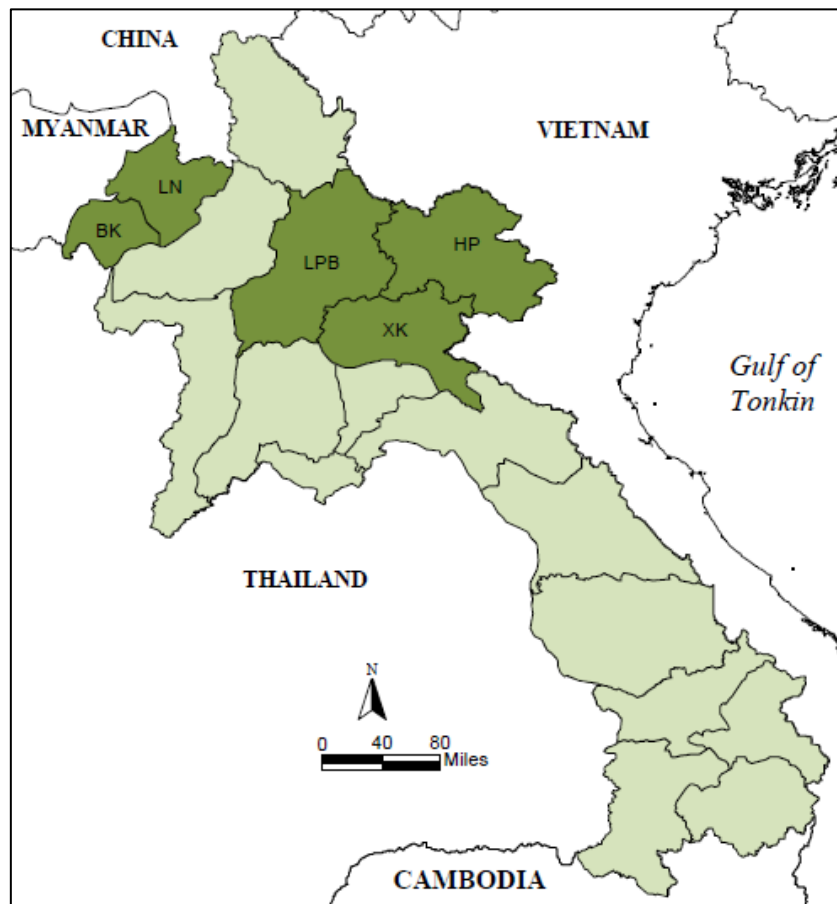
It was concluded that both *T.vitulorum* and *F.gigantica* are endemic in northern Laos and contribute to substantial production losses in this area. Smallholder farmers still keep large ruminants mostly as an asset and there is also a large knowledge gap amongst smallholder producers about internal parasites, their health and production effects and effective control methods. This knowledge gap and the lack of commercial driver contribute to the deficit of widespread adoption of parasite control methods by smallholder farmers despite their availability and known effectiveness. These results suggest, especially for *T.vitulorum* that if recommended control methods were widely adopted, large ruminant production output from smallholder farming systems could be increased through reduced calf morbidity and mortality rates.

# **CHAPTER 1**

## LITERATURE REVIEW

## 1.1 Laos and the study area

Laos or officially the Lao People's Democratic Republic (PDR) is a land linked socialist republic of South-East Asia sharing a 5,083km border with Cambodia (541km), China (423km), Myanmar (235km), Thailand (1,754km) and Vietnam (2,130km). In the west of Laos the Mekong River forms most of the border with Thailand and to the east the Annamite Chain forms much of the border with Vietnam. Laos covers an area of 236,800km<sup>2</sup> that is divided into 17 provinces, which are further subdivided into 129 districts and around 12,000 villages (Department of Statistics 2010). A map of Laos is presented (Figure 1.1) with the study area highlighted.



**Figure 1.1 Map of the study area in northern Laos**  
(Bokeo (BK), Luang Namtha (LN), Luang Prabang (LPB),  
Houaphan (HP) Xieng Khuang (XK) provinces)

The history of Laos traces back to a Kingdom which existed from the 14<sup>th</sup> to the 18<sup>th</sup> century when it split into three separate Kingdoms. In 1893, it became a French protectorate, with the three Kingdoms of Luang Prabang, Vientiane and Champasak uniting to form what is now known as Laos.

Laos briefly gained independence in 1945 after Japanese occupation but returned to French rule until it was granted autonomy in 1949. Laos then a constitutional monarchy became independent in 1953 and withdrew from the French union in 1954. Shortly afterwards a long civil war started and the monarchy ended in 1975 when the communist Pathet Lao movement came to power. Whilst not officially involved in the Vietnam War, Laos was heavily bombed between 1965 and 1973 by American forces and unexploded ordnances and defoliants still impact its people and agriculture (Evans 2002). These conflicts also led to large human capital loss, with about half the population leaving the country during the Indochina conflict and another 10-15% between 1975 and 1985 when the Pathet Lao governed (Evans 2002).

Today Laos is a single-party socialist republic with a (semi) free market economy of around 8 billion USD per annum and an annual growth rate averaging around 7% over the past decade. Laos is a member of the Asia Pacific Trade Agreement (APTA), Association of South-East Asian Nations (ASEAN), East Asia Summit and since 2013 the World Trade Organization (WTO). However, Laos has been dependant on foreign assistance for over 50 years due to its historical, geopolitical and geographical situation (Phraxayavong 2009).

Laos, with a population of around 6.83 million people is the least populated country in the Mekong region. The population is culturally and socially diverse with the major ethnic groups being Lao (55%), Khmou (11%) and Hmong (8%) and over 40 minority ethnic groups. Around 33% of Laos' population live in urban areas and 67% in rural areas (Department of Statistics 2010).

Despite rapid economic growth in recent years, Laos still remains one of the world's least developed and poorest nations with northern Laos in particular considered an economic poor region (Thongmanivong & Fuljita 2006). In 2013 Laos had a United Nations Human Development Index (HDI)<sup>1</sup> of 0.543, which gives Laos a ranking of 138 out of 187 countries with comparable data, and falls below the regional average of 0.683 (UNDP 2013).

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<sup>1</sup> The HDI is an alternative to conventional measures of national development, such as level of income and the rate of economic growth and provides a broader definition of well-being by giving a composite measure of three basic dimensions of human development: health, education and income.



The climate in Laos is tropical with a dry season that is split into a cool to cold period from November to February and a hot period from March to April. The wet season lasts from May to October and is dominated by heavy and frequent rains. Mean monthly rainfall ranges from 17.6mm to 336.3mm and mean monthly temperatures from 19.3°C to 26.3°C based on data from 1901 to 2009 (World Bank 2011).

Agricultural production, including livestock in Laos is influenced by its location, climate, history, political system and economics. Agricultural development, as in many developing nations faces numerous constraints. These include land availability, traditional agricultural and livestock production methods, little opportunity for producers and livestock extension personnel to increase their knowledge, endemic livestock diseases, low capacity animal health systems, poor infrastructure and poorly developed markets. Further, investment to advance agriculture is hampered by many other concurrent priorities with continually increasing investment urgently needed in health and education in particular.

## **1.2 Smallholder farming systems & large ruminant production**

Around 85% of Laos' population depend on agriculture for their livelihood (ADB 2005) and agriculture accounts for 52% of the GDP with livestock and fisheries contributing 18%. The majority (95%) of agricultural product is produced by smallholder farmers. The Government's strategic vision for the agricultural sector (GOL 1999) recognizes two distinct agro-economic zones in Laos: (1) the Mekong Corridor and (2) the Sloping Lands Zone.

The Mekong Corridor includes most of the flatland areas of Laos with irrigated (paddy) rice, a basic road network, credit facilities, access to local and regional markets and a flow of agricultural technology from regional markets. Agriculture in these areas is becoming more market oriented with market forces driving the process of agricultural intensification and diversification. This zone includes districts bordering the Mekong and its tributaries in most central and southern provinces and some valleys in the northern regions.

The Sloping Lands Zone includes the upland and mountainous areas. These are characterized by rugged mountainous terrain, poor infrastructure, very limited

agricultural technology flows, poor market access, little access to credit and predominantly subsistence agriculture. They include many districts in the northern regions of Laos but also some districts in the central and southern regions bordering Vietnam and Cambodia (Stür, Gray & Bastin 2002).

Smallholder farming systems in northern Laos are mixed enterprises producing mainly crops (foremost dry land rice) and keeping livestock (pigs, cattle, buffalo, goats and poultry) using basic traditional farming methods. They predominantly operate at subsistence levels and are vulnerable to climatic and economic shocks. Large ruminants have an important role with 68% of households owning at least one buffalo and 35% at least one cow (Wilson 2007). Cattle and buffalo are important assets, providing manure for fertilising crops, meat, draft power and most importantly a cash reserve. Animals are mostly sold when the household requires cash rather than for optimal returns. In northern Laos, smallholders typically own 6-7 cattle and/or buffalo (Nampanya et al. 2010) and may have access to between 1.1 and 1.8 ha of unirrigated land (Department of Statistics 2010). Large ruminants that are raised within these farming systems are indigenous *Bos indicus* type brown/yellow cattle and pink or black Asian water buffalo (*Bubalus bubalis*) (Figure 1.2).



**Figure 1.2 Asian water buffalo (*Bubalus bubalis*) and indigenous yellow cattle (*Bos indicus*), Ban Nong, Xieng Khuang province, northern Laos**  
(L Rast, December 2010)

The cattle are small with males weighing up to 350kg and females up to 250kg (Kennard, Phatlamchanh & Sithivong 1996, Hansen 1997). Growth rates under the current low input feeding system are low with animals taking 4-6 years to

reach mature weights. The buffalo are larger than cattle with males reaching up to 450kg and females up to 350kg. Reported mean large ruminant production benchmarks for the Mekong region are presented (Table 1.1).

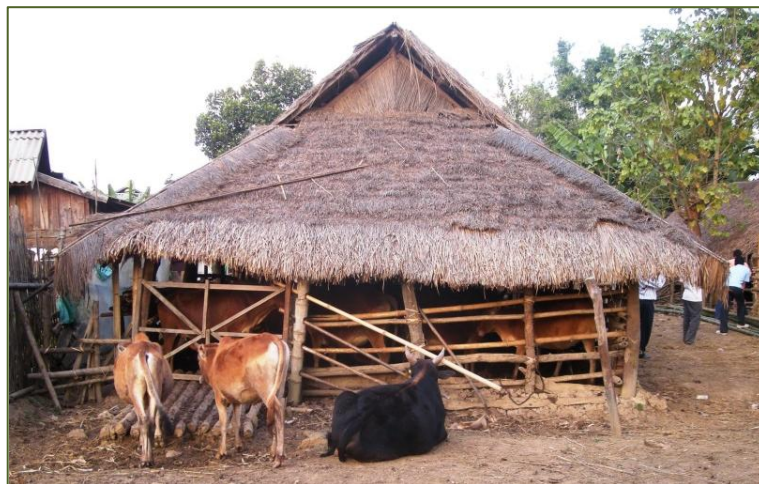
**Table 1.1 Mean production benchmarks of cattle and buffalo in the Mekong region**  
(Teufel et al. 2010)

Parameter	Buffalo	Cattle
Calf birth weight (kg)	-	10-15
Age at first parturition (months)	47.2	29.9
Weight of mature cow (kg)	376.6	302.0
Weight of mature bull (kg)	397.3	338.3
Calf mortality risk (%) <sup>1</sup>	29.5	9.0
Fertility rate <sup>2</sup>	0.63	0.75

<sup>1</sup> Standardised to 12 months and calculated:  $1-(1-\text{Mortality rate})^s/b$ ; with  $s=12$  and  $b=\text{observation period}$ .

<sup>2</sup> Number of parturitions per adult female per year.

Smallholder farmers in Laos mostly use basic traditional methods for large ruminant raising and rarely keep written records of production inputs and outputs. Cattle and buffalo are kept together and allowed to roam freely through naturally vegetated areas along roads or crop fields near the village during the day. They are brought back to the house or a communal livestock shelter at night (Figure 1.3).



**Figure 1.3 Communal cattle house in Ban Nong, Xieng Khuang province, northern Laos**  
(L Rast, December 2010)

Fencing is uncommon and therefore cattle and buffalo that are not used for draft are often taken to communal land or forests away from villages between planting and harvesting times, so they cannot destroy crops. There they spend months roaming freely and are often unattended. In some areas there are communal grazing areas allocated to villages by government decree where large ruminant owners keep and graze their cattle and buffalo most of the year.

Large ruminant reproduction is not managed for optimal outcome. Castration of male animals is not practised and males and females are run together resulting in year-round births with a natural peak between October and February. Pregnancy testing is not done and pregnant females are not observed daily even when close to parturition. Calves are naturally weaned and infertile cows or bulls are not culled.

Growth of vegetation is abundant from around June to September in the wet season, but growth stops and remaining vegetation dries out for the rest of the year. Supplementary feeding rarely occurs and if it does, consists mostly of rice straw which has poor nutritional value (Stür et al. 2002b, Wilson 2007). As a result most cattle and buffalo have a body condition score of  $\geq 2.5$  out of 5 in the wet season but  $< 2$  throughout the dry season.

Animal health inputs such as regular vaccination, internal parasite control or biosecurity are not routinely practiced or available and often limited to short term project funded interventions. Vaccination of large ruminants for haemorrhagic septicaemia (HS) is an exception as a vaccine is produced in Laos and distributed annually in November on the national livestock vaccination day. However vaccination rates and probably effective vaccine delivery to individual animals remain low and HS outbreaks reports continue (personal communication, Khounsy S 2012).

### **1.3 Animal health system and capacity in Laos**

The legislative foundation for Laos' animal health system is contained in a bill on livestock and veterinary matters approved by the government in 2008. However this is considered to be inadequate in providing the basis for an animal health system that meets international standards to ensure world class food safety and animal health (OIE 2011).

Public veterinary services are the responsibility of the Ministry of Agriculture, Forestry and Fisheries (MAFF), which is comprised of eight Departments, including the Departments of Livestock and Fisheries (DLF) and Agriculture and Forestry (DAF). The DLF has four divisions and four centres. The public veterinary services fall within the National Animal Health Centre (NAHC), which has five units: (1) diagnostic and laboratory, (2) veterinary supply, (3) meat inspection, (4) epidemiology and disease control, and (5) administrative and information. Independent from the DLF other than for technical advice are the 17 provincial and 141 district Agriculture and Forestry Offices (PAFO and DAFO). These provide veterinary, livestock and fisheries services at the provincial or district level and are administered by the provincial and district governments respectively.

The national Agriculture and Forestry Extension Services (NAFES) is responsible for delivering extension services in agriculture, livestock, fisheries and forestry, whilst the national Agriculture and Forestry Research Institute (NAFRI) has responsibility for all agricultural and forestry research including livestock, but not veterinary research; this remains with the DLF.

In 2010 only 13 official veterinarians delivered services at the provincial level and none at the district level, with most, if not all veterinary positions at national level involved in administration or laboratory diagnosis. Approximately six private veterinary surgeries operate across the country, most in urban centres, especially in Vientiane. Most veterinarians working in Laos at present are graduates from the former Soviet Union states, Thailand, Vietnam, Malaysia and occasionally, Cuba. Implementation of advances in technology is rare with limited opportunities for self-learning or other means of professional development.

Laos has legally defined village veterinary workers (VW) as part of the veterinary services. These VWs are comparable to community based animal health workers in other countries, with similar training, responsibilities and activities. VWs have mostly been trained within various international aid projects, varying from five days to three months. Officially (NAHC Annual report 2010), there are 11,571 VWs, but it is estimated that less than 20% remain active. The animal health system depends on VWs to be de-facto government agents, carrying out basic disease control programs such as HS vaccination and providing information on outbreaks of animal diseases. However there appears to be little incentive to report disease incidents and the performance of the VW system varies (OIE 2011).

The national veterinary laboratory in Vientiane has diagnostic capacities for about half of the notifiable diseases in Laos. A new multistorey veterinary laboratory opened in late 2012. In addition there are four provincial laboratories covering the 17 provinces, three in the north (Luang Prabang, Oudomxay and Luang Namtha) and one in the south (Champasak). The turnover of services in the provincial laboratories is very low, with diagnostic services limited to egg-counts for gastro-intestinal parasites, rapid diagnostic tests for Avian Influenza and Classical Swine Fever plus storing, packing and forwarding samples to the national laboratory in Vientiane.

Supply of veterinary medicines is unreliable, minimally regulated and unmonitored, with VWs, district DLF employees or private individuals supplying animal medicinal and biological products to farmers for their livestock.

Five agricultural colleges governed by MAFF throughout Laos offer certificates or degrees at different levels and lasting three to five years. Degrees in agronomy, livestock, fisheries and agribusiness are offered. MAFF commenced a strategy of reform in the agriculture and forestry colleges in 2008. In northern Laos there is one college, the Northern Agriculture and Forestry College (NAFC). With the support of a foreign funded project (SURAFCO 2009) NAFC commenced the MAFF reform process in 2009. By 2020 NAFC aims to be acknowledged as a centre of excellence in sustainable upland farming systems, offering skills based and market oriented education, training and services. Since 2010, the National

University of Laos (NUOL) Nabong campus near Vientiane has offered a veterinary degree in addition to their degree in animal science.

Funding for the public animal health system is limited. Despite budget increases over the last few years, there is little evidence that this has reached district levels. District level employees often rely on income from provision of private sector services (i.e. selling animal pharmaceuticals) with government salary structures below the cost of living (OIE 2011, personal communication). Despite their lack of formal veterinary training, district personnel frequently perform specialist tasks including meat inspection, inspection of food products and livestock at border posts, vaccination services, clinical diagnostics and treatment and control of diseases in farm animals. NAFRI, NAFES, DAFO and PAFO all have similar budgetary and capacity constraints.

But most importantly there is little evidence of a younger generation of experienced animal health professionals seeking employment in Laos or of these positions being resourced to attract a new generation of professionals.



**Figure 1.4 Veterinary laboratory Luang Prabang, northern Laos**  
(L Rast, 2010)



## 1.4 *Toxocara vitulorum*

### 1.4.1 Introduction

There is a paucity of recent published studies on *T.vitulorum* in the international scientific literature. This probably reflects that this parasite is more problematic in buffalo and cattle calves in developing countries in the tropics and subtropics, where animal health capacities including research skills are limited. In Asia, most published research is from studies in India, Sri Lanka and Pakistan, involving buffalo in dairy production where calf mortalities severely impact production through loss of herd replacements.

In South-East Asia, dairy production in most countries, including Laos is a minor priority. Nonetheless, *T.vitulorum* infection is very likely to also negatively impact buffalo and cattle productivity, especially if associated calf morbidity and mortality rates are high. With red meat demand in the region growing rapidly and consistently, opportunities for smallholder farmers to provide animals to this expanding market may be compromised by calf losses due to *T.vitulorum*.

### 1.4.2 Epidemiology

*T.vitulorum* is a member of the Ascaridoidea family and is a large roundworm with a translucent and soft body surface. Female worms measure 15-40cm in length, male worms 10-27cm. Detailed morphology has been described (Starke-Buzetti 2006) and an image of an adult worm is presented (Figure 1.5).



**Figure 1.5 Adult *Toxocara vitulorum* worm**

(L Rast, March 2011)

The major hosts of *T.vitulorum* are buffalo (*Bubalus bubalis*) and cattle (*Bos* species) in the humid tropics of Asia, Africa and South America.

*T.vitulorum* infection has been described occasionally in non-tropical areas including England (Jones et al. 2009), Japan (Taira et al. 1991), Belgium (Thienpont & Keyser 1981) and Australia (Warren & Needham 1969) and in other species including bison (*Bison bison*) in Canada and the USA (Goossens et al.

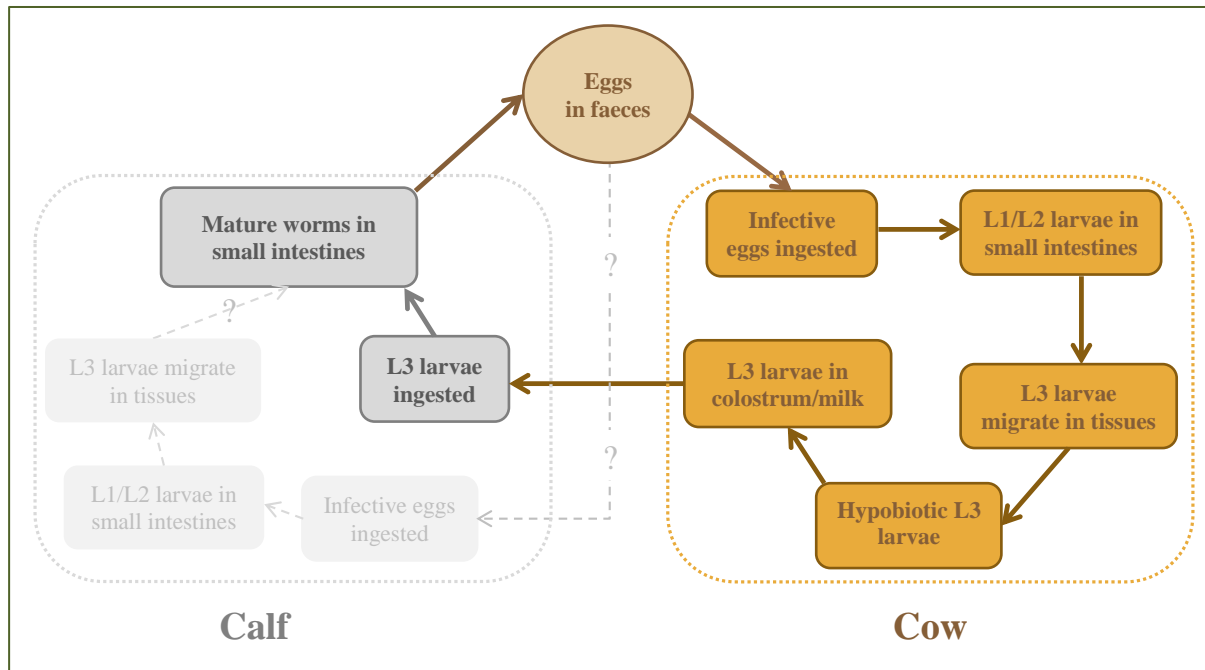


2007, Woodbury et al. 2012), sheep (*Ovis aries*; Mattoff & Vassilev 1959, Cvetković & Nevenić 1960) and a range of experimentally infected species. These include guinea pigs (*Cavia porcellus*; Refuerzo et al. 1952), rabbits (*Oryctolagus cuniculus*; Barriga & Omar 1992), mice (*Mus musculus*; Warren 1971, Amerasinghe et al. 1992, Paula et al. 2004), rats (*Rattus norvegicus*; Chauhan et al. 1974) and chickens (*Gallus gallus*; Chauhan et al. 1974).

Whether infection of goats (*Capra hircus*) with *T.vitulorum* occurs is uncertain with conflicting reports in the literature. The presence of eggs in faeces (Shanmugalingam 1956) and the finding of immature infection (Vassilev 1959) have been reported, although other reports have failed to confirm caprine infection (Warren 1971, Roberts 1989).

*T.cati* and *T.canis* have been reported to cause visceral larvae migrans in humans, which can lead to eosinophilia, hepatomegaly, recurrent pneumonia or an ocular syndrome (Soulsby 1983, Macpherson 2013). Thus migration of infective *T.vitulorum* larvae in human tissues has been suggested by several authors (Fernando et al. 1970, Roberts 1993, Starke-Buzetti 2006), yet to date there is no published record of confirmed human infection with larvae of *T.vitulorum*. A more specific diagnostic test for studies in humans is required as the existing ELISA test cannot differentiate between *T.vitulorum*, *T.cati* and *T.canis* antibodies (Page et al. 1991, Macpherson 2013).

*T.vitulorum* has a unique life cycle amongst the Ascaridoidea with patent infection only occurring in young ruminant calves. As calves reach around six months of age, adult worms parasitising the small intestines commence to die, are expelled and calves become resistant to developing patent infections if they ingest larvae (Roberts 1990b, Roberts 1993). A summary of the life cycle is presented (Figure 1.6). Adult worms live in the small intestines of calves and eggs are deposited into the environment in the faeces of infected calves. Adult ruminants ingest eggs when grazing, larvae hatch, develop to L3 larvae migrating through various tissues. Larval development in adult cattle and buffalo is arrested at the L3 stage (hypobiosis). In females, L3 larvae migrate to mammary tissue shortly before parturition and are excreted in the colostrum and milk for 2-22 days (Tongson 1971, Warren 1971, Mia et al. 1975, Gautam et al. 1976, Roberts, Fernando & Sivanathan 1990).



**Figure 1.6 Life cycle of *Toxocara vitulorum*** (adapted from Starke-Buzetti 2006)

Hypobiosis allows larvae to persist in maternal host tissues for at least two pregnancies (Roberts 1992). Adult *T. vitulorum* has been found in the intestines of calves within six days of birth (Roberts 1990b) and between 6-29 days after birth (Starke-Buzetti 1992). Uterine transmission has been reported through the uterine cotyledons and the chorio-allantoic fluid into the abomasum and small intestines of the foetus (Mozgovoi & Shakhmatova 1969, Mozgovoi & Shikhov 1971, Mozgovoi et al. 1973). However, other studies have shown the trans-mammary transmission to be the main and most likely the sole transmission route (Warren & Needham 1969, Warren 1971, Mia et al. 1975, Roberts 1990b, Roberts, Fernando & Sivanathan 1990).

Several studies have attempted to demonstrate a direct life cycle, by exposing buffalo calves to infective eggs (Cvetković & Nevenić 1960, Enyenihi 1970, Mozgovoi & Shikhov 1971, Akyol 1993) but failed to produce infection in the calves. This included only one study (Mia et al. 1975) that controlled possible transmission from dams by weaning calves at birth.

*T. vitulorum* eggs are round with a thick typically pitted mosaic or honeycomb pattern on the surface. Reported egg size varies between 60 and 120µm (Doughty 1972, Srivastava & Sharma 1981, Roberts 1993). Their thick shell renders them resistant to different climatic environmental conditions, with

reports of prolonged egg survival of at least 17 months in Sri Lanka (Roberts 1989) and more than two years in Belgium (Thienpont & De Keyser 1981). Egg output peaks around 35-62 days after birth of buffalo calves, with the peak lasting 5.5 (+/-2.5) days with 98,000 (+/-63,700) eggs per gram of faeces (Starke-Buzetti 2006).

Prolonged survival of eggs in the environment and larvae in tissues of the maternal host, as well as lactogenic transmission contribute to the life cycle of *T.vitulorum* being largely independent of climatic influences. This and the high fecundity of adult worms provide special challenges for persistent control of this parasite in large ruminants.

#### **1.4.3 Impact of toxocariasis on large ruminant production**

Clinical effects of *T.vitulorum* relate to the large number of adult worms becoming compacted in the duodenum and anterior parts of the jejunum. Average worm burdens of 95 (+/-65) adult worms (range 20-208) in buffalo calves aged 24-28 days have been reported (Roberts, Fernando & Sivanathan 1990). There is uncertainty on the level of worm burden that is pathogenic as determined by consideration of eggs per gram of faeces (EPG), with a review of literature finding EPG above a range of 500 to 20,000 considered as indicating a pathogenic worm burden by different authors (Roberts 1993).

Reported clinical signs of *T.vitulorum* infection are: diarrhoea resembling white scours, intermittent colic, tympanism, constipation, anorexia, dehydration, steatorrhea, butyrous odour of breath, weight loss, hypo-proteinaemia, skin eczema and the loss of glossiness of the coat. Autopsy findings of haemorrhagic enteritis, pneumonia, pleural and pericardial effusions, intestinal obstructions, intussusception and perforation of small intestines have been described (Das & Singh 1955, Roberts 1993, Starke-Buzetti 2006).

The pathogenicity of the infection appears to be more serious when associated with poor nutritional status with an inverse relationship between worm burden and appetite and weight gain reported (Enyenihi 1969). Infected bovine calves had only half the weight of treated controls at ten weeks of age and after

deworming the formerly infected calves gained weight at the same rate as the controls but without compensatory weight gain.

Infected calves may show a marked anaemia, in addition to significant leucocytosis with neutrophilia, basophilia and lymphopenia. Hepatic enzyme levels (serum alkaline phosphatase, glutamic oxaloacetic and glutamic pyruvic transaminase) may be increased, possibly due to larval migration in the liver, hepatic toxins produced by the metabolism of larvae or substances formed through the decomposition of adult worms. Increased levels of serum urea and creatinin have also been observed and may indicate renal impairment (El-Albdin et al. 1975). Increased serum histamine levels have been described (Gupta et al. 1976) and could indicate an allergic reaction to the worms or larvae.

Microscopic lesions in the intestinal mucosa reported include infiltration of lymphocytes, eosinophils, fibroblasts and macrophages (Srivastava 1963) and hyperplasia of mast cells and eosinophils in the duodenum and jejunum during the peak of infection (Neves et al. 2003, Starke-Buzetti 2006).

*T.vitulorum* antibodies against larval excretory–secretory antigen (ES) (Rajapakse et al. 1994), larval soluble extract antigen (Ex) and larval ES (Starke-Buzetti et al. 2001), adult peri-enteric fluid (Pe) and Ex antigens (de Souza et al. 2004) have been demonstrated in serum and colostrum of buffalo cows and calves naturally infected with *T.vitulorum*. This is indicative of the parasitic stimulation of host immunity and lead to the suggestion (Starke-Buzetti 2006) that vaccination could be used in dams to kill larvae in tissues before they are transferred to calves. Alternatively, future studies could aim to identify vaccine molecules and adjuvants for mucosal administration to induce protection against *T.vitulorum* infection in calves. This hypothesis is based on observations that adult worms are expelled by calves after the peak of egg output is reached and that calves appear to be resistant to subsequent reinfection, which could be due to ageing of worms and reduced fecundity of the parasite (Roberts 1993). Another possibility is that particularly at the peak of infection an inflammatory response is elicited by *T.vitulorum* and an intestinal cellular immune response manifests (Neves et al. 2003, Starke-Buzetti 2006).

There is a perception in the published literature that buffalo calves are clinically more severely affected than cattle calves and prevalence surveys investigating both species show higher prevalence in buffalo. In Brazil, prevalence of close to 100% in calves by three months of age was reported (Starke et al. 1983, Barbosa et al. 1992). In Guadeloupe (Mahieu & Naves 2008) prevalence of 77.0% in well fed Creole calves with no clinical signs and effect on growth rates was reported. In Egypt (Selim & Tawfik 1974) prevalence of 70.9% and in Turkey 34.4% in cattle calves <6 months old were reported (Aydin et al. 2006). In India prevalence of 42.9% in large ruminants was reported with buffalo calves having higher prevalence (53.2% and 41.6%) than cattle calves (34.1% and 38.3%) in separate studies (Devi et al. 2000, Kumari et al. 2004). A prevalence of 25.6% in cattle calves and 35.5% in buffalo calves was found in Pakistan (Raza et al. 2007). All these prevalence studies used different methodologies and often included older calves (>12 weeks old) thus probably underestimate the true prevalence (Roberts 1993).

Calf mortality rates as high as 80% due to *T.vitulorum* infection have been reported (Das & Singh 1955) although more recent reports fail to quantify morbidity and mortality rates attributable to infection other than stating that they are high. This is probably because determining the cause of mortality and morbidity attributable to a single aetiology in calves is difficult particularly in developing countries where research and diagnostic capacities are low.

#### **1.4.4 Control and management of *Toxocara vitulorum* infection**

It was estimated that infected calves can excrete around  $70 \times 10^6$  or more eggs in their faeces during the patent period (Roberts 1990a). Therefore measures that reduce environmental contamination with eggs are important, particularly as *T.vitulorum* eggs are relative resistance to environmental influences. Control of *T.vitulorum* is theoretically an ideal example where strategic anthelmintic treatment could be effective (Barger 1999) as a single treatment with pyrantel of calves when 14-21 days old was shown to control *T.vitulorum* infection effectively, with 97.0% efficacy against mature and immature parasites (Roberts 1989). Other anthelmintics trialled had much lower efficacies (<90%) and were frequently ineffective against immature stages of the parasite. Some reports present conflicting results, with ivermectin showing high efficacy based on egg

counts (Gill et al. 1989) but considered ineffective in another trial (Hassanain & Degheidy 1990). A recent study measured the efficacy of subcutaneously administered ivermectin, doramectin and moxidectin in Brown Swiss dairy calves in Turkey (Avcioglu & Balkaya 2011) and found all anthelmintics to be 100.0% effective in reducing faecal egg counts 12 days after treatment. However this does not indicate if the trialled anthelmintics are effective against immature *T.vitulorum*.

Treatment of adult female ruminants with compounds that kill migratory and hypobiotic larvae prior to partition would effectively stop transmission of infection to calves. Larvicidal chemicals tested in laboratory mice against migratory stages of *T.canis* include levamisole, albendazole, febendazole and ivermectin. However a large proportion (38%) of larvae remained alive after 13 days of treatment and the larvae resumed development after treatment ceased (Abo-Shehada & Herbert 1984, Carrilo & Barriga 1987). Further, anthelmintic treatments of adult cows is currently considered impractical in developing countries as the time of parturition is often not known, handling facilities and weight scales are absent and anthelmintics for dosing adults are either prohibitively expensive for many smallholder farmers or not available for purchase.

Other control measures such as grazing management and preventing the access of calves to infected colostrum are considered impractical, particular for the smallholder farming systems that prevail in South-East Asia.

## **1.5 *Fasciola gigantica***

### **1.5.1 Introduction**

Fasciolosis is a global parasitic infection caused by *Fasciola hepatica* in more temperate climates and *Fasciola gigantica* in tropical climates, although both species have been recorded in Africa and Asia (Mas-Coma et al. 2005). *F.gigantica* is a digenetic leaf shaped trematode which requires a freshwater lymnaeid snail as an intermediate host. The parasite invades the liver and mature fluke are located in the bile ducts. *F.gigantica* infects mostly ruminants but can also occur in other species, such as horses, pigs and in humans (Bargues & Mas-Coma 2005, Mas-Coma et al. 2009). Fasciolosis is potentially a substantial human health issue (Torgerson & Macpherson 2011) although the true extent is unknown, with large epidemiological studies only carried out in a few countries (Mas-Comas et al. 2005).

Fasciolosis is considered as one of the most important diseases impacting on cattle and buffalo production in humid tropical regions (Boray 1985, Partoutomo et al. 1985, Fabiyi 1987, Murrell 1994, Harrison et al. 1996, Roberts & Suhardono 1996, Malone 1997, ACIAR 2008) and mostly associated with irrigated rice growing areas and presence of waterways. Substantial investment in research on liver fluke in South-East Asia has occurred and initial findings documented (ACIAR 2008). This confirmed the presence of *F.gigantica* in the region and estimated substantial financial impacts on cattle and buffalo production (Copeman & Copeland 2008).

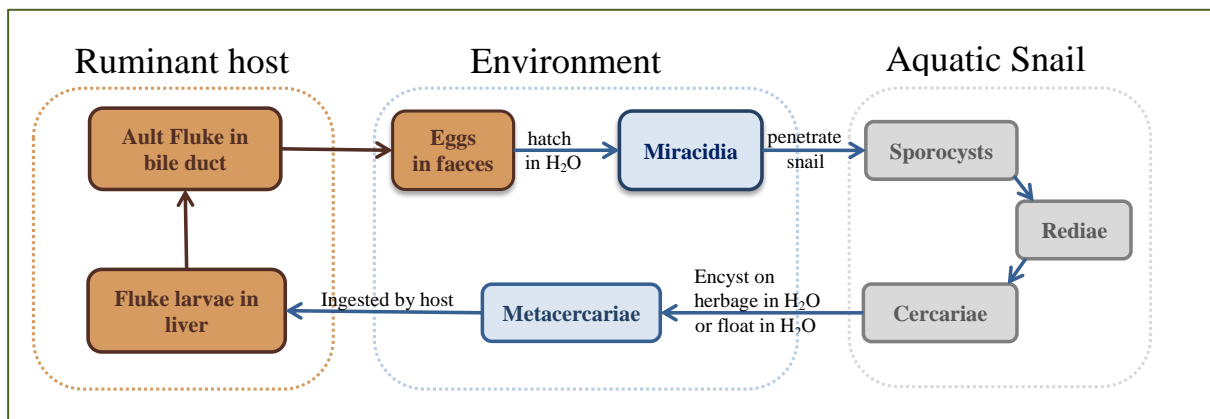
There is very limited published information on fasciolosis in large ruminants in mountainous regions of South-East Asia including northern Laos. A survey of several slaughterhouses in 2004 in the two southern cities of Vientiane and Savannaketh found 17-57% prevalence of *F.gigantica* in cattle and buffalo by detecting fluke in livers during post mortem examination (Duong Quang et al. 2008). Another slaughterhouse survey in Vientiane Capital between September 2010 and February 2011 sampled 172 cattle and found a prevalence of 11.6% (faecal examination) and 94.7% (serology) (Phomhaksa et al. 2012). As no permanent livestock identification system exists in Laos the source of slaughtered ruminants is uncertain and these surveys may or may not have included cattle or buffalo originating from northern Laos.

In northern Laos there are ample smaller waterways and water bodies and some rice paddies, especially in valleys or on plateaus. This, combined with free grazing of large ruminants used by most smallholder farmers, are conducive to the life cycle of *F.gigantica* by enabling prolonged contact with the infective metacercariae. The presence of the parasite is further supported by anecdotal reports of presence of fluke in livers of slaughtered buffalo and cattle from slaughterhouses located in the northern part of the country.

### 1.5.2 Epidemiology

Much of the sparse published research on *F.gigantica* epidemiology (in comparison to published *F.hepatica* research) has been concentrated in areas where infection occurs through ingestion of metacercariae in water or on vegetation growing at peripheries of waterways or water bodies. The large potential of infection from post-harvest rice paddies has only recently been recognised (Spithill, Smooker & Copeman 1999, Suhardono & Copeman 2008).

The life cycle of *F.gigantica* is the same as for *F.hepatica* which has been reviewed and described in detail (Andrews 1999) and a summary is presented (Figure 1.7).



**Figure 1.7 Life cycle of *Fasciola gigantica***

(adapted from Andrews, 1999)

The entire life cycle takes around 12-16 weeks to complete and adult fluke live in the bile ducts for around a year but sometimes three to four years (Hammond & Sewell 1975).



The intermediate host for *F.gigantica* are aquatic snails belonging to the species *Lymnaea auricularia sensu lato* (Kendall 1965). This snail species breeds throughout the year in lowland tropical areas in favourable habitats, which include the fringes of clear slow or stagnant water ways with high oxygen levels and abundant aquatic vegetation (Kendall 1959, Chartier et al. 1990). *F.gigantica* does not appear to occur naturally outside the habitat range of *L.auricularia sensu lato* therefore it is likely that the contribution of other snails to the epidemiology of this parasite is minor (Spithill et al. 1999). In the tropics the habitat of snails in or adjacent to natural waterways and water bodies is influenced by annual rainfall patterns. During the wet season waterways flood and disperse the snail populations which then concentrate again once the water levels stabilise. In contrast the snail populations in irrigated rice fields are not directly influenced by rainfall but rather by the availability of water for irrigation and the stage of crop growth. Snails and eggs can survive from previous crops and colonise a new crop or they enter through the irrigation water (Suhardono & Copeman 2008).

The survival time of the different development stages of *F.gigantica* outside its definitive hosts and within the intermediate snail hosts is mostly influenced by air temperature. Higher temperatures (25-30°C) result in a more rapid life cycle, with the various stages developing more quickly and with shorter survival periods. Lower temperatures (15-20°C) slow the life cycle, with hatching or release of different development stages occurring over longer periods and survival time prolonged (Dinnik 1956, Dinnik 1963, Dinnik 1964, Guralp 1964, Asanji 1988, Suhardono et al. 1996). Infective metacercariae attach to herbage just below water level or float freely. Definitive hosts become infected by ingesting vegetation that contain attached metacercariae or by drinking water containing floating metacercariae. The proportion of floating metacercariae is higher for *F.gigantica* than for *F.hepatica* (Dreyfuss & Rondelaud 1997). This could suggest that infection of livestock, through drinking from infested water sources rather than eating infested vegetation is more important for *F.gigantica*. Also, waterborne spread of metacercariae into host populations some distance away along waterways is possible. A review of literature concluded that in lowland tropical regions aquatic habitats were clear of infection around two months after snails were removed and in cooler upland areas after up to 6 months (Spithill, Smooker & Copeman 1999). Metacercariae on vegetation just

below water level become non-infectious about five weeks after water levels recede in lowland areas or on hay and can survive up to four months in cooler upland areas.

The longevity of adult fluke, prolonged infectiousness of the different development stages of fluke and the intermediate snail host, as well as varying local farming practices and seasons all contribute to different and changing contamination rates of the environment with fluke eggs and metacercariae. Thus transmission of fluke to large ruminants varies dependant on these factors. Any control practises promoted need to consider the complexity, including seasonal and local variations of *F.gigantica's* life cycle.

### **1.5.3 Diagnosis of *Fasciola gigantica***

*F.gigantica* infection in large ruminants is commonly diagnosed by examining faeces for fluke eggs using microscopy. Sedimentation concentration techniques of fluke eggs (Boray & Pearson 1960, Taira et al. 1983) have been described. As these techniques have low sensitivity and repeatability particularly in bovines, a more sensitive and reliable method using Tween20 solution instead of water for faeces suspension has been recommended (Suhardono et al. 2006). Even this method only detects about 30% of eggs present. The negative predictive value of single egg counts has been estimated at 45% (Anderson et al. 1999). In developing countries herd treatment is prohibitively expensive and a better diagnostic test for individual animals could assist improved control, by identifying individual animals with infection early (Piedrafita et al. 2010). However it is likely that development and field application of such tests will take many more years.

Immunodiagnostic methods with higher test sensitivity for *F.gigantica* exist and have been used in some surveys in South-East Asia (Anderson et al 1999, Bui et al. 2003, Nguyen et al. 2012). Their use for routine diagnosis of *F.gigantica* infection in livestock however remains limited in developing countries due to high costs, low laboratory capacities and transport limitations for getting samples to laboratories.

#### **1.5.4 Impact of *Fasciola gigantica* on large ruminant production**

In ruminants, *F.gigantica* infection is associated with chronic production losses, decreased carcass quality and yield, and less commonly overt clinical disease and death. The chronic production losses are attributed to reduced weight, and lowered reproduction and draft performance. As *F.gigantica* infection mainly leads to chronic symptoms and does not affect transboundary trade, it is often a disease neglected by owners and livestock workers in developing countries where there are low animal health inputs (Gray & Copland 2008). There is still incomplete information on the magnitude of production impacts caused by *F.gigantica* infection in cattle and buffalo, particularly within the common mixed predominately rain fed smallholder farming systems of upland South-East Asia.

In Laos, although accurate and timely data on prevalence and impact are lacking, annual economic losses due to *F.gigantica* infection in livestock were estimated between AUD 50-60 million for a high prevalence estimate of 26% and AUD 29-34 million for a low prevalence estimate of 15% (Copeman & Copland 2008).

The immediate consequences of infection are tissue damage, which results in haematological and biochemical changes and an immune response (Molina 2008). Several studies have suggested the pathological processes of infection result in reduced weight gain, fertility, lactation, draft power and some mortality (Spithill, Smooker & Copeman 1999, Copeman & Copland 2008, Molina et al. 2008). Compensatory weight gain once nutritional levels were adequate in sheep infected with *F.gigantica* in Egypt and bovines in Africa has been observed (Nour 1979). An abattoir study on *F.gigantica* infection in cattle in Cambodia found significantly lower weights in infected bullocks >6 years old and females aged 3-4 years and infected female cattle had significantly lower body condition scores compared to uninfected animals (Sothoeun et al. 2006). In Indonesia a study found that experimentally infected cattle around nine months of age had lower daily weight gain (228 and 323g/day for the two breeds studied) compared to uninfected controls (404g/day). In the same study infected buffalo had similar weight gains compared to uninfected animals (Wiedosari et al. 2006).

There is some evidence that infection reduces draft performance probably due to associated anaemia. A 7-15% reduction in work output was estimated in infected buffalo (Roberts, Fernando & Sivanathan 1990). This is supported by a study in Indonesia where farmers reported using cattle twice as many days for preparing fields for rice planting when their cattle were treated with triclabendazole once a year for two years compared to those that were not. The same study also found significantly lower inter-calving intervals in cows that were treated with triclabendazole each July for two years compared to cows that were not treated (Suhardono 1991). As it is recognised that sexual maturity of female large ruminants is determined by weight rather than age, reduced weight gain of young females infected with *F.gigantica* is likely to prolong the time to reach maturity.

### **1.5.5 Human fasciolosis**

In recent years the adverse human health impact of fasciolosis has received increasing recognition (Esteban et al. 1998, De et al. 2003, Mas-Comas et al. 2005, Duong Quang et al. 2008, Piedrafita et al. 2010, Torgerson & Macpherson 2011). In 2005 it was estimated that around 17 million (18.7%) of 91 million exposed people were infected (Keiser 2005). As there are diagnostic limitations especially in developing countries and human fasciolosis is not a notifiable disease it is probable that the true prevalence of human infection is higher than estimated so far. There are recognised human endemic regions (i.e. South America, France, Vietnam) and epidemics or isolated cases in animal endemic areas. Interestingly the expected correlation between animal and human fasciolosis is not a consistent finding (Mas-Comas 2005). Maybe this reflects very diverse local human dietary preferences, livestock husbandry practices and living conditions but possibly also limited collaborations between veterinary and human health professionals.

In Laos potential for human infection is large, especially in the more remote north where it is common for the rural human population to drink untreated water and uncooked aquatic plants and meat, including liver, are a part of the diet. Cattle and buffalo live in close proximity or within the villages and their manure is used for fertilising crops, thus potentially continued risk for environmental contamination with fluke eggs and human infection exists. A small

survey in six villages in southern Laos in 2004 found a faecal egg count prevalence of 2.4% and a serology prevalence of 13.8% in the human population (Duong Quang et al. 2008). No such surveys have been reported from northern Laos.

Acute fasciolosis in humans leads to fever, abdominal pain and eosinophilia, whereas chronic fasciolosis is asymptomatic (Tolan 2011). As the clinical signs of acute human fasciolosis are the same as for many other diseases and human health facilities including diagnostic capacity in northern Laos are very limited, it is probable that human infection if present remains mostly undetected. Human fasciolosis can be successfully treated with triclabendazole, but appearance of triclabendazole resistance in *F.hepatica* despite some questions about reliable techniques for resistance testing (Fairweather 2011) remains a concern for the future (Mas-Comas 2005).

#### **1.5.6 Control & management of *Fasciola gigantica* infection**

Control options for *F.gigantica* in large ruminants are based around disrupting its life cycle (Figure 1.6) and include (1) elimination of mature and immature fluke stages in cattle or buffalo using chemicals, (2) prevention of environmental contamination with fluke eggs by controlling faecal contamination, (3) elimination of the intermediate snail host using chemicals or biological methods and (4) prevention of ingestion of metacercariae in water or on herbage by large ruminants through controlling their grazing.

The most effective chemical compound against mature and immature stages of *F.gigantica* is triclabendazole. In buffalo triclabendazole requires twice the dose rate recommended for cattle as the clearance is more rapid (Estuningsih et al. 1990, Sanyal & Gupta 1996, Gupta & Singh 2002). In most developing countries in South-East Asia triclabendazole is not commonly available or too expensive for smallholder farmers plus handling facilities required for weighing and drenching large ruminants efficiently and safely are absent. Additionally, the epidemiology of fluke may vary from location to location as demonstrated in a study in Cambodia (Suon et al. 2006). Cattle in riverbank villages acquired infection between August and November from grazing on plants near water containing metacercariae. Thus the incidence of infection increased between December and

April, and then stopped in May concurrent with local flooding seasons when cattle were moved to higher areas. On the other hand, in villages away from rivers, infection occurred between September and March when rice was harvested and cattle were fed stubble containing metacercariae. It was concluded that cattle in river bank villages should be treated in May when cattle were moved away from flood waters, and in March in villages located away from rivers coinciding with the finish of rice harvest.

Widespread and sustainable use of anthelmintics for the control of *F.gigantica* in large ruminants in South-East Asia is currently impractical due to the constraints outlined above. This was demonstrated in the Philippines where a national control program of fasciolosis in cattle and buffalo was designed and implemented. The program used chemicals as the main control method and covered 16 provinces where *F.gigantica* was found to be endemic. Initial findings were that greater efficacy was obtained in cattle than buffalo and that mass treatment programs can work but need sufficient funding, efficacious flukicides and skilled ongoing technical support (Copland & Skerratt 2008).

In smallholder farming systems in South-East Asia environmental contamination including habitats of snails with large ruminant faeces occurs uncontrolled as large ruminants often graze freely around villages. In addition, manure from large ruminants is often used to fertilize crops especially rice. Therefore, controlling the egg content in manure for example by heating (composting) or drying (Copland & Skerratt 2008) or by restricting grazing near snail habitats provide fasciolosis control options. Both practices are not widely used, perhaps because they are impractical or smallholder producers lack the knowledge, time and incentives to adopt them on a larger scale.

Eliminating snails either by using molluscicides or predators such as ducks, geese or fish have been reviewed (Copland & Skerratt 2008, Roberts & Suhardono 2008). None have been widely adopted probably as they may be impractical, ineffective or too costly within smallholder farming systems in South-East Asia.

Controlling the ingestion of metacercariae by ruminants either through drinking infested water or ingesting infested vegetation could be an effective control

method. This requires a sound knowledge of the local epidemiology and ecology pertaining to the fluke life cycle. For example in Indonesia a study confirmed that metacercariae on dried rice stalks kept at around 28°C for one month or exposed to sunlight for eight hours were killed (Suhardono 2006). The same study concluded that metacercariae were only present on the lower parts of the plant that had been immersed in water hence not feeding these parts to ruminants prevents infection. From this follows that in areas where infection occurs from herbage after flood waters recede, preventing cattle or buffalo grazing until metacercariae have died controls infection.

In Cambodia the risk of fasciolosis in cattle and buffalo was mapped using a geographic information system and using different determinants for infection or transmission (Tum et al. 2004). This study aimed to assist in identifying high risk areas to allow limited resources, including extension personnel to train smallholder farmers, to be concentrated in areas of most need. Once it is confirmed if this information has been applied and led to improved control, this could be applied in other parts of the region.

Vaccination against liver fluke could provide an ideal disease control option, preventing the pathology caused by migration of immature fluke and providing an alternate management technology in the face of the increasing probability of anthelmintic resistance. Antibody generating surface proteins of fluke have been identified by molecular techniques in an effort to gain a better understanding of the immune mechanism especially in apparent resistant species or breeds of ruminants. This may eventually lead to vaccine development or improved diagnostic methods. However, practical application of such research may be many years way and will require considerable investment and commitment (Piedrafita et al. 2010).

It is evident that the control of *F.gigantica* is complex and needs to consider its life cycle and epidemiology, as well as locally different infection patterns. This provides for a challenging extension message that needs adapting for different local conditions and audiences. It is likely that with fasciolosis being recognised as an important human health risk and with production demand for high quality

red meat increasing, that there will be more pressure to control liver fluke in large ruminants in South-East Asia in the future.

## **1.6 Other internal parasites of large ruminants in tropical climates**

There are only a few recent studies that have assessed the prevalence and impact of gastro-intestinal parasites in ruminants in smallholder farming systems in South-East Asia.

A study in Cambodia using faecal egg counts detected infections in cattle from smallholder farms with a range of internal parasite species (*Cooperia* spp., *Oesophagostomum* spp., *Haemonchus* spp., *Trichostrongylus* spp., *Mescistocirrus* spp., *Bunostomum* spp., *Fasciola* spp. and *Paramphistomum* spp.). The study further found an association between parasite infection and low body condition score. It was concluded that despite the vast range of helminth species found, there was little evidence of their clinical impact and further work to determine the relationship between poor nutrition and parasitism in cattle in Cambodia was recommended (Dorny et al. 2011).

Another study in Pakistan (Khan et al. 2010), also using faecal egg counts found significantly higher gastrointestinal parasite prevalence in sheep compared to goats, cattle and buffalo, with prevalence higher in animals less than two years of age in all species. A significant determinant for prevalence of parasites was using ponds, rivers or canals as drinking water. Helminths identified included *F.gigantica*, *F.hepatica*, *Haemonchus contortus*, *T.vitulorum*, *Trichostrongylus* spp., *Oesophagostomum* spp., *Ostertagia* spp., *Cooperia* spp., *Strongyloides* spp., *Moniezia* spp. and *Trichuris* spp., with prevalence per species ranging from 4.6-25.6% in cattle and buffalo. Clinical impact or production impacts were not evaluated in this study.

A study of helminth prevalence in water buffalo in Hunan province in China examined adult buffalo at slaughter by examining different organs for adult worms. It found a prevalence of 24.9% and 44.7% for *F.hepatica* and *F.gigantica* respectively and a 61.8% prevalence of *Haemonchus contortus*. In addition a range of other trematode and nematode species were identified with prevalence ranging from 1.7-23.5% (*Homalogaster paloniae*, *Eurytrema* spp, *Fishoederius*



*elongates*, *Paramphistomum cervi*, *Schistosoma japonicum*, *Setaria labiatopapillosa* and *Thelazia spp*). The authors concluded that the prevalence of nematode and trematode infections in buffalo was quite severe and may pose some significant human health risks. They recommended integrated strategies to control helminthic infections (Liu et al. 2009).

In contrast, a study in buffalo in Sri Lanka concluded that there was no adverse clinical or production effect due to internal parasites, and treatment other than for *T.vitulorum* in young calves was not recommended (Roberts, Fernando & Sivanathan 1990).

The lack of more studies on this topic probably reflects the difficulties of field research in developing countries, but also that to date there is little evidence that gastrointestinal parasites other than *T.vitulorum* and *F.gigantica* have substantial clinical or production impact on large ruminant production.

## **1.7 Objectives of the research presented in this thesis**

Laos is a developing nation where agriculture is important and widely practiced. Agricultural products are mostly produced within smallholder farming systems that operate at subsistence levels. The increasing demand for red meat in the region driven by growing economies and urbanisation provide challenges and opportunities for smallholder farmers. Challenges include producing consistent quality and quantity of livestock products with the current constraints, whereas the expansion of the economy does provide opportunities to increase farming income and to contribute to food security in the region.

In South-East Asia, including northern Laos, there are considerable anecdotal reports on production limiting diseases. Based on these reports it is evident that *T.vitulorum* and *F.gigantica* are likely to be endemic and are the main internal parasites of importance causing production limiting disease in large ruminants in the region. Despite substantial past research investment on both parasites, there is a paucity of quantitative and timely studies on disease prevalence and impact plus little evidence that sustained and effective control methods are adopted and applied by smallholders. Yet if smallholder farmers wish to contribute to, and benefit from the market demand for large ruminant products in the region, they

require technologies and drivers that enable them to optimise their production outputs to supply consistent and good quality products.

Accurate and timely prevalence data and baseline production benchmarks are required to assess the clinical and financial impact of diseases as well as gauge the effectiveness of new technologies implemented within smallholder farming systems.

The research presented in this thesis aimed to address the current knowledge gap on the clinical and financial impact of *T.vitulum* and *F.gigantica* on large ruminant production in smallholder farming systems in northern Laos. Importantly the research also aimed to determine key drivers for sustained and widespread adoption of control and management of these parasites by smallholder farmers, extension personnel, government and other stakeholders involved in cattle and buffalo production.

In particular the research objectives were to:

- (1) Determine the prevalence of *T.vitulum* in buffalo and cattle calves ( $\leq 3$  months old) and *F.gigantica* in adult ( $\geq 12$  months old) cattle and buffalo in northern Laos.
- (2) Assess farmer knowledge and practices related to *T.vitulum* and *F.gigantica* and their control.
- (3) Assess the effectiveness of locally available anthelmintics to control *T.vitulum* and *F.gigantica*
- (4) Assess the clinical and financial impact of *T.vitulum* and *F.gigantica* on cattle and buffalo production within smallholder farming systems in northern Laos.
- (5) Assess the drivers and inhibitors of adopting efficient and sustainable control methods for *T.vitulum* and *F.gigantica* within smallholder farming systems.

# **CHAPTER 2**

## GENERAL MATERIALS AND METHODS

## 2.1 Training and capacity building of animal health personnel and farmers

### 2.1.1 Workshop training of DLF extension personnel

A series of seven 2 or 3 day workshops covering topics of large ruminant health and production were developed and delivered over a two year period by a team of Australian and Lao livestock production experts including the author (Table 2.1).

**Table 2.1 Summary of the DLF livestock production extension personnel workshop series held in Laos, including topics and dates**

Workshop Title	Topics	Date
Initial training	Introduction on large ruminant health and production, project methodology	29-30 Sep 2008
Animal health	Diseases (FMD, HS, Blackleg, internal and external parasites), epidemiology, outbreak investigation, sample collection	6-7 Feb 2009
Nutrition	Forage establishment, quality and quantity assessment, body condition scoring, feed requirements	6-8 Apr 2009
Biosecurity	Disease risk assessment and management and basic village level biosecurity measures	21-22 Jul 2009
Reproduction	Reproduction physiology, breeding soundness examination, reproduction management	16-18 Mar 2010
Nutrition & marketing	Forage conservation, large ruminant nutrition, value assessment for different markets, reproduction & marketing interventions	15-17 Jun 2010
Extension & village-level biosecurity	Development of extension strategies from all topics covered in previous workshops	6-8 Dec 2010

The workshops were presented using theory and practical sessions as well as interactive case-studies and problem solving sessions in small groups. Attendees included 28 district and provincial DLF employees who were working with the LDP (ADB 2007) and the BPHH projects (Windsor 2006) and a teacher employed by the NAFC.

### 2.1.2 Field training of extension personnel and farmers

Extension personnel applied and strengthened their knowledge, both previous and new knowledge gained from the workshop series through implementation of project activities with collaborating farmers by applied field research and on the job training. Over a four year period (late 2008 to the end of 2012) project activities included regular data and sample collection, record keeping, large

ruminant handling, farmer training, farmer interviews and disease outbreak investigations.

### **2.1.3 Farmer Training**

In three villages, a formal farmer training program was delivered by DLF employees. This included a series of half day training sessions that covered large ruminant production topics, including diseases, biosecurity, best practice husbandry, reproduction management, marketing and nutrition. Additionally, farmer exchange visits were organised, with small groups of farmers visiting another village to exchange large ruminant production practices and techniques (i.e. fattening cattle or silage making) with each other (ACIAR 2013).

## **2.2 Prevalence surveys for *Toxocara vitulorum* and *Fasciola gigantica***

### **2.2.1 Study area and villages participating in projects**

To achieve optimal internal and external validity it would have been necessary to select a random sample of villages, households and animals from all villages that raise large ruminants in northern Laos. This was not possible due to lack of records, requirement of official authority to visit villages, lack of all year road access to some villages and limitation of resources. These constraints are typical for developing tropical countries and the chosen sampling method had to accommodate local rules, infrastructures and resources. The sampling method used may have led to some selection bias as the sampling frame included villages selected for other projects with pre-set selection criteria and did not include all villages with large ruminants. However, we chose a large sample size and the geographic distribution reflected a cross-section of mixed rain fed smallholder farming systems in northern Laos.

The sampling frame for our prevalence surveys included all 198 villages that had more than 20 cattle or buffalo and were part of a foreign funded livestock development project plus all six villages that were part of a smaller research project. These projects worked in collaboration with MAFF and DAF and were (1) 'The Northern Region Sustainable Livelihood through Livestock Development Project' (LDP), a development project running from July 2007 to December 2013

and working in upland areas of the five northern provinces of Bokeo, Houaphan, Luang Namtha, Luang Prabang and Xieng Khuang (ADB 2007) and (2) the 'Best Practice Health and Husbandry in cattle and buffalo, Lao PDR' (BPHH)(Windsor 2006) research project running from July 2008 to December 2012 and working in six villages in the three northern provinces of Houaphan, Luang Prabang and Xieng Khuang. This sampling frame ensured necessary authority to visit selected villages, having data on large ruminant populations within these villages and having established relationships between the local DAF extension personnel and the smallholder farmers.

Village selection criteria for inclusion in the LDP were: (i) high level of poverty, (ii) 50% of poor households which rear livestock, (iii) interested to join project, (iv) all year road access, (v) located within a cluster of villages and (vi) a traditional not a resettled village (ADB 2007). For the BPHH project, the villages were originally selected based on the following selection criteria: (i) having all year road access, (ii) willingness of village authority and farmers to participate, (iii) large ruminant population of >250 animals (iv) evidence of forages for supplementary feeding and (v) located at least 10km apart (Windsor 2006).

### **2.2.2 Pilot surveys**

During 2009, two small pilot surveys were conducted in the six villages of the BPHH project to establish if the internal parasites of interest were present and test the feasibility of conducting larger surveys under local conditions and with assistance of DLF employees. The results of these pilot surveys confirmed the presence of both parasites (Ambler 2009, Lee 2009) and assisted in developing the protocols and sampling methods for the subsequent cross-sectional studies to determine prevalence, farmer knowledge and practices and impact of *F.gigantica* and *T.vitulorum* on large ruminant production.

### **2.2.3 Sampling methods**

For the formal surveys, a sample size of 68 villages and 10 animals per village was calculated using Survey Toolbox Software (Cameron 1999) with 95% level of confidence, 0.075 error and expected prevalence of 10-30% based on results of the pilot surveys.

As stated above, the 198 villages enrolled in the LDP project that had more than 20 cows and/or buffalo formed the sampling frame for selection of villages.

As training and improving capacity of local personnel was an essential priority for both the LDP and BPHH projects, it was necessary to ensure that all participating DLF district employees had at least two villages where they could assist in the survey activities. Hence in each of the 18 districts in which the LDP project was active two to four villages were selected randomly from the sampling frame using a random number calculator (<http://www.random.org>). In addition all six villages that were enrolled in the BPHH project were also selected.

For the *T.vitulorum* prevalence survey, calves  $\leq 90$  days old were sampled as this is the patent period of infection (Roberts 1993, Starke-Buzetti 2006). Calving times and calf numbers could not be established prior to visiting villages as controlled breeding and pregnancy testing are not practised, resulting in all year calving with a natural peak between October and February. For this reason the *T.vitulorum* prevalence survey commenced in September 2009 to coincide with the natural calving peak. For each selected village, the village authorities were informed about the sampling team's intended visit date and farmers were asked to bring cows and calves to a central village point. The farmers were asked the age of presented calves and all calves  $\leq 90$  days old were sampled until a minimum of 10 and a maximum of 20 faecal samples were obtained in each selected village.

For the *F.gigantica* prevalence survey, buffalo and cattle  $\geq 12$  months old were sampled. Farmers were asked to present their adult cattle or buffalo on the days the sampling team visited the village and animals were sampled until a minimum of 10 and a maximum of 20 faecal samples were obtained in each selected village.

### 2.2.4 Faecal sample collection

DLF employees trained at the workshop series in faecal sample collection and animal restraint (Table 2.1) from the relevant district offices were instructed to collect the required faecal samples and data in the villages. A sampling kit was distributed and included:

- One page of background information and instructions in Lao language
- Data collection sheets in Lao language
- Name of the selected villages to sample
- Plastic bags for faecal samples
- A permanent marker pen
- 500ml 3% formalin solution
- Latex gloves.

In addition, DLF personnel were given verbal instructions on sample collection during the workshops (Table 2.1) and encouraged to contact the author or the Lao LDP and BPHH project leader if they had any questions. Faecal sample collection commenced in September 2009 and finished in June 2010 for the *T.vitulorum* prevalence survey and was from August 2010 to December 2010 for the *F.gigantica* survey.

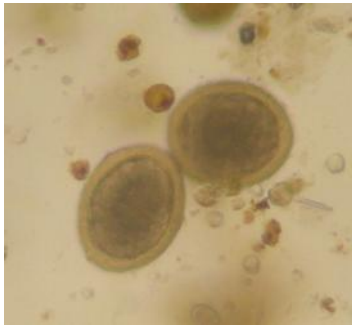
As faecal samples could not be analysed within 24 hours and refrigeration was not available in most district offices, 5ml of 3% formalin was added to each sample for preservation at the time of collection. Samples were stored at the local district offices until all selected villages were sampled and then sent to Luang Prabang by bus or delivered personally by DLF employees.

### 2.2.5 Faecal egg count methods

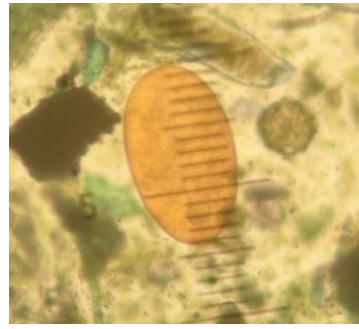
Processing of samples was completed at the local veterinary laboratory in Luang Prabang by the author with the assistance of three DLF laboratory personnel (Figure 1.4). Limited capacities and basic facilities plus the need to preserve the faecal samples with formalin prevented consideration of more sensitive diagnostic methods such as for example copro-antigen ELISA for *F.gigantica*.

For *T.vitulorum*, floatation and a modified McMaster's egg counting technique as described by Hansen & Perry (1994) was used. *T.vitulorum* eggs were identified (Figure 2.1), counted and recorded for each sample.

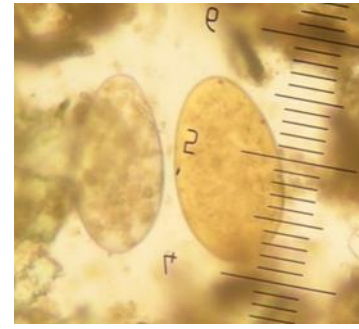




**Figure 2.1** *Toxocara vitulorum* eggs



**Figure 2.2** *Fasciola gigantica* egg



**Figure 2.3** *Fasciola gigantica* egg (right), *Paramphistomum* spp. egg (left)

40 x magnification (L Rast, 2010)

For *F.gigantica* faecal egg counts (FEC) were performed using the simple sedimentation method (Happich & Boray 1969) with the modification of only analysing 0.25 ml of sediment per sample, rather than the whole sediment due to resource constraints. *F.gigantica* eggs were identified (Figures 2.2 & 2.3), counted and recorded for each sample.

### **2.2.6 Data collection for potential risk factors**

At the time of faecal sample collection, initial large ruminant productivity and morbidity data was collected. For the *T.vitulorum* survey this included owner, village and district names and calf details (species, sex, date of birth and if the owners considered the calves being sick or healthy). The researchers assessed the body condition score, the coat condition and any evidence of scouring in the sampled calves. For the *F.gigantica* survey data collected included owner and village name, age and monetary value of animal provided by the owner plus species, gender, body condition, coat condition, morbidity (including clinical signs) and faecal consistency assessed or observed by the researchers.

Data collection sheets were designed in English then translated into Laos and completed by the local DLF employees trained in data recording. Completed data sheets were sent to the central office at Luang Prabang, translated into English by an independent translator and entered into a spread sheet (Excel Microsoft 2003) by the author.

### 2.3 Slaughterhouse surveys

The objective of the surveys was to determine the prevalence of *F.gigantica* at slaughter and assess the liver damage due to *F.gigantica* in slaughter animals.

In northern Laos, slaughter of large ruminants occurs in small basic privately owned slaughter facilities (Figure 2.4) during the night, for sale of meat products at local markets a few hours later (Figure 2.5). In all facilities visited, 5-15 large ruminants were killed and processed each night by different trader or butcher teams who had purchased the animals from farmers.



**Figure 2.4 Slaughter facilities in Luang Namtha and Sam Nua northern Laos**



**Figure 2.5 Meat market Sam Nua**  
(L Rast, 2011)

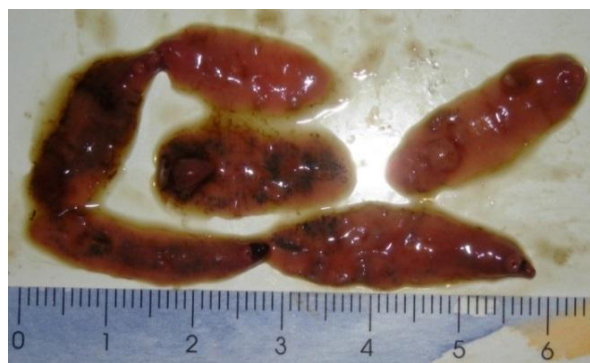
A pilot slaughterhouse survey was conducted at three slaughter houses in Luang Prabang during April and May 2009 to test the feasibility of conducting larger

surveys in local conditions and with assistance of DLF employees (Ambler 2009). Each facility was visited for two to three nights over a four week period.

For the main slaughterhouse survey between March and June 2011, the principal slaughterhouse in each provincial capital of Bokeo (Huaxay), Houaphan (Sam Nua), Luang Namtha (Luang Namtha), Luang Prabang (Luang Prabang) and Xieng Khuang (Phonsavanh) was visited for three to five consecutive nights by the author and a Lao research assistant. At each location, DLF employees organised necessary authorities to visit the facilities and sometimes accompanied the research team, usually on the first night of the surveys. Slaughtered animals were examined pre and post slaughter by the author, with special attention on livers. Any clinical signs and gross lesions observed were recorded on record sheets and with digital images. Faecal samples were collected post slaughter and analysed for *F.gigantica* eggs as described for the prevalence survey.

#### 2.4 Phylogenetic analysis of northern Laos liver fluke

Genetic analysis of liver fluke present in large ruminants in Laos was conducted to determine the species present. Fluke specimens (Figure 2.6) from 14 cattle and buffalo collected during the slaughterhouse survey from each of the five provincial slaughterhouses were preserved in 70% ethanol in sterile plastic screw top containers. They were individually labelled with animal identification, date and place of collection and the specimens were sent to a research team at Iwate University (Ueda, Morioka, Iwate, Japan) for phylogenetic analysis using a PCR-RFLP method (Ichikawa & Itagaki 2010). Results of the analysis are presented (Appendix 1).



**Figure 2.6 *Fasciola gigantica* collected from buffalo at slaughter in Luang Prabang**

(L Rast, June 2011)

## 2.5 Farmer surveys

Two separate farmer surveys were completed using face-to-face interviews in villages that had been selected for the previous prevalence surveys for *T.vitulorum* and *F.gigantica*. The farmers interviewed had participated in the relevant prevalence survey, and this survey collected data to assess farmer knowledge and practices related to *T.vitulorum* or *F.gigantica* and control, and to obtain baseline large ruminant production data on reproductive performance and adult and calf morbidity and mortality rates.

### 2.5.1 Questionnaire development

The questionnaires were developed in English with the assistance of two local experts and translated into Lao. They were tested with two district DLF extension employees to ensure questions were clear and unambiguous and questions subsequently adapted. The final questionnaire contained eleven open and ten closed questions for the *T.vitulorum* farmer interview and five open and ten closed questions for the *F.gigantica* farmer interview.

### 2.5.2 Study population and sampling

For the *T.vitulorum* farmer survey, the village sampling frame was the 69 villages that had participated in the prevalence survey 1-10 months earlier and that had eight or more households with cattle or buffalo calves tested for *T.vitulorum* in that survey. From this, 34 villages were randomly selected and then eight farmers per village were randomly selected from the list of all farmers who had participated in the prevalence survey. Similarly for the *F.gigantica* survey, the village sampling frame was the 75 villages that participated in the prevalence survey and that had eight or more households with adult cattle or buffalo tested for *F.gigantica*. Here random selection was implemented to select 40 villages and then eight to nine farmers per village from the list of farmers that participated in the prevalence survey.

This sample size provided 95% confidence and 5% precision of estimation of proportion for characteristic with expected prevalence  $\leq 20\%$  amongst households and 95% confidence and 5% precision of estimation of proportion for characteristic with expected prevalence  $\leq 50\%$  amongst cattle and buffalo (Thrusfield 2007).

### **2.5.3 Interview process**

For the *T.vitulorum* farmer survey the interview team consisted of the author, a Lao research assistant and one or two DLF district employees for each of the 17 districts where interviews were conducted. The research assistant was experienced in farmer interviews, and was trained on the purpose of the survey and interview process by the author prior to the interviews.

Provincial and district DLF employees arranged interview times with the village headman in each selected village between July and August 2010. The author and the Lao research assistant met with the local district personnel on the day prior to the interviews and trained them on the interview process and questionnaire. On the day of the interviews, the village headman, the selected farmers (person responsible for the household's cattle and buffalo) and the interview team met in the village meeting hall, school or village headman's house. The author explained the purpose of the interview to the producers through direct translation by the research assistant. At commencement of the interviews the identity of the farmers were checked and compared with the records from the prevalence survey and the unique farmer ID of that survey noted on the questionnaire forms.

The Lao research assistant conducted the first one or two interviews with the DLF employees observing, and afterwards they continued interviews independently. All interviews were conducted in Lao with answers recorded in Lao by the interviewers on the questionnaire forms. The author observed the interviews, checked that a consistent process was followed and was available to answer any queries by farmers or interviewers. Each interview took between 45-60 minutes.

For the *F.gigantica* farmer survey, the questionnaires were sent to the same DLF employees who completed the *T.vitulorum* interviews with a list of selected villages and farmers and instructions to follow the identical interview process as for the *T.vitulorum* interviews. The interviews were completed between January and February 2011 and interview records sent to a central office in Luang Prabang.

## **2.6 Field treatment trials**

### **2.6.1 Introduction**

Two simple field treatment trials were conducted to establish that (1) DLF employees and farmers were able and willing to use oral anthelmintic application (including weighing animals, calculating dose rate, restraining animals and applying anthelmintic orally) (2) assess the efficacy of locally available anthelmintics and (3) assess the effect of treatments on weight gain. Local field conditions and capacities limited the trial methods.

### **2.6.2 *Toxocara vitulorum***

#### **2.6.2.1 Trial sites**

The trial was located in four villages (Ban Hard Pang and Ban Hard Khor in Pakou district of Luang Prabang province; Ban Nakud and Ban Tham La in Vienthong district of Houaphan province). Ban Hard Pang and Ban Nakud, both project sites of the BPHH project, were nominated as treatment villages and Ban Hard Khor and Ban Tham La as control villages.

#### **2.6.2.2 Trial design and procedures**

Buffalo and cattle calves were selected if they were <50 days old on the day the research team visited for the first time. The calves remained under the care of their owners during the 12 week trial period and were identified with numbered ear tags. At the start of the trial (week 0) in October 2011, selected cattle and buffalo calves in the two treatment villages were weighed using locally available mechanical scales, and then given pyrantel (Pharmaceutical Factory no 2, Vientiane Lao PDR) orally at a dose of 10mg/kg bodyweight. In the two control villages the enrolled calves were only weighed.

#### **2.6.2.3 Data and sample collection**

At week 0 the date, ear tag number, sex, species, trial group and village name each enrolled calf was recorded. Faecal samples (2-5g) were collected per rectum from all calves enrolled in the trial at week 0 (before pyrantel administration), 4 and 12 of the trial. The samples were stored and FECs done as described for the prevalence survey.

### **2.6.3 *Fasciola gigantica***

#### **2.6.3.1 Trial site**

The trial was located in one of the BPHH project villages, Ban Nong, in Xieng Khuang province in northern Laos. This village was selected as it had a known high prevalence ( $\geq 50\%$ ) of *F. gigantica* as determined by FEC.

#### **2.6.3.2 Trial design and procedures**

In July 2011 (week 0), farmers enrolled in the BPHH project were asked to present their adult ( $\geq 12$  months) cattle and buffalo and the 26 cattle and 27 buffalo presented were randomly allocated to two treatment groups and a control group. All animals were weighed using electronic scales (EC2000B TruTest, New Zealand) and faecal samples collected. The treatment groups were treated once with either triclabendazole oral drench (Fasinex®, Novartis Animal Health Australia Inc.), which was imported for the trial as it is not commercially available in Laos, or triclabendazole/albendazole tablets (Han-Detрил-B, Hanvet, Vietnam) which could be purchased in Laos. The animals were dosed according to their live weight; cattle at rates recommended by the manufacturer (12mg/kg) and buffalo were dosed with triclabendazole at a dose rate of 24 mg/kg as recommended (Sanyal & Gupta 1996). Weighing and faecal sample collection was repeated on all animals available at week four (August 2011), eight (September 2011) and twelve (October 2011) of the trial.

All enrolled animals were identified with individually numbered plastic ear tags and vaccinated against FMD and HS as part of the BPHH research project and remained in the care of their owners throughout the trial period. They mostly grazed in and around the village on common natural grazing land, around rice fields or in forested areas. During the night animals were usually brought back to the village and kept in cattle shelters (Figure 1.3) or tethered in the owner's house yard.

#### **2.6.3.3 Data and sample collection**

Animal identification, species, age, gender, weight and owner details were recorded at week 0. At each further sample collection animal identification and weight were recorded. Faecal samples were taken per rectum from each animal at each of the four data collections and processed as describe for the prevalence

survey. However the entire sample was inspected for eggs by microscope, and the number of *F.gigantica* eggs per gram (EPG) were determined using the technique described (Happich & Boray 1969). Data record sheets were sent to a central office at Luang Prabang and translated into English and entered into a database created in Excel (Microsoft 2003).

## **2.7 Data storage, management and analyses**

For the prevalence surveys, completed data collection sheets were sent to the central office in Luang Prabang, translated by an office assistant and entered into a separate spread sheet (Excel Microsoft 2003) for *F.gigantica* and *T.vitulorum* by the author. For each individual farmer record a unique numeric farmer ID was created. Results of the FECs were entered into the same spread sheets.

For the slaughter house surveys, data collection sheets were in English and completed by the author and entered into an Excel database (Microsoft 2003).

The originals of the completed questionnaires of both farmer surveys were kept at the central office in Luang Prabang and copies were sent to an independent translator for translation into English. The author entered translated data into a customised Access (Microsoft 2003) database in English.

The completed data collection sheets of both treatment trials were entered into separate spread sheets (Excel Microsoft 2003) by research assistants.

All data were analysed using Excel (Microsoft 2003) and SAS version 9.3 (© 2002–2003 by SAS Institute Inc., Cary, NC, USA). Details of statistical methods used are described in each relevant chapter.



## **CHAPTER 3**

### *TOXOCARA VITULORUM* IN CATTLE AND BUFFALO IN NORTHERN LAOS

## **PART 1:**

### **PREVALENCE AND CLINICAL IMPACT OF *TOXOCARA VITULORUM* IN CATTLE AND BUFFALO CALVES IN NORTHERN LAO PDR**

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**Statement of contribution of co-authors**

Luzia Rast, Jenny-Ann Toribio, Syseng Khounsy and Peter Windsor collaborated on the conceptualisation and design of this study. Shing Lee, Sonevilay Nampanya and Luzia Rast designed the pilot survey and conducted the field work. Shing Lee analysed the faecal samples and data from the pilot survey. Luzia Rast analysed the faecal samples and data from the main field surveys and prepared the paper for publication. Shing Lee, Sonevilay Nampanya, Jenny-Ann L. M. L. Toribio, Syseng Khounsy and Peter A. Windsor contributed to reviewing and editing the manuscript.



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Peter A. Windsor

### 3.1 Abstract

This study was completed to determine the prevalence and distribution of *Toxocara vitulorum* infection in cattle and buffalo calves and investigate its clinical impact in northern Lao PDR (Peoples Democratic Republic). The results aim to assist decisions on disease control measures that can contribute to increasing cattle and buffalo productivity within smallholder farming systems in tropical areas. A prevalence survey for *T.vitulorum* in buffalo and cattle calves aged <3 months was conducted between September 2009 and June 2010 in five provinces of northern Laos using a two-stage sampling technique to select 69 villages and 899 calves, with faecal samples collected and examined for *T.vitulorum* eggs at a local laboratory. At the time of sampling, data on calf morbidity and anthelmintic treatment was also collected. Factors potentially associated with infection and severity of infection were analysed at univariable and multivariable levels, using *T.vitulorum* status (positive/negative) and on the positive calves only, faecal egg count levels as outcome variables. The estimated prevalence of *T.vitulorum* in northern Laos was 22.6% (95 % confidence interval [CI], 0.17–0.28), and 76.8% of villages had at least one positive calf. Province was the only significant ( $p < 0.05$ ) variable investigated associated with calf infection status. Species (buffalo) was the only variable significantly ( $p < 0.05$ ) associated with higher egg per gram of faeces levels among infected calves. Prevalence in calves aged 1–21 days, the reported prepatent period, was 17.5% (CI 0.11–0.24). Treatment levels were very low (8.2%) and if treatment occurred it was mostly unsuccessful. The high and widespread infection of *T.vitulorum* in cattle and buffalo calves identified in this survey is likely to result in suboptimal cattle and buffalo productivity. Improved management of *T.vitulorum* infection in cattle and buffalo calves in northern Laos is indicated to reduce potential negative production impacts and enable more efficient development of large ruminant livestock industry as a pathway out of rural poverty for smallholder farmers in northern Laos. In addition to quantifying this disease problem in calves, the conduct of this applied participatory research study provided an important opportunity to improve animal health services by increasing the parasite, large ruminant handling and research knowledge and capacity of government animal health employees and farmers.

**Keywords:** *Toxocara vitulorum*, Cattle, Buffalo, Calves, Prevalence survey, Developing country, Lao PDR, Animal health capacity

### 3.2 Introduction

*Toxocara vitulorum* is a pathogenic gastrointestinal nematode of cattle and buffalo in tropical and subtropical regions, and if not controlled, the prevalence can approach 100%. The parasite's life cycle and epidemiology has been described by Starke-Buzetti (2006) and Roberts (1993) and a brief summary is provided here. Adult worms, located in the duodenum of calves 3–12 weeks of age (patent period), produce large numbers of eggs and adult cattle and buffalo ingest infective eggs from the environment although the parasite does not mature in adult animals. *T.vitulorum* larvae hatch, migrate through tissues and then persist in a hypobiotic state in female animals until late gestation when they are reactivated, migrate to the mammary glands and are excreted in the colostrum and milk for 7–8 days post-partum, infecting calves via the lactogenic route. Larvae in tissues of female adult ruminants can survive several years and have the potential to infect calves over 1–3 parturitions. Ingested larvae mature in the calf within 20–25 days (prepatent period) and calves older than 8 weeks have rapidly declining faecal egg output, most likely due to development of immunity and ageing of the parasite. The adult worms are large (106–400mm long) and worm burdens ranging from 14 to 1,025 worms per calf have been reported. The bulk of worms occur in the duodenum and their nutrient requirements retard the passage of food, impairing the assimilation of nutrients. Infected calves may develop diarrhoea or constipation, anorexia, loss of body condition, stunted growth, colic, loss of coat glossiness and skin tone with eczema and signs of dehydration. In severe infestations, intestinal obstruction, intussusceptions, volvulus and intestinal perforation can occur and cause mortalities, with losses of 30–80% estimated in 2 to 3 months old calves.

Driven by increasing urbanization of the human population and improved incomes, a change in dietary preferences is occurring in South-East Asia resulting in increasing demand for red meat products that is expected to increase at 3% per annum between 1993 to 2020 (Delgado et al. 1999). Lao Peoples Democratic Republic (Lao PDR), surrounded by expanding market demands of Thailand, China and Vietnam, is situated in a prime area to leverage on this growth with opportunities to develop their livestock industry (Stür et al. 2002,

Wilson 2007). Improving livestock productivity appears a feasible way of addressing poverty in northern Laos and other developing countries in the region in a sustainable manner and several research and development projects are addressing this issue (Windsor 2011).

Laos is a developing country with a predominantly rural society where 85% of people are involved with agricultural activities and agriculture contributes to 52% of GDP (Wilson 2007, UNDP 2009, Nampanya et al. 2010). Rural poverty is a major challenge, particularly in the upland and mountainous areas of northern Laos predominated by smallholder producers, owning 6–7 cattle and/or buffalo per household and having access to between 1.1 and 1.8ha of land relying mainly on subsistence farming. Large ruminants are an important asset, providing manure for fertilising crops, a meat source, draft power and importantly, a cash reserve. The value of large ruminants is comparatively high and they can be sold when the household needs larger amounts of cash. Despite the importance of large ruminants, farmers are not able to maximise yields from this resource as husbandry methods and reasons for raising livestock are entrenched in traditional practices, with low input and hence low output (Stür et al. 2002, Wilson 2007). Large ruminants are usually grazed, roaming in forests surrounding the villages, on road sides and on harvested rice fields or on common village grazing land, with supplementary feed rarely given. Reproductive management is not practiced. Male animals are not castrated, and bulls and cows run together throughout the year, resulting in all year calving with a natural seasonal peak into the dry season between October and January. Disease management such as drenching or vaccination is not routinely practiced despite many diseases, including *T.vitulorum* infection in calves, reported to be endemic. *T.vitulorum* infection in calves is identified mostly based on clinical signs (such as distension of the abdomen, white diarrhoea and rough coats) and not confirmed by laboratory diagnostics, and farmers do not routinely treat calves.

Recent reviews of the Laos livestock sector identified internal parasites especially *T.vitulorum* and *Fasciola gigantica* as production limiting diseases of importance for cattle and buffalo, resulting in production losses for smallholder producers (Stür et al. 2002). However the prevalence and distribution of *T.vitulorum* in Laos as well as its clinical and financial impact is unknown.

The objective of this study was to determine the prevalence and distribution of *T.vitulorum* infection in cattle and buffalo calves and investigate its clinical impact in northern Laos. This study was part of a larger research project examining the clinical and financial impact of internal parasites in large ruminants in northern Laos. The research aims to assist decisions on disease control measures that can contribute to increasing cattle and buffalo productivity within smallholder farming systems in tropical regions.

### **3.3 Materials and methods**

A pilot survey was conducted in January and February 2009 followed by a cross-sectional survey between September 2009 and June 2010.

#### **3.3.1 Study area**

The study area included villages located in the five northern Laos provinces of Bokeo, Houaphan, Luang Namtha, Luang Prabang and Xieng Khuang. The north of Laos is mountainous with peaks reaching 2,800m and sparsely populated with human population densities ranging from 16-26 people/km<sup>2</sup>. The climate is tropical dominated by a wet/cool and a dry/hot season. Some areas and villages are not accessible by car all year plus access to villages requires local government authority. Both, the smaller bilateral research project entitled 'Best Practice Health and Husbandry in cattle and buffalo, Lao PDR' (BPHH) (Windsor 2006) and the larger multilateral development project entitled 'Northern Region Sustainable Livelihoods through Livestock Development Project' (LDP) (ADB 2007) operate in the five provinces selected for the study area with both projects aiming to facilitate improved rural incomes through enhancing livestock productivity. They are managed and implemented by the same Lao Department of Livestock and Fisheries (DLF) staff and villages enrolled in these two projects provided the primary sampling unit as they had all year road access by car, and authority for the research team to work in the villages was provided through these projects.

### **3.3.2 Selection of villages and calves**

#### **3.3.2.1 Pilot survey**

The six villages enrolled in the five year (2008–2012) BPHH research project were selected with two each located in the three northern provinces of Luang Prabang, Xieng Khuang and Houaphan. Village selection for this project was based on following criteria: (1) more than 250 head of cattle and/or buffalo in the village, (2) all year road access and (3) willingness of village authorities, farmers and local DLF staff to participate. An additional two villages in Luang Prabang and six neighbouring villages in Xieng Khuang provinces were included to increase sample size and access untreated animals as some villagers had treated calves for *T.vitulorum* infection as part of the BPHH research project. Visits to the villages were arranged with the district authorities of DLF and the village headmen, and all cattle and buffalo calves <6 months old not previously treated for *T.vitulorum* presented on the day of the research team visit were sampled. A total of 39 buffalo and 46 cattle calves were included in the study, with 14 animals from Houaphan, 30 animals from Xieng Khuang and 41 animals from Luang Prabang provinces.

#### **3.3.2.2 Cross-sectional survey**

A sample size of 68 villages and ten calves per village was calculated using Survey Toolbox Software (Cameron 1999) and information from the pilot survey, with 95% confidence and error of 0.075. A two-stage sampling technique was used and the 198 villages enrolled in the LDP project that had more than 20 cows and/or buffalo formed the sampling frame for selection of villages as the primary sampling unit. Village selection criteria for the LDP project consisted of: (1) high level of poverty, (2) 50% of poor households which rear livestock, (3) interested to join project, (4) all year road access, (5) located within a cluster of villages and (6) a traditional not a resettled village. The names of all villages which met these criteria were entered into Excel (Microsoft 2003) and two to four villages in each of the 17 districts were randomly selected. All six villages enrolled in the BPHH research project were also selected. Faecal samples from 899 calves, consisting of 566 cattle and 333 buffalo calves, from 69 villages (including one extra village than statistically necessary) in 17 districts in five Northern provinces were collected and analysed for the presence of *T.vitulorum* eggs.



The sampling and data collection teams from the DLF district offices organised visits to the selected villages with local authorities including the village headmen between September 2009 and June 2010, which included the months of the annual natural calving peak in northern Laos. In each selected village the sampling team sampled all calves <90 days of age that were presented at each visit until at least ten calves were sampled. All animals sampled were local indigenous yellow cattle (*Bos indicus*) or Asiatic water buffalo (*Bubalus bubalis*).

### **3.3.3 Faecal sample collection and analysis**

Samples were collected directly from the rectum by hand using latex gloves, with calf restraint provided by the farmer. Samples were placed in zip-lock plastic bags, and labelled with calf identification number, species, date, village and farmer names. Unless samples could be analysed within 24h, 5ml of 3% formalin was added to preserve the samples during transport to the laboratory in Luang Prabang, which could take several days to weeks after collection and refrigeration was not always available. Labelled samples were placed into a second clear plastic bag and sealed with elastic bands. Floatation method and a modified McMaster's egg counting technique as described by Hansen and Perry (1994), with faeces to water dilution of 1:20 and a universal Whitlock four chamber egg counting slide (JA Whitlock & Co., Australia) were used to identify *T.vitulorum* eggs and obtain quantitative estimates of egg numbers per gram of faeces.

### **3.3.4 Data collection and management**

#### **3.3.4.1 Pilot survey**

At faecal sampling, data obtained included: owner, village and district names and calf details (species, sex, date of birth and if the owners considered the calves being sick or healthy). The researchers assessed the body condition score, presence of scouring, mucous membrane colour and coat condition of sampled calves and measured calf height at the wither. A translator who spoke English and Lao communicated with farmers in Lao and provided answers to the researchers in English, who recorded data on a field record form.

### **3.3.4.2 Cross-sectional survey**

Data record sheets used in the pilot survey were adapted and translated into Lao language. District staff of the Lao DLF collected the faecal samples and the same data as in the pilot survey with exception that the current health status (healthy or sick as assessed by the owner) of the dam of sampled calves was also obtained, that mucous membrane colour of calves was not assessed and that sampled calves were not measured due to resource constraints. Completed data record sheets were sent to a central office in Luang Prabang, translated into English and entered into a spread sheet created in Microsoft Excel 2003. Birth dates provided by farmers, often included the month and year only. In these cases the 15<sup>th</sup> day of the month was the assumed birth date to calculate the age of the calf in days at the time of faecal sampling.

### **3.3.5 Statistical analyses**

Prevalence and its confidence interval were calculated using EpiTools software (Sergeant 2009). Descriptive statistics, logistic and linear regression analyses of the data were carried out using SAS Macros for Statistical Modelling (Dhand 2009) and SAS statistical software (© 2002–2003 by SAS Institute Inc., Cary, NC, USA). Descriptive analysis was performed on each variable using frequency tables and charts for categorical variables and mean, median and range for continuous variables.

Univariable logistic regression was performed to determine the association between the binary outcome variable of faecal egg count result (positive/negative) with ten explanatory variables (Table 3.1). The models were checked for missing values and interaction between variables. From the likelihood ratio chi-square analysis, the odds ratios of significant explanatory variables were examined to determine the extent as well as positive or negative association with the presence of infection. Subsequently, explanatory variables with a p-value of  $\leq 0.25$ , <10% missing values and Spearman rank coefficient of  $< 0.7$  (when variables checked for collinearity in pairs) were included in a multivariable logistic regression model and tested using a stepwise approach. Variables with likelihood ratio chi-square  $p \leq 0.05$  were retained in the final multivariable model. For the pilot survey the calves were subdivided into three age categories:  $\leq 20$ , 21–70 and  $\geq 71$ –180 days. For the cross-sectional survey

the age categories used were:  $\leq 21$ , 22–49, 50–90 and 91–120 days to better account for the different stages of the life cycle of *T.vitulorum*.

Eggs per gram of faeces (EPG) were calculated and presumed to indicate the severity of adult *T.vitulorum* infection, with higher EPG suggesting higher intestinal worm burdens. The EPG data of positive calves was log transformed to ensure validity of assumptions of normality and univariable linear regression analysis carried out to determine the association between EPG and the ten explanatory variables described previously. Subsequently, explanatory variables with a p-value of  $\leq 0.25$ ,  $< 10\%$  missing values and a Spearman rank coefficient of  $< 0.7$  (when variables checked for collinearity in pairs) were included in the multivariable linear regression model and tested using a step-wise approach and variables with F-test p-values  $\leq 0.05$  at each step retained in the final multivariable model. The average EPG counts for buffalo were 7,573.3 (range 10–110,000) and for cattle 2,795.3 (range 10–63,320). Summary statistics and univariable regression results are presented (Table 3.1 and 3.3).

### **3.3.6 Improving animal health and research capacity of Lao farmers and livestock extension staff**

As capacity and knowledge among Lao smallholder farmers and DLF staff were initially very limited, staff training occurred by delivering a series of seven 2 to 3 day training workshops during 2009 and 2010, covering large ruminant production and health topics, data collection including sampling methods, farmer interview techniques and record keeping. Staff then applied and increased their capacities throughout the study period through active participation. Farmers assisted in sampling and data collection and through this participatory approach were introduced to improved livestock handling and husbandry techniques.

**Table 3.1 Contingency table and univariable logistic regression results for association of explanatory variables with *Toxocara vitulorum* positive or negative faecal egg counts for 899 calves in northern Laos, 2009/10**

Variable Categories	<i>T.vitulorum</i> result		OR	95% CI	p-value
	Negative (%)	Positive (%)			
<i>Province</i>					< 0.0001
Bokeo	78 (72.2)	30 (27.8)	1.00		
Houaphan	161 (67.4)	78 (32.6)	1.26	0.77, 2.10	
Luang Namtha	63 (67.0)	31 (33.0)	1.28	0.70, 2.34	
Luang Prabang	296 (89.2)	36 (10.8)	0.32	0.18, 0.55	
Xieng Khuang	98 (77.8)	28 (22.2)	0.74	0.41, 1.35	
<i>Sampling time</i>					0.044
Sep/Oct	132 (80.0)	33 (20.0)	1.00		
Nov/Dec	242 (72.9)	90 (27.1)	1.49	0.95, 2.36	
Jan/Feb	302 (79.5)	78 (20.5)	1.03	0.66, 1.65	
Mar-Jun	20 (90.9)	2 (9.1)	0.40	0.06, 1.47	
<i>Species</i>					0.107
Buffalo	248 (74.5)	85 (25.5)	1.00		
Cattle	448 (79.2)	118 (20.9)	0.77	0.56, 1.06	
<i>Age of calves</i> <sup>1</sup>					0.117
1 - 21 days	174 (82.5)	37 (17.5)	1.00		
22 - 49 days	258 (74.4)	89 (25.7)	1.62	1.06, 2.51	
50 - 90 days	225 (76.8)	68 (23.2)	1.42	0.91, 2.24	
91 - 120 days	38 (82.6)	8 (17.4)	0.99	0.40, 2.20	
<i>Is calf sick?</i> <sup>1</sup>					0.022
No	587 (79.1)	155 (20.9)	1.00		
Yes	107 (70.4)	45 (29.6)	1.59	1.07, 2.34	
<i>Was calf treated for <i>T.vitulorum</i>?</i> <sup>1</sup>					0.154
No	632 (76.8)	191 (23.2)	1.00		
Yes	62 (83.8)	12 (16.2)	0.64	0.32, 1.17	
<i>Gender of calf</i> <sup>1</sup>					0.256
Female	377 (78.9)	101 (21.1)	1.00		
Male	311 (75.7)	100 (24.3)	1.20	0.88, 1.65	
<i>Is mother of calf sick?</i> <sup>1</sup>					0.963
No	640 (77.4)	187 (22.6)	1.00		
Yes	54 (77.1)	16 (22.9)	1.01	0.55, 1.77	
<i>Does calf have diarrhoea?</i> <sup>1</sup>					0.915
No	569 (77.5)	165 (22.5)	1.00		
Yes	118 (77.1)	35 (22.9)	1.02	0.67, 1.54	
<i>Coat condition of calf</i> <sup>1</sup>					0.704
Normal	550 (77.6)	159 (22.4)	1.00		
Abnormal	138 (76.2)	43 (23.8)	1.08	0.07, 1.57	

<sup>1</sup> Data were missing for 2-12 calves.

### 3.4 Results

#### 3.4.1 Pilot survey

The estimated prevalence of *T.vitulorum* infection was 6.3% in calves <20 days old, 37.5% in calves 21–70 days old and 4.4% in calves aged ≥71–180 days. Logistic and linear regression analysis results showed that only age category (3–10 weeks) was significantly associated with infection.

#### 3.4.2 Cross-sectional survey

Sampled calves ranged from 1 to 120 days old with a mean and median age of 43.1 and 62 days, respectively. While the aim was to sample calves <90 days old, 46 calves aged between 91 to 120 days were also sampled and included in analysis. Overall prevalence was 22.6% (CI 0.17–0.28).

76.8% of sampled villages (n= 69) had at least one calf with a positive faecal egg count result. Variables investigated and univariable logistic regression results are presented in table 3.1 and results of the final logistic regression model are shown in table 3.2. Province was the only variable significantly associated with the outcome and all other variables investigated were not significantly ( $p > 0.05$ ) associated with presence or absence of *T.vitulorum* infection.

**Table 3.2 Final logistic regression models for *Toxocara vitulorum* infection in a cross-sectional survey of 882 calves in northern Laos, 2009–2010**

Variable Category	b	SE(b)	OR	95% CI	p-value
<i>Province</i>					<0.001
Bokeo			1		
Houaphan	0.24	0.26	1.27	0.77-2.13	
Luang Namtha	0.25	0.31	1.29	0.70-2.36	
Luang Prabang	-1.13	0.28	0.32	0.19-0.56	
Xieng Khuang	-0.29	0.31	0.75	0.41-1.37	

**Table 3.3 Summary statistics and univariable linear regression results for association of explanatory variables with *Toxocara vitulorum* EPG levels in 202 calves with positive faecal samples, Laos, 2009-2010**

<b>Variable Category</b>	<b>N</b>	<b>Min.</b>	<b>Q1</b>	<b>Mean</b>	<b>Median</b>	<b>Q3</b>	<b>Max.</b>	<b>p-value</b>
<i>Species</i>								0.001
Buffalo	85	10	270	7573.3	1830	7750	110000	
Cattle	117	10	60	2795.3	493	2187	63320	
<i>Age<sup>1</sup></i>								0.043
1 - 21 days	37	10	160	3772.9	1480	4440	24070	
22 - 49 days	89	10	260	5753.2	1413	4095	110000	
50 - 90 days	67	10	30	4656.9	547	2613	63320	
91 -118 days	8	10	50	714.1	103	394	4610	
<i>Province</i>								0.048
Bokeo	30	10	30	7124.9	425	10710	63320	
Houaphan	78	10	220	6372.7	1287	5130	110000	
Luang Namtha	31	10	93	3164.6	1250	3890	21851	
Luang Prabang	35	20	160	2448.3	590	2310	18250	
Xieng Khuang	28	10	17	2720.4	100	3183	24070	

<sup>1</sup>Data was missing for one calf

Linear regression on the subset of data that were positive for *T.vitulorum* in the final model indicated that 'species' was the only variable significantly associated ( $p = 0.001$ ) with an increase of EPG levels, with buffalo having a  $\beta$  value of 1.12 (intercept  $\beta$  value = 6.02).

### 3.5 Discussion

In this study the prevalence of *T.vitulorum* in cattle and buffalo calves aged 1–120 days from five northern Laos provinces was estimated to be 22.6% (CI 0.17–0.28), which is similar to estimates reported in other studies in the tropics. In the Red River Delta region of Vietnam, prevalence was estimated to be 35.1% in cattle calves (Holland et al. 2000); in Turkey 40.2% in cattle calves aged <3 months (Aydin et al. 2006); and in India 28.6% in cow and buffalo calves in one study (Kumari et al. 2004) and 34.1% in cattle calves and 53.2% in buffalo aged <4 months (Devi et al. 2000). Comparison of these prevalence studies is difficult given differences likely to influence results exist in age group classification, husbandry, species, season of sampling as well as calf selection

and diagnostic methods and more recent studies in the South-East Asia region are lacking. It is possible that *T.vitulorum* prevalence varies between geographical areas, due to different climates, large ruminant husbandry practices, cattle and buffalo stocking density, or even natural resistance of the local indigenous cattle and buffalo breeds. Our study sampled a large ruminant population belonging to smallholder producers, managed extensively with minimal husbandry inputs, whereas most other reported studies were done in large ruminant populations from research stations or in populations with more intensive farming practises.

There are limitations with using a flotation method to analyse faecal samples for presence of parasite eggs in a single sample to determine presence of infection and test sensitivity and specificity of this method for *T.vitulorum* has not been reported. False positives could occur with coprophagia and false negatives could occur if the animals are sampled during the pre-patent period, if there is intermittent shedding or low egg output. The likelihood of false negative results was considered to be limited in this study as our calves were sampled at an age when *T.vitulorum* infection if present was patent (Roberts 1993, Starke-Buzetti 2006). *T.vitulorum* is reported to be a prolific egg producer with an egg output of 110,000 eggs per day and faecal volume produced by calves is low, hence false negative results due to low egg output are unlikely. Further, for all samples all four chambers of the counting chamber were examined to reduce the chance of false results. Patent infection was not confirmed in this field study as no calves were available for necropsy.

The significant higher prevalence in the age group category of 3–10 weeks found in the pilot survey reflects the life cycle of *T.vitulorum*, with sampling of calves older than 3 months then limited for the main survey. However the prevalence in calves aged 1–21 days was unexpectedly high at 17.5%, as this occurs within the reported prepatent period. This could indicate intrauterine infection, although presence of eggs in the faeces due to coprophagia, cross contamination of samples in the field or in the laboratory or inaccurate age records may also be possible. Cross contamination of samples was considered unlikely as local staff was well trained and hygienic procedures preventing cross contamination were consistently implemented in the field and laboratory.

Although previous research on the life cycle of *T.vitulorum* found the intrauterine path of infection was not conclusively disproved, the balance of evidence was considered to support only the lactogenic route of infection as occurring (Roberts 1993). In northern Laos, cattle and buffalo are often not observed daily and age records used were provided by farmers who could sometimes only provide the month the calf was born and not the exact day and in these situations the calf age was calculated assuming birth occurred on the 15<sup>th</sup> day of the month, which may have resulted in inaccurate age estimations. The mean and median within this age category were 12 and 10.5 days, respectively, indicating that observations in this age category were not close towards the reported end of the prepatent period (i.e. towards 20 days). Further studies to determine if presence of *T.vitulorum* eggs in calves less than 3 weeks old as found in this study is due to patent infection are suggested as well as determining the sensitivity of using faecal egg count analysis of a single or multiple faecal samples as diagnostic test for *T.vitulorum* infection.

Prevalence estimates were significantly different between the provinces with Luang Prabang the lowest at 10.8% and Luang Namtha the highest at 33.0%. A reason for this could include higher treatment rates as 56.1% of calves treated were in Luang Prabang province and no treatments were recorded in calves from Luang Namtha province. Treatment rates were very low (8.2%) and often ineffective, with 16.2% of treated calves having a positive FEC for *T.vitulorum*. Different husbandry practices not recorded could affect transmission or different climatic conditions affecting survival of *T.vitulorum* eggs in the environment and thus also contribute to the apparent geographical difference in prevalence in northern Laos, but these were not investigated.

Clinical signs including poor coat condition with eczema, stools resembling white scour, inappetance with intermittent colic and tympany have been reported as indicators of toxocariasis especially in buffalo calves (Roberts 1993) and we found this a common view amongst Lao animal health staff and farmers. However, more recent studies have described that calves with toxocariasis could have either pale coloured or black diarrhoea, or could appear clinically normal (Starke-Buzetti 2006, Jones et al. 2009). Our study supports these more recent studies, finding that 17.0% of sampled calves showed clinical signs of general sickness, 17.2% diarrhoea and 20.3% abnormal coat, but none of these factors were significantly associated with *T.vitulorum* infection status.



These clinical signs are non-specific and could occur with many other diseases or husbandry deficiencies, including external parasites, bacterial, protozoan or viral infections, other internal parasitic diseases or suboptimal nutrition. These disorders cause high calf morbidity and are likely to contribute significantly to negative production impacts in affected calves. Further studies will be required to determine the aetiology of these clinical signs and the relative contribution of *T.vitulorum* towards the overall clinical and financial impact of neonatal calf disorders.

When comparing the prevalence of infection between species, 25.5% of buffalo and 20.9% of cattle calves were found to be positive. Although the difference between species was not statistically significant in this survey ( $p = 0.107$ ), the trend for higher prevalence among buffalo calves than cattle calves aligns with literature and anecdotal reports from Lao farmers and livestock extension staff that suggest toxocariasis is more common in buffalo calves (Roberts 1993, Stür et al. 2002, Starke-Buzetti 2006). In Laos, the husbandry practices tend to expose both species to similar levels of environmental contamination as they are regularly grazed or confined together, and this may lead to more similar prevalence between species than reported elsewhere. However the severity of infection (as determined by EPG count analysis and assuming that higher EPG means higher worm burden) was clearly found to be higher among buffalo calves in our study ( $p < 0.001$ ). The reported pathogenic levels of EPG range from 500 to 30,000. This large range has arisen because the stage of infection was not considered when trying to determine pathogenic EPG levels (Roberts 1993). *T.vitulorum* egg output is not consistent throughout the patent period, peaking when the calf is about 5–7 weeks of age and tapering off due to a natural ageing process of the parasite as well as the maturing of the humoral and cell mediated immune response of the calf (Roberts 1993, Neves et al. 2003). None of the clinical signs investigated in this study were associated with infection status or level of infection. This may indicate that pathogenic effects producing clinical signs in Laos large ruminant calves requires higher egg counts, clinical signs manifest in older calves than we investigated or clinical signs are different than those we investigated (e.g. weight loss, reduced growth rates).

Nevertheless our findings indicate that it is inadequate to only treat calves showing clinical signs that may indicate a worm problem or only buffalo calves

due to the inconsistency of clinical signs and species with presence and level of *T.vitulorum* infection. Our study identified that it is possible that calves have patent infections at an early age (<21 days) or that farmers are uncertain about the correct age of their calves and therefore determining the correct age of calves is important as anthelmintics are considered an effective control by a single treatment of pyrantel between 14 to 21 days of age (Roberts 1993).

It is important to note that 76.8% of villages (n = 69) from which animals were sampled and tested had at least one positive calf, indicating widespread infection in northern Laos. Widespread infection and a prevalence of 22.5% at individual animal level are significant and likely to contribute to negative production impacts as well as continuous environmental contamination with eggs if left unmanaged. Toxocariasis is reported to cause large mortalities in calves (Starke-Buzetti 2006) and it is necessary to assess mortalities associated with *T.vitulorum* infection to estimate the full clinical and economic impact. Further studies have been completed by the same authors to assess calf mortality in northern Laos to better quantify the cost and impact of this disease. These studies are considered essential to enable more efficient development of a large ruminant livestock industry as a pathway from rural poverty for smallholder farmers in northern Laos.

### **3.6 Acknowledgments**

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## **PART 2:**

### **WHY IS A SIMPLE CONTROL OPTION FOR *TOXOCARA VITULORUM* NOT IMPLEMENTED BY CATTLE AND BUFFALO SMALLHOLDER FARMERS IN SOUTH-EAST ASIA?**

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**Statement of contribution of co-authors**

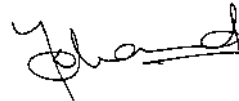
Luzia Rast, Jenny-Ann Toribio, Syseng Khounsy and Peter Windsor collaborated on the conceptualisation and design of this study. Luzia Rast conducted the field work and analysed the data with collaboration of Navneet Dhand. Luzia Rast prepared the paper for publication. Jenny-Ann L. M. L. Toribio, Navneet Dhand and Peter A. Windsor contributed to reviewing and editing the manuscript.



Luzia Rast



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### 3.7 Abstract

*T.vitulorum* infection in large ruminants is endemic in many tropical countries and particularly in South-East Asia. A single treatment of calves with pyrantel at 14-21 days of age effectively controls the parasite. Despite this treatment being readily available, *T.vitulorum* infection remains common and widespread. To understand drivers of effective control of *T.vitulorum* infection, we examined treatment practices and knowledge of smallholder farmers of this parasite plus determined annual calf morbidity and mortality and identified potential risk factors for these estimates. Interviews were conducted with 273 smallholder farmers who had calves tested for *T.vitulorum* 4-6 months earlier. Reproductive rates of 0.6 and 0.4 calf per annum in cattle and buffalo respectively, and annual calf morbidity and mortality of 42.6% (CI 0.38-0.47) and 37.3% (CI 0.33-0.42) respectively, were identified. Interviewed farmers had either none (80.6%) or only minimal (19.4%) knowledge about *T.vitulorum* and only 2.5% of the farmers treated their calves for *T.vitulorum* using the recommended control regime. Multivariable logistic regression analyses with random effects showed that the number of adult cattle per household, *T.vitulorum* infection status of the household herd and farmer knowledge of *T.vitulorum* were significantly ( $p < 0.05$ ) associated with calf morbidity and mortality. Financial analysis using partial budgeting showed a net benefit of USD 3.69, 7.46, 11.09 or 14.86 per calf when treating calves with pyrantel and attributing 25%, 50%, 75% or 100% of morbidity and mortality to *T.vitulorum* infection. The study identified that poor reproduction, high calf morbidity and mortality combined with very limited farmer knowledge and effective control of endemic toxocariasis contribute to suboptimal large ruminant production in mixed smallholder farming systems in South-East Asia. The large net benefit per calf achievable by a single pyrantel treatment should drive implementation of this intervention by smallholder farmers, especially as demand for livestock products continues to increase in this region and forces a change to more production oriented farming rather than keeping large ruminants as an asset. To support this, continued capacity building that ensures knowledge transfer of best practice *T.vitulorum* control to smallholder farmers is required.

**Key words** *Toxocara vitulorum*, Cattle, buffalo, calf morbidity, calf mortality, smallholder farming systems, partial budget analysis, Lao PDR, developing countries

### **3.8 Introduction**

In South-East Asia but especially in the less developed countries of the region, agricultural production is dominated by smallholder farming systems that are mixed enterprises producing crops (predominantly rice) and livestock. The farmers use basic traditional farming methods operating at subsistence levels. They face many constraints, including low land availability, isolation, poor infrastructure, limited knowledge of livestock production and animal health practices, endemic diseases, low capacity animal health systems, poorly developed market systems and widespread poverty. These constraints are evident in the mountainous north of Lao PDR (Lao People's Democratic Republic, or Laos). Smallholders typically own 6-7 cattle and/or buffalo (Nampanya et al. 2010), may have access to between 1.1 and 1.8 ha of unirrigated land and rely on farming for their livelihood (Lao Department of Statistics 2010). Cattle and buffalo are an important asset, providing manure for fertilising crops, meat, draft power and most importantly a cash reserve. Animals are sold when the household requires cash rather than for optimal returns. Despite the importance of large ruminants, animal health inputs are minimal and supplementary feeding or routine animal health preventative measures such as vaccination are rarely practised. Records are seldom kept resulting in a lack of baseline production information (including reproductive performance, calf morbidity and mortality) to inform understanding of the clinical and financial impact of diseases such as *T.vitulorum*.

There is an increasing demand for animal products including red meat in South-East Asia. This is driven by the rapidly growing regional economies and urbanisation, both of which are projected to continue (Delgado 1999, Steinfeld et al. 2006). An opportunity exists for smallholder farmers in the region to increase large ruminant productivity and their income by the provision of more and better quality large ruminants to this market (Windsor 2011). However this requires a change from traditional low input/low output livestock production systems to more productive livestock farming using modern technologies that enable livestock to generate income rather than being an asset for storage of wealth.

*Toxocara vitulorum* is an endemic nematode infection of young (< 3-4 months) cattle and buffalo calves in tropical regions. A major impact of *T.vitulorum*

infection is the associated mortalities as well as morbidity, leading to uncompensated stunted growth in calves that survive (Starke-Buzetti 2006). Control of *T.vitulorum* may provide a simple intervention for smallholder farmers to increase productivity of their large ruminants. This is especially as a single treatment of pyrantel given to calves when they are 14-21 days old effectively controls the parasite and reduces environmental contamination with eggs (Roberts 1993). Pyrantel is readily available in South-East Asia and is relative cheap with a dose for a calf costing USD 0.25-0.37. However widespread effective and continuous control of *T.vitulorum* is lacking, with a survey conducted between September 2009 and June 2010 in northern Laos reporting prevalence of 22.6% in cattle and buffalo calves <3 months of age and widespread infection with 76.8% of villages where calves were sampled having at least one positive calf (Rast et al. 2013).

The objective of this study in northern Laos was to (1) assess smallholder farmer knowledge and practices of large ruminant production and management of animal diseases especially *T.vitulorum*, (2) assess calf morbidity and mortality and its association with *T.vitulorum* infection and farmer knowledge and practices and (3) analyse the financial impact of *T.vitulorum* treatment of calves.

## **3.9 Materials and methods**

### **3.9.1 Study design and area**

Face-to-face interviews were conducted with 273 or 45.0% of smallholder farmers that owned calves tested for *T.vitulorum* 4-6 months earlier, located in 34 villages in 17 districts of the five northern Laos provinces of Bokeo, Houaphan, Luang Namtha, Luang Prabang and Xieng Khuang. Villages were randomly selected using a random generator calculator (<http://www.random.org>) from those that had participated in the prior prevalence survey and had more than eight households with calves tested for *T.vitulorum*. In each selected village, eight households were randomly selected from the list of households that had calves sampled previously. Lao Department of Livestock and Fisheries (DLF) district staff arranged permission to visit the village with the local authorities and the village headmen.

### **3.9.2 Questionnaire and interviews**

The questionnaire was designed in English, translated into Lao and pilot tested with two DLF district extension staff to ensure appropriate local terminology especially related to *T.vitulorum* was used. The final questionnaire contained 21 open and closed questions that aimed to elicit data on the location of the household; household income for the past five years; number, age, gender, species and monetary value of large ruminants owned; number of calves born to each female whilst owned; calf morbidity and mortality over the last 12 months; and knowledge and practices of farmers about *T.vitulorum* and control. A copy of the questionnaire is available from the author. Interviews were conducted face-to-face in Lao with the household member responsible for the large ruminants in July and August 2010. Interviews took approximately one hour per farmer to complete and responses were recorded in Lao. The interview team consisted of the same two researchers for all interviews, plus one or two different DLF district staff in each of the 17 districts where interviews were conducted. The local DLF staff members of each district were trained on the purpose of the study, the interview process and the questionnaire on the day before the interviews were conducted in their district. Prior to the interview, an explanation was provided to the farmers about the survey purpose and the importance of providing accurate answers to the questions and stating if they were unsure or did not know an answer.

### **3.9.3 Data entry and management**

Completed interviews were translated into English by an independent translator and data entered into a customised database in Microsoft Access 2003 (Microsoft Cooperation, Redmond, WA, USA) by the senior author.

### **3.9.4 Estimation of reproductive performance, calf mortality & morbidity**

Smallholder farmers in northern Laos generally do not keep written records of their large ruminant production inputs and outputs. Annual calving rate for the 12 months preceding the interview was estimated from the reported number of calves born and the number of female cattle and buffalo  $\geq 36$  months old owned by the interviewed farmers on day of interview, as the youngest age at first



calving reported was 36 months for cattle and buffalo and annual large ruminant numbers were not available.

Annual calf morbidity and mortality was computed by dividing the total number calves born by the number of calves reported to have been sick or to have died respectively in the previous 12 months.

### **3.9.5 Statistical analyses**

Statistical analyses were conducted using SAS version 9.3 (© SAS 2002-2010, SAS Institute Inc., Cary, NC, USA). A descriptive analysis was performed on all variables using frequency tables. Associations were separately investigated between 10 categorical explanatory variables (Table 3.4) and the three binary outcome variables of (1) household herd *T.vitulorum* status (positive/negative), (2) household herd calf morbidity over last 12 months (yes/no) and (3) household herd calf mortality over the last 12 months (yes/no). *T.vitulorum* status and household calf morbidity were also explanatory variables investigated for household calf morbidity and mortality or household *T.vitulorum* status and mortality outcomes respectively. The *T.vitulorum* status of each household large ruminant herd was determined positive if more than one cattle or buffalo calf had a positive faecal egg count for *T.vitulorum* in the calf prevalence study preceding the farmer interviews. Calf morbidity and mortality status of the household herd were determined from the farmer responses in the interviews and classed as 'yes' if more than one cattle or buffalo calf was reported ill or dead, respectively in the last 12 months.

For each outcome, unconditional association was assessed based on the likelihood ratio chi-square tests using univariable logistic regression models with UniLogistic SAS Macro (Dhand 2009). For explanatory variables with an unconditional p-value of  $\leq 0.25$  and  $< 10\%$  missing values, pairs were tested for collinearity using Spearman rank coefficient; and for significant associations (coefficient  $> 0.7$ ) one variable of a collinear pair was selected for further investigation based on biological plausibility. Subsequently a multivariable logistic regression model was built using manual forward stepwise approach employing MultiLogistic SAS Macro (Dhand 2009) and variables with p-values  $\leq 0.05$  were retained. To assess the effect of clustering among outcome observations from the same province, district or village, each were added as

random effect terms to the final model for each outcome and the model refitted using the GLIMMIX procedure in SAS.

**Table 3.4 Frequency of explanatory variables investigated for the three binary outcome variables of smallholder household status of *Toxocara vitulorum* infection (n=264)<sup>1</sup>, calf morbidity (n= 251)<sup>1</sup> and calf mortality (n=240)<sup>1</sup> in northern Laos, 2010**

Variable Categories	Household <i>T.vitulorum</i> infection <sup>2</sup>		Household calf morbidity		Household calf mortality	
	Yes (%)	No (%)	Yes (%)	No (%)	Yes (%)	No (%)
<i>Farmer has heard about T.vitulorum</i>						
No	31 (11.8)	66 (25.0)	41 (16.3)	51 (20.3)	29 (12.1)	59 (24.6)
Yes	65 (24.6)	102 (38.6)	78 (31.1)	81 (32.3)	57 (23.8)	95 (39.6)
<i>Farmer T.vitulorum knowledge<sup>3</sup></i>						
No knowledge	74 (28.0)	143 (54.2)	106 (42.2)	101 (40.2)	77 (32.1)	120 (50.0)
Minimal knowledge	21 (8.0)	26 (9.8)	13 (5.2)	31 (12.4)	9 (3.8)	34 (14.2)
Good knowledge	0	0	0	0	0	0
<i>Farmer assessment of T.vitulorum status of own calves</i>						
Unsure	30 (11.4)	55 (20.8)	38 (15.1)	40 (15.9)	29 (12.1)	45 (18.8)
Infected	27 (10.2)	52 (19.7)	31 (12.4)	47 (18.7)	21 (8.8)	54 (22.5)
Not infected	38 (14.4)	62 (23.5)	50 (19.9)	45 (17.9)	36 (15.0)	55 (22.9)
<i>Does farmer treat for T.vitulorum</i>						
No	42 (15.9)	62 (23.5)	56 (22.3)	40 (15.9)	41 (17.0)	49 (20.4)
Yes	53 (20.1)	107 (40.5)	63 (25.1)	92 (36.7)	45 (18.7)	105 (43.8)
<i>Correct treatment for T.vitulorum<sup>4</sup></i>						
No	94 (35.6)	163 (61.7)	117 (46.6)	127 (50.6)	84 (35.0)	149 (62.1)
Yes	1 (0.4)	6 (2.3)	2 (0.8)	5 (2.0)	2 (0.83)	5 (2.1)
<i>Household calf morbidity<sup>5</sup></i>						
No	51 (19.3)	69 (26.1)	-	-	0	132 (55.0)
Yes	44 (16.7)	100 (37.9)	-	-	86 (35.8)	22 (9.2)
<i>Number of adult cattle per household</i>						
None	16 (6.1)	28 (10.6)	18 (7.2)	24 (9.6)	12 (5.0)	27 (11.3)
<3	27 (10.2)	44 (16.7)	23 (9.2)	47 (18.7)	15 (6.3)	54 (22.5)
3-5	29 (11.0)	51 (19.3)	33 (13.1)	42 (16.7)	24 (10.0)	49 (20.4)
>5	23 (8.7)	46 (17.4)	45 (17.9)	19 (7.6)	35 (14.6)	24 (10.0)

Variable Categories	Household <i>T.vitulorum</i> infection <sup>2</sup>		Household calf morbidity		Household calf mortality	
	Yes (%)	No (%)	Yes (%)	No (%)	Yes (%)	No (%)
<i>Number of adult buffalo per household</i>						
None	36 (13.6)	66 (25.0)	41 (16.3)	56 (22.3)	29 (12.1)	67 (27.9)
<3	26 (9.8)	36 (13.6)	27 (10.8)	35 (13.9)	19 (7.9)	39 (16.3)
3-5	18 (6.8)	47 (17.8)	35 (13.9)	26 (10.4)	26 (10.8)	31 (12.9)
>5	15 (5.7)	20 (7.6)	16 (6.4)	15 (6.0)	12 (5.0)	17 (7.1)
<i>Species of large ruminants in household</i>						
Cattle only	35 (13.3)	65 (24.6)	41 (16.3)	54 (21.5)	29 (12.1)	65 (27.1)
Buffalo only	15 (5.7)	27 (10.2)	18 (7.2)	22 (8.8)	12 (5.0)	25 (10.4)
Cattle and buffalo	45 (17.0)	77 (29.2)	61 (24.3)	55 (21.9)	46 (19.2)	63 (26.3)
<i>Average household income for 2005-2009</i>						
< 312USD	36 (13.9)	61 (23.6)	40 (16.3)	53 (21.5)	31 (13.2)	58 (24.7)
313-615USD	20 (7.7)	40 (15.4)	28 (11.4)	31 (12.6)	20 (8.5)	36 (15.3)
616-1000USD	22 (8.5)	31 (12.0)	26 (10.6)	23 (9.3)	15 (6.4)	32 (13.6)
> 1000USD	15 (5.8)	34 (13.1)	21 (8.5)	24 (9.8)	17 (7.2)	26 (11.1)
<i>Household <i>T.vitulorum</i> infection<sup>4</sup></i>						
Negative	-	-	68 (27.1)	92 (36.7)	45 (18.8)	110 (45.8)
Positive	-	-	51 (20.3)	40 (15.9)	41 (17.1)	44 (18.3)

<sup>1</sup> *T.vitulorum* infection status of 264 interviewed households could be determined by matching against records from the calf prevalence survey between September 2009 and June 2010. Complete data for herd calf morbidity was recorded for 251 households and for mortality from 240 households.

<sup>2</sup> Household *T.vitulorum* infection was categorised as positive if  $\geq 1$  calf had a positive faecal egg count test in the prevalence survey completed between September 2009 and June 2010 (Rast et al. 2013)

<sup>3</sup> Farmers answers to what they knew about *T.vitulorum* were categorised as 'no knowledge' if farmers answered nothing or only knew the name of the parasite or disease or had wrong knowledge (i.e. stated it was a viral disease); as 'minimal knowledge' if their answers showed they knew it to be a parasite of cattle and buffalo calves or some clinical signs or that control methods were available ; and as 'good knowledge' if the farmers knew the aetiology and major clinical signs and control methods and basic epidemiology features.

<sup>4</sup> Answers were categorised a 'correct treatment' if pyrantel was used when calves were 14-21 days old at a dose rate of 125mg/10kg bodyweight any other answers were categorised as 'incorrect treatment'

<sup>5</sup> Household calf morbidity was categorised as 'yes' if farmers reported  $\geq 1$  calf as having been sick in the preceding year.

### 3.9.6 Financial analysis

Partial budgeting (PB) was used to estimate the net benefit of treating calves for *T.vitulorum* by comparing no treatment for *T.vitulorum* with the alternate practice of treating all calves with a single dose of pyrantel at 14-21 days old. To calculate the net benefit for the alternate practice, we calculated (1) additional returns, (2) reduced costs, (3) returns forgone and (4) additional costs using a purpose built spread sheet model in Microsoft Excel (Microsoft 2010) with input values and source presented (Table 3.5).

**Table 3.5 Input values of the partial budget model for single pyrantel treatment of large ruminant calves in northern Lao smallholder farming systems, 2010**

Input value (unit)	Value	Lower quartile	Upper quartile	Median	Source
<i>T.vitulorum</i> prevalence (%)	22.6	-	-	-	Rast et al. 2013
Annual calf morbidity (%)	42.6	-	-	-	Farmer interviews
Annual calf mortality (%)	37.3	-	-	-	Farmer interviews
Value per kg calf live weight (USD)	1.50	-	-	-	Phonvisay 2012
Pyrantel cost/calf (USD)	0.30	-	-	-	DLF purchasing unit
Labour cost/hour (USD)	1.25	-	-	-	Farmer interviews
Labour requirement for sick calves (h/day)	1.5	-	-	-	Farmer interviews
Labour requirement pyrantel application (h/calf)	0.016	-	-	-	Farmer interviews
Daily weight gain of calves (kg)	0.18	-	-	-	Phonvisay 2012
Duration of illness (days)	14.7 <sup>a</sup>	3	15	8	Farmer interviews
Medication cost for treatment of sick calf per day (USD)	1.78 <sup>a</sup>	0	0.89	0.25	Farmer interviews
Calf (<12 months old) value (USD)	117 <sup>a</sup>	75	125	100	Farmer interviews

<sup>a</sup> mean

Additional returns, being returns obtained when treating calves against *T.vitulorum* infection with pyrantel, were the value of the surviving calves (product of the mean calf value, *T.vitulorum* prevalence and annual mortality), and the value of healthier and heavier calves (product of mean duration of illness, estimated mean daily weight gain, live weight value per kg, annual morbidity and *T.vitulorum* prevalence) attributable to the treatment.

Reduced costs, being costs of treating illnesses of *T.vitulorum* infected calves avoided by calf pyrantel treatment, were the value of calf treatments (product of mean duration of illness, daily medication cost per calf, annual calf morbidity and

*T.vitulorum* prevalence), and the labour cost (product of average duration of illness, hourly labour cost, mean time per day to care for sick calves, annual morbidity and *T.vitulorum* prevalence).

Returns forgone, being the returns from not treating calves with pyrantel that will not be received when treating calves, were considered nil as farmers did not sell or consume dead calves hence there was no salvage value. Additional costs, being the cost of treating calves with pyrantel and of other costs incurred subsequent to pyrantel treatment not incurred when there is no treatment for *T.vitulorum* (product of the value of pyrantel calculated to dose a 25 kg calf and labour cost for administration). Feed and animal health costs for extra calves were considered zero as currently cattle and buffalo do not receive supplementary feed or routine preventative animal health inputs such as vaccination.

The net benefit was calculated as follows: (additional returns + reduced costs) – (returns forgone + additional costs). Assumptions were that pyrantel treatment was 100% effective in reducing *T.vitulorum* faecal egg counts or worm burden (Roberts 1993, Starke-Buzetti 2006) supported by a field treatment trial in four northern Laos villages (unpublished), that all calves infected with *T.vitulorum* showed clinical disease, and that the level of mortality and morbidity caused by *T.vitulorum* was constant over the year.

The aetiology of calf morbidity and mortality is generally multifactorial and it is difficult to determine and quantify the contribution of each. This study did not allow us to quantify the level of calf morbidity and mortality caused by *T.vitulorum* or reduced by pyrantel treatment and there is a paucity of literature on the subject, suggesting mortality rates between 40-80% (Starke-Buzetti 2006). Hence a sensitivity analysis was applied, assuming rates of 25, 50, 75 and 100% of morbidity and mortality being due to *T.vitulorum* infection. Further, monetary value of calves <12 months old, daily medication cost per calf used by farmers other than pyrantel, and duration of illness, were dynamic measures and lower and upper quartiles were used to conduct a sensitivity analysis for each of these inputs. Monetary data was collected in local currency of Lao Kip (LAK) and an exchange rate of 1 US Dollar (USD) = 8000 LAK was used.

### **3.10 Results**

#### **3.10.1 Reproductive performance, calf mortality and morbidity**

The 273 interviewed farmers reported a total of 517 calves born to 959 female cattle and buffalo  $\geq 36$  months old in the 12 months prior to the interviews. The 362 female buffalo had a total of 146 calves or 0.40 calf annually per female buffalo and the 597 female cattle had a total of 371 calves or 0.62 calf annually per female cow.

Of the 517 live born calves, 51 (9.9%) were found dead and 220 (42.6%) became ill showing a range of clinical signs. Data on the outcome were recorded from 200 ill calves, with 142 (71.0%) dead, 52 (26.0%) fully recovered, and 6 (3.0%) partially recovered. Annual calf morbidity was 42.6% (CI 0.38-0.47) and annual calf mortality was 37.3% (CI 0.33-0.42).

Clinical signs reported in sick calves were: diarrhoea in 94 (42.7%); anorexia in 53 (24.1%); weight loss in 35 (15.9%); depression/lethargy in 19 (8.6%); swollen neck in 14 (6.4%); dermatitis in 10 (4.6%); unhealthy rough coat in 8 (3.6%); lameness or distended abdomen in 7 (3.2%); vesicular lesions on feet or mouth in 6 (2.7%); fever in 3 (1.4%); constipation in 2 (0.9%), and respiratory signs in 1 (0.5%). Farmers reported unspecific signs such as 'unwell', 'sick calf' or 'not observed' for 45 (20.5%) sick calves. Multiple clinical signs were recorded in 83 sick calves and a single clinical sign (mostly diarrhoea) was recorded in 128 sick calves.

#### **3.10.2 Farmer knowledge and practices**

Information on treatment was available from 186 sick calves belonging to 66 farmers. Of these sick calves, 106 (57.0%) were treated and 80 (43.0%) were not. Records on the outcome of 103 treated calves were available; 51 (49.5%) died, 46 (44.7%) recovered fully and 6 (5.8%) recovered partially. Of the 76 untreated calves with outcomes recorded; 70 (92.1%) died, 4 (5.2%) recovered fully and 2 (2.6%) recovered partially. Types of medication used in sick calves were: traditional medicines/plants in 29 (27.4%); antibiotics in 13 (12.3%); anthelmintics in 2 (1.9%); and vitamins in 1 (0.9%). Notably for 61 (57.5%) calves reported as treated, the owner was unsure what medication was used.

The different aetiologies of disease farmers suspected in sick calves were: *T.vitulorum* infection in 29 (13.2%); wildlife attack/accident in 11(5.0%); foot and mouth disease or haemorrhagic septicaemia in 6 (2.7%) for each; malnutrition in 4 (1.8%); dystocia and snake bite in 2 (0.9%) for each; and cold weather, dermatitis and external parasites in 1 (0.5%) for each. For 157 (71.4%) sick calves, farmers were uncertain of the aetiology. Disease confirmation through submission of diagnostic samples and laboratory testing did not occur for any of the ill calves. The mean duration of illness per calf reported was 14.7 days (n=174; range 1-365 days) and the mean daily treatment cost was USD 1.78 per calf (range USD 0-31.25).

Of the 273 interviewed farmers, 225 (80.6%) had only heard of or had no knowledge of toxocariasis in cattle and buffalo. The remaining 48 (19.4%) farmers had minimal knowledge (mostly some knowledge of clinical signs). None of the interviewed farmers displayed good basic knowledge of aetiology, that it affects young cattle and buffalo calves, causes significant morbidity and mortality and that a single anthelmintic treatment controls infection. When asked if they treated their calves for *T.vitulorum*, 160 (60.6%) farmers answered affirmatively with 104 (39.4%) replying negatively. The farmers who answered 'yes' were then asked what was the name of the medication used was; 55 (33.0%) did not know, 49 (29.3%) used pyrantel, 31 (19.4%) mebendazole; 10 (6.0%) bendazole, 8 (4.8%) children's de-wormer, 3 (1.8%) santonin, 2 (1.2%) antibiotics, 2 (1.2%) white chalk, and 4 (2.4%) coconut bark, fermented fish or an unidentified tree fruit. Only 7 (2.5%) farmers used pyrantel at recommended dose (125mg/10kg) and age (14-21 days) of the calf to control *T.vitulorum*.

### **3.10.3 Univariable and multivariable logistic regression analyses**

Frequencies of investigated variables (Table 3.4) and the results of the final logistic regression models (Table 3.6 and 3.7) are presented. Farmer records from the earlier prevalence study and the farmer interviews were linked and resulted in 264, 251 and 240 complete records available for investigating the three outcomes of household herd *T.vitulorum* status, calf morbidity and calf mortality respectively. For the multivariable conditional analysis adult number of cattle, farmer knowledge about *T.vitulorum* and household herd *T.vitulorum* status were significantly ( $p<0.05$ ) associated with both household herd calf morbidity and mortality.

Calf morbidity was the only variable significantly ( $p=0.045$ ) associated with *T.vitulorum* status however after the random effect terms were added to the model the association became non-significant ( $p=0.075$ ).

**Table 3.6 Results of final logistic regression models for the outcome variable of large ruminant household calf morbidity in a farmer survey in northern Laos, 2010 (n=251)**

Explanatory variables	Categories	b	SE(b)	OR	95% CI	p-value
Intercept		-0.29	0.43			
Household <i>T.vitulorum</i> infection						0.035
	Negative	0.00		1.00		
	Positive	0.65	0.31	1.91	1.05, 3.48	
Farmer <i>T.vitulorum</i> knowledge						0.036
	None	0.00		1.00		
	Minimal	-0.85	0.40	0.43	0.19, 0.95	
Adult cattle in household herd						0.030
	0	0.00		1.00		
	1 to 2	-0.38	0.44	0.68	0.29, 1.63	
	3 to 5	0.02	0.44	1.02	0.43, 2.45	
	$\geq 6$	0.86	0.48	2.36	0.91, 6.12	

Covariance parameter estimates (standard errors): Province= 0.13 (0.23); District = 0.09 (0.23); Village = 0.32 (0.31)

**Table 3.7 Results of final logistic regression models for the outcome variable of large ruminant household calf mortality in a farmer survey in northern Laos, 2010 (n=238)**

Explanatory variables	Categories	b	SE(b)	OR	95% CI	p-value
Intercept		-1.00	0.48			
Household <i>T.vitulorum</i> infection						0.004
	Negative	0.00		1.00		
	Positive	0.96	0.33	2.60	1.35, 4.99	
Farmer <i>T.vitulorum</i> knowledge						0.042
	None	0.00		1.00		
	Minimal	-0.93	0.46	0.40	0.16, 0.97	
Adult cattle in household herd						0.008
	0	0.00		1.00		
	1 to 2	-0.54	0.51	0.59	0.21, 1.60	
	3 to 5	0.07	0.50	1.07	0.40, 2.87	
	$\geq 6$	1.03	0.53	2.81	0.99, 8.03	

Covariance parameter estimates (standard errors): Province= 0.084 (0.21); District = 0.12 (0.30); Village = 0.48 (0.39)



### 3.10.4 Financial analysis

The results of the partial budget are presented (Table 3.8). The analysis indicated that treating calves with a single dose of pyrantel returned a positive net benefit of USD 14.86, 11.09, 7.46 or 3.69 per calf to the smallholder farmer assuming 100%, 75%, 50% or 25% of morbidity and mortality was due to *T.vitulorum* infection respectively. This represents a return on invested funds (additional costs) of 4469, 3336, 2243 or 1110% respectively. The results of partial budget sensitivity analysis using the upper and lower quartile of the dynamic input values (daily medicine value, duration of illness and calf value) showed that the net benefit remained positive.

**Table 3.8 Partial budget analysis results (net profit in USD/calf) and sensitivity analysis for treating cattle and buffalo calves in northern Laos with a single dose of pyrantel when 14-21 days old in 2010**

Input value of three dynamic variables set at:	Percentage of calf mortality and morbidity attributed to <i>T.vitulorum</i> infection			
	100%	75%	50%	25%
Mean	14.86	11.09	7.46	3.69
Lower quartile	6.56	4.88	3.21	1.52
Upper Quartile	14.55	10.87	7.30	3.62
Median	9.89	7.38	4.91	2.40

### 3.11 Discussion

To our knowledge this study is the first to quantify calf morbidity and mortality in smallholder farming systems on a large scale in this region, identifying annual calf morbidity of 42.6% and mortality of 37.3% in calves up to 12 months old which reflects a substantial production impact. This impact is exacerbated by the low reproductive performance of 0.4 and 0.6 calf per year for buffalo and cattle respectively. The magnitude of low reproductive rates is probably mostly attributable to minimal and poor reproductive management rather than diseases or genetics. Controlled breeding, castration of male animals and separation of males and females in the non-breeding season are not practised, resulting in year-round births with a peak between October and February. Pregnancy testing is not done and large ruminants are often kept on common grazing land or in forests away from villages where pregnant females are not observed daily even when close to parturition. Calves are naturally weaned and infertile cows or bulls are not culled.

A recent assessment of livestock production in the Mekong region (Teufel et al. 2010) collated results from a small number of studies and reported calf (aged  $\leq 12$  months) mortalities of 9.0% (cattle) and 30.0% (buffalo), fertility rates of 0.6 (buffalo) and 0.8 (cattle) calf per year, and first calving age of 4 years (buffalo) and 2.5 years (cattle). A small study in two regions of northern Laos (Dideron et al. 2000) found calf mortality of 16.5% (cattle) and 31.5% (buffalo), fertility rates of 0.5 calf (buffalo) and 0.7 calf (cattle) per year, with first calving age of 5 years (buffalo) and 4 years (cattle). Our study showed higher calf mortality and similar fertility rates but lower first calving ages, especially for buffalo. There is a paucity of published information on large ruminant production in comparable production systems and climates of South-East Asia and the few reported studies used varied methodologies, making comparison difficult. This probably reflects the challenges in conducting research in developing countries and lack of production benchmark recording.

Lack of village livestock population data, production records and limited staff, resources and access to villages as well as all year calving restricted the approach to sampling that could be implemented for the prevalence survey and subsequently for this study. Villages were selected from a list of villages that had been selected to participate in a large livestock development project by local experts which might have introduced some selection bias. However, random selection of villages for this study would have ensured that our sample is representative of the sampling frame, although not that of all villages in the selected districts. Households and their calves within these villages were selected dependent on having calves of specified age ( $< 90$  days) during the prevalence survey period between September 2009 and June 2010 (Rast et al. 2013). This may have also led to some selection bias by excluding households that had no young large ruminant calves during the prevalence survey period or villages and households that did not meet the original selection criteria for the livestock development project (i.e. high level of poverty, traditional villages, all year road access). However as we conducted interviews with a large sample of farmers (273) selected randomly from the prevalence study participants from across northern Laos, their geographic distribution reflected a cross-section of mixed rain fed smallholder farming systems in northern Laos. Data were collected through a single farmer survey and as Lao farmers rarely keep records of farm production, misclassification of some variables by farmers providing inaccurate

values is possible. As infrastructure, livestock production and animal health capacity continues to improve in developing countries, future surveys should be able to apply sampling methods that lead to improved validity of results. Longitudinal studies would be required to further define production parameters and especially reproductive performance.

Calf morbidity and mortality are usually multifactorial in aetiology and also influenced by husbandry practices, diagnostic capacities and farmer knowledge. Lack of knowledge on diseases and diagnostic capacity is clearly evident with 71.4% of farmers being uncertain about the aetiology of calf morbidity within their herds and no laboratory diagnostic confirmation. Diarrhoea was the most common (42.7%) reported clinical sign in sick calves and is a clinical sign produced by numerous conditions including infectious diseases, internal parasites and nutrition. Further studies to determine the various causes of calf diarrhoea in the region are needed.

Whilst the mortality of sick calves that were treated was almost half that of untreated calves (49.5% versus 92.1%), treatment of sick calves was still unsuccessful in almost half of treated calves. Reasons most likely include lack of farmer knowledge about calf diseases, lack of animal health personnel, and little or no diagnostic capacities. It is also likely that effective medication is not readily available in some villages, that smallholder farmers are reluctant to purchase medications as they are not aware of the benefits of effective treatments, and that some farmers prefer to use traditional treatment methods that may or may not be effective. This is supported by 27.4% of farmers reporting their use of traditional medicinal plants for treatment of sick calves and 57.5% of farmers not knowing what medication was used to treat their sick calves. In this latter situation, farmers probably sought advice from their local DLF government extension staff, local village animal health worker or a local pharmacist who prescribed a treatment but without proper instruction about medication and dosage. This reinforces the need for continued efforts in capacity building and ensuring knowledge transfer to all livestock production stakeholders. The relative high survival rate of treated calves compared to untreated calves may also reflect supportive treatment (i.e. water, nutrition, shelter) these calves received.

Despite *T.vitulorum* being a common and widespread disease in northern Laos our study confirms that smallholder farmers have very limited knowledge of the disease, with none of the interviewed farmers having good knowledge, 19.4% having minimal knowledge and 80.6% having no knowledge or only awareness of the disease name. Most importantly there was a lack of knowledge on effective *T.vitulorum* control with only 2.5% of interviewed farmers using the recommended control of 125mg/10kg pyrantel when calves are 14-21 days old (Roberts 1993). A recent study to assess changes in farmer knowledge over time in northern Laos (Nampanya et al. unpublished) provided some evidence of improved knowledge on cattle and buffalo diseases among extension staff and farmers that participated in training programs over a three year period. However farmers in this study did not report changed practices despite having been part of a prevalence survey that included several visits by DLF extension staff and survey results made available to DLF staff for reporting to farmers. This demonstrates the lack of knowledge transfer especially to farmer level. Continued training and capacity building for all stakeholders and institutions involved in large ruminant production is required.

The logistic regression model results support that *T.vitulorum* infection contributes significantly to calf illness and death with results showing 1.91 or 2.60 times higher odds of calf morbidity and mortality when the household herd had a positive *T.vitulorum* status. Whilst it was not possible to quantify *T.vitulorum* associated morbidity and mortality in our study, farmers in four villages where calves were treated for *T.vitulorum* as part of a large ruminant health and production research project (Windsor 2006) noted reduction of calf mortalities up to 100% compared to when calves were not treated.

Species was significantly ( $p < 0.05$ ) associated with calf morbidity and mortality in the household herd; and households having higher numbers of cattle had around two to three times higher risk of calf illness or mortality. Higher cattle densities combined with poor husbandry practices (including poor hygiene) common in northern Laos probably increase disease susceptibility for calves. The models further indicated that even minimal farmer knowledge about *T.vitulorum* decreased the risk of calf morbidity and mortality by about half (OR 0.43 and 0.40). It is feasible that farmers with some knowledge were likely to treat sick calves; and whilst only a small proportion (2.5%) of farmers used the

recommended *T.vitulorum* treatment, the alternate treatments used or the supportive care given to ill calves probably contributed to the reduced risk of morbidity and mortality.

Partial budget (PB) analysis quantifies financial consequences of a change in farming management and as such is a tool that aims to identify practices that will maximise returns of farming. Our PB analysis indicated a considerable net benefit per calf and return on investment indicating that *T.vitulorum* treatment of calves has a positive financial consequence if adopted. Increased financial benefit as a driver for change will only lead to changes in large ruminant management if income generation is a predominant reason for keeping these animals. As economic growth and increased demand for red meat is predicted to continue, pressure on smallholder farming systems to supply this market is likely to increase as well. Therefore it is feasible that economic drivers will eventually assist in production improvements, including adoption of disease control within smallholder livestock production systems. Our results identified the lack of knowledge transfer to farmers about effective *T.vitulorum* treatment despite local extension employees working with farmers, the chemical readily available and a positive net benefit if used. This indicates that drivers that lead to sustained adoption need to be explored and implemented to ensure current high calf morbidity and mortality is addressed for better production. We suggest that the *T.vitulorum* extension message could provide an ideal entry point to engage smallholder farmers in large ruminant production improvement as it is a technology that has large potential financial benefits, is simple to use and readily available.

### **Conflict of interest**

None

### **3.12 Acknowledgments**

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## **CHAPTER 4**

### *FASCIOLA GIGANTICA* IN CATTLE AND BUFFALO IN NORTHERN LAOS

## **PART 1:**

### ***FASCIOLA GIGANTICA* PREVALENCE AND CLINICAL IMPACT IN LARGE RUMINANTS IN NORTHERN LAO PDR: FIELD AND SLAUGHTERHOUSE SURVEYS**

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**Statement of contribution of co-authors**

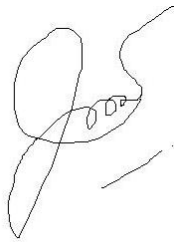
Luzia Rast, Jenny-Ann Toribio, Syseng Khounsy and Peter Windsor collaborated on the conceptualisation and design of this study. Verity Ambler, Sonevilay Nampanya and Luzia Rast designed the pilot survey and conducted the field work. Luzia Rast analysed the faecal samples and data from the main field surveys and prepared the paper for publication. Verity Ambler, Sonevilay Nampanya, Jenny-Ann L. M. L. Toribio and Peter A. Windsor contributed to reviewing and editing the manuscript.



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## 4.1 Abstract

Slaughterhouse and field surveys for evidence of *Fasciola gigantica* infection were conducted in five northern provinces of Laos during 2010 and 2011. The field survey of single faecal samples from 1262 cattle and buffalo ( $\geq 12$  months old) from 75 randomly selected villages, examined for fasciola eggs using a sedimentation method identified that infection was widespread with 73.3% of villages having at least one faecal egg count positive animal. The slaughterhouse survey examined 125 animals pre- and post-slaughter with faecal samples collected, livers examined for gross lesions and fluke presence. Prevalence estimates for the field survey were 17.2% (CI 0.14-0.21) and for the slaughterhouse survey 34.1% (CI 0.26-0.42). Of the 123 examined livers at necropsy 87 (70.7%) had gross liver and bile duct lesions characterised as mild (22.8%), moderate (17.9%) or severe (30.1%) pathology consistent with *F.gigantica* infection. Higher rates of gross hepatopathy were observed in buffalo compared to cattle, being 95.6% (n=68) and 40.0% (n=55) respectively. Of note 50 (63.3%) animals had liver damage (36 or 72% buffalo and 14 or 28% cattle) but were not found to have *F.gigantica* eggs in the faeces. Regression analysis was performed on data collected during the field and slaughterhouse surveys. Province was significantly associated ( $p < 0.0001$ ) with *F.gigantica* infection status, suggesting geographical differences in prevalence of *F.gigantica* in northern Laos. Cattle showed moderately lower risk (OR 0.7) of being infected in the field survey and lower risk of having liver damage (OR 0.02) in the slaughterhouse survey compared to buffalo. Whilst suboptimal production is caused by a multitude of factors our findings suggest that control of *F.gigantica* in cattle and buffalo should be considered as the levels of infection found are likely to result in substantial production losses and pose potential human health risks through continued and widespread contamination of the environment with *F.gigantica* eggs. Our slaughterhouse surveys confirmed this approach as a practical and convenient surveillance method in a country with limited animal health capacity, providing useful disease information despite the inevitable bias associated with a slaughterhouse population. As slaughterhouse surveys are easily implemented across a broad geographical area, they may be more appropriate for surveillance for other diseases in developing countries than is generally acknowledged.

## 4.2 Introduction

In the Lao People's Democratic Republic (Lao PDR, Laos), agriculture employs about 85% of the labour force, with around 95% of agricultural output from smallholder production. Livestock production contributes to 20% of the agricultural GDP and is distributed widely through the population who are predominately smallholder farmers, with 68% of households owning at least one buffalo and 35% at least one cow (Wilson 2007). Large ruminants provide a cash asset, draft power, manure used as fertiliser and a source of meat (Millar & Phoutakhoun 2008). Livestock production in Laos is low input and output as there are many constraints to best practice livestock production with optimal productivity. Constraints include: endemic parasitism (toxocariasis, fasciolosis) and infectious diseases (haemorrhagic septicaemia, foot and mouth disease), suboptimal husbandry and management practices (nutrition, reproduction and general husbandry), land degradation and availability for livestock production, transportation and communication difficulties especially in mountainous northern Laos, and lack of funds and capacity to carry out broad scale disease control and surveillance activities (Windsor 2006, Wilson 2007, Millar & Phoutakhoun 2008).

Laos is strategically placed in South-East Asia to capitalise on the growing demand for meat products in the region especially from the larger market demands in Thailand, Vietnam and China. Increasing beef and buffalo meat production by smallholder farmers to meet this demand could help in addressing the widespread rural poverty in Laos, particularly in the north (Delgado 1999, Wilson 2007, Millar & Phoutakhoun 2008).

Fasciolosis is a global parasitic infection caused by *Fasciola hepatica* in more temperate climates and *F. gigantica* in tropical climates. It affects mostly ruminants but can also occur in other animal species, such as horses and pigs and in humans. Fasciolosis is regarded as one of the most important diseases impacting on cattle and buffalo production in humid tropical regions. In ruminants, infection is associated with chronic production losses, decreased carcass quality and yield, and less commonly, overt clinical disease and death. As it causes mainly chronic symptoms and does not affect transboundary trade, fasciolosis is often neglected by owners and livestock workers in developing countries where there are low animal health inputs (Gray & Copland 2008). In

Laos, although accurate prevalence data is lacking, annual economic losses due to fasciolosis were estimated between AUD50-60 million for a high prevalence estimate of 26% and AUD29-34 million for a low prevalence estimate of 15% (Copeman & Copland 2008).

Human fasciolosis in Laos is a potential notable disease burden (Torgerson & Macpherson 2011) although as in many developing countries its true extent is unknown, with large epidemiological studies only carried out in a few countries (Mas-Comas et al. 2005). In southern Laos in 2004 a survey of a number of slaughterhouses in the two cities of Vientiane and Savannaketh found 17-57% prevalence of *F.gigantica* in cattle and buffalo and in a survey of six villages 2.4% (faecal examination) and 13.8% (serology) prevalence in humans (Duong Quang et al. 2008). The climate of northern Laos is conducive to the parasite's life cycle as is the management system of free grazing of large ruminants used by most farmers that potentially enables prolonged contact with the infective metacercariae. There is a generalised lack of awareness and absence of any control of the disease in northern Laos, suggesting fasciolosis may be a significant livestock production issue and potential human health risk. Yet, to date no prevalence studies in northern Laos have been published and there is a lack of comprehensive countrywide prevalence surveys for fasciolosis in the region (Copeman & Copeland 2008).

The objective of this study was to determine the prevalence of *F.gigantica* in cattle and buffalo in northern Laos and provide an initial assessment of its clinical impact. This information is of use in decisions on context appropriate management and control of fasciolosis in the region that may potentially increase cattle and buffalo productivity within the Lao smallholder livestock systems.

### **4.3 Materials and methods**

A field survey and a slaughterhouse survey were conducted, each preceded by smaller pilot surveys.

### **4.3.1 Study area**

The study area included villages located in the five provinces of Bokeo, Houaphan, Luang Namtha, Luang Prabang and Xieng Khuang in northern Laos. The north of Laos is mountainous, sparsely populated with human population densities between 16-26 people/km<sup>2</sup> (Lao Ministry of Planning and Investment 2010). The large ruminant population consists of around 201,000 cattle and 205,000 buffalo in 2200 livestock holdings with an average size of 1.3 ha (Lao Agricultural Census Office 2000). The climate is tropical, dominated by a wet/hot and a dry/cool season. Some areas and villages are not accessible by car all year and access to villages requires local government authorisation. The research project 'Best practice Health and Husbandry in Cattle and Buffalo, Lao PDR' (BPHH) (Windsor 2006) was working in the study area alongside the multilateral development project 'Northern Region Sustainable Livelihoods through Livestock Development Project' (LDP) (ADB 2007), with both projects aiming to facilitate improved rural incomes through enhancing livestock productivity. Both projects were managed and implemented by the same Lao Department of Livestock and Fisheries (DLF) employees. Villages enrolled in these two projects provided the sampling frame for site selection, having a year round road access by car and authorisation for the research team to work in the villages.

### **4.3.2 Selection of provinces, villages and animals**

For the pilot survey all six villages enrolled in the five year (2008-2012) BPHH project were selected with two each located in the three northern provinces of Houaphan, Luang Prabang and Xieng Khuang. Village selection for the BPHH project was based on following criteria: (i) more than 250 head of cattle and/or buffalo in the village; (ii) all year road access and (iii) willingness of village authorities, farmers and local DLF employees to participate. In March and April 2009, DLF staff collected faecal samples from 30 randomly selected cows and buffalo >12 months old in each village.

For the main prevalence survey, a sample size of 68 villages and 10 animals per village was calculated using Survey Toolbox Software (Cameron 1999) with 95% level of confidence, 0.075 error and expected prevalence of 10-30% based on results of the pilot survey. A two stage sampling technique was used to select villages and animals. The sampling frame for random selection of 2-4 villages per

district (n=18) was a list of the 198 villages with more than 20 cows and/or buffalo and enrolled in the LDP. They were located in the northern provinces of Bokeo, Houaphan, Luang Namtha, Luang Prabang and Xieng Khuang. In addition all BPHH project villages were also selected. Criteria for village selection in the LDP were (i) high level of poverty, (ii) 50% of poor households which rear livestock, (iii) interested to join project, (iv) all year road access, (v) located within a cluster of villages and (vi) a traditional not a resettled village.

DLF district personnel organised visits to the selected villages with local authorities and the village headmen between August and December 2010 and collected faecal samples from animals >12 months old presented by farmers on the days of visit until 10-20 faecal samples per village were obtained. All large ruminants sampled were either Asiatic water buffalo (*Bubalis bubalus*) or local indigenous cattle (*Bos indicus*).

#### **4.3.3 Faecal sample collection and analysis**

Faecal sample and data collection was completed by 25 DLF district employees, trained at a series of seven 2 or 3 day workshops held during 2009 and 2010, covering topics including large ruminant diseases and husbandry, sample collection, record keeping and farmer interview techniques. The same staff had then applied their knowledge in practice through participating in a prevalence survey for *Toxocara vitulorum* in calves in northern Laos during 2009 and 2010 (Rast et al. 2013).

About 10g of faeces were collected per rectum using a latex glove and put in a small zip-lock plastic bag, labelled with the animal identification, village name and date of collection. Samples were preserved by adding 5ml of 3% formalin to each sample as transport to the laboratory in Luang Prabang usually took several days and refrigeration was not always available. Faecal egg counts (FEC) were performed using the simple sedimentation method described by Happich and Boray (1969) with modification of only analysing 0.25 ml of sediment per sample rather than the whole sediment due to resource constraints.

*F.gigantica* eggs were differentiated from *Paramphistomum* spp. eggs using microscopy with *F.gigantica* eggs being oval, operculated, golden brown and with a more homogenous and darker content than *Paramphistomum* spp. Eggs (Figures 2.2-2.3). The latter are oval, operculated, lighter brown, sometimes

appearing a blue or pink shade with more granular contents and slightly smaller. Using more sensitive testing methods that are also able to detect immature or pre-patent infections such as copro-antigen ELISA or indirect serum ELISA were considered for use but requires more resources and capacity than were currently available in Laos.

#### **4.3.4 Data collection and management**

The data collection forms used in the pilot survey were adapted and translated into Lao for the main field survey and data recorded included owner and village name, age and monetary value of animal provided by the owner, and species, gender, body condition, coat condition, morbidity (including clinical signs) and faecal consistency assessed or observed by the researchers. Completed data record sheets were sent to a central office in Luang Prabang, translated into English and data entered into a spread sheet (Excel Microsoft 2003).

#### **4.3.5 Slaughterhouse surveys**

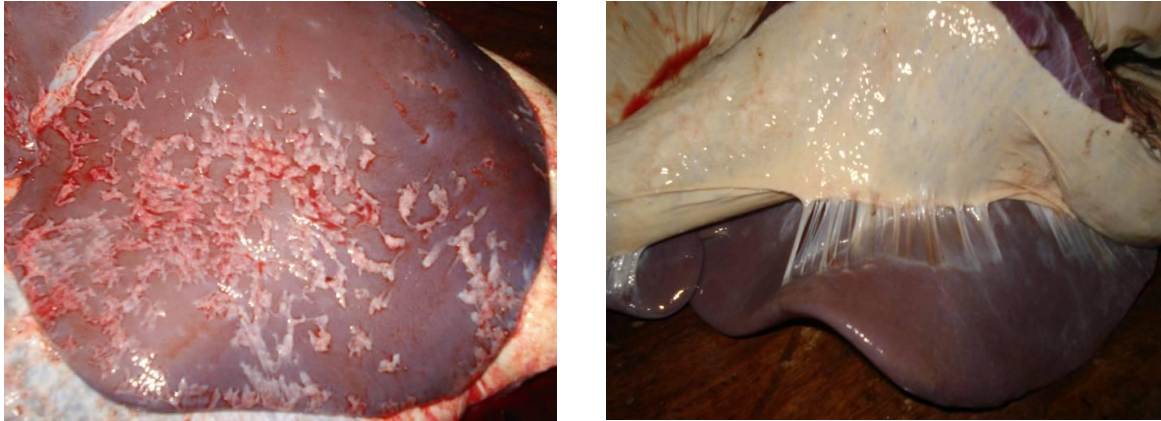
In northern Laos, slaughter of large ruminants occurs in small, basic, privately owned slaughter facilities during the night for sale of meat products at local meat markets a few hours later. In all facilities visited, 5-15 large ruminants were killed and processed each night by different trader and butcher teams who had purchased the animals.

The pilot slaughterhouse survey was conducted at three slaughter houses in Luang Prabang during April and May 2009 and each facility visited on 2-3 nights over a four week period. For the main slaughterhouse survey between March and June 2011, the principal slaughterhouse in each provincial capital of Bokeo (Huaxay), Houaphan (Sam Nua), Luang Namtha (Luang Namtha), Luang Prabang (Luang Prabang) and Xieng Khuang (Phonsavanh) provinces was each visited for three to five consecutive nights.

Visits were arranged with the local DLF authorities and the slaughterhouse owners and the facility visited about half an hour before the start of slaughter each night until the last ruminant was slaughtered.

All animals were examined pre-slaughter and findings recorded. Due to lack of safe restraining facilities and light, examinations were mostly limited to a close

visual assessment. Post mortem examination and sample collection (faeces and tissue) were done from as many animals as possible without interrupting the slaughter process. This involved several animals being slaughtered and processed at any one time on the ground by different butcher teams. Post mortem examinations were limited due to the standard of the facilities, with minimal space and light potentially compromising the safety of the survey team. Species, sex, body condition score (BCS), coat condition and any obvious abnormalities were determined or observed and recorded at the pre-slaughter examination. The source (province and district) of the animal was ascertained when possible from the slaughter point owners or trader. BCS was scored according to a five point scale (1-emaciated to 5-obese). During post-mortem examination the animal's age was determined according to dentition (FAO 2002) and the liver and some other internal organs examined for any gross abnormalities and findings recorded on data collection sheets and by digital images. Livers were grossly assessed as normal or with mild, moderate or severe liver lesions based on categories as described (Molina et al. 2006) and presented (Figures 4.1-4.3). Although livers were also examined for liver fluke, minimal dissection of liver and bile ducts was possible as both were highly valued commodities. No liver condemnations were observed. Any flukes found were preserved in 70% ethanol in sterile plastic screw top jars and individually labelled for later phylogenetic analysis (Appendix 1). Faecal samples were collected per rectum and put in individually labelled zip-lock plastic bags. When analysis was not possible within 24 hours, 5ml 3% formalin was added to preserve the samples.



**Figure 4.1** Gross lesions of liver damage classed as mild. Small amounts of surface damage such as fibrin tags and fibrous adhesions to adjacent organs or between liver lobes, small superficial fibrous patches, few white foci in the parenchyma



**Figure 4.2** Gross lesions of liver damage classed as moderate. Evidence of fibrosis around the bile ducts, more numerous white foci in the parenchyma, larger or deeper surface fibrous areas



**Figure 4.3** Gross lesions of liver damage classed as severe. Marked fibrosis of bile ducts and parenchyma with evidence of calcification, necrosis, haemorrhage and distortion of the liver

(Images by V Ambler 2009)



### **4.3.6 Statistical analyses**

Apparent prevalence of animals infected with *F.gigantica* and confidence limits were calculated for the field and slaughterhouse surveys using EpiTools epidemiological calculators (Sergeant 2009). Descriptive statistics, logistic and linear regression analysis of the data were carried out using SAS Macros for statistical modelling (Dhand 2009) and SAS statistical software (© 2002–2003 by SAS Institute Inc., Cary, NC, USA).

#### **4.3.6.1 Field surveys**

*F.gigantica* faecal egg count results provided two outcome variables, (1) infection status (positive or negative), and for the positive cattle and buffalo (2) level of infection as determined by the number of eggs per gram (EPG) of faeces. EPG were log transformed for analysis to normalize the data. For both outcomes explanatory variables investigated were animal origin (province), species (cattle, buffalo), gender (male, female), coat condition (normal, abnormal), diarrhoea (yes, no), general illness at time of sampling (yes, no), age, BCS and monetary value of animals. Age in years reported by owners was analysed as a continuous and categorical variable (<3 years, 3-5 years, >5 years) to enable any differing statistical significance to be determined. BCS was re-categorised from 1 (emaciated) to 4 (fat). Descriptive analysis was performed on each variable using frequency tables and charts for categorical variables and mean, median and range for continuous variables. Univariable logistic regression analysis was then performed to investigate the association between infection status and the explanatory variables using the likelihood ratio qui square test. From the likelihood ratio chi-square analysis, the odds ratios of explanatory variables were examined to determine the extent as well as positive or negative association with the presence of infection. For the main survey only, the correlation between pairs of explanatory variables was checked using the Spearman rank test and variables with a Spearman rank coefficient of <0.7, with a p-value of  $\leq 0.25$  and <10% missing values were included in a multivariable logistic regression models and each tested using a stepwise approach and accounting for clustering within village using Proc GLIMMIX of SAS (2002-3). Variables with a p-value  $\leq 0.05$  were considered statistically significant and retained in the final model, which was tested for interaction between variables.

### **4.3.6.2 Slaughterhouse surveys**

For both the pilot and main slaughterhouse surveys three outcome variables used were (1) *F.gigantica* infection status (positive or negative), (2) amount of liver damage (Figures 4.1-4.3) and for cattle and buffalo with positive faecal egg counts (3) level of infection as determined by the number of eggs per gram (EPG) of faeces. EPG were log transformed for analysis to normalize the data. *F.gigantica* infection status was defined as positive if an animal had a positive faecal sedimentation and/or if flukes were found in the liver. Explanatory variables and categorisations used were the same as in the field surveys except body condition score (categorised: 1= BCS 1-1.5; 2= BCS 2-2.5; 3= BCS >3) and weight which was obtained for some animals at the main slaughterhouse survey using a weight band developed for Lao cattle and buffalo (unpublished).

Kappa analysis (<http://faculty.vassar.edu/lowry/kappa.html>) was performed to determine the level of agreement between faecal analysis and liver examination at necropsy for diagnosing infection (Fleiss et al. 1969).

Univariable regression analysis was conducted to investigate the association between each outcome variable and the explanatory variables using the likelihood-ratio chi-squared test. For the main slaughterhouse survey only, this was followed by a multivariable analysis using the same criteria for explanatory variables as cut off for inclusion described for the field survey analysis. Variables with a p-value  $\leq 0.05$  were considered statistically significant and retained in the final model.

Level of infection was analysed using the faecal egg count positive subset of data only as described for the field survey.

## **4.4 Results**

### **4.4.1 Prevalence estimations**

The apparent prevalence (AP) estimation for the pilot field survey was 5.7% (CI 0-0.16) and for the slaughter house survey 30.7% (CI 0.22-0.40).

The AP estimation for the main field survey was 17.2% (CI 0.14-0.21) and the slaughterhouse survey 34.1% (CI 0.26-0.42).

#### 4.4.2 Field surveys

For the pilot survey 70 cattle and 106 buffalo from Houaphan (56), Luang Prabang (60) and Xieng Khuang (60) provinces were tested of which 1.8% were positive in Houaphan, 3.3% in Luang Prabang and 11.7% from Xieng Khuang. Univariable analysis showed that there was a significant difference ( $p=0.05$ ) between *F.gigantica* positive status and province.

For the main field survey 1268 large ruminants  $\geq 12$  months old were sampled consisting of 462 buffalo and 806 cattle from 75 villages. Of the animals sampled, 68.5% were female and 31.5% were male. Among the sampled villages, 55 (73.3%) had at least one animal with a positive faecal sedimentation test. Prevalence varied from 12.9% in Luang Prabang to 24.7% in Houaphan province. At sampling 32 (2.5%) of the animals sampled were reported as sick and 15 (46.9%) of the sick animals had positive faecal egg count results. Average faecal egg counts were 132.6 EPG in cattle and 158.9 EPG in buffalo. Results of the univariable analysis are presented (Table 4.1). Multivariable analysis showed that province, being sick, gender (female) and species (buffalo) were significantly ( $p<0.05$ ) associated with *F.gigantica* infection status (Table 4.2).

**Table 4.1 Final logistic regression model for *Fasciola gigantica* infection status in a field survey of 1261 cattle and buffalo >12 months old in northern Laos, 2010/11**

Variable Category	b	SE(b)	OR	95% CI	p-value
Intercept	-1.49	0.27			
<i>Province</i>					< 0.0001
Bokeo	-	-	1		
Houaphan	0.65	0.27	1.93	1.15, 3.40	
Luang Namtha	-0.06	0.33	0.95	0.49, 1.83	
Luang Prabang	-0.12	0.30	0.89	0.50, 1.62	
Xieng Khuang	0.05	0.35	1.06	0.53, 2.11	
<i>Is animal sick?</i>					<0.0001
No	-	-	1		
Yes	1.43	0.38	4.17	1.98, 8.73	
<i>Species</i>					0.023
Buffalo	-	-	1		
Cattle	-0.36	0.16	0.70	0.51, 0.95	
<i>Gender</i>					0.006
Female	-	-	1		
Male	0.50	0.18	0.61	0.42, 0.86	

The final linear regression model for level of infection among positive cattle and buffalo indicated that being sick was the only variable significantly associated ( $p=0.0001$ ) with EPG levels, with healthy animals having a  $\beta$ -value of -0.59 (intercept  $\beta$ -value=5.17).

**Table 4.2 Contingency table and univariable logistic regression results for association of explanatory variables with *Fasciola gigantica* infection status in a field survey of 1268 cattle and buffalo  $\geq 12$  months old in northern Laos, 2010/11**

Variable Category	Infection status		OR	95% CI	p-value
	Negative (%)	Positive (%)			
<i>Province</i>					<0.0001
Bokeo	116 (85.9)	19 (14.1)	1.00		
Houaphan	327 (75.4)	107 (24.7)	2.00	1.20, 3.49	
Luang Namtha	155 (86.1)	25 (13.9)	0.95	0.49, 1.83	
Luang Prabang	298 (87.1)	44 (12.9)	0.92	0.51, 1.71	
Xieng Khuang	154 (87.0)	23 (13.0)	1.06	0.54, 2.12	
<i>Is animal sick?<sup>1</sup></i>					<0.0001
No	1032 (83.6)	203 (16.4)	1.00		
Yes	17 (53.1)	15 (46.9)	4.49	2.18, 9.14	
<i>Gender<sup>1</sup></i>					0.001
Female	698 (80.5)	169 (19.5)	1.00		
Male	349 (87.7)	49 (12.3)	0.58	0.41, 0.81	
<i>Species</i>					0.002
Buffalo	362 (78.4)	100 (21.7)	1.00		
Cattle	688 (85.4)	118 (14.6)	0.62	0.46, 0.84	
<i>Coat condition</i>					0.004
Normal	958 (83.9)	184 (16.1)	1.00		
Abnormal	92 (73.0)	34 (27.0)	1.92	1.25, 2.91	
<i>Age of animal<sup>1</sup></i>					0.043
$\leq 3$ years	305 (82.2)	66 (17.8)	1.00		
3-5 years	495 (85.3)	85 (14.7)	0.79	0.56, 1.13	
$\geq 5$ years	248 (78.7)	67 (21.3)	1.25	0.85, 1.83	
<i>Sampling time</i>					0.222
August	245 (84.5)	45 (15.5)	1.00		
September	769 (82.9)	159 (17.1)	1.13	1.79, 1.63	
October	17 (65.4)	9 (34.6)	2.88	1.17, 6.74	
November	5 (71.4)	2 (28.6)	2.18	0.31, 10.45	
December	14 (82.4)	3 (17.7)	1.17	0.26, 3.75	
<i>Body condition</i>					0.224
BCS 1	45 (75.0)	15 (25.0)	1.00		
BCS 2	406 (82.7)	85 (17.3)	0.63	0.34, 1.21	
BCS 3	561 (83.1)	114 (16.9)	0.61	0.34, 1.17	
BCS 4	38 (90.5)	4 (9.5)	0.32	0.08, 0.96	

<sup>1</sup> 1-2 missing observations

#### **4.4.3 Slaughterhouse surveys**

During the pilot slaughterhouse survey 99 animals (12 cattle and 87 buffalo) from three slaughter houses in Luang Prabang were examined. At necropsy 59.4% of examined animals had liver damage (mild, moderate or severe), with 80.6% of animals determined as infected with *F.gigantica* displaying liver damage and 50% of uninfected animals displaying hepatic or biliary tract pathology respectively.

For the main slaughterhouse survey 125 animals, comprising of 11 large ruminants in Bokeo, 40 in Houaphan, 15 in Luang Namtha, 14 in Luang Prabang and 45 in Xieng Khuang main provincial slaughterhouses were examined and sampled. A majority (66.3%) of animals slaughtered were >5 years old. Of the 123 examined livers, 87 (70.7%) had gross liver lesions ranging from mild (22.8%) to moderate (17.9%) and severe (30.1%). Buffalo had higher rates of hepatic and biliary tract pathology with 95.6% (n=68) having grossly abnormal liver compared to 40.0% (n=55) of cattle. Of the 44 *F.gigantica* positive animals where livers could be examined, 37 (84.1%) had hepatic and biliary tract pathology, with 29 (78.4%) of these being buffalo and 8 (21.6%) cattle. Interestingly of the 79 animals determined not infected with *F.gigantica* using FEC, 50 (63.3%) had hepatic and biliary tract pathology with 36 (72.0%) of these being buffalo and 14 (28.0%) cattle. A summary of the locations, number and species of large ruminants examined, results of liver and faecal examinations and the estimated prevalence are presented (Table 4.3).

**Table 4.3 Summary of results of the slaughterhouse surveys in northern Laos in 2009 and 2011 for *Fasciola gigantica* and gross liver pathology in cattle and buffalo**

Slaughterhouse Location (Province: Town)	Pilot survey (March-April 2009)				Main survey (March-June 2011)							
	Luang Prabang: Luang Prabang		Bokeo: Huaxay		Houaphan: Sam Nua		Luang Namtha; Luang Namtha		Luang Prabang: Luang Prabang		Xieng Khuang: Phonsavanh	
	cattle	buffalo	cattle <sup>2</sup>	buffalo	cattle	buffalo	cattle	buffalo	cattle <sup>2</sup>	buffalo	cattle	buffalo
No. animals examined	12	87	-	11	14	26	7	8	-	14	35	10
No. with positive FEC (%)	3 (25.0)	22 (25.3)	-	1 (9.1)	4 (28.6)	14 (53.8)	1 (14.3)	1 (12.5)	-	5 (35.7)	9 (25.7)	3 (30.0)
No. with <i>F. gigantica</i> found in liver (%)	3 (25.0)	27 (31.0)	-	3 (27.3)	0	2 (7.7)	0	3 (37.5)	-	2 (14.3)	2 (5.7)	2 (20.0)
Prevalence <sup>1</sup> (%)	25.0	31.0	-	27.3	28.6	53.8	14.3	37.5	-	35.7	25.7	50.0
No. with normal liver	10 (83.3)	29 (33.3)	-	0	10 (71.4)	2 (7.7)	3 (42.9)	0	-	0	20 (57.1)	1 (10.0)
No. with mild liver pathology	1 (8.3)	23 (26.4)	-	2 (18.2)	4 (28.6)	4 (15.4)	4 (57.1)	0	-	5 (35.7)	8 (22.9)	1 (10.0)
No. with moderate liver pathology	1 (8.3)	26 (29.9)	-	1 (9.1)	0	10 (38.5)	0	1 (12.5)	-	5 (35.7)	2 (5.7)	3 (30.0)
No. with severe liver pathology	0	9 (10.3)	-	8 (72.7)	0	9 (34.6)	NA	4 (28.6)	-	7 (87.5)	4 (11.4)	5 (50.0)

<sup>1</sup> based on positive FEC, presence of fluke or both<sup>2</sup> nil cattle slaughtered at this location during survey period

Univariable analysis for the pilot slaughterhouse survey showed that province was significantly ( $p < 0.05$ ) associated with *F. gigantica* infection status, and that province, species, age and *F. gigantica* infection status were significantly ( $p < 0.05$ ) associated with liver damage. Statistical analysis of the main slaughterhouse survey data showed that liver damage and low BCS were the only variables tested significantly associated ( $p < 0.05$ ) with *F. gigantica* infection status. Source of the animals could not be further analysed as data was missing from  $> 10\%$  of animals. The final model using amount of hepatic and biliary tract pathology as outcome is presented (Table 4.4), showing that species (buffalo), age ( $\geq 3$  years) and *F. gigantica* infection status (positive) were significantly ( $p < 0.05$ ) associated with liver damage.

**Table 4.4 Final logistic regression model for gross liver pathology of cattle and buffalo in a slaughterhouse survey of 116 animals in northern, Laos, 2011**

Variable Category	b	SE(b)	OR	95% CI	p-value
<i>Species</i>					<0.0001
Buffalo	-	-	1		
Cattle	-4.14	0.98	0.02	0.00, 0.09	
<i>F. gigantica status</i>					<0.0001
Negative	-	-	1	-	
Positive	1.43	0.42	4.16	1.81, 9.96	
<i>Age</i>					<0.0001
< 3 years	-	-	1		
3-5 years	1.71	0.91	5.52	1.16, 33.70	
< 5 years	1.26	0.58	3.53	1.16, 11.03	

Linear regression results indicated that none of the factors investigated were associated with the level of *F. gigantica* faecal egg counts.

Kappa result for comparison of diagnosing *F. gigantica* infection by either faecal egg count or assessing liver damage was 0.36.

## 4.5 Discussion

Apparent prevalence estimates found in this study of 17.2% for the main field survey and 30.7% and 34.1% in the pilot and main slaughterhouse surveys respectively fall within the range of unpublished data on fasciola prevalence in Laos by Vongthilath and Douangngeun (cited in Copeman & Copeland 2008). They estimated prevalence of 9-23% in cattle and 21-38% in buffalo with a combined prevalence of 26% in 700 animals sampled. A survey of the main slaughterhouses in the cities of Vientiane and Savannaketh in southern Laos in 2004 (Duong Quang et al. 2008) reported prevalence of 17-57%. Further, a small number of studies in the bordering countries of Cambodia, Vietnam, Thailand and China reported prevalence ranging between 12% and 76% (Copeman & Copland 2008). Different study methodologies used, season of surveys and possibly host differences among animals surveyed in these studies make comparisons difficult. The pilot field survey prevalence estimate of 5.7% in our studies is likely an underestimation as the sample size was small and sampling method may have been biased towards healthier and heavier animals.

Our prevalence estimates are likely to be an underestimation as testing for *F.gigantica* infection by analysing a single faecal sample has a sensitivity of 43-64% depending on quantity of faeces used (Charlier et al. 2008) but could be improved to 90% when two or three serial samples are examined (Rapsch et al. 2006). This option was not available in our study due to transport and resource constraints. Limited resources at the local laboratory such as the unavailability of sequential sieves which is described as a more sensitive method (Kleiman et al. 2005) may have further contributed to underestimation of prevalence. The limitation of faecal sedimentation as a test for diagnosing *F.gigantica* infection are particularly evident in the slaughter house surveys where 50% (pilot survey) and 64.2% (main survey) of animals classed as uninfected had hepatic and biliary tract pathology likely to indicate a prepatent or previous infection. As flukes do not enter the bile ducts until eight weeks post infection and eggs are produced 13-14 weeks after initial infection, faecal egg identification and fluke identification in liver may have missed a significant number of infected animals.



The method of cutting up the liver and macerating through sieves as well as collecting the gall bladder contents would enable counting of both immature and mature flukes (Molina et al. 2005a, Wiedosari et al. 2006). This could not be done in our studies as liver and bile were highly valued products. Even where pathology was present, the liver was never condemned and resource constraints prevented their purchase for study. As evidenced by the positive faecal egg results from seven animals with livers that appeared grossly normal, it is likely that small localised lesions and flukes may have been missed. Infection with immature *F.gigantica* has been reported to cause a significant amount of liver pathology, resulting in poor production (Sothoeun et al. 2006).

It is important to note that 73.3% of survey villages (n=75) had at least one positive large ruminant indicating widespread infection across northern Laos. The main field survey data indicated significant differences of infection status between provinces ( $p < 0.0001$ ). It is probable that spatial variation of *F.gigantica* infection occurs within limited geographical areas as its life cycle is dependent on environmental factors, particularly the topography, rainfall and temperature that influence the presence and survival of the intermediate snail host and metacercariae and hence the prevalence of fasciolosis (Copeman & Copeland 2008). Determining what environment the animal spent most of its time in may have been useful but was undetermined in our current surveys.

The slaughterhouse prevalence estimates in our surveys are higher than the field survey prevalence estimates. It is probable that the slaughterhouse study population is biased, reflecting the farmers' culling or selling practices and transport limitations rather than being representative of the general large ruminant population in northern Laos. Lao farmers tend to sell animals when there is a sudden need for cash for the household (i.e. health emergencies, ceremonies, education costs) rather than for maximum profit when the animal is in good condition. They may choose older, 'sick' or 'poorer' animals to sell despite it being discouraged by authorities. Smallholder farmers often cannot afford treatment or they fear their animals may die both impacting on household assets. Some evidence of this practice was apparent with 65.3% of animals at the main slaughterhouse surveys being >5 years old and with some likely to be much older than they could be aged from dentition due to extensive teeth wear.

Identification of risk factors for *F.gigantica* infection may help in developing control strategies.

Some previous studies have hypothesised that buffaloes may have greater resistance to infection than cattle and suggest that techniques to manage the parasite in buffaloes may be less important (Wiedosari et al. 2006). Our study found that cattle were less likely to be infected (OR 0.7) in the field survey and less likely to have hepato-biliary pathology (OR 0.02) in the slaughterhouse survey. The average FEC in cattle was lower at 132.6 EPG compared to buffalo at 158.9 EPG. These results contrast with Molina et al. (2005a, 2005b) who found a significantly higher prevalence of fluke infection but lower fluke burden per animal in buffalo compared with cattle and Wiedosari et al. (2006) who reported significantly lower FEC and fluke counts (mature and immature) in buffalo than cattle. Further studies on differences in susceptibility between these species are lacking and it is likely that host-parasite relationships differ between different countries and animal species sampled in studies to date. Whilst our models showed a significant ( $p < 0.0001$ ) difference between species with cattle apparently less susceptible, when the amount of hepato-biliary pathology observed at necropsy was compared, regardless of their infection status, buffaloes were more likely to have hepato-biliary lesions than cattle and for that pathology to be more severe (Table 4.4). This may indicate that buffalo in this study were more susceptible to infection, that they experience greater infective doses because of their predilection for swampy areas or that damage from past infections is cumulative and tissue regeneration poor. Alternatively, it may be possible that another aetiology causes similar gross liver lesions in buffalo, particularly as 92.3% of buffalo classed as not infected with *F.gigantica* had hepato-biliary lesions.

Nevertheless our study suggests that control is important in both species as buffaloes may suffer greater growth and production losses from the increased severity of hepato-biliary pathology and especially as farmers in Laos keep both species in close contact and keep their animals until they are quite old before selling.

Age of the host is an important consideration in *F.gigantica* infection. Previous studies indicate that if infected with high doses, young animals can suffer very poor growth (Mehra et al. 1999) and that significant performance losses occur in the pre-patent period of infection. This means that to reduce production losses most effectively, treatment is required as early in the infection as possible (Sothoeun et al. 2006, Ganga et al. 2007). In our field survey 17.8% (66) of sampled animals <3 years old were found to be positive for *F.gigantica* (Table 4.1). Infection was highest in animals aged >5 years in both the field and slaughterhouse surveys and hepato-biliary pathology highest in animals >5 years old. Our study suggests that in northern Laos large ruminants can become infected with *F.gigantica* at a young age and that infection and liver pathology accumulates with increasing age, indicating that control of *F.gigantica* should commence in animals <3 years old.

Being sick was significantly ( $p < 0.0001$ ) associated with *F.gigantica* infection in the field survey and lower BCS was significantly associated with *F.gigantica* infection in our slaughterhouse survey. Clinical signs for fasciolosis are non-specific, including weight loss, oedema, inappetance, lethargy, anaemia and jaundice, and are poor indicators for diagnosis or treatment decisions. BCS is a subjective assessment of weight and weighing and measuring animals would increase accuracy in future studies but requires better handling facilities and weight scales or weight tapes, all currently not commonly used or available in northern Laos.

The gross pathology findings at the slaughterhouse surveys in infected livers were largely in agreement with previous studies (Phiri et al. 2006, Wiedosari et al. 2006, Molina et al. 2008). Gross findings differing from these studies were that some livers exhibited well defined localised pathology with the rest of the liver appearing normal. This was seen as a distinct area, mostly appearing as a bulging surface or raised nodule, which contained fibrotic ducts, sometimes calcification and flukes, with the rest of the liver appearing normal, perhaps indicating an ability of some animals to localise infections to a small area of the liver. Comparison of diagnosis of *F.gigantica* infection at slaughter by gross liver pathology assessment or faecal analysis and fluke identification, showed moderate agreement ( $\kappa = 0.37$ ). More detailed studies to determine the

sensitivity and specificity of both these diagnostic methods would be beneficial as gross liver pathology assessment would provide a simple, low cost diagnostic tool for *F.gigantica* infection at slaughter.

In our field survey male animals were less likely to be infected (Table 4.2). It is possible that bulls have an increased level of resistance to parasite infection as they have better body condition and nutrition levels compared to females. This probably reflects the impact of lactation during the extensive period of the dry season when energy is limited, plus male animals, particularly draft animals, are considered more valuable, receiving the often limited feed resources available.

Our study confirmed that *F.gigantica* is an important parasite in Laos as it is widespread with 73.3% of tested villages infected, has significant prevalence of 17.3% using a test with low sensitivity and causes frequent and severe liver pathology. These findings suggest that control should be considered as these levels of infection are likely to result in substantial production losses and pose a potential human health risk through continued and widespread contamination of the environment with *F.gigantica* eggs. Faecal analysis is likely to continue to be the main diagnostic method available in live animals and countries with low animal health capacities, thus means of increasing test sensitivity (i.e. serial faecal sampling) need to be considered.

Analysis of factors associated with *F.gigantica* infection in our study indicated that there is a significant difference of prevalence across different provinces and that it is important to target both cattle and buffalo and animals of all ages in control strategies. Widespread and severe hepatic and biliary tract pathology found in both cattle and buffalo but more severe and frequent in buffalo, supports that early intervention to control *F.gigantica* may have significant benefits in increasing large ruminant production.

Our slaughterhouse surveys in northern Laos confirmed this method to be a practical surveillance method in areas with limited animal health capacities for collecting regional or provincial disease information. This surveillance method could easily be implemented regularly and be expanded geographically and for other diseases (i.e. clinical foot and mouth disease, other internal parasites) and production benchmarks such as weight, body condition and reproductive status, providing the possible bias of the slaughterhouse population is considered before making policy decisions based on information obtained from slaughterhouse surveys. Further, slaughterhouse surveillance could also provide an opportunity for implementing a system of quality control.

#### **Conflict of interest statement**

None

#### **4.6 Acknowledgments**

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## **PART 2:**

### **CONTROL OF *FASCIOLA GIGANTICA* INFECTION IN SMALLHOLDER LARGE RUMINANT FARMING SYSTEMS IN DEVELOPING COUNTRIES: A CASE STUDY FROM LAO PDR**

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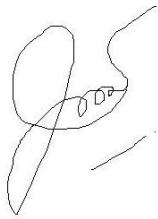
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**Statement of contribution of co-authors**

Luzia Rast, Jenny-Ann Toribio, Syseng Khounsy and Peter Windsor collaborated on the conceptualisation and design of this study. Luzia Rast, Sonevilay Nampanya and Jenny Hanks conducted the field work. Luzia Rast and Jenny Hanks analysed the faecal samples and data from the treatment trial. Luzia Rast and Navneet Dhand analysed the data of the farmer surveys. Luzia Rast prepared the paper for publication. Jenny Hanks, Sonevilay Nampanya, Jenny-Ann Toribio, Peter Rolfe and Peter Windsor contributed to reviewing and editing the manuscript.



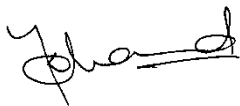
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## 4.7 Abstract

In many tropical developing countries costs of estimated production losses due to *F.gigantica* infection in large ruminants are high and in recent years fasciolosis has been increasingly recognised as a human health risk. For many regions of South-East Asia but especially in upland areas there is little information on the prevalence and impact of the disease plus management of the parasite is rare. Using faecal egg count analysis we previously found an apparent prevalence of 17.2% in cattle and buffalo in northern Laos, with infection in 73.3% of the villages where animals were tested. In the survey reported here of smallholder farmer knowledge and practices (n=326) we identified that 93.1% of farmers had no knowledge and 6.9% minimal knowledge of fasciolosis despite 20.6% of farmers reporting sighting of leaf shaped parasites in the liver of their cattle or buffalo when slaughtered in the past. A field treatment trial on *F.gigantica* during a period of declining nutrition and using imported triclabendazole and a locally available triclabendazole/albendazole combination, identified that both anthelmintics were effective, with >90% faecal egg count reduction at 4, 8 and 12 weeks post treatment in cattle and buffalo, although no significant treatment effect on weight was determined (p=0.6) between the treatment and control groups over the trial period. With predictions of increasing demand for red meat in the region over the next decades, opportunities exist for poor smallholder farmers to supply this market and increase their income. This requires improved large ruminant production outputs from smallholder farming systems including addressing the knowledge and practice gaps on fasciolosis identified in our studies. Most of interviewed farmers (95.4%) indicated a desire to learn more about the disease and improved knowledge and control of fasciolosis in this region may significantly improve rural livelihoods through increased livestock productivity, addressing regional rural poverty and food security. In addition control of *F.gigantica* infection in livestock will assist reducing the risk of increasing human fasciolosis.

### Keywords

*Fasciola gigantica*, smallholder farming systems, farmer knowledge, treatment trial, cattle, buffalo



## 4.8 Introduction

Fasciolosis is a global parasitic infection caused by *Fasciola hepatica* in temperate and *F.gigantica* in tropical climates, with overlapping of their geographical distribution in some Asian and African countries (Mas-Comas et al. 2005). The parasite mostly affects domesticated ruminants but can also occur in other species including horses, pigs and humans. Fasciolosis is considered one of the most important diseases of cattle and buffalo in humid tropical regions (Copeman & Copland 2008) and has recently been recognised as an important zoonosis in developing countries (Mas-Comas et al. 2005, Soliman 2008, Torgerson & Macpherson 2011).

In large ruminants fasciolosis is associated with chronic production losses, decreased carcass quality and yield, and less commonly, overt clinical disease and death. As it causes mainly chronic symptoms and does not affect transboundary trade, fasciolosis is often neglected by owners and livestock workers in developing countries where animal health inputs are low (Gray & Copland 2008). In much of South-East Asia, high rates of fasciolosis are associated with areas where the intermediate snail hosts thrive and sustain the parasite's life cycle, including areas of rice cultivation, where animals graze recently harvested crops and in low lying areas close to rivers and lakes (Suon 2006, Suhardono & Copeman 2008). Large areas of South-East Asia are mountainous upland areas where mostly rain fed agriculture is practiced including growing of dry land rice. There is a paucity of published information on the occurrence and prevalence of fasciolosis in these upland areas, although the climate is conducive for the maintenance of *F.gigantica*. For example in northern Laos annual rainfall is around 1500mm and many small rivers, lakes or dams exist near villages where smallholder farmers co-graze their cattle and buffalo. A prevalence survey in late 2010 in five northern provinces of Laos using faecal egg count analysis of 1262 cattle and buffalo, identified an overall apparent prevalence of 17.2% (95% CI 0.14-0.21) with 73.3% of villages (n=75) having at least one positive animal. In addition, slaughterhouse surveys conducted in March to June 2011 in the same five provinces identified 95.6% and 40.0% of slaughtered buffalo and cattle respectively with gross hepatic lesions consistent with liver fluke infection.

The apparent prevalence as determined by faecal egg count analysis of this slaughterhouse population was 34.1% (95% CI 0.26-0.42) (Rast et al. 2013a). In southern Laos in 2004 a survey of slaughterhouses in the two cities of Vientiane and Savannaketh found a prevalence of 17-57% of *F.gigantica* in cattle and buffalo, with a survey of six villages indicating a prevalence in humans of 2.4% (by faecal examination) and 13.8% (serology) (Duong Quang et al. 2008). These findings suggest that *F.gigantica* infection in the upland and potentially the lowlands of Laos and similar environments in other countries in the region is widespread and common. Fasciolosis is likely to contribute substantially to suboptimal large ruminant production and is potentially a serious human health risk in the region.

The geographical location of Laos in South-East Asia makes it an increasingly important contributor to meeting the rising demand for livestock and red meat in the region, which is driven by the growing economies and urbanisation in the more developed countries (Delgado 1999, Steinfeld et al. 2006). Laos is a poor country with the majority of agricultural outputs produced by smallholder farmers contributing 31% of the GDP (World Bank 2011). As in other parts of the region, smallholder farms are mostly mixed enterprises with farmers using traditional low input farming methods and operating at subsistence levels. Large ruminants are used for draft power, a source of meat and manure as fertilizer, and importantly, are kept as an asset store and sold when the household needs larger amounts of cash, rather than for optimal returns. In the north of Laos the farming population is very poor, with most villages remote due to poor road infrastructure, leading to limited market access. In addition, access to modern technologies in agriculture and livestock production is lacking (Millar & Phoutakhoun 2008). The increased demand for red meat and livestock products provides opportunities for smallholder farmers to improve their incomes by providing animals to this market as access improves, although improved productivity is required. To increase large ruminant production the many constraints that currently inhibit optimal production need addressing and importantly smallholder farmers need to change from being large ruminant keepers to large ruminant producers.

Constraints include endemic transboundary and production limiting diseases, limited farmer knowledge and access to information on modern production practices, as well as difficulties of market access and other deficiencies in market chains.

This study had the objective to assess the (1) farmer knowledge of fasciolosis in cattle and buffalo, (2) impact of *F.gigantica* on cattle and buffalo in northern Laos, and (3) the effectiveness of anthelmintic treatments used in the field for reducing *F.gigantica* egg output in faeces of cattle and buffalo and improving weight gain. It is expected that these findings contribute to better understanding of the drivers for sustained parasite control that can lead to improved large ruminant productivity in the region's smallholder farming systems.

## **4.9 Materials and methods**

### **4.9.1 Farmer Interviews**

Face-to-face interviews were conducted in January and February 2011 with 45.6% (326) smallholder farmers who had participated in a prevalence study of *F.gigantica* one to five months earlier across 75 villages of the five northern Laos provinces of Bokeo, Houaphan, Luang Namtha, Luang Prabang and Xieng Khuang (Rast et al. 2013a). All villages included in the earlier prevalence study and this study, were enrolled in a large livestock development project (ADB 2007) and in a smaller research project on large ruminant health and production (Windsor 2006). Department of Livestock and Fisheries (DLF) personnel involved in both of these projects provided authority to access the villages and assisted in data collection. For this study 37 villages were randomly selected from the list of 75 villages where animals had previously been sampled for *F.gigantica*, and then eight households per village were randomly selected from the list of households that had participated in the prevalence survey. The interviews were conducted by DLF district employees that had been trained and implemented similar interviews for a *Toxocara vitulorum* study six months earlier (Rast et al. 2013). Interviews took about an hour per farmer to complete and responses were recorded in Lao on the questionnaire forms.

### **4.9.2 Questionnaire**

The questionnaire was designed in English, translated into Lao, tested for clarity and modified with assistance from two DLF district extension employees. The final questionnaire contained 15 open and closed questions to capture data on the location of the household, number of household members, number, age, gender, species, weight and monetary value of large ruminants owned, morbidity and mortality of large ruminants (>12 months old) over the last 12 months, plus knowledge and practices of farmers about *F.gigantica*.

### **4.9.3 Field treatment trial**

The trial was located in the village of Ban Nong in Xieng Khuang province in northern Laos, which was one of the project sites for the research project examining interventions to increase cattle and buffalo productivity through improved health, nutrition, husbandry, reproduction and marketing (Windsor, 2006). Interventions commenced in late 2008 and included vaccination for foot and mouth disease and haemorrhagic septicaemia once or twice a year, anthelmintic treatment of young calves with pyrantel against *T.vitulorum* infection, establishment of forage plots in a small number of households to provide supplementary nutrition for large ruminants, and participatory training of farmers in large ruminant production techniques (Nampanya et al. 2010). In addition, targeted disease surveys were conducted and included faecal sample collection and analysis from 30 randomly selected cattle and buffalo per village in April 2009 and October 2010 for *F.gigantica*. Ban Nong was selected for this field treatment trial as 50% of the faecal samples in these surveys were positive for *F.gigantica* eggs.

The trial commenced in July 2011 with 26 cattle and 27 buffalo randomly selected and allocated to two treatment groups and a control group (Tables 4.5 and 4.6). Each of the selected animals was weighed using electronic scales (EC2000B, TruTest, New Zealand) and a faecal sample collected. Treatment group animals were given either triclabendazole (TCB) oral drench (Fasinex®, Novartis, Animal Health Australia Inc.) imported for the trial as it is not commercially available in Laos, or triclabendazole/albendazole (TCBA) tablets (Han-Detрил-B, Hanvet, Vietnam) purchased in Laos.

The animals were dosed according to their live weight and manufacturer's recommendation with TCB for buffalo dosed at 24mg/kg bodyweight as recommended (Sanyal & Gupta 1996). Both anthelmintics were applied orally using a calibrated drench gun with the TCBA tablets first dissolved in 20ml of water. Weighing and faecal sample collection were repeated on all animals presented at week 4, 8 and 12 (August to October 2011) of the trial, with data recorded including animal identification, species, age, gender, weight and owner details at each collection. After the final faecal sample collection all animals were treated with triclabendazole.

**Table 4.5 Gender, age (years), body condition score (BCS) , weight (kg) and species of field treatment trial animals at start of the trial in Ban Nong, Xieng Khuang province, Laos, July 2011.**

Treatment Group	Cattle					Buffalo				
	Gender		Age	BCS	Weight	Gender		Age	BCS	Weight
	M	F	years	1-5	kg	M	F	years	1-5	kg
ABZ/TBZ <sup>1</sup> (SD)	0	6	7.3 (0.5)	3.1 (0.5)	182.833 (10.108)	1	6	5.4 (2.6)	3.5 (0.3)	328.714 (29.568)
TBZ <sup>2</sup> (SD)	1	7	6.0 (1.2)	2.7 (0.5)	174.438 (23.548)	1	7	6.1 (2.5)	3.4 (0.6)	356.250 (73.315)
Control (SD)	1	11	5.5 (1.8)	2.9 (0.6)	166.542 (17.474)	0	12	6.0 (2.0)	3.4 (0.4)	357.818 (65.525)

<sup>1</sup>Han-Dertil-B, Hanvet, Vietnam; <sup>2</sup> Fasinex<sup>®</sup> Novartis Animal Health, Australia  
SD = standard deviation

Faecal samples were taken per rectum from each animal and put in small individually labelled zip-lock plastic bags, and 5ml of 3% formalin was added for sample preservation as analysis at the veterinary laboratory in Luang Prabang could not be completed within 24 hours. The sedimentation technique was used to detect *Fasciola* eggs using microscopy and the number of *F. gigantica* eggs per gram of faeces (EPG) was determined using the technique described (Happich & Boray 1969). Owners assisted with drench application and weighing but were not given records of type and dose of drench applied and laboratory personnel not informed of the anthelmintic treatment status of the animals for which faecal samples were analysed. All trial animals remained in the care of their owners during the trial period and were managed using the locally established practices, mostly free-grazing in and around the village on common natural grazing land, around rice fields or in forested areas with minimal supplementary feeding.

During the night some animals were brought back to the village and stabled in shelters (Figure 1.3) or tethered in their owner's house yard, with cattle and buffalo often together.

Cattle and buffalo included in the trial were chosen from farmers who had established forage plots as part of the large ruminant productivity research project to ensure the animals were as well fed as possible to reduce the likelihood of poor nutrition as a cause of poor weight gain. Reproductive management of large ruminants is not practised in northern Laos. Male animals are not castrated and are run together with females throughout the year, resulting in all year calving with a natural peak between October and February. Pregnancy testing is not practiced and calves are weaned naturally, therefore the reproductive status could not be accounted for in the weight analyses.

#### **4.9.4 Data entry and management**

Completed interviews were translated into English by an independent translator and the data was entered into a customised database in Microsoft Access 2003 (Microsoft Cooperation, Redmond, WA, USA) by the senior author. Basic data manipulations were conducted in this database and Excel (Microsoft 2003 and 2010). Data record sheets from the treatment trial were sent to a central office at Luang Prabang and translated into English and entered into a database created in Excel (Microsoft 2010).

#### **4.9.5 Statistical analyses**

Statistical analyses were conducted using SAS version 9.3 (© SAS 2002-2010, SAS Institute Inc., Cary, NC, USA). Data on numbers of large ruminants per household and farmer knowledge on *F.gigantica* were obtained by open questions and answers were categorised for analysis (Table 4.6). Farmer knowledge was categorised as: 0 (no knowledge) if the answer indicated the farmer had heard about liver fluke but had no further knowledge; as 1 (minimal knowledge) if the answer indicated that the farmer knew that it was a parasite of cattle or buffalo, or that it affected the liver, or caused chronic disease or could list some of the clinical signs (including oedema, weight loss, anorexia, anaemia, jaundice); or as 2 (good knowledge) if the answer indicated that the farmer had

basic knowledge about the aetiology, epidemiology, impact (clinical signs) and control or management options of liver fluke.

**Table 4.6 Frequency of the explanatory variables investigated for association with the two binary outcome variables of (1) *Fasciola gigantica* faecal egg count status (positive or negative) (n=306) and (2) morbidity over the past 12 months (yes or no) (n=304) of cattle and/or buffalo  $\geq 12$  months in smallholder households in northern Laos, 2011**

Variable Categories	<i>F.gigantica</i> FEC status of household herd		Morbidity of household herd in past 12 months <sup>1</sup>	
	Pos. (%)	Neg. (%)	Yes (%)	No (%)
<i>Forage plot established</i>				
No	29 (9.5)	113 (36.9)	21 (6.9)	119 (39.1)
Yes	52 (17.0)	112 (36.6)	20 (6.6)	144 (47.4)
<i>Liver fluke knowledge</i>				
None	79 (25.8)	206 (67.3)	36 (11.8)	247 (81.3)
Minimal <sup>2</sup>	2 (0.7)	19 (6.2)	5 (1.6)	16 (5.3)
<i>Fluke seen in cattle or buffalo liver when slaughtered</i>				
No	56 (18.3)	187 (61.1)	26 (8.6)	215 (70.7)
Yes	25 (8.2)	38 (12.4)	15 (4.9)	48 (15.8)
<i>Cattle or buffalo <math>\geq 12</math> months old sick in household herd in past 12 months<sup>3</sup></i>				
No	63 (20.7)	200 (65.8)	-	-
Yes	18 (5.9)	23 (7.6)	-	-
<i>Cattle <math>\geq 12</math> months old sick in household herd in past 12 months<sup>3</sup></i>				
No	45 (20.5)	149 (67.7)	-	-
Yes	12 (5.5)	14 (6.4)	-	-
<i>Buffalo <math>\geq 12</math> months old sick in household herd in past 12 months<sup>3</sup></i>				
No	48 (25.8)	120 (64.5)	-	-
Yes	7 (3.8)	11 (5.9)	-	-
<i>Number of large ruminants per household<sup>3</sup></i>				
1	5 (1.6)	30 (9.9)	3 (1.0)	31 (10.2)
2-3	24 (7.9)	77 (25.3)	8 (2.6)	93 (30.7)
4-5	24 (7.9)	39 (12.8)	9 (3.0)	54 (17.8)
$\geq 6$	28 (9.2)	77 (25.3)	21 (6.9)	84 (27.7)

Variable Categories	<i>F.gigantica</i> FEC status of household herd		Morbidity of household herd in past 12 months <sup>1</sup>	
	Pos. (%)	Neg. (%)	Yes (%)	No (%)
<i>Number of cattle per household</i> <sup>3</sup>				
0	23 (7.6)	56 (18.4)	7 (2.3)	71 (23.4)
1-2	19 (6.3)	66 (21.7)	8 (2.6)	77 (25.4)
3-5	24 (7.9)	50 (16.4)	13 (4.3)	61 (20.1)
≥ 6	15 (4.9)	51 (16.8)	13 (4.3)	53 (17.5)
<i>Number of buffalo per household</i> <sup>3</sup>				
0	26 (8.6)	88 (29.0)	13 (4.3)	100 (33.1)
1-2	19 (6.3)	62 (20.5)	11 (3.6)	70 (23.2)
3-5	31 (10.2)	46 (15.2)	12 (4.0)	65 (21.5)
≥ 6	5 (1.7)	26 (8.6)	5 (1.7)	26 (8.6)
<i>Species in household herd</i> <sup>3</sup>				
Cattle only	26 (8.6)	88 (29.0)	13 (4.3)	101 (33.3)
Buffalo only	23 (7.6)	55 (18.2)	7 (2.3)	71 (23.4)
Cattle and buffalo	32 (10.6)	79 (26.1)	21 (6.9)	90 (29.7)

<sup>1</sup> Household herd refers to animals aged >12 months; <sup>2</sup> Interview answer indicated that the farmer knew that it was a parasite of cattle or buffalo, or that it affected the liver, or caused chronic disease or could list some of the clinical signs (including oedema, weight loss, anorexia, anaemia, jaundice); <sup>3</sup> 1-5 records missing

For the farmer interviews a descriptive analysis was performed on all variables using frequency tables. Associations were separately investigated between 10 categorical explanatory variables (Table 4.6) and the two binary outcome variables of household herd *F.gigantica* status (positive/negative) and household herd morbidity in adult (≥12 months old) animals over the last 12 months (yes/no). The *F.gigantica* status of each household's large ruminant herd (animals ≥12 months old) was determined positive if ≥1 large ruminant had a positive faecal egg count for *F.gigantica* in the prevalence study preceding the farmer interviews (Rast et al. 2013a). Household large ruminant herd morbidity of adult animals was determined from answer farmers gave in the interview to the question if they had sick cattle or buffalo ≥12 months old over the last 12 months. Unconditional association between the outcome variables and each explanatory variable was assessed based on the likelihood ratio chi-square using univariable logistic regression and using UniLogistic SAS Macro (Dhand 2009). For explanatory variables with an unconditional p-value of ≤0.25 and <10% missing values, pairs were tested for collinearity using Spearman rank coefficient and for significant associations (coefficient >0.7) one variable of the collinear



pair was selected for further investigation based on biological plausibility. Subsequently a multivariable logistic regression model was built using manual forward stepwise approach employing MultiLogistic SAS Macro (Dhand 2009).

Variables with p-values  $\leq 0.05$  were retained in the final model for each outcome. To assess the effect of clustering among outcome observations from the same province, district or village each were added as random effect terms to the final model for each outcome and the model refitted using the GLIMMIX procedure in SAS.

For the treatment trial, analyses were performed on data of 43 animals that were present at all four collection points and that had a positive FEC at the commencement of the trial. Of the ten excluded animals, five were not present at one of the data collection points and five had a negative FEC at the commencement of the trial. Weight differences between the groups and the sampling points were evaluated using the MIXED procedure in SAS, with treatment group, species and collection date (week 0, 4, 8 and 12) as fixed effect and animal as random effect. Prior to statistical analyses weight was log-transformed to stabilise variance. The anthelmintic efficacy was calculated from faecal egg count reduction (FECR) at week 4, 8 and 12 for each treatment group and for cattle and buffalo separately using the method described (Dash et al. 1988). The formula used was:

$$\text{FECR} = 100 \times (1 - [T_x/T_0] [C_0/C_x])$$

where  $T_x$  and  $C_x$  = arithmetic mean eggs per gram (EPG) of faeces count of each treatment and control group at week 4, 8 and 12 and  $T_0$  and  $C_0$  = arithmetic mean count of EPG of faeces of each treatment or control group at week 0.

## 4.10 Results

### 4.10.1 Farmer interviews

Farmers (n=326) from 37 villages in 20 districts were interviewed across the five northern provinces of Bokeo, Luang Namtha, Luang Prabang, Houaphan and Xieng Khuang. The interviewed farmers reported morbidity in 39 (5.7%) of their adult ( $\geq 12$  months old) buffalo and 89 (8.2%) of their adult cattle with a mean duration of illness of 25.9 days (SD 23.3). The mean age of sick animals was 52.9 months (SD 33.2) and clinical signs and frequency that farmers reported for cattle and buffalo are listed (Table 4.7).

The treatment rate of sick animals was 68.0% and medications used included traditional medicines (56 animals), salt (6), antibiotics (11; penicillin-streptomycin or oxytetracycline), anthelmintics (5; ivermectin or levamisole), human antidiarrheal medication (1) and petrol (1). Seven owners were uncertain what medication was used. Of the 110 sick animals where the outcome was reported, 96.4% (106) recovered, 2.7% (3) died and one was sold.

**Table 4.7 Clinical signs reported by 326 interviewed farmers in January 2012 in 128 sick cattle and buffalo  $\geq 12$  months old in northern Laos over the preceding 12 months**

Clinical signs	Cattle	Buffalo
FMD lesions on mouth, feet, udder, salivation	46	4
Diarrhoea	26	6
Swollen neck/sore throat	9	6
Weight loss	4	6
Lameness	0	10
Sore eyes	1	6
External parasites	0	5
Depression and anorexia	4	0
Vaccination abscess	0	2
Red urine	2	0
Trauma (wildlife snare)	1	0

Note: in 10 sick animals farmers reported more than 1 clinical sign

Among the 306 farmers who answered the questions regarding their knowledge about liver fluke, 93.1% (285) had never heard of or knew anything about liver fluke, 6.9% (21) had minimal knowledge of fasciolosis in large ruminants and

none had good knowledge. Farmers were asked if they had seen leaf shaped worms in cattle or buffalo livers when animals were slaughtered in the past and 20.6% (63) had seen some. Farmers were also asked if they thought their own cattle or buffalo had liver fluke, with 73.4% (174) and 78.1% (157) unsure of their cattle and buffalo liver fluke status respectively, 6.4% (12) and 5.2% (17) believing their cattle or buffalo respectively were infected and the remainder classing their animals as uninfected. The majority of farmers expressed a desire to learn more about liver fluke with 95.4% (292) wanting to know more including its aetiology, epidemiology, life cycle, control and prevention. Only one farmer wanted to know about its economic impact and 13 farmers did not respond to this question.

The results of the final multivariable logistic regression models are presented (Tables 4.8 and 4.9). Farmer sighting of fluke like worms in livers of cattle or buffalo when slaughtered was significantly associated ( $p < 0.023$ ) with liver fluke status and morbidity in the last 12 months at household herd level.

**Table 4.8 Final logistic regression model for household herd *Fasciola gigantica* status in a survey of 303 households in northern Laos, 2011**

Variable Category	b	SE(b)	OR	95% CI	p-value
<i>Fluke seen in cattle or buffalo liver when slaughtered</i>					
No			1.00		
Yes	1.25	0.35	3.48	1.76, 6.92	0.023

Covariance parameter estimates (standard errors):  
Province= 0.20 (0.33); District = 0.48 (0.56); Village = 0.59 (0.46)

**Table 4.9 Final logistic regression model for household herd morbidity status in a survey of 303 households in northern Laos, 2011**

Variable Category	b	SE(b)	OR	95% CI	p-value
<i>Fluke seen in cattle or buffalo liver when slaughtered</i>					
No			1.00		
Yes	0.84	0.39	2.33	1.11, 4.76	0.023

Covariance parameter estimates (standard errors):  
Province= 0.16 (0.61); District = 0.82 (0.77); Village = 0.56 (0.54)

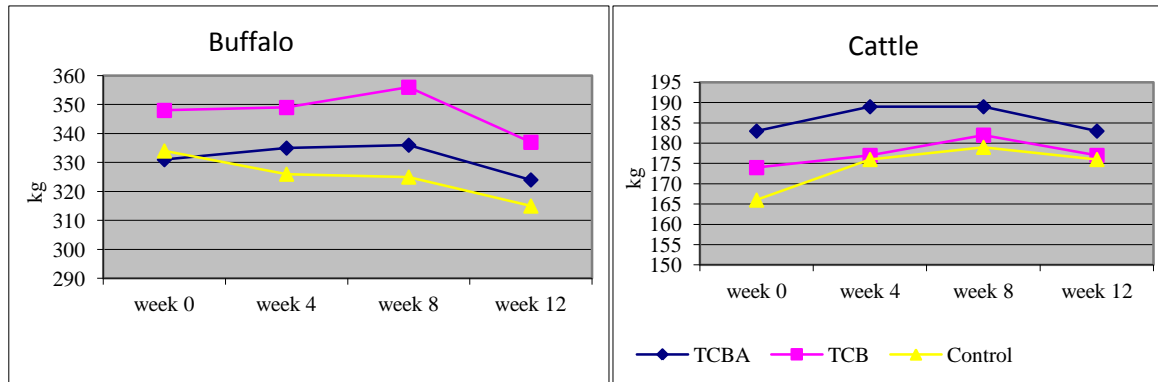
#### 4.10.2 Treatment trial

Of the 43 animals presented at all four data collection points and having a positive FEC for *F.gigantica* at the trial start, 19 were buffalo (18 female and 1 male) and 24 cattle (22 female and 2 male). The age of cattle ranged from 3 to 8 years with a mean age of 6.0 years (SD 1.6) and of buffalo from 3 to 11 years with a mean age of 6.1 years (SD 2.3). FECR of the two treatment groups is presented (Table 4.10), with efficacies ranging between 95.4% to 100.0% in cattle and 90.2% to 100.0% in buffalo for either treatment between weeks 4 and 12 post treatment. Mean weights for each group and species at weeks 0, 4, 8 and 12 of the trial are presented (Figure 4.4), showing a trend of increased weight gain in treatment groups especially buffalo. Weight was not significantly ( $p=0.6$ ) different between the trial groups over the 12 week trial period.

**Table 4.10 Number of positive faecal egg count (FEC) results, mean FEC per gram of faeces (EPG) and FEC reduction (FECR) percentage for each treatment group over the three month trial period in Ban Nong, northern Laos, 2011**

Week	Variable	Treatment Group					
		ABZ/TBZ <sup>1</sup>		TBZ <sup>2</sup>		Control	
		Cattle (n=6)	Buffalo (n=7)	Cattle (n=8)	Buffalo (n=8)	Cattle (n=12)	Buffalo (n=12)
0	No. FEC +ve	6.00	7.00	8.00	8.00	10.00	9.00
	Mean EPG	20.17	190.00	39.75	14.00	5.70	10.57
4	No. FEC +ve	1.00	0.00	3.00	3.00	9.00	8.00
	Mean EPG	0.33	0.00	0.38	1.00	4.00	7.71
	FECR	97.67	100.00	98.64	90.21	-	-
8	No. FEC +ve	0.00	2.00	4.00	2.00	8.00	10.00
	Mean EPG	0.00	1.00	1.38	0.86	4.30	7.00
	FECR	100.00	92.05	95.40	90.72	-	-
12	No. FEC +ve	0.00	1.00	2.00	1	7	7.00
	Mean EPG	0.00	0.40	0.50	0.29	2.40	4.14
	FECR	100.00	94.62	97.01	94.71	-	-

<sup>1</sup>Han-Dertil-B, Hanvet, Vietnam; <sup>2</sup>Fasinex<sup>®</sup> Novartis Animal Health, Australia



TCBA=triclabendazole/albendazole; TCB=triclabendazole; control=no treatment

**Figure 4.4 Average weight changes of the three buffalo and cattle trial groups for *Fasciola gigantica* treatment over the 12 week trial period from July to October 2011 in Ban Nong, Laos**

## 4.11 Discussion

### 4.11.1 Farmer knowledge

There has been significant past investment into *F.gigantica* research in South-East Asia yet there is little evidence that the knowledge gained has led to widespread and sustained control and management of the parasite (ACIAR 2008, Gray et al. 2012). Our study confirmed the almost complete lack of knowledge among farmers in northern Laos about *F.gigantica* in large ruminants and its impact on production. As the surveyed farmers had been part of a prevalence survey for liver fluke involving faecal sample collection from their animals a few months earlier and had been regularly visited by trained DLF extension personnel for 2-3 years prior to this survey either knowledge of fasciolosis amongst extension staff was still poor or their ability to transfer this knowledge was deficient. We think it is more likely that the farmer knowledge gap identified probably reflects lack of knowledge transfer. This may be due to a number of reasons, including cultural and communication challenges (i.e. different ethnic groups and languages), remoteness of many villages from DLF district centres in larger towns where some training and information is more readily available, plus the low capacity in and minimal resources of the Lao animal health and livestock production extension system. The desire of farmers for more information on the topic was evident with 95.4% of the interviewed farmers wanting more information on liver fluke especially on its epidemiology, clinical signs, control and prevention.

Only one of the interviewed farmers wanted to know about the economic impact of fasciolosis in cattle and buffalo, probably reflecting that large ruminant smallholder farming in northern Laos is predominantly at subsistence levels and that economic considerations are not yet a driver for farmers to use parasite control in their mixed farming enterprises.

The need for education of farmers and for them to have access to relevant information is evident from the observations that of the interviewed farmers who had no liver fluke knowledge a large proportion reported seeing leaf shaped worms in the livers of slaughtered cattle (18.4%) and buffalo (17.7%) respectively. This further indicates that smallholder farmers did not associate the presence of parasite in livers of cattle and buffalo with any adverse effect on large ruminant health and production. Although it is possible that the leaf shaped structures farmers reported sighting were not *F.gigantica*, we consider this unlikely as extensive slaughterhouse surveys at the same time in the same areas did not reveal the presence of any other trematode or similar looking parasites in the examined livers (Rast et al. 2013a).

Statistical modelling results showed that farmer sighting fluke like worms at slaughter in the liver of their large ruminants was significantly ( $p < 0.023$ ) associated with positive *F.gigantica* FEC status of their herd. This provides some evidence that farmers could determine the infection status of their herd at slaughter and presence of fluke at slaughter could be used as an indicator for the need to treat for liver fluke in the herd of origin. However further investigation on production impact (such as weight gain) is needed to evaluate preventative versus therapeutic treatment as it is likely that avoidable production losses occur much earlier in the disease process. This is supported by our treatment trial results that showed a trend of increased weight gain in the treated buffalo compared to the untreated group (Figure 4.4). Statistical analyses also showed reported morbidity in the household large ruminant herd (animals  $\geq 12$  months old) in the past 12 months was significantly ( $p = 0.023$ ) associated with farmers having seen fluke in livers at slaughter. This could indicate that fasciolosis eventually leads to obvious clinical signs and production losses or that farmer tend to slaughter sick or lighter animals.

Our study did not allow determination of the aetiology of morbidity reported in adult cattle and buffalo by the interviewed farmers as the clinical signs described were of a general nature (not considered pathognomonic for any diseases other than for FMD) and diagnostic investigation of sick animals with submission of samples did not occur.

Treatment rates of sick animals were considered high with 68.0% of ill animals treated, mostly using traditional medicines or plants but also some anthelmintics, antibiotics and human medications. This is of potential concern for food safety and sustainability as development of resistance to antibiotics and anthelmintics due to uncontrolled use of therapeutics is possible and with the widespread unavailability of weight scales suited for adult large ruminants in villages, it is probable that animals are regularly dosed incorrectly.

Villages for the prevalence survey were selected from a list of villages that had been selected to participate in a large livestock development project (ADB 2007) by local experts and did not include all villages in the selected districts, which might have introduced some selection bias. However, random selection of villages for this study would have ensured that our sample is representative of this sampling frame. In the prevalence study not all adult animals in each herd were sampled and *F.gigantica* status was determined using a faecal egg counts, which has low sensitivity. Both could have led to some misclassification of herd status. As we conducted interviews with a large sample of farmers (326) selected randomly from the prevalence survey participants across northern Laos, their geographic distribution reflected a cross-section of mixed rain fed smallholder farming systems in northern Laos. As infrastructure, livestock production and animal health capacity continues to improve in developing countries, future surveys should be able to apply sampling methods that lead to improved validity of results.

#### **4.11.2 Field treatment trial**

Although there is no global accepted standard, generally an anthelmintic is considered effective if there is  $\geq 90\%$  reduction of faecal eggs 14 days post treatment (Fairweather 2011). FECR  $>90\%$  were achieved in our field trial for both anthelmintics used in both cattle and buffalo (Table 4.10). The FECR results

for TCBA were slightly higher and both anthelmintics showed higher efficacy in cattle than in buffalo. This is consistent with previous research that showed lower anthelmintic efficacies at the recommended rates for cattle of triclabendazole (12mg/kg) in buffalo due to more rapid clearance of triclabendazole in buffalo than in cattle. Further, dose rates of 24mg/kg and 36mg/kg triclabendazole given by intra-ruminal injection were 100% effective in buffalo (Sanyal and Gupta 1996).

In our trial 100% efficacy for triclabendazole in buffalo using a dose rate of 24mg/kg was not achieved, although as an oral application was used, it is possible that not all buffalo swallowed the full dose of the anthelmintic due to difficulties of restraining buffalo compared to the much smaller local indigenous cattle. The lowered FECR at week 8 compared to week 4 and then again higher at week 12 post treatment for buffalo treated with TCBA and cattle treated with TCB, could be due to maturation of young flukes or possibly, that fluke eggs were stored in the gall bladder for some time after treatment and flukes were killed and expelled around the time of the faecal collection at week 8.

In cattle the trends in weight gain were similar in treatments and the control group with an initial increase and then loss over the last four weeks of the trial. However in buffalo both treatment groups showed a trend of higher weight gain compared to the control group up to week 8 and then weight loss in all groups over the last four weeks of the trial (Figure 4.4). Weight differences of the groups were not statistically significant ( $p=0.6$ ) over the trial period probably due to inadequate nutrition. Weight loss observed in all groups between week 8 and 12 (October) is likely to be the result of insufficient nutrition to maintain weight at the beginning of the dry season when available grazing feed is of declining quality and quantity, including the available forages grown by some farmers. Although the trial identified successful reduction of the *F.gigantica* egg burden using anthelmintics, for this to improve production at a time of declining pasture quality requires sufficient supplementation of nutrition beyond what is currently available in northern Laos. Resolving nutritional constraints through further experimental research and extension on forages technology in particular may then enable assessment of whether treatment for fasciolosis is able to provide production benefits in the infected animals in the region.



Despite the limitations, this field trial provided evidence that triclabendazole and albendazole/triclabendazole are effective options for management of fasciolosis in smallholder farming systems as they showed high efficacy against *F.gigantica* and were accepted by farmers and local government extension employees.

However, sustainable control of *F.gigantica* needs to be context appropriate and consider farming systems, knowledge, practices of farmers and other large ruminant production stakeholders, plus identify drivers for sustained uptake of control strategies. Sustained widespread internal parasite control is currently not practiced in Laos and many other tropical developing countries for a variety of reasons, including low availability, high cost and low knowledge of farmer and extension workers (Sani et al. 2004) plus the lack of a sound understanding of farmer needs and requirements within the different farming systems (Gray et al. 2012). Parasite management in livestock usually involves a combination of anthelmintics, grazing and husbandry management that is underpinned by good knowledge about the local ecology and epidemiology of the parasite. For farmers, knowledge of the epidemiology, diagnosis, impact and control methods of the parasite, including correct dosage and application, are important. Whilst the drivers for sustained *F.gigantica* control in smallholder farming systems are likely to be complex and require adaptation to different regions and farming systems, awareness of the parasite and knowledge of control amongst stakeholders especially the producers is an essential driver for sustained and widespread control and management. This appears to require continued training of animal health extension personnel, farmers and other livestock production stakeholders implementing extension methods and capacities that allow knowledge transfer to producers.

#### **Conflict of interest**

None

#### **4.12 Acknowledgments**

We thank ACIAR for assistance with funding and all Lao Department of Livestock and Fisheries personnel and farmers who assisted with the study and data collection.

# **CHAPTER 5**

## **DISCUSSION**

## **IMPROVING LARGE RUMINANT PRODUCTION WITHIN SMALLHOLDER FARMING SYSTEMS IN SOUTH-EAST ASIA FOR BETTER FOOD SECURITY: CONTROL OF INTERNAL PARASITES AS AN ENTRY POINT?**

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**Statement of contribution of co-authors**

Luzia Rast prepared this paper. Jenny-Ann Toribio, Syseng Khounsy and Peter Windsor contributed to reviewing and editing the manuscript.



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Peter Windsor

## 5.1 Abstract

*Toxocara vitulorum* and *Fasciola gigantica* are causes of endemic endoparasitism in large ruminants in South-East Asia but effective and sustained control is largely absent. Recent field surveys in northern Laos identified apparent prevalence for *T.vitulorum* in calves and *F.gigantica* in adult cattle and buffalo of 22.6% and 17.2% respectively, with over 70% of sampled villages having at least one animal positive for each parasite using faecal egg counts. In addition a slaughterhouse survey found 34.1% prevalence for *F.gigantica* in slaughtered cattle and buffalo. We surveyed knowledge and treatment practices of smallholder farmers (n=273 and n=326) plus the clinical and financial impact of *T.vitulorum* and *F.gigantica* in two separate studies. Annual calf morbidity and mortality rates were high with 42.6% and 37.3% respectively; as was morbidity of adult large ruminants at 7.8%. Financial analysis showed a large positive net benefit of USD 3.70-14.90 per calf if treated for *T.vitulorum* and farmers estimated the value of sick ruminants as 3.8-33.5% less than that of healthy animals. Farmers had either no (82.4%) or minimal (17.6%) knowledge of *T.vitulorum*. Only 2.5% of the farmers treated their calves with the recommended regime of a single pyrantel treatment when calves are 14-21 days old, despite anecdotal reports that this intervention is highly valued by farmers. Although 20.6% of farmers reported having seen *F.gigantica* in livers of slaughtered cattle or buffalo, they had either none (93.1%) or only minimal (6.9%) knowledge of *F.gigantica* and its impacts, with 95.4% of farmers indicating a desire to learn more about the disease. None of the interviewed farmers practised any *F.gigantica* control in their cattle or buffalo. With increasing red meat demand in the region and evidence that control of *T.vitulorum* in calves is a very cost effective strategy to increase calf survival, plus increasing recognition of fasciolosis as a major animal and human health risk, increased efforts to strengthen 'parasitism extension' capacity is required to ensure that smallholder farmers can optimise their potential contribution to regional food security.

### Keywords

Smallholder farming systems, cattle, buffalo, *Fasciola gigantica*, *Toxocara vitulorum*, farmer knowledge and practices, financial impacts

## 5.2 Introduction

Over the last few decades some countries in South-East Asia (e.g. China, Thailand and Vietnam) have experienced rapidly growing economies and changing political and social structures. This has led to more developed infrastructure and industrial capacities, including agriculture and livestock production and health. However, in parts of these countries and in some other countries of the region (e.g. Laos, Cambodia and Myanmar) rural development in particular has been slow and the dominant smallholder farming population remains poor, and isolated from many of the recent advances in technical and economic capacity.

Increasing wealth and urbanisation has led to a sustained increased demand for red meat that is predicted to grow over coming decades (Delgado 2003, Steinfeld et al. 2006). This offers opportunities and challenges for smallholder farmers in the Greater Mekong Subregion (GMS). Opportunities include providing large ruminants and their products to the expanding red meat market, generating increased household income and potentially alleviating poverty (Windsor 2011). Challenges include reducing the many constraints that impede optimal large ruminant production. Of particular concern is the presence of endemic levels of animal diseases, poor market development, and the low capacity animal health and production systems that predominate in the GMS.

In developing countries smallholder production contributes most of the national agricultural output (Thomas et al. 2002, McDermott 2010). Smallholder farming systems are diverse (Steinfeld et al. 2006) but generally are mixed livestock and cropping enterprises. Large ruminants are traditionally regarded as a wealth store and sold when cash for emergency household expenses is needed, rather than a commodity that can be traded for optimal returns to provide regular income generation. The smallholder farming systems in the GMS of South-East Asia, including northern Laos, mostly operate at subsistence levels with low inputs and outputs, hence are very prone to shocks such as disease outbreaks (Rast et al. 2010) or weather extremes (Khounsy et al. 2012). In the short term, large scale commercialisation of livestock production is often not considered possible or is not supported by governments as capacities are low, land availability is problematic and markets are insufficiently developed. Therefore it

is more beneficial to optimise the production potential of current smallholder livestock systems, rather than introducing new production systems including non-indigenous livestock breeds that are poorly adapted to the GMS environment.

There has been substantial research input in internal parasites in South-East Asia in the past and technologies for control are known (ACIAR 2008, Gray 2012). However more recent studies and particularly research on production impact of parasites and sustained adoption of effective control measures by smallholder farmers are lacking.

We present a summary of studies from northern Laos that determined large ruminant production benchmarks (annual morbidity, mortality, calving rates), *T.vitulorum* and *F.gigantica* prevalence, their clinical and financial impact as well as farmer knowledge and practices about internal parasites plus their control. The objective was to identify constraints to and drivers for improved sustainable internal parasite control in cattle and buffalo within smallholder farming systems.

## **5.3 Material and methods**

### **5.3.1 Study area and villages**

The study area included the five northern Laos provinces of Bokeo, Houaphan, Luang Namtha, Luang Prabang and Xieng Khuang. A cattle and buffalo research project entitled 'Best Practice Health and Husbandry in cattle and buffalo, Lao PDR' (BPHH) (ACIAR 2013) and a larger development project entitled 'Northern Region Sustainable Livelihoods through Livestock Development Project' (LDP) (ADB 2007) operated in these provinces. Both projects aimed to facilitate improved rural incomes through enhancing livestock productivity and were locally managed and implemented by the same Lao Department of Livestock and Fisheries (DLF) employees. The primary sampling unit were 204 villages enrolled in either of these two projects and with more than 20 large ruminants per village. The authority for the research team to work in these villages was provided through the two projects and they had all year road access by car.

### **5.3.2 Improving animal health and research capacity of livestock extension personnel and farmers**

DLF extension employees (28) who worked with the two livestock projects attended a series of seven 2 or 3 day training workshops between 2009 and 2010. The workshops covered large ruminant production and health topics plus basic research and extension techniques. The workshops were delivered by Lao and foreign experts using didactic teaching, practical session as well as small group sessions for problem solving. DLF employees then applied and increased their capacities throughout the study period from 2009 to 2012 by involvement in several related research studies on large ruminant health and production. Collaborating farmers assisted in sampling and data collection, and some participated in training meetings led by extension personnel and farmer exchange visits to other villages during 2012. At the end of the workshop series all DLF extension personnel were assessed on their knowledge by a written examination on the topics covered. The same examination was used to assess the base knowledge of 238 farmers in 2009 (Nampanya et al. 2010) in six villages in three northern provinces (Luang Prabang, Houaphan and Xieng Khouang) and repeated in 2011 and 2012 to assess the change of knowledge (ACIAR 2013).

### **5.3.3 Cross-sectional surveys for *Toxocara vitulorum* and *Fasciola gigantica***

Two separate surveys, based on faecal egg counts were conducted to determine the apparent prevalence of each parasite. At the time of faecal sample collection, additional data was obtained to assess the clinical impact of the parasites.

For the *T.vitulorum* survey, faecal samples and clinical data were collected and analysed from 899 cattle and buffalo calves <90 days old from 69 villages between September 2009 and June 2010 (Rast et al. 2013). The extended survey period was necessary to get a large enough sample of calves young enough for testing. In northern Laos smallholder farmers manage reproduction of their large ruminants minimally. Male and female cattle and buffalo are run together permanently resulting in all year calving with a natural peak between September and February. In addition, to obtain most accurate prevalence estimates using faecal egg counts calves need to be tested when <90 days old



as in older calves patent infection with *T.vitulorum* rapidly decreases (Roberts 1993).

For the *F.gigantica* survey faecal samples and clinical data were collected and analysed from 1268 cattle and buffalo  $\geq 12$  months old from 75 villages between August and December 2010 (Rast et al. 2013a). More accurate testing methods such as the copro-antigen ELISA were considered, but limited local laboratory capacity, transport and resource constraints prevented their use.

#### **5.3.4 Slaughterhouse survey for *Fasciola gigantica***

Across northern Laos, slaughtering of livestock occurs at night in relative small privately owned slaughter facilities where 5-15 large ruminants are killed and processed each night, with products sold at local meat markets (Figure 2.5) the next morning. The main provincial slaughterhouse of each study province was visited on three to five consecutive nights between March and June 2011. All cattle and buffalo presented for slaughter were examined pre and post slaughter. Faecal samples were collected and livers were examined closely for presence of gross lesions indicative of *F.gigantica* infection and when possible the parasite itself, by incising biliary ducts (Rast et al. 2013a).

#### **5.3.5 Field treatment trials**

A small field treatment trial was conducted in Ban Nong village in, Xieng Khuang province where >50% of sampled animals had fluke eggs detected in faeces within the last 12 months to examine the effectiveness of imported triclabendazole oral drench (Fasinex®, Novartis Animal Health Australia Inc.) and locally available triclabendazole/albendazole tablets (Han-Detril-B, Hanvet, Vietnam). The trial animals were administered either one of the anthelmintics or left untreated and body weight and faecal egg counts were monitored on four occasions a month apart over the 12 week trial period from August to October 2011 (Rast et al. 2013c), with faecal egg count reduction calculated for week 4, 8 and 12 post treatment.

Treatment of *T.vitulorum* in calves using pyrantel when they are 14-21 days old has been recommended (Roberts 1993) as pyrantel is effective on mature and immature parasites and treating calves less than three weeks old is prior to

patency of *T.vitulorum* in calves. A field treatment trial to ensure the effectiveness of locally produced pyrantel (Pharmaceutical Factory no. 2, Vientiane, Lao PDR) was conducted between October 2011 and March 2012. Cow and buffalo calves <50 days old (n=41) from four villages in two northern Lao provinces (Luang Prabang and Houaphan) were enrolled in the trial and individually identified with ear tags. In each province one of the two villages and their calves were allocated as treatment group and all calves were weighed and given pyrantel at a dose rate of 125mg/10kg bodyweight at the start of the trial. In the two control villages calves were weighed only. Faecal samples (2-5 g) were collected per rectum from all calves present at the start, week four and week twelve of the trial. Faecal egg counts were performed at the local veterinary laboratory in Luang Prabang and faecal egg count reduction was calculated for week 4 and 12 after treatment. At the end of the trial, all calves in the control villages were also treated with pyrantel at recommended dose.

### **5.3.6 Farmer knowledge and practices assessment**

Two separate farmer surveys were conducted using face-to-face semi structured interviews with a randomly selected number of farmers (n=273 and n=326) that had their cattle and/or buffalo sampled for either *T.vitulorum* or *F.gigantica* one to six months earlier. Data on farmer knowledge and treatment practices for internal parasites in large ruminants, husbandry and production practices and benchmarks as well as financial data were collected. Details of surveys and analysis methods using multivariable logistic regression have been reported (Rast et al. 2013b, Rast et al. 2013c). Farmer knowledge was assessed by asking farmers an open question on their knowledge of either *T.vitulorum* or *F.gigantica* in the respective farmer surveys. Answers were categorised as 'no knowledge' (farmers stated they had no knowledge or knew only the parasite's name or had incorrect knowledge), 'minimal knowledge' (farmers knew the aetiology or some clinical signs or control techniques) or as 'good knowledge' (farmers knew the aetiology and some clinical signs and control techniques and basic epidemiology).

### **5.3.7 Data analyses**

Descriptive, logistic and linear regression analyses of data were conducted using SAS version 9.3 (© SAS 2002-2010, SAS Institute Inc., Cary, NC, USA) and are

described in detail in referenced papers by the senior author. Descriptive analyses of financial data for *F.gigantica* was performed and for *T.vitulorum* a partial budget analysis was carried out using a custom built model in Excel (Microsoft 2010, Redmond, WA USA) to determine the net benefit of treating calves when 14-21 days old with pyrantel compared to not treating them.

## 5.4 Results

### 5.4.1 Prevalence of *Toxocara vitulorum* and *Fasciola gigantica*

The estimated apparent prevalence of both parasites in northern Laos was high with 22.6% (CI 0.17-0.28) for *T.vitulorum* in cattle and buffalo calves <90 days old and 17.2% (CI 0.14-0.21) for *F.gigantica* in cattle and buffalo  $\geq$ 12 months old. Additionally, infections were widespread with 76.8% and 73.3% of villages where animals were sampled having at least one faecal egg count positive animal for *T.vitulorum* or *F.gigantica* respectively (Rast et al. 2013, Rast et al. 2013a).

### 5.4.2 Clinical impact *Toxocara vitulorum*

Overall reported annual calf morbidity was 42.6% (CI 0.38-0.47) and annual calf mortality was 37.3% (CI 0.33-0.42). Morbidity in calves at sampling was assessed as 'general sickness' (17.0%), 'having diarrhoea' (17.2%) and having an 'abnormal coat' (20.3%). Being sick, having diarrhoea and an abnormal coat were not significantly associated with *T.vitulorum* positive faecal egg counts ( $p>0.05$ ) of individual calves. The difference of positive faecal egg count results between cattle (20.9%) and buffalo (25.5%) calves was not statistically significant ( $p=0.107$ ), however the number of eggs in positive buffalo calves was significantly ( $p=0.001$ ) higher compared to cattle calves.

### 5.4.3 Clinical impact *Fasciola gigantica*

Overall reported annual morbidity of cattle and buffalo  $\geq$ 12 months old was 7.4% (CI 0.06-0.09) and annual mortality 2.8% (CI 0.003-0.05). Morbidity by species and gender is presented (Table 5.1). Province, being sick, gender (female) and species (buffalo) were significantly ( $p<0.05$ ) associated with the *F.gigantica* infection status of individual animals. Further, positive liver fluke status and morbidity in large ruminants  $\geq$ 12 month old during the past year in

the household herd was significantly ( $p < 0.023$ ) associated with farmer sighting fluke like worms in livers of cattle or buffalo when slaughtered in the past.

**Table 5.1 Number of large ruminants by species, gender and health status, mean age, monetary value and fluke status of cattle and buffalo  $\geq 12$  months old, northern Laos, 2010-2011**

Variable	Buffalo (n=613)				Cattle (n=956)			
	Female (n=435)		Male (n=178)		Female (n=688)		Male (n=268)	
	Healthy	Sick	Healthy	Sick	Healthy	Sick	Healthy	Sick
Number (%) of large ruminants	401 (92.2)	34 (7.8)	173 (97.2)	5 (2.8)	631 (91.7)	57 (8.3)	248 (92.5)	20 (7.5)
Mean age (months)	74.0	75.0	43.0	36.0	68.0	64.0	39.0	31.0
Mean value (USD)	497.61	478.57	468.79	365.63	292.85	246.55	350.52	232.95

#### 5.4.4 Slaughterhouse surveys

The apparent prevalence in adult animals at slaughter of *F. gigantica* using faecal egg counts was 34.1% (CI 0.26-0.42). Of the 123 livers examined at necropsy 87 (70.7%) had gross liver and bile duct pathology consistent with *F. gigantica* infection (including fibrin tags and fibrous adhesions to adjacent organs or between liver lobes, white foci in the parenchyma, fibrosis around the bile ducts, calcification of bile ducts, necrosis, haemorrhage in parts of the liver and distortion of the liver). Higher rates of gross pathology were observed in buffalo compared to cattle, being 95.6% (n=68) and 40.0% (n=55) respectively.

Additional findings at post mortem examination were that 44% of female cattle and 47% of buffalo were found to be pregnant with gestation lengths ranging from first to last trimester based on foetal size and 9.8% of cattle and buffalo had lesions on the tongue or oral mucosa indicative of FMD.

All parts of the animals including internal organs and fluids were processed and sold at the local meat markets for human consumption two to five hours after slaughter. No condemnation of any products by authorities was observed.

### 5.4.5 Farmer knowledge and practices

A summary of farmer knowledge and practices about *T.vitulum* and *F.gigantica* in cattle and buffalo is presented (Table 5.2). Notably, the awareness about *T.vitulum* in calves was moderate with 62.3% of farmers having heard about the parasite, however more in depth knowledge was lacking with 17.6% of farmers having minimal knowledge and the remainder none. Whilst 60.4% of farmers reported treating calves for *T.vitulum*, only 2.5% used the recommended treatment regime of 125mg pyrantel/10kg bodyweight when calves were 14-21 days old. All farmers interviewed expressed a desire to learn more about *T.vitulum*.

Awareness of *F.gigantica* in cattle and buffalo was low with only 9.2% of farmers having heard about fasciolosis in cattle or buffalo. There was an almost complete lack of knowledge about *F.gigantica* and fasciolosis with 7.4% of farmers having minimal knowledge and the remainder none. This was despite 20.6% of the interviewed farmers having observed the parasite in livers of cattle or buffalo during slaughter. Most farmers (95.4%) interviewed expressed a desire to learn more about *F.gigantica* in large ruminants.

**Table 5.2 Summary of farmer knowledge and practices on *Toxocara vitulum* and *Fasciola gigantica* in large ruminants in northern Laos from 273 and 326 farmer interviews in 2010-2011**

Variable		<i>T.vitulum</i> (%)	<i>F.gigantica</i> (%)
Awareness of parasite	Yes	62.3	9.2
	No	37.7	90.8
Knowledge of parasite	None	82.4	92.6
	Minimal	17.6	7.4
Treatment of parasite	Yes	60.4	0
	No	39.6	0
Effective treatment	Yes	2.5	-
	No	97.4	-
Wanting more parasite knowledge and training	Yes	100.0	95.4
	No	0	4.6

#### **5.4.6 Financial impact**

Partial budget analysis showed a positive net benefit of USD 3.70- 14.90 per calf (attributing 25%, 50%, 75% or 100% of morbidity and mortality to *T.vitulorum* infection) when treating calves with pyrantel once only when 14-21 days old compared to not treating them (Rast et al. 2013b).

Adult ( $\geq 12$  months old) cattle and buffalo that were sick had 3.8%-33.5% lower mean monetary values than healthy animals in their same species and gender class (Table 5.1), with heavier male animals losing more of their value when sick compared to females.

#### **5.4.7 Knowledge of farmers and extension personnel on large ruminant production, health and husbandry**

Initial farmer and extension personnel knowledge was low with a mean knowledge score of 15.7 out of a possible 42 (Nampanya et al. 2010). Knowledge scores of extension employees were increased at the end of the workshops with an average score of 32.6. A proportion of farmers that were tested again in 2011 and 2012 showed higher mean knowledge scores of 26.1 and 24.8 respectively, indicating that some knowledge was gained and retained (ACIAR 2013).

#### **5.4.8 Treatment trials**

The field trial using triclabendazole and triclabendazole/albendazole combination showed that both anthelmintic preparations were effective to control *F.gigantica* by reducing faecal egg counts  $>90\%$  four to twelve weeks post treatment in buffalo and cattle (Rast et al. 2013c).

The field trial using locally produced pyrantel in cattle and buffalo calves showed faecal egg count reduction for *T.vitulorum* of  $\geq 95\%$  at four and twelve weeks post treatment in both species (unpublished).

### **5.5 Discussion**

Our field surveys confirmed high apparent prevalence rates of *T.vitulorum* and *F.gigantica* in cattle and buffalo of 22.6% and 17.2% respectively. Suboptimal large ruminant production output in the participating herds is apparent with

concurrent high annual morbidity of 42.6% in calves and 7.8% in adults, plus mortality rates in calves of 37.3% and 2.8% in adults. In addition, low annual reproductive rates of 0.6 calf per adult female per year for cattle and 0.5 calf for buffalo (Rast et al. 2013b) contributed further to suboptimal production outputs. Further studies will be required to quantify contribution of *T.vitulorum* or *F.gigantica* to overall morbidity and mortality rates of large ruminants in northern Laos and the GMS region, as such studies require greater diagnostic capacities and resources than were available for our surveys.

Our studies on endoparasitism also identified large knowledge gaps amongst smallholder farmers, especially the more in depth knowledge, such as clinical impact and control or prevention. This was demonstrated with 60.4% of farmers reporting to treat calves for *T.vitulorum*, but more in depth questioning revealing that only 2.5% of interviewed farmers used effective treatment of calves (correct anthelmintic, dose rate and time) to control the parasite. Awareness and knowledge of *F.gigantica* was even lower with only 9.2% of interviewed farmers having heard about fasciolosis, and 92.6% of farmers having no knowledge and none treating for the disease. This is despite 20.6% of farmers identifying that they had previously observed *F.gigantica* at slaughter (Rast et al. 2013c) and some having been involved in livestock development and research projects in recent years that included the epidemiology and control of internal parasites infection in large ruminants.

The identified knowledge gap is more likely due to a lack of knowledge transfer rather than lack or unavailability of knowledge and technologies per se as extension personnel and some farmers had been part of recent research studies and training (ADB 2005, Windsor 2006, ACIAR 2008, ACIAR 2013). Limited capacities within the Lao DLF extension system, with employees having very limited resources for extension activities and continued training beyond what is funded by external projects, remoteness of some villages with little interaction between farmers (i.e. farmer groups or associations) plus farmers in northern Laos belonging to various ethnic groups with different traditions, language, values and beliefs are probably factors that contribute to the challenges and effectiveness for effective knowledge transfer.

The large positive net benefit identified in our partial budget analyses of USD 3.70-14.90 per calf if treating with pyrantel for *T.vitulum* (Rast et al. 2013c) plus the estimated reduction in monetary values of 3.8-33.5% per sick large ruminant (Table 5.1) provide an important extension message. Financial impacts of preventable diseases are likely to become more important if smallholder farming systems are to respond to market demands for increased red meat production. For smallholder farmers this will mean changing from the keeping of large ruminants as an assets store to their production as a commodity providing more regular income generation and presents a cultural change. It is likely that once such a change has occurred, commercial drivers become of greater importance to achieve adoption of preventative animal health investments such as regular internal parasite control by smallholder farmers.

Notably a much larger knowledge gap on *F.gigantica* in large ruminants amongst smallholder farmers and DLF extensions personnel than for *T.vitulum* was identified in our studies. Clearly more basic knowledge is required before effective and sustainable adoption of any control measures can be expected. Knowledge transfer on fasciolosis provides a challenge due to its complex epidemiology and control methods. However with increasing recognition of fasciolosis as an important human health issue (Mas-Comas et al. 2005, Soliman 2008, Torgerson & Macpherson 2011) control and prevention of fasciolosis in large ruminants (and other hosts) becomes a higher priority.

A feature of most developing countries is that there is minimal regulation on the quality, availability and use of pharmaceuticals. Antimicrobials and anthelmintics for livestock are usually procured by smallholders without prescription or professional advice. This was confirmed in our studies with 60.2% of interviewed farmers treating their sick calves using antimicrobials, anthelmintic and other preparations, but only 2.5% of the farmers using recommended dosage regime for *T.vitulum*. Further 68.0% of adult sick cattle and buffalo were given treatment by the farmers using an assortment of chemicals including traditional medicines, salt, antibiotics, anthelmintics, human antidiarrheal medication and petrol (Rast et al 2013b, Rast et al 2013c).



Correct use and dosage of anthelmintic (and other therapeutics) in livestock is an urgent priority to ensure effective control of targeted parasites or pathogens in the first place, but also to protect food safety and quality. This is becoming more important as red meat demand and consumption in the region is increasing. Further, with likely reliance on chemicals for internal parasite control, at least in the more immediate future, correct dosage is important to delay the development of anthelmintic resistance.

Addressing the production deficits caused by internal parasites, especially *T.vitulorum* provides an ideal entry point to increase large ruminant production as it results in increased calf survival and growth. It also provides a relative quick and visible positive outcome to farmers in return for an investment that is simple and requires few resources. Whilst our studies showed an increase in farmer knowledge over the period extension personnel were active in the villages, our research also showed a lack of correct adoption of this technology by farmers. This provides evidence that knowledge transfer requires continued and maybe additional support and monitoring. It is also important to identify any failure of recommended interventions before sustainable use by farmers is being discouraged possibly by lack of evident beneficial results for them or for other reasons. It has been suggested that long term changes can only be achieved and sustained if strong multi-disciplinary and participatory approaches with research are developed (Millar & Phoutakhoun 2008, Windsor 2011, Catley et al. 2012, Gray et al. 2012). Our results supports this but also indicate that to commence this process a knowledge base among smallholder farmers that is currently lacking needs to be built by ensuring knowledge transfer to farmers occurs and is sustained.

**Conflict of interest**

None

**5.6 Acknowledgments**

Sonevilay Nampanya and Bounthom Khounsy are thanked for their assistance in fieldwork and translation. Funding support was gratefully provided by the Australian Centre for Agricultural Research and the Australian Crawford Fund.

## **CHAPTER 6**

### RECOMMENDATIONS FOR FUTURE WORK

## 6.1 Introduction

As confirmed in our studies, large ruminants within smallholder farming systems in northern Laos are still predominantly raised using traditional low input methods. Notably, cattle and buffalo are raised and kept as assets and sold when the household needs cash for unexpected larger expenses. In addition many constraints to increase large ruminant production outputs exist. Constraints include endemic diseases, and an animal health system with low capacities and lack of knowledge and knowledge transfer on modern large ruminant production techniques. In addition and especially in remoter areas, market are still poorly developed providing little commercial incentives for smallholder producers. Therefore smallholder farmers have little opportunity to benefit from the increased regional demand for red meat that is driven by urbanisation and increasing wealth in some parts of the region.

In Laos, as in many developing countries, there are multiple concurrent high priority constraints to improved wellbeing of the population, which makes decisions on allocation of scarce resources difficult. Improving animal health and livestock production are important as they contribute towards many desirable objectives in the region, including better food security and food safety, and reduction of rural poverty.

Our studies have enhanced knowledge on the clinical and financial impact of *T.vitulorum* and *F.gigantica* and on methods to enhance awareness and improve control of these parasites, and also highlighted continuing large knowledge gaps that warrant future attention and research.

## 6.2 *Toxocara vitulorum* and calf morbidity

In our surveys we found high calf morbidity rates and diarrhoea was the most commonly reported clinical sign. Yet an analysis showed that calves having diarrhoea was not significantly associated with *T.vitulorum* infection. Further research is necessary to determine the different aetiologies of calf morbidity and mortality, especially of factors leading to diarrhoea in young calves. Such studies would also assist in better quantification of different syndromes' contribution to

morbidity and mortality, allowing more considered decisions on how to best reduce calf morbidity and mortality.

Goats are a common livestock species within smallholder farming systems in Laos. Therefore research to clarify their role as well as that of other livestock species in the epidemiology of *T.vitulorum* is needed.

Further research into effective treatment of *T.vitulorum* hypobiotic stages in adult females is needed. This becomes more important as inter-calving intervals are likely to decrease with better husbandry and productivity and the risk of females infecting 2-3 subsequent calves increases. Ideally chemical treatment effective for several parasite species and stages (i.e. *T.vitulorum* and *Fasciola spp*) would provide most benefit.

Larvae migrans in humans of *T.cati* and *T.canis* have been described and human infection with *T.vitulorum* has been suggested (Fernando et al. 1970, Roberts 1993, Starke-Buzetti 2006, Macpherson 2013). Further studies to identify (1) the significance of larvae migrans in the human population in rural South-East Asia including *T.vitulorum* and (2) diagnostic methods, especially serology that differentiates between *Toxocara spp.* are indicated.

Finally, to strengthen the extension message for *T.vitulorum* control in large ruminants, the financial and production benefit of *T.vitulorum* control requires further studies that quantify treatment effects on specific production parameters such as weigh gain and growth rates of large ruminant calves.

### **6.3 *Fasciola gigantica***

With identified high and widespread prevalence of *F.gigantica* across northern Laos and indications that human fasciolosis is increasing globally, surveys to quantify prevalence in humans plus to identify risk factors for human infection in South-East Asia are indicated. This will require collaboration between professionals in human and veterinary medicine as well as agricultural and social sciences.

The epidemiology and ecology of *F.gigantica* in different regions including intermediate snail hosts needs further research as this may identify opportunities for non-chemical control, which is likely to become more important as anthelmintic resistance is increasing globally.

#### **6.4 Diagnostic methods**

While local animal health systems lack capacity, including diagnostic capacity, regular whole herd (household or village level) treatment for *T.vitulorum* (calves only) and *F.gigantica* (adult animals) is indicated to achieve optimal control. As diagnostic and surveillance capacity increase, targeted treatment of infected herds or areas using currently available diagnostic methods of faecal egg counts should be possible. The practicality and field application of more specific and sensitive tests, such as copro-antigen or antibody ELISA tests for *F.gigantica*, needs investigation to enable more targeted treatment and control of these parasites.

#### **6.5 Slaughterhouse surveillance and meat inspection**

Our slaughterhouse surveys found this to be an efficient surveillance method to estimate *F.gigantica* prevalence and liver damage due to fasciolosis. The surveys also assisted in identifying other diseases (i.e. FMD), production and farmer practices (i.e. slaughter of a large proportion of pregnant females and older animals), and slaughter and processing practices. As the increasing meat demand in the region is likely to continue to drive large ruminant production volume, ensuring food safety and quality become higher priorities. This will require an effective meat inspection system to identify and condemn poor quality or contaminated products considered unsafe for human consumption. Whilst such a system is needed to increase food safety it would also assist in providing important disease and production surveillance data that could assist in driving disease control at smallholder farmer level and improve animal welfare. However to enable effective feedback to producers a livestock identification system plus a better resourced animal health system is also required.

## **6.6 Knowledge transfer and sustained adoption of new livestock husbandry practises**

Our research identified lack of knowledge transfer to smallholder producers even with a simple technology such as *T.vitulorum* treatment, leading to an absence of effective and sustained adoption by farmers. This demonstrates that just offering a new technology does not necessarily lead to widespread adoption.

More research is indicated to identify methods that sustainably engage smallholder producers of different ethnic backgrounds and with varied opportunities and methods to access information to develop and adopt new technologies.

Further, monitoring to ensure that extension messages engage producers and lead to increased livestock production are also needed. This sociological research is particularly important as a large shift or cultural change is necessary for smallholder producers to become large ruminant producers rather than being large ruminant keepers. Once identified and understood, drivers for such a change need to be well supported.

Importantly, for sustained livestock production to contribute to future regional food security, interdisciplinary collaborations need to be achieved to ensure sustained regional and global food security, food safety, and human and animal health and welfare.

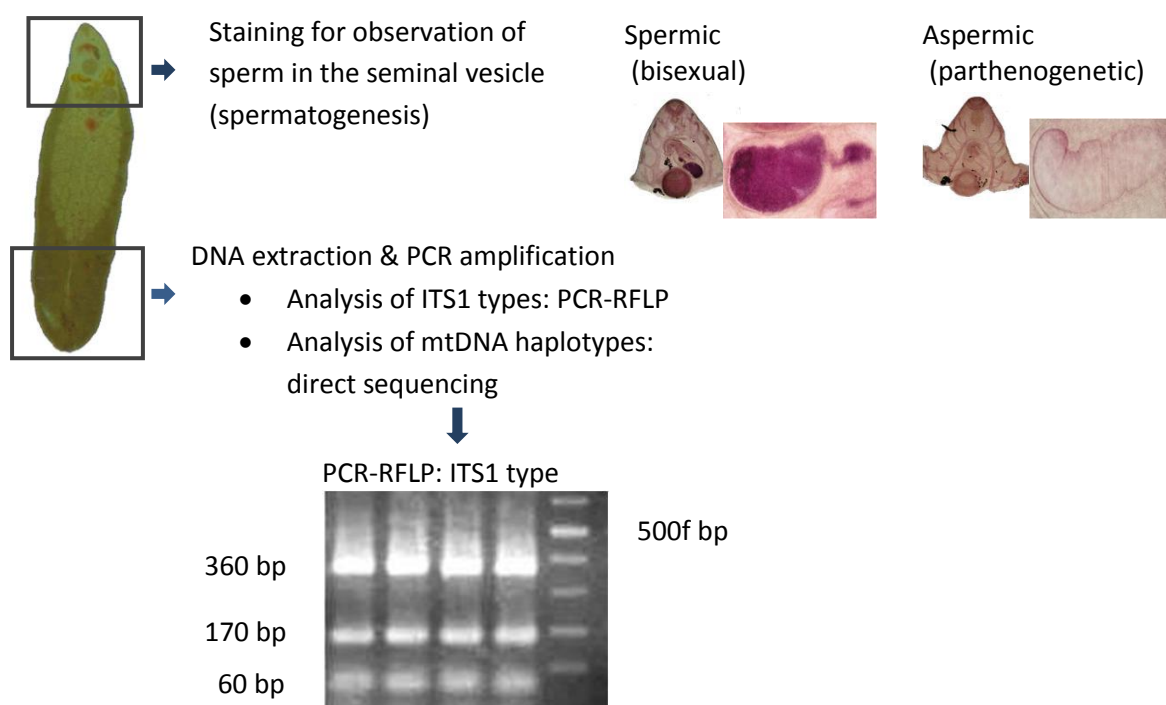
## APPENDIX

All work presented in this appendix was completed by:

Madoka Ichikawa-Seki, DVM, PhD, A/Professor  
Parasitology Laboratory, Faculty of Agriculture, Iwate University  
Ueda3-18-8, Morioka, Iwate 020-8550, Japan

Specimens for the analysis were collected by Luzia Rast during the slaughterhouse surveys in northern Laos between March and June 2011.

## Phylogenetic Analyses of *Fasciola* flukes

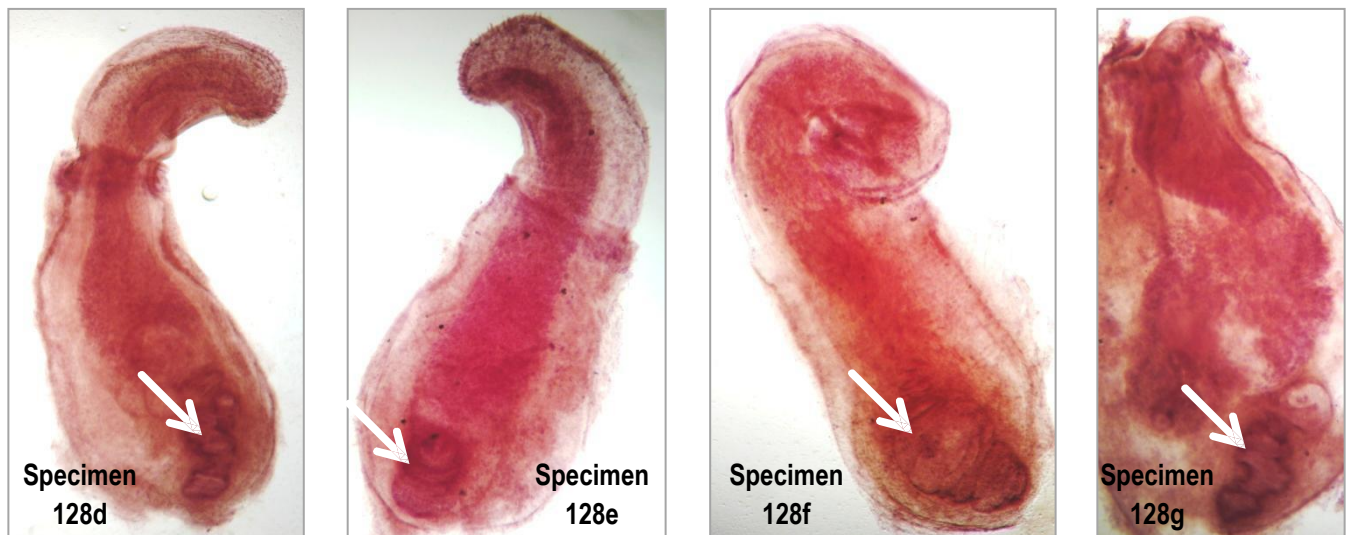
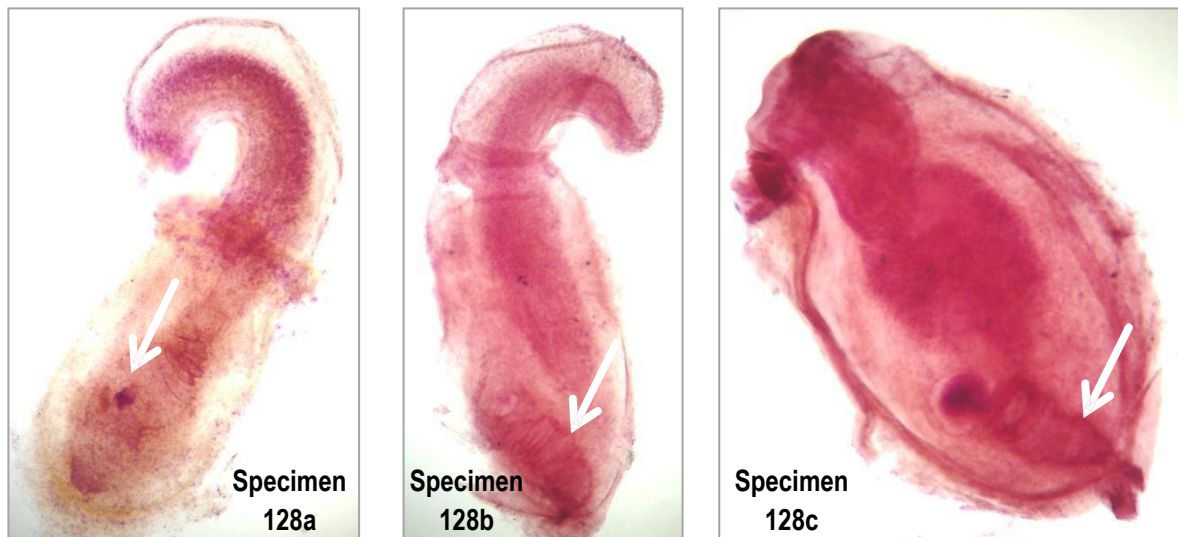
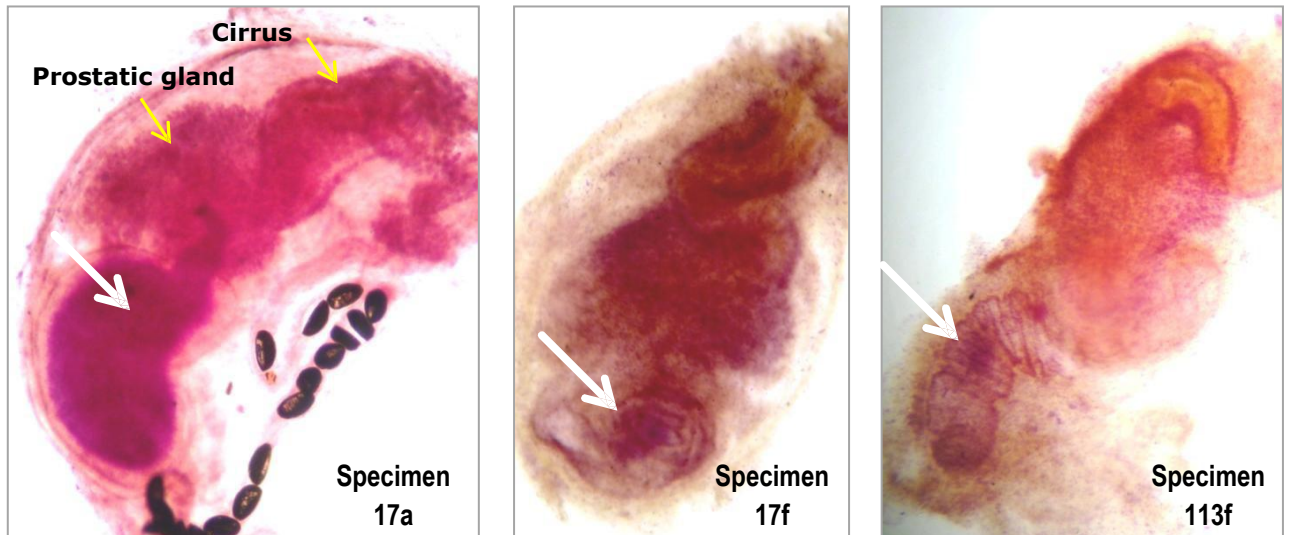


**Table A1 Results of phylogenetic analysis of *Fasciola* flukes from Lao, 2012**

Origin of Animal	Host Species	Number of fluke	Average size (mm)		Spermatogenesis		ITS1 type	<i>Fasciola</i> species
			Length	Width	Spermic	Aspermic		
Phonsavanh	Buffalo	6	24.0	8.8	5	1	Fg	<i>F.gigantica</i>
Phonsavanh	Cattle	3	24.6	6.5	1	2	Fg	<i>F.gigantica</i>
Phonsavanh	Buffalo	4	25.0	8.4	4	0	Fg	<i>F.gigantica</i>
Phonsavanh	Cattle	3	25.3	9.2	2	1	Fg	<i>F.gigantica</i>
Huaxay	Buffalo	6	31.0	9.0	6	0	Fg	<i>F.gigantica</i>
Huaxay	Buffalo	1	28.0	8.5	1	0	Fg	<i>F.gigantica</i>
Huaxay	Buffalo	1	28.0	10.0	0	1	Fg	<i>F.gigantica</i>
Luang Namtha	Buffalo	9	29.0	9.7	8	1	Fg	<i>F.gigantica</i>
Luang Namtha	Buffalo	2	30.0	10.0	2	0	Fg	<i>F.gigantica</i>
Luang Namtha	Buffalo	1	23.7	7.0	0	1	Fg	<i>F.gigantica</i>
Sam Nua	Buffalo	19	24.5	9.5	15	4	Fg	<i>F.gigantica</i>
Sam Nua	Buffalo	3	28.0	12.0	2	1	Fg	<i>F.gigantica</i>
Luang Prabang	Buffalo	7	-	-	2	5	Fg	<i>F.gigantica</i>
Luang Prabang	Buffalo	2	20.0	6.0	2	0	Fg	<i>F.gigantica</i>



**Sperm in the seminal vesicles of *Fasciola* flukes from Lao**

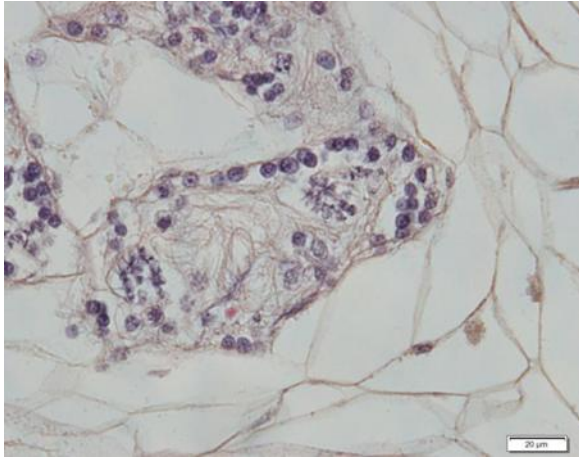


Seminal vesicles are indicated with white arrows

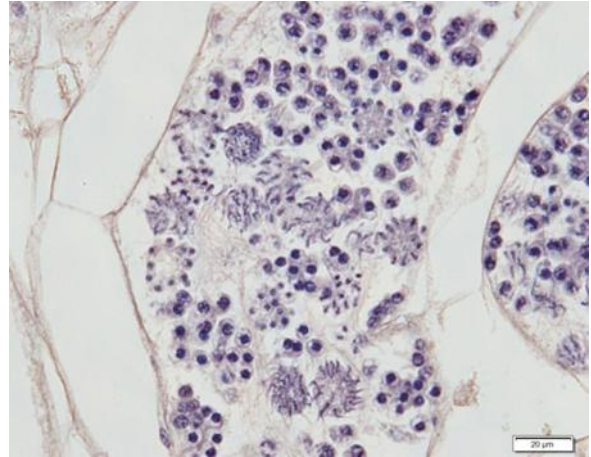
17a: filled with mature sperm (a typical example of spermic flukes)

17f, 113f, 128a-g: containing few or no sperm

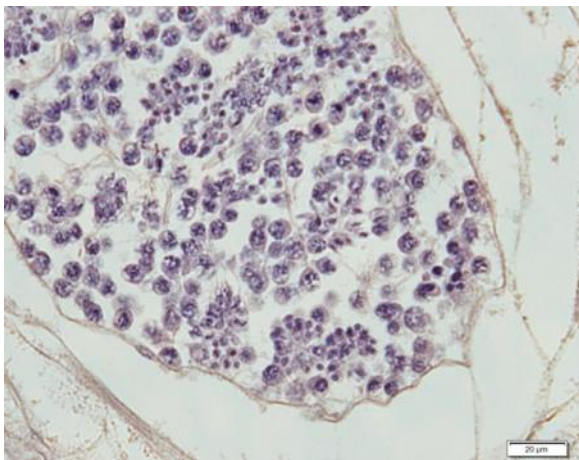
**Histology of Lao *Fasciola gigantica* specimens with few or no sperm**



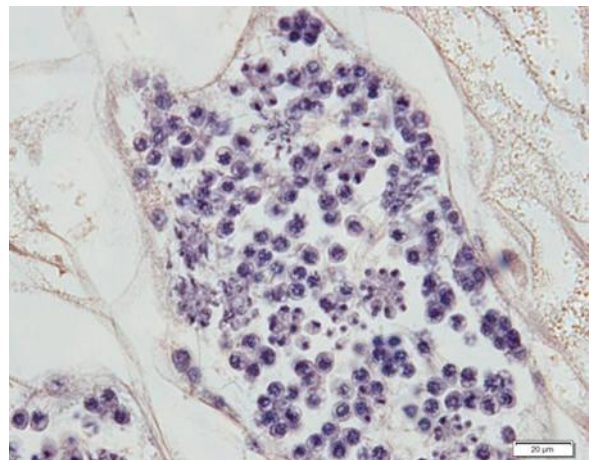
Lao *F.gigantica* specimen 17f



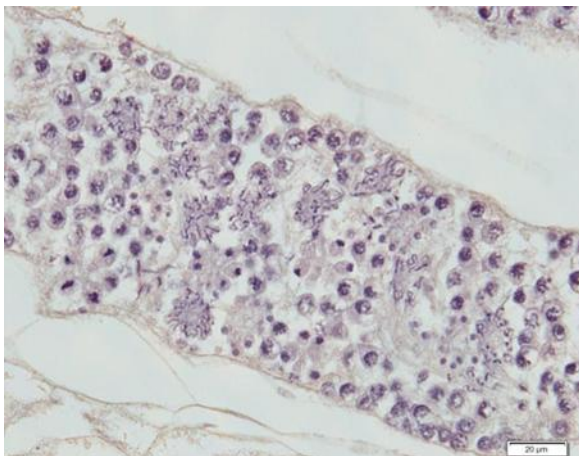
Lao *F.gigantica* specimen 113f



Lao *F.gigantica* specimen 128a

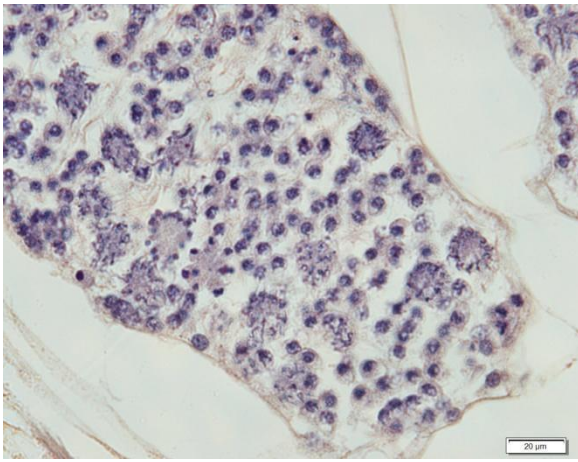


Lao *F.gigantica* specimen 128b

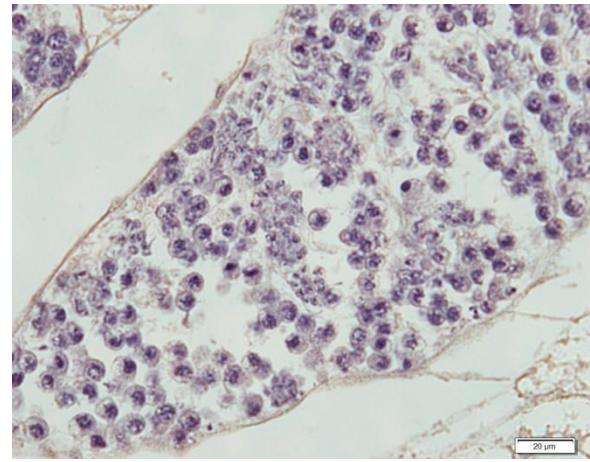


Lao *F.gigantica* specimen 128c

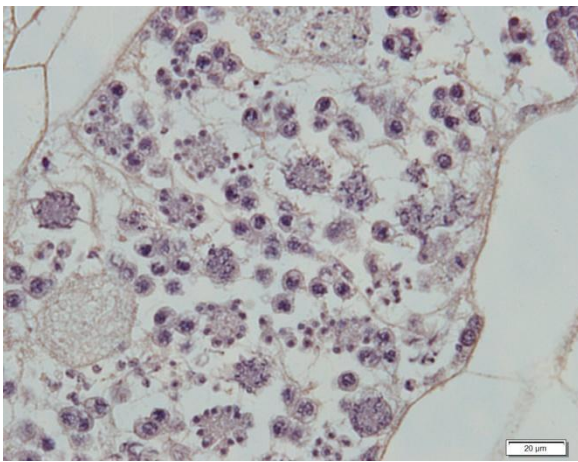




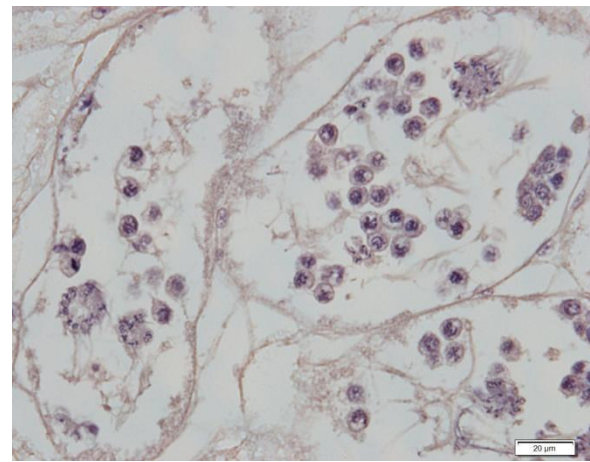
Lao *F.gigantica* specimen 128d



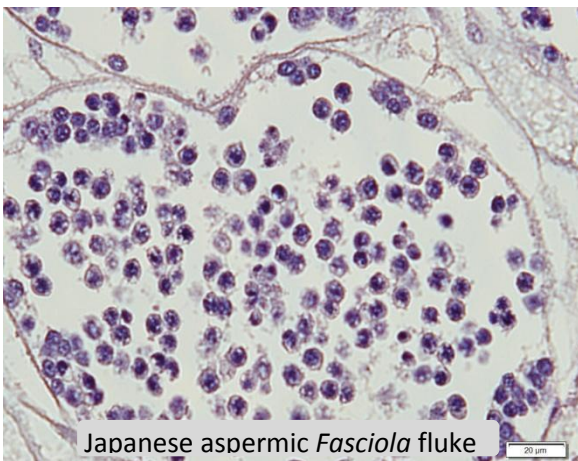
Lao *F.gigantica* specimen 128e



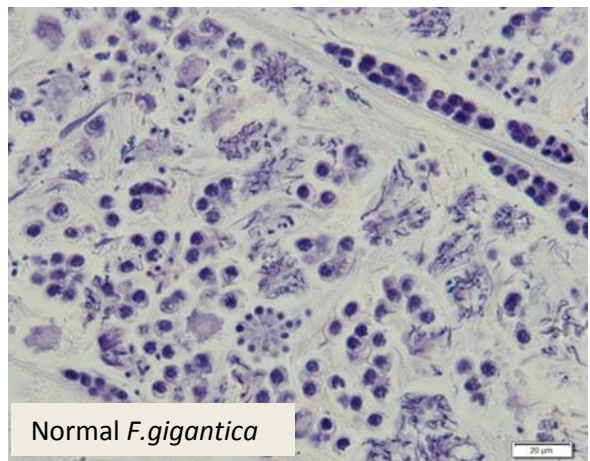
Lao *F.gigantica* specimen 128f



Lao *F.gigantica* specimen 128g



Japanese aspermic *Fasciola* fluke

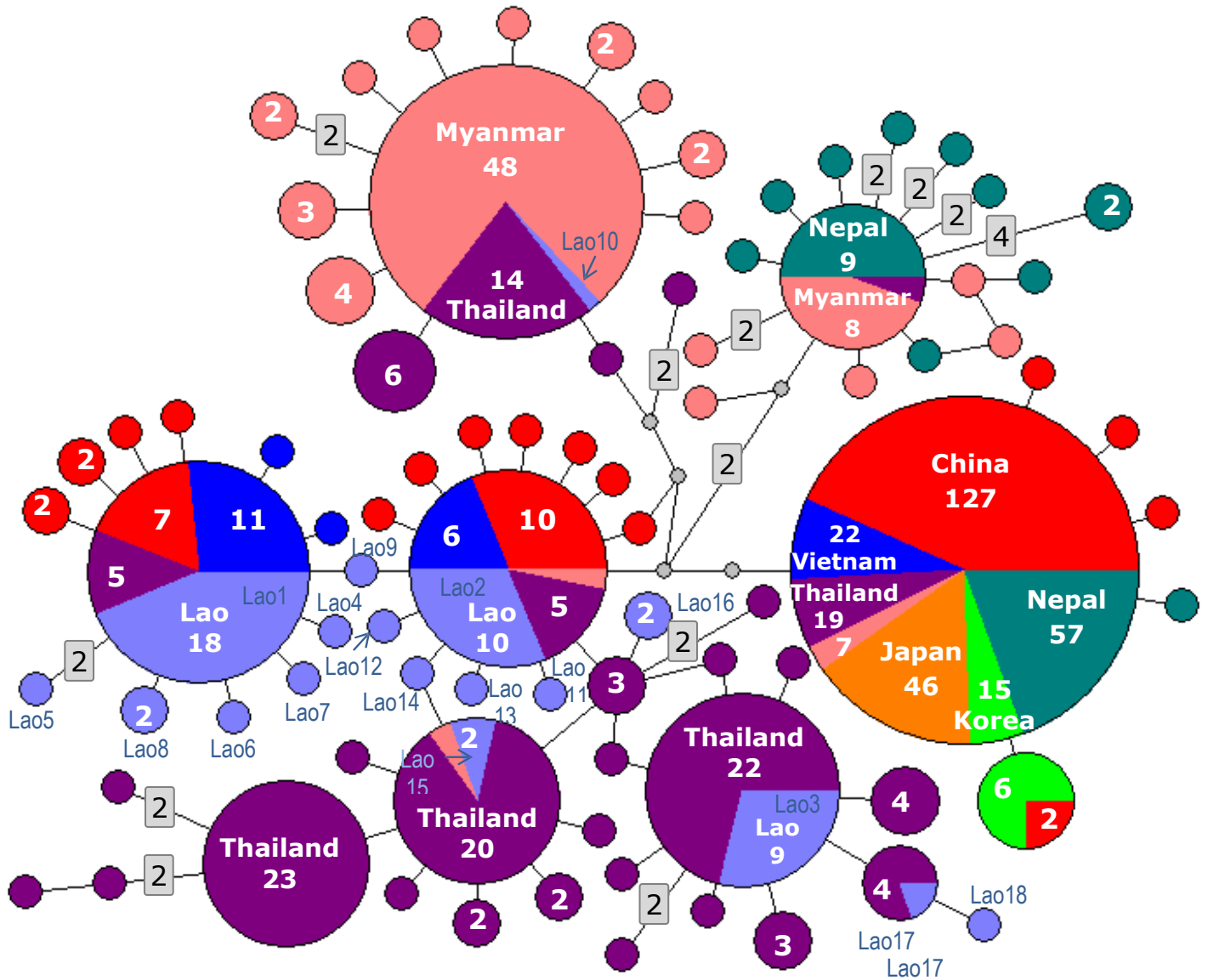


Normal *F.gigantica*

113f, 128a-f: Lack mature spermatozoa, other stages are same as normal *Fasciola gigantica*.

17f, 128g: Few cells in their testis

**Reduced-median network on the basis of mitochondrial *nad1* haplotypes from Asian *Fasciola* flukes (*F.gigantica* haplogroup)**



- Lao
- Myanmar
- Thailand
- Nepal
- median vectors
- China
- Vietnam
- Japan
- Korea

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