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.

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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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Publications and presentations

Publications arising from this thesis


Other publications related to surra


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. 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, 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. 21st 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, 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 (x10⁻⁶)
-  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/ T. evansi
CI  confidence interval
CMU  Central Mindanao University
CMU-DP  Dairy Project of Central Mindanao University
Cyt b  cytochrome b
DA Department of Agriculture
DEAE diethylaminoethyl
DME direct microscopic examination
DNA deoxyribonucleic acid
EDTA ethylenediamine-tetraacetic acid, tri potassium salt
et al. and others
g unit of gravitational field
GPS global positioning system
h hour
HRP-PG horseradish peroxidise 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
p 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
sp.  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
General Introduction

Surra (animal trypanosomosis) is a disease of animals caused by infection with *Trypanosoma evansi*, which is a protozoan parasite of the blood and tissues (Damayanti *et al.*, 1994; Antoine-Moussiaux and Desmecht, 2008). Surra is an economically important disease because it has a broad geographical distribution and can infect a broad range of domestic and wild mammalian hosts causing severe disease and mortality. *Trypanosoma evansi* is endemic throughout Asia, Africa, and Central and South America where it affects livestock productivity in terms of high mortality, low milk and meat production, poor carcass quality, reduced reproduction performance, decreased animal draught output, and high costs of treatment (Luckins, 1988; Manuel, 1998; Reid, 2002). Surra may pose a threat to human health following the first confirmed human surra in India in 2004 (Brun, 2005; Joshi *et al.*, 2005; Joshi *et al.*, 2006).

Surra continues to spread to other free areas because of unregulated animal movement and the ubiquity of its main tabanid vector. In recent years incursions have occurred in France and Spain through importation of infected camels from Africa (Molina *et al.*, 2000; Desquesnes *et al.*, 2009b; Tamarit *et al.*, 2010).

There is limited information on the epidemiology and impact of surra in endemic countries particularly on: its impact on buffalo population dynamics and demographics; the economic losses due to the disease and the benefits of various treatment strategies; the prevalence and risk factors of surra in different livestock; the presence or absence of
RoTat 1.2 amongst local *T. evansi* isolates; the association of surra with reproductive problems in buffalo cows; the impact and risks of infection with fasciolosis in buffaloes; and, the potential insect vectors of *T. evansi*, their abundance and potential hosts. These pieces of information are essential for the development of rational control programmes in countries such as the Philippines where surra is the most important cause of mortality in livestock. Mindanao, the second largest island in the Philippines has experienced the most number of serious epidemics resulting in high mortalities in horses, water buffaloes, cattle and goats (Roeder, 1994; Reid, 2002; Mercado *et al.*, 2004).

Epidemiological studies were conducted to: a) Estimate the impact of surra on buffalo population dynamics and demographics; (b) Estimate losses from surra and evaluate various treatment options including its benefits; (c) Determine the prevalence and the risks of infection with *T. evansi* in buffalo and other livestock; (d) Screen local *T. evansi* isolates for presence of RoTat 1.2 gene using molecular markers; (e) Investigate the cause/s of reproduction problems and early calf mortality in buffaloes; (f) Determine the association, impact and risks of natural infections with surra and fasciolosis; and, (g) Identify potential insect vectors of *T. evansi*, their seasonal abundance and potential hosts in Mindanao, Philippines.

The impact of surra on buffalo population was estimated using data from cross-sectional surveys conducted in Mindanao during 2002 until 2006. A bio-economic infectious disease model in different livestock hosts was developed using these data and additional information from succeeding field surveys to estimate losses from surra and the financial benefits of various treatment options for the disease. A series of follow up surveys were performed to collect additional epidemiological data and examine the
interaction of surra with other endemic livestock diseases such as fasciolosis. Investigation of the presence of *Neospora* and *Brucella spp.* in buffalo cows with poor reproductive performance was undertaken to determine the potential impact of each pathogen. One hundred and sixty eight local isolates of *T. evansi* collected during the surveys were tested using a PCR to determine if they possess the RoTat 1.2 gene to ensure that CATT (Card agglutination test for trypanosomosis/*T. evansi*), which is based on this antigen can be used in Mindanao. In addition, a study was performed to trap local tabanids in high- and low-surma risk areas of Mindanao to provide data on the species present, their seasonal abundance and host preference.
2.1 Introduction

This review covers the general features and biology of *Trypanosoma evansi*, with emphasis on its epidemiology in Southeast Asia particularly in the Philippines. It will cover biology of *T. evansi*, transmission, hosts, geographical distribution, clinical manifestations and pathology, diagnosis, chemotherapy, economics and application of modelling to assess impact of the disease and the benefits of control. The aim of this review is to identify important issues that need to be addressed to improve the understanding of surra and to enhance surveillance and control strategies that are applicable not only in the Philippines but also to other countries where the disease is endemic.

2.2 Surra, the disease

Surra, a disease caused by *Trypanosoma evansi*, was the first trypanosomosis identified by Dr. Griffith Evans in India in 1880. In Hindi and Marathi languages, surra means “rotten” and the “sound of heavy breathing through the nostrils”, respectively. The disease is also known by various local terms throughout the world including “mal das cadeiras” or “mal de caderas” in Brazil (Aquino *et al.*, 1999; Davila *et al.*, 1999); “derrengaderra” in Spanish speaking countries of South America (Monzon *et al.*, 1990; Silva *et al.*, 1995; Seidl *et al.*, 2001); “murrina” in Panama (Rodrigues *et al.*, 2009); “heyam” in Saudi Arabia (Al-Qarawi *et al.*, 2004); “dbab” which means fly in Morocco (Atarhouch *et al.*, 2003); “el debab” in Northern Africa; “mbori” and “guffar” in Sudan (El Rayah *et al.*, 1999); “guifar” or “dioufar” in Chad; “gandi”, “salaf” or “dukan” in Somalia (Dirie *et al.*, 1989); “yuleye” in Kenya (Dirie and Abdurahman, 2003); and,
“bayawak” in the northern Philippines. Surra can be acute or subacute depending on the susceptibility of the host and virulence of the isolate, with affected animals dying within weeks or months in contrast to chronically-infected animals, which may survive for years (Losos, 1980; Brun et al., 1998).

2.3 *Trypanosoma evansi* and its biology

2.3.1 Classification and origin

*Trypanosoma evansi* is a salivarian trypanosome belonging to the Family Trypanosomatidae, Order Kinetoplastida. This tissue parasite is thought to have evolved from *T. brucei* and is closely related to the subspecies: *T. brucei brucei*, *T. brucei rhodesiense* and *T. brucei gambiense*, the aetiologic agents of animal and human trypanosomoses in Africa, respectively (Brun et al., 1998). The current hypothesis is that when *T. brucei*-infected camels were moved outside the tsetse fly zone in Africa via travelling caravans, trypanosomes were spread by other haematophagous flies to other animal species. Recent molecular evidence supports the close genetic relationship between *T. brucei* and *T. evansi*, leading others to suggest that *T. evansi* is a subspecies of *T. brucei* (Jensen et al., 2008; Lai et al., 2008).

2.3.2 Morphology of *T. evansi*

*Trypanosoma evansi* is morphologically indistinguishable from the slender form of *T. brucei*. It is generally monomorphic in its slender form, although stumpy and intermediate forms may be observed in some isolates (Losos, 1980). *Trypanosoma evansi* is 14-33 µm long and 1.5-2.2 µm wide, with a free flagellum and a subterminal kinetoplast (Brun et al., 1998). Isolates without kinetoplast have been identified in livestock from Brazil (Ventura et al., 2000) and after prolonged *in vitro* cultivation
(Zweygarth et al., 1990), or after exposure to dyes or trypanocide treatment (Schnaufer et al., 2002). The key ultrastructural features of *T. evansi* are displayed in Figure 2.1.

Figure 2.1  Detailed ultrastructure of *Trypanosoma evansi* by electron microscopy (Vickerman, 1977 as cited by Baron, 1996).

2.3.3 Life cycle and multiplication of *T. evansi*

The life cycle of *T. evansi* is simple and direct. It does not undergo any development or multiplication in its insect vectors. The parasite multiplies by binary fission only in the animal host (Brun et al., 1998). *Trypanosoma evansi* does not develop in its insect vectors because it lacks maxicircle DNA that is necessary for development in vectors (Borst et al., 1987; Lun and Desser, 1995; Ventura et al., 2000).

2.3.4 Transmission of *T. evansi*

Mechanical transfer by haematophagous flies, particularly tabanids (horseflies, March flies), is the main mode of transmission of *T. evansi* from an infected to another host. Tabanids are effective transmitters of *T. evansi* because they are large aggressive
feeders that induce significant avoidance behaviour in hosts. Host avoidance leads to transfer of half-fed tabanids between hosts. The duration between meals of tabanids is a crucial factor in the transmission of *T. evansi* because trypanosomes do not live long in the mouthpart of flies (Losos, 1980). *Trypanosoma evansi* would not survive for more than 7 minutes in the mouthpart of *Stomoxys niger niger* (Sumba *et al.*, 1998). Likewise, successful transmission of *T. evansi* by *Tabanus striatus* has been observed not to occur beyond 15 minutes of feeding interval between animals (Mitzmain, 1914). The efficiency of transmission is significantly affected by the level of parasitaemia in the infected host. Tabanids have large mouthparts that trap a significant volume of blood after an interrupted feed. After intermittent feeding, approximately 6.12 and 12.5 nanolitres (nL) of blood is trapped on the mouthparts of *Chrysops fuliginosus* and *Tabanus fuscicostatus*, respectively (Knaus *et al.*, 1993). Therefore, for successful transmission of *T. evansi* to occur between hosts, the parasitaemia in the infected host must be at least 1 trypanosome per 6 nL of blood. Other biting insects (*Stomoxys, Lyperosia, Haematopota*) may also transmit *T. evansi* but they are thought to be unlikely significant because they tend to complete their blood meals from a single animal (Luckins, 1998b). Nieschulz working in Indonesia between 1925 and 1929 (LeClerq, 1952, cited by Kettle, 1984) and Sumba *et al.* (1998) had successfully demonstrated transmission of *T. evansi* with *Stomoxys spp.* However, studies in the Philippines and India have shown *Stomoxys calcitrans* as unimportant in the transmission dynamics of *T. evansi* (Mitzmain, 1912; 1913c; Choudhuri *et al.*, 1966).

Oral transmission of *T. evansi* is also possible amongst carnivores and has been successfully demonstrated by experiment in dogs and mice. Dogs and mice fed with meat and blood of a *T. evansi*-infected goat kid developed parasitaemia between 8 to 16 days and 2 to 10 days, respectively (Raina *et al.*, 1985). The mechanism of infection is
still unclear but it is likely that trypanosomes invade the buccal or gastrointestinal mucosa (Raina et al., 1985). *Trypanosoma evansi* is principally a tissue parasite (Finol et al., 2001; Reid et al., 2001b; Dargantes et al., 2005a) which suggests that viable trypanosomes are present in meat after slaughter. This mode of transmission has potential implication in remote areas in developing countries where there are no designated abattoirs. In most rural villages in Asia, farmers often sell or slaughter animals with surra to salvage some value (Davison et al., 2000). In these areas, poor abattoir facilities increase the likelihood of carnivorous infections in dogs and cats that may access blood or meat from freshly killed animals. In South America, blood-sucking vampire bats that feed on livestock are also potential transmitters (Losos, 1980; Quieroz et al., 2000).

Transplacental transmission of *T. evansi* during pregnancy and through coitus has also been proposed, however, the evidence is circumstantial and further studies are required (Uche and Jones, 1992; Cresencio et al., 1994). Trypanosomes have been observed in vulvar tissues of infected female rabbits (Uche and Jones, 1992). However, this does not confirm that sexual transmission occurs. Similarly the detection of trypanosomes in the blood of a 43-hour old calf born to a cow with a high parasitaemia may imply transplacental infection but further investigation is needed (Cresencio et al., 1994). Conversely, iatrogenic transmission through infected needles is likely to happen during mass treatment, vitamin supplementation or vaccination of animals (Reid, 2002). Often in the field hypodermic needles are reused to save time and money. Approximately about 8.8± 1.0 nL of residual blood is left in a 22-gauge needle after intramuscular injection (Knaus et al., 1993), which is sufficient to transfer trypanosomes between animals (Uilenberg, 1998).
2.3.5 Antigenic variation

Trypanosomes undergo antigenic variation that enables them to evade the host’s immune system (Vickerman, 1978; Jones and McKinnell, 1984; Barry and Turner, 1991; Baron, 1996; Donelson et al., 1998). Antigenic variation occurs when a new generation of trypanosomes arises that expresses a different variable surface glycoprotein (VSG). The VSG is a glycoprotein with a molecular weight of about 65,000 that forms a 12-15 nm thick external monolayer over the plasma membrane and is attached to it by a glycosyl-phosphatidyl inositol (GPI) anchor (Blum et al., 1993; Pays, 2006). It covers the entire parasite including the flagellum presumably as a protective shield against host antibodies (Englund et al., 1982; Pays et al., 2001; Machado et al., 2006). This protective coat determines the antigenic features of the parasite, is easily recognised by the host and is highly immunogenic.

Once a host is infected with a trypanosome manifesting a specific antigen, known as variable antigen type (VAT), the host responds by producing specific antibodies to eliminate the proliferating trypanosomes. Successive waves of parasitaemia occur when a new VAT becomes predominant. These cyclical waves of parasitaemia are characteristic of trypanosome infections and are associated with clinical signs of pyrexia (Losos, 1980; Damayanti et al., 1994; Dargantes et al., 2005b). Two or several VATs may be present during the second and subsequent parasitaemic waves (Horchner et al., 1984; Jones and McKinnell, 1984; 1985; Machado et al., 2006). Whilst some authors believe that the host’s immune system triggers antigenic variation, others believe that antibodies selectively regulate rather than initiate VSG changes. This is because VSG switches have been observed in vitro in the absence of antibodies, in immuno-compromised animals and in early infections before antibodies are produced (Englund et al., 1982). There are over 1,000 genes encoding different VSGs but only
one gene coding for a particular VSG is expressed at any point in time (Baron, 1996; Graham and Barry, 1996; Uzcanga et al., 2004). The expression of a new VSG may also involve genomic rearrangement (Englund et al., 1982; Pays, 2006).

2.4 Host range

*Trypanosoma evansi* has the widest range of mammalian hosts amongst pathogenic trypanosomes (Lun and Desser, 1995). Whilst this is partly related to the wide feeding preference of its haematophagous insect vectors, the varying affinity of transferrin receptors of this parasite to different host transferrins may also have a significant influence (Bitter et al., 1998). Infection with *T. evansi* is often fatal in horses, dogs and Australian marsupials (Singh et al., 1993; Monzon et al., 1995a; Silva et al., 1995; Reid et al., 2001b). Other animals that are susceptible to *T. evansi* include: cattle (Singh and Misra, 1988; Payne et al., 1993; Partoutomo et al., 1995), water buffaloes (Damayanti et al., 1994), donkeys (Jani et al., 1992), goats (De Villa et al., 1991; Ngeranwa et al., 1993; Dargantes et al., 2005b), sheep (Onah et al., 1996; Audu et al., 1999), pigs (Arunasalam et al., 1995), cats (Wongyouni et al., 1990; Tarello, 2005), capybaras (Franke et al., 1994), elephants, tapirs, mongooses, ocelots, deer, sambars, (Levine, 1985; Singh, 1998; Reid et al., 1999), bandicoot rats (Biswa et al., 2001), guanacos (Kinne et al., 2001), rabbits (Chandra et al., 1999), guinea pigs (Monzon and Villavicencio, 1990), rats (Al-Mohammed, 2006; Omer et al., 2007) and mice (Fernández et al., 2009). The infection is usually chronic in most animals which serve as reservoir hosts, however high mortalities have been reported in buffalo (Löhr et al., 1986; Manuel, 1998; Reid, 2002), cattle (Payne et al., 1988; Payne et al., 1990), camels (Dirie et al., 1989), goats (Ngeranwa et al., 1993; Dargantes et al., 2005b), sheep (Audu et al., 1999), pigs (Arunasalam et al., 1995) and deer (Nurulaini et al., 2007). These
deaths are probably associated with the high pathogenicity of the parasite involved, poor health status of the animals, stress factors and concurrent infections.

*Trypanosoma evansi* is considered to have limited zoonotic potential. Recently, a case of human trypanosomosis due to *T. evansi* was reported in a farmer in Nagpu, central India (Joshi et al., 2005). The affected farmer showed signs of intermittent fever, chilling and sweating for two weeks as well as disorientation, violent behaviour and sensory deficit (Joshi et al., 2005). The case was successfully treated with suramin (Joshi et al., 2006). The mode of transmission was unclear but it was speculated that the patient was infected by contact with *T. evansi*-infected animal blood through a wound (Joshi et al., 2005). Molecular analysis of the isolate suggested that it was a typical Type A (based on minicircles) *T. evansi*, with RoTat 1.2 gene, absence of serum resistant associated (SRA) gene and was closely related to an isolate collected from a buffalo in Vietnam (Truc et al., 2007). The patient was subsequently found to have a rare deficiency in apolipoprotein L-I (APOL1) (Vanhollebeke et al., 2006) that is required to kill pathogenic trypanosomes, including *T. evansi* (Vanhamme and Pays, 2004; Pays and Vanhollebeke, 2008; Otto et al., 2010). Other primates such as orangutan, mangabeys, mandrills and baboons also possess TLFs in their sera (Lugli et al., 2004). Nevertheless, some trypanosome species (*T. rhodesiense*) have developed anti-trypanosome lytic factor (TLF) known as serum resistance associated protein (SRA) which is a lysosomal protein that prevents lysis of the parasite by the human serum (Gibson et al., 2002; Njiru et al., 2004c). A follow up serological survey on the human population in the area where this Indian farmer lived showed that 5 to 23% of them had antibodies against *T. evansi*, implying previous exposure to the parasite. Fortunately, these CATT-positive individuals were asymptomatic and aparasitaemic (Shegokar, 2006).
2.5 Epidemiology of surra

Despite widespread distribution of the disease, information on the extent of infection with *T. evansi* amongst animals in endemic areas, particularly in Southeast Asia, is limited (Ozawa, 1998). Only a few well planned studies have been conducted on a larger scale using more sensitive diagnostic procedures and employing proper sampling techniques probably due to high financial costs involved and the lack of technical knowledge (Luckins, 1988; Luckins, 1998a).

2.5.1 Geographical distribution of *T. evansi*

Surra is the most widespread amongst the pathogenic trypanosomoses. It is present in Africa, Central and South America, Asia and parts of Europe (Luckins, 1988; Lun and Desser, 1995; Gutierrez *et al.*, 2000; Reid, 2002; Desquesnes *et al.*, 2008). *Trypanosoma evansi* has been spread amongst countries by the movement of infected animals (Fèvre *et al.*, 2006). The movement of infected African camels to areas where mechanical vectors are present led to the spread of the disease to Africa, the Middle East and Asia, and India, in particular. Importation of livestock from India was believed to be the cause of spread of *T. evansi* throughout Asia (Losos, 1980). Introduction to Latin America occurred through infected horses imported from West Africa in the 16th century (Wells, 1984). In 1995, an incursion of surra occurred on the Canary Islands in Spain because of the importation of infected camels from Africa (Gutierrez *et al.*, 2000; Molina *et al.*, 2000). Thirteen years later, the disease was detected amongst camels, horses and donkeys in a farm in Alicante Province in eastern mainland Spain (Tamarit *et al.*, 2009). In 2006, *T. evansi* was detected in Aveyron Region in France when infected camels were introduced from the Canary Islands (Desquesnes *et al.*, 2008; Desquesnes *et al.*, 2009b). There has also been a case of *T.*
*T. evansi* infection in a dog in the Netherlands which was imported from Nepal in the 1980s (Hellebrekers and Slappendel, 1982).

### 2.5.2 Surra in Asia

In Asia, surra has been reported in Bhutan, Cambodia, China, India, Indonesia, Iran, Laos, Mongolia, Myanmar, Nepal, Pakistan, Philippines, Soviet Asia, Thailand, Vietnam and some of the Arabian countries in Western Asia (Ozawa, 1998; Abo-Shehada *et al.*, 1999; Al-Qarawi *et al.*, 2004; Zhou *et al.*, 2004). It is believed that the parasite has spread in these areas within the last century (Luckins, 1988; Lun *et al.*, 1993), and may have entered trypanosomiosis-free areas due to the uncontrolled movement of infected livestock (Luckins, 1998a; Ozawa, 1998).

In Indonesia, surra is endemic in the entire archipelago with the exception of Papua (Payne *et al.*, 1990; Payne *et al.*, 1991b; Luckins, 1998a; Reid and Copeman, 2000). Uncontrolled movement of animals and the abundance of insect vectors facilitated the spread of the disease throughout the region (Luckins, 1998a). In 1991 an extensive nationwide survey was conducted in Indonesia using an antibody-detection ELISA (Payne *et al.*, 1991b). The results of the survey showed that 52% of the animals sampled (*n* = 2,145) were seropositive to *T. evansi*, with 48, 43 and 1.7% of buffalo, cattle and horses were seropositive, respectively. Higher proportions (>70%) of buffalo were seropositive in southern Sumatra and Central Java than in Kalimantan and Sumba (<30%) (Payne *et al.*, 1991b). Despite the widespread occurrence of surra in Indonesia, only sporadic localised outbreaks occur, which may imply that the parasite has established enzootic stability (Payne *et al.*, 1991b).
Surra is a notifiable disease in Thailand and is believed to have been introduced into the
country through imported mules from Algeria in 1916. To date, the disease is endemic
in most areas of Thailand and sporadic outbreaks occur from time to time (Löhr et al.,
1985; Jittapalapong et al., 2008). In an epidemiological survey in north-eastern
Thailand, 20% of buffaloes were tested positive with *T. evansi* (Löhr et al., 1985). The
disease is common during the rainy season and aggravated by concurrent infections
with fasciola and stress (Löhr et al., 1985). *Trypanosoma evansi* infection has been
associated with late-term abortions and stillbirths (Löhr et al., 1986). In 1990,
outbreaks of surra on 13 cattle farms in Pethaboon Province were associated with high
mortality, neurological signs (aggressive behaviour, circling, convulsions and
excitability) and late-term abortions (Tuntasuvan et al., 1997). The sera and blood from
affected animals were tested using IFAT and MIT, respectively, to confirm the presence
of *T. evansi*. *Trypanosoma evansi* was detected in impression smears and in the mice
inoculated with brain tissue from three cows that died with neurological signs
(Tuntasuvan et al., 1997). In separate investigations involving 500 newly introduced
dairy cows in northeast Thailand, late-term abortions were also observed (Pholpark et
al., 1999). A follow up study on these cattle revealed that *T. evansi*-positive cows (by
MHCT) produced 27% less milk than uninfected cows (Pholpark et al., 1999).
Recently, *T. evansi* was detected in rodents (*Rattus, Leopoldamys* and *Bandicota*
species) using molecular techniques but its importance in the epidemiology of surra
amongst domestic animals in Thailand is unclear (Jittapalapong et al., 2008).

Surra occurs in Vietnam, where it is considered to be the most important and
widespread blood parasite affecting livestock, in particular buffalo (My et al., 1998a;
Thu et al., 1998). Prevalence estimates of 30 and 8% were observed in buffalo in north
and south Vietnam, respectively, in a large-scale cross-sectional survey conducted
between 1988 and 1995 (Phuc, 1994). The high prevalence of surra amongst buffalo in the north was further confirmed by My et al. (1998) who reported a 35% seroprevalence amongst animals sampled (n=698) using the card agglutination test for trypanosomosis (CATT). They observed that cases of surra were significantly higher during the wet than the dry season (My et al., 1998a), which was probably associated with the abundance of tabanids during this period.

In China, surra is also a disease of great economic importance affecting mostly buffalo, cattle and horses in the eastern, southern and south-western regions, respectively, whilst it is a problem in camels in northern and western parts (Lun et al., 1993).

2.5.3 Surra in the Philippines

2.5.3.1 History and current status of surra in the Philippines

Surra has occurred in the Philippines for more than 100 years. It is believed to have been introduced into the archipelago through infected cavalry horses imported from China by the American forces during the American-Spanish war in the early 19th century (Manuel, 1998). The first cases occurred in horses in Manila (Luzon Island) in 1901. This was followed by several outbreaks in neighbouring provinces in Luzon in succeeding years with high mortality mostly in horses (Manresa, 1935; Randall and Schwartz, 1936; Manuel, 1998). From 1934 to 1937, a series of nationwide serological surveys using the complement fixation test (CFT) showed that CFT-positive reactors were present in all 41 provinces surveyed, with provinces in Luzon showing more positive reactors (as high as 100%) than those in Visayas and Mindanao (as low as 0.4%). From those surveys, an overall apparent prevalence of 9.7% (n= 4,985) was detected amongst animals sampled with the highest proportion of positive reactors in
water buffalo (15.3%, 406/2,658) followed by cattle (3.8%, 75/1,998) and horses (0.6%, 2/323) (Topacio and Acevedo, 1938).

Surra is the second most economically important disease of livestock in the Philippines after fasciolosis (Manuel, 1998; Reid, 2002). However, its real economic impact on livestock is unknown and epidemiological information is incomplete. Based on the available data, financial losses due to surra from 1989 to 1997 were estimated at 4.9 million Philippine pesos (130,000 US dollars) per year (Manuel, 1998). This is likely to be an underestimation of the total losses because the amount is only based on reported deaths. Sources of economic losses from surra include mortality, weight loss, reduced milk yield and draught capacity and low reproductive performance (Losos, 1980; Mahmoud and Gray, 1980). In the past decade, serious outbreaks of surra with high mortalities have occurred in horses, buffalo, cattle and even small ruminants (R. Mercado pers. comm., 2006). Clinical disease and mortalities have also been reported in pigs (Batolos and Somoray, 1989; Manuel, 1998). Whilst mortalities in draught buffalo with surra could be partly associated with stress of overwork, other factors such as malnutrition, concurrent infections and adverse climatic conditions may contribute to reduced resistance and higher susceptibility of animals to the disease (Löhr et al., 1985; Manuel, 1998).

Currently, T. evansi infection is believed to be present in all regions in the Philippines (Manuel, 1998). Data from the Philippine Animal Health Centre (PAHC) suggest that the predominant disease foci are in Regions 3, 4 and 5 in Luzon, Regions 6 and 8 in Visayas and Regions 11 and 13 in Mindanao (PAHC, unpublished data). Official government records from 1988 to 1989 showed that the nationwide percentage mortality attributable to surra was 8.1% (747 deaths out of 9,254 cases), with the
highest mortality in Luzon (8.3%, 459 deaths out 5,547 cases), followed by Mindanao (8.2%, 243 deaths out 2,259 cases) and Visayas (6%, 45 deaths out 748 cases). The mortality was highest in horses (10.1%), followed by water buffalo and cattle with 7.3% and 6.1%, respectively (Manuel, 1998). Given the limitations in making an accurate diagnosis, lapses in the reporting system, possible non-reporting by farmers and the remoteness of affected villages, it is believed that the real figures for animal deaths from surra are probably 5 to 10 times higher (Manuel, 1998).

From 2001 to 2005, *T. evansi* was detected in 2.5% (n=32,426 samples) of stained blood smear samples submitted to PAHC (PAHC, unpublished data). In October 1993, a herd of 220 water buffalo were dispersed to six municipalities (towns) in Eastern Visayas (Samar). Within six months of dispersal (April 1994), 52 of the dispersed animals had died. Another 1,016 of the 11,753 (8.6%) water buffalo present in 9 towns (6 received dispersed water buffalo) in that province died (Roeder, 1994). From 1999 to 2001, at least six municipalities experienced similar serious outbreaks of surra in central and western Visayas affecting horses, buffalo and cattle with mortality ranging from 20 to 35% (Roeder, 1994; Manuel, 1998).

This series of outbreaks in the Philippines seems to differ from the situation in Indonesia where only a few small sporadic outbreaks have been reported in recent years and may suggest that the Philippine strain of *T. evansi* is highly pathogenic (Manuel, 1998; Reid, 2002). A preliminary comparative evaluation on the pathogenicity between Mindanao *T. evansi* isolates and an Indonesian isolate proved the hypothesis to be correct with mice infected with isolates from Mindanao dying earlier than those infected with the Indonesian isolate (Dargantes *et al.*, 2003, unpublished data).
2.5.3.2 The Mindanao surra experience

The recent outbreaks of surra in Mindanao have been outlined in a report by Mercado et al. (2004). The first case of surra in the island was confirmed in 1989 from an outbreak in Barangay Pangyan of Sarangani Province in Region 12 (Figure 2.2), where 38% of horses and cattle died (8/21 cases). Similar outbreaks with high mortality in horses and other farm species (buffalo, cattle, goats and sheep) were reported in adjacent provinces in the following years, particularly in Davao del Sur in 1990, Davao City in 1992, General Santos City and South Cotabato in 1993, and North Cotabato in 2000 to 2001. Serious outbreaks also occurred in the distant provinces of Agusan del Sur, Surigao del Sur and Surigao del Norte between 1994 and 1996, and Zamboanga del Sur in 2000, in the northern and western parts of Mindanao, respectively. Between 2001 until 2007 more serious sporadic outbreaks have occurred in Mindanao particularly in Agusan del Sur, Surigao del Sur, Surigao del Norte, Davao del Sur, Davao del Norte, and South Cotabato. Outbreaks have also occurred in more than 14 villages in Agusan del Norte and four villages in Magsaysay, Misamis Oriental between March to June 2008 (Drs. J. Dargantes and C. Ramosa, pers. comm., 2008), and in Barangay Malinao in Magsaysay, Misamis Oriental in August 2009 (Dr. R. Gulfo, pers. comm., 2009). Mortality in goats and sheep were also confirmed in an outbreak in General Santos City (Mercado et al., 2004). The outbreak in South Cotabato involved nine municipalities where 9% (1,500/17,000) of buffalo died (Roeder, 1994). In the outbreak in Agusan del Sur, 2% (276/14,300), 4% (26/633) and 1.4% (20/1,381) of the buffalo, horses and cattle, respectively, died (Manuel, 1998). In Zamboanga, a mortality of 5% was observed and 20% of the animals affected (6,405 horses, 8,846 buffalo, and 15,237 cattle) (Reid, 2002).
Figure 2.2 A map of Mindanao in the Philippines showing the provinces and years where and when outbreaks of surra have occurred (1989-2009). There were no reported outbreaks of surra in some provinces (shaded pink) (Dr. J. Dargantes and J. Alforque pers. comm., 2010).
To reduce the number of cases of surra in Mindanao, the Philippine Government implemented the Mindanao Unified Surra Control Approach (MUSCA) programme which primarily involved the Department of Agriculture (DA) and selected government universities. The programme focussed on the use of chemotherapy to control surra and to reduce deaths, particularly during outbreaks. In addition, an information campaign was conducted through mass media, symposia and conferences to educate different stakeholders (farmers, veterinarians) and local policy-makers. Other activities included disease monitoring and surveillance, training of veterinarians and laboratory technicians, and, enhancing regional diagnostic laboratories to improve the diagnosis and reporting of surra (Mercado et al., 2004).

2.5.3.3 Potential insect vectors of *T. evansi* in the Philippines

Early studies suggested that horseflies (*Tabanus spp.*), buffalo flies (*Haematobia exigua*), stable flies (*Stomoxys calcitrans*), mosquitoes and even non-biting houseflies can mechanically transmit *T. evansi* from infected to susceptible hosts in the Philippines (Mitzmain, 1913b; c; 1914). However, a subsequent study showed that only *Tabanus spp.* particularly *Tabanus striatus*, was found to transmit *T. evansi* (Kelser, 1927). Approximately 50 species of Tabanidae, mostly under the genus Tabanus, have been identified in the Philippines (Philip, 1959). There is no information on the diversity and abundance of different *Tabanus spp.* relative to the season, vegetation, topography and hosts. Only one small study conducted in Luzon has provided information on the monthly abundance of tabanids in the Philippines (Manresa and Mondoñedo, 1935). This study may not be representative of the situation in Mindanao where climatic and topographic (i.e., elevation, land cover) conditions are different. Studies of this kind are essential in understanding the monthly, seasonal and geographic variations in the
abundance of tabanids as has been done with other tabanid species (Dia et al., 1998; McElligott and Lewis, 1998; Krčmar, 2005).

2.6 Pathogenesis, clinico-pathology of surra and impact of co-infections

2.6.1 Pathogenesis

The pathogenesis and establishment of *T. evansi* in the skin of the host was described by Luckins et al. (1991). Initial multiplication of *T. evansi* occurs at the inoculation site, particularly in the dermal collagen, which triggers the development of a cutaneous necrotic oedematous lesion called as chancre, accompanied by an intense local immune reaction and cellular infiltration (Luckins et al., 1991). Proliferating trypanosomes then enter the circulation and other tissues (e.g., skeletal muscles, liver, heart, brain, digestive system, kidney, testes) where inflammatory lesions are produced. The cellular pathological lesions due to infection with *T. evansi* are mainly considered as sequelae of the immunological reaction triggered by trypanosomal antigens rather than the direct effects of the parasite (Quinones Mateu et al., 1994; Dargantes et al., 2005a). Immunologic products such as immune complexes, cytokines (i.e., interferons, interleukins, chemokines and tissue necrotic factors) and nitric oxide produced in response to presence of trypanosomes may mediate cellular damage in infected host (Sharafeldin et al., 2000; Baetselier et al., 2001; Al-Qarawi et al., 2004; Baral et al., 2007; Saleh et al., 2009). In addition, both humoral and cellular immunity may be suppressed in later stages of *T. evansi* infection leading to immune exhaustion and complications with other infections (Ngeranwa et al., 1993; Onah et al., 1997; 1998; Sharma et al., 2000b; Cox, 2001; Dargantes et al., 2005a). Anaemia which could also be immunologic by nature may further aggravate organ failure and ultimately death of
the host (Audu et al., 1999; Dargantes et al., 2005a; Dargantes et al., 2005b; Hilali et al., 2006).

2.6.2 Prepatent period and parasitaemic waves

The prepatent period varies from species to species and depends on the susceptibility of the host, pathogenicity of the isolate, route and dose of infection and the test used to detect parasitaemia. The prepatent period in mice infected with approximately $10^4$ trypanosomes was 4 to 12 hours, 1 day and 2 to 5 days post-infection using polymerase chain reaction (PCR), micro-haematocrit centrifugation technique (MHCT) and direct blood examination, respectively (Ijaz et al., 1998; Fernández et al., 2009). Likewise, data from experimental studies indicated that trypanosomes could be detected in blood as early as 24 hours in horses (Wernery et al., 2001), 48 hours in goats and cattle (Payne et al., 1993; Dargantes et al., 2005b), 3 days in Rusa deer, pigs and sheep (Audu et al., 1999; Reid et al., 1999), 6 days in Australian wallabies (Reid et al., 2001b), and 14 days in buffalo (Holland et al., 2001a) by MHCT. The prepatent period is 3 days in buffalo if mouse inoculation test (MIT) is used (Holland et al., 2001a). The prepatent period of as early as 2 days have been reported in South American coati (Herrera et al., 2002), 5 days in rats (Al-Mohammed, 2006), and 9 days in dogs (Aquino et al., 1999) after intravenous infection with T. evansi. Raina et al. (1985) observed a prepatent period of 8 to 12 days and 2 to 10 days, respectively, following oral challenge of dogs and mice with T. evansi.

The characteristic fluctuating parasitaemia in animals infected with T. evansi is due to the interplay of the immune response of the host and the ability of the parasite to evade it by antigenic variation. These waves of parasitaemia are directly related to the rise and fall of the body temperature of the host and occur at intervals of 6 to 7 days in cattle.
(Payne et al., 1993), 8.3 days in sheep (Audu et al., 1999) and 13 to 19 days in goats (Dargantes et al., 2005b). At times when parasitaemia is low, detection of the parasite is difficult, even when using highly sensitive tests, making diagnosis a problem.

2.6.3 Clinical signs

The clinical signs of surra are non-specific and will likely be confused with other diseases. The clinical signs seem to vary amongst hosts depending on the virulence of the isolate, the susceptibility of the hosts and the presence of concurrent infections, stress, malnutrition or adverse climatic conditions (Hoare, 1972; Losos, 1980; Luckins, 1998a; Reid, 2002). The most common signs amongst infected animals include progressive anaemia, intermittent fever, poor body condition, weakness, anorexia, oedema, lymphadenopathy, nervous signs, low reproduction and death (Losos, 1980; Brun et al., 1998).

In horses and camels, where the disease is usually fatal, signs of anorexia, intermittent fever, poor body condition, general anaemia, oedema of the hind legs and ventral abdomen, conjunctivitis, testicular enlargement, urticarial plaques, marked depression, dullness and sudden death are common clinical manifestations (Silva et al., 1995; Wernery et al., 2001; Enwezor and Sackey, 2005). Neurological signs such as circling, blindness, hyper-excitability, head tilting and paddling have also been observed in horses (Rodrigues et al., 2009) and abortions may occur in pregnant mares (Silva et al., 1995; Tuntasuvan et al., 2003; Gutierrez et al., 2005; Tibary et al., 2006). The acute disease syndrome may last for 2 to 20 days, whilst animals with the chronic form may survive for months but will ultimately die if not treated (Silva et al., 1995; Wernery et al., 2001; Enwezor and Sackey, 2005).
In domestic ruminants, signs of general anaemia and poor body condition, fluctuating fever, anorexia, weakness and death are commonly observed (Ngeranwa et al., 1993; Payne et al., 1993; Damayanti et al., 1994; Audu et al., 1999; Dargantes et al., 2005b; Hilali et al., 2006). The disease is usually chronic but acute cases may also occur with high mortality. In addition, abortion, infertility, testicular enlargement, diarrhoea, coughing and ocular lesions have been reported in goats (Jacquiet et al., 1993; Dargantes et al., 2005b; Morales et al., 2006). Anoestrous, late stage abortion, stillbirth, decreased milk yield and nervous signs of circling, lethargy, jumping, convulsion and aggressive behaviour have also been observed in cattle and buffalo with naturally acquired surra (Löhr et al., 1986; Payne et al., 1993; Tuntasuvan et al., 1997; Manuel, 1998; Pholpark et al., 1999; Jittapalapong et al., 2009). Corneal lesions occur in cats and dogs (Wongyounoi et al., 1990; Singh et al., 1993; Arora and Pathak, 1995) and alopecia, respiratory symptoms and oedema of the eyelids, face and nostrils have been reported in rabbits (Chandra et al., 1999). In pigs, whilst they may not show clinical signs in early infection (Reid et al., 1999; Holland et al., 2003), fever, anorexia, poor body condition, depression, abortion, death and paralysis may occur in late stages of the disease (Dargantes et al., unpublished data; Batolos and Somoray, 1989; Arunasalam et al., 1995).

Haematological changes in *T. evansi*-infected animals include decreased packed cell volume (PCV), haemoglobin and red blood cell count suggestive of a normocytic, normochromic anaemia in goats, buffalo and horses (Damayanti et al., 1994; Dargantes et al., 2005b; Hilali et al., 2006; Rodrigues et al., 2009) or in some cases macrocytic anaemia in goats (Sharma et al., 2000a). Likewise, leucocytosis with monocytosis and lymphocytosis amongst infected animals has been observed in infected animals (Damayanti et al., 1994; Onah et al., 1996; Wernery et al., 2001; Dargantes et al.,...
Leucopaenia may occur in prolonged infection (Holland et al., 2003; Dargantes et al., 2005b) which suggests that the immune system of the host has failed (Dargantes et al., 2005a).

2.6.4 Pathology

The pathological manifestations of surra are also not pathognomonic (Umeda and Isoda, 1984; Uche and Jones, 1992; Chandra et al., 1999; Rossi et al., 1999; Finol et al., 2001). The pathology of *T. evansi* has been thoroughly investigated in water buffalo (Damayanti et al., 1994) and in goats (Dargantes et al., 2005a). The most common gross lesions in buffalo are emaciation, hydropericardium, pericardial haemorrhages, serous fat atrophy, oedematous lymphadenopathy and hyperplastic bone marrow (Damayanti et al., 1994). Consistent microscopic lesions include interstitial myocarditis and pneumonia, splenic necrosis, interstitial myositis and hyperplastic bone marrow (Damayanti et al., 1994). Similar lesions have been observed in *T. evansi*-infected goats (Dargantes et al., 2005a). In addition, consolidation of the lungs, testicular enlargement and aspermia, splenic hyperplasia, enteritis, haemosiderosis in the spleen, liver, bone marrow and lungs, renal glomerular hypercellularity and cell depopulation on the spleen, bone marrow and lymph nodes in the late stage of infection have been observed (Dargantes et al., 2005a). Pericarditis, splenomegaly, ulcerative gastritis and enteritis, serous fat atrophy, and mononuclear infiltration of major internal organs but without distinct cellular lesions have been observed in Australian wallabies (Reid et al., 2001b). In horses showing nervous signs, lesions involving severe to moderate necrotising meningoencephalitis with oedema, haemorrhage and perivascular cuffing have been observed (Rodrigues et al., 2009). Trypanosomes were subsequently demonstrated by immunohistochemistry in the Virchow-Robin spaces and neuropils (Rodrigues et al., 2009).
2.6.5 **Immunosuppression due to surra and effects of concurrent infections**

There is good evidence that active *T. evansi* infection is immunosuppressive. Local cellular activity and both cellular and humoral responses were significantly decreased in *T. evansi*-infected buffalo given human serum albumin as heterologous antigen and *P. multocida* vaccine, respectively (Holland *et al.*, 2001b). The antibody response to classical swine fever (CSF) vaccination was lower in *T. evansi*-infected pigs compared to uninfected control group and was not sufficiently protective to prevent experimental infection (Holland *et al.*, 2003). In sheep, infection with *T. evansi* reduced the local lymphocytic cellular response at the site of deposition of *P. multocida* vaccine suggesting a lowered immune response (Onah *et al.*, 1997).

Leucopaenia and cellular depopulation in the spleen, bone marrow and lymph nodes in the late stages of infection is further evidence of the immunosuppressive effect of the parasite on the host (Dargantes *et al.*, 2005a). This immunosuppressive impact of *T. evansi* may increase the susceptibility of infected livestock in surra-endemic areas to other infections such as haemorrhagic septicaemia, nematodosis and fasciolosis in water buffalo and cattle. Concurrent infections of goats with *T. evansi* and *H. contortus* resulted in higher mortality and more serious pathological lesions (Sharma *et al.*, 2000b).

**2.7 Diagnosis**

The accurate identification of animals infected with *T. evansi* is a key factor in the success of any epidemiological surveillance or control programme for surra. Unfortunately, diagnosis of surra is often difficult because infected animals do not often show pathognomonic clinical and pathological signs, thus laboratory diagnostic tools are necessary to facilitate a fast, accurate diagnosis.
2.7.1 Parasitological tests for detection of *T. evansi*

The demonstration of trypanosomes in tissues or blood of hosts is the definitive diagnosis for surra. It is affected by the level of parasitaemia and limited survival of trypanosomes in blood or tissue samples. Therefore, samples should be processed and examined shortly (except for stained blood smears) after collection and should not be exposed to direct sunlight (Holland *et al.*, 2001a). Several field-based, simple parasitological tests are available to detect trypanosomes in blood including (in ascending order of sensitivity): direct microscopic examination (DME) of fresh or Giemsa-stained blood smears (GSS, thick or thin), micro-haematocrit centrifugation technique (MHCT), the miniature anion-exchange centrifugation technique (MAECT) and the rodent or mouse inoculation test (MIT). When parasitaemia is high, parasitological tests are generally efficient in detecting *T. evansi* amongst infected animals but diagnosis is problematic when parasitaemia is at a low level particularly in animals with chronic disease (Nantulya, 1990; Luckins, 1992; Ijaz *et al.*, 1998; Davison *et al.*, 1999).

2.7.1.1 Examination of blood smears

Direct microscopic examination (DME) of blood smears is a cheap, rapid but insensitive method of detecting trypanosomes. In buffalo, the limit of detection of fresh blood smear is about 12,500 trypanosomes mL\(^{-1}\) (My *et al.*, 2000). The sensitivity of direct blood examination has been estimated to be 45.4, 48.3 and 53.8% in naturally infected camels, buffalo and horses, respectively (Monzon *et al.*, 1990; Pathak *et al.*, 1997; Singh *et al.*, 2004), which is similar to the sensitivity of examining Giemsa-stained smears (45.6%) (Soodan *et al.*, 1998). However, subsequent estimates of DME have been significantly lower. The sensitivity of the DME and GSS were only 24 and 27% in naturally-infected camels, respectively, when PCR (highly sensitive) was used.
as the gold standard of infection (Singh et al., 2004). In an experimental infection in buffalo, even with the addition of sodium dodecyl sulphate (lyses red cells to facilitate microscopic examination) in blood smears, the DME had a sensitivity of only 25.1% (Holland et al., 2001a).

### 2.7.1.2 The micro-haematocrit centrifugation technique (MHCT)

This MHCT is based on the principle that centrifugation of anti-coagulated blood separates less dense trypanosomes from the blood cells, which are observed microscopically at the plasma-buffy coat interface (Bennet, 1962; Worth, 1964). This technique is cheap, fast and easy to perform under field conditions and several samples can be processed within a short time. However, the sensitivity of MHCT is adversely affected by exposure of blood samples to direct sunlight and heat. Holland et al. (2001) showed that trypanosomes could not be detected by MHCT 30 minutes after exposure of blood to direct sunlight. They further reported that trypanosomes in samples from animals with high (>10⁴ trypanosomes mL⁻¹ of blood) and low parasitaemia (250 trypanosomes mL⁻¹ of blood) could not be detected beyond 8 and 3 hours after storage at 4°C to 27°C, respectively. In contrast, other reports suggest that *T. evansi* can still be detected using the MHCT in horse blood stored on ice 24 to 36 hours after collection (Monzon et al., 1995b).

The MHCT is able to detect a parasitaemia of 250 parasites mL⁻¹ of blood in water buffalo and 31 parasites mL⁻¹ of blood in mice under experimental conditions (My et al., 2000; Reid et al., 2001a). *Trypanosoma evansi* was detected using the MHCT within 14 to 28 days after experimental infection of buffaloes in Vietnam (Holland et al., 2001a). In contrast, trypanosomes have been detected earlier in horses using MHCT, within 1 to 3 days after experimental infection (Wernery et al., 2001). The
estimated sensitivity of the MHCT is 53 and 60% in experimentally infected pigs and mice, respectively (Thekisoe et al., 2005; Fernández et al., 2009). Monzon et al. (1990) showed that *T. evansi* could be detected in 71% of horses naturally infected with *T. evansi* using the MHCT.

Several modifications to the MHCT have been undertaken to improve the sensitivity of detection of *T. evansi*. For example, addition of glycerol to separate red blood cells from trypanosomes or microscopic examination of the buffy coat from cut centrifuge tubes (buffy coat method, BCM). However, these changes result in longer test and involve more effort. In fact, BCM has lesser sensitivity compared to the conventional MHCT for detecting trypanosomes in horses (63.4% vs. 71.1%) and in buffalo (38.6% vs. 69.6%) (Monzon et al., 1990; Holland et al., 2001a). The simplest way to improve the sensitivity of the MHCT is to test two or more tubes from each sample (Holland et al., 2001a; Reid et al., 2001a; Dargantes et al., 2005b).

### 2.7.1.3 Miniature-anion exchange centrifugation technique (MAECT)

Trypanosomes can be separated from blood by anion-exchange chromatography using diethylaminoethyl cellulose (DEAE-cellulose). The DEAE-cellulose adsorbs the more negatively charged blood components whilst the less negatively charged trypanosomes pass through the column and into the eluate (Lanham and Godfrey, 1970). This method was modified by centrifugation of the eluate to concentrate the trypanosomes prior to examination by microscopy, that is, the miniature-anion exchange centrifugation technique (MAECT) (Lumsden et al., 1979). The MAECT has been largely used to diagnose human trypanosomosis but recently it has also been applied in the diagnosis of animal trypanosomosis. In buffalo infected with *T. evansi*, this method was shown to have a combined sensitivity estimate of 64% and detected trypanosomes within 14 to 45
days of infection (Holland et al., 2001a). In goats, it was found to be most sensitive than the MHCT and wet blood examination (Gutierrez et al., 2004). Further modification of the test by using theuffy coat of centrifuged blood samples improved the test’s sensitivity to detect a minimum parasitaemia of 5 trypanosomes mL$^{-1}$ of blood or as low as 1.25 trypanosomes in 2 mL (or 1 trypanosome mL$^{-1}$) of blood (Reid et al., 2001a).

### 2.7.1.4 Rodent inoculation

Rodents (i.e., rats and mice) are highly susceptible to infection with *T. evansi*. Mice (mouse inoculation test, MIT) are often used because they are small, cheap and easily available. Approximately 0.2 to 0.5 mL of blood is inoculated intra-peritoneally into a mouse or rat. Blood drawn from the tail-tip of the inoculated rodent is then examined by microscopy for the presence of trypanosomes for a period of 14 to 60 days after inoculation (Gutierrez et al., 2000; Verloo et al., 2000; Holland et al., 2001a; Njiru et al., 2004a). This test is considered to be the most sensitive of all parasitological tests for detecting infection with *T. evansi*, particularly when the Buffy coat is used instead of whole blood (Reid et al., 2001a), and is regarded as the “gold standard” for detecting infection with *T. evansi*. It is also the confirmatory test for *T. evansi* since it differentiates this parasite from non-pathogenic *T. theileri* and *T. vivax*. *Trypanosoma vivax* is variably infective to rodents (De Gee et al., 1982; Ventura et al., 2001), with some African strains infecting rodents (Moloo, 1981; Dirie et al., 1993) in contrast to South American isolates which do not grow in rodents (Osório et al., 2008).

The detection limit of the MIT is 3 trypanosomes mL$^{-1}$ of buffalo blood (My et al., 2000) to as low as 1.25 trypanosomes per 4 mL of blood when the buffy coat is used as the inoculum (Reid et al., 2001a). Therefore, it is useful for detecting intermediate to
high parasitaemias with detection of parasites as early as 3 to 4 days post-inoculation in buffalo (Holland et al., 2001a; Hilali et al., 2004). The conventional MIT (using whole blood) has a sensitivity of 74% in buffalo (Holland et al., 2001a), 88% in horses (Monzon et al., 1990) and 87% in camels (Pathak et al., 1997). The effect of cold storage on the infectivity of T. evansi to mice was evaluated by Reid et al. (2001a). Bovine blood containing 25 trypanosomes mL\(^{-1}\) remained infective after 21 hours of storage in a cool box and refrigerator (Reid et al., 2001a).

### 2.7.2 Immunoassays for detection of infection with T. evansi

Several immunodiagnostic tests have been developed to increase the probability of detection of T. evansi in animals. The common methods in this category are the card agglutination test for trypanosomosis (CATT/T. evansi), the enzyme-linked immunosorbent assay (ELISA), indirect immunofluorescence antibody test (IFAT) and the latex agglutination test (LAT). The complement fixation (CFT), direct agglutination (DAT), double immunodiffusion (DID), slow agglutination (SAT) and turbidity tests are also occasionally used for the diagnosis of surra. The main drawback of these tests is the inability to differentiate current from past infections because antibodies may remain detectable in the host even after successful cure.

#### 2.7.2.1 Card agglutination test for trypanosomosis/T. evansi (CATT/T. evansi)

The CATT is a rapid agglutination test, originally developed to detect infection with Trypanosoma brucei gambiense in humans (CATT, Testryp\textsuperscript{®}) that was later applied to the diagnosis of animal trypanosomosis. The test uses a freeze-dried, Comassie-stained whole trypanosome antigen representing a single VAT. AnTat 1.8 and LiTat 1.3 of T. b. gambiense are used to diagnose sleeping sickness in humans (Van Meirvenne et al., 1977; Magnus et al., 1978) and Rode Trypanozoon antigen Type (RoTat 1.2) is used
for surra (Bajyana Songa and Hamers, 1988). It is able to detect anti-\textit{T. evansi} antibodies in livestock 7 to 9 days after experimental infection with \textit{T. evansi} (Wernery \textit{et al.}, 2001; Hilali \textit{et al.}, 2004). The VAT RoTat 1.2 antigen was originally cloned from an isolate of \textit{T. evansi} collected from a buffalo in Indonesia in 1982. It is known to be specific (Claes \textit{et al.}, 2002) and predominant among \textit{T. evansi} worldwide (Verloo \textit{et al.}, 2001). Recent studies have demonstrated that a small number of isolates of \textit{T. evansi} from camels in Kenya did not possess the RoTat 1.2 VSG gene and that animals infected by these isolates were negative when tested using the CATT (Ngaira \textit{et al.}, 2004; Ngaira \textit{et al.}, 2005). However, RoTat 1.2 antigen has been demonstrated in two isolates of \textit{T. evansi} from Mindanao (Verloo \textit{et al.}, 2001; Njiru \textit{et al.}, 2006).

A number of studies of varying quality have been conducted to validate the CATT in different livestock species in different countries. Davison \textit{et al.} (1999) estimated that the sensitivity and specificity of the CATT was 77-79\% and 100\%, respectively, when used to test sera from Indonesian buffalo infected with \textit{T. evansi} (confirmed using MHCT and MIT) and uninfected Australian buffalo. In a similar study, Reid and Copeman (2003) showed that the sensitivity and specificity of the CATT at 1:4 dilution of serum was 83\% and 96\% respectively when used to test sera from Indonesian cattle infected with \textit{T. evansi} and uninfected Australian cattle from Townsville. In addition, the sensitivity and specificity of the CATT for use in horses have been estimated at 80.2\% and 98.5\%, respectively (Claes \textit{et al.}, 2005). Different studies have estimated the sensitivity and specificity of the CATT for use in camels. Estimates of the sensitivity range from 65.5\% and 68.6\% in Kenya (Ngaira \textit{et al.}, 2003; Njiru \textit{et al.}, 2004a), 72.2\% in India (Pathak \textit{et al.}, 1997) and 87\% in Mauritania (Dia \textit{et al.}, 1997). Estimates of the specificity of the CATT are 100\% in Kenya (Ngaira \textit{et al.}, 2003; Njiru \textit{et al.}, 2004a) and 83\% in Mauritania (Dia \textit{et al.}, 1997).
2.7.2.2 Enzyme-linked immunosorbent assay (ELISA)

Antigen- (Ag-ELISA) and antibody-detecting ELISAs (Ab-ELISA) have been developed for the diagnosis of surra. Antibody-ELISAs for *T. evansi* are more commonly used because they are generally more accurate (Davison *et al.*, 1999; Reid and Copeman, 2003; Singh *et al.*, 2004). A rigorous evaluation of an antibody- and two monoclonal-based antigen-ELISAs demonstrated that the Ab-ELISA was more efficient than the Ag-ELISA for detecting *T. evansi* infection in buffalo in Indonesia (Davison *et al.*, 1999). Specifically, the Ab-ELISA had higher point estimates of the sensitivity and specificity (89 and 92%, respectively), compared with the 2G6 Ag- (71 and 81%, respectively) and Tr7 Ag-ELISA (75 and 78%, respectively) (Davison *et al.*, 1999).

An antibody-ELISA using a 40 to 50% fraction of the detergent (ammonium sulphate) extract of *T. evansi* had a sensitivity of 81% when used to test sera from Indonesian cattle infected with *T. evansi* and the specificity was 99% when used to test sera from *T. evansi-*uninfected Australian cattle from Townsville where other blood parasitic infections such as babesiosis, anaplasmosis, *Theileri buffeli* and *Trypanosoma theileri* are endemic (Reid and Copeman, 2003). However, antibody-ELISAs also have drawbacks because they cannot differentiate antibodies from an active infection from those remaining from previous infections cured by treatment. Antibodies to infections may persist in horses for as long as one year after successful treatment (confirmed by MHCT and MIT) with quinapyamine sulphate (Monzon *et al.*, 2003).

The lower sensitivity of the antigen-ELISAs could be due to the presence of undetectable levels of antigen in the host due to the fluctuating parasitaemia or that the infection is recent and the amount of antigen is low. In addition, the sensitivity of the Ag-ELISAs could be reduced by the presence of antigen-antibody complexes that may
inhibit the binding of serum antigen and test antibody (Nantulya et al., 1989a; Kashiwazaki et al., 1998; Thammasart et al., 2001). Antigen-ELISAs are, however, likely to complement other parasitological tests to detect active infections in animals with undetectable parasitaemia in the blood (Nantulya et al., 1989b).

2.7.2.3 Latex agglutination test

Two rapid and simple latex-based tests have been developed to detect *T. evansi*: the antibody-detecting LATEX/*T. evansi* and the antigen-detecting Suratex®. The former utilises purified variable surface glycoprotein antigen of RoTat 1.2 *T. evansi* (Verloo et al., 1998; Verloo et al., 2000) whilst the latter uses monoclonal antibody (mab) to *T. evansi* to capture trypanosomal antigen (Nantulya, 1994). Due to its simplicity, Suratex® has been more applied widely in the field. However, its diagnostic efficacy is variable. It is claimed to detect trypanosomal antigens in the blood as early as 3 days in horses (Wernery et al., 2001) and as late as 2 to 3 weeks in camels (Olaho-Mukani et al., 1996) after infection with *T. evansi*. The reported sensitivity varies from 59 to 88% in camels (Olaho-Mukani et al., 1996; Ngaira et al., 2003) and 91 to 95% in cattle (Chen et al., 1986; Reid and Copeman, 2003). The specificity in cattle and camels has been reported to range from 95 to 100% (Chen et al., 1986; Olaho-Mukani et al., 1996; Nantulya and Diall, 1998). However, Suratex® was reported to have a specificity of only 41% when used to test *T. evansi*-free Australian cattle (Reid and Copeman, 2003). It is possible that the test cross-reacted with other blood parasites known to infect Australian cattle such as *Anaplasma marginale*, *Babesia bigemina*, *B. bovis*, *Theileria buffeli* and *Trypanosoma theileri* (Reid and Copeman, 2003).
2.7.3 Molecular methods to diagnose infection with *T. evansi*

2.7.3.1 Polymerase chain reaction (PCR)

The PCR is able to detect *T. evansi* in the circulation of mice and calves as early as four and 12 hours after experimental infection, respectively (Ijaz *et al.*, 1998; Omanwar *et al.*, 1999; Fernández *et al.*, 2009). The detection limit of the PCR is as low as 0.5 pg of parasite DNA or one to five trypanosomes in 10 μL (1 to 5 x 10³ mL) of blood (Wuyts *et al.*, 1994; Omanwar *et al.*, 1999). Therefore, it may be a useful test for diagnosing *T. evansi* infection in animals with a low parasitaemia. The reported sensitivity ranged from 78.2% in experimentally-infected buffalo (Holland *et al.*, 2001a) to as high as 97 to 100% in naturally-infected camels (Njiru *et al.*, 2004a; Singh *et al.*, 2004).

The PCR is also a useful tool for monitoring the efficacy of drugs against surra. Trypanosomal DNA was no longer detected in the blood of *T. evansi*-infected buffalo by PCR 24 hours after treatment with diminazene (Holland *et al.*, 2001a). The PCR may also be utilised to detect trypanosomal DNA in sera, and consequently could be useful for retrospective studies using stored sera (Desquesnes, 1997). Unlike most parasitological tests which require immediate examination after collection, PCR analysis of samples can be delayed as long as 180 days (Holland *et al.*, 2001a). Blood samples on filter paper or micro-slides can also be examined for *T. evansi* DNA using a PCR (Wuyts *et al.*, 1995; Pereira de Almeida *et al.*, 1998), which is a more convenient method for use during field collection, particularly in remote areas.

Variation of the diagnostic sensitivity of the PCR depends mainly on the types of primer used. Primers that are designed to amplify multiple-copy genes (e.g., kinetoplastid minicircles) give higher levels of sensitivity compared to genes with fewer copies. For instance, PCRs using TBR1/2 (Masiga *et al.*, 1992) and ESAG6/7 (Holland *et al.*, 2001a)
primers had a sensitivity of 80% for the detection of *T. evansi* in experimentally infected mice compared to PCRs using primers 21/22-mer (Wuyts *et al.*, 1995) and ITS1 (Njiru *et al.*, 2005) that had only 66 and 60% sensitivity, respectively (Fernández *et al.*, 2009). The first three primers are subgenus Trypanozoon-specific and target multicopy genes of a mini-chromosome satellite repeated monomers (TBR1/2), whilst the fourth primer (ITS1) is genus-species specific and amplifies the more conserved internal transcribed spacers present in the ribosomes of trypanosomes (Njiru *et al.*, 2005). However, the ITS1 primer with the lowest reported sensitivity is still useful because it can distinguish between different species of pathogenic trypanosomes because of variation in the size of the ITS region (Njiru *et al.*, 2005).

In Africa, PCR has also been applied to detect pathogenic trypanosomes in insect vectors (Solano and Amsler-Delafosse, 1995; Malele *et al.*, 2003; Adams *et al.*, 2006) and to identify the hosts of these insects (Njiokou *et al.*, 2004; Konnai *et al.*, 2008a). In Zambia for example, Konnai *et al.* (2008) were able to identify the hosts of *T. brucei*-infected tsetse flies using PCR as humans, elephants, buffalo, antelope, kudu, warthogs and goats. This information has widened the knowledge on the host preferences of tsetse flies and the likely role of these animals in the dynamics of African trypanosomoses.

### 2.7.3.2 Loop-mediated isothermal amplification (LAMP)

Loop-mediated isothermal amplification (LAMP) is a recent modification of PCR technology that can be used in the field or in simply-equipped laboratories. It amplifies target DNA using *Bacillus stearothermophilus* (Bst) DNA polymerase within an hour at isothermal temperatures (maximum of 80°C) using a common water bath or heat block. Unlike Taq polymerase in conventional PCR, Bst polymerase is not affected by
common PCR inhibitors such as haemoglobin, host DNA and myoglobin (Notomi et al., 2006; Thekiso et al., 2007). LAMP appears to be suited for use in surra-endemic countries where finances limit the establishment of more advanced molecular laboratories because it does not require expensive equipment (Iseki et al., 2007). Initial evaluation of the LAMP in pigs experimentally infected with T. evansi showed that its sensitivity (45%) was higher than MHCT (38%) and the conventional PCR (33%), but lower than the MIT (65%) (Thekiso et al., 2007).

2.8 Prevention and control of surra

There are no vaccines available for control of infection with T. evansi. Preventive measures are directed towards reducing the exposure of susceptible livestock to infected animals and mechanical vectors. These measures include the use of fly-proof pens, fogging, fly repellents, fly traps and targets and by restricting animal movements from endemic into non-endemic areas (Mitzmain, 1913c; Lun et al., 1993; Luckins and Dwinger, 2004; Fèvre et al., 2006). Trypanocidal drugs are mainly used for treatment, and less for prophylaxis due to the emergence of drug-resistance and the high costs involved in dealing with large herds of animals (Brun et al., 1998; Luckins, 2000; Holmes et al., 2004; Luckins and Dwinger, 2004).

2.8.1 Fly control to lessen transmission of T. evansi

Complete fly control is not feasible due to the complexity of the biology of fly vectors (i.e., mainly Tabanids) and the difficulty in implementation. Application of fly repellents to livestock, selective and separate grazing, confinement, and utilisation of baits and traps (Day and Sjogren, 1994; Foil and Hogsette, 1994; Hall et al., 1998b; Mihok et al., 2006) to reduce the fly population would appear feasible. However, due to the potential environmental impact of insecticides, their use has not been widely
adopted, and confinement of livestock is impractical except for small numbers. Hence, more research is necessary to identify cheap, nature friendly and effective repellents, and to enhance trapping and baiting techniques for target fly species. Separating infected from susceptible animals by more than 50 metres may also be effective in preventing transmission of *T. evansi* in surra-endemic areas (Allan *et al.*, 1987; Barros and Foil, 2007). This is based on the observation that during intermittent feeding of tabanids, these blood-sucking flies did not transfer to complete their feeding between horses placed 50 metres apart (Barros and Foil, 2007). Whether this scenario is also true with other animals such as buffalo and cattle is unknown, and in localities where pasture area is limited this option seems not to be feasible.

2.8.2 Vaccination against surra

The possibility of developing a vaccine against surra appears remote because of the ability of *T. evansi* to evade the host’s immune system by antigenic variation (Horchner *et al.*, 1984; Jones and McKinnell, 1984; 1985; Barry and Turner, 1991; Pays, 2006). Early attempts to produce a vaccine against *T. evansi* were unsuccessful with only around 60% protection provided in mice (Ryu, 1975). Recently, the use of beta-tubulin as antigen for trypanosomal vaccines has been explored. Beta-tubulin is present in microtubules of trypanosomes which are components of the surface membrane, flagellum, paraflageller rod, and mitotic spindle apparatus (Stieger *et al.*, 1984; Gallo and Precigout, 1988; Gallo *et al.*, 1988; Woods *et al.*, 1989). As a stable biological component of trypanosomes, it is considered an ideal target for chemotherapy and a probable novel antigen for vaccine (Lubega *et al.*, 2002; Li *et al.*, 2007). Use of native tubulin and other related cytoskeleton proteins of *T. brucei* (a close relative of *T. evansi*) as vaccine antigens conferred immunity in mice in separate trials (Balaban *et al.*, 1995; Rasooly and Balaban, 2004). Similarly, a recombinant *T. evansi* beta-tubulin vaccine
successfully protected 83.3% of mice from challenge with pathogenic *T. evansi* (Li et al., 2007). The same type of vaccine afforded 70 and 76.7% protection in mice challenged with *T. equiperdum* and *T. brucei*, respectively (Li et al., 2007).

### 2.8.3 Chemotherapy of surra

The main component of control programmes for surra is the use of trypanocidal drugs (Brun *et al.*, 1998; Luckins, 2000; Reid, 2002; Luckins and Dwinger, 2004). Suramin, an effective treatment for African human trypanosomosis (Powar *et al.*, 2006) and an anti-cancer drug (Barrett and Barrett, 2000), has been the treatment of choice for surra in all animal species for over half a century. However, its production for animal use is limited because of its narrow margin of safety, potential problems with drug-resistance (Gill, 1971; Payne *et al.*, 1994a; El Rayah *et al.*, 1999) and difficulty in administering the drug through mass treatment. Furthermore, it is no longer manufactured commercially.

Diminazene aceturate is widely used in various countries including Asia because it is safe and effective (Kashiwazaki *et al.*, 1998; Seidl *et al.*, 1998). Diminazene inhibits the kinetoplast DNA of trypanosomes and it is able to cross the blood-brain barrier (Kellner *et al.*, 1985; Onyeyili and Anika, 1991; Mamman and Peregrine, 1994) but its concentration within the brain tissues is approximately 3 to 4 times lower than the plasma level (Mamman and Peregrine, 1994). The lower concentration of diminazene in the central nervous system may be insufficient to eliminate trypanosomes and might lead to relapse of infection (Jennings *et al.*, 1979; Jennings and Gray, 1983; Whitelaw *et al.*, 1988; Tuntasuvan *et al.*, 2003). Diminazene has no prophylactic effect because it is readily metabolised by the body (Mamman and Peregrine, 1994).
Quinapyramine is effective against *T. evansi*, *T. congolense*, *T. brucei* and *T. vivax* and it has been used in Africa and in Asia for several decades. It is highly effective in treating trypanosomosis by inhibiting growth and cell division (Brander and Pugh, 1971). It is the drug of choice for surra in India (Luckins, 2000) and is usually marketed as quinapyramine chloride alone, which is mainly therapeutic or in combination with quinapyramine sulphate which has prophylactic properties. The combination of both quinapyramine compounds protected donkeys and ponies against surra for 30 and 60 days, respectively (Gill, 1972; Suryanarayana et al., 1985). The potential toxic side effects associated with quinapyramine treatment (salivation, trembling, sweating, recumbency, and even death) and the emergence of resistance (Maina et al., 1996; El Rayah et al., 1999) and cross resistance with other drugs such as diminazene, isometamidium and homidium have limited its use (Gill, 1971).

The recently developed water-soluble trivalent arsenical drug, melarsomine hydrochloride (MelCy) marketed as Cymelarsan® (Berger and Fairlamb, 1994) was originally produced to treat surra in camels (Otsyula et al., 1992; Van Gool et al., 1992; Touratier, 1993; Musa et al., 1994; Gutierrez et al., 2005). However, it was also found to be highly effective and safe for the treatment of surra in other domestic animals (Lun et al., 1991; Otsyula et al., 1992; Zweygarth et al., 1992; Payne et al., 1994a; Tarello, 2005; Youssif et al., 2008), with effective dose ranging from 0.25 mg kg\(^{-1}\) in camels to 0.75 mg kg\(^{-1}\) in cattle and buffaloes (Lun et al., 1991; Musa et al., 1994; Payne et al., 1994b). Its mode of action is unknown, but it is presumed to be related to its effect on glycolysis. Melarsomine is usually given as a single intramuscular dose. It is rapidly absorbed and plasma concentrations peaks within 15 minutes of administration and with clearance from the circulation within six hours of administration (Bujon, 1990). Melarsomine has no prophylactic activity but clears parasitaemia in a short time. It is
likely effective in chronic cases of *T. evansi* infection involving nervous signs because it is believed to cross the blood-brain-barrier (Raynaud *et al.*, 1998, as cited by Animal Health Australia, 2006). Melarsomine is toxic at higher doses because of its arsenic component. For instance, single and multiple doses of 1.25 and of 0.125 mg kg\(^{-1}\) bodyweight, respectively, were toxic to goats and resulted in dysentery, haematuria, nervous signs, tachycardia, alopecia, paralysis, recumbency and death (Youssif *et al.*, 2008). In buffalo, scleroma and tissue necrosis were observed at the site of injection after administration of 1.5 to 3.0 mg kg\(^{-1}\) doses, respectively (Lun *et al.*, 1991).

Isometamidium (Samorin\(^{®}\)) has also been used for surra but its toxic effects on treated animals and low efficacy have limited its widespread adoption (Toro *et al.*, 1983; Ali and Hassan, 1986; Payne *et al.*, 1991c). Isometamidium is mainly used as a prophylactic drug against *T. congolense*, *T. vivax* and *T. brucei* in Africa, but not for *T. evansi* (Toro *et al.*, 1983; Stevenson *et al.*, 1995; Luckins, 2000; Stevenson *et al.*, 2000; Awa and Ndamkou, 2006). Its prolonged effect is attributed to its extensive binding to tissues at the injection site and other body organs (Kinabo and McKellar, 1990; Eisler, 1996).

### 2.8.4 Resistance of *T. evansi* to drugs

Resistance of *T. evansi* to trypanocides is a worldwide problem yet its magnitude has not been properly evaluated. Resistance to suramin has been reported in China (Zhang *et al.*, 1991; Zhou *et al.*, 2004), Indonesia (Payne *et al.*, 1994a), Kazakhstan (Petrovskii and Khamiev, 1974), Kenya and Sudan (Boid *et al.*, 1989; Maina *et al.*, 1996; Wesongah *et al.*, 1997; El Rayah *et al.*, 1999). Resistance to isometamidium has also been documented in Kenya (Stevenson *et al.*, 2000), China (Zhang *et al.*, 1991; Brun
and Lun, 1994) and Vietnam (My et al., 1998b), and to quinapyramine in India, Sudan and Kenya (El Rayah et al., 1999).

Drug resistance may develop following repetitive and widespread use of a particular drug at a sub-curative level, coupled with immunosuppression due to medication, stress or concurrent infections (Mutugi et al., 1994; Yuan et al., 1995; Holmes et al., 2004). In the field where the weight of animals treated are usually estimated, underdosing may occur (Besier and Hopkins, 1988). The mechanism of drug resistance is thought to be correlated with reduced drug uptake by the trypanosomes which is probably associated with adaptation of the parasite by mutation (Mäser et al., 2003). The standardised in vivo screening test in mice offers a simple, cheap and convenient method to detect drug resistance (Eisler et al., 2001; Mamoudou et al., 2008). Recently, the use of molecular markers to determine resistance of trypanosomes to a particular drug has also been explored (Witola et al., 2005; Maina et al., 2007; Delespaux et al., 2008a; Mamoudou et al., 2008) but requires a well established molecular laboratory to run the assay.

Resistance of trypanosomes to diminazene aceturate is widespread in a number of African countries (Codjia et al., 1993; Rowlands et al., 1994; Waitumbi et al., 1994; Afewerk et al., 2000; Geerts et al., 2001; Delespaux et al., 2008b) and in Asia (Prastyawati, 1983; Zhang et al., 1991; My et al., 1998b; Tuntasuvan et al., 2003). In the Philippines, particularly in Mindanao where diminazene has been extensively used for more than two decades, there is anecdotal evidence that drug resistance to diminazene exists.
2.8.5 Financial benefits of controlling surra

There is a lack of information on the economic impact of the disease on water buffalo and other farm animals. Consequently, there is a need to estimate the financial benefits of controlling surra in domestic animals in areas such as Mindanao where the disease is widespread and causes a significant problem in the livestock industry.

In surra-endemic area in Pantanal, Brazil where the cattle industry is significant and horses are used for herding livestock, it has been estimated that losses of US$ 2.4 million occur each year in horses. Year-long monitoring and treatment of horses with diminazene aceturate has been shown to be the most economical treatment option of six control strategies evaluated, with a total net benefit of more than US$2 million per year (Seidl et al., 2001). Nevertheless, this return assumed that the drug was 100% effective against *T. evansi* which is unlikely to be the case particularly in areas where resistance exists.

2.9 Potential use of mathematical simulation modelling to surra

Modelling is a means of simulating the progression of a particular disease at particular periods of time given the biological features of that disease (infectivity, hosts), its determinants and expected consequences (mortality). The first recorded use of models in veterinary medicine was by Farr who used a model to forecast the epidemic of rinderpest in Great Britain in 1865 (Hurd and Kaneene, 1993). The classification, features and potential applications of different modelling schemes in veterinary epidemiology have been thoroughly reviewed by several authors (Sørensen, 1990; Hurd and Kaneene, 1993; De Jong, 1995; Ferguson, 2005; Thrusfield, 2007). Simulation modelling has been increasingly applied to most infectious diseases to simulate the progression of epidemics and the dynamics of endemic diseases (Furniss and Hahn,
1981; Cherry et al., 1998; De Koeijer et al., 1998; Thrusfield, 2007; Chowell et al., 2006; Mitchell et al., 2008) and also to estimate the economic gains of a particular control option (Hall et al., 1998a; Torgerson, 2006; Castillo-Riquelme et al., 2008).

The basic principle of disease simulation modelling is the Kermack-McKendrick susceptible-infected-resistant/recovered (SIR) model (Allen and Burgin, 2000; Roy and Holt, 2008; Wang et al., 2010). This is based on the hypothesis that if an infectious agent infects a given population, individuals or animals could be proportioned as susceptible (S), infected (I) and resistant/recovered (R) sub-groups. In some instances, this model has been modified to suit the biology of the disease under investigation and the outcome of infection. For example, in diseases where resistance is not possible the model is limited to a susceptible-infected (SI) framework (West and Thompson, 1997), or a susceptible-infected-susceptible (SIS) model where animals recover from the disease but are still prone to re-infection (Zhou, 1995; Allen and Burgin, 2000; Xiao and Chen, 2001; Jacob and Viet, 2003).

Several papers that deal with the application of modelling to depict the epidemiology and control of both human (Gettinby, 1989; Milligan, 1989; Rogers, 1989; Jusot et al., 1995; Schofield and Maudlin, 2001; Jennings et al., 2002) and animal trypanosomoses (Agur and Mehr, 1997; Kristjanson et al., 1999) have been published. Coen et al. (2001) described a model of infection in buffalo in Indonesia using a susceptible-infected-susceptible (SIS) model based on an age-structured prevalence as determined by two antigen-ELISAs: 2G6 and Tr7 ELISAs. This model assumed that buffalo recover from surra by treatment or self-cure but remained susceptible to further infection. Based on the results from testing buffalo with the Tr7 antigen-ELISA, the model estimated that the incidence rate of surra increased with age with 1.31 to 2.08 per
buffalo year at risk. This estimate was close to the direct estimate of the incidence of 0.44 per buffalo year at risk that was generated from a separate longitudinal survey amongst buffalo in Indonesia using the same antigen-ELISA (Davison et al., 2000). The model also predicted that infected buffalo would be free of infection within 16.8 months either by chemotherapy or self-cure.
Estimating the impact of *Trypanosoma evansi* infection (surra) on buffalo population dynamics in southern Philippines using data from cross-sectional surveys

3.1 Introduction

*Trypanosoma evansi* (Trypanosomatidae, Kinetoplastida), the cause of surra, has the widest geographical distribution and host range of all pathogenic trypanosomes. It is endemic throughout Central and South America, Africa and Asia where it is an important constraint to the productivity of livestock, and poses a high risk of spreading to other free countries through animal movement and trade. Surra is an acute disease with high mortality in susceptible animal species such as horses, dogs and wallabies (Aquino *et al.*, 1999; Reid *et al.*, 2001b; Seidl *et al.*, 2001) and a chronic but invariably fatal disease in most other livestock species (Mahmoud and Gray, 1980; Luckins, 1988). Buffaloes and cattle are probably the main reservoir hosts for *T. evansi* because other infected species tend to either rapidly die (horses, wallabies, dogs) or experience a brief/low parasitaemia (pig, deer) in tissues which impede transmission (Luckins, 1988; Reid *et al.*, 1999; Reid *et al.*, 2001b).

Surra is an economically important livestock disease in the Philippines. Several large epidemics have occurred, particularly in the islands of Visayas and Mindanao during the past two decades. The outbreaks were characterised by high morbidity and mortality in horses, cattle, goats and buffaloes (Roeder, 1994; Manuel, 1998; Reid, 2002). However, the impact of *T. evansi* on host population dynamics has not been evaluated. The impact of surra on the fertility, mortality and immigration/emigration of
domestic buffalo populations was estimated from data collected during a 4-year cross-sectional survey conducted in various provinces in Mindanao, southern Philippines.

3.2 Materials and methods

3.2.1 Prevalence survey

3.2.1.1 Study area

Surveys to detect *T. evansi* infection in buffaloes were conducted in 71 villages in five provinces in Mindanao from 2002 to 2006. Mindanao (8°000 North, 125°000 East) is the second largest island in the Philippines, located in the south with a total land area of 94,630 sq km. It receives more than 2,000 mm of rain annually, a mean annual temperature of 26.6°C and a humidity of 71–85%. Livestock industry plays an important role in the region’s economy where most small-hold farmers own one to two buffaloes for their farm. Buffaloes are raised in a semi-extensive farming system and are usually tethered with other farm animals in a common pasture area.

3.2.1.2 Animals sampled

A total of 1,732 local swamp buffaloes (*Bubalis bubalis*), 205 cattle, 43 horses and 38 goats owned by small-hold farmers were sampled during 2002–6 (Table 3.1). Animal owners were informed in advance of the survey through their local government officials and they were requested to bring their animals to one or two sampling sites in each village. Paired whole blood (with and without the anticoagulant ethylenediamine-tetraacetic acid (EDTA)) was taken from each of the animals brought to the sampling site. Blood samples in EDTA were placed inside a cooler box until examination and blood without anticoagulant was allowed to clot for one hour at room temperature and placed inside the cooler box until the serum was separated. This study was performed
with approval from the Murdoch University Animal Ethics Committee, permit No. R881/01.

### 3.2.1.3 Parasitological tests

The microhaematocrit centrifugation technique (MHCT) and mouse inoculation test (MIT) were used to detect *T. evansi* in blood samples. Tests were carried out within 6–8 h after collection using published protocols with slight modification (Holland *et al.*, 2001a; Reid *et al.*, 2001a; Wernery *et al.*, 2001). Briefly, two heparinised microcapillary tubes (75-1.5 mm) were filled with EDTA-treated blood, sealed with clay and centrifuged at 12,000g for 15 min. Each tube was microscopically examined at 100–400x magnification for the presence of motile trypanosomes at the buffy coat–plasma interface. Blood samples positive to MHCT and those with low packed cell volumes (PCVs < 25%) were then tested using the MIT. Approximately 0.3–0.5 mL of uncoagulated blood was injected into the peritoneal cavity of a locally-bred, 4–6 week-old Swiss white mouse. The development of parasitaemia was monitored daily for 30–40 days after inoculation by wet smear examination of tail-tip blood. A sample that was positive for either MHCT or MIT was counted as positive.

### 3.2.1.4 Serological test

The card agglutination test for trypanosomosis/*T. evansi* (CATT/*T. evansi*) (Institute for Tropical Medicine, Antwerp, Belgium) was used to determine presence of circulating anti-trypanosome antibodies in the sera as per the manufacturer’s instructions. Briefly, 25 uL of serum (1:4 dilution) was mixed with 45 uL of CATT reagent on a reaction card, gently agitated for 5 min with a card rotator and inspected visually for the presence of agglutination. A sample was considered positive when blue agglutinates were visible (Verloo *et al.*, 2000). The true prevalence of *T. evansi* infection in buffaloes and other host animals was calculated using the published sensitivity and
specificity estimates for the CATT at 1:4 serum dilution, 83 and 96%, respectively, from Reid and Copeman (2003) using the formula from Rogan and Gladen (1978). The corrected seroprevalence (CATT) was used to estimate the impact of the disease on buffalo populations in Mindanao. Seroprevalence data from buffaloes and other host animals were aggregated across areas and years that had similar seroprevalence. It was necessary to pool data for this comparison because too few non-buffalo hosts were sampled from each site.

3.2.2 Birth rates
From the age structured survey data, summarized in Figure 3.1, calving percentages/year were estimated for each province and time by dividing the number of animals less than a year old by the number of females between 3 and 12 years old. Animals aged from 1 to 2 years could have been included in this estimation but were not as there was no way to determine how many had been imported or exported. This is also true for animals less than a year old. However, suckling calves are less likely to be removed for sale or consumption and thus should provide a less biased estimate. Pearson correlation and simple linear regression analyses (Zar, 1984; Dawson and Trapp, 2001; Quinn and Keough, 2002) were used to quantify the relationship between the calving rate and corrected surra seroprevalence and to predict the calving performance based on the corrected surra seroprevalence, respectively. The linear regression was fitted to the estimates of calving percentage for each sample time and province. Fitted values were also obtained by fixing the birth rates at 47.5% and 15% for individual uninfected and infected animals, respectively. The proportion in each category was then set according to the sample seroprevalence for the province/time and then the mean fertility of the pooled population was calculated. These values were chosen because 47.5% was the mean calving rate for Bukidnon (the only province
sampled that has no history of outbreaks of surra) and areas with 100% corrected seroprevalence had estimated birth rates of 15%.

3.2.3 Removal rates

The removal rate represents the net removal due to death, export and slaughter for consumption. Removal rates for high and low risk areas were estimated using the pooled data from Figure 3.1. Animals aged 0–2 years were omitted from the analysis because there were more 2–4 year olds than younger animals in the high risk areas, which implies animals were imported from low risk areas to maintain herd size (breeding capacity) in the high risk areas. This analysis thus assumes that relatively few animals over 2 years old are imported. The data were also pooled into 2-year age categories (i.e., 0–2, 2–4, . . .) to provide some smoothing over time/age. Removal rates were fitted separately to the female (Figure 3.3) and male (Figure 3.4) buffalo populations from the two risk areas. Two models were fitted to the data: (1) a single removal rate for all ages; and, (2) different removal rates for animals aged 2–8 and over 8.
Table 3.1  The prevalence of *T. evansi* infection in buffaloes in Mindanao determined using MHCT/MIT and CATT (corrected prevalence) from 2002-6. The geographical provinces are classified by the historical risk of surra and the proportion of female animals is presented.

<table>
<thead>
<tr>
<th>Province</th>
<th>Year</th>
<th>Surra risk</th>
<th>n</th>
<th>% Prevalence (MHCT/MIT)</th>
<th>% Corrected prevalence (CATT)</th>
<th>Province female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Overall</td>
<td>Female</td>
<td></td>
</tr>
<tr>
<td>Agusan del Sur</td>
<td>2002</td>
<td>High</td>
<td>104</td>
<td>12.6</td>
<td>99.6</td>
<td>93.4</td>
</tr>
<tr>
<td>Agusan del Sur</td>
<td>2005</td>
<td>High</td>
<td>294</td>
<td>2.7</td>
<td>13.0</td>
<td>15.4</td>
</tr>
<tr>
<td>Agusan del Sur</td>
<td>2006</td>
<td>High</td>
<td>103</td>
<td>19.4</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Compostela Valley</td>
<td>2002</td>
<td>High</td>
<td>303</td>
<td>0.0†</td>
<td>81.8</td>
<td>81.8</td>
</tr>
<tr>
<td>Surigao del Norte</td>
<td>2004</td>
<td>High</td>
<td>128</td>
<td>8.6</td>
<td>27.6</td>
<td>25.8</td>
</tr>
<tr>
<td>Surigao del Norte</td>
<td>2005</td>
<td>High</td>
<td>228</td>
<td>3.5</td>
<td>52.1</td>
<td>40.1</td>
</tr>
<tr>
<td>Surigao del Sur</td>
<td>2006</td>
<td>High</td>
<td>274</td>
<td>13.9</td>
<td>100.0</td>
<td>95.9</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td></td>
<td></td>
<td></td>
<td><strong>11.1</strong></td>
<td><strong>67.7</strong></td>
<td><strong>67.6</strong></td>
</tr>
<tr>
<td>Bukidnon*</td>
<td>2004</td>
<td>Low</td>
<td>39</td>
<td>0.0</td>
<td>20.9</td>
<td>7.6</td>
</tr>
<tr>
<td>Bukidnon*</td>
<td>2005</td>
<td>Low</td>
<td>220</td>
<td>0.0</td>
<td>5.9</td>
<td>10.5</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td></td>
<td></td>
<td></td>
<td><strong>0.0</strong></td>
<td><strong>13.4</strong></td>
<td><strong>73.0</strong></td>
</tr>
</tbody>
</table>

†MHCT only; animal mortalities due to surra were reported in this province

*No case of surra was reported/confirmed in this area
Figure 3.1  Frequency histograms of the number of buffaloes by age and sex sampled in high- (a: n = 1,446) and low- (b: n = 257) surra risk areas.
3.2.4 Age-structured transition state model

A population projection analysis (Caswell, 1989) was performed using PopTools (Hood, 2009) in Excel (Microsoft Inc., USA) to fit female immigration/emigration rates to the same data used in Section 3.2.3. Data from the 0–2 year olds were also included because they represent the age group most likely to be moved among provinces. It was assumed that the observed population structure from the surveyed population was representative of the two risk areas and that the areas have similar size buffalo populations. For each area, a single birth rate was estimated rather than fit birth and death rate parameters for each age cohort, which would over-parameterize the model. For young (3 to 4 years) and old (over 14 years) buffaloes, the common birth rate was halved to simulate reduced fertility in these groups. In each area, separate death rates were fitted for animals less than 9 or over 9 years old. Parameters were estimated by minimizing the residual sums of squares for the proportion of animals in each age cohort in each area. Because there were few animals 19 years or older, this group was pooled as a single cohort for analysis. To estimate the goodness of fit of the model, the total chi-square across both areas was determined from the observed and fitted number of animals in each age cohort in each area.

3.3 Results

3.3.1 Prevalence survey

Table 3.1 shows the apparent prevalence based on parasitological tests (combined MHCT and MIT) and the corrected seroprevalence (CATT) for various provinces. Trypanosomes were detected by MHCT or MIT (Figure 3.2) in all provinces regarded as high risk for surra except for Compostela Valley. A comparison of corrected seroprevalence for buffaloes and other hosts is given in Table 3.2.
3.3.2 Birth rates

There was a significant negative correlation between the estimated calving rate and the corrected seroprevalence in buffaloes ($p=0.042$, $R^2=0.469$). The fertility rate in uninfected buffaloes was estimated to be 40.4% (the intercept of the trend line with 0% surra seroprevalence (Figure 3.3). Similar fitted values to the regression model were obtained by setting birth rates to 47.5% and 15% for uninfected and infected animals, respectively, and then calculating the mean fertility of the pooled population (shown in Figure 3.2). If 40% was used, instead of 47.5%, then the “predicted” value would be the same as that determined by the fitted linear model. The mean calving rate for areas of historically high prevalence was 20.4%. A number of areas had 100% seroprevalence with estimated calving rates of 15%.
Table 3.2  Corrected observed seroprevalence of *T. evansi* infection in different host species in Mindanao, Philippines.

<table>
<thead>
<tr>
<th>Province</th>
<th>Year</th>
<th>Host</th>
<th>Seroprevalence</th>
<th>Buffalo seroprevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agusan del Sur &amp; Compostela Valley</td>
<td>2002, 2006</td>
<td>Horse</td>
<td>92</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>90</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>510</td>
</tr>
<tr>
<td>Agusan del Sur &amp; Compostela Valley</td>
<td>2002</td>
<td>Goat</td>
<td>68</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>86</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>407</td>
</tr>
<tr>
<td>Agusan del Sur, Compostela Valley &amp; Surigao del Sur</td>
<td>2002, 2006</td>
<td>Cattle</td>
<td>58</td>
<td>76</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>92</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>644</td>
</tr>
<tr>
<td>Agusan del Sur &amp; Surigao del Norte</td>
<td>2005</td>
<td>Cattle</td>
<td>11</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>30</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>522</td>
</tr>
<tr>
<td>Bukidnon</td>
<td>2005</td>
<td>Cattle</td>
<td>10</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>220</td>
</tr>
</tbody>
</table>

Figure 3.3  Relationship between calving percentage/year of 3-12 years old buffalo cows and the corrected seroprevalence of *T. evansi* estimated using the card agglutination test for trypanosomosis/*T. evansi* (CATT)
3.3.3 Removal rates

The best fit to the female populations was obtained by fitting separate removal rates for animals aged 2–8 and over 8 years (Figure 3.4). The total chi-square was 21 ($p=0.013$) and 15 ($p=0.093$) for the high- and low-surra risk areas, respectively. Fitting a single removal rate in both cases more than doubled the chi-square ($p<0.001$). The removal rates for 2–8 year olds were 0.091 and 0.001 for the high- and low-surra risk areas, respectively. The removal rates for the older females were 0.251 and 0.231 for the high- and low-surra risk areas, respectively. A single removal rate of approximately 0.266 for all ages in the high- and low-surra risk areas best fitted the data for male animals (Figure 3.4). The total chi-squares were 5 ($p=0.915$) and 12 ($p=0.263$) for the high- and low-surra risk areas, respectively. Fitting the additional removal rate gave a poorer fit for the male data, i.e. slightly increased total chi-square and reduced $p$.

3.3.4 Age-structured transition state model

Figure 3.5 shows the fitted values from the age-structured state transition model. The model estimated that 28% of female calves may be moved from the low- to the high-surra risk areas to maintain the observed age structure in both areas. The total chi-square across both areas was 49 ($p<0.001$). In the high-surra risk area, the 15- and 16-year old cohorts contributed over half of the total chi square (32), so the total chi was re-estimated by excluding this value to give 17 with $p=0.103$. The model produced the following parameter estimates: birth rates of 29% and 38%; death rates for females less than 9 years old were 10% and 0% for high- and low-surra risk areas, respectively; and, for animals over 9 years old, the removal rate was 30% (in both areas).
Figure 3.4  Estimated removal rates (single: --- ; different: — ) and rate of export/import (×) of female buffaloes in the high- (a) and low- (b) surra risk areas in Mindanao, Philippines (♦ shows the category mid-point). Removal rates of buffalo cows aged 2-8 and more than 8 years old are shown.
Figure 3.5  Estimated removal rates (single: --- ; different: — ) and rate of export/import (×) of male buffaloes in the high- (a) and low- (b) surra risk areas in Mindanao, Philippines (♦ shows the category mid-point).
3.4 Discussion

All buffalo populations sampled in the high-surra risk provinces of Mindanao had more 2-year old than 1-year old calves. However, in the low risk area (sampled on two occasions) there were more 1-year old calves compared to 2-year old calves as would normally be expected in a stable or growing population. These observations suggest that these populations are stable with one maintained by imports and the other by exports. Coen et al. (2001) reported a seroprevalence of about 70% from buffalo populations in Indonesia with pooled results from animals over 7 years old and not segregated by sex. Data from this study showed that there were also more animals aged 1–2 than 0–1 year old (Table 3.3), which is similar to the observations from this study in the high-surra risk areas of Mindanao. The observation that a model with different removal rates for young and old animals fitted the population age structure than a single removal rate for all ages (Coen et al., 2001) is in agreement with the results of the current study. It is not possible to determine from Coen et al. (2001) what the principal cause of animal removal was, i.e. death (disease or natural) or harvest (sale or food). In high-surra risk areas in Mindanao, farmers tend to sell or slaughter their buffaloes quickly if they show signs of surra, even at a low price, to salvage financial investment (Dr. P. Calo, Dr. J. Dargantes, pers. comm., 2007). This practice has also been observed in surra-endemic regions in Indonesia (Davison et al., 2000).

Restricting the Philippine data to females is likely to provide an estimate of death or removal attributed to surra, particularly in the young female population that is highly valued and kept for work and breeding. For males in particular, the harvest rate probably dominates the combined removal rate, since owners would generally maintain females for breeding. In this study, the demographic inferences were derived from data which was primarily a prevalence survey in Mindanao on high risk areas where there
was sporadic drug treatment. Whilst a longitudinal survey over many years would best serve to estimate such demographic parameters, its cost would be prohibitive. Therefore, estimating parameters from cross-sectional survey data is justified.

The halving of the calving rate of buffaloes in high-surra risk areas compared with those from the low-surra risk area (Bukidnon) may be attributed to several factors including poor nutrition and management and infectious diseases. However, the consistently low calving rate of buffaloes in high-surra risk areas suggests a strong association between the disease and poor reproductive performance. Surra has been associated with reproductive problems (i.e., abortions, stillbirths) in buffaloes (Löhr et al., 1986) and in other animals (Arunasalam et al., 1995; Kashiwazaki et al., 1998; Suteeraparp et al., 1999; Gutierrez et al., 2006). Reduced calving rate of buffaloes in high-surra risk areas could also be attributed to reduced fertility of infected bulls. Whilst there is no available information on the effect of surra on male buffalo fertility, T. evansi-infected bucks and dromedary bulls showed evidence of testicular enlargement, poor semen quality and even aspermia that may indicate infertility (Ngeranwa et al., 1991; Al-Qarawi et al., 2004; Dargantes et al., 2005a). Loss of weight and anaemia associated with surra (Löhr et al., 1985; Damayanti et al., 1994; Manuel, 1998) may have also contributed to low reproduction performance of buffalo cows. In Indonesia, weight loss due to surra was believed to cause cessation of oestrus in one T. evansi-infected heifer (Payne et al., 1993). In high-surra risk areas in Mindanao, buffalo cows are commonly in poor body condition and anaemic.

It is unlikely that management and nutrition factors have caused the observed lower calving performance in the high-surra risk areas. This is because buffaloes sampled from this study were from provinces with similar farming and management practices.
Forage is abundant all year round. Other infectious diseases (e.g., brucellosis, neosporosis) that can cause low reproductive performance in buffaloes may also be implicated. However, a recent survey in Luzon showed no association between abortion in buffaloes with these diseases (Konnai et al., 2008b). Other pathogenic trypanosome species (*Trypanosoma vivax, T. congolense*) have also been reported to cause reproductive problems in animals elsewhere (Ogwu et al., 1986; Llewelyn et al., 1987; Okech et al., 1996). However, they are not present in the Philippines and only *T. evansi* was confirmed in this study based on parasitological examination and molecular analysis (Chapter 5).

Results from this study suggest that the higher death rate of buffaloes in areas where *T. evansi* infection is endemic compared to buffaloes in villages where clinical surra has not been detected is attributed to surra. The high death rate in young buffalo cows (2-8 years) due to surra has the greatest impact to farmers because females of this age are highly valued for draught power and breeding. Death of these buffalo cows during their most productive stage implies a shortening of their life expectancy (almost half). Surra has been proven to cause mortality in buffalo after experimental (Damayanti et al., 1994) or natural infection (Löhr et al., 1985; Payne et al., 1991c; Lun et al., 1993; Manuel, 1998). Surra in buffalo and in other *T. evansi*-infected animals is believed to be aggravated by poor nutrition, stress due to overwork (for draught animals), unfavourable climatic conditions or concurrent diseases (Löhr et al., 1985) that may lead to death. Animals infected with *T. evansi* are more likely to be susceptible to other infections because of the immunosuppressive effect of the parasite as demonstrated in buffaloes (Holland et al., 2001b), goats (Ngeranwa et al., 1993; Sharma et al., 2000b; Dargantes et al., 2005a), pigs (Holland et al., 2003) and sheep (Onah et al., 1997; 1998) experimentally infected with *T. evansi*. It is also likely that the Philippine isolates of
*T. evansi* are highly pathogenic as suggested by Manuel (1998) and Reid (2002) causing deaths in buffaloes and other livestock in high-surra risk areas in Mindanao and in other parts of the country (Manuel, 1998).

Generally, the negative reproductive impact and the high mortality in buffaloes caused by surra represent a considerable economic loss for small-scale farmers in surra-endemic areas who expect to produce surplus animals for sale as additional income or for consumption. Losses of farmers from surra are not only restricted to a reduced income but also from low draught output, decreased milk yield and cost of treatment of affected livestock (Payne *et al*., 1991a; Manuel, 1998; Reid, 2002). The need to import replacement stock from other sources is an additional financial burden for marginally low income farmers.

The age-structured state transition model proved useful in estimating the population dynamics of buffaloes as affected by infection with *T. evansi* particularly in terms of birth and death rates and the number of female animals needed to be imported to maintain the buffalo population in the high-surra risk areas. The parameter estimates derived from the model and the findings of this study will provide the inputs for the development of a simulation model to estimate the overall losses from surra and the subsequent financial benefits of its control in buffaloes and other domestic animals. This will be explored in the succeeding chapter.
Models for \textit{Trypanosoma evansi} (surra), its control and economic impact on small-hold livestock owners in the Philippines

4.1 Introduction

The epidemiology of \textit{Trypanosoma evansi} (surra) is a complicated interaction amongst the wild and domestic animals, the main arthropod-vector (\textit{Tabanus} spp. of biting flies), the climate and the pathogenicity of the parasite (Luckins, 1998a; Reid, 2002). In the high-surra risk areas in Mindanao, tabanids are abundant throughout the year (Chapter 7) facilitating transmission of \textit{T. evansi} amongst susceptible village buffaloes and other host animals that are usually tethered together in common pasture areas. Stress, due to farm work and concurrent diseases, assists the survival of the parasite in animal hosts. The constant movement of animals within the island due to trade, government dispersal programme and transmigration of people facilitates geographic spread of the disease.

Surra is one of the most economically important parasitic diseases of livestock in Southeast Asia, including the Philippines. The disease is highly fatal to horses but mortality may also occur in buffaloes, cattle, small ruminants (Löhr \textit{et al.}, 1985; Manuel, 1998; Reid, 2002; Mercado \textit{et al.}, 2004) and pigs (Batolos and Somoray, 1989; Arunasalam \textit{et al.}, 1995). Apart from mortality, losses due to \textit{T. evansi} infection may also result from low production (loss of weight, decreased draught and milk output), cost of diagnosis and treatment and reproduction failure (Mahmoud and Gray, 1980; Payne \textit{et al.}, 1991a; Manuel, 1998; Reid, 2002). Reduced calving performance of buffaloes was observed in high-surra risk areas in Mindanao (Dargantes \textit{et al.}, 2009) and abortions in affected livestock were reported in Thailand (Löhr \textit{et al.}, 1986; Kashiwazaki \textit{et al.}, 1998; Jittapalapong \textit{et al.}, 2009). However, despite the impact of
*Trypanosoma evansi* infection on animal productivity, financial losses due to the disease are potentially underestimated because of: insufficient data, misdiagnosis, remoteness of the affected areas, and reluctance of the farmers to report mortalities.

Surra can only be effectively controlled by chemotherapy. Of the drugs available only melarsomine hydrochloride (Cymelarsan®) and diminazine aceturate (Berenil®, Surraplex®, Trypan®) are considered safe for use in all animal species. However, only the former drug has satisfactory efficacy against *T. evansi* infection in Southeast Asia (Lun *et al*., 1991; Payne *et al*., 1994a) but it is not currently marketed in the Philippines. Diminazene products are currently used in Mindanao but despite massive treatments, the sporadic epidemics of surra have continued. There is, therefore, a need to assess the economic benefits of the present chemotherapy regimen and other practical treatment options in order to justify the continuation of a more cost-effective, sustainable control strategy against the disease. In this study, a disease model for *T. evansi* infection in village buffalo and other animal hosts was developed to estimate losses due to the disease and evaluate financial benefits of six treatment regimens. Data from cross-sectional surveys of *T. evansi* infection in livestock in Mindanao were used to estimate parameters for the host population demographic and the infectious disease models. Treatment costs and the benefits gained by animals achieving their full reproductive potential were included in the model as a measure of the productivity of livestock from village/smallholder farms in the Philippines.

### 4.2 Materials and methods

#### 4.2.1 Development of an infectious disease model for *T. evansi* in buffaloes

*Trypanosoma evansi* infection in buffaloes was modelled using the susceptible-infectious-subclinical (SIC) infection framework, modified from the susceptible-
infected-susceptible (SIS) disease model where there is incomplete immunity against the disease and re-infection may occur (Coen et al., 2001; Keeling and Eames, 2005). Data from a 4-year (2002-2006) field survey of the prevalence of *T. evansi* infection in village buffaloes and other animal hosts from five provinces in Mindanao, Philippines were used to define the model framework (Dargantes et al., 2009). Additional data from recent surveys of buffaloes and other livestock (Chapter 5) were also used to estimate the relevance of each host species in the epidemiology of *T. evansi* infection. A simplified diagram of the model is shown in Figure 4.1 with two assumed cohorts: the ‘Innate Resistant’ and ‘Innate Susceptible’ groups. The ‘Innate Resistant’ is an infected cohort of the population but is more resistant to infection compared to the ‘Innate Susceptible’ cohort. Within each cohort are three nodes: *o*, *i* and *c* indicating uninfected-susceptible, infectious (clinical infection) and subclinical-infected animals, respectively. If the innate-resistant-fraction (denoted “Innate”) is set to zero then the model simplifies to the lower four nodes in the diagram (i.e., *Yo*, *So*, *Si* and *Sc*). Death and harvest rates for each stage are included in the model but are not shown, and the model was not age structured for simplicity. The transition between the infection states in the model was dependent on vector capacity, prevalence, host resilience and chemotherapy as described below.
Figure 4.1 Diagram of the susceptible–infectious–subclinical (SIC) model of *T. evansi* infection in buffaloes. In the model, *o*, *i* and *c* indicate uninfected-susceptible, infectious (clinical infection) and subclinical-infected animals, respectively. Lines pointing back to ‘*Yo*’ indicate reproduction rate, lines cycling within a cohort indicate survival rate in that stage and lines connecting stages indicate survival and transition rate from stage to stage.

4.2.1.1 Transition states

Tables 4.1 and 4.2 show the details of the transitions in the SIC model for buffaloes. There is no transition from the two infected states (infectious (*i*) and subclinical (*c*)) to the uninfected-susceptible state, which is a common transition for some bacterial and viral diseases (i.e., susceptible-infectious-resistant-susceptible (SIRS) models). The simulation model allows for drug treatment and in this case the number of infected animals successfully treated in a time step are moved to the appropriate uninfected cohorts (*So* or *Ro*). Animals cannot transit directly to a subclinical cohort without first becoming infectious (clinically infected). The simulation model time step was set for one month. The parameters used in the model including costs of treatment, diagnosis,
labour and sale of full-grown livestock are detailed in Table 4.3. However, cost parameters were fixed in the model as the effect of the disease on productivity was the primary concern of the study rather than accounting for the impact of fluctuations in market price.

Table 4.1  Simplified difference equations for resulting change (Δ) in cohort size in one time step but not showing birth of calves and removal of dead and/or harvested animals from the cohorts.

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Change</th>
<th>Uninfected * proportion</th>
<th>- losses</th>
<th>+ gains</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninfected</td>
<td>ΔSo =</td>
<td>((1-Innate)<em>Yo)/(1-V</em>StoI)</td>
<td>V<em>StoI</em>So</td>
<td></td>
</tr>
<tr>
<td>Uninfected</td>
<td>ΔRo =</td>
<td>(Innate<em>Yo)/(1-V</em>RtoI)</td>
<td>V<em>RtoI</em>Ro</td>
<td></td>
</tr>
<tr>
<td>Clinical</td>
<td>ΔSi =</td>
<td>((1-Innate)<em>Yo+So)/(V</em>StoI)</td>
<td>Si*ItoC</td>
<td>Sc*CtoI</td>
</tr>
<tr>
<td>Clinical</td>
<td>ΔRi =</td>
<td>(Innate<em>Yo+Ro)/(V</em>RtoI)</td>
<td>Ri*ItoC</td>
<td>Rc*CtoI</td>
</tr>
<tr>
<td>Sub-clinical</td>
<td>ΔSc =</td>
<td></td>
<td>Sc*CtoI</td>
<td>Si*ItoC</td>
</tr>
<tr>
<td>Sub-clinical</td>
<td>ΔRc =</td>
<td></td>
<td>Rc*CtoI</td>
<td>Ri*ItoC</td>
</tr>
</tbody>
</table>

Cohort size is represented by So, Ro, Si, Ri, Sc, Rc & Yo (calves), where S and R stand for innate susceptible and resistant adults, respectively. Parameter definitions are given in Table 4.2.
Table 4.2  Parameters, definitions and symbols used in the buffalo SIC-model and Table 4.1. Rate parameters are given as annual rates. The range indicates the allowable input of minimum and maximum limits for the parameters which are sufficiently wide to allow simulation of a variety of hosts.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Range</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Innate</td>
<td>0.1</td>
<td>0, 1</td>
<td>Proportion with innate resistance</td>
</tr>
<tr>
<td>StoI</td>
<td>0.8</td>
<td>0, 1</td>
<td>Infection success for uninfected innate susceptible</td>
</tr>
<tr>
<td>RtoI</td>
<td>0.2</td>
<td>0, 1</td>
<td>Infection success for uninfected innate resistant</td>
</tr>
<tr>
<td>ItoC</td>
<td>1.0</td>
<td>0, 1</td>
<td>Transition proportion from infectious to subclinical</td>
</tr>
<tr>
<td>CtoI</td>
<td>0.5</td>
<td>0, 1</td>
<td>Transition proportion from subclinical to infectious</td>
</tr>
<tr>
<td># Sex</td>
<td>0.7</td>
<td>0, 1</td>
<td>Female proportion of the population</td>
</tr>
<tr>
<td>Birth Rates</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\mu_{ro}$</td>
<td>0.475</td>
<td>0, 40</td>
<td>Uninfected resistant-Ro female</td>
</tr>
<tr>
<td>$\mu_{so}$</td>
<td>0.475</td>
<td>0, 40</td>
<td>Uninfected normal-So female</td>
</tr>
<tr>
<td>$\mu_i$</td>
<td>0.150</td>
<td>0, 40</td>
<td>Infectious Si &amp; Ri females</td>
</tr>
<tr>
<td>$\mu_c$</td>
<td>0.150</td>
<td>0, 40</td>
<td>Sub-clinical infected Sc &amp; Rc females</td>
</tr>
<tr>
<td>Death Rates</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\lambda_y$</td>
<td>0.115</td>
<td>0, 1</td>
<td>Calf uninfected Yo</td>
</tr>
<tr>
<td>$\lambda_{ro}$</td>
<td>0.115</td>
<td>0, 1</td>
<td>Uninfected resistant Ro</td>
</tr>
<tr>
<td>$\lambda_{so}$</td>
<td>0.115</td>
<td>0, 1</td>
<td>Uninfected normal So</td>
</tr>
<tr>
<td>$\lambda_i$</td>
<td>0.250</td>
<td>0, 1</td>
<td>Infectious Si &amp; Ri</td>
</tr>
<tr>
<td>$\lambda_c$</td>
<td>0.175</td>
<td>0, 1</td>
<td>Sub-clinical infected Sc &amp; Rc</td>
</tr>
<tr>
<td>Drug treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TreatI</td>
<td>0.95</td>
<td>0, 1</td>
<td>Proportion of infectious animals treated</td>
</tr>
<tr>
<td>TreatC</td>
<td>0.20</td>
<td>0, 1</td>
<td>Proportion of sub-clinical animals treated</td>
</tr>
<tr>
<td>TreatY</td>
<td>0.03</td>
<td>0, 1</td>
<td>Proportion of uninfected animals treated</td>
</tr>
<tr>
<td>$^a$Efficacy</td>
<td>0.99</td>
<td>0, 1</td>
<td>Proportion of treated animals rendered uninfected</td>
</tr>
<tr>
<td>$^a$Drug</td>
<td>12</td>
<td>0, 12</td>
<td>Number of drug treatments/year</td>
</tr>
<tr>
<td>Infection dynamics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$^a$minN</td>
<td>33</td>
<td>5, 50</td>
<td>Minimum population for disease stability</td>
</tr>
<tr>
<td>$^a$maxMonth</td>
<td>5</td>
<td>1, 12</td>
<td>Month in which the maximum fly intensity occurs</td>
</tr>
<tr>
<td>$^a$Vi</td>
<td>2 to 5</td>
<td>0, 10</td>
<td>Arbitrary measure of vector Intensity</td>
</tr>
<tr>
<td>Scinfect</td>
<td>0.2</td>
<td>0, 1</td>
<td>Proportion of subclinical animals that are infectious</td>
</tr>
<tr>
<td>$^a$V=Vi*$I/N$</td>
<td></td>
<td></td>
<td>Vector transmission (for $N \geq minN$)</td>
</tr>
<tr>
<td>$^a$V=Vi*$/minN$</td>
<td></td>
<td></td>
<td>Vector transmission (for $N &lt; minN$)</td>
</tr>
<tr>
<td>$^a$N=</td>
<td></td>
<td></td>
<td>Total buffalo population</td>
</tr>
<tr>
<td>$^a$I = Si+Ri+ Scinfect$</td>
<td></td>
<td></td>
<td>Number of infectious animals</td>
</tr>
</tbody>
</table>

Note: All clinically sick animals were assumed to be infectious and a proportion of sub-clinical animals were infectious. $^a$Parameters/quantity were not subjected to Monte Carlo simulation (stochastic) variation.
Table 4.3  Parameters used in the SIC model to simulate *T. evansi* infection in buffaloes and other hosts. Parameters for the cost benefit analysis of *T. evansi* infection and its treatment are also given.

<table>
<thead>
<tr>
<th>Parameter or value</th>
<th>Buffalo</th>
<th>Cattle</th>
<th>Horse</th>
<th>Sheep/Goat</th>
<th>Pig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of hosts (<em>N</em>) per village</td>
<td>80</td>
<td>40</td>
<td>15</td>
<td>150</td>
<td>200</td>
</tr>
<tr>
<td>Relative host-vector ratio</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td>Birth rate</td>
<td>0.475</td>
<td>0.500</td>
<td>0.40</td>
<td>1.50</td>
<td>18</td>
</tr>
<tr>
<td>Infected birth rate</td>
<td>0.150</td>
<td>0.150</td>
<td>0.10</td>
<td>0.30</td>
<td>5</td>
</tr>
<tr>
<td>Death rate</td>
<td>0.115</td>
<td>0.080</td>
<td>0.07</td>
<td>0.14</td>
<td>0.1</td>
</tr>
<tr>
<td>Clinical death rate</td>
<td>0.250</td>
<td>0.330</td>
<td>0.80</td>
<td>0.50</td>
<td>0.4</td>
</tr>
<tr>
<td>Subclinical death rate</td>
<td>0.175</td>
<td>0.175</td>
<td>0.80</td>
<td>0.50</td>
<td>0.4</td>
</tr>
<tr>
<td>Infection rate ‘Innate Susceptible’ host</td>
<td>0.2</td>
<td>0.1</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Infection rate ‘Innate Resistant’ host</td>
<td>0.8</td>
<td>0.5</td>
<td>0.9</td>
<td>0.8</td>
<td>0.8</td>
</tr>
</tbody>
</table>

*Cost/Benefit Parameters (Pesos)*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Buffalo</th>
<th>Cattle</th>
<th>Horse</th>
<th>Sheep/Goat</th>
<th>Pig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnostic cost per test</td>
<td>300</td>
<td>300</td>
<td>300</td>
<td>300</td>
<td>300</td>
</tr>
<tr>
<td>Drug cost per treatment</td>
<td>300</td>
<td>300</td>
<td>300</td>
<td>30</td>
<td>200</td>
</tr>
<tr>
<td>Labour cost per treatment</td>
<td>300</td>
<td>300</td>
<td>300</td>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td>Sale or purchase price of replacement host</td>
<td>25,000</td>
<td>25,000</td>
<td>10,000</td>
<td>1,500</td>
<td>5,000</td>
</tr>
</tbody>
</table>

*Parameters/values were not subjected to Monte Carlo simulation (stochastic)*
4.2.1.2 Transmission of *T. evansi* by insect vectors

*Trypanosoma evansi* is mechanically transmitted between buffaloes and other host species by tabanid flies. Infection success is dependent on the fly intensity, susceptibility of the host, disease prevalence and level of parasitaemia in infected animals (Desquesnes *et al.*, 2009a). Tabanid intensity or attack rate (arbitrarily set with potential range of 0 to 10) was assumed to be seasonal (Manresa and Mondoñedo, 1935) and was defined by a cosine-curve (Figure 4.2) with three parameters: maxMonth, max and min which fix the month of maximum fly intensity and the maximum and minimum levels, respectively. The minimum level of fly intensity was assumed to occur six months after the maximum level, with *Vector Intensity* (*Vi*) defined by the cosine-curve as: 

\[ Vi = min + [(max - min) * (1 + \cos(\text{angle})) / 2]; \]

where *angle* is the *month* transformed to an angle by: 

\[ \text{angle} = (\text{month} - \text{maxMonth}) * 360/12 \]

and *month* was 1, 2, 3... for January, February, March, etc. The angle was converted to radians before use in the cosine function. The parameters *StoI* and *RtoI* are the probability that infection will result from a bite from a fly carrying the parasite for the innate susceptible and resistant cohorts, respectively. The transition from susceptible to infectious determined by the equations shown in Tables 4.1 and 4.2 is similar to the ‘frequency-dependent transmission’ defined for host-microparasite relationship models (Begon *et al.*, 2002). However, *I/N* represents the proportion of infectious animals, not prevalence, because it was assumed that a proportion of subclinical animals do not have a parasitaemia high enough to allow biting tabanids to acquire trypanosomes. The proportion of subclinical animals that contribute to the total pool of infectious animals was determined from the parameter *SCinfect* (Table 4.2). To estimate transmission success the proportion of infectious animals (*I/N*) was further amplified or decreased by *Vi* to yield *V* (i.e., *V*=*Vi* *I/N*), which was used in the model as shown in Table 4.1. It is not unreasonable for *V* to exceed one as this would indicate multiple contacts with flies carrying *T. evansi*. However, if *V* *StoI* or *V* *RtoI* exceeds one, which is possible at high prevalence and
high vector intensities, then this product is reset to one as only 100% of uninfected hosts can be converted to infectious. \( V_i \) is a parameter that combines a number of parameters associated with the vector such as: fly–host contact rate, the probability that a fly acquires a trypanosome(s) from an infected animal, the probability of successfully transferring a viable trypanosome to an uninfected host (vector competence) and fly intensity. Measurement of these parameters is currently not possible so the above seasonally defined \( V_i \) between set levels substitutes for a more comprehensive exploration of vector competence. The parameter \( \text{min} N \) was included to represent a pool of uninfected non-buffalo hosts (in buffalo equivalents) that are also tabanid targets but generally maintain a lower seroprevalence of \( T. \) evansi infection than buffaloes (e.g., goats, pigs, etc.). For large buffalo populations or simulations that included these hosts, this factor was not applied. However, for small populations \( N \) was increased to the minimum value as necessary.

![Scaled flies/buffalo/hour](chart)

**Figure 4.2** Observed tabanids caught on buffaloes in open pasture (Manresa and Mondoñedo, 1935). The raw data (Tabanids/host/hour ranges from 3 to 42) was scaled by dividing the observed values by 42 (shown as “●”). The line shows the cosine-curve when the three parameter values were: \( \text{max} \text{Month} = 4, \text{max} = 0.6 \) and \( \text{min} = 0.07. \)
4.2.1.3 Summary of potential transmission of *T. evansi* between livestock by tabanids

Changes in vector populations, normally subject to climate temperature and moisture variables, were modelled in a simplistic way by assuming that tabanid abundance changes smoothly between preset peak season abundance and minimum levels, which occurred six months after the peak. An arbitrary scale of 0 to 10 was chosen to represent vector intensity (*Vi*). Seasonal fluctuations between the maximum and minimum levels of *Vi* (set by the user) was defined by a cosine-curve which provides the fly intensity for a particular month. The *Vi* value was then reduced by the current proportion of infectious animals (*I/N*) to give *V* (the monthly transmission coefficient). However, if *N* was less than min*N* the proportion infectious was set to *I/minN*. That is, *V* = *Vi* *I/minN* becomes the monthly transmission coefficient for small herds. Finally, the proportion of uninfected animals that became infected in a particular month was *V* *Stol* and *V* *RtoI* for the innate susceptible and resistant hosts, respectively.

4.2.1.4 Drug treatment, animal harvest and import

The efficacy of curative drug, the proportion of each cohort treated and the frequency of treatment were control/user-defined management parameters (Table 4.2). The proportion of animals successfully treated (e.g., *Efficacy*TreatI) in a particular time step was removed from the infected groups and returned to the uninfected groups; no immunity was assumed to be conferred by prior infection (Donelson *et al.*, 1998). If the number of drug treatments/year were set at 0.5, 1 or 2... the simulation treatment would be scheduled once in 24, 12 or 6...months, etc. Annual birth and death rates were converted to monthly rates. At each time step the number of dead animals was removed from each cohort, and excess males (or females, depending on the set sex ratio) were removed (harvested) as calves to maintain the initial sex ratio. If the resulting population exceeded the initial population then additional animals were “harvested”, or if necessary animals were imported to
maintain the original herd size. Any animals imported were assumed to be uninfected. The model was developed in Excel (Microsoft Inc., USA).

4.2.1.5 Development of infectious disease models for *T. evansi* infection in other non-buffalo hosts

The SIC model described above (for buffalo) was also used to simulate *T. evansi* infection in non-buffalo hosts (i.e., cattle, horse, sheep/goat and pig) by changing the relevant parameters in Table 4.3. Separate Excel workbooks for each host species were opened simultaneously to exchange information on host numbers and vector intensity. A relative host–vector ratio was defined to convert different hosts to buffalo equivalents for the purpose of estimating vector transmission and the ratio of infectious to uninfected animals for all host species at risk in an area. This ratio was defined as the number of non-buffalo animals that attracted the same number of flies that were on one buffalo. For example, if a buffalo had attracted 60 flies whilst a cow attracted 30 flies then two cows were equivalent to one buffalo and the cow ratio would be set at two. The proportion of infectious animals (*I/N*) was determined by converting all hosts to buffalo equivalents using the relative ratios shown in Table 4.3. The composite estimate of *I/N* was used as described above. The relative ratios were also used to scale the attack rate per host species (e.g., if the vector intensity (*Vi*) was 5 for buffaloes then it was scaled to 2.5 and 0.5 for cattle and goats, respectively) using the ratios from Table 4.3. However, *I/N* remained common for all species. Like *Vi*, the relative host–vector ratio simplifies a number of complex issues into a single parameter, e.g. host size, tabanid host-specificity, host defensive activities and animal management practices. The estimates used in this model were based on anecdotal observations by veterinarians visiting surra outbreaks in Mindanao (expert opinion) and have taken into account the local management practices used for different host species.
4.2.2 Evaluation of different treatment regimens for *T. evansi* infection using the SIC model

The SIC model was used to assess the success of different treatment regimens in a typical village of moderate/high surra-risk. Buffaloes, goats/sheep, pigs, horses and cattle were included because these are the animals of major economic or production importance. Simulations were run for 15 years with results focused on the first five years after intervention. Six drug treatment regimens were simulated: (1) twice yearly treatment of all animals; (2) twice yearly treatment of horses, cattle and buffaloes (goats and pigs remained untreated); (3) targeted treatment of all animals showing clinical signs, monitored monthly; (4) targeted treatment of horses, cattle and buffaloes showing clinical signs (goats and pigs remained untreated), monitored monthly; (5) annual diagnostic testing of buffaloes, cattle and horses, and only treating seropositive animals; (6) no treatment of any hosts. For regimens 1 to 5, drug efficacy was assumed to be 99% (i.e., for melarsomine or Cymelarsan®). Regimen 5 was also simulated with the efficacy set to 80% (regimen 5a) to represent the use of diminazene aceturate. Regimens 1 to 4 were considered realistic practical options for Mindanao. Regimen 5a is currently applied in Mindanao but in practice additional treatments are also applied in response to a disease outbreak. To simulate these regimens, additional assumptions were made.

When estimating the total number of drug treatments required for regimens 1 to 5, drug treatment was stopped for a host species when no new clinical cases occurred in that species. For regimens 1 and 2, it was assumed that 100% of the host species targeted for treatment received treatment. The true cohort of clinically sick animals is shown in Table 4.1. Allowance was made for incorrect diagnosis at the time of treatment for regimens 3 and 4. Thus the drug treatment parameter values given in Table 4.3, for regimens 3 and 4, are the proportions of animals actually treated (when aiming to treat all clinically sick animals), which were set at 0.95, 0.2 and 0.03 for clinical, subclinical and uninfected animals, respectively. For regimen 5, the efficacy of treatment of seropositive animals is dependent on the sensitivity and specificity of the diagnostic test used. The
proportion of clinical, subclinical and uninfected animals treated was set to 0.83, 0.83 and 0.04, respectively, assuming the CATT has a sensitivity and specificity of 83% and 96%, respectively (Reid and Copeman, 2003). Model parameters for the proportion of animals treated, birth, death and transition rates were randomly varied for 500 iterations using Monte Carlo simulation method to estimate mean model results and their 95% confidence limits. Random selection for the parameters given in Table 4.3 or as described above was generated using the Pert (using “expert opinion”) distribution. The impact of drug resistance or using a low efficacy drug in regimens 1 to 4 was examined by setting drug efficacy to 100%, 99%, 95%, 90%, 80%, 70%...0% in additional simulations for these regimens. Random variables and Monte Carlo simulations were generated using PopTools (Hood, 2009) within Microsoft Office Excel version 2003 (Microsoft Inc., USA).

4.2.3 Cost benefit analysis of the different treatment options for T. evansi infection and estimation of the financial losses due to the disease

The net-benefit for each drug regimen was obtained by subtracting the cost/benefit result for the untreated simulation (regimen 6) from the cost/benefit result for the drug treatment regimens (1 to 5). This was done to simplify the cost/benefit analysis by ignoring common costs and benefits. All species were maintained at a fixed level in the simulations, therefore some common costs/benefits were assumed to be approximately equal. That is, let $T$, $M$ and $R$ represent animal Treatment, Maintenance and Replacement costs, respectively, and $D$, $C$ and $E$ represent benefits from animal Draught-power, Consumption and Export, respectively. If $Bt$ and $Bu$ are the benefits from treated and untreated regimens, respectively, then the net-benefit for a treatment regimen ($Bt - Bu$) is given by: $Bt - Bu = Dt - Du + Ct - Cu + Et - Eu - Tt - Mt + Mu - Rt - Ru$. This reduces to:

$Bt - Bu = Et - Eu - Tt - Rt + Ru$, if we assume that under both regimens: (a) draught-power requirements are sufficient; (b) consumption of animals are similar and (c) maintenance costs are similar. Thus, the simplified cost/benefit analysis for each regimen needs only to account for: $T$
(drug purchase, application and test costs), \( R \) (purchase of replacement animals when needed) and \( E \) (sale of excess animals when available) if we only estimate the net-benefit for each treatment regimen.

The mean benefit/host (mean of the benefit of the five host types) in Philippine Pesos or US$ was determined for each drug treatment regimen and the untreated hosts (i.e., \( \frac{\sum B_i}{5} \) where \( B_i \) was the mean benefit/year for host type \( i \) and \( i=1...5 \)). The total benefit for a treatment regimen was estimated using the formula:

\[
\text{Total benefit} = \sum N_i \times B_i ; \quad \text{where} \quad N_i \text{ was the number of animals of host type } i \text{ and } i=1...5
\]

The total financial losses in all animals in a village due to \( T. evansi \) infection were estimated by subtracting the total benefit (sum of the benefit for all animals in a village) for the untreated (regimen 6) from the total benefit obtained with the most effective treatment regimens (1 or 3).

4.3 Results

4.3.1 Development of infectious disease models for \( T. evansi \) infection in buffaloes and other host species

Table 4.4 shows the long-term predicted seroprevalence in untreated animals for a range of vector intensities with populations (Table 4.3) assumed to be maintained by births and imports. Using the observed seroprevalence in buffaloes from Table 3.2 in Chapter 3 as a reference point, a comparison between long-term modelled seroprevalence (Table 4.4) and observed seroprevalence for other host species was made. The observed seroprevalence in goats/sheep was significantly lower compared to observed buffalo seroprevalence. The predicted seroprevalence estimates in goats/sheep and pigs were also significantly lower compared to a broad range of buffalo seroprevalence at different vector intensities \( (p<0.05) \). The modelled seroprevalence for horse is
similar to cattle at low and higher vector intensities. This result was based on the need in the model for sustained import of horses to maintain the population in high vector intensity areas. If imports cease, then horse populations rapidly die, which has been observed in high-surra risk areas of Mindanao (Dr. P. Calo, pers. comm., 2007).

### 4.3.2 Evaluation of different treatment regimens for T. evansi infection using the SIC model

The number of drug treatments required to prevent clinical disease (in the first 5 years) under each regimen for each host species (deterministic result using the most likely parameter value and stochastic results) are shown in Table 4.5. Significantly fewer treatments were required for regimens 1 and 2 compared to regimens 3 and 4 as evidenced by the absence of overlapping in their respective 95% confidence intervals (Table 4.5). Approximately five times more buffaloes need to be treated under the two treatments/year regimen (1 versus 2) and twofold more under the targeted treatment regimen (3 versus 4) if pigs, sheep and goats are not treated (Table 4.5). The predicted mean prevalence of T. evansi infection over the first 5 years for each host species and control regimen is summarised in Table 4.6. For regimen 5, the prevalence of T. evansi infection was approximately halved (depending on host species) whilst regimens 1 to 4 reduced the prevalence of T. evansi infection to less than 6%.
Table 4.4  The effect of increasing vector intensity on the long term seroprevalence of *T. evansi* infection in untreated hosts as predicted by the model.

<table>
<thead>
<tr>
<th>Fly intensity</th>
<th>Buffalo</th>
<th>Cattle</th>
<th>Horse</th>
<th>Goat/sheep</th>
<th>Pig</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>95% CI</td>
<td>%</td>
<td>95% CI</td>
<td>%</td>
</tr>
<tr>
<td>0 to 2</td>
<td>44.4</td>
<td>7.3, 71.8</td>
<td>13.4</td>
<td>2.8, 25.9</td>
<td>13.8</td>
</tr>
<tr>
<td>Set at 1.6</td>
<td>58.9</td>
<td>27.2, 76.7</td>
<td>24.5</td>
<td>10.6, 38.0</td>
<td>23.9</td>
</tr>
<tr>
<td>2 to 5</td>
<td>73.7</td>
<td>59.2, 82.0</td>
<td>44.9</td>
<td>31.9, 57.1</td>
<td>44.2</td>
</tr>
<tr>
<td>5 to 8</td>
<td>79.3</td>
<td>70.8, 84.4</td>
<td>57.2</td>
<td>47.3, 64.6</td>
<td>59.6</td>
</tr>
<tr>
<td>8 to 10</td>
<td>81.2</td>
<td>75.1, 85.0</td>
<td>62.0</td>
<td>54.3, 67.7</td>
<td>64.7</td>
</tr>
</tbody>
</table>
4.3.3 Cost benefit analysis of different treatment options for *T. evansi* infection and estimation of the financial losses due to the disease

The net-benefit from the six drug treatment regimens by comparison with untreated hosts are shown in Figure 4.3. Treatments with 95% net-benefit confidence intervals that do not overlap with zero (x-axis) are significantly different from the untreated animals. The mean benefits/host (averaged over benefit for host type) for regimens 1 to 5a assuming a 100% drug efficacy were 11,527, 10,318, 11,527, 10,731, 8,960 and 6,355 pesos respectively (231, 206, 231, 215, 179 and 127 in US$, respectively; assuming 5,000 Philippine pesos (PhP) is equivalent to US$100). The mean benefits for regimens 1 and 3 were both 81% larger than the benefit for the untreated animals. The current practice of annual treatment of seropositive buffaloes, cattle and horses only yielded a 40% improvement. Figure 4.4 shows the increased mean benefit from untreated animals for regimens 1 to 4 at various drug efficacies. As drug efficacy declines the benefit for animals treated twice each year, regardless of disease status, declined steadily whilst little loss of benefit occurred for animals monitored monthly and treated if clinically sick. The mean benefit/host was 8,935, 8,110, 11,182 and 10,370 pesos (US$179, 162, 224, 207, respectively) for regimens 1 to 4, respectively, when drug efficacy was 50%. Approximately, half the gain provided by regimens 1 and 2 was lost whilst less than 10% of the gain made by regimens 3 and 4 was lost due to reduction of drug efficacy to 50%. In terms of the value added to the domestic livestock this was estimated to be US $88, $84, $151, $7, $114 per animal per year for buffaloes, cattle, horses, goats/sheep and pigs, respectively (deterministic result).

The total benefits (sum of the benefit for all animals in a village) for the untreated (regimen 6) and effectively treated (1 or 3) regimens were 1.5 and 9.4 million Philippine pesos (PhP) per year, respectively (US $30,000 and $188,000 per year,
respectively). Therefore, the total net-benefit lost or the total financial loss to a village due to *T. evansi* infection was 7.9 million pesos per year or US $158,000 per year.

Table 4.5  Total and mean number of drug treatments (95% CI) required in the first five years for each treatment option to prevent clinical case of surra.

<table>
<thead>
<tr>
<th>Host</th>
<th>Number of drug treatments$^+$</th>
<th>Number of drug treatments$^+$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Model$^{**}$ Mean LCI UCI</td>
<td>Model$^{**}$ Mean LCI UCI</td>
</tr>
<tr>
<td></td>
<td>Regimen 1</td>
<td>Regimen 2</td>
</tr>
<tr>
<td>Buffalo</td>
<td>156 271 154 790</td>
<td>783 715 462 790</td>
</tr>
<tr>
<td>Cattle</td>
<td>78   79   77  80</td>
<td>77   198   77  397</td>
</tr>
<tr>
<td>Horse</td>
<td>28   33   26   59</td>
<td>86   100   25   150</td>
</tr>
<tr>
<td>Sheep/Goat</td>
<td>289  311  285  577</td>
<td>0     0     0     0</td>
</tr>
<tr>
<td>Pig</td>
<td>322  350  310   628</td>
<td>0     0     0     0</td>
</tr>
<tr>
<td>Total</td>
<td>873 1044 852 2134</td>
<td>946 1013 564 1337</td>
</tr>
<tr>
<td></td>
<td>Regimen 3</td>
<td>Regimen 4</td>
</tr>
<tr>
<td>Buffalo</td>
<td>71   76   66   110</td>
<td>151  161   93   227</td>
</tr>
<tr>
<td>Cattle</td>
<td>27   27   26   29</td>
<td>27    28    26    41</td>
</tr>
<tr>
<td>Horse</td>
<td>4    5    1    8</td>
<td>5     6     2     13</td>
</tr>
<tr>
<td>Sheep/Goat</td>
<td>59   61   55   68</td>
<td>0     0     0     0</td>
</tr>
<tr>
<td>Pig</td>
<td>70   70   62   78</td>
<td>0     0     0     0</td>
</tr>
<tr>
<td>Total</td>
<td>231 239 210 293</td>
<td>183 195 121 281</td>
</tr>
<tr>
<td></td>
<td>Regimen 5</td>
<td>Regimen 5a (80% efficacy)</td>
</tr>
<tr>
<td>Buffalo</td>
<td>157 166 81 252</td>
<td>189 188 106 258</td>
</tr>
<tr>
<td>Cattle</td>
<td>40   42   25   62</td>
<td>48    50    34    69</td>
</tr>
<tr>
<td>Horse</td>
<td>19   18   4    37</td>
<td>22    21    6     40</td>
</tr>
<tr>
<td>Sheep/Goat</td>
<td>0    0    0    0</td>
<td>0     0     0     0</td>
</tr>
<tr>
<td>Pig</td>
<td>0    0    0    0</td>
<td>0     0     0     0</td>
</tr>
<tr>
<td>Total</td>
<td>216 226 110 351</td>
<td>259 258 146 367</td>
</tr>
</tbody>
</table>

$^+$Generated from 500 iterations in Monte Carlo simulation with 95% confidence intervals (LCI, UCI)

$^{**}$Deterministic result of the model
Table 4.6  Mean prevalence of *T. evansi* infection in each host species, for each treatment regimen, over the first 5 years of treatment.

<table>
<thead>
<tr>
<th>Treatment regimen</th>
<th>Mean prevalence (%) of <em>T. evansi</em> infection for years 1-5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Buffalo</td>
</tr>
<tr>
<td>Initial starting prevalence</td>
<td>70.0</td>
</tr>
<tr>
<td>1</td>
<td>4.4</td>
</tr>
<tr>
<td>2</td>
<td>5.8</td>
</tr>
<tr>
<td>3</td>
<td>5.4</td>
</tr>
<tr>
<td>4</td>
<td>5.6</td>
</tr>
<tr>
<td>5</td>
<td>25.6</td>
</tr>
<tr>
<td>5a</td>
<td>38.1</td>
</tr>
<tr>
<td>6</td>
<td>74.9</td>
</tr>
<tr>
<td>(no treatment)</td>
<td></td>
</tr>
</tbody>
</table>
Figure 4.3  Mean and 95% Monte Carlo confidence intervals of the net-benefit for the six drug treatment regimens using drugs with 99% and 80% (5a) efficacy.
Figure 4.4  The effect of declining drug efficacy on the benefit obtained for drug treatment regimens 1 to 4. The relative benefit is shown as the percentage improvement in mean benefit per host compared with untreated animals (regimen 6). The regimen shown by × indicates only buffalo, cattle and horses were treated, ▲ indicates the treatment of all host species, the broken line (---) indicates 2 drug treatments per year and the solid line (——) shows the regimen for the targeted treatment of clinically sick animals when monitored monthly.

4.4 Discussion

Results showed that the infection models developed in this study are useful to predict the epidemiological dynamics and outcome (i.e., prevalence, mortality, morbidity, demographic impact) of *T. evansi* infection in buffaloes and other livestock species in high-surra risk villages in Mindanao and probably in other areas where surra is endemic. The mathematical models, with the incorporation of treatment parameters and costs have also been shown as essential tools in estimating financial losses from *T. evansi* infection and the subsequent financial benefits of its control, necessary to evaluate different control strategies. Indeed, these models of *T. evansi* infection would serve as important guide to government decision-makers in formulating a more efficient and cost-effective control measure against surra in Mindanao and in other surra-endemic provinces in the country. The usefulness of utilising mathematical simulation
models in predicting infection and evaluating treatment options has also been successfully demonstrated in other animal diseases (Van Nes et al., 1998; French et al., 1999; Lesnoff et al., 2004; Häsler et al., 2006; Mitchell et al., 2008; Gilioli et al., 2009) including trypanosomosis (Baker, 1992; Coen et al., 2001). The advantage of using Microsoft Office Excel to model an infectious disease is that different parameters regarding the disease and treatment regimens can be easily introduced to the simulation study by changing the column value associated with the parameter. Indeed, this would allow the use of these models in other geographical locations where surra dynamics might be different. Likewise, the simultaneous modelling of a number of host species in Excel allows the assessment of the total benefit of surra control at the village, provincial or national level. With few changes in the parameters, the present models could be used to forecast the outcome of epidemics of surra in different species of domestic and wild animals in surra-endemic areas or in free regions/countries should incursion occur.

The financial losses due to *T. evansi* infection in livestock and the benefits of treatment estimated by these models are likely to be conservative because they do not account for weight loss and reduced milk yields associated with surra (Joshi et al., 1993; Damayanti et al., 1994; Manuel, 1998; Dargantes et al., 2005b). However, the estimated monetary losses from *T. evansi* infection in this study are significantly higher than the previous estimates of losses with surra in the Philippines which was only about PhP5 million (US$ 0.1 million) per year in the whole country (Manuel, 1998). The previous estimates were probably lower because losses were mainly based on reported mortalities due to surra whilst the present study considers more parameters: (a) mortality and (b) reproduction losses estimated from cross-sectional surveys in Mindanao, and (c) costs of diagnosis and (d) treatment of the disease. It is also most
likely that the reported surra cases were not reflective of the actual field cases of surra in the country (Manuel, 1998) because of potential misdiagnosis, inaccessibility of the affected villages, lack of communication facility and the tendency of farmers not to consult government authorities. It is interesting to note however, that the estimated financial losses from \textit{T. evansi} infection in the present study (US$158,000 per village per year) are comparable to the estimated losses (mainly based on horse mortality) in Pantanal, Brazil of about US$2,143 per ranch per year (Seidl \textit{et al.}, 1998). This is despite the fact that horses are highly susceptible to \textit{T. evansi} infection compared to any other livestock like buffaloes, cattle, goats (Losos, 1980; Mahmoud and Gray, 1980; Payne \textit{et al.}, 1990; Manuel, 1998) and the pathogenicity of \textit{T. evansi} might have differed between the two countries. The significantly high financial losses caused by \textit{T. evansi} infection in livestock in Mindanao have a great economic and social impact on poor farmers who are dependent on their livestock for farm activities and income. Therefore, the control of \textit{T. evansi} infection in Mindanao and other surra-endemic provinces in the country must be given top priority by livestock owners and concerned government authorities. The substantial financial benefits using curative drugs to control \textit{T. evansi} infection in livestock estimated in this study justify such control measures. The results of the modelling suggest that the greatest variation in net-benefit for surra control will likely be observed in pigs because small changes in fertility due to the disease will be amplified by the relatively large litter size and the short gestation. Horses may also show large variation because of their relatively high mortality when exposed to \textit{T. evansi} infection (Manuel, 1998; Tuntasuvan \textit{et al.}, 2003; Rodrigues \textit{et al.}, 2009) and their high replacement costs.

In recent years, the Philippine government has implemented an extensive Mindanao-wide control programme known as the Mindanao Unified Surra Control Approach
(MUSCA). It was designed to reduce the incidence of *T. evansi* infection in endemic areas to avoid further economic losses, and prevent its spread to other provinces. The programme which mainly involved annual testing of livestock (mainly buffaloes, horses and cattle) for *T. evansi* infection and chemotherapy using diminazene also included educating farmers on the biological aspects and impact of the disease, capability-building of technicians and regional laboratories for better disease diagnosis, supportive treatment and disease monitoring. The endeavour has been successful in reducing the damage due to the disease (Dr. R.T. Mercado pers. comm., 2007) but the project was stopped due to lack of funding. The sporadic epidemics of surra that occurred in Mindanao in the last three years (Dr. P. Calo, Dr. J. Dargantes, Dr. A. Gonzales, pers. comm., 2007-2009) and the high prevalence of the disease in livestock particularly in high-surra risk areas in Mindanao (Chapters 3, 5 and 6) indicate that control efforts against surra should be continued, intensified and be sustained. However, there is a need to enhance the control strategy implemented in Mindanao in order to address the present situation of surra in the island. The models of the present study predict that regular monitoring and targeted treatment of all clinically sick animals (including sheep/goats and pigs) using a highly effective drug (i.e., Cymelarsan®) is the best treatment option in Mindanao, requiring few treatments to produce a low prevalence (Tables 4.5 and 4.6). A similar approach of curative treatment of sick animals all year-round has been identified as the most economical treatment strategy against *T. evansi* infection in horse population in Pantanal, Brazil where surra is also endemic (Seidl et al., 1998). In practice this strategy would require education of farmers regarding the economic significance of *T. evansi* infection and its symptoms and a commitment by stock owners to report the disease to authorities promptly. In addition, the regimen assumes that local livestock services can deliver the trypanocides quickly. Treatment of all animals twice per year provides similar net benefits to targeted treatment without the
need for constant monitoring of animals at risk. This strategy needs an initial high input cost sufficient to treat all animals and is dependent on using a drug with high efficacy. Another disadvantage of this option is that systematic mass chemotherapy will increase the risk of selection for drug resistance. Massive drug treatment has been associated with the emergence of drug resistance in agents causing human malaria (Payne, 1988; Von Seidlein and Greenwood, 2003) and may also lead to the development of resistance to drugs in trypanosomes (Uilenberg, 1998). Drug resistance is a problem in trypanosomes in Africa (El Rayah et al., 1999; Afewerk et al., 2000; Assefa and Abebe, 2001; Mamoudou et al., 2008; Miruk et al., 2008) and other Asian countries (Zhang et al., 1991; Payne et al., 1994a; Tuntasuvan et al., 2003; Zhou et al., 2004) and may emerge in Mindanao under such a regimen. Data from modelling the low drug efficacy showed that if drug resistance develops, the best option is to regularly monitor and treat only clinically sick animals. Treating pigs and sheep/goats is important for surra control because leaving them untreated prolongs the period of new clinical cases in other hosts. However, there is little financial benefit from the treatment of sheep/goats if considered in isolation from other hosts.

In conclusion, T. evansi infection in Mindanao causes high significant economic losses on affected animals. Treating all animals (including sheep/goats and pigs) twice per year or regular monitoring and targeted treatment of any clinically sick animals with a drug of high efficacy (i.e., Cymelarsan®) would provide a substantial benefit. Of these options, targeted treatment is recommended because of reduced input costs, less labour and reduced risk of selection for drug resistance.
Trypanosoma evansi infection (surra) in village buffaloes and other livestock in Mindanao, Philippines

5.1 Introduction

Water buffaloes (Bubalus bubalis) are mainly used as draught animals in Asia. They are highly susceptible to infection with Trypanosoma evansi, as evidenced by serious outbreaks with high infection rates and mortalities (Löhr et al., 1985; Payne et al., 1991c; Singh and Joshi, 1991; Lun et al., 1993; Laha and Sasamal, 2009), and high production and reproduction losses (Löhr et al., 1986; Dargantes et al., 2009). The detection of T. evansi is problematic. The card agglutination test for trypanosomosis/T. evansi (CATT/T. evansi or CATT), is a reliable and accurate serological test that has been used in the Philippines (Mercado et al., 2004). However, there is little information on whether the local isolates of T. evansi express the RoTat 1.2 variable surface glycoprotein (VSG) gene, which is the basis of the diagnostic antigen of CATT (Bajyana Songa and Hamers, 1988). In Kenya, several isolates of T. evansi do not express the RoTat 1.2 VSG gene and camels infected with non-RoTat 1.2 isolates of T. evansi were negative to CATT (Ngaira et al., 2005). There is a need therefore to confirm that local isolates of T. evansi in Mindanao possess the RoTat 1.2 gene.

More information is required to determine the causes of poor reproduction performance of buffaloes in surra-endemic provinces which was identified in field studies conducted as part of Chapter 3. Trypanosoma evansi has been implicated as a cause of abortion, early calf mortality and anaestrous in livestock in other surra-endemic countries (Löhr et al., 1986; Payne et al., 1993; Arunasalam et al., 1995; Gutierrez et al., 2005; Jittapalapong et al., 2009). This study was carried out to: (a) determine the prevalence
and risks associated with *T. evansi* infection in buffaloes and other livestock species; (b) confirm that local isolates of *T. evansi* possess the RoTat 1.2 gene; and, (c) identify the cause/s of reproductive problems and early calf deaths in buffalo cows in selected high- and low-surra risk provinces in Mindanao.

### 5.2 Materials and Methods

The use of animals in this study and interviews with farmers were approved by the Murdoch University Committees on Animal and Human Ethics, respectively (Permit numbers R881/01 and 2007/034, respectively).

#### 5.2.1 Study areas

During 2007 to 2008, a cross-sectional survey to detect infection with *T. evansi* in village buffaloes and other livestock was conducted in five provinces in Mindanao, southern Philippines. The provinces were located in high- (Agusan del Norte, Agusan del Sur, Surigao del Norte and Surigao del Sur) and low- (Bukidnon) surra risk areas (Figure 5.1).

The provinces of Agusan del Norte (9°10’ North, 125°30’ East), Agusan del Sur (8°30’ North, 125°50’ East), Surigao del Norte (9°40’ North, 125°38’ East) and Surigao del Sur (8°40’ North, 126°00’ East) are located in north-eastern Mindanao in the Caraga Region (Region XIII). Bukidnon (7°55’ North, 125°05’ East) is in northern Mindanao (Region X). Surra has occurred for more than two decades in the Caraga Region whilst it has never been reported in Bukidnon. The climate and the topography of the provinces in the Caraga Region are similar (detailed in Chapter 6), except that Surigao del Norte has more rugged, mountainous areas. All provinces in the Caraga Region have abundant wooded areas due to timber, coconut and rubber plantations and forests.
A majority of the villages in Surigao del Norte and Surigao del Sur are located along the sea coast. The Agusan Marsh in Agusan del Sur is one of the largest wetlands in the country covering 89,359 hectares of freshwater marshes and watercourses of lakes, rivers and ponds. The climatic and geographical features of Bukidnon are described in Chapter 6.

Figure 5.1 A map of Mindanao showing the five provinces sampled for surra.

5.2.2 Sampling of buffaloes and other livestock

Whole blood and serum were collected from a total of 2,383 buffaloes, 290 goats, 226 cattle, 151 pigs and 35 horses from 73 villages in 27 municipalities from the five provinces. The areas sampled represented 32.5% and 4.6% of the total number of municipalities and villages in the five provinces, respectively, as presented in Table 5.1. A two-stage random sampling strategy (Cameron, 1999; Cameron et al., 2003) was employed. The minimum number of villages (n=91) and buffaloes per village (n=10) required for the study was computed using the Survey Toolbox software version 1.0
(Cameron, 1999; Cameron et al., 2003) assuming: an estimated prevalence of 25% with 5% and 10% variances between and within villages, respectively; a total of 1,558 villages; an average village buffalo population of 109 (Table 5.1); an estimated survey cost of US$300 per village and US$6 per animal; and, a 95% level of confidence. However, only 73 villages were sampled due to the inaccessibility (i.e., rough terrain, heavy rains and security reasons) of some remote villages. To maintain the desired sample size, the number of buffaloes sampled from each village was increased from 10 to an average of 33 buffaloes per village (range: 13-90). Where possible, buffaloes were randomly selected in each village as described in Chapter 6. However, in most cases more buffaloes were sampled than was required per village due to requests from farmers. Thus, the total number of buffaloes sampled (n = 2,383) was 2.6 times more than the minimum number of buffaloes required in the survey (n=910). All other livestock (cattle, pigs, goats, horses) brought to the collection sites were also sampled. Data on age, gender, history of disease, animal movement, husbandry management, reproductive performance and problems including early calf mortality were obtained from each farmer-owner. Each animal was physically examined to determine its overall body condition, identify abnormal clinical signs and if the farmer was uncertain of the animal’s age then age was assessed by dentition as described in Chapter 6.

5.2.3 Packed cell volume

The packed cell volume (PCV) of each animal was determined as described in Chapter 3.
Table 5.1  Population data of buffaloes and other livestock in the provinces surveyed in Mindanao, Philippines.

<table>
<thead>
<tr>
<th>Province</th>
<th>Number of towns (villages)</th>
<th>Estimated buffalo population</th>
<th>Average buffalo population per town (village)</th>
<th>Cattle</th>
<th>Goats</th>
<th>Pigs</th>
<th>Horses‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bukidnon</td>
<td>20 (464)</td>
<td>64,745</td>
<td>3,465 (149)</td>
<td>94,447</td>
<td>377,430</td>
<td>43,485</td>
<td>16,900</td>
</tr>
<tr>
<td>Agusan del Sur</td>
<td>14 (314)</td>
<td>46,142</td>
<td>3,289 (147)</td>
<td>3,410</td>
<td>113,690</td>
<td>33,312</td>
<td>1,320</td>
</tr>
<tr>
<td>Surigao del Sur</td>
<td>18 (309)</td>
<td>25,252</td>
<td>1,417 (83)</td>
<td>1,684</td>
<td>135,180</td>
<td>13,540</td>
<td>31</td>
</tr>
<tr>
<td>Agusan del Norte</td>
<td>11 (166)</td>
<td>22,108</td>
<td>1,359 (90)</td>
<td>7,341</td>
<td>70,560</td>
<td>38,921</td>
<td>33</td>
</tr>
<tr>
<td>Surigao del Norte</td>
<td>20 (335)</td>
<td>14,889</td>
<td>1,116 (67)</td>
<td>2,078</td>
<td>84,640</td>
<td>5,761</td>
<td>52</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>83 (1,588)</strong></td>
<td><strong>173,136</strong></td>
<td><strong>2,086 (109)</strong></td>
<td><strong>108,960</strong></td>
<td><strong>781,500</strong></td>
<td><strong>135,019</strong></td>
<td><strong>18,336</strong></td>
</tr>
</tbody>
</table>

†Data from the Provincial Veterinary Office of each province surveyed

‡Bureau of Agricultural Statistics, 2008

‡‡Mindanao Unified Surra Control Approach (MUSCA) data (J. Alforque, pers. comm., 2007)
5.2.4 Detection of *Trypanosoma evansi*

5.2.4.1 Parasitology

MHCT and MIT were used to detect active *T. evansi* infections as described in Chapter 3. All blood samples from buffalo and other livestock were examined by MHCT whilst 1,333 buffalo and all blood samples from other livestock were each inoculated into mice and examined for propagation of trypanosomes for 30 days. Seven buffaloes infected with *Trypanosoma theileri* were also tested using the MIT.

5.2.4.2 Serology

Each serum sample from the buffaloes and other livestock was tested using the CATT following the standard protocol as described in Chapter 3. The corrected seroprevalence was calculated based on the formula by Rogan and Gladen (1978).

5.2.4.3 Extraction of genomic DNA and amplification *T. evansi* target DNA

Whole genomic DNA was extracted from the blood of each of the 887 buffaloes using the MasterPure™ DNA Purification kit (EPICENTRE® Biotechnologies, USA) and the Prepman Ultra (Applied Biosystems, USA) (Taylor *et al*., 2008) as per the manufacturer’s instructions and frozen at -20°C until used.

The genomic DNA samples were tested for the presence of a *Trypanozoon*-specific gene by PCR using primers (pMURTec F: 5’-GCAGACGACCTGACGCTACT-3’; pMURTec R: 5’-CTCCTAGAAGCTTCGGTG TCCT-3’) designed by Wuyts *et al.* (1994) that produce a 227 bp fragment. The modified protocol by Njiru *et al.* (2001) was followed for DNA amplification except that the DNA template used was 1 µL. The parameters for amplification of the target DNA included an initial pre-incubation step at 94°C for 5 min to denature the DNA, followed by 40 cycles at 94°C for 30 s, 60°C for 30 s and 72°C for 30 s min. DNA extension was allowed to occur at 72°C for 7 min and
the DNA products were kept at 14°C until analysis. The PCR products were analysed as described in Section 5.2.4.

5.2.5 Molecular screening of Mindanao *T. evansi* isolates

5.2.5.1 Sources of *T. evansi* isolates and extraction of genomic DNA

A total of 168 isolates of *T. evansi* (Table 5.2) were collected and individually propagated (Chapter 3) in mice (MIT). Approximately 0.5-1.0 mL of whole blood was collected from the tail-tip or by intra-cardiac puncture from each infected mouse with patent parasitaemia, mixed with EDTA and frozen at -20°C until DNA extraction was performed. Whole genomic DNA was extracted from each thawed blood sample using the MasterPure™ DNA Purification kit (EPICENTRE® Biotechnologies, USA) as per the manufacturer’s instructions. The purified DNA samples were frozen at -20°C until needed.

Table 5.2 The origin of *T. evansi* isolates collected from various animal hosts in Mindanao, Philippines.

<table>
<thead>
<tr>
<th>Province of isolation</th>
<th>No. of villages involved</th>
<th>Animal host/Number of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Buffalo Goat Pig Horse Total</td>
</tr>
<tr>
<td>Agusan del Sur</td>
<td>28</td>
<td>119   2   1   0   122</td>
</tr>
<tr>
<td>Surigao del Sur</td>
<td>11</td>
<td>30    0   0   3   33</td>
</tr>
<tr>
<td>Agusan del Norte</td>
<td>4</td>
<td>9     0   0   1   10</td>
</tr>
<tr>
<td>Surigao del Norte</td>
<td>3</td>
<td>3     0   0   0   3</td>
</tr>
<tr>
<td>Total</td>
<td>46</td>
<td>161   2   1   4   168</td>
</tr>
</tbody>
</table>
5.2.5.2 Amplification of RoTat 1.2 VSG and *Trypanozoon* DNA targets

Purified genomic DNA was tested for the presence of a portion of the *Trypanozoon* genome according to Wuyts *et al.* (1994) as described above. A 488 bp fragment of the gene encoding the RoTat 1.2 VSG was amplified using the PCR protocol described by Urukawa *et al.* (2001) and modified by Ngaira *et al.* (2004) using ILO7957 (5′-GCCACCACGGCGAAAGAC-3′) and ILO8091 (5′-TAATCAGTGTGGGTGC-3′) primers. The PCR was performed in a total reaction volume of 25 µL with 1 µL of purified DNA as template. Target DNA was amplified using an automated thermal cycler (Applied Bio Systems, Singapore) at temperature conditions of 94°C for 30 s, 56°C for 30 s, 72°C for 30 s min in 40 cycles and 72°C for 7 min to extend the product. PCR products were analysed by electrophoresis (Bio-Rad, USA) on a 1.5% agarose gel stained with SYBR® Safe (Invitrogen, USA) and were visualised using ultraviolet (UV) light transillumination (Transilluminator®, Fisher Biotec, Australia). A 100 bp DNA ladder (Promega, USA) was included in each gel as a marker for molecular weight.

5.2.5.3 Confirmation of the PCR products by sequencing

Four bands from the RoTat 1.2 PCR and three bands from the *Trypanozoon* PCR were excised from the agarose gel and purified using the UltraClean™ 15 DNA purification kit (MO BIO Laboratories, Inc., USA). They were amplified using their respective forward primers and sequenced using an ABI Prism™ Terminator Cycle Sequencing kit (Applied Biosystems, USA) on a 3730 DNA Analyser (Applied Biosystems, USA) according to standard protocols of the manufacturer. The nucleotide sequences were checked and edited using the Chromas Lite version 2.01 (Technelysium Propriety Limited, Australia; http://www.technelysium.com.au) and Nucleic Acid Sequence Massager (http://www.attotron.com/cybertory/analysis/seqMassager.htm). The identities of the PCR products were confirmed by comparing their nucleotide sequences.
with known sequences available in the GenBank using the Basic Local Alignment Search Tool (BLAST) (http://www.ncbi.nlm.nih.gov/).

5.2.5.4 **Determination of the sensitivity and specificity of the tests to detect *T. evansi* infection in buffaloes**

Deterministic estimates of the sensitivity and specificity and the 95% binomial confidence interval of each test were calculated as described by Thrusfield (2007). Samples from buffaloes from high-surra risk areas that were positive to either the MCHT or MIT, or to either the MHCT, MIT or PCR were used as “gold standard” references for estimating the sensitivity. Samples from buffaloes from the low-surra risk province that were negative to either the MHCT, MIT or PCR were used as “gold standard” references for estimating the specificity. Likewise, Bayesian methods were attempted to estimate the sensitivity and specificity of the tests but were shown to be unsuitable for the available data.

5.2.6 **Investigating the aetiology of the reproductive problems and early calf mortality in buffalo cows**

A total of 110 buffalo cows from Agusan del Sur, Surigao del Sur and Bukidnon (low surra-risk) with a history of reproductive problems (i.e., abortion, stillbirth, infertility) and at least one calf dying early after birth were identified. Irradiated sera (detailed in Chapter 6) and genomic DNA from blood samples from these animals were tested to detect evidence of infection with *Brucella abortus*, *Neospora caninum* and *T. evansi*.

5.2.6.1 **Sero logical examination for *Brucella abortus***

Eighty-two buffalo serum samples were tested for antibodies against *Brucella abortus* at the Australian Animal Health Laboratory (AAHL) in Geelong, Victoria, Australia
using the indirect antibody-ELISA following the standard diagnostic protocol of the laboratory (Corner, 1987).

5.2.6.2 Serological and molecular detection for *Neospora caninum*

A commercial competitive antibody-ELISA kit (BioK 218; BioX-Diagnostics, Belgium) was used to detect antibodies to *N. caninum*. The test kit has an estimated sensitivity and specificity of 91% and 93%, respectively (Dr. P. Coppe, BioX-Diagnostics, Belgium, pers. comm., 2009). Briefly, 100 µL of diluted (1:20) buffalo and control reference sera were placed into microplate wells pre-coated with recombinant *N. caninum* P38 antigen. The microplate was incubated for two hours at 37°C and washed three times with the wash buffer. A total of 100 µL of horseradish-peroxidase-labelled *N. caninum* P38 conjugate was added to each well and the plate incubated for 30 minutes at 37°C and washed as before. Tetramethyl-benzidine (TMB; 100 µL) was added to each well and the plate incubated for 30 minutes at 37°C. The reaction was stopped with 50 µL 1M phosphoric acid and the absorbance of each well was determined using a microplate reader (Expert 96, ASYS Hitech GmbH-British Biochrom, UK) at 450 nm. Each sample was considered positive if the inhibition percentage was greater than 34% (Dr. P. Coppe, pers. comm., 2009).

The whole genomic DNA extracted from the blood samples of the same buffalo cows was tested for the presence of *N. caninum* using a nested-PCR that produced a 224 bp product according to McInnes *et al.* (2006). The PCR products were analysed and selected bands were sequenced as described in Section 5.2.5.3.
5.2.6.3 Serological and molecular testing for *T. evansi*

Serum samples were tested for antibodies for *T. evansi* using an antibody-ELISA following the methods (Reid and Copeman, 2002; 2003) described in Chapter 6. The molecular detection of *T. evansi* on the extracted genomic DNA from buffalo cows under investigation followed the protocol described in Section 5.2.5.2.

5.2.7 Management and analyses of data

Data were encoded into Excel (Microsoft Inc., USA). The degree of agreement and significant differences between tests were determined using the kappa statistic (κ) and the McNemar’s chi-squared test for paired data (Dawson and Trapp, 2001; Thrusfield, 2007), respectively, calculated using EpiTools (Sergeant, 2009). Prevalence estimates, odds ratios and their 95% binomial confidence intervals stratified by host factors (age, gender and species) and geographical location were calculated. The statistical significance of any difference was determined using Pearson’s chi-square test for independence or two-tailed Fisher’s exact test, respectively (Zar, 1984; Dawson and Trapp, 2001; Thrusfield, 2007). The significant differences in the mean PCVs of each group were determined using Student’s *t* test (pig data) or one-way analysis of variance (ANOVA) with Tukey’s honestly significant difference (Tukey’s HSD) test (Zar, 1984; Dawson and Trapp, 2001; Quinn and Keough, 2002). A statistical significant difference was accepted when the *p* value was less than 0.05. Statistical calculations and data analyses were carried out using either Microsoft Excel, EpiTools or Statistical Package for Social Sciences version 17.0 (SPSS Inc., USA).
5.3 Results

5.3.1.1 Animal-level apparent prevalence of *T. evansi* infection in buffaloes and risk of infection

The apparent prevalence of *T. evansi* infection in buffaloes from the high- and low-surra risk zones in Mindanao, as estimated by parasitological (MHCT/MIT), molecular and serological tests are presented in Table 5.3. The overall apparent prevalence estimates (with 95% CIs) were: 15.7% (14.3, 17.2) by MHCT/MIT; 23.9% (21.2, 26.9) by PCR; and, 41.8% (39.8, 43.8%) by CATT. Results from the MHCT and MIT were used in parallel because MIT was only performed on samples with a PCV <25% and on MHCT-positive samples. The apparent prevalence estimates differed significantly using different tests ($\chi^2$=410.3; $p<0.001$). A significantly higher proportion of samples were positive after testing with CATT than PCR ($\chi^2$=88.5, $p<0.001$), with CATT than MHCT-MIT ($\chi^2$=395.2, $p<0.001$) and with PCR than MHCT/MIT ($\chi^2$=29.6, $p<0.001$).

A higher proportion of buffaloes from the high- compared to the low-surra risk provinces were seropositive using CATT ($\chi^2$=286.0, $p<0.001$). *Trypanosoma evansi* was not detected in any buffaloes from the low-surra risk areas using either MHCT/MIT or PCR.

A significantly higher proportion of buffaloes were positive to *T. evansi* infection in Agusan del Sur compared to buffaloes from Surigao del Sur or Surigao del Norte and in Surigao del Sur or Agusan del Norte compared to Surigao del Norte using MHCT/MIT, CATT or PCR ($p<0.05$). There was no significant difference in the proportion of *T. evansi*-positive buffaloes in Surigao del Sur compared to Agusan del Norte using either of the three tests. A village buffalo from either Agusan del Sur, Surigao del Sur, Agusan del Norte and Surigao del Norte was more likely to be seropositive with *T. evansi* infection compared to a buffalo raised in Bukidnon (ORs
(95% CIs): 97.9 (43.3, 221.2), 60.1 (26.3, 137.2), 56.8 (24.6, 131.0) and 16.1 (6.6, 39.4), respectively).

5.3.1.2 Village-level apparent prevalence of *T. evansi* infection in buffaloes in Mindanao

Overall the village/herd level surra apparent prevalence estimates were: 64.4% (95% CI: 52.5, 75.3%; n=73), 72.7% (95% CI: 59.0, 83.9%; n=55) and 86.3% (95% CI: 76.2, 93.2%; n=73) using MHCT-MIT, PCR and CATT, respectively. In the high-surra risk provinces, 78.3% (95% CI: 65.8, 87.9%) of the villages had at least one buffalo infected with *T. evansi* (MHCT/MIT-positive) whilst 78.4% (95% CI: 64.7, 88.7%) and 96.7% (95% CI: 88.5, 99.6%) of the villages had at least one buffalo that was positive when tested with the molecular and serological tests.

5.3.1.3 Agreement and performance of tests in detecting *T. evansi* infection in buffaloes

Overall, there was substantial agreements between the results from the MHCT and MIT ($\kappa$= 0.76; 95% CI: 0.72, 0.80), MHCT and PCR ($\kappa$= 0.63; 95% CI: 0.57, 0.69) and MIT and PCR ($\kappa$= 0.77; 95% CI: 0.72, 0.82). There was only slight agreements between the results from the MHCT and CATT ($\kappa$= 0.19; 95% CI: 0.16, 0.22), MIT and CATT ($\kappa$= 0.25; CI: 0.21, 0.29) and PCR and CATT ($\kappa$= 0.21; 95% CI: 0.15, 0.27).

The estimated sensitivity and specificity of PCR (78% and 100%; 95% CIs: 72.0, 83.2 and 99.1, 100, respectively) for the detection of *T. evansi* infection in buffaloes were not significantly different from CATT (77.3% and 98.6%; and 95% CIs: 72.7, 81.4 and 96.9, 99.5, respectively) based on MHCT/MIT as “gold standard” ($p>0.05$). Using MHCT/MIT/PCR as “gold standard”, the estimated sensitivity and specificity of CATT were 76.6% (95% CI: 72.2, 80.6) and 98.6% (95% CI: 96.9, 99.5), respectively.
Table 5.3  Apparent prevalence of *T. evansi* infection in buffaloes from high- and low-surra risk areas in Mindanao using MHCT/MIT, CATT and PCR.

<table>
<thead>
<tr>
<th>Surra risk/Province</th>
<th>Number tested (n)</th>
<th>MHCT/MIT</th>
<th>CATT</th>
<th>PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>% positive†</td>
<td>95% CI</td>
<td>% positive</td>
</tr>
<tr>
<td><strong>High risk</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Agusan del Sur</td>
<td>1003</td>
<td>26.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.1, 29.7</td>
<td>58.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Surigao del Sur</td>
<td>473</td>
<td>13.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.8, 17.2</td>
<td>46.7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Agusan del Norte</td>
<td>322</td>
<td>10.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.2, 14.1</td>
<td>45.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Surigao del Norte</td>
<td>168</td>
<td>4.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.7, 8.4</td>
<td>19.0&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Subtotal</strong></td>
<td><strong>1,966</strong></td>
<td><strong>19.0</strong></td>
<td><strong>17.3, 20.8</strong></td>
<td><strong>50.3††</strong></td>
</tr>
<tr>
<td><strong>Low risk</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bukidnon</td>
<td>417</td>
<td>0.0</td>
<td>0.0, 0.9</td>
<td>1.4</td>
</tr>
</tbody>
</table>

†Proportions within a column with different letters are significantly different (p<0.05)

††Significantly higher compared to the proportion in the low-surra risk province (p<0.05)
5.3.1.4 Gender- and age-specific apparent prevalence of *T. evansi* infection in buffaloes

The gender- and age-specific apparent prevalence estimates from the high- and low surra risk areas are shown in Table 5.4. In high-surra risk areas, a significantly higher number of female buffaloes were seropositive for *T. evansi* than male buffaloes ($\chi^2=5.7$, $p=0.017$). There was no significant difference in the prevalence between the two sexes using either MHCT/MIT ($\chi^2=1.3$, $p=0.262$) or PCR ($\chi^2=0.1$, $p=0.760$). Female buffaloes were more likely to be seropositive to *T. evansi* infection than male buffaloes in high-surra risk areas (OR: 1.26 (95% CI: 1.0, 1.5)).

The average age of buffaloes sampled and tested for *T. evansi* was 5.2 years, ranging from one month to 27 years. Buffaloes were classified into three age groups: young: <3 years; mature: 3-6 years; and, old: >6 years of age. *Trypanosoma evansi* was detected in all age groups as early as three months using MIT, PCR and CATT in high-surra risk areas. *Trypanosoma evansi* was not detected in any age group by MHCT/MIT or PCR in the low-surra risk areas where less than 2% were seropositive using CATT in any age group.

In high-surra risk areas, statistical differences were observed in the proportions of buffaloes in each age group that were serologically ($\chi^2=28.0$, $p<0.001$) and parasitologically ($\chi^2=9.2$, $p=0.010$) positive with *T. evansi*. However, there was no statistical difference in the proportion of buffaloes that were PCR-positive in each age group ($\chi^2=1.3$, $p=0.535$). The seroprevalence of *T. evansi* infection was significantly higher in old buffaloes compared to mature and young buffaloes ($\chi^2=27.9$, $p<0.001$, $\chi^2=6.7$, $p=0.009$, respectively) and higher in mature compared to young buffaloes ($\chi^2=9.3$, $p=0.002$). The proportion of MHCT/MIT-positive buffaloes was significantly higher in old compared to young buffaloes ($\chi^2=6.3$, $p=0.012$) and mature buffaloes.
compared to young buffaloes ($\chi^2=4.1$, $p=0.043$). The old and mature buffaloes were more likely to be seropositive with *T. evansi* infection than the young buffaloes (ORs (95% CIs): 1.9 (1.5, 2.3) and 1.4 (1.1, 1.7), respectively). Old and mature buffaloes were more likely to be parasitologically positive with *T. evansi* than the young ones (ORs (95% CIs): 1.5 (1.2, 2.0) and 1.3 (1.0, 1.8), respectively.

### 5.3.1.5 Apparent prevalence of *T. evansi* infection in other livestock in Mindanao

The results of the parasitological and serological examinations for *T. evansi* in cattle, goats, horses and pigs are shown in Tables 5.5 and 5.6. Two cows (aged 3 and 10 years) of the 226 cattle examined (0.9%) from two high-surra risk villages were shown to be infected with *T. evansi* (MHCT and MIT positive). Both were in poor body condition, anaemic and the 10-year old cow had a calf that died early after calving. Nine of the 290 goats (3.1%) and two of the 151 pigs (1.3%) sampled were shown to be infected with *T. evansi* using the MHCT and MIT. Four of 10 horses examined in Banahao, Lianga, Surigao del Sur were infected with *T. evansi*. One horse had been treated with diminazene two weeks prior to sampling. All four parasitologically positive horses were showing poor body condition, conjunctivitis, weakness and testicular enlargement (in one stallion).
Table 5.4  Gender- and age-specific apparent prevalence estimates of *T. evansi* infection in buffaloes in Mindanao using MHCT/MIT, CATT and PCR.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Surra risk/Specific class</th>
<th>Number of buffaloes tested (n)</th>
<th>MHCT/MIT</th>
<th>CATT</th>
<th>PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>% positive</td>
<td>95% CI</td>
<td>% positive</td>
</tr>
<tr>
<td>Gender</td>
<td>High risk</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>1310</td>
<td>18.3</td>
<td>16.3, 20.5</td>
<td>52.2*</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>656</td>
<td>20.4</td>
<td>17.4, 23.7</td>
<td>46.5</td>
</tr>
<tr>
<td></td>
<td>Low risk</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>304</td>
<td>0.0</td>
<td>0.0, 1.2</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>113</td>
<td>0.0</td>
<td>0.0, 3.2</td>
<td>1.8</td>
</tr>
<tr>
<td>Age</td>
<td>High risk</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Young</td>
<td>561</td>
<td>15.5*</td>
<td>12.6, 18.8</td>
<td>42.1*</td>
</tr>
<tr>
<td></td>
<td>Mature</td>
<td>782</td>
<td>19.8</td>
<td>17.1, 22.8</td>
<td>50.5</td>
</tr>
<tr>
<td></td>
<td>Old</td>
<td>623</td>
<td>21.2</td>
<td>18.0, 24.6</td>
<td>57.5</td>
</tr>
<tr>
<td></td>
<td>Low risk</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Young</td>
<td>140</td>
<td>0.0</td>
<td>0.0, 2.6</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>Mature</td>
<td>171</td>
<td>0.0</td>
<td>0.0, 2.1</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>Old</td>
<td>106</td>
<td>0.0</td>
<td>0.0, 3.4</td>
<td>1.9</td>
</tr>
</tbody>
</table>

*Significantly different (p<0.05)
5.3.1.6 Comparison of prevalence and risk of *T. evansi* infection in buffaloes and other livestock in high-sura risk provinces in Mindanao

The parasitological prevalence of *T. evansi* infection in buffaloes was higher compared to goats, cattle or pigs (*p*<0.05) but did not differ compared to horses (*p*=0.697) (Figure 5.2). The proportion of horses with detectable parasitaemia was higher compared to goats, cattle or pigs (*p*<0.05). There were no significant differences in the proportion of parasitaemic cattle, goats and pigs (*p*>0.05). The corrected seroprevalence of *T. evansi* infection was higher in buffaloes compared to goats, cattle or pigs (*p*<0.05). There was no significant difference between the seroprevalence in buffalo compared to horses and in the seroprevalence in cattle, goats and pigs (*p*>0.05). Buffaloes, horses, goats and cattle were more likely to be MHCT-MIT positive (ORs (95% CIs): 17.6 (4.3, 71.2), 14.2 (24.0, 82.3), 2.7 (0.6, 12.5) and 1.1 (0.1, 7.7), respectively) and more likely to be seropositive with *T. evansi* than pigs in high-sura risk areas (ORs (95% CIs): 3.2 (2.2, 4.8), 1.8 (0.7, 4.4), 1.5 (0.9, 2.3) and 1.8 (1.1, 3.1), respectively.

5.3.1.7 Packed cell volume (PCV) of *T. evansi*-infected and uninfected buffaloes, cattle, goats and pigs in Mindanao

The mean PCVs (±SEM) of buffaloes and of horses, cattle and goats from the high- and low-sura risk provinces in Mindanao grouped according to the results of each diagnostic test are presented in Figures 5.3 and 5.4, respectively. There were significant differences in the mean PCVs of buffaloes (*F*$_{4,2290}$=198.0, *p*<0.001). All buffaloes that were positive using MHCT/MIT and PCR (D), using all tests (E) and using only the CATT (C) had mean PCV values below 25% and were significantly lower compared to the seropositive and uninfected buffaloes (Figure 5.3). There were also significant differences in the mean PCVs of horses (*F*$_{3,31}$=7.3, *p*<0.001), cattle (*F*$_{2,220}$ =21.8, *p*<0.001) and goats (*F*$_{3,285}$=131.1, *p*<0.001) (Figure 5.4). The mean PCVs of horses and goats that were parasitologically positive to *T. evansi* using MHCT/MIT were
significantly lower compared to the seropositive and seronegative animals ($p<0.05$). Seropositive cattle had significantly lower mean PCVs compared to seronegative cattle ($p<0.05$). There was no significant difference in the mean PCVs between seropositive and seronegative pigs ($29.8\pm0.9$ vs. $30.1\pm0.3$ % SEM; Student’s $t$ test, $df=149$, $p=0.684$). The parasitologically positive cattle ($n=2$; 21.5% mean PCV) and pigs ($n=2$; 30% mean PCV) were excluded in the statistical analysis.
<table>
<thead>
<tr>
<th>Surra risk/ Province</th>
<th>Cattle</th>
<th></th>
<th></th>
<th></th>
<th>Goats</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n tested</td>
<td>MHCT/MIT</td>
<td>CATT</td>
<td>n tested</td>
<td>MHCT/MIT</td>
<td>CATT</td>
<td>n tested</td>
<td>MHCT/MIT</td>
</tr>
<tr>
<td></td>
<td>% pos</td>
<td>95% CI</td>
<td>% pos</td>
<td>95% CI</td>
<td>% pos</td>
<td>95% CI</td>
<td>% pos</td>
<td>95% CI</td>
</tr>
<tr>
<td>High risk</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Agusan del Sur</td>
<td>88</td>
<td>1.1</td>
<td>0.0, 6.2</td>
<td>42.0</td>
<td>31.6, 53.0</td>
<td>217</td>
<td>3.7</td>
<td>1.6, 7.1</td>
</tr>
<tr>
<td>Surigao del Sur</td>
<td>23</td>
<td>0.0</td>
<td>0.0, 14.8</td>
<td>30.4</td>
<td>13.2, 52.9</td>
<td>8</td>
<td>0</td>
<td>0.0, 36.9</td>
</tr>
<tr>
<td>Agusan del Norte</td>
<td>31</td>
<td>3.2</td>
<td>0.1, 16.7</td>
<td>25.8</td>
<td>11.9, 44.6</td>
<td>35</td>
<td>2.9</td>
<td>0.1, 14.9</td>
</tr>
<tr>
<td>Subtotal</td>
<td>142</td>
<td>1.4</td>
<td>0.2, 5.0</td>
<td>36.6</td>
<td>28.7, 45.1</td>
<td>35</td>
<td>3.5</td>
<td>1.6, 6.5</td>
</tr>
<tr>
<td>Low risk</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bukidnon</td>
<td>84</td>
<td>0.0</td>
<td>0.0, 4.3</td>
<td>3.6</td>
<td>0.7, 10.1</td>
<td>30</td>
<td>0</td>
<td>0.0, 3.6</td>
</tr>
</tbody>
</table>

Table 5.5 Test estimates of the prevalence of *T. evansi* infection in cattle and goats from the high- and low-surra risk provinces in Mindanao.
Table 5.6  Test estimates of the prevalence of *T. evansi* infection in horses and pigs from the high- and low-surra risk provinces in Mindanao.

<table>
<thead>
<tr>
<th>Surra risk/Province</th>
<th>Horses</th>
<th>Pigs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n tested</td>
<td>MHCT/MIT</td>
</tr>
<tr>
<td></td>
<td>% pos</td>
<td>95% CI</td>
</tr>
<tr>
<td>High risk</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Agusan del Sur</td>
<td>6</td>
<td>0.0</td>
</tr>
<tr>
<td>Surigao del Sur</td>
<td>14</td>
<td>28.6</td>
</tr>
<tr>
<td>Agusan del Norte</td>
<td>5</td>
<td>0.0</td>
</tr>
<tr>
<td><strong>Subtotal</strong></td>
<td>25</td>
<td>16.0</td>
</tr>
<tr>
<td>Low risk</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bukidnon</td>
<td>10</td>
<td>0.0</td>
</tr>
</tbody>
</table>

NS- no samples
Figure 5.2  Estimated prevalence and corrected seroprevalence (95% CIs in error bars) of surra in buffaloes and other livestock from the high-surra risk provinces in Mindanao, Philippines.

Figure 5.3  Mean packed cell volume (PCV, ±SEM) values of buffaloes from the high- and low-surra risk provinces grouped according to test results (A= low risk, negative; B= high risk negative; C= CATT positive, MHCT/MIT & PCR negative; D= MHCT/MIT & PCR positive; E= all tests positive; *means with different letters are significantly different, p<0.05).
Figure 5.4 Mean packed cell volume (PCV, ± SEM) values of non-buffalo livestock from the high- and low-surra risk provinces infected or uninfected with *T. evansi* (*Means within each species with different letters are significantly different, p<0.05); ‡ excluded in the analysis due to limited number of cases).

5.3.1.8 Apparent clinical signs in buffaloes and other livestock with surra

Eighty-two percent and 67% of buffaloes that were parasitologically positive based on MHCT/MIT and PCR tests (n=410) or seropositive (n= 989), respectively, were in poor body condition (Figure 5.5). All (n=2) and 60% (n=52) of the parasitologically positive or seropositive cattle, respectively were in poor body condition (Figure 5.6). Fifty-seven percent (n=9) and 23% (n=82) of the parasitologically positive or seropositive goats were in poor body condition, respectively. Seventy-five percent and 44% of the horses that were parasitologically positive (n=4) or seropositive (n=9) were in poor body condition, respectively. Only 14% (n=35) of the CATT-positive pigs were in poor body condition whilst two parasitologically positive pigs were apparently healthy.
Other common apparent signs that were observed amongst buffaloes and other ruminants (i.e., cattle and goats) with surra in Mindanao included: anaemia (pale mucous membranes), enlargement of superficial lymph nodes, recumbency, anorexia, coughing, diarrhoea, reduced tolerance to work (for draught animals), urticarial plaques and small wounds due to tabanid bites, and alopecia (i.e., tail, ears or/and body). Other signs such as circling, inflammation of the joints, laboured breathing, sudden death, testicular enlargement, abortion and death of newborn calves/kids were also reported by farmers. In horses, clinical signs included weight loss, anaemia, ventral abdominal oedema, conjunctivitis with probable blindness, weakness, recumbency, testicular enlargement and death (Figure 5. 7).

Figure 5.5  A buffalo with *T. evansi* infection from Agusan del Sur, Mindanao.
Figure 5.6  A cow infected with *T. evansi* from Agusan del Sur, Mindanao.

Figure 5.7  A typical case of surra in a horse from Agusan del Sur, Mindanao showing ventral abdominal oedema and enlargement of testicles (inset).
5.3.2 Molecular detection of RoTat 1.2 gene in isolates of *T. evansi* from Mindanao

All 168 isolates of *T. evansi* collected from Mindanao were positive to *Trypanozoon-* and the RoTat 1.2-gene PCRs (Figure 5.8). There was 100% homology between the representative nucleotide sequences of both PCR products and the published sequences for RoTat 1.2 (Figure 5.9) and *Trypanozoon* genes.

5.3.3 Investigation of the aetiology of reproductive problems and early calf mortality in buffalo cows from high- and low-surra risk zones in Mindanao

A total of 110 cases of buffalo cows with a history of reproductive problems and neonatal mortality from the high- (106) and low- (4) surra risk areas were investigated. The majority of these buffalo cows were in poor body condition (75.5% (95% CI: 66.3, 83.2), $\chi^2=58.0, p<0.001$) at the time of sampling. Of the buffalo cows with a history of abortion (n=58), 87% aborted once, 10% aborted twice or thrice and 3% aborted four or five times during the last trimester of pregnancy. One buffalo cow had both aborted and had three neonatal deaths. Buffalo cows with a history of neonatal mortality (n=38), had either one (81.6%), two (15.8%) or three (2.6%) buffalo calf deaths. Deaths of buffalo calves occurred as early as three days after parturition.

![Figure 5.8](image-url) RoTat 1.2 PCR products (480 bp) of *Trypanosoma evansi* isolates from Agusan del Sur (lanes 1-4), Surigao del Sur (lanes 5-8) and Surigao del Norte (lanes 9-10), and the positive control (lane 11) on 1.5% agarose gel stained with SYBR® Safe DNA stain (Invitrogen, USA).
Figure 5.9  Nucleotide sequence alignment of one *T. evansi* isolate from Mindanao with a published *Trypanosoma evansi* RoTat1.2 variable surface glycoprotein sequence (Accession code: gb|AF317914.1|AF317914).

*Trypanosoma evansi* was detected in 34% of the cases using MHCT-MIT (Table 5.7). A significantly higher proportion of buffalo cows were seropositive and PCR-positive for *T. evansi* compared to *N. caninum* ($\chi^2$=39.1, $p<0.001$, $\chi^2$=21.2, $p<0.001$, respectively). A higher proportion of cows with a history of abortion/stillbirth were seropositive for *T. evansi* compared to *N. caninum* ($p<0.05$). *Trypanosoma evansi* was detected by PCR in a higher proportion of buffalo cows with abortion/stillbirth (37.2 vs. 16.3%; $\chi^2$=4.8, $p=0.028$), infertility (87.5 vs. 12.5%; $\chi^2$=9.0, $p=0.003$) and early calf mortality (48.4 vs. 9.7%; $\chi^2$=11.3, $p=0.001$) compared to *N. caninum*. No buffaloes were tested positive to *B. abortus*. 
Table 5.7  Diagnostic examination of samples from buffalo cows from high- and low-surra risks areas in Mindanao with a history of reproductive problems and early calf death.

<table>
<thead>
<tr>
<th>Surra status/Problem</th>
<th>Number of cases</th>
<th>MHCT/MIT-\textit{T. evansi}</th>
<th>\textit{T. evansi}-ELISA</th>
<th>\textit{N. caninum}-ELISA</th>
<th>\textit{T. evansi}-PCR</th>
<th>\textit{N. caninum}-PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>% pos (95% CI)</td>
<td>n</td>
<td>% pos (95% CI)</td>
<td>n</td>
<td>% pos (95% CI)</td>
</tr>
<tr>
<td>High risk††</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abortion/stillbirth</td>
<td>58</td>
<td>58</td>
<td>36.2 (24.0, 49.9)</td>
<td>58</td>
<td>55.2(a) (41.5, 68.3)</td>
<td>48</td>
</tr>
<tr>
<td>Infertility</td>
<td>10</td>
<td>10</td>
<td>40.0 (12.2, 73.8)</td>
<td>10</td>
<td>90.0(a) (55.5, 99.7)</td>
<td>8</td>
</tr>
<tr>
<td>Neonatal death</td>
<td>38</td>
<td>38</td>
<td>28.9 (15.4, 45.9)</td>
<td>38</td>
<td>78.9(a) (62.7, 90.4)</td>
<td>29</td>
</tr>
<tr>
<td>Subtotal</td>
<td>106</td>
<td>106</td>
<td>34.0 (25.0, 43.8)</td>
<td>106</td>
<td>67.0(a) (57.2, 75.8)</td>
<td>85</td>
</tr>
<tr>
<td>Low risk</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abortion/stillbirth</td>
<td>3</td>
<td>3</td>
<td>0.0 (0.0, 70.8)</td>
<td>3</td>
<td>0.0 (0.0, 70.8)</td>
<td>3</td>
</tr>
<tr>
<td>Neonatal death</td>
<td>1</td>
<td>1</td>
<td>0.0 (0.0, 97.5)</td>
<td>1</td>
<td>0.0 (0.0, 97.5)</td>
<td>1</td>
</tr>
<tr>
<td>Subtotal</td>
<td>4</td>
<td>4</td>
<td>0.0 (0.0, 60.2)</td>
<td>4</td>
<td>0.0 (0.0, 60.2)</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>110</td>
<td>110</td>
<td>32.7 (24.1, 42.3)</td>
<td>110</td>
<td>64.5(a) (54.9, 73.4)</td>
<td>89</td>
</tr>
</tbody>
</table>

\(††\) Proportions within a row with different superscripts are significantly different (\(p<0.05\))
5.4 Discussion

The present study confirms previous observations (Chapter 3) that *T. evansi* infection (surra) is prevalent in livestock in the low-altitude, high-surra risk zones in Mindanao. In addition, the absence of *T. evansi* in the animals sampled in the current survey in the high-altitude, low-surra risk province of Bukidnon is a continuing evidence of the area’s freedom from surra. The difference observed between these two zones is probably attributed to the high tabanid population in the high-surra risk areas and the low population or absence of tabanids in high-altitude areas. During the present survey, farmers in the high-surra risk provinces noted the high abundance of tabanids biting both humans and animals, whilst farmers in Bukidnon (low-risk) never identified tabanids as a problem. In addition, a one-year tabanid trapping study (Chapter 7) using unbaited Nzi traps in these two zones has confirmed the observations of the farmers. It is widely accepted that the haematophagous tabanid flies are the main transmitters of *T. evansi* in surra-endemic regions (Luckins, 1988; Lun *et al*., 1993; Enwezor and Sackey, 2005) including the Philippines (Mitzmain, 1913a; b; 1914; Manresa and Mondoñedo, 1935; Yutuc, 1949). Most of the tabanid flies prefer low-lying, swampy and wooded habitats, which are the features of the high-surra risk areas in Mindanao, as their larvae could be aquatic, semi-aquatic or terrestrial (Service, 1986; Strother, 1999). Likewise, it is the general observation that the presence of surra in an area is associated with the presence of these large biting flies (Dirie *et al*., 1989; Dia *et al*., 1997), and the increase on their activity had been linked to the upsurge of high surra cases or epidemics (Mahmoud and Gray, 1980; Löhr *et al*., 1985; Tuntasuvan *et al*., 1997; Barros, 2001; Reid, 2002).

The movement of infected animals within and across geographical zones is the main cause of the spread of surra within Asia and in most countries where the disease is
present (Losos, 1980; Wells, 1984; Luckins, 1988; Reid, 2002; Tamarit et al., 2010). In Mindanao, and in most provinces in the Philippines, this uncontrolled movement of animals may be related to: animal dispersal projects, human transmigration, trading for food and replacement stock, work-related activities that involve draught animals, movement of breeders, entertainment purposes and indigenous rituals. Amongst these, the dispersal of livestock (buffaloes, cattle, goats) by the government and some private organizations to small-hold farmers has been identified as a significant contributor to the spread of surra in most provinces in Mindanao (Dr. R. Mercado, pers. comm., 2007).

The preferred diet particularly in terms of meat of the local inhabitants in different areas also influences the movement of animals in Mindanao. For example, the local inhabitants in Surigao del Sur and Surigao del Norte prefer to consume buffalo meat especially during festivities (Dr. P. Calo, F. Oralisa, pers. comm., 2007). They often import buffaloes from neighbouring provinces like Agusan del Sur, Compostela Valley and as far as South Cotabato where surra is endemic (Mercado et al., 2004; Dargantes et al., 2009). Data from the interviews conducted with farmers show that they purchase replacement stock from other villages within or in another province without knowing the status of the animals with regard to *T. evansi*. This is likely because animals infected with *T. evansi* may not show clinical signs of surra (Payne et al., 1991b; Holland et al., 2004). In addition, the common practice of using buffaloes, cattle or horses to carry farm produce to common markets or to processing industries (i.e., for rice, corn or coffee) facilitates transmission of *T. evansi* between hosts. Such scenario was exemplified during the 2008 outbreak in Agusan del Norte where 108 animals died (50 buffaloes, 31 horses, 15 cattle and 8 goats) (Dr. J. Dargantes, pers. comm., 2008). The source of the outbreak was believed to be a buffalo used to carry corn products to a
milling station in another town, where it probably became infected with *T. evansi* from a subclinically infected animal. Similarly, the introduction of infected horses into Madura island in Indonesia from East Java initiated a severe outbreak of surra in local livestock (Payne *et al.*, 1990). Within the villages sampled the common practice of tethering animals in one common pasture along irrigation canals, vacant farm lots, newly-harvested crop plantations, along the river banks or allowing them to wallow (for buffaloes) in a common pool enhances the risk of transmission of *T. evansi*. Barros and Foil (2007) suggested that tabanids would not travel more than 50 metres to re-initiate feeding. Therefore, if tethering in a common grazing ground can not be avoided, they suggest that animals should be kept separated from each other as far as 200 metres apart (Foil, 1983; Barros and Foil, 2007), which would prevent tabanids moving between hosts and the transmission of *T. evansi* by tabanids.

Water buffaloes showed the highest infection percentage with *T. evansi*, and the presence of clinical signs (i.e., poor body condition, anaemia, conjunctivitis, etc.). The apparent prevalence and seroprevalence estimates in buffaloes in the present survey are higher compared to the results of previous surveys in the Philippines. In other countries, the prevalence of *T. evansi* infection in buffaloes is lower: <1% to 2% by MIT, 6% by PCR, 22.3% by ELISA and 36% by CATT in separate surveys in Vietnam (My *et al.*, 1998b; Verloo *et al.*, 2000; Holland *et al.*, 2004); 1.4% by MHCT and 48% by CATT in Indonesia (Payne *et al.*, 1991b); and, 5% by MHCT/MIT/GSS and 17% by ELISA in Thailand (Löhr *et al.*, 1985).

The presence of clinical disease in a number of *T. evansi*-positive buffaloes in Mindanao concurs with the observations made by Löhr *et al.* (1985) in Thailand but contradicts the findings in Indonesia and Vietnam (Payne *et al.*, 1991b; Davison *et al.*, 2007).
Interestingly, buffaloes with clinical surra were mostly observed in areas where recent surra outbreaks had occurred. In Thailand, the buffaloes that showed clinical surra were also mostly from outbreak villages (Löhr et al., 1985). The reasons for the variations in the epidemiology of surra in buffaloes in different geographical locations are unknown but could probably be due to the differences in: the pathogenicity of *T. evansi* isolates, husbandry practices amongst countries, concurrent infections, immunity and the level of stress as draught animals (Löhr et al., 1985; Luckins, 1998a; My et al., 1998a; Reid, 2002). Whilst tolerance to trypanosomosis has been documented in some domestic animals in Africa (Goossens et al., 1997a; Hill et al., 2005; Naessens, 2006; Geerts et al., 2009), this phenomenon has not been observed in buffaloes in Asian countries. Nevertheless, the pathogenicity of *T. evansi* isolates may vary within and across regions (Dia, 1995; Queiroz et al., 2000; De Menezes et al., 2004). Indeed, unpublished data show that mice infected with isolates of *T. evansi* from Mindanao had significantly reduced survival compared to mice infected with Indonesian isolates (Reid and Husein, unpublished; Dargantes et al. unpublished).

The role of other species of large livestock (horses, cattle) in the epidemiology of surra varies. Surra is widely considered as highly fatal to horses (Monzon et al., 1995a; Silva et al., 1995; Seidl et al., 2001), whereas cattle are considered as reservoir hosts because they are mildly affected (Mahmoud and Gray, 1980). The high susceptibility of horses to *T. evansi* infection in the high-surra risk villages in Mindanao is evidenced by the small number of horses available for sampling in the present study. Records obtained in the field show that up to 70% of horses in the villages that have experienced outbreaks of surra have died (Dr. M. Samar, Dr. J. Dargantes, Dr. P. Calo, Dr. G. Yparraguirre, Dr. A. Gonzales, pers. comm., 2007-2009). The results of this study suggest that cattle are more resilient to *T. evansi* infection compared to horses and buffaloes. This is
because a high seroprevalence was observed with only few cattle showing patent parasitaemia during the surveys, which agrees with the findings of Payne et al. (1991a) in Indonesia. However, mortality (as high as 20%) amongst cattle has also been observed in the high-surma risk provinces (Dr. M. Samar, Dr. J. Dargantes, Dr. P. Calo, pers. comm., 2007-2009). High mortality in cattle due to surra has also been reported in Thailand and Indonesia (Payne et al., 1988; Payne et al., 1990; Tuntasuvan et al., 1997).

The detection of *T. evansi* in goats (approximately as many as 200 to more than 500 trypanosomes per capillary tube by MHCT in two goats) in the present study demonstrates the possible importance of small ruminants in the dynamics of *T. evansi* infection in Mindanao. This is interesting because the role of small ruminants in the epidemiology of surra is unclear. Some reports suggest that small livestock do not contribute to the transmission of trypanosomes (Griffin, 1978; Abo-Shehada et al., 1999; Simukoko et al., 2007). However, the susceptibility of goats and sheep (Ngeranwa et al., 1993; Manuel, 1998; Audu et al., 1999; Sharma et al., 2000a) and pigs to *T. evansi* in experimental trials and in field outbreaks (Batolos and Somoray, 1989; Sirivan et al., 1989; Arunasalam et al., 1995; Holland et al., 2003; Holland et al., 2005) suggest otherwise. Indeed, several surveys have reported infection with *T. evansi* in these species (Boid et al., 1981; Singh, 1998; Reid et al., 1999; Herrera et al., 2005; Holland et al., 2005). Mortality due to surra has also been reported in goats in Mindanao (Dr. P. Calo, Dr. J. Dargantes, pers. comm., 2008). In addition, the high susceptibility of goats to a Mindanao isolate of *T. evansi* has been proven experimentally (Dargantes et al., 2005a; Dargantes et al., 2005b). Epidemiologically, small ruminants have been regarded as important sources of pathogenic trypanosomes for other animals in Africa (Osaer et al., 1999; Kalu et al., 2001; Dinka and Abebe,
and as potential reservoir hosts for *T. vivax* in South America (Batista *et al.*, 2009). In addition, goats together with pigs are considered potential reservoir hosts of human African trypanosomosis (Njiru *et al.*, 2004c; Musa *et al.*, 2005; Simo *et al.*, 2008).

The observation that pigs were infected with *T. evansi* is also interesting because there are few reports of clinical disease or published surveys to determine the prevalence in domestic populations. In a survey in Thailand, Holland *et al.* (2005) showed that 2.6 and 36% of pigs owned by small-scale farmers were infected with *T. evansi* using MHCT and antibody-ELISA, respectively, which are comparable to the figures obtained in this study. In addition, other pathogenic trypanosomes have been detected amongst apparently healthy pigs in Africa (Njiru *et al.*, 2004c; Musa *et al.*, 2005) and wild pigs have been incriminated as maintenance hosts of both *T. evansi* and *T. cruzi* in Brazil (Herrera *et al.*, 2008). Furthermore, pigs are resilient to *T. evansi* infection as evidenced by mild clinical signs during experimental infections (Reid *et al.*, 1999; Holland *et al.*, 2003). Muzari *et al.* (2010) recently demonstrated that pigs do not exhibit strong defensive responses to the bite from tabanid flies but that their thick skin extends the time taken to complete a blood meal (Muzari *et al.*, 2010). This suggests that tabanid behaviour, and the subsequent risk of transmission of *T. evansi*, is likely to differ when they feed on pigs (Muzari *et al.*, 2010). However, the results of the molecular analysis on tabanids captured in the Caraga Region suggest that tabanids do regularly feed on pigs (Chapter 7). This is important in the context of Mindanao because of the common practice of tethering pigs close to other animals (e.g., buffaloes) or allowing free range to forage.
The prevalence of *T. evansi* infection is associated with age indicating that the risk of *T. evansi* infection in buffaloes increases with age. This observation corroborates with findings of *T. evansi* infection in buffaloes and cattle in Indonesia (Davison *et al.*, 2000), and in camels in Chad, Kenya and Canary Islands in Spain (Gutierrez *et al.*, 2000; Delafosse and Doutoum, 2004; Njiru *et al.*, 2004a). This is most likely due to the chronic nature of the infection and the longer exposure of adult buffaloes over time. In addition, it may also partly reflect local management practices. In the Philippines, buffaloes are only used for draught when they are mature and after proper training, which commences when they are about two to three years of age (The 2002 Carabao Production Committee, 2003). This means that younger animals are likely to spend more of their time near buildings and shelters where tabanid activity and animal density are lower. In addition, tabanids are attracted to bigger rather than smaller objects (Phelps and Vale, 1976; Gibson and Torr, 1999). Old animals may also exhibit lessened defensive responses against tabanid attacks than young animals. Indeed, a higher number of tabanids were observed to attack an adult compared to a young cattle (Konstantinov and Ul’ianov, 1988; Mohamed-Ahmed and Mihok, 2009) and Foil *et al.* (1985) also reported that more tabanids are likely to feed on a mare than a foal.

Results of the parasitological (mouse inoculation), serological and the molecular analyses of 168 isolates of *T. evansi* from Mindanao have confirmed that *T. evansi* is the only pathogenic trypanosome infecting livestock in southern Philippines. The other trypanosome present in the island is *T. theileri*, which has been detected in a small proportion of buffaloes (0.3%; 7/2,383) and cattle (0.4%; 1/226) by MHCT and in trapped tabanids that were examined using molecular techniques (Chapter 7). *Trypanosoma theileri* is a common parasite of buffaloes and cattle in Asia (Samad and Shahidullah, 1985; Siswansyah and Tarmudji, 1989) and in other countries worldwide.
(Farrar and Klei, 1990; Greco et al., 2000; Rodrigues et al., 2003). It is generally regarded to be non-pathogenic (Bose et al., 1987) although it has been detected in a small number of cases in cattle showing clinical disease (Doherty et al., 1993; Seifi, 1995).

The detection of the gene encoding RoTat 1.2 VSG in all isolates of *T. evansi* from Mindanao examined in this study agrees with previous observations that the VSG of RoTat 1.2 variable antigen type (VAT) is thought to be present amongst all *T. evansi* isolates worldwide (Verloo et al., 2001). These findings support the suitability of the CATT and other RoTat 1.2-based tests (i.e., PCR, ELISA) for the diagnosis of *T. evansi* infection in the Philippines. However, further evaluation is required for the use of the CATT (and probably RoTaT 1.2-based ELISA) in Mindanao because it is unknown whether RoTat 1.2 gene is expressed by all local isolates, and at what stage of infection it is expressed by the isolates which could also be detected by the host’s immune system. In fact, 15% of the isolates screened for RoTat 1.2 VSG gene were collected from CATT-negative buffaloes, and that 23% of all parasitologically positive buffaloes (n=374) in the present survey were CATT-negative. The most likely causes of the false negative results are that these animals were only recently infected with *T. evansi* and that there was insufficient time for development of an antibody response, or, that the RoTat 1.2 VSG had not yet been expressed in the infecting trypanosome population (Ngaira et al., 2004). Previous studies suggest that the former is the most likely explanation. For instance, the average sensitivity of the CATT was only 63% (range: 22-89%) within the first seven weeks after experimental infection of 18 buffaloes (Holland et al., 2002). Holland et al. (2002) found that the CATT was only able to identify all infected buffalo eight weeks after infection. Similarly, antibodies to RoTat 1.2 VSG were not detected in rabbits infected with Chinese and Philippine isolates,
during the first wave of parasitaemia or early stage of infection occurring within a month after experimental infection with *T. evansi* (Verloo et al., 2001). This may also explain the slight agreement between the results of the CATT and parasitological tests (MHCT or MIT), and the CATT and PCR in detecting *T. evansi* infection in buffaloes. Nevertheless, the diagnostic sensitivity and specificity estimates of CATT for buffaloes in this study are comparable with the results of Reid and Copeman (2003) who estimated that the sensitivity and specificity of the CATT for the detection of *T. evansi* in cattle in Indonesia was 83% (95% CI: 76, 88) and 96% (95% CI: 82, 98), respectively. This is higher than the estimates obtained when CATT was used to detect *T. evansi* in camels in Kenya (69%) (Ngaira et al., 2003; Njiru et al., 2004a). The lower sensitivity of CATT in Kenyan camels may also be partly due to the presence of *T. evansi* isolates that do not possess the RoTat 1.2 gene (Ngaira et al., 2003; Ngaira et al., 2004).

The PCR is considered to be highly sensitive in detecting *T. evansi* infection (Wuyts et al., 1994; Holland et al., 2001a; Davila et al., 2003; Njiru et al., 2004a; Fernández et al., 2009). The sensitivity of the PCR is dependent to some extent on the sequence of the primers used and the number of repeated nucleotide sequences in the target genome of the trypanosome (Desquesnes and Davila, 2002; Pruvot et al., 2010). Recent evaluation of the diagnostic performance of PCR using different primer sets (Pruvot et al., 2010) revealed that the sensitivity of some of the primers including pMURTec primers (Wuyts et al., 1994) is only between 100 to 1,000 trypanosomes mL\(^{-1}\) in rat blood. This is similar to the MHCT which detects 31 to 250 trypanosomes mL\(^{-1}\) in cattle blood (Reid et al., 2001a) but less than the MIT, which can detect three trypanosomes mL\(^{-1}\) of blood (Reid et al., 2001a). The performance of the PCR protocol used in this study could have been adversely affected by degradation of
trypanosomal DNA during the storage of blood samples after collection. However, the relative proportion of buffaloes that were positive using the PCR compared to MIT/MHCT is similar to previous studies (Pruvot et al., 2010).

The clinical signs observed and reported by farmers during this study are in agreement with observations from experimental and natural infections with *T. evansi* in buffaloes (Löhr et al., 1985; Damayanti et al., 1994; Hilali et al., 2006), cattle (Payne et al., 1992; Tuntasuvan et al., 1997; Manuel, 1998), horses (Mahmoud and Gray, 1980; Silva et al., 1995; Seidl et al., 2001; Laha and Sasmal, 2008) and goats (De Villa et al., 1991; Ngeranwa et al., 1993; Dargantes et al., 2005b). The absence of apparent clinical signs in parasitologically positive pigs is in agreement with the findings of other studies (Reid et al., 1999; Herrera et al., 2005). The observed clinical signs are not pathognomonic of surra. However, in high-surra endemic areas, the combination of poor body condition, anaemia, reduced work tolerance in draught animals, poor appetite, testicular swelling, abortion, early calf death, infertility, coughing, diarrhoea and alopecia is likely to be a fairly accurate case definition for purposes of control and treatment. In addition, high mortality in horses and considerable deaths in ruminants would also be indicative of surra.

The results of the investigation into poor reproductive performance in buffalo provides good evidence that it is associated with *T. evansi* infection in high-surra risk areas. Whilst a number of other pathogens may cause reproductive problems in buffaloes there is no reason to expect that their prevalence would be sufficiently different to account for the observations made in this study. These results support earlier observations in surra-endemic areas of Mindanao (Chapter 3) (Dargantes et al., 2009) and other endemic countries (Löhr et al., 1986; Payne et al., 1993; My et al., 1998a; Jittapalapong et al.,
These reproductive losses have a significant impact on buffalo production and on the livelihood of marginalised buffalo owners in Mindanao who depend much on their animals for livelihood (Chapters 3 and 4). The negative impact of surra on buffalo reproduction may also affect the government-initiated genetic programme to improve buffalo production in Mindanao through crossbreeding with selected imported Murrah and Bulgarian buffalo stocks (Dr. E. Paraguas, Philippine Carabao Centre, pers. comm., 2008).

The detection of *N. caninum* in an aborting buffalo from the low-surra risk area and amongst some aborting buffaloes from the high-surra risk areas merits further study. *Neospora caninum* is a protozoan parasite of companion and livestock (Dubey and Lindsay, 1996; Guarino *et al.*, 2000; Serrano-Martínez *et al.*, 2007; Howe *et al.*, 2008; Spilovská *et al.*, 2009) including buffaloes (Huong *et al.*, 1998; Guarino *et al.*, 2000; Campero *et al.*, 2007; Konnai *et al.*, 2008b) that has been associated with abortion (Dubey and Lindsay, 1996; Dubey *et al.*, 2006). It is possible that co-infections with *T. evansi* and *N. caninum* may result in more severe pathological effects in the pregnant buffalo and foetus, leading to a higher risk of abortion. Serological evidence of concurrent infections with neosporosis and other abortion-causing diseases (i.e., brucellosis, toxoplasmosis, listeriosis) has been documented in a dairy cattle herd with a high prevalence of abortion (Yildiz *et al.*, 2009).

In conclusion, surra is endemic and widespread in Mindanao particularly in the Caraga Region where it has a significant impact on the productivity and reproductive performance of buffaloes and other livestock species. It remains a problem in the region despite the concerted control efforts of the government mainly using chemotherapy. The current control strategy needs to be reviewed in consideration of
the following: 1) inclusion of cattle, goats and pigs in the prevention and control strategy; 2) investigation of potential resistance of local isolates of *T. evansi* to diminazene and isometamidium; 3) evaluation of the efficacy of alternative trypanocide/s; 4) incorporate control options targeting the insect vectors and strategies to limit the exposure of animals to tabanids and infected animals; 5) enactment of local ordinance and stricter implementation to control animal movement between non-endemic and endemic, and within the endemic areas; and, 6) activities to improve the awareness of farmers and other stakeholders in the importance and impact of surra, its basic biology and control.

As *T. evansi* is the only pathogenic trypanosome in Mindanao, and all Mindanao isolates screened possess the RoTat 1.2 gene, the use of the RoTat 1.2 antigen-based tests (e.g., PCR- and ELISA-RoTat 1.2 or CATT) will be useful in the island. However, further evaluation is needed to optimise the sensitivity of these tests in detecting *T. evansi* infection amongst various animals in Mindanao. The application of these tests in combination with other parasitological techniques (MHCT, modified MIT) will likely improve the utility of diagnosis of surra in Mindanao and will enhance the surveillance and control efforts of the government against the disease.
Seroprevalence, risk and health impact of natural infections with *Trypanosoma evansi* and *Fasciola gigantica* in village buffaloes in southern Philippines

6.1 Introduction

Water buffaloes (*Bubalus bubalis*) locally known as “carabaos” are the main draught animals in the Philippines where agriculture is the principal activity. Most of the 3.5 million buffaloes in the country (The 2002 Carabao Production Committee, 2003; Bureau of Agricultural Statistics, 2008) are raised by economically marginalised village farmers for draught, milk, meat and as a means of additional income. Hence, buffaloes are regarded as indispensable assets for Filipino farmers and buffalo health is of high importance. However, buffaloes are continuously at risk from infectious diseases including surra, an economically important disease of livestock that has been endemic in the country for more than a century. Surra significantly impacts on buffalo productivity and population dynamics due to high mortality, decreased fertility, weight loss, reduction of draught power and high treatment costs (Manuel, 1998; Reid, 2002; Dargantes *et al.*, 2009; Dobson *et al.*, 2009). Surra may cause immunosuppression, which consequently lowers immune response of infected animals to vaccination (Onah *et al.*, 1997; Holland *et al.*, 2001b; Holland *et al.*, 2003; Singla *et al.*, 2009) and resistance against other infectious disease-causing agents (Sharma *et al.*, 2000b). One parasitic disease that may also infect buffaloes is *Fasciola gigantica* (tropical liver fluke), which is the most common helminth parasite of buffaloes in lowland areas in the tropics (Spithill *et al.*, 1999; Molina *et al.*, 2005; Molina and Skerratt, 2005). There are no available data on the prevalence and risks and the potential impact of
combined/concurrent infections between surra and fasciolosis in areas where they are both endemic. This study was undertaken to determine the apparent seroprevalence and impact of combined/concurrent infections of *T. evansi* and *F. gigantica* in village buffaloes in Mindanao, Philippines.

### 6.2 Materials and Methods

Research protocols involving the use and handling of animals in this study were approved by the Murdoch University Committee on Animal Ethics with permit number R881/01. The survey was carried out between April and December 2007.

#### 6.2.1 Study sites

Two provinces in southern Philippines (Agusan del Sur and Surigao del Sur) where surra and fasciolosis are endemic (high risk) and one province (Bukidnon) where fasciolosis is present but surra has not been reported (low risk) were purposely chosen (Chapter 5, Figure 5.1). The current status of surra and fasciolosis in buffalo populations were obtained using expert opinions of the heads and staff of the Provincial Veterinary Offices of each province and from previous studies (Intong *et al.*, 2003; Dargantes *et al.*, 2009).

Agusan del Sur (8°30 North, 125°50 East) and Surigao del Sur (8°40 North, 126°00 East) are located in the north-eastern part of Mindanao in the Caraga Region where surra has been endemic for more than two decades. Both provinces largely depend on rice, corn, timber products and aquaculture products for income. There is no pronounced dry season in these provinces and heavy rainfall occurs between November and January. During 2007, the average monthly rainfall was 304 and 353 mm for Agusan del Sur (average value from five weather stations) and Surigao del Sur (average...
value from three weather stations), respectively. Most of the villages in these two provinces are low-lying and water bodies are present, which favour the proliferation of tabanids, the vectors of surra. The first major outbreak of surra in the region occurred in Agusan del Sur in 1994 when more than 200 buffaloes died followed by serious outbreaks in Surigao del Sur (Roeder, 1994; Manuel, 1998). To date, the disease is endemic in most villages in both provinces and sporadic outbreaks with high mortality occur in horses, water buffaloes and small ruminants (Drs. P. Calo, G. Yparraguirre and M. Samar, pers. comm., 2007). Fasciolosis is also prevalent in these provinces particularly in low-lying areas where rice production is common (Drs. M. Samar and G. Yparraguirre, pers. comm., 2007).

Bukidnon (7°55’ North, 125°05’ East) is located on a plateau at an average altitude of 915 metres above sea level. There have been no reported clinical cases of surra in the province and recent surveys found no evidence of *T. evansi* infection using different diagnostic tests (Chapter 5). Fasciolosis occurs especially in rice-producing villages (Drs. B. Gascon, S. Medidas and I. Miquiabas, pers. comm., 2007) (Intong et al., 2003). Bukidnon is relatively dry from November to April and wet during the rest of the year. In 2007, it received an average monthly rainfall of 205 mm (CMU and Malaybalay PAG-ASA Meteorological Weather Stations).

### 6.2.2 Survey design and sampling

Whole blood, sera and faeces were collected from 1,163 water buffaloes owned by small-hold farmers from a total of 32 villages (13 to 58 animals per village) (Table 6.1). Buffaloes were selected using a two-stage random sampling technique with villages and buffaloes as the primary and secondary sampling units, respectively (Cameron, 1999; Cameron *et al.*, 2003). At least 10 villages were randomly selected from each of the
two “high risk” provinces and 10 villages were chosen from the “low risk” province. Permission for each survey was obtained from the respective regional, provincial, municipal and village head executives. Farmers were informed about the survey 3-4 weeks prior to each visit through their respective village heads and animal health technicians. To encourage participation by farmers, vitamins and anthelmintics and consultation on animal health concerns were provided free of charge on each sampling occasion. In addition, information on the biology and impact of surra and fasciolosis and the importance of a survey for disease diagnosis was provided to farmers. A minimum of 13 buffaloes were randomly selected using a lottery system from each village. The sample size was calculated using Win Episcope 2.0 (Thrusfield et al., 2001) to be sufficient to detect at least one infected buffalo with 95% confidence with a prevalence of 25% and an average buffalo population of 126 per village (Table 6.1) using a test with 100% sensitivity and specificity.

Paired blood samples were collected into two vacuum blood collection tubes from the jugular vein of each buffalo for serum and whole blood (EDTA) (Vacuette®, Greiner Bio-One, Austria). Blood in plain tubes was allowed to clot for an hour at room temperature and then placed in a cooler box for transportation to a laboratory facility. Tubes were then centrifuged at 1,163×g for five minutes (Adams™ Compact II Centrifuge, USA) and the sera aspirated and stored in 1.5 mL-“o” ring tubes at -20ºC until required. The whole blood was processed for PCV determination and examination for motile trypanosomes using the MHCT (Chapter 3). Faecal samples were collected from buffaloes per rectum and kept on ice during transport to the laboratory facilities. Faecal samples were examined for the presence of trematode eggs using the modified sedimentation-sieving technique (Buenviaje et al., 2003).
Data on gender, age and history of any health problems for each animal were obtained from the individual animal-owners. A secondary estimate of the age of each buffalo was made by examining the dentition of the animal (The Beef Cattle Production Committee, 1994; Dyce et al., 1996).

Table 6.1  Population, disease status, number sampled and origin of water buffaloes examined for surra and fasciolosis in Mindanao, Philippines.

<table>
<thead>
<tr>
<th>Province</th>
<th>Disease status</th>
<th>Estimated buffalo population</th>
<th>Average population per village</th>
<th>No. of villages sampled</th>
<th>No. of buffaloes examined</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bukidnon</td>
<td>No cases</td>
<td>Endemic</td>
<td>69,295</td>
<td>149</td>
<td>10</td>
</tr>
<tr>
<td>Agusan del Sur</td>
<td>Endemic</td>
<td>Endemic</td>
<td>46,040</td>
<td>147</td>
<td>12</td>
</tr>
<tr>
<td>Surigao del Sur</td>
<td>Endemic</td>
<td>Endemic</td>
<td>25,509</td>
<td>83</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>140,844</td>
<td>126</td>
<td>32</td>
</tr>
</tbody>
</table>

*Bureau of Agricultural Statistics, 2008

6.2.3 Assessment of the body condition of buffaloes

The body condition of each buffalo was determined visually. A simplified binary body scoring scheme, modified from the methods for dairy cattle (Edmonson et al., 1989) and for buffaloes (The 2002 Carabao Production Committee, 2003), was used to classify animals as: (a) good (healthy) = smooth or slight depressions between the hooks and between pins and hooks, fleshy rump and well-muscled body; and, (b) poor (slight to severe) = moderate to severe depressions between hooks and between pins and hooks, rump not fleshy and thin body.
6.2.4 Gamma-irradiation treatment of sera

All sera were gamma-irradiated with a dose of 50 kilogram (kGray) (E. Backhouse, AQIS, pers. comm., 2009) in Victoria, Australia to conform to the requirements for importation into Australia.

6.2.5 Antibody-detection ELISA for *T. evansi*

An antibody-detection ELISA using a crude *T. evansi* antigen was performed according to Reid and Copeman (2003) with slight modification. The optimal dilutions of antigen and the horseradish peroxidase conjugated protein G (HRP-PG) (Jackson ImmunoResearch Laboratories, USA) were determined by checkerboard titration using control sera from *T. evansi*-infected and uninfected buffaloes from Mindanao, Philippines. A pooled sample of 10 sera from buffaloes in surra-endemic villages in Agusan del Sur, confirmed to be positive with surra using MHCT and MIT (Chapter 3) were used as positive controls. Ninety serum samples from buffaloes from villages in Bukidnon known to be free of surra were used as negative controls.

Briefly, 96-well, “U-bottom” microtitre ELISA plates (Sarstedt, Australia) were coated with *T. evansi* antigen diluted (1:1,000) in 0.05 M carbonate-bicarbonate buffer (pH 9.6; Sigma) by incubating overnight at 4°C in a sealed humid chamber (Reid and Copeman, 2003). The antigen solution was discarded and plates were washed three times with Tris-EDTA-sodium chloride-Tween 20 (TEN-T) (0.05 M Tris; 0.006 M EDTA; 0.003 M sodium chloride; and, 0.05% Tween 20; pH 8.0). The test and control (positive and negative) sera were diluted at 1:100 with TEN-TC (TEN-T with 0.2% casein) (TropBio, JCU) and 100 µL of each 45 serum samples were added into each well of the plate in duplicates. The plates were placed in a closed humid chamber and incubated for 60 min at 37°C. The plates were then washed three times as above and
100 μL of HRP-PG (Jackson Immunoresearch Laboratories, USA) diluted (1:2,000) in TEN-TC was added to each well and incubated in a humid chamber for 60 min at 37°C. The plates were again washed three times and 100 μL of 2,2'-azino-di-3-ethyl-benzthiazoline-6-sulfonate (ABTS®) peroxidase substrate (Kirkegaard and Perry Laboratories, USA) was added to each well and the plates were incubated in a humid chamber at room temperature for 90 min. The absorbance of each well, expressed as optical density (OD), was measured using an ultraviolet (UV) microplate reader (BioRad, USA) at dual wavelengths of 412 and 490 nm. A sample was considered positive if the computed mean OD value was greater than the mean OD plus two standard deviations (2SD) of the negative control sera (Payne et al., 1991b; Kashiwazaki and Thammasart, 1998)

6.2.6 Antibody-detection ELISA for *F. gigantica*

Anti-*F. gigantica* antibodies were detected in each serum sample using an antibody-ELISA protocol (Hansen et al., 1999; Raadsma et al., 2007; 2008) with minor changes. The antigen preparation was kindly provided by Dr. David Piedrafita from Monash University, Australia. The protein concentration of the crude *F. gigantica* whole worm extract antigen lysate was determined using a NanoDrop® ND-1000 Spectrophotometer (USA) at 260 nm. A pooled sample of 10 sera from swamp buffaloes from a fasciolosis-endemic village in Bukidnon, confirmed to be positive with fasciolosis by the modified sedimentation-sieving technique (Buenviaje et al., 2003) were used as positive controls. Forty sera from Bulgarian water buffaloes from a herd kept in closed and relatively well-drained paddocks at the Philippine Carabao Centre (PCC) of Central Mindanao University (CMU) in Bukidnon, were used as negative controls. The herd was confirmed free of infection with *F. gigantica* by ante-mortem inspection of a number of animals from the herd (Dr. E. Paraguas, pers. comm., 2007). The negative
control animals were further tested for infection with *F. gigantica* using the modified sedimentation-sieving technique (Buenviaje *et al.*, 2003) three weeks before and on the day of blood collection.

One-hundred (100) µL *F. gigantica* antigen (10 µg mL⁻¹) diluted in a carbonate-bicarbonate coating buffer (pH 9.6) was added to wells of a 96-well, “U-bottom” microtitre plate (Sarstedt, Australia) and incubated overnight at 4°C. The antigen solution was discarded and plates were washed two times using phosphate-buffered-saline (NaCl, KCl, KH₂PO₄ and Na₂HPO₄) with 0.05% Tween 20 (PBS/0.05% Tween 20). Each well was blocked with 200 µL of 5% skimmed milk in PBS/0.05% Tween 20 and the plates were incubated for one hour at 37°C. The plates were washed three times and 50 µL of diluted (1:200) test sera in 1% skimmed milk- PBS/0.05% Tween 20 were added into each well in duplicates and incubated for an hour at 37°C. The plates were then washed six times as above and 50 µL of HRP-PG (Jackson ImmunoResearch Laboratories, USA) diluted (1:8,000) in 1% skimmed milk- PBS/0.05% Tween 20 was added into each well and incubated for an hour at 37°C. The plates were again washed eight times and 100 µL of 3,3',5,5'-tetramethylbenzidine substrate (TMB One Solution, USA) was added into each well and the plates were incubated at room temperature for 10 minutes with occasional shaking. The reaction was stopped by adding 25 µL of 1M H₂PO₄ into each well. The absorbance of each well, expressed as optical density (OD), was measured using an ultraviolet (UV) spectrophotometer ELISA reader (Expert 96, ASYS Hitech GmbH-British Biochrom, UK) at 450 nm filter. A sample was considered positive if the computed mean OD value was greater than the mean OD plus two standard deviations (2SD) of the negative control sera (Guobadia and Fagbemi, 1996; Arias *et al.*, 2007)
6.2.7 Statistical analysis

The apparent seroprevalence of *T. evansi* and *F. gigantica* infections, individually and together, and their 95% binomial confidence intervals (Dawson and Trapp, 2001) were calculated for buffaloes in high- and low-surra risk areas. Data were stratified based on a number of grouping variables such as age, gender and location. The statistical significance of any observed differences in the seroprevalence in each category was determined using the chi-square or Fisher exact tests with 95% confidence (Zar, 1984). The statistical significance of any differences in continuous variables (e.g., PCV) was determined using a one-way analysis of variance (ANOVA) with Tukey’s honestly significantly difference test at 95% level of confidence. The degree of correlation between the ODs of the two ELISAs was determined by calculating the Pearson’s correlation coefficient. Odds ratios (OR) and 95% CIs were calculated for each variable using dichotomous seroprevalence data (Zar, 1984; Dawson and Trapp, 2001; Quinn and Keough, 2002). Data processing and statistical computations were conducted using Excel (Microsoft Inc., USA) and the Statistical Package for Social Sciences version 17.0 (SPSS Inc., USA).

6.3 Results

The results of the serological tests to detect exposure of buffaloes to combined and single *T. evansi* and *F. gigantica* infections, stratified by location, gender and age, are shown in Table 6.2. The results of the faecal analysis were inconclusive.

6.3.1 Apparent seroprevalence and risk of combined or single infections with *T. evansi* and *F. gigantica* in buffaloes based on location

*Tryptosoma evansi* was not detected in buffaloes from Bukidnon using parasitological diagnostic methods. The apparent seroprevalence was low with 95% CIs including zero. The overall apparent seroprevalence of *T. evansi* infection was significantly
higher in buffaloes in Agusan del Sur compared to buffaloes in Surigao del Sur ($\chi^2=10.6$, $p<0.01$) and Bukidnon ($\chi^2=424.1$, $p<0.001$). Buffaloes in Agusan del Sur were more likely to be seropositive for *T. evansi* infection compared to buffaloes in Surigao del Sur (OR (95% CI): 1.7 (1.2, 2.3)). The apparent seroprevalence of single *T. evansi* infection was significantly higher in buffaloes in Agusan del Sur compared to buffaloes in Surigao del Sur ($\chi^2=29.2$, $p<0.001$). Buffaloes in Agusan del Sur were more likely to be seropositive with single *T. evansi* infection than buffaloes from Surigao del Sur (OR (95% CI): 4.3 (2.4, 7.7)).

The overall apparent seroprevalence of *F. gigantica* infection was significantly higher in buffaloes in Surigao del Sur compared to buffaloes in Agusan del Sur ($\chi^2=4.3$, $p=0.038$) and Bukidnon ($\chi^2=24.0$, $p<0.001$). Buffaloes in Surigao del Sur and Agusan del Sur were more likely to be seropositive with *F. gigantica* than buffaloes in Bukidnon (ORs (95% CIs): 2.4 (1.7, 3.4), and 1.7 (1.2, 2.3), respectively). The apparent seroprevalence of single *F. gigantica* infection in buffaloes was significantly higher in low- than in high-surra risk areas ($\chi^2=232.7$, $p<0.001$). Buffaloes from Bukidnon were more likely to be seropositive of single *F. gigantica* infection than those buffaloes from the high-surra risk provinces (OR (95% CI): 8.1 (6.1, 10.8)). There was no significant difference in the apparent seroprevalence of single infection with *F. gigantica* in buffaloes from the high-surra risk provinces ($\chi^2=2.5$, $p=0.112$).

There was no significant difference in the apparent seroprevalence of combined (*T. evansi*/*F. gigantica*) infections in buffaloes from the two high-surra risk areas ($\chi^2=0.3$, $p=0.600$). The apparent seroprevalence of combined infections was significantly higher ($\chi^2=324.9$, $p<0.001$) in buffaloes from the high-surra risk areas compared to the low-surra-risk areas. Buffaloes from the high-surra risk areas were more likely to be
seropositive with the combined infections than those buffaloes from the low-surra risk areas (OR (95% CI): 46.7 (25.2, 86.5)). In high-surra risk provinces, there were significantly more buffaloes seropositive for combined infections with T. evansi and F. gigantica than with a single infection only of either parasite ($\chi^2=812.7$, $p=0.000$). Buffaloes raised in the high-surra risk provinces were more likely to be seropositive for both T. evansi and F. gigantica infections (OR (95% CI): 12.9 (9.9, 16.6)) and a sole infection with F. gigantica (OR (95% CI): 1.6 (1.2, 2.1)) than with T. evansi only.

There was a significant positive correlation between the OD values for the overall T. evansi and F. gigantica infections in buffaloes in high-surra risk areas ($r= 0.41$; $p<0.001$). Buffaloes that were seropositive to T. evansi infection were also more likely to be seropositive to F. gigantica infection than buffaloes seronegative to T. evansi infection (OR (95% CI): 3.3 (2.4, 4.7)).

### 6.3.2 Gender-specific apparent seroprevalence and risk of concurrent and single infections with T. evansi and F. gigantica in buffaloes

There was no significant difference in the overall apparent seroprevalence of T. evansi infection in males and females from high- and low-surra risk areas ($p>0.05$). The overall apparent seroprevalence with F. gigantica infection was significantly higher in female than in male buffaloes in the high-surra risk areas ($\chi^2=13.1$, $p<0.001$). Female buffaloes were more likely to be seropositive with F. gigantica infection (OR (95% CI): 1.8 (1.3, 2.6)) compared to males in high-surra risk areas.
Table 6.2 Proportions of buffaloes seropositive with *T. evansi* and *F. gigantica* (overall, combined or individually) and seronegative buffaloes in Mindanao, stratified by location, gender and age.

<table>
<thead>
<tr>
<th>Factor/ Saura status</th>
<th>Specific class</th>
<th>n</th>
<th>Overall infection (%)</th>
<th>Combined or single infection and uninfected (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>T. evansi (Tev)</em></td>
<td><em>F. gigantica (Fg)</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>%†</td>
<td>95% CI</td>
</tr>
<tr>
<td>1. Location</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High risk</td>
<td>Agusan del Sur</td>
<td>482</td>
<td>77.2†</td>
<td>73.2, 80.9</td>
</tr>
<tr>
<td></td>
<td>Surigao del Sur</td>
<td>352</td>
<td>67.0</td>
<td>61.9, 71.9</td>
</tr>
<tr>
<td>Low risk</td>
<td>Bukidnon</td>
<td>329</td>
<td>3.6</td>
<td>1.9, 6.3</td>
</tr>
<tr>
<td>2. Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High risk</td>
<td>Female</td>
<td>510</td>
<td>72.2</td>
<td>68.0, 76.0</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>324</td>
<td>74.1</td>
<td>68.9, 78.8</td>
</tr>
<tr>
<td>Low risk</td>
<td>Female</td>
<td>229</td>
<td>4.8</td>
<td>2.4, 8.4</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>100</td>
<td>1.0</td>
<td>0.0, 5.4</td>
</tr>
<tr>
<td>3. Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High risk</td>
<td>Young (&lt;3 yo)</td>
<td>230</td>
<td>65.2†</td>
<td>58.7, 71.4</td>
</tr>
<tr>
<td></td>
<td>Mature (3-6 yo)</td>
<td>344</td>
<td>73.3</td>
<td>68.2, 77.9</td>
</tr>
<tr>
<td></td>
<td>Old (&gt;6 yo)</td>
<td>260</td>
<td>79.2</td>
<td>73.8, 84.0</td>
</tr>
<tr>
<td>Low risk</td>
<td>Young (&lt;3 yo)</td>
<td>116</td>
<td>1.7</td>
<td>0.2, 6.1</td>
</tr>
<tr>
<td></td>
<td>Mature (3-6 yo)</td>
<td>125</td>
<td>4.8</td>
<td>1.8, 10.2</td>
</tr>
<tr>
<td></td>
<td>Old (&gt;6 yo)</td>
<td>88</td>
<td>4.5</td>
<td>1.3, 11.2</td>
</tr>
</tbody>
</table>

†Proportions in a column within the location factor and within each surra status for age and gender are significantly different (*p*<0.05)
There was no significant difference in the apparent seroprevalence of combined *T. evansi* and *F. gigantica* infections in male and female buffaloes from high- ($\chi^2= 1.8$, $p=0.185$) and low-surra risk areas (Fisher’s exact test, $p=0.183$). There was also no significant difference in the apparent seroprevalence of either *T. evansi* or *F. gigantica* single infections in male and female buffaloes in low-risk areas. In high-surra risk areas, the apparent seroprevalence of single *T. evansi* infection was significantly higher in male compared to female buffaloes ($\chi^2=8.4$, $p=0.004$). The apparent seroprevalence of single *F. gigantica* infection was significantly lower in males compared to females ($\chi^2=4.98$, $p=0.026$). Male buffaloes were more likely to be seropositive with single *T. evansi* infection (OR (95% CI): 1.9 (1.2, 1.9)) than females, whereas female buffaloes were more likely to be seropositive with a single infection of *F. gigantica* (OR (95% CI): 1.6 (1.1, 2.3)) compared to male buffaloes in high-surra risk areas.

### 6.3.3 Age-specific apparent seroprevalence and risk of combined and single infections with *T. evansi* and *F. gigantica* in buffaloes

The overall apparent seroprevalence of *T. evansi* infection was significantly lower in young compared to mature ($\chi^2=4.2$, $p=0.039$) and old buffaloes ($\chi^2=12.1$, $p=0.001$) in high-surra risk areas. Mature and old buffaloes were more likely to be seropositive with *T. evansi* infection than young buffaloes in the high-surra risk areas (ORs (95% CIs): 1.5 (1.0, 2.1) and 2.0 (1.4, 3.0), respectively). There was no significant difference in the overall apparent seroprevalence of *F. gigantica* infection in the different age groups in high-surra risk areas ($p>0.05$). In contrast, the overall apparent seroprevalence of *F. gigantica* infection was significantly higher in mature than in young buffaloes ($\chi^2=6.7$, $p=0.01$) but not significantly different between mature and old ($p>0.05$), and between young and old buffaloes ($p>0.05$) in low-surra risk areas. Mature buffaloes were more likely to be seropositive with *F. gigantica* infection than young buffaloes in low-surra risk areas (OR (95% CI): 2.0 (1.2, 3.5)).
In high-surma risk provinces, age has an influence on the apparent seroprevalence of combined infections \( (\chi^2=8.1, \ p=0.017) \). The lowest proportion of combined infection was observed in young (<3 years old) than mature (3 to 6 years old; \( \chi^2=4.4, \ p=0.037 \)) and old (>6 years old; \( \chi^2=7.6, \ p=0.006 \)) buffaloes. There was no significant difference in the number of seropositive animals with combined infections of *T. evansi* and *F. gigantica* between the mature and old buffaloes \( (\chi^2=0.77, \ p=0.379) \). In addition, the proportions of animals with single infection of *F. gigantica* in the three age classes differed significantly \( (\chi^2=7.9, \ p=0.019) \). Young animals had significantly higher apparent seroprevalence with single infection of *F. gigantica* than mature \( (\chi^2=6.9, \ p=0.008) \) and older \( (\chi^2=4.4, \ p=0.037) \) buffaloes. There was no significant difference between the apparent seroprevalence with single infection of *F. gigantica* in mature and old animals \( (\chi^2=0.59, \ p=0.443) \). Old and mature buffaloes were more likely to be seropositive to combined *T. evansi* and *F. gigantica* infections \( (\text{ORs (95% CIs)}: 1.7 (1.2, 2.4) \text{ and } 1.4 (1.0, 2.0), \text{ respectively}) \), and to a single infection of *F. gigantica* \( (\text{ORs (95% CIs)}: 1.9 (1.2, 3.0) \text{ and } 1.2 (0.1, 1.9), \text{ respectively}) \) than young buffaloes in high-surma risk areas. Buffaloes that were young, old and mature in the low-surma risk areas were likely to have a single infection with *F. gigantica* than buffaloes in a similar age groups in the high-surma risk areas \( (\text{ORs (95% CIs)}: 4.4 (2.7, 7.1), 10.7 (6.1, 18.8) \text{ and } 11.4 (7.1, 18.4), \text{ respectively}) \).

### 6.3.4 Packed cell volume

The mean packed cell volume (PCV) of buffaloes sampled from the high- and low-surma risk areas grouped based on age and the ELISA results for *T. evansi* and *F. gigantica* are presented in Table 6.3. Overall, buffaloes from the high-surma risk areas had significantly lower mean PCVs than from low-surma risk areas \( (23.9\pm4.0 \text{ vs. } 29.0\pm4.4) \).
SD %; $p<0.001$). There was no significant difference in the mean PCVs of the male and female buffaloes between and within high- and low-surra risk areas ($p>0.05$).

There was no significant difference in the mean PCVs of buffaloes from the two high-surra risk provinces of Agusan del Sur and Surigao del Sur ($p>0.05$). In high-surra risk areas, there was a significant difference in the mean PCVs of uninfected buffaloes and buffaloes with either combined or single infection with *T. evansi* or *F. gigantica* ($F_{3, 830}= 71.4; p<0.001$). Buffaloes seropositive to *F. gigantica* had significantly lower PCVs than uninfected buffaloes ($p<0.05$) and significantly higher PCVs in buffaloes seropositive to *T. evansi* only and combined infections ($p<0.05$).

### 6.3.5 Body condition of buffaloes

Table 6.4 shows that almost two-thirds of buffaloes sampled in high-surra risk areas were in poor body condition compared to buffaloes examined in low-surra risk areas (62% vs. 14%; $\chi^2= 212.5; p<0.001$). Buffaloes in high-surra risk areas were more likely to be in poor body condition (Figure 6.1) compared to buffaloes in low-surra risk areas (OR (95% CI): 10.2 (7.2, 14.5)). There was significantly higher proportion of buffaloes in severely poor body condition in high- than in low-risk areas (26.4% vs. 0.9%; $\chi^2= 98.7; p<0.001$).
Table 6.3  Mean packed cell volume (PCV) values of seronegative buffaloes and buffaloes seropositive of combined or single infection of *T. evansi* and *F. gigantica* in Mindanao.

<table>
<thead>
<tr>
<th>Surra risk/Test result</th>
<th>n</th>
<th>Mean PCV (±SD), %†</th>
<th></th>
<th>Mean PCV (±SD), %</th>
<th></th>
<th>Mean PCV (±SD), %</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Young</td>
<td></td>
<td>Mature</td>
<td></td>
<td>Old</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>n</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High risk</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tev+ &amp; Fg+</td>
<td>515</td>
<td>22.8± 3.5c</td>
<td>125</td>
<td>24.1± 3.2c</td>
<td>217</td>
<td>22.8± 3.7c</td>
<td>173</td>
</tr>
<tr>
<td>Tev+ &amp; Fg-</td>
<td>93</td>
<td>23.1± 3.1c</td>
<td>25</td>
<td>23.6± 2.8c</td>
<td>35</td>
<td>23.0± 2.7c</td>
<td>33</td>
</tr>
<tr>
<td>Tev- &amp; Fg+</td>
<td>141</td>
<td>25.8± 3.9b</td>
<td>52</td>
<td>26.3± 3.5b</td>
<td>54</td>
<td>25.6± 4.1b,c</td>
<td>35</td>
</tr>
<tr>
<td>Tev- &amp; Fg- (Uninfected)</td>
<td>85</td>
<td>28.1± 3.4a</td>
<td>28</td>
<td>28.9± 2.2a</td>
<td>38</td>
<td>27.5± 3.8ab</td>
<td>19</td>
</tr>
<tr>
<td>Low risk‡‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fg+</td>
<td>205</td>
<td>28.2± 4.4b</td>
<td>65</td>
<td>29.1± 3.8b</td>
<td>85</td>
<td>28.2± 4.7b</td>
<td>55</td>
</tr>
<tr>
<td>Tev- &amp; Fg- (Uninfected)</td>
<td>112</td>
<td>30.5± 4.2a</td>
<td>49</td>
<td>30.6± 3.7a</td>
<td>34</td>
<td>30.4± 4.7a</td>
<td>29</td>
</tr>
</tbody>
</table>

†Means in a column within high- and low-surra risk status with different superscripts are significantly different from each other (*p*<0.05)

‡‡Excluding the 11 *T. evansi/F. gigantica* seropositive and one *T. evansi* seropositive buffaloes in the analysis.
### Table 6.4

Proportions of buffaloes seropositive to *T. evansi* and *F. gigantica*, combined or singly, and seronegative buffaloes with poor body condition in Mindanao.

<table>
<thead>
<tr>
<th>Surra status</th>
<th>Test result</th>
<th>n</th>
<th>% Poor</th>
<th>95% CI</th>
<th>p value</th>
<th>Odds ratio (OR)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>High risk</td>
<td><strong>Overall</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1. <em>T. evansi</em> (Tev) +</td>
<td>608</td>
<td>73.7</td>
<td>70.0, 77.1</td>
<td>0.009 (1 vs. 2)</td>
<td>1.4</td>
<td>1.1, 1.8</td>
</tr>
<tr>
<td></td>
<td>2. <em>F. gigantica</em> (Fg) +</td>
<td>656</td>
<td>66.9</td>
<td>63.2, 70.5</td>
<td></td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Combined, single</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3. Tev+ &amp; Fg+</td>
<td>515</td>
<td>75.1</td>
<td>71.2, 78.8</td>
<td>0.000 (3 vs. 5)</td>
<td>15.3</td>
<td>8.4, 28.1</td>
</tr>
<tr>
<td></td>
<td>4. Tev+ &amp; Fg-</td>
<td>93</td>
<td>65.6</td>
<td>55.0, 75.1</td>
<td>0.054 (3 vs. 4)</td>
<td>9.7</td>
<td>4.7, 19.8</td>
</tr>
<tr>
<td></td>
<td>5. Tev- &amp; Fg+</td>
<td>141</td>
<td>36.9</td>
<td>28.9, 45.5</td>
<td>0.000 (4 vs. 5)</td>
<td>3.0</td>
<td>1.5, 5.8</td>
</tr>
<tr>
<td></td>
<td>6. Tev- &amp; Fg- (Uninfected)</td>
<td>85</td>
<td>16.5</td>
<td>9.3, 26.1</td>
<td>0.001 (5 vs. 6)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Subtotal</strong></td>
<td>834</td>
<td>61.6</td>
<td><strong>58.2, 64.9</strong></td>
<td></td>
<td><strong>10.2</strong></td>
<td><strong>7.2, 14.5</strong></td>
</tr>
<tr>
<td>Low risk‡‡</td>
<td>7. Tev- &amp; Fg+</td>
<td>205</td>
<td>18.5</td>
<td>13.5, 24.5</td>
<td></td>
<td>4.9</td>
<td>1.9, 12.8</td>
</tr>
<tr>
<td></td>
<td>8. Tev- &amp; Fg- (Uninfected)</td>
<td>112</td>
<td>4.5</td>
<td>1.5, 10.1</td>
<td>0.000 (7 vs. 8)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Subtotal</strong></td>
<td>317</td>
<td>13.6</td>
<td><strong>10.0, 17.8</strong></td>
<td><strong>0.000 (vs. high risk)</strong></td>
<td>1.0</td>
<td></td>
</tr>
</tbody>
</table>

1. 95% confidence interval
2. Excluding the 11 *T. evansi/F. gigantica* seropositive and one *T. evansi* seropositive buffaloes in the analysis because *T. evansi* was not detected in Bukidnon using MHCT/MIT
The overall proportion of buffaloes with poor body condition in high-surra risk areas was significantly higher in buffaloes seropositive to *T. evansi* than to *F. gigantica* infection ($\chi^2 = 6.9; p<0.01$). Buffaloes in high-surra risk areas seropositive with *T. evansi* were more likely to lose weight than those buffaloes seropositive with *F. gigantica* (OR (95% CI): 1.4 (1.1, 1.8)). In high-surra risk areas, a significantly higher proportion of buffaloes in poor body condition were seropositive to both *T. evansi* and *F. gigantica* compared to *F. gigantica* alone ($\chi^2 =73.2; p<0.001$), to *T. evansi* alone compared to *F. gigantica* alone ($\chi^2 =18.5; p<0.001$) and to *F. gigantica* alone compared to uninfected buffaloes ($\chi^2 =10.7; p<0.01$). Buffaloes in high-surra risk areas seropositive for both *T. evansi* and *F. gigantica*, and *T. evansi* or *F. gigantica* only were more likely to be in poor body condition than uninfected buffaloes (ORs (95% CIs): 15.3 (8.4, 28.1), 9.7 (4.7, 19.8) and 3.0 (1.5, 5.8), respectively). Likewise, buffaloes in low-surra risk areas seropositive with a single infection of *F. gigantica* were more likely to be in poor body condition than those uninfected buffaloes (OR (95% CI): 4.9 (1.9, 12.8)).

Figure 6.1  A water buffalo with combined infections of *T. evansi* and *F. gigantica* from Agusan del Sur, Philippines showing poor body condition.
6.4 Discussion

This is the first detailed study of the extent and possible impact of combined infections with *T. evansi* and *F. gigantica* in livestock in Mindanao. The results of this study show that there is a high apparent seroprevalence of combined *T. evansi* and *F. gigantica* infections in village buffaloes in high-surra risk areas in Mindanao, and that *F. gigantica* is highly endemic. The observed high apparent seroprevalence of combined infections with these parasites is not surprising because *T. evansi* and *F. gigantica* are endemic in the Philippines (Tongson, 1978; Manuel, 1998). Tongson (1978) showed that between 35 to 100% of buffaloes in the Philippines are infected with *F. gigantica*. In addition, the higher prevalence of *F. gigantica* infection was observed in animals resident in low-lying areas. Recent surveys showed that 47% and between 65 to 100% of buffaloes are infected with *F. gigantica* in southern Mindanao and Bukidnon, respectively (Intong et al., 2003; Molina et al., 2005). Recent serological surveys have also shown that 20% and 78% of buffaloes in southern and northern Mindanao, respectively, are infected with *T. evansi* (Buenviaje et al., 2003; Dargantes et al., 2009).

The observation that the majority of buffaloes in high-surra risk areas are infected with both *T. evansi* and *F. gigantica* is probably due to the presence of risk factors for both infections. Buffaloes are likely to come in contact with infected animals and with the intermediate hosts of the causative agents of both diseases when they are at work (e.g., rice fields), tethered in the pasture or in the wallowing pools. The intermediate snail host of *F. gigantica* in Southeast Asia, the *Lymnaea auricularia rubiginosa*, proliferates in shallow, slow moving water with sufficient vegetation and oxygen level (Kendall, 1954, cited by Spithill et al., 1999). The main mechanical vectors of *T. evansi*, the tabanid flies, are also present in areas with permanent water bodies (Fominykh and
Eremina, 1984; Service, 1986). Rainfall is abundant throughout the year in Agusan del Sur and Surigao del Sur, and most villages sampled in these provinces are low-lying (<100 metres above sea level) where water is abundant in creeks, rivers, rice paddies, lakes and in Agusan del Sur, a wide marshland. During hot weather, buffaloes are allowed to cool down in common wallowing pool because of their poor body thermo-regulatory mechanism (Das et al., 1999; Marai and Haeb, 2010). They are also usually tethered in close contact with other animals in wet, swampy pastures at night, early morning or late afternoon when the temperature is low (Dr. P. Calo, pers. comm., 2007).

The observed high seroprevalence of combined *T. evansi* and *F. gigantica* infections in buffaloes in high-surra risk areas agrees with Buenviaje et al. (2003) who found buffaloes in Mindanao that were seropositive to *T. evansi* infection were more likely to be seropositive to *F. gigantica* than seronegative buffaloes (OR: 1.4). The odds for surra-seropositive buffaloes to be infected with *F. gigantica* in high-surra risk areas in this study was more than twice (OR: 3.3) compared to the observed odds in Buenviaje et al. (2003). This difference is probably due to the lower prevalence of surra and the limited number of samples tested in the previous study (n=170). Buenviaje et al. (2003) also reported that buffaloes seropositive to *T. evansi* infection were more likely to be infected with *Pasteurella multocida* (haemorrhagic septicaemia) compared to *T. evansi*-seronegative buffaloes (OR: 6.0). Whilst this aspect was not explored in the present study, the information is important because haemorrhagic septicaemia is a fatal endemic disease of livestock in the Philippines and other Asian countries (Molina et al., 1994; Khan et al., 2006). Haemorrhagic septicaemia has been suspected to complicate and contribute to higher mortality during surra outbreaks (Manuel, 1998), making surra
more severe in livestock than any other parasitic diseases in southern Philippines and in areas where it is endemic.

The high apparent seroprevalence of combined infections with *T. evansi* and *F. gigantica* in mature and old buffaloes is consistent with previous observations that occurrence of these diseases in livestock is age-related. Higher apparent seroprevalence of *T. evansi* has been observed in adults than in young animals (Davison *et al.*, 2000; Gutierrez *et al.*, 2000; Coen *et al.*, 2001; Atarhouch *et al.*, 2003; Njiru *et al.*, 2004a) and with *F. gigantica* infection in cattle and buffaloes (Maqbool *et al.*, 2002; Molina *et al.*, 2005; Pfukenyi *et al.*, 2005; Phiri *et al.*, 2005). This is probably because mature and adult buffaloes are often used for draught which provides them more exposure to vectors of both diseases than young buffaloes. This could also be attributed to the chronic nature of both infections and persistence of antibodies even after treatment (Davison *et al.*, 1999; Spithill *et al.*, 1999; Njiru *et al.*, 2004a; Desquesnes *et al.*, 2009b). Likewise, lowered resistance in old animals due to stress from environmental factors (Maqbool *et al.*, 2002) and farm activities may also favour infection.

The influence of gender in the occurrence of both surra and fasciolosis is variable. The prevalence of *F. gigantica* infection has been observed to be either significantly higher in females (Asanji and Williams, 1984; Phiri *et al.*, 2005) or similar in both sexes in cattle and buffaloes (Maqbool *et al.*, 2002; Molina *et al.*, 2005; Khan *et al.*, 2009). A significant number of male buffaloes in this study were found infected with single *T. evansi* than females in agreement with the observations of Njiru *et al.* (2004) who detected higher prevalence of surra in male than in female camels in Kenya (Njiru *et al.*, 2004a). Nevertheless, a higher proportion of female buffaloes were seropositive with *T. evansi* from a larger epidemiological survey in Mindanao (Chapter 5). Whilst the
reasons for the potential susceptibility of a particular gender to both diseases are unknown, it is possible that host (i.e., genetics, hormonal changes, behaviour and stress) and environmental factors that may affect host-vector-parasite interplay may influence such a phenomenon (Asanji and Williams, 1984; Spithill et al., 1999; Njiru et al., 2004a).

The low PCV values and high prevalence of poor body condition in buffaloes with combined/concurrent infections with *T. evansi* and *F. gigantica* are in agreement with previous studies (Griffin et al., 1981b; Kaufmann et al., 1992; Goossens et al., 1997b; Sharma et al., 2000b). Trypanosomosis is believed to enhance the establishment of other parasitic infections within susceptible hosts and potentiate their pathogenicity. For example, experimental challenge of goats with *T. evansi* and later with *Haemonchus contortus* resulted to: a shorter prepatent period of *H. contortus* (17 vs. 23 days, on average); development of more infective larvae in the abomasum as reflected in the high number of recovered adult worms from the abomasum; and, more severe anaemia, weight loss and higher mortality than those infected singly with either *T. evansi* or *H. contortus* (Sharma et al., 2000b).

Similar findings of the deleterious impact of combined infections were also demonstrated between African trypanosomosis and *H. contortus*. Goats experimentally infected with *T. congolense* and *H. contortus* also showed a higher level of mortality, lower PCV values and severe weight loss compared to goats with single infections of either parasite (Griffin et al., 1981a; Griffin et al., 1981b). These effects were also observed in naturally trypanotolerant breeds of African Djallonke sheep and N’Dama cattle which showed severe anaemia, loss of weight and high deaths, whereas animals with single infections only of either parasite remained healthy (Kaufmann et al., 1992;
Goossens et al., 1997b). Furthermore, African N’Dama and Gobra cattle and their crosses with natural trypanosomosis (T. congolense, T. vivax, T. brucei) were observed to be more highly susceptible to tick infestations as evidenced by high tick burden (Mattioli et al., 1998). Similarly, higher susceptibility to anaplasmosis amongst Australian swamp buffaloes imported to Indonesia was attributed to a concurrent T. evansi infection which resulted to an increased parasitaemia with A. marginale and a high (as high as 73%) number of deaths (Payne et al., 1991c). There was no report however on the status of the body conditions of the affected buffaloes which could have been an interest to the present study as basis for comparison.

The increased susceptibility of T. evansi-infected buffaloes to another infection like F. gigantica and the synergistic negative impact of T. evansi and F. gigantica combined/concurrent infections on affected animals could be associated to immunosuppression. Suppression of the immune capacity of the host is one of the important pathological features of pathogenic trypanosomes, including T. evansi (Urquhart, 1980; Van Dam et al., 1981; Dempsey and Mansfield, 1983; Flynn et al., 1994). Decreased immune responses after vaccination with other disease agents (Onah et al., 1997; 1998; Onah and Wakelin, 2000; Holland et al., 2001b; Holland et al., 2003; Singla et al., 2009), and higher susceptibility to other pathogens like H. contortus have been observed experimentally (Sharma et al., 2000b).

The observation that more buffaloes seropositive to combined infections of T. evansi and F. gigantica or single T. evansi had poor body condition compared to buffaloes that were only seropositive with single infection of F. gigantica is consistent with observations from other studies. Weight loss is one of the adverse clinical symptoms of animals with surra (Losos, 1980; Ngeranwa et al., 1993; Audu et al., 1999; Dargantes et
al., 2005b) and fasciolosis (Spithill et al., 1999). Buffaloes experimentally infected with *T. evansi* and those with natural infection show weight loss (Löhr et al., 1985; Damayanti et al., 1994; Manuel, 1998). Similarly, buffaloes with experimental or natural *F. gigantica* infection lose weight during the course of infection (Yadav et al., 1999; Molina et al., 2005; Ganga et al., 2007). It was necessary to develop a simple scoring system for this study because a method suitable for field use in Mindanao was not available. This was achieved by combining the body scoring methods in dairy cattle and buffaloes (Edmonson et al., 1989; The 2002 Carabao Production Committee, 2003) into a binary score that was easy to use and interpret.

It was expected that concurrent/combined infections of buffaloes with *T. evansi* and *F. gigantica* would cause more severe anaemia compared to infection with either parasite alone. This is because surra and fasciolosis are both characterised by varying degrees of anaemia in infected animals (Sangwan et al., 1993; Damayanti et al., 1994; Hilali et al., 2006). The aetiology of trypanosomal anaemia is multifactorial and includes: haemodilution, haemolysis and inhibition of erythropoiesis or dyshaemopoiesis (Jenkins et al., 1980; Sharma et al., 2000a; Dargantes et al., 2005a; Stijlemans et al., 2008; Da Silva et al., 2009; Mijares et al., 2010). Anaemia in fasciolosis is mainly caused by the migration of immature flukes during the early stage of infection that causes organ damage and haemorrhage, and to the blood-sucking activity of adult flukes in chronic infections (Ogunrinade and Anosa, 1981; Yadav et al., 1999). It is estimated that one adult fluke consumes about 0.2 to 0.5 mL of the host’s blood everyday (Dawes and Hughes, 1970). Blood loss is further aggravated by a hormonal imbalance due to pathological lesions in the thyroid and adrenal glands caused by the flukes (Ganga et al., 2007) and increased erythrocyte lysis due to toxic products produced by adult flukes.
In this context, an effective, sustainable and holistic prevention and control strategy should be implemented against *T. evansi* and *F. gigantica* infections in buffaloes in high-surra risk areas in Mindanao to avoid further economic losses. The control of fasciolosis includes drug treatment, biological control of snails and limiting exposure of livestock (e.g., avoidance of swampy pasture) to the snail intermediate hosts of *F. gigantica* (Spithill *et al.*, 1999; Intong *et al.*, 2003; Suhardono *et al.*, 2006). The control of surra relies much on chemotherapy because of limited information on the identity, biology and abundance of the major mechanical insect transmitters (mainly tabanid flies) of its aetiological agent in endemic areas in Mindanao.
Potential tabanid vectors of *Trypanosoma evansi* in Mindanao, Philippines: identification, abundance and molecular studies

7.1 Introduction

The medium to large, blood-sucking tabanid flies known as March flies or horse flies (Diptera, Tabanidae) are vectors of diseases relevant to human and animal health (Fallis, 1980; Foil, 1989; Gillott, 2005). Tabanids have been implicated in the transmission of viruses (Oshima *et al.*, 1981; Issel and Foil, 1984; Issel *et al.*, 1988; Manet *et al.*, 1989; Foil and Issel, 1991; Hasselschwert *et al.*, 1993; Carn, 1996), bacteria (Davies, 1983; Hugh-Jones and Blackburn, 2009; Foley and Nieto, 2010) and parasites (Hawkins *et al.*, 1982; Otte and Abuabara, 1991; Desquesnes and Dia, 2003; 2004; Hornok *et al.*, 2008), including *Trypanosoma evansi*, the cause of surra (Losos, 1980; Mahmoud and Gray, 1980; Luckins, 1988; Desquesnes *et al.*, 2009a). Tabanids are efficient transmitters of *T. evansi* amongst livestock because they have large mouthparts and they have persistent and intermittent feeding habit (Krinsky, 1976; Luckins, 1988; Lun *et al.*, 1993; Luckins and Dvinger, 2004; Gubler, 2009). In the Philippines, there is insufficient and outdated information about tabanids. Earlier studies on tabanids have been conducted in the northern part of the country (Mitzmain, 1913a; c; Manresa and Mondoñedo, 1935; Yutuc, 1949). However, the exact distribution and diversity of tabanids in the entire archipelago, particularly in the southern part (Mindanao) where most of the outbreaks of surra have occurred in recent years (Manuel, 1998; Reid, 2002) are unknown. It is therefore important to study the species composition, diversity and dynamics of tabanids as well as their potential role as mechanical transmitters of *T. evansi* amongst livestock in Mindanao.
In this study, tabanids were collected over a one year period using Nzi traps from high- and low-surra risk areas in Mindanao, southern Philippines. The identity, abundance and correlation to environmental determinants were determined. Trypanosomes in the flies and the sources of the blood meals of the tabanids were identified using PCR-based tests.

7.2 Materials and Methods

7.2.1 Tabanid collection sites
Tabanids were trapped from six villages located in high- and low-surra risk provinces in Mindanao (Figure 7.1). The sites were chosen based on altitude, tabanid sightings and history of surra (Table 7.1). The description of the climate and topography of the provinces are described in Chapters 5 and 6. The history of surra in the provinces was based on the results of the epidemiological surveys (Chapters 3, 5 and 6) and the expert opinion of local veterinarians.

7.2.2 Field collection of tabanids using Nzi traps
Tabanid flies were trapped from August 2007 to July 2008 using 18 unbaited Nzi traps (Mihok, 2002). The traps were sewn using Sunbrella® Pacific blue and black coloured fabrics (Glen Raven Inc, USA) and UV-treated polyethylene-acrylic horticultural netting (Econet L®, Ludvig Svensson, Sweden) following instructions from Mihok (2007). These coloured fabric materials have been proven to last for long periods of time in the field without fading (Mihok et al., 2006) and the netting material has also been proven durable (Dr. S. Mihok, pers. comm., 2007). Round timbers were utilised as posts for the Nzi trap and empty plastic bottles were used as fly collection tubes (Figure 7.2). The traps were washed and the posts were replaced, as necessary, every four months during the duration of the study.
Figure 7.1 Trapping sites for tabanids in Mindanao, Philippines.
Table 7.1 The altitude, coordinates and surra history of villages in Mindanao where tabanids were trapped.

<table>
<thead>
<tr>
<th>Town and province</th>
<th>Surra history of province</th>
<th>Village</th>
<th>Altitude, above sea level (m)</th>
<th>Coordinates</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Veruela, Agusan del Sur</td>
<td>Present</td>
<td>San Gabriel</td>
<td>27</td>
<td>08.10068</td>
<td>125.93893</td>
</tr>
<tr>
<td>Tagbina, Surigao del Sur</td>
<td>Present</td>
<td>Sta. Fe</td>
<td>33</td>
<td>08.48367</td>
<td>126.12553</td>
</tr>
<tr>
<td>Esperanza, Agusan del Sur</td>
<td>Present</td>
<td>Duangan</td>
<td>35</td>
<td>08.60884</td>
<td>125.71511</td>
</tr>
<tr>
<td>Tubay, Agusan del Norte</td>
<td>Present</td>
<td>Telesfora†</td>
<td>81</td>
<td>09.19720</td>
<td>125.57717</td>
</tr>
<tr>
<td>Maramag, Bukidnon</td>
<td>Absent</td>
<td>Dologon (CMU-DP)</td>
<td>321</td>
<td>07.86646</td>
<td>125.05368</td>
</tr>
<tr>
<td>Maramag, Bukidnon</td>
<td>Absent</td>
<td>Dologon (PCC-CMU)</td>
<td>410</td>
<td>07.88430</td>
<td>125.05764</td>
</tr>
</tbody>
</table>

†Trypanosoma evansi infection was not parasitologically detected from animals in this village (Chapter 3)
Three traps were placed in the animal grazing areas (grassland) at each site. The traps were spaced 50 metres apart on a straight line, facing due west, approximately 20 to 40 metres from a wooded area or under coconut trees (Sta. Fe and Telesfora) and approximately 50-200 metres away from water bodies. Fly traps were placed on pasture areas of the Dairy Project of Central Mindanao University (CMU-DP) and the Philippine Carabao Centre at CMU (PCC-CMU) in Dologon, Bukidnon. Traps were placed along the periphery of fenced pasture paddocks and enclosed with barbed wire to prevent destruction by grazing animals. The ~50-hectare pasture area at the CMU Dairy Project accommodates about 120 dairy animals and at least six horses. Approximately 100 sheep and goats are herded nearby. At the PCC, 200 Bulgarian Murrah buffaloes (Bubalis bubalis) have been pastured in rotation within a 47-hectare pasture area and four horses and 10 goats were pastured nearby.

Tabanids were collected weekly from the traps at each site and other flies were discarded. Flies were either placed in collection bottles with 80% ethanol or individually pinned as dried specimens in insect boxes (Gullan and Cranston, 2005). They were grouped according to the area and month of collection until identification and counting. For the molecular study, random samples of 10-20 flies per species from two surra-endemic villages (Sta. Fe and Duangan) were individually preserved in 95% ethanol in small plastic vials or kept as dried specimens until analysis. Stored flies from each study site were collected monthly and taken to CMU laboratory for identification and counting. Rainfall data were obtained from the weather station nearest to each collection site and altitude was recorded using a handheld global positioning system (GPS) navigator (eTrex® H, Garmin International Incorporated, Taiwan).
7.2.3 Identification of tabanids

Tabanids were identified morphologically based on published taxonomic keys for Philippine tabanids (Philip, 1959), *Tabanus striatus* complex (Burger and Thompson, 1981) and *Chrysops* species of Australasia (Burger and Chainey, 2000). The identities of the tabanids were confirmed by a local entomologist from CMU in the Philippines and entomologists from James Cook University in Queensland, the Australian Quarantine and Inspection Service (AQIS) in Darwin, Australia and Kasetsart University in Bangkok, Thailand. Specimens of *T. striatus* collected from Thailand (courtesy of Dr. M. Desquesnes, Kasetsart University, Thailand) were used to confirm the specimens of *T. partitus* in the present study.

7.2.4 Extraction of whole genomic DNA from tabanids

Molecular analysis was performed on 60 tabanids (10-20 per tabanid species) caught in the same trapping period (October-December 2007) from Sta. Fe in Tagbina, S organo.
del Sur and Duangan in Esperanza, Agusan del Sur. These sites were chosen because *T. evansi* had been identified in livestock in these villages by parasitological, serological and molecular tests during the cross-sectional surveys (Chapters 3, 5 and 6).

After removal of the wings and extremities, each fly was washed three times with distilled water, minced and homogenised in a 1.5 mL centrifuge tube containing phosphate buffered solution (PBS). Whole genomic DNA was extracted using the MasterPure™ DNA Purification kit (EPICENTRE® Biotechnologies, USA) following the manufacturer’s instruction. The DNA concentration (ng/µL) and the purity of each sample extract were estimated using a NanoDrop® ND-1000 Spectrophotometer (NanoDrop Technologies, USA) at 260 nm. Samples were stored at -20°C until analysed.

### 7.2.5 Detection and identification of trypanosomes in tabanids

Conventional and nested polymerase chain reactions (PCRs) using primers specific for *Trypanozoon* (Wuyts *et al.*, 1994) and Trypanosome 18s genes (Maslov *et al.*, 1996; McInnes *et al.*, 2009), respectively, were used to detect trypanosomal DNA in tabanids (Table 7.2). The conventional PCR for *Trypanozoon* was carried out as described in Chapter 5 whilst the nested PCR for 18s trypanosome gene was performed following the published protocol (McInnes *et al.*, 2009). The primers, target genes and PCR product sizes are summarised in Table 7.2. For the nested PCR, the 18s trypanosome gene in flies was initially amplified in a total reaction volume of 25 µL mixture of reagents and 1 µL of fly genomic DNA, as summarised in Table 7.3. For the second PCR, 1 µL of DNA product from the first PCR was used as template. The amplifications of DNA were performed using an automated thermal cycler (Applied
BioSystems, Singapore) employing the thermal conditions for each PCR system (Table 7.4).

7.2.6 Molecular detection of the sources (hosts) of blood meal of tabanids

The detection of host species was achieved by amplification of the mammalian cytochrome \( b \) gene from undiluted and diluted (1:10, 1:20 or 1:40 with distilled water) whole genomic extracts from each fly. The PCR was performed as previously described (Konnai et al., 2008a) except that amplification was carried out in a 50\( \mu \)L reaction mixture. The primers, reagents and thermal conditions are given in Tables 7.2, 7.3 and 7.4. PCR products (4 \( \mu \)L each) were analysed and results documented as described in Chapter 5.

7.2.7 Purification, cleaning, sequencing of amplicons and sequence analysis

Amplicons from each of the PCRs were purified, cleaned and sequenced as described in Chapter 5. The bands from the agarose gel of both PCRs were individually and separately excised with sterile scalpel blades on a Dark Reader Transilluminator\(^\circledR\) (Clare Chemical Research, USA). Purified DNA product (1–6 \( \mu \)L) was used to amplify target DNA using the forward primer of each PCR system. Individual nucleotide sequences of the amplified products were analysed and compared with the existing sequences in the GenBank as described in Chapter 5 to identify the species of trypanosomal DNA amplified from the tabanids and the host species.

7.2.8 Statistical analysis

Monthly rainfall and total tabanid catch per month were transformed by \( \log_{10} \) (rainfall, mm) and \( \log_{10} \) (catch + 1), respectively (Mihok, 2002; Van Hennekeler, 2007). Significant differences between tabanid catches in different locations in high-surra risk
provinces, and between the two surra-risk areas and between the two collections sites in Bukidnon were determined using one-way analysis of variance (ANOVA) with Tukey’s HSD post hoc test, and Student’s t test, respectively at a 95% confidence level (Quinn and Keough, 2002). The relationship between the number of tabanids trapped and rainfall and altitude was determined by Pearson correlation and regression analysis (Dawson and Trapp, 2001; Quinn and Keough, 2002). A multiple regression equation was fitted to the data to predict monthly tabanid catch from monthly rainfall and altitude. Significant differences between the proportions of tabanids infected with T. evansi or T. theileri were analysed using a chi-square test ($\chi^2$) (Dawson and Trapp, 2001; Quinn and Keough, 2002). Statistical analyses were run using the Statistical Package for Social Sciences version 17.0 (SPSS Inc., USA) or PopTools (Hood, 2009).

Table 7.2 List of primers employed to detect trypanosomes and animal hosts in tabanids and the expected PCR products.

<table>
<thead>
<tr>
<th>Target gene</th>
<th>Product size (bp)</th>
<th>Primer sequence (5’-3’)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trypanozoon</td>
<td>227</td>
<td>pMURTec F: TGCAGACGACCTGACGCT</td>
<td>Wuyts et al., 1994</td>
</tr>
<tr>
<td></td>
<td></td>
<td>pMURTec R: ACTCTCCTAGAAGCTTCG GTGTCTCT</td>
<td></td>
</tr>
<tr>
<td>Trypanosome 18s rRNA</td>
<td></td>
<td>1st PCR: SLF5 F: GCTTGGTTTCAAGGACTTAGC</td>
<td>Maslov et al., 1996; McInnes et al., 2009</td>
</tr>
<tr>
<td>(nested PCR)</td>
<td>−900</td>
<td>S762 R: GACTTTTGCTTCCTCTTAATG</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2nd PCR: S825 F: ACCGTTTCGGCTTTTGTGG</td>
<td></td>
</tr>
<tr>
<td></td>
<td>−904</td>
<td>S662 R: GACTACAATGGTGCTCTTAATC</td>
<td></td>
</tr>
<tr>
<td>Cytochrome b (vertebrate host)</td>
<td>359</td>
<td>Cyt b F: CCATCCAACATCTCAGCATGATGAAA</td>
<td>Steuber et al., 2005; Konnai et al., 2008</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cyt b R: GCCCCTCAGAATGATATTGT CCTCA</td>
<td></td>
</tr>
</tbody>
</table>
### Table 7.3 Reagents and the final volume of PCR mixture in each PCR system.

<table>
<thead>
<tr>
<th>Component</th>
<th>Trypanozoon (µL)</th>
<th>Trypanosoma 18s 1st (µL)</th>
<th>Trypanosoma 18s 2nd (µL)</th>
<th>Cytochrome b (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>16.3</td>
<td>16.35</td>
<td>10.35</td>
<td>20.8</td>
</tr>
<tr>
<td>Buffer, 10x</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>5.0</td>
</tr>
<tr>
<td>MgCl</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>4.0</td>
</tr>
<tr>
<td>dNTPs (dATP, dTTP, dCTP and dGTP)</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Forward primer</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Reverse primer</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Cresol red</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tth Plus DNA polymerase</td>
<td>0.2</td>
<td>0.15</td>
<td>0.15</td>
<td>0.2</td>
</tr>
<tr>
<td>DNA template</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Total Volume (µL)</td>
<td>25.0</td>
<td>25.0</td>
<td>25.0</td>
<td>50.0</td>
</tr>
</tbody>
</table>

### Table 7.4 Thermal cycling conditions for each PCR used to detect trypanosomes in tabanids and the hosts of the flies.

<table>
<thead>
<tr>
<th>PCR Steps</th>
<th>Trypanozoon</th>
<th>Trypanosoma 18s (nested) 1st PCR</th>
<th>Trypanosoma 18s (nested) 2nd PCR</th>
<th>Cytochrome b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial DNA denaturation</td>
<td>94°C, 5 min</td>
<td>94°C, 5 min</td>
<td>94°C, 5 min</td>
<td>94°C, 4 min;</td>
</tr>
<tr>
<td>Primers annealing</td>
<td>--</td>
<td>50°C, 2 min</td>
<td>50°C, 2 min</td>
<td>--</td>
</tr>
<tr>
<td>DNA extension</td>
<td>--</td>
<td>72°C, 4 min</td>
<td>72°C, 4 min</td>
<td>--</td>
</tr>
<tr>
<td>Number of cycles</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>DNA denaturation</td>
<td>94°C, 30 s</td>
<td>94°C, 30 s</td>
<td>94°C, 30 s</td>
<td>94°C, 30 s</td>
</tr>
<tr>
<td>Primers annealing</td>
<td>60°C, 2 min</td>
<td>52°C, 30 s</td>
<td>52°C, 30 s</td>
<td>50°C, 3 min</td>
</tr>
<tr>
<td>DNA extension</td>
<td>72°C, 30 s</td>
<td>72°C, 2.2 min</td>
<td>72°C, 2.2 min</td>
<td>72°C, 30 s</td>
</tr>
<tr>
<td>Number of cycles</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>45</td>
</tr>
<tr>
<td>Final extension</td>
<td>72°C, 7 min</td>
<td>72°C, 7 min</td>
<td>72°C, 7 min</td>
<td>72°C, 7 min</td>
</tr>
<tr>
<td>Holding</td>
<td>14°C, ∞</td>
<td>14°C, ∞</td>
<td>14°C, ∞</td>
<td>14°C, ∞</td>
</tr>
</tbody>
</table>
7.3 Results

7.3.1 Species and relative abundance of tabanids caught by Nzi traps

A total of 14,851 tabanids were collected using Nzi traps within one year from six villages in Mindanao (Table 7.5). Five species belonging to genera Tabanus and Chrysops were identified (Figure 7.3): Tabanus partitus Walker, T. ceylonicus Schiner, T. philippinensis Kröber, T. reducens Walker and Chrysops cinctus Bigot with total relative abundances of 61.9, 34.6, 1.1, 1.0 and 1.3%, respectively. A greater number of species was trapped in the Caraga Region (3-5 species) compared to Bukidnon (1 species).

The mean monthly number of tabanids caught per trap varied significantly between collection sites in the high-surra risk provinces (F$_{3, 140}$= 250.4; p<0.001). Overall, the average number of tabanids caught per trap per week was: 47, 32, 16, 7, 2 and 1 tabanid from San Gabriel, Duangan, Sta. Fe, Telesfora, CMU-PD and PCC-CMU, respectively. A significantly higher number of tabanids were trapped from San Gabriel, Veruela, Agusan del Sur compared to any of the collection sites in the Caraga Region (p<0.05).

In Bukidnon (low-risk), more tabanids were caught at CMU-DP compared to PCC-CMU (p<0.001).

There was a significant negative correlation between altitude and monthly tabanid catches ($R^2$= 0.902; p<0.001) (Figure 7.4). A significantly higher number of tabanids were trapped every month from the Caraga Region (low altitude, high-surra risk) compared to Bukidnon (high altitude, low-surra risk province) (101±6.3 vs. 5±0.4 SEM; p<0.001).
Figure 7.3 Tabanid species collected in Mindanao (length in mm). a. *Chrysops cinctus* Bigot; b. *T. ceylonicus* Schiner; c. *Tabanus partitus* Walker; d. *T. reducens* Walker; and, e. *T. philippinensis* Kröber.

Table 7.5 Number of tabanids caught using Nzi traps from various locations in Mindanao during August 2007 until July 2008.

<table>
<thead>
<tr>
<th>Surra risk status/site</th>
<th>Altitude, above sea level (m)</th>
<th>Total tabanids caught</th>
<th>Mean tabanids caught/trap/month (SEM)†</th>
<th>Number &amp; tabanid species††</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>High risk</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>San Gabriel</td>
<td>27</td>
<td>6,760</td>
<td>188±13.0</td>
<td>3 (a, b, c)</td>
</tr>
<tr>
<td>Duangan</td>
<td>35</td>
<td>4,601</td>
<td>128±5.3</td>
<td>3 (a, b, c)</td>
</tr>
<tr>
<td>Sta. Fe</td>
<td>33</td>
<td>2,310</td>
<td>64±2.3</td>
<td>5 (a, b, c, d, e)</td>
</tr>
<tr>
<td>Telesfora</td>
<td>81</td>
<td>940</td>
<td>26±1.8</td>
<td>4 (a, b, c, d)</td>
</tr>
<tr>
<td><strong>Low risk</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMU-DP</td>
<td>321</td>
<td>231</td>
<td>6±0.6</td>
<td>1 (c)</td>
</tr>
<tr>
<td>PCC-CMU</td>
<td>410</td>
<td>101</td>
<td>3±0.3</td>
<td>1 (c)</td>
</tr>
<tr>
<td><strong>Total/Grand mean</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>14,943</strong></td>
<td><strong>70±5.2</strong></td>
<td>5 (a, b, c, d, e)</td>
</tr>
</tbody>
</table>

†Means in a column are significantly different (*p*<0.05)
†† (a) *Chrysops cinctus* Bigot; (b) *T. ceylonicus* Schiner; (c) *Tabanus partitus* Walker; (d) *T. reducens* Walker; and, (e) *T. philippinensis* Kröber
7.3.2 Abundance of tabanids as affected by rainfall

The total monthly abundance of tabanids in relation to monthly rainfall in each collection site is shown in Figures 7.5 and 7.6. Overall, there was a significant positive correlation between the total monthly catch of tabanids and total monthly rainfall in different sites in high-surra risk areas ($R^2=0.14; p<0.01$). There was also a significant correlation between the total monthly catch of tabanids and total monthly rainfall in San Gabriel ($R^2=0.86; p<0.001$), Duangan ($R^2=0.46; p<0.05$), Sta. Fe ($R^2=0.51; p<0.01$) and Telesfora ($R^2=0.56; p<0.01$). There was no significant correlation between the total monthly catch of tabanids and total monthly rainfall in Bukidnon (low-surra risk) ($R^2=0.05; p=0.307$).
Figure 7.5  Monthly total tabanid catches using Nzi traps (n=3 per site) and total rainfall from different villages in the Caraga Region (high-surra risk) (a. San Gabriel, Veruela; b. Duangan, Esperanza; c. Sta. Fe, Tagbina; and, d. Telesfora, Tubay).
7.3.3 Multiple linear regression model to predict tabanid catches

Given the preliminary simple linear regression analyses, the function fitted was:

$$\log_{10}(\text{catch+1}) = b_0 + b_1 \times (\log_{10}(\text{rainfall})) + b_2 \times \text{altitude}.$$  

All three parameters ($b_0$-$b_2$; Table 7.6) were highly significant ($R^2=0.894$; $p<0.001$) and are thus included in the final regression model which becomes:

$$\log_{10}(\text{catch+1}) = 1.693179 + 0.356064 \times (\log_{10}(\text{rainfall})) - 0.003819 \times \text{altitude}$$
Table 7.6  Results of multiple linear regression analysis to predict log$_{10}$ (total) monthly tabanid catches from log$_{10}$ (total) monthly rainfall (mm) and altitude (m). Parameters fitted to the data were: intercept $b_0$ and coefficients $b_1$ and $b_2$ for rainfall and altitude, respectively.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimated value</th>
<th>Standard deviation</th>
<th>$t$ test</th>
<th>$p$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$b_0$</td>
<td>1.693179</td>
<td>0.3112250</td>
<td>5.440</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>$b_1$</td>
<td>0.356064</td>
<td>0.1273490</td>
<td>2.796</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>$b_2$</td>
<td>-0.003810</td>
<td>0.0001763</td>
<td>-21.613</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

7.3.4 Molecular detection of trypanosomes in tabanids

*Trypanosoma evansi* and *T. theileri* were detected in at least one fly from each tabanid species examined using the nested PCR (McInnes *et al.*, 2009) but not using the conventional PCR with pMUTEC primer (Wuyts *et al.*, 1994). *Trypanosoma theileri* was detected in a significantly higher proportion of tabanids (63.3%; 95% CI: 49.9, 75.4) compared to *T. evansi* (28.3%; 95% CI: 17.5, 41.4) ($\chi^2=16.3$, $p<0.001$). There were no significant differences in the proportion of each tabanid species that contained *T. evansi* ($\chi^2=2.7$, $p=0.604$) compared to *T. theileri* ($\chi^2=6.4$, $p=0.172$) (Figure 7.7).
7.3.5 Molecular detection of animal hosts of tabanids

The vertebrate cytochrome $b$ gene was detected in all tabanids (n=60) examined from the Caraga Region using a PCR. Five different host species (including humans) were identified as sources of the blood meals for these tabanids (Table 7.7). Almost half of the tabanids examined had fed on humans and a quarter had fed on chickens. A higher proportion of tabanids had fed on buffaloes compared to goats and pigs ($\chi^2=15.42$, $p<0.001$).
Table 7.7 Host species of tabanids in Mindanao based on molecular analysis of their blood meals for vertebrate cytochrome b.

<table>
<thead>
<tr>
<th>Host species</th>
<th>T. reducens</th>
<th>T. philippinensis</th>
<th>C. cinctus</th>
<th>T. ceylonicus</th>
<th>T. partitus</th>
<th>Total (n=60)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Host species</td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td>Human (Homo sapiens)</td>
<td>5</td>
<td>50</td>
<td>3</td>
<td>30</td>
<td>3</td>
<td>30</td>
<td>4</td>
</tr>
<tr>
<td>Buffalo (Bubulus bubalis)</td>
<td>2</td>
<td>20</td>
<td>4</td>
<td>40</td>
<td>3</td>
<td>30</td>
<td>2</td>
</tr>
<tr>
<td>Chicken (Gallus gallus)</td>
<td>2</td>
<td>20</td>
<td>2</td>
<td>20</td>
<td>4</td>
<td>40</td>
<td>4</td>
</tr>
<tr>
<td>Goat (Capra hircus)</td>
<td>1</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pig (Sus scrofa)</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

† 20 tabanids were examined
7.4 Discussion

This is the first extensive study conducted in southern Philippines to determine the local tabanid fauna and their abundance, and the application of molecular techniques to detect and identify trypanosomes in tabanids and the sources of blood meals of these flies. Tabanids are a highly diverse group of flies with more than 4,000 species distributed worldwide in different geographical habitats (Foil, 1989; Gillott, 2005). At least 50 species of tabanids have been identified in the Philippines (Philip, 1959) but only five species were caught using Nzi traps in Mindanao in the present study. Nzi traps have been shown to be efficient for catching tabanids in different habitats in: Australia (Van Hennekeler et al., 2008), North America (Mihok et al., 2006), Africa (Mihok, 2002; Desquesnes and Dia, 2004) and South America (Martins et al., 2008). It is likely that there are other tabanid species in Mindanao that were absent in the sites where traps were set because tabanid fauna may vary from one habitat to another (Mullens and Gerhardt, 1980; Barros, 2001). In this study, *T. partitus* was caught at each sampling site, *T. reducens* and *T. philippinensis* in forested areas and *C. cinctus* was only trapped in low-lying sites. The variation of tabanid fauna in different habitats was also observed in Japan (Hayakawa, 1986). Hayakawa (1986) reported that the composition of tabanid species differed between the lowland compared to the mountainous areas. Furthermore, 19 species of tabanids were caught in an open grassland whilst only 17 species were caught from the savannah habitat in Brazil (Barros, 2001). Therefore, a wider survey of tabanids will be necessary to ascertain the complete fauna in Mindanao and the likely variation in species composition in various habitats.

There is some confusion in the classification of *T. partitus* and *C. cinctus* in the Philippines. *Tabanus partitus* was the most predominant species caught by Nzi traps in this study. It was erroneously reported as *T. striatus* by Mitzmain (1913, 1914), Kröber
(1924, as cited by Burger and Thompson, 1981) and Philip (1959) but was re-classified by Burger and Thompson (1981) after samples were re-examined. There are only a few morphological differences between *T. partitus* and *T. striatus*. The most distinctive features are: absence or evanescent median pale stripe on the second tergum over the dorsal abdomen on a black abdominal background, and clear, untainted costal cell for *T. striatus* whilst *T. partitus* has complete median pale stripe on the second tergum over the dorsal abdomen on a dark brown to brown black abdominal background, and a yellow tinted costal cell (Burger and Thompson, 1981). *Tabanus partitus* is widely distributed in Thailand, Indonesia, Micronesia, Myanmar, Malaysia, India and the Philippines (Burger and Thompson, 1981; Veer et al., 2002). It (reported as *T. striatus*) has been confirmed to transmit *T. evansi* to susceptible animals in the Philippines (Mitzmain, 1913c; 1914). In contrast, *Tabanus striatus* is common in Pakistan, India, Sri Lanka, China (Burger and Thompson, 1981; Veer et al., 2002), and in Thailand (Boonchit et al., 1996). On the other hand, *Chrysops cinctus* was earlier identified as *C. signifera* by Philip (1959) but was also re-classified by Burger and Chainey (2000) following the original description by Bigot (1892, as cited by Burger and Chainey, 2000). *Chrysops cinctus* is common in the Philippines and in parts of Sabah, Malaysia whilst *C. signifer/signifera* occurs in Indonesia (Burger and Chainey, 2000).

Most species of tabanids prefer low-lying, swampy or wet zones, close to forests or trees. This is because their larvae are aquatic or semi-aquatic requiring water for optimum development and the adult female haematophagous flies usually wait on the leaves of trees for passing hosts on which to feed (Service, 1986; Kettle, 1992; McElligott and Lewis, 1996; Krčmar et al., 2006b). This may explain the high abundance of tabanids in the two collection sites in Agusan del Sur, which are characterised by habitats that include numerous water bodies traversing villages, vast
marshland, rice fields and abundance of small forests and timber plantations. Furthermore, rainfall is well distributed throughout the year in the Caraga Region which favours the development of the larval stages of these flies. *Chrysops cinctus* were only caught in low-altitude sites because most *Chrysops* spp. favour low-lying and swampy habitats (Service, 1986). However, fewer *C. cinctus* were caught than expected because they are less attracted to unbaited traps (Hansens *et al.*, 1971; Allan *et al.*, 1987).

The high abundance of tabanids at the collection sites in the Caraga Region and presumably most areas in the region, may explain the high prevalence of *T. evansi* infection in livestock in the region (refer to Chapters 3, 5 and 6). As a consequence, livestock reared in or imported into these low-lying zones will have a high risk of becoming infected with *T. evansi*. In contrast, the low tabanid abundance in high-altitude areas of Bukidnon supports the inference that surra is absent in the province because of its high altitude. The observed lower abundance of tabanids in low-altitude compared to high-altitude collection sites is in agreement with the results of studies in Europe (Davies *et al.*, 1971). In addition, few tabanids were trapped using Manitoba traps in high altitude (1,050 m, asl) sites in the Switzerland (Auroi, 1983). However, it is not possible to completely ignore the potential presence of tabanids in high altitude areas. Published studies have shown that *T. nipponicus*, *T. chrysurus*, *T. rufidens*, *Atylotus bivitrateinus* and *T. rufidens* are present in mountainous areas (880 m, asl) of Japan (Ito and Matsumura, 1987) and *T. infestus* in mountainous areas in Armenia and Azerbaijan, as high as 1,700 m above sea level (Dolin and Andreeva, 1983). A number of other studies in Japan (Hayakawa, 1986), Croatia (Hackenberger *et al.*, 2009) and the USA (Mullens and Gerhardt, 1980) support these observations. The observation that *T. partitus* is present in low and high altitude areas in Mindanao may suggest that the altitude of Bukidnon is not sufficiently high to select for specific populations of tabanid.
The observation that tabanids are abundant throughout the year in the Caraga Region with peak populations associated with high rainfall is important because it corresponds well to most of the large outbreaks that occurred in this region in recent years. For example, the large outbreaks of surra in Agusan del Norte and Misamis Oriental in 2008 started in March and April after the high rainfall in January and February (Dr. J. Dargantes, Dr. A. Ramosa, pers. comm., 2008). High number of cases of surra in Agusan del Sur and Surigao del Sur also occurred during or immediately after the rainy months (Dr. P. Calo, Dr. M. Samar and Dr. G. Yparragueirre, pers. comm., 2007). In other surra-endemic countries, outbreaks of surra were also observed when tabanid populations were high particularly during the rainy season (Löhr et al., 1985; Dirie et al., 1989).

Seasonal fluctuations in the abundance of tabanids have been documented from earlier studies in the northern Philippines (Manresa and Mondoñedo, 1935, data re-evaluated by Dobson et al., 2009) and in several countries in north (Wright et al., 1984; White et al., 1985; Strickler and Walker, 1993; McElligott and Lewis, 1998) and south America (Barros and Foil, 1999; Barros, 2001; Koller et al., 2003; Oliveira et al., 2007); Europe (Krčmar, 2005), Africa (Dia et al., 1998; Ahmed et al., 2005; Sinshaw et al., 2006); Australia (Van Hennekeler, 2007); and, Asia (Ito and Matsumura, 1987; Boonchit et al., 1996; Suh et al., 2005). Tabanids need water for reproduction and development (Fominykh and Eremina, 1984; Kettle, 1992; Krčmar et al., 2006b) therefore their abundance during the rainy season is logical. Other climatic factors (i.e., temperature, humidity) have been reported to influence tabanid abundance (Davies et al., 1971; Dale and Axtell, 1975; McElligott and Galloway, 1991; Gorayeb, 1993; Strickler and Walker, 1993; Polyakov, 1994). These factors may be less important in tropical countries like the Philippines where there is less climatic variation. Information on the
seasonal abundance of tabanids in surra-endemic areas of Mindanao would enable the
development of more focussed control programmes with surveillance and preventive
activities scheduled to occur prior to the high risk periods (i.e., prior to the onset of the
rainy season).

The predictive multiple linear regression model for tabanid abundance developed in this
study using altitude and rainfall as inputs will allow the development of maps of
environmental suitability for tabanids in Mindanao. Such data may enhance the
forecasting capacity of control programmes and hence the ability of local livestock
agencies to more effectively prioritise surveillance and control activities. However,
additional data on tabanid abundance at different altitudes (90 to 300 m, asl) are needed
to validate the model as the current data do not include observations at these mid-level
elevations. Other factors that may significantly favour tabanid proliferation in
Mindanao such as presence of water bodies, vegetation (wooded areas) and host
populations (Fominykh and Eremina, 1984; Foil and Hogsette, 1994; Krčmar, 2005;
Krčmar et al., 2006b) should also be investigated and be included in the model to
enable prediction of tabanid abundance at a certain locality with more certainty. The
use of this predictive model in other areas with different geo-climatic features from
Mindanao should be dealt with caution because tabanid species and their dynamics may
vary between different habitats (Mullens and Gerhardt, 1980; Ito and Matsumura, 1987;
Van Hennekeler, 2007; Butt et al., 2008).

Unbaited Nzi traps were used in this study to collect tabanids. The Nzi trap was chosen
because it is simple to manufacture using local resources, robust and relatively efficient
(Mihok, 2002; Van Hennekeler et al., 2008). It is likely that a higher number of
tabanids would have been caught in the traps if carbon dioxide or odorous bait were
used (Krčmar et al., 2005; Krčmar et al., 2006a; Van Hennekeler et al., 2008). However, this was considered impractical in the field sites chosen for this study. In addition, recent studies have shown that the diversity of species caught in baited and unbaited Nzi traps does not differ (Mihok et al., 2007; Van Hennekeler et al., 2008).

Tabanids locate their target hosts using olfactory and visual cues (Allan et al., 1987; Phelps and Holloway, 1992). The former is necessary to detect hosts at a distance whilst specific location of animal targets depends on the latter (Gibson and Torr, 1999). Tabanids are more attracted to moving, large, dark-coloured (black, blue and red) than light objects (white, yellow) (Bracken and Thorsteinson, 1965; Phelps and Vale, 1976; Phelps and Holloway, 1992; Sasaki, 2001) or animals (Manresa and Mondoñedo, 1935; Thompson and Pechuman, 1970; Konstantinov and Ul’ianov, 1988; Horváth et al., 2010a). Recent evidence suggests that tabanids are polarotactic (Horváth et al., 2010b) that is, they find their hosts by the extent of polarisation of reflected light emanating from the surface of the hosts or objects. Dark coloured objects or animals produce high horizontal polarised light and readily attract tabanids (Kriska et al., 2009; Horváth et al., 2010b). This is the reason why Nzi and other tabanid traps are made up of dark coloured backgrounds (Hribar and Foil, 1994; Sasaki, 2001; Mihok, 2002; Mihok et al., 2006). Several natural (carbon dioxide, animal urine) and synthetic substances (octenol, phenols, acetone) have been shown to lure tabanids and increase trap catches (Schreck et al., 1993; Foil and Hribar, 1995; Krčmar et al., 2005; Krčmar et al., 2006a; Krčmar, 2007; Mihok et al., 2007; Van Hennekeler et al., 2008).

Microscopic examination of the mouthparts and gut of insect vectors is the conventional method of detecting trypanosomes in flies (Baylis, 1997; Msangi et al., 1998; Tarimo-Nesbitt et al., 1999; Waiswa et al., 2006). However, this requires the examination of freshly caught flies. This was not possible in this study because flies were caught using
unattended traps and were mostly dead when collected for preservation. In recent years, molecular techniques using specific genetic markers have been developed to study the insect vectors of other pathogenic trypanosomes in Africa (Solano and Amsler-Delafosse, 1995; Morlais et al., 2001; Malele et al., 2003; Njokou et al., 2004; Njiru et al., 2004b; Steuber et al., 2005; Konnai et al., 2008a). There is limited published information in the application of these techniques to detect *T. evansi* in tabanids. In this study, the use of a nested PCR for 18s rRNA (McInnes et al., 2009) provided more useful information compared to the Trypanozoon-specific PCR (Wuyts et al., 1994; Wuyts et al., 1995). This is because the nested PCR is more sensitive than the simple one-step PCR in detecting trypanosomes (Ribeiro-Dos-Santos et al., 1999; Aradaib and Majid, 2006) and all trypanosome species could be identified by the non-species specific primers (18s rRNA) that were used. The observation that a higher proportion of tabanids contained the non-pathogenic *T. theileri* compared to *T. evansi* is not surprising because the former develops in tabanids (Bose and Heister, 1993) whilst the latter does not (Losos, 1980; Mahmoud and Gray, 1980; Luckins, 1988). Whilst the amount of *T. evansi* DNA detectable by the nested-PCR would largely depend on the number of *T. evansi* trapped in the mouthpart of tabanids, the potential degradation of DNA of *T. evansi* and the presence of PCR inhibitors in flies such as haemoglobin, other cellular proteins and debris may also affect DNA amplification of *T. evansi* (Penchenier et al., 1996; Boid et al., 1999). The number of *T. evansi* trapped in the mouthpart of tabanids depends on the parasitaemia of the host and the amount of blood that could be taken by tabanids during feeding. There is no data on the amount of blood that the species of tabanids from Mindanao could imbibe but other tabanid species such as *Chrysops fuliginosus, Tabanus nigrovittatus* and *T. fuscicostatus* ingest an estimated maximum of 6.12, 10.8 and 12.5 nanolitres (nL) of blood during a 15-second interrupted feeding, respectively (Knaus et al., 1993).
The molecular detection of *T. evansi* DNA in tabanids implies that these flies have been feeding on *T. evansi*-infected animals and further confirms the endemicity of surra in these villages (collection sites for tabanids) as discussed in Chapters 3, 5 and 6. This finding may not directly indicate transmission of *T. evansi* by these tabanid species. However, it is highly probable that these flies have a significant role in the transmission and spread of *T. evansi* in livestock in Mindanao because tabanids, particularly the *Tabanus spp.*, are generally considered as efficient transmitters of *T. evansi* (Mitzmain, 1914; Losos, 1980; Luckins, 1998a). Of the five species identified, *T. ceylonicus* and *T. partitus* have been proven to be efficient transmitters of *T. evansi* in Indonesia (Luckins, 1998a). In addition, the latter species (reported as *T. striatus*) has also been shown to transmit *T. evansi* in the Philippines (Mitzmain, 1913b; 1914). The success of transmission of *T. evansi* by tabanids between hosts would depend on the pathogenicity and viability of the trypanosome, parasitaemia of bitten hosts and the time interval between tabanid meals because trypanosomes do not survive long in the mouthpart of the tabanids (Losos, 1980; Luckins, 1998a).

The amplification and sequence analysis of a small fragment of the highly conserved mitochondrial vertebrate cytochrome *b* was successfully used to identify the sources of blood meals of tabanids in this study in agreement with other studies. The species of host in the blood meals of other haematophagous arthropods such as tsetse flies (Steuber *et al.*, 2005; Konnai *et al.*, 2008a), sandflies (Sant'Anna *et al.*, 2008), mosquitoes (Ngo and Kramer, 2003; Kent and Norris, 2005; Molaei *et al.*, 2006; Oshaghi *et al.*, 2006a), lice (Lord *et al.*, 1998) and ticks (Kirstein and Gray, 1996) have been identified by molecular analysis of the cytochrome *b* gene. Cytochrome *b* gene of hosts could be detected by PCR as long as 36 hours and 4 days after experimental blood meals in mosquitoes (Oshaghi *et al.*, 2006b) and tsetse flies (Steuber *et al.*, 2005),
respectively. Cytochrome $b$ gene is also used as a molecular target in identifying animal origin in human food products (De Pancorbo et al., 2004; Aida et al., 2005; Lin and Hwang, 2007; Zhang et al., 2007), animal feeds (Bellagamba et al., 2001; Prado et al., 2002) and in forensic investigations (Zehner et al., 1998; Bravi et al., 2004; Matsuda et al., 2005; Nakaki et al., 2007).

The results from this study showed that tabanids feed on a variety of different host species. Several species of tabanids are known to feed on large mammals (Hollander and Wright, 1980; Barros, 2001), reptiles (Medem, 1981; Ferreira et al., 2002), birds (Limeira-de-Oliveira et al., 2002; Ferreira and Rafael, 2004) and humans (Strother, 1999). This wide preference of hosts of tabanids facilitates transmission of $T. evansi$ to a diverse species of domestic and wild mammalian hosts and makes the epidemiology of surra and its control more complex. Cattle and horses were not identified in the blood meal of the tabanids examined in this study because they were not present in the two locations where tabanids were caught. Further studies in Mindanao that include areas where a variety of domestic and wild animals are present are required to confirm the host preferences of tabanids in the field. This information may assist in further interpretation of the results obtained from survey activities described in Chapters 3 and 5.

The detection of humans as one source of blood meal for tabanids in Mindanao confirms claims by local farmers that they are frequently bitten by tabanids. In this study, tabanids were trapped from sites close to human residences where the probability of flies feeding on humans is highest. In general, humans are not the main hosts for tabanids yet these flies also attack and bite humans (White, 1977; Thomas, 1978; Strickler and Walker, 1993; Pechuman and Dearborn, 1996; Strother, 1999). Tabanid
bites cause nuisance, irritation, allergy, anaphylaxis, blood loss (Hollander and Wright, 1980; Hemmer et al., 1998; Strother, 1999; Hrabak and Dice, 2003) and transmission of disease pathogens in humans (Service, 1986; Cheke et al., 2003; Nigrovic and Wingerter, 2008; Padgett and Jacobsen, 2008; Mullen et al., 2009). Humans are generally resistant to *T. evansi* infection. However, there is a risk that humans may become infected with *T. evansi* in areas where there are a small number of individuals that are immuno-compromised or who have deficiencies in apolipoprotein L-I (APOL1) similar to the farmer in India (Joshi et al., 2005; Powar et al., 2006; Vanhollebeke et al., 2006).

The complex biology of tabanids (involving aquatic stage) makes their control difficult. Therefore, a more holistic approach to decrease contact between animals and vectors and prevent transmission of *T. evansi* would involve: use of repellents on animals (Foil et al., 1991), confinement and sheltering, selective grazing of animals in pastures far from wooded areas and water bodies, using protective rugs and hoods, separating animals (~200 m) in pasture (Mitzmain, 1913c; Foil, 1989; Foil and Hogsette, 1994; Barros and Foil, 2007) and use of baited dark-coloured traps and fly targets impregnated with insecticides (Pavlova, 1988; Day and Sjogren, 1994; Hall et al., 1998b; Mihok et al., 2006). The use of fly traps baited with aged animal urine as natural fly attractant (Krčmar et al., 2005; Krčmar et al., 2006a) could be a practical, cheap, sustainable and environment-friendly option of reducing tabanid populations in common pastures in villages in Mindanao. This can be applied as part of a community-based control strategy for surra. However, there is a need to explore what natural odour attractant is best for tabanid species in Mindanao. This is because the response of tabanids to a particular odour attractant varies from species to species (Krčmar et al., 2006a; Mihok et al., 2007). In addition, as tabanids were observed to be mostly abundant during late
afternoon in this study and in previous studies (Manresa and Mondoñedo, 1935; Burton, 1978), pasturing/tethering animals before this period, at night or early morning may lessen tabanid attacks. Further studies are also required to verify the observation by local farmers that mud sticking to the bodies of buffaloes after wallowing protects their animals from tabanid bites.
General Discussion

The aims of this project were to: determine the extent and impact of *T. evansi* infection in village livestock, estimate losses and financial benefits of treatment of the disease (surra), determine the impact of combined infection with *F. gigantica* and investigate potential insect vectors of *T. evansi* in Mindanao, southern Philippines. There is insufficient information on the epidemiological aspects and impact of surra on livestock in the Philippines despite its occurrence for more than a century. This information is required to enable the development of more efficient, economical and practical control programmes for *T. evansi* infection in Mindanao and other areas in the country where it is endemic.

Surra is an economically important disease limiting the productivity of livestock in Mindanao. It has a significant negative impact on the buffalo population in surra-endemic areas due to high mortality, in particular of young buffalo cows in their productive years, and low reproduction output. Losses from infection with *T. evansi* may also include: reduced draught capacity, loss of bodyweight, reduction of market value, the cost of replacement stock, the cost of treatment, and loss of opportunity to improve the quality of breeding stock. The evidence of reduced reproduction in buffaloes has great implication but has been previously underestimated.

The outputs of the bio-economic model that was developed to determine the outcome of infection with *T. evansi* in buffaloes and other livestock will enable the development of rational and cost-effective control strategies for surra in Mindanao. This is because it provides accurate estimates of the economic and production impacts of surra on
populations of livestock and predicts the likely outcome and financial benefits from different control strategies. The present control regimen in Mindanao of annual treatment of seropositive buffaloes, cattle and horses with diminazene was shown to have poor cost-effectiveness mainly because of its poor efficacy in the field. Constant monitoring of livestock (by animal owners) and targeted treatment of all sick animals (including goats/sheep and pigs) throughout the year with a highly effective drug such as melarsomine is the most practical and economically viable strategy to control *T. evansi* infection in livestock in Mindanao. Twice a year treatment of all animals provides similar net financial benefits but the probability of developing drug resistance from mass chemotherapy is a disadvantage of this option. The use of diminazene as treatment for *T. evansi* infection in livestock in Mindanao should be discontinued and the extent of resistance against this drug amongst Mindanao isolates of *T. evansi* should be investigated.

The results of surveys in the Caraga Region of Mindanao show that *T. evansi* infection is highly endemic despite massive control efforts. The prevalence of *T. evansi* was lower in cattle, goats and pigs compared to buffaloes in the Caraga Region of Mindanao. However, it is expected that these populations of animals would still suffer significant production losses when other risk factors are present (i.e., poor nutrition and proper management, concurrent infections, etc.). These results also imply that small ruminants and pigs may play an important role in the epidemiology of surra as reservoirs for *T. evansi* for other susceptible livestock.

The strong association observed between *T. evansi* infection and reproductive problems and early calf mortality in buffalo cows confirms observations of reduced calving output of buffaloes in high-surra risk areas in Mindanao. It further supports the
inference that surra significantly reduces the productivity of buffaloes in high-surra risk areas in Mindanao, where farmers import replacement stocks as breeding and working animals. Results from this study indicate that *Brucella* and *Neospora* spp. do not contribute significantly to the reproductive losses observed in high-surra risk areas. It is possible that other infections (e.g., leptospirosis, toxoplasmosis) may play a role, which requires further investigation.

The isolates of *T. evansi* collected in Mindanao possess the RoTat 1.2 gene. This suggests that the CATT and other RoTat 1.2-based tests (e.g., PCR, ELISA) could be used to diagnose *T. evansi* infection in livestock in Mindanao. There is still a need however, to evaluate further the performance of the CATT and to improve diagnosis of *T. evansi* infection in the island and, the application of molecular techniques should be explored.

The observation that there was a strong association between infection with *T. evansi* and *F. gigantica* in buffaloes is expected because both parasites share some common environmental determinants. Combined infections of *T. evansi* and *F. gigantica* in buffaloes resulted to more severe negative impact compared to individual infections as evidenced by poor body condition of infected animals. This information implies that animal health improvement programmes in Mindanao must include strategies to reduce infections with both *T. evansi* and *F. gigantica*. This further indicates the need to explore the impact of concurrent *T. evansi* infection with other important infections (e.g., haemorrhagic septicaemia, haemonchosis, etc.) present in Mindanao and in areas where surra occurs.
Only five species of tabanids were identified in this study as potential vectors of *T. evansi*. This study showed that the abundance and possibly the diversity of tabanids in Mindanao are related to rainfall and altitude. This is important because it appears that outbreaks of surra in Mindanao have largely occurred in low-lying areas with significant rainfall. In general *T. evansi* infection is highest in livestock in low-altitude areas (<100 m) where tabanids are highly abundant and absent in animals in high-altitude areas (>300 m) where tabanid abundance is considerably lower. Tabanid control using cheap and sustainable means (i.e., using fly traps and targets baited with natural attractants, repellents, reducing contact of livestock to tabanids) should be investigated in Mindanao to complement effective chemotherapy of *T. evansi* infection.

The control of *T. evansi* infection in livestock in Mindanao should be given high priority by the government and other animal stakeholders. However, the present surveillance and control strategy for *T. evansi* infection in Mindanao should be re-evaluated and improved to make it more economically viable and sustainable. Control efforts should be prioritised in areas where the risk of surra is high so that the impact of limited resources are maximised. There is also a need to continue educating animal farmers on the impact of *T. evansi*, its biology, transmission and control. They should be empowered to actively take part in the implementation of an integrated, village-based, sustainable, and cost-effective control programme for *T. evansi* infection in Mindanao.


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