

**Epidemiology, control and potential insect vectors of  
*Trypanosoma evansi* (surra) in village livestock  
in southern Philippines**

Thesis presented by

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## **Declaration**

I declare that this thesis is my own account of my research and contains as its main content work which has not been previously submitted for a degree at any tertiary education institution.

**Alan Payot Dargantes**

## Abstract

The objective of this project was to determine the extent and impact of infection with *Trypanosoma evansi* in livestock in Mindanao, Philippines, evaluate economic benefits of control options and determine its vectors. The project was undertaken because of insufficient knowledge on the dynamics and impact of surra in livestock in the island and because sporadic serious epidemics have occurred in recent years despite the implementation of control measures.

Data from cross-sectional surveys conducted in 2002-6 involving more than 2,000 animals were utilized to estimate the impact of *T. evansi* infection in buffalo populations. A bio-economic infectious disease model was also developed using these data and data from follow up surveys to evaluate economic losses and benefits of control of *T. evansi* in different animal hosts. *Trypanosoma evansi* infection caused significant negative impact on buffalo populations with high mortality and reproductive losses. The estimated financial losses from *T. evansi* infection are high. However, targeted treatment of all sick animals throughout the year using a highly effective drug would have substantial benefits. The estimated annual total financial net benefit from an effective surra control for a typical village in a moderate/high-surra risk area in Mindanao was US \$158,000. The value added to buffaloes, cattle, horses, goats/sheep and pigs as a result of this control was US \$88, \$84, \$151, \$7, \$114 per animal per year, respectively.

Follow up surveys were conducted in 2007-8 to determine the prevalence of *T. evansi* infection in 2,383 buffaloes and other animals (290 goats, 226 cattle, 151 pigs and 35 horses) from 73 villages in Mindanao, investigate associations between *T. evansi* and other pathogens (*Neospora caninum* and *Brucella abortus*) with reproductive failure

and calf mortality in buffalo cows, and to confirm the presence of RoTat 1.2 gene in 168 local isolates of *T. evansi*. *Trypanosoma evansi* was detected using MHCT, MIT, PCR and CATT in livestock in a number of high-surra risk areas with 59%, 41%, 41%, 35% and 25% seroprevalence in buffaloes, cattle, horses, goats and pigs, respectively. *Trypanosoma evansi* was associated with reproductive failure and early calf mortality in buffalo cows. The RoTat 1.2 gene was detected in all 168 local isolates of *T. evansi* tested but was probably not expressed in all cases.

The seroprevalence and impact of combined infections of *T. evansi* and *F. gigantica* were determined in 1,163 buffaloes from 32 villages in high- and low-surra risk areas in Mindanao. *Fasciola gigantica* infection was highly prevalent in buffaloes in both areas and combined infections of *T. evansi* and *F. gigantica* were highly prevalent in high-surra risk villages. Buffaloes that were seropositive to *T. evansi* infection were more likely to be seropositive with *F. gigantica* than uninfected buffaloes and combined infections were associated with poor body conditions and low PCV.

Trapping of tabanids was conducted in 2007-8 in selected villages in high-and low-surra risk provinces to determine the local tabanid fauna and their abundance, detect trypanosomes in tabanids and determine the hosts of the flies using genetic markers. All five species of trapped tabanids were more abundant in low- than high-altitude areas and abundance was significantly associated with high rainfall. *Trypanosoma evansi* and *T. theileri* were detected from at least one fly of every tabanid species caught. Buffaloes, pigs, goats, humans and chickens were identified as hosts of tabanids in Mindanao. There is a need to identify tabanid fauna in other areas in Mindanao and confirm their active role in the transmission of *T. evansi* in livestock.

Results support the conclusions that: (a) *Trypanosoma evansi* infection causes significant economic losses in livestock in Mindanao but its effective control would provide substantial financial benefits; (b) *Trypanosoma evansi* infection is highly prevalent in livestock in Mindanao which is highly associated with poor reproduction performance in buffaloes; (c) RoTat 1.2 based tests (PCR and CATT) are applicable in the diagnosis of surra in Mindanao but the value of the CATT still requires further evaluation; (d) Fasciolosis needs to be included in the control strategy for surra in high risk areas; and, (e) Tabanids identified in Mindanao are potential transmitters of *T. evansi* and their control should be explored. There is a need, therefore, to sustain surveillance and implement an integrated and more effective control programme against *T. evansi* infection in livestock in Mindanao.

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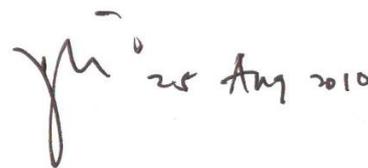
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A handwritten signature in black ink, appearing to read 'Alan P. Dargantes', with the date '25 Aug 2010' written to the right of the signature.

**Alan P. Dargantes**  
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## Publications and presentations

### Publications arising from this thesis

**Dargantes AP**, Mercado RT, Dobson RJ and Reid SA. 2009. Estimating the impact of *Trypanosoma evansi* infection (surra) on buffalo population dynamics in southern Philippines using data from cross-sectional surveys. *International Journal of Parasitology*, 39: 1109-1114.

Dobson RJ, **Dargantes AP**, Mercado RT and Reid SA. 2009. Models for *Trypanosoma evansi* (surra), its control and economic impact on small-hold livestock owners in the Philippines. *International Journal of Parasitology*, 39: 1115-1123.

### Other publications related to surra

Konnai S, Mekata H, Mingala NM, Abes NS, Gutierrez CA, Herrera JRV, **Dargantes AP**, Witola WH, Cruz LC, Inoue N, Onuma M and Ohashi K. 2009. Development and application of a quantitative real-time PCR for the diagnosis of Surra in water buffaloes. *Infection, Genetics and Evolution*, 9 (4): 449-452.

Njiru ZK, Ouma JO, Enyaru JC and **Dargantes AP**. 2010. Loop-mediated isothermal amplification (LAMP) test for detection of *Trypanosoma evansi* strain B. *Experimental Parasitology*, 125: 196–201.

**Dargantes AP**, Reid SA and Copeman DB. 2005. Experimental *Trypanosoma evansi* infection in the goat. I. Clinical signs and clinical pathology. *Journal of Comparative Pathology*, 133:261-266.

**Dargantes AP**, Campbell RSF, Copeman DB and Reid SA. 2005. Experimental *Trypanosoma evansi* infection in the goat. II. Pathology. *Journal of Comparative Pathology*, 133:267-276.

## **Presented papers**

### **A. Oral papers**

Reid SA, **Dargantes AP**, Mercado RT and Dobson RJ. 2010. The utility of data from cross-sectional serological surveys (or some surprises we found in our cross-sectional data). Annual College Science Week of the Australian College of Veterinary Scientists, 1-3 July 2010, Gold Coast, Australia.

**Dargantes AP**, Dobson RJ, Robertson ID and Reid SA. 2010. The Mindanao experience of surra surveys. FAO Training and Workshop on Influenza Diseases in Asia, 19-22 April 2010, Manila, Philippines.

**Dargantes AP**. 2009. Important animal diseases and zoonoses in the Philippines: updates and research prospects. CIRAD-GREASE Meeting, 14 December 2009, Bangkok, Thailand.

**Dargantes AP**, Dobson RJ, Robertson ID and Reid SA. 2009. Surra in the Philippines: impact on buffalo population, economic losses and benefits of control. Scientific Meeting of the Faculty of Veterinary Medicine, Kasetsart University, 22 October 2009, Bangkok, Thailand.

**Dargantes AP**, Dobson RJ, Mercado RT and Reid SA. 2009. Seroprevalence and the impact of *Trypanosoma evansi* infection (surra) on reproduction and population dynamics of backyard carabaos in southern Philippines. 76th National Scientific Conference and Annual Convention of the Philippine Veterinary Medical Association (PVMA), 18-20 February 2009, Davao City, Philippines.

**Dargantes AP**, Dobson RJ, Mercado RT and Reid SA. 2009. The financial benefits of various control strategies against *Trypanosoma evansi* infection (surra) in backyard livestock in Mindanao, Philippines. 76th National Scientific Conference and Annual Convention of the Philippine Veterinary Medical Association (PVMA), 18-20 February 2009, Davao City, Philippines.

Dobson RJ, **Dargantes AP**, Mercado RT and Reid SA. 2008. Modelling outbreaks of *Trypanosoma evansi* (surra) and its impact on host populations. Australian Biosecurity Cooperative Research Centre (AB-CRC) National Workshop, 24-26 June 2008, Bangkok, Thailand.

Taylor T, Boyle D, Colling A, Reid S and **Dargantes AP**. 2008. Development and validation of a Taqman assay for the detection of *Trypanosoma evansi*, the agent of surra. Australian Biosecurity Cooperative Research Centre (AB-CRC) National Workshop, 24-26 June 2008, Bangkok, Thailand.

Dobson RJ, **Dargantes AP**, Reid SA, Hood GM and Mercado RT. 2007. Estimating the impact of *Trypanosoma evansi* (surra) on buffalo populations in the Philippines using data from cross-sectional surveys. 21<sup>st</sup> International Conference of the World Association for the Advancement of Veterinary Parasitology (WAAVP), 19-23 August 2007, Ghent, Belgium.

## **B. Posters**

**Dargantes AP**, Dobson RJ, Robertson ID, Piedrafita D and Reid SA. 2010. Natural infections with surra and fasciolosis in southern Philippines: seroprevalence, risks and impact on buffalo health. International Conference for Parasitology (ICOPA), 15-20 August 2010, Melbourne, Australia.

**Dargantes AP**, McInnes LM, Dobson RJ, Robertson ID and Reid SA. 2009. Reproductive failure and early calf mortality associated with *Trypanosoma evansi* infection (surra) amongst water buffaloes in the Philippines. Poster Day at Murdoch University, 13 November 2009, Murdoch, Western Australia.

**Dargantes AP**, McInnes LM, Dobson RJ, and Reid SA. 2009. Molecular detection of RoTat 1.2 diagnostic antigen amongst *Trypanosoma evansi* isolates infecting livestock in Mindanao, Philippines. 76th National Scientific Conference and Annual Convention of the Philippine Veterinary Medical Association (PVMA), 18-20 February 2009, Davao City, Philippines (**Best Scientific Poster**).

## Symbols and abbreviations

### Symbols

~	approximately
°C	degrees Celsius
=	equals
>	greater than
≥	greater than or equal to
<	less than
μ (prefix)	micro (x10 <sup>-6</sup> )
-	negative
%	percent
+	positive

### Abbreviations

Ab-ELISA	antibody-detecting enzyme-linked immunosorbent assay
Ag-ELISA	antigen-detecting enzyme-linked immunosorbent assay
ABTS	2,2'-azino-di-3-ethyl-benzthiazoline-6-sulfonate
AQIS	Australian Quarantine and Inspection Service
asl	above sea level
bp	base pair
CATT	card agglutination test for trypanosomosis/ <i>T. evansi</i>
CI	confidence interval
CMU	Central Mindanao University
CMU-DP	Dairy Project of Central Mindanao University
Cyt <i>b</i>	cytochrome <i>b</i>

DA	Department of Agriculture
DEAE	diethylaminoethyl
DME	direct microscopic examination
DNA	deoxyribonucleic acid
EDTA	ethylenediamine-tetraacetic acid, tri potassium salt
<i>et al.</i>	and others
<i>g</i>	unit of gravitational field
GPS	global positioning system
h	hour
HRP-PG	horseradish peroxidase conjugated protein G
LAMP	loop-mediated isothermal amplification
m	metre
M	molar concentration
MHCT	micro-haematocrit centrifugation technique
min	minute
MIT	mouse inoculation test
mL	millilitre
mm	millimetre
MUSCA	Mindanao Unified Surra Control Approach
NaCl	sodium chloride
nL	nanolitre
nm	nanometre
OD	optical density
OR	odds ratio
<i>p</i>	probability of an event due to chance alone
PBS	phosphate-buffered-saline

PCC	Philippine Carabao Centre
PCR	polymerase chain reaction
PCV	packed cell volume
pers. comm.	personal communication
pH	negative log of hydrogen ion concentration
PhP	Philippine peso
SD	standard deviation
SEM	standard error of the mean
SIC	susceptible-infectious-subclinical
SIRS	susceptible-infectious-resistant-susceptible
SIS	susceptible-infected-susceptible
<i>sp.</i>	species
sq km	square kilometre
TEN-T	tris-EDTA-sodium chloride with 0.05% Tween 20 (v/v)
TEN-TC	TEN with 0.05% Tween 20 (v/v) and 0.2% casein (w/v)
UV	ultraviolet
VAT	variable antigen type
VSG	variable surface glycoprotein
v/v	volume in volume (%)
w/v	weight in volume (%)
x	times