

Epidemiology of Zoonotic and Neglected Tropical Diseases in the Lao People's Democratic Republic

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For Finn and Alice

"If the misery of the poor be caused not by the laws of nature, but by our institutions, great is our sin".

Charles Darwin

"As is a tale, so is life: not how long it is, but how good it is, is what matters".

Lucius Annaeus Seneca

Declaration

I declare that this thesis is my own account of my research, with due acknowledgement made to the contribution of others, and contains as its main content work that has not previously been submitted for a degree at any other tertiary educational institution.

.....

James V Conlan

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Abstract

Laos is one of the poorest and least developed countries in Southeast Asia and living conditions, livestock production and cultural practices place large proportions of the population at risk of exposure to a range of parasitic and viral zoonoses. Surveys of humans, pigs and dogs were conducted to determine the prevalence of and risk factors associated with the transmission of *Taenia solium* and related *Taenia* species, *Trichinella* spp., soil-transmitted helminths (STH), and viral zoonoses including Japanese encephalitis virus (JEV), hepatitis E virus (HEV), swine influenza virus (SIV) and Nipah virus (NiV). Surveys were conducted in villages and slaughterhouses in four ethnically diverse provinces of northern Laos.

The human, pig and dog populations studied had a very high prevalence of parasite infection and zoonotic transmission between humans and animals was apparent for multiple species including taeniasis/cysticercosis, trichinellosis and hookworms. Cysticercosis in the human population was relatively rare with a prevalence of less than 2%, although a focal distribution and concentration of cases in a small number of villages was evident. *Taenia saginata* was the dominant *Taenia* species infecting people and *T. hydatigena* was the dominant species infecting pigs. *Trichinella spiralis* was the only species detected in pigs and we found serological evidence that human exposure to *Trichinella* larvae was common. STH infections were very common and the poorest members of the survey population and people of the Mon-Khmer ethnic group were at greatest risk of having an STH infection.

JEV was identified as being hyper-epizootic in northern Laos and remains an unmanaged threat to human health. The hemagglutination inhibition seroprevalence of JEV in the pig population was 74.7% and IgM seroprevalence of 2.3 % peaked in the monsoonal wet season months. Seroprevalence of HEV was 21.1% and the molecular characterisation of HEV isolates from village pigs demonstrated genetic homogeneity with human HEV isolates from China.

This thesis presents new data on a wide range of neglected tropical diseases, ranging from parasitic infections associated with poverty and poor sanitation through to non-discriminating zoonotic viruses. The zoonotic and neglected tropical diseases circulating in Laos are, undoubtedly, a major burden on public health and wellbeing and initiatives to prevent transmission are urgently required.

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I wish to finally acknowledge the support of family and friends, in particular my wife, Iwona, for her love, patience, encouragement and interest in science of all denominations.

List of publications, and work in progress, included in this thesis

Chapter 2

Conlan, J.V., Vongxay, K., Khamlome, B., Dorny, P., Sripa, B., Elliot, A., Blacksell, S.D., Fenwick, S. and Thompson, R.C.A. (2012) A cross-sectional study of *Taenia solium* in a multiple taeniid-endemic region reveals competition may be protective. *American Journal of Tropical Medicine and Hygiene* 87(2): 281-291.

Chapter 3

Conlan, J.V., Khamlome, B., Vongxay, K., Elliot, A., Pallant, L., Sripa, B., Blacksell, S.D., Fenwick, S. and Thompson, R.C.A. (2012) Soil-transmitted helminthiasis in Laos: A community-wide cross-sectional study of humans and dogs in a mass drug administration environment. *American Journal of Tropical Medicine and Hygiene* 86(4): 624-634.

Chapter 4

Conlan, J.V., Vongxay, K., Khamlome, B., Gomez-Morales, M.A., Pozio, E., Blacksell, S.D., Fenwick, S. and Thompson, R.C.A. Patterns and risks of trichinellosis in pigs and humans in northern Laos. *For submission to journal*

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Chapter 6

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List of other publications related to this thesis

Review articles:

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3. Willingham, A.L., Wu, H-W, Conlan, J. and Satrija, F. (2010) Combating *Taenia solium* Cysticercosis in Southeast Asia: An Opportunity for Improving Human Health and Livestock Production. *Advances in Parasitology* 72: 235-266.
4. Thompson, R.C.A. and Conlan, J.V. (2011) Emerging issues and parasite zoonoses in the Southeast Asian and Australasian region. *Veterinary Parasitology* 181(1): 69-73.
5. Conlan, J.V., Sripa, B., Attwood, S. and Newton, P.N. (2011) A review of parasitic zoonoses in a changing Southeast Asia. *Veterinary Parasitology* 182(1): 22-40.

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Statement of human and animal ethics approval

The protocol for surveys involving human subjects was reviewed and approved by the Murdoch University Human Ethics Committee (Project number: 2008/266) and the Lao Ministry of Health National Ethics Committee for Health Research (Number: 239/NECHR) prior to commencing field activities.

For the studies involving dogs and pigs, the protocols were reviewed and approved by the Murdoch University Animal Ethics Committee (project number: R2108/07).

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List of abbreviations

AAHL	Australian Animal Health Laboratory
ACIAR	Australian Centre for International Agricultural Research
AOR	Adjusted odds ratio
CI	Confidence interval
DALY	Disability adjusted life years
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphates
ELISA	Enzyme linked immunosorbent assay
ES	Excretory secretory
FECT	Formalin-ether-concentration technique
HEV	Hepatitis E virus
HN	Haemagglutinin – neuraminidase (influenza virus membrane glycoproteins)
JEV	Japanese encephalitis virus
LFNC	Lao Front for National Construction
LECS	Lao economic and consumption survey
MDA	Mass drug administration
NiV	Nipah virus
NTD	Neglected tropical disease
OIE	World Organisation for Animal Health
ORF	Open reading frame
RNA	Ribonucleic acid
SIV	Swine influenza virus
STH	Soil transmitted helminth
WHO	World Health Organisation

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Introduction to thesis

Thesis structure

This thesis is presented as a compilation of inter-related published papers. At the time of submission, all but one of the papers have been published in high quality peer reviewed journals. The published papers (Chapters 2, 3, 5 and 6) are reproduced in this thesis as re-formatted copies of the corrected proofs. The style and format is consistent throughout the thesis with all references, figures, tables, funding sources, acknowledgements and author declarations reproduced as published after the main body of text in each chapter. As such, there is some duplication of material, particularly in the materials and methods section of each chapter and references.

Historical context

The research and results described and discussed in this thesis were assembled during the course of a project funded by the Australian Centre for International Agricultural Research (ACIAR), Project Number AH2006/161. The Australian organisation commissioned to manage the project was the School of Veterinary and Life Sciences, Murdoch University, Perth, Australia. The project entitled “*Management of pig associated zoonoses in the Lao People’s Democratic Republic*” commenced in January 2008 and was successfully completed in December 2010. As might be ascertained from the project title, the central objective of the project was to establish the evidence base for a range of pig associated zoonoses in Laos and identify public health interventions to prevent pathogen transmission between pigs and humans. Very early in the project, the carriage of *Taenia hydatigena* in village dogs was identified as a crucial factor in the ecology of *Taenia* cysticercosis in pigs in northern Laos. As such, surveys were also conducted in the village dog populations selected for the human and pig surveys. During the analysis of human and dog faecal samples, a very high prevalence of soil-transmitted helminths was recognised. The importance of these pathogens in the overall host-pathogen ecology could not be ignored and the research took a direction toward the broader zoonotic and neglected tropical diseases, rather than focusing solely on pig-associated zoonoses.

Thesis contents

Chapter 1 introduces the reader to Laos and the varied neglected tropical diseases and zoonoses examined in this thesis. Thereby setting the context for the epidemiological risk factors explored in this thesis, including geography, demographics, education, economic indicators and poverty,

ethnic diversity, general health and sanitation. The final section of Chapter 1 outlines the research objectives and specific questions addressed in this thesis.

Chapters 2 to 4 report the findings of the parasitic zoonoses and neglected tropical diseases surveys in multiple hosts. The epidemiology of *Taenia* spp. in humans, pigs and dogs is presented in Chapter 2. The epidemiology of soil transmitted helminths in humans and dogs, including hookworm, *Ascaris lumbricoides*, *Trichuris trichiura* and *Strongyloides stercoralis* is presented in Chapter 3. The epidemiology of trichinellosis in pigs and humans is presented in Chapter 4.

Chapters 5 and 6 report the findings of the viral zoonoses surveys in pigs. The sero-epidemiology of Japanese encephalitis virus, hepatitis E virus, swine influenza virus and Nipah virus are presented in Chapter 5. The molecular characterisation of hepatitis E virus in village pigs is presented in Chapter 6.

Chapter 7 brings together the results and discussion of the preceding chapters to present an overall synopsis of the zoonotic and neglected tropical diseases in Laos, including a discussion of the limitations of the research presented, an outline of future research needs and proposals for the sustainable control of zoonotic and neglected tropical diseases in northern Laos.

Appendix 1 details the consent form provided to participants prior to being asked to take part in the survey.

Appendix 2 is the questionnaire used in the village surveys together with a brief description of the field trial process.