

**EPIDEMIOLOGICAL STUDIES ON CAMEL
TRYPANOSOMOSIS (SURRA) AND ITS CONTROL AND
ECONOMIC IMPACT IN SOMALILAND**

By

**Abdirahman A. Salah
(DVM & MVS)**

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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 previously been submitted for a degree at any tertiary education institution.

Abdirahman Salah

Abstract

Surra is a disease caused by the pathogenic trypanosome, *Trypanosoma evansi*, and is distributed throughout Africa, Asia and South America. The study outlined in this thesis was conducted to determine the epidemiology of trypanosomosis in camels, its economic impact on camels raised under a traditional pastoral production system and potential vectors that could transmit the disease between camels in Somaliland. Prior to this study there was limited information on the distribution and impact of surra in camels in Somaliland, although field reports indicated significant losses of production and consequently impact on the livelihood of pastoralists.

In this study 2,575 camels were sampled from 144 herds and tested with the CATT/*T. evansi*. The animal level test seroprevalence observed was 26.4% (95% CI 24.8, 28.2) (real prevalence after adjusting for test sensitivity and specificity was 38.2%; 95% CI: 36.0, 40.0). The seroprevalence varied significantly between regions ($p < 0.05$) with a higher level (37.2%) in Sahil than in Awdal (19.3%) or Waqoyi Galbed (17.4%).

A susceptible-infectious-subclinical (SIC) model was constructed in order to determine criteria for successful disease control by mass and targeted chemotherapy. This was used to simulate and estimate the economic benefits of four different control options against surra in camels. Adopting biannual treatment of all camels, monthly targeted treatment of clinically sick camels or biannual targeted treatment of seropositive camels was estimated to result in a benefit of US\$141,431, 170,577 and 114,625 per village (80 camels) for a five year period in the study area, respectively.

The prevalence after five years of control was predicted to be 7.4, 6.4 and 6.7% for the biannual treatment of all camels, monthly targeted treatment of clinically sick camels or biannual targeted treatment of seropositive camels, respectively compared with 72.2% if no treatments were applied. The annual revenue lost in the studied camel herds was estimated at US\$404,630 (20159 camels studied) if no treatment was administered. The greatest loss was associated with decreased milk yield (US\$314,630).

As part of this research Nzi and biconical traps were set to trap tabanids responsible for transmitting trypanosomosis. Three genera of tabanids were trapped (*Philoliche*, *Tabanus* and *Haematopota*) and these flies were recognised as potential vectors of trypanosomosis in camels. *Philoliche* species were the most widely distributed and abundant biting flies in the area. The activity of the biting flies differed throughout the day, with the highest activity observed in the middle of the day and the lowest in the afternoon. There was a significant difference between the alighting sites of biting flies on camels, with the lower body and belly of camels being the preferred sites compared to the upper body (head, neck and hump). In this study on average 0.87 ± 0.34 flies of the *Philoliche* genus alighted on the lower body and belly of camels in the middle of the day.

The results of this study strongly support the need for implementation of surveillance and control programs for trypanosomosis in camel herds in Somaliland.

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Table of Contents

Declaration	ii
Abstract	iii
Acknowledgements	v
Conference Presentations (Oral)	xi
Poster Presentations	xii
List of tables	xiii
List of figures	xv
CHAPTER ONE	1
GENERAL INTRODUCTION	1
1.1 Background.....	1
1.1.1 Somaliland’s Historical, Geographic and Demographic Features.....	1
1.1.2 Pastoral productionsystem.....	5
1.2 The purpose of the current study	7
1.3 The study	8
CHAPTER TWO	9
Review of the epidemiology of <i>Trypanosoma evansi</i> (surra), its vectors and economic impact on camels in Somaliland	9
2.1 Introduction.....	9
2.2 Biology and ecology of <i>Trypanosoma evansi</i>	11
2.2.1 Classification and origin	11
2.2.2 Morphology of <i>T. evansi</i>	11
2.2.3 History of the disease	13

2.3	Epidemiology of <i>T. evansi</i>	14
2.3.1	Clinical signs	16
2.3.2	Mechanical transmission	17
2.3.3	Vectors.....	20
2.3.4	Distribution of <i>T. evansi</i>	22
2.3.5	Pathology and pathogenesis.....	24
2.4	Surra in pastoral communities	26
2.5	The influence of seasonal animal movements on the transmission of <i>T. evansi</i>	27
2.6	Socio-economic importance of livestock in Somalia and Somaliland	29
2.7	Economic impact of surra.....	33
2.8	Diagnosis	34
2.8.1	Parasitological tests	36
2.8.2	Serological and antigenic tests	38
2.9	Prevention and control of surra	46
CHAPTER THREE		49
Prevalence and distribution of <i>Trypanosoma evansi</i> in camels in Somaliland		49
3.1	Introduction.....	49
3.2	Materials and methods	50
3.2.1	Sampling sites.....	50
3.2.2	Study design and sampling	52
3.2.3	Serological test	54
3.2.3.1	Card agglutination test (CATT/ <i>T.evansi</i>)	54
3.2.4	Statistical analyses	55
3.3	Results	55
3.2.1	Seroprevalence test analysis	55
3.3.2	Distribution of surra.....	56
3.3.3	Ecological zones	58
3.3.4	Age and gender.....	59
3.3.5	Body condition score	60
3.4	Discussion.....	62

CHAPTER FOUR.....	67
Modelling the potential benefits of different strategies to control infection with <i>Trypanosoma evansi</i> in camels in Somaliland	67
4.1 Introduction.....	67
4.2 Materials and methods	68
4.2.1 Susceptible-infectious-subclinical (SIC) disease model for surra.....	68
4.2.1.1 SIC transition states model.....	70
4.2.1.2 Mechanical transmission	74
4.2.2 Evaluation of the SIC model for surra control regimens in Somaliland ..	77
4.2.3 Cost-benefit analysis.....	80
4.3 Results.....	82
4.3.1 Surra/camel SIC model for control regimens	82
4.3.2 Cost benefit analysis	83
4.4 Discussion.....	85
CHAPTER FIVE.....	91
Estimating the economic impact of <i>T. evansi</i> on pastoral camel production in Somaliland	91
5.1 Introduction.....	91
5.2 Materials and Methods.....	93
5.2.1 Description of the Study Area	93
5.2.2 Study Design.....	93
5.2.3 Assessment of the economic loss from surra.....	93
5.2.4 Direct financial costs and benefits of <i>T. evansi</i> treatment strategies.....	96
5.2.5 Risk of <i>T. evansi</i> infection.....	96
5.2.6 Components of the treatment strategies to control infection with <i>T. evansi</i>	98
5.2.7 Data Analysis.....	99
5.3 Results.....	100
5.3.1 Economic loss assessment.....	101
5.3.2 Benefit/cost ratios of treatment strategies	103
5.4 Discussion.....	104

CHAPTER SIX.....	109
The alighting and activity patterns of potential vectors of <i>T. evansi</i> on camels in Somaliland	109
6.1 Introduction.....	109
6.2 Materials and Methods.....	111
6.2.1 Study area	111
6.2.2 Study design	111
6.2.3 Field observations on the daily activity and alighting pattern of tabanids.....	112
6.2.4 Wing length measurement	113
6.2.5 Data analysis.....	114
6.3 Results.....	114
6.3.1 Daily activity and alighting pattern of tabanids on camels	115
6.3.2 Wing length measurement	117
6.3.3 Effect of weather variables on total weekly catch.....	120
6.4 Discussion.....	121
CHAPTER SEVEN.....	129
General Discussion	129
REFERENCES	138
APPENDICES	152

Publications and presentations

Publications

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List of tables

Table 2.1 Number of livestock exported from 2000 to 2010 through Berbera Port (Ahmed Heybe, personal communication)	32
Table 2.2 Livestock prices at local and international markets (Personal communication with different livestock dealers).....	32
Table 2.3 Tax per head on animals exported from Somalia (Ahmed Matan, personal communication).....	33
Table 3. 1 Location of camels sampled from six regions of Somaliland (2010 to 2011)	53
Table 3.2 Seroprevalence of <i>T. evansi</i> infections in regions in Somaliland (2011 to 2012)	57
Table 3.3 Seroprevalence of <i>T. evansi</i> infection in ecological zones	59
Table 3.4 Effect of age-class on the seroprevalence of <i>T. evansi</i> using the CATT/ <i>T. evansi</i>	59
Table 3.5 The seroprevalence to surra in camels with different body condition scores	61
Table 4.1 Parameters and formulas used in the SIC model for <i>T. evansi</i> in camels	71
Table 4.2 Input parameters and symbols used for the SIC model to assess the four control regimens.....	73
Table 4.3 Model input parameters used for four different control options for surra in camels.....	79
Table 4.4 The results of the deterministic model and the mean (total number of treatment regimens 1-3) of drug treatments used in the first five years for three different regimens 1 to 3	82
Table 5.1 Definition of effect, population size, production loss and benefits of treatment against surra	96
Table 5.2 Components of the estimated benefit-cost analysis for evaluating treatment strategies for surra	98
Table 5.3 Control strategies against <i>Trypanosoma evansi</i> evaluated in camels from Somaliland.....	99
Table 5.4 Comparison of the revenue generated when surra was present or absent..	102

Table 5.5 Economic effects of surra on camel production and the benefits of treatment	102
Table 5.6 Benefit-cost ratios of different treatment strategies for <i>T. evansi</i> in camels	104
Table 6.1 Activity pattern of Tabanids feeding on camels during the day in the field	116
Table 6.2 Alighting sites of horse flies on camels during the field study	117
Table 6.3 Weather variables and flies collected over the four weeks	119
Table 6. 4 Values of the mean flies caught and correlation coefficient of different weather variables	120

List of figures

Figure 1.1 Map of Somalia and regional political powers (Soumare et al., 2007)	3
Figure 1.2 Map of Somaliland regions (Somaliland Mission, 2000).....	7
Figure 2.1 Fine structure of <i>T. evansi</i> by electron microscopy of thin sections as cited by (Yaeger, 1996). (Adapted from: Vickerman, 1977).....	13
Figure 2.2 Map indicating global distribution of surra for the period January – Jun 2016 (OIE, 2017).	23
Figure 2.3 Map indicating the distribution of surra in Somalia January - Jun 2016 (OIE, 2017).	27
Figure 2.4 Production of camel milk by different countries in 2011(FAO, 2011).	31
Figure 2.5 Production of camel meat by different countries in the world in 2011 (FAO, 2011).	31
Figure 3.1 Geographic coordinates for the sites sampled in Somaliland	51
Figure 3.2 Ecological zones in Somaliland (Sommerlatte and Umar, 2000).....	54
Figure 3.3 Geographical distribution of seroprevalence	58
Figure 4.1 Susceptible -infectious-subclinical (SIC) model framework of <i>T. evansi</i> infection (Dobson et al., 2009).....	70
Figure 4.2 The number of Tabanids caught on camels in open fields over the year (Mohamed, 2011).....	76
Figure 4.3 The mean seroprevalence predicted during the first five years of treatments	83
Figure 5.1 Camels included in the study to determine the impact of <i>T. evansi</i> on camel production	95
Figure 6.1 Photo of Nzi (a) and biconical traps for fly collection (b).....	112
Figure 6.2 Distribution of Tabanids caught using traps and hand nets during the field observations.....	115
Figure 6.3 <i>Philoliche</i> species feeding on the lower flank (a) and belly of a camel (b)	116
Figure 6.4 <i>Philoliche</i> (a, b and c) and <i>Tabanus</i> genus (d and e) - the potential vectors for transmission of surra on camels in Somaliland	118

Acronyms and abbreviations

Ab-ELISA	Antibody-detecting enzyme-linked immunosorbent assay
Ag-ELISA	Antigen-detecting enzyme-linked immunosorbent assay
B/C	Benefit cost ratio
CATT	Card agglutination test for <i>Trypanosoma evansi</i>
CI	Confidence interval
CL	Confidence limit
DNA	Deoxyribonucleic acid
FAO	United Nations Organisation for Food and Agriculture
GDP	Gross Domestic Product
GPS	Global Positioning System
INT	International Prices
LAMP	loop-mediated isothermal amplification
L	Litre
m	metre
ML	millilitre
MHCT	Micro-haematocrit centrifugation techniques
MT	Metric tonnes
PCR	polymerase chain reaction
PCV	packed cell volume
SAHSP	Somali Animal Health Services Project
ISTVS	IGAD Sheikh Technical Veterinary School
spp	species
VAT	variable antigen type

CHAPTER ONE

GENERAL INTRODUCTION

1.1 Background

1.1.1 Somaliland's Historical, Geographic and Demographic Features

Somaliland gained independence on the 26th June 1960 from the British Protectorate and united with the Italian protectorate Somalia on the 1st July 1960 to form the Democratic Republic of Somalia. The country was governed democratically until 1969 when the elected civilian government was overthrown by a military regime in a bloodless coup. From 1969 until 1991 the country was under the rule of General Mohammed Siad Barre who appointed himself as President. In 1977 Somalia invaded the Ogaden region of Ethiopia, which was populated with Somali clans. By the end of 1978 Somalia suffered defeat in their invasion of the Ogaden region. This event further weakened the state's authority and national respect for the military, leading to the military no longer providing a unifying force to the Somali state. In 1991 the military dictatorship of President Barre was toppled by rival clans and a violent civil war broke out throughout the country (Cantelon, 2010).

In response to the breakdown of the northwest region, the former British colony of Somaliland declared independence on the 18th May 1991 and formed a separate government. Demographically Somalia is a clan-based society and the clans cross over the administrative district boundaries. To the north and east the districts have grouped

together to form a semi-autonomous region known as Puntland. In the remainder of Somalia an extensive civil war and instability resulted in the destruction of most of the state's infrastructure (Cantelon, 2010).

Somaliland borders are based on the former colonial boundaries and it has its own currency, flag and national policies. The security situation improved after the separation, mainly due to clan elders working hard to maintain peace by solving internal conflicts during the reconciliation process. People of Somaliland have somewhat recovered from the war and have moved towards developing and rebuilding their country.

The country is located in the Horn of Africa; bordering the Red Sea to the north, Somalia to the east, Ethiopia to the south and Djibouti to the northwest (Figure 1.1). Somaliland has an estimated area of 137,600 km² with a coastline of 850 km. It lies between latitudes 8°00' and 11°27' north and longitudes 42°30' and 49°00' east.

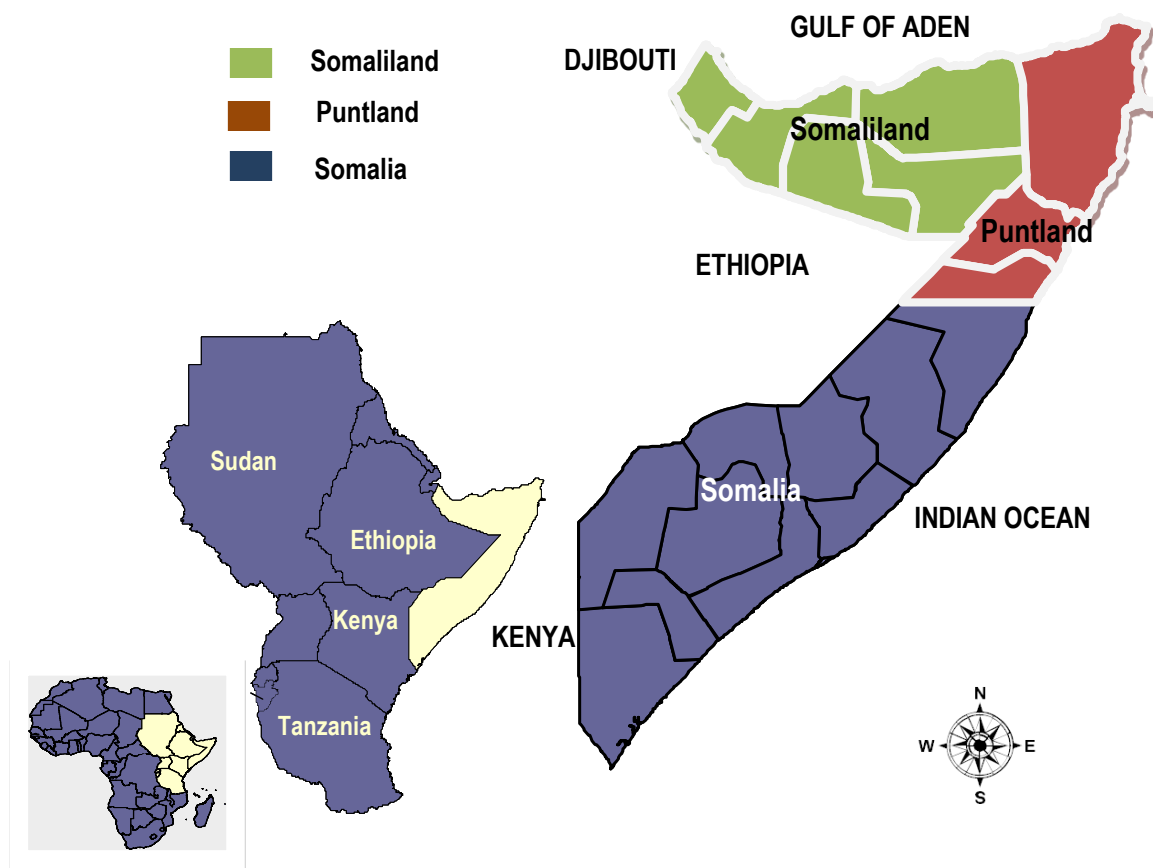


Figure: 1. 1 Map of Somalia and regional political powers

The country is divided into six regions: Togdheer, Sahil, Awdal, Waqoyi Galbed, Sanaag and Sool (Fig. 1.2). The six regions are further subdivided into 24 districts and 20 sub-districts. Somaliland receives between 100 and 300mm of rain per annum. The country is mainly arid and semi-arid, and rainfall is irregular and unevenly distributed both spatially and temporally (FAO, 2004).

Somaliland pastoralists recognise three ecological zones within the country:

1. The *Guban* – the coastal plains. ‘Guban’ literally means ‘burnt’ desiccation of much of the coast;

2. The *Ogo*–watershed mountain highlands immediately adjacent to the coastal plains;
3. The *Haud* –the higher plateau extending across the Ethiopian border.

The economy of Somaliland primarily depends upon livestock production. However, livestock production is hampered by rangeland degradation, disease, limited delivery of animal health services, shortage of pastures and water and improper land use and surface water runoff. As a result of these, livestock production has failed to provide increased income for residents or greater food security. This has led to increased vulnerability to poverty and consequently increased migration to urban centers where there are limited employment opportunities (FAO, 2004).

According to the 1997 census, the population of Somaliland was estimated to be 3.5 million with an estimated population density of 22persons per Km² (Anonymous, 2007; Soumare, 2006). Approximately 55% of the population lives a nomadic or agro-pastoral life while the remaining 45% live in cities. Somaliland’s main exports are sheep, goats, camel and cattle to Saudi Arabia, Yemen, Oman and the United Arab Emirates (UAE), through the port of Berbera (Soumare, 2006). The Somaliland economy is linked directly and indirectly to livestock trade and it is an important part of the economy and culture. The country has benefited from the stability provided by the regional government which has allowed for a functioning local economy and reasonable control over resources contained within the area (Cantelon, 2010).

1.1.2 Pastoral production system

Nomadic and agro-pastoralism are the two types of production or management systems practiced in Somaliland; however harsh environmental conditions have made nomadic pastoralism the dominant mode of production. Pastoralism describes the extensive mobile grazing of livestock on communal rangelands in response to the erratic rainfall pattern. The community is highly reliant on livestock as the only practical means of transforming pasture into food and income. A pastoral household is defined as one that obtains greater than 50% of its gross income from mobile livestock rearing on unimproved communal pastures (Rass, 2006). The pastoralists are confined to the extensive drier areas of the coastal plains and mountain valleys and the *Haud* plateaus over most of the country (Baumann and Zessin, 1992; FAO, 2004).

The types of livestock herded in Somaliland are the result of adaptation to environmental conditions, and nomads typically keep mixed herds in order to utilize different livestock products, to maximise their returns and minimise the impact of animal disease (Baumann and Zessin, 1992). Livestock in Somaliland are the major repository of individual and national wealth. Sheep, goats, camels (dromedary) and cattle are the main species of livestock reared by Somali people. Sheep, goats and camels are herded in the largest numbers, but the level of animal productivity is generally low due to disease, poor management and inadequate veterinary services (FAO, 2004). The livestock sector is threatened by the recurrent imposition of export trade bans by importing countries due to suspicions of or actual outbreaks of trade limiting animal diseases. Improvement of the livestock productivity and pastoral production system of Somaliland would provide opportunities for expansion and

diversification of trade in livestock and livestock commodities. However reduction of the risk of mortality requires a thorough understanding of the diseases present and the determinants of infection. Information on camel diseases in the country is minimal and there is also a lack of epidemiological investigations into diseases affecting livestock in Somaliland. The camel is, however, affected by many viral and bacterial diseases with the most important diseases including camel pox, brucellosis, pasteurellosis and anthrax (Baumann and Zessin, 1992).

Surra, caused by the protozoan parasite *Trypanosoma evansi*, is the most important disease of camels because it is responsible for considerable reduced camel production and economic losses (Enwezor and Sackey, 2005). The disease has a morbidity of up to 30% and a mortality of approximately 3% (Enwezor and Sackey, 2005). Cases of surra occur throughout the year and are found in most regions of Somalia (Dirie et al., 1989). Surprisingly this disease has not been recognised as an important disease in Somaliland since an outbreak in 1983 in camels in the Daragodle area of the Sahil region. This outbreak resulted in significant mortality (up to 30%) in camels (Dirie et al., 1989). Subsequently fatalities ceased when camels were moved from the affected area with the only biting fly caught in the area being a tabanid (Diptera: Tabanidae) (Dirie et al., 1989). Subsequently it became clear that trypanosomosis in camels only occurred in places where suitable vegetation for tabanids was found (Dirie et al., 1989). A survey on infection of livestock with trypanosomes was carried out in Somalia in 1985 with 3,000 blood samples from camels examined. Of these 160 (5.3%) were found to be infected with *T. evansi* (Dirie et al., 1989), however only 19 animals (1%) were infected from the herds examined in the northern regions of Somaliland.



Figure: 1.2 Map of Somaliland regions (Somaliland Mission, 2000)

More recently, field investigations on *T. evansi* in camels were conducted from 2006 to 2007 in the coastal plains (*Guban*) - Sahil region of Somaliland (Figure 1.2). The study targeted camels with apparent clinical signs and of 59 suspected infected camels, 7 were positive on stained blood smears, while a formol-gel test (biochemical test) detected infection in 22 of 44 camels tested (Mohamed, 2007).

1.2 The purpose of the current study

In Somalia there has been no systematic research on trypanosomosis in camels and its vectors to assess the impact of the disease on the production of camels. There is a lack of information on the epidemiology of the disease and its principal vector species and their ability to transmit *T. evansi*. This information is needed to understand the

dynamics of surra, its impact on pastoralists and the measures that can be adopted to control the disease.

1.3 The study

The aims of the research outlined in this thesis were to investigate the epidemiology of *T. evansi*, its impact on camels in Somaliland and the vectors that may be involved in its transmission. In Chapter Two the literature available on trypanosomosis in camels caused by *T. evansi* and the vectors responsible for transmission of the disease are discussed. In Chapter Three epidemiological studies to determine the prevalence and distribution of the disease in Somaliland are described. This is followed in Chapter Four with a discussion of a model developed to determine the impact of different control strategies on the disease in Somaliland. In Chapter Five the economic impact of surra on camels is evaluated and the benefit of control considered. The potential vectors for surra in camels in Somaliland are identified and their preferred landing sites on camels and their activity patterns described in Chapter Six. The thesis concludes with an overall General Discussion where the results of this thesis are compared and contrasted to those of other studies.

CHAPTER TWO

Review of the epidemiology of *Trypanosoma evansi* (surra), its vectors and economic impact on camels in Somaliland

2.1 Introduction

The purpose of this review is to summarise the epidemiology of *T. evansi* in camels. The biology and ecology of *T. evansi*, risk factors for the disease, knowledge of trypanosomosis in camels in Somaliland and its impact on camels under a pastoral production system will be discussed. The ultimate aim is to use this information to develop and establish more effective ways of controlling the disease in Somaliland.

The aetiological agent of surra, *Trypanosoma evansi*, belonging to the subgenus *Trypanozoon*, was discovered by Evans in 1880 in camels from the Punjab Province in the then British India (Dirie et al., 1989). 'Surra' literally means 'emaciated' in the local Indian language and is the local name for the disease (Al-Rawashdeh et al., 2000). Many researchers have shown that the agent of surra is related to the African trypanosomes and is believed to have evolved from *T. brucei* (Eyob and Matios, 2013).

Interestingly, *T. evansi* can infect most species of livestock and wild animals resulting in a polymorphic disease across a large geographical area in Africa, Asia and South and Central America (Dargantes et al., 2009; Desquesnes et al., 2013; Njiru et al., 2006). Infection with *T. evansi* can take two forms, chronic or acute, although it usually occurs as a chronic wasting disease. The acute form, when *T. evansi* is easily detectable in the blood, is almost always fatal (Ngaira et al., 2003; Njiru et al., 2004).

The agent, *T. evansi*, is mechanically transmitted between animals by various species of biting flies and infection is characterized by remittent fever resulting in emaciation, anaemia, oedema, debility and in coordinated movements (Ngaira et al., 2003; Tamarit et al., 2010). Animal trypanosomosis endemic in arid and semi-arid countries in the Horn of Africa where it causes “surra” in camels (Dirie et al., 1989; Njiru et al., 2004). In Asia and in south and central America a different range of animal species are affected, including horses, cattle and buffalo (Dargantes et al., 2009; Enwezor and Sackey, 2005).

Recently, the introduction of camel trypanosomosis into France and Spain has resulted in severe outbreaks of disease and was associated with the importation of camels from the Canary Islands (Desquesnes et al., 2009; Gutierrez et al., 2010). The disease has a major effect on the efficiency of livestock production resulting in significant mortality and considerable economic losses due to a decrease in milk and meat production and premature births or abortions (Gutierrez et al., 2005; Ngaira et al., 2003).

Horse flies and other tabanids (*Tabanidae*) are incriminated as vectors of *T. evansi* in Somalia. They require specific conditions, such as suitable vegetation and water points, to survive (Dirie et al., 1989). In Somalia, some of the tabanids (*Philoliche zonata* and *P. magretti*) only appear during the two rainy seasons. These two species appear approximately 10 days after the first rains and persist for up to 45 days into the following dry seasons (Dirie et al., 1989). Prior to the study reported in this thesis no survey had been conducted to determine the seroprevalence of surra in camels in Somaliland/northern regions of Somalia.

2.2 Biology and ecology of *Trypanosoma evansi*

2.2.1 Classification and origin

Trypanosoma evansi belongs to the subgenus *Trypanozoon*, which belongs to the genus *Trypanosoma*, Family of *Trypanosomatidae* and the order of *Kinetoplastida*. The subgenus *Trypanozoon* is divided into three species: *T. evansi*, *T. brucei* and *T. equiperdum* (Gibson, 2003). These species of the *Kinetoplastida* are characterized by the presence of Kinetoplast DNA (Lun and Dessler, 1995). *Trypanosoma evansi* most probably evolved from Africa in the north of the tsetse belt and is morphologically identical to long slender forms of the tsetse transmitted *T. brucei* and *T. equiperdum* (Lun and Dessler, 1995).

2.2.2 Morphology of *T. evansi*

Trypanosoma evansi is a small, actively motile trypanosome and is generally described monomorphic member of the *T. brucei* species (Zhang and Baltz, 1994). It measures 14 - 33 μm in length and is 1.5 - 2.2 μm wide, has a free flagellum and a small terminal or sub-terminal kinetoplast (Brun et al., 1998). It is morphologically and chemically indistinguishable from *T. brucei* and *T. equiperdum* but can be distinguished on molecular level (Njiru et al., 2006; Zhang and Baltz, 1994), with no apparent differences between strains from different regions or host species (Brun et al., 1998). *Trypanosoma evansi* and *T. equiperdum* multiply in their mammalian hosts by longitudinal binary fission, Brun et al. (1998) described that *T. evansi* and *T. equiperdum* are more closely related and indistinguishable morphologically at the light or electron microscope level.

Trypanosomes transmitted by tsetse fly must undergo replication and cyclical development within the vector (Lun and Dessler, 1995) and both *T. evansi* and *T. equiperdum* are capable of cyclical development (Gibson, 2003). *Trypanosoma evansi* does not have a functional kinetoplast. For the morphological transformation of trypanosomes in the vector, a functional kinetoplast must be present (Lun and Dessler, 1995). The absence of morphological transformation in the vector is believed to correspond with the lack of maxicircle kDNA in the kinetoplast and consequently this parasite is unable to develop in its vector and must multiply in its mammalian hosts through longitudinal binary fission (Njiru et al., 2006; Zhang and Baltz, 1994). The lack of maxicircle kDNA could be associated with the utilization of a wide range of mechanical vectors (Lun and Dessler, 1995).

Trypanosoma evansi shows dyskinetoplastic forms which appear after treatment with various trypanocidal drugs (Brun et al., 1998). *Trypanosoma evansi* isolated from an infected mouse has been demonstrated to show similar ultrastructural properties as the long slender forms of *T. brucei brucei* (Brun et al., 1998). The parasite is believed to be a mutant lineage of *T. b. brucei* that evolved in the north of the African tsetse belt by adaptation to a non-cyclic transmission (Luckins, 1988; Njiru et al., 2006). The ultrastructural features of *T. evansi* are shown in Figure 2.1.

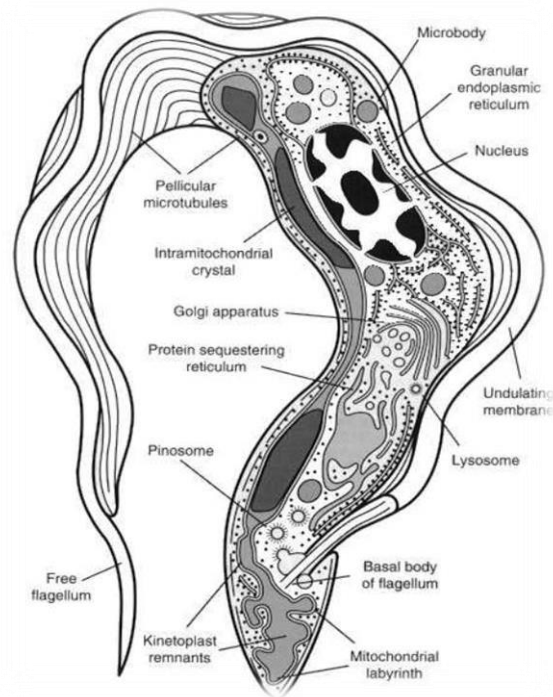


Figure: 2.1 Fine structure of *T. evansi* by electron microscopy of thin sections as cited by (Yaeger, 1996). (Adapted from: Vickerman, 1977)

2.2.3 History of the disease

The history of the *T. evansi* and its spread throughout the world was described by Hoare (1972). Among pathogenic trypanosome species, *T. evansi* has the widest host range and geographical distribution, affecting the health of livestock in many regions of the world (Herrera et al., 2004; Zhang and Baltz, 1994). It has been postulated that the parasite originated in Africa and spread to Asia and South America through the exportation of camels, horses and mules (Tamarit et al., 2010).

Trypanosoma evansi is considered to be the most important disease of dromedary camels in Africa and the Middle East. Apart from the name surra, which originates from India, there are many local terms for the disease. In Arabic speaking countries in North Africa it is known as “el debad”(FAO, 1998); “gufar” in Sudan (El Rayah et al., 1999);

“dbab” in Morocco (Atarhouch et al., 2003); “heyam” in Saudi Arabia (Al-Qarawi et al., 2004); “tribersa” in India and “salaf, dukan or maliig” in Somalia (Dirie et al., 1989).

2.3 Epidemiology of *T. evansi*

The outcome of a disease is dependent on the characteristics of a pathogen, its host, reservoir, vectors and interaction between their environments. Surra is the most widespread and multispecies disease in which these characteristics can exhibit highly complex epidemiology (Desquesnes et al., 2013). Consequently, the incidence and the severity of *T. evansi* infections observed in different regions varies due to the strains of parasite and species affected (Losos, 1980). In Africa, the principal host species of *T. evansi* infection are camels, whilst in Central and South America the horse is principally affected, and in Asia a various host species is involved, including cattle, buffalo and pigs (Eyob and Matios, 2013).

Of all the animal trypanosomosis, *T. evansi* is the sole cause of camel trypanosomosis, however camels kept close to the tsetse belt area have been reported with *T. brucei brucei* infection. Studies have described that *T. evansi* infection is restricted to the northern region of Africa and parts of East Africa, the northern most limits of the tsetse belt (Adeiza et al., 2010; Desquesnes et al., 2013). It has been suggested that the spread of surra from North Africa and India has resulted in significant losses to livestock and their owners (Losos, 1980; Reid, 2002).

The disease is an important constraint to productivity, particularly in camels which are the principal source of income and food for the large pastoral community in the Horn of Africa. The disease in camels is governed by the seasonal vector activity and the times when animals are stressed due to food or water shortage (Dia et al., 1997). The disease is more common during the rainy season when the number of blood-sucking insect specks (Desquesnes et al., 2013). A lack of knowledge about the epidemiology of surra and appropriate control measures has resulted in the parasite becoming endemic in this region (Desquesnes et al., 2013; Dia et al., 1997; Gutierrez et al., 2010).

In Somalia, the disease is present throughout the country and appears most commonly during the rainy seasons “Gu” - *autumn* (April to June) and “Deyr”- *spring* (October to November). Occasionally outbreaks result in high mortality among infected camels (Dirie et al., 1989). Infection of *T. evansi* in camels results in an acute and a chronic form of the disease. Somali pastoralists associate the acute form with the bites of tsetse fly and the chronic form with other biting flies. It later became clear that the disease was transmitted by *T. evansi* (Dirie et al., 1989). The chronic form is more common and is likely to present an association with secondary infection due to immune-suppression caused by *T. evansi* (Eyob and Matios, 2013; Olaho-Mukani et al., 1993). It results in a low parasitaemia with animals affected by surra potentially remaining carriers for several years (Dirie et al., 1989). In the acute form up to 90% of camels can die if they are not treated (Ahmed et al., 2004). There is the possibility of an increase in parasitaemia at the time of stress-induced immune suppression and animals infected with trypanosomosis could develop concurrent infections and even fatal bacterial, viral

or protozoal disease (Derakhshanfar et al., 2010). Animals that recover from the acute form are often normal and have a low parasitaemia (Ijaz et al., 1998).

2.3.1 Clinical signs

A large range of hosts are susceptible to infection with *T. evansi*. There are considerable differences in the severity of clinical signs induced by *T. evansi* in different geographical areas and these are dependent upon the virulence of the infecting strain and the susceptibility of the host (Herrera et al., 2004). However, anaemia is the main outcome of infection. Documented clinical signs of surra include pyrexia leading to emaciation, progressive anaemia, loss of body condition and weakness. Infected animals show recurrent episodes of fever and intermittent parasitaemia during the course of the disease (Atarhouch et al., 2003; Enwezor and Sackey, 2005; Ijaz et al., 1998).

In camels, surra is manifested by anaemia, oedema of the dependent body parts especially the abdomen and neck, lacrimation and sometimes late-term abortions and still births (Brun et al., 1998). Urticarial plaques and petechial haemorrhages in serous membranes and enlargement of the lymph nodes may also be observed (Atarhouch et al., 2003; Enwezor and Sackey, 2005). Nervous signs, including marked depression, circling, in coordination and dullness, have been observed in camels infected with *T. evansi* (Enwezor and Sackey, 2005). The neurological signs appear in the chronic stage of the disease and may be the result of damage of brain tissue by the parasites (Brun et al., 1998; Enwezor and Sackey, 2005).

In Africa camels are the most economically important host, however in other parts of the world horses, buffalos and cattle are affected, although other animals, including wildlife, are also susceptible (Desquesnes et al., 2013; Enwezor and Sackey, 2005). Surra is more chronic in camels than horses (Biswas et al., 2001), causing significant losses through reduced production of milk and meat and death can occur within weeks of infection if animals are left untreated (Ijaz et al., 1998; Ngaira et al., 2003).

In east Africa, surra mostly occurs in the chronic form, and is characterized in camels by the presence of a rough coat, intermittent fever, progressive emaciation, loss of hair from the tail, a characteristic fetid odour, disappearance of the hump, severe muscle atrophy of the rear quarters, oedema of the dependent parts, corneal opacity, diarrhea and sexual excitement (Boid et al., 1985; Dirie and Abdurahman, 2003). The characteristic urine smell is due to ketones produced after the breakdown of amino acids by the parasite in infected animals (Njiru et al., 2004).

2.3.2 Mechanical transmission

Trypanosoma evansi is transmitted mechanically between animals by various species of *Tabanus* and *Stomoxys*, and other blood-sucking flies such as Hippoboscids (*H. equina* and *H. camelina*) (Brun et al., 1998; Desquesnes et al., 2013; Dirie et al., 1989) without any development or multiplication of the agent in the vector (van Hennekeler, 2007).

The events that lead to the mechanical transmission of *T. evansi* are:

1. Vector initiates feeding on an infected host
2. The vector's feeding is interrupted

3. The vector moves to a susceptible host, transporting the agent on or within the body parts that routinely contact the new host
4. The vector feeds upon the susceptible host and introduces the agent via the bite wound (Foil, 1989; van Hennekeler, 2007).

The characteristics of a good mechanical vector are that it is: frequently interrupted in feeding; highly mobile; and has large mouthparts to transfer agents (Foil, 1989).

The most important mechanical vectors of surra in camels are the horse flies (tabanids). It has been demonstrated experimentally that more than 20 species of *Tabanus* are capable of transmitting the disease (Enwezor and Sackey, 2005; Reid, 2002). Despite mechanical transmission being well established, the dynamics of transmission are not completely understood. Thus, studies of the effect on the host species, the duration of infection in the host and the level of parasitaemia are important for understanding the efficiency of different vector species and the dynamics of the mechanical transmission (Eyob and Matios, 2013; Obihiro, 1998). A mathematical model on the transmission of trypanosomes has indicated that the transmission competence by tabanids is related to parasitaemia and the number of biting flies around the susceptible hosts (Desquesnes et al., 2009).

The mode of transmission of *T. evansi* is direct, but the role of vector transmission and the pathogenic effect in different species may vary between geographical areas (Brun et al., 1998; Reid, 2002). In Africa, camels and horses are the most susceptible species to infection with *T. evansi* (Desquesnes et al., 2009) with outbreaks of disease occurring where reservoir hosts, vectors and susceptible animal species are all located (Enwezor

and Sackey, 2005; Reid, 2002). In Southeast Asia, horses, dogs and camels are the most susceptible species for *T. evansi* infection, and buffalo, cattle and pigs are believed to be the reservoir hosts for the parasite (Reid, 2002).

Trypanosoma evansi has also been shown to be pathogenic in sheep and goats and these animals may also play a role as reservoir hosts (Mahmoud and Gray, 1980).

In Sudan *T. evansi* infection has been reported in these species (goats and sheep) (Enwezor and Sackey, 2005). Parasites have been shown to persist for 19 days in goats, and infection may last for years in goats. The disease has also been demonstrated in dogs, donkey and cattle after experimental inoculation (Mahmoud and Gray, 1980). In south and central America transmission by vampire bats has been reported and these animals can serve as both vectors and reservoir hosts for *T. evansi* (Brun et al., 1998). The ingestion of infected carcasses by carnivores is reported to play a role in the transmission to susceptible animals (Enwezor and Sackey, 2005). Iatrogenic spread is also another potential route of transmission and could occur during vaccination campaigns if needles are reused between individuals (Reid, 2002).

Tabanids are the natural vectors of surra because of their large mouthparts and aggressive feeding behaviour, which often means animals disrupt the flies before they can finish their blood meal. This increases the likelihood that multiple animals will be fed upon, resulting in an increased efficiency for transmission (Brun et al., 1998; Enwezor and Sackey, 2005). Conversely if the feeding is completed on a single animal, the chance of transmission is reduced (Foil, 1989).

The efficiency of transmission is also dependent upon the interval between two successive feeds and the intensity of biting flies (Enwezor and Sackey, 2005). The shorter the interval between two feeds, the greater the chances of successful transmission and as short as five seconds makes possible for an infection to occur (Luckins, 1988). Other determinant factors that might influence transmission are the level of parasitaemia, the presence of infected camels and horses and the herding of animals together (Luckins, 1988).

2.3.3 Vectors

Foil (1989) reviewed the role of tabanids for the spread of disease in humans and other animals. Tabanids have been described as mechanical vectors of viruses, bacteria, protozoa and helminths of livestock (Foil, 1989; Muzari et al., 2010). Horse flies and other tabanids affect the health of livestock throughout the world as mechanical vectors of disease such as surra, caused by *T. evansi*, and nagana, caused by *T. vivax* (Mohamed-Ahmed and Mihok, 2009). Many studies carried out on tabanids have shown that they can experimentally transmit *T. evansi* to susceptible animals (Enwezor and Sackey, 2005).

Surveys conducted in different tropical areas have also indicated an association between seasonal outbreaks of surra and an increase in the number of tabanids during the rainy season (Enwezor and Sackey, 2005). However, the most important factors influencing mechanical transmission are the high level of biting flies and their size, and the volume of blood transferred from one host to another (Desquesnes et al., 2013). *Trypanosoma evansi* infection has been associated with the presence of tabanids and high density of

biting fly, resulting in weight-loss and reduced milk production (van Hennekeler, 2007; Veer et al., 2002). Other biting flies, such as *Haematopota*, *Lyperosia* and *Chrysops* are also capable of experimentally transmitting *T. evansi*, although their role in field situations is not known (van Hennekeler, 2007).

The comparison of vector efficiency of different tabanids species has been documented and van Hennekeler (2007) reported that *Tabanus* spp. were more efficient vectors than *Haematopota* and *Chrysops* and that all tabanids were better vectors than mosquitoes and other biting flies, such as *Stomoxys*. The mechanical transmission of surra by *Stomoxys*, *Lyperosia* and *Haematopota* in a single animal reduces their effectiveness as vectors in the field (van Hennekeler, 2007). It has been estimated that four to five bites of horses are required by most *T. townsvilli* to reach engorgement to give a high probability of transmission (Muzari et al., 2010).

In Somalia some of the tabanids (*P. zonata* and *P. magretti*) are seasonal, appearing only during the two rainy seasons (Dirie et al., 1989). These tabanids adapt easily to the harsh conditions present in the northern part of the country. In the Sahdeer district of Sool region in north Somalia it has been demonstrated that there are two periods of high biting activity (08:00 to 12:00 and 15:00 to 17:30) (Dirie et al., 1989). In contrast herdsman have reported that there is no peak biting activity on cool cloudy days (Dirie et al., 1989). The flies are numerous in coastal and low land regions of Somalia, but numbers decrease greatly and are focally distributed around permanent water sources and swamps during the dry season. There is a paucity of information to determine whether transmission may be more or less likely during the wet season. The Somali nomads can identify the species of biting flies and have a local name for each species

with the species varying from region to region (Dirie et al., 1989). It has also been reported that *T. evansi* can be transmitted by a large number of blood sucking flies (Lun and Desser, 1995). However, many aspects of the biology of these species, their distribution, transmission and seasonal variation remain unknown.

In the riverine regions of Somali, conditions suitable for tabanid activity persist through the dry seasons. The four common tabanids in the riverine area are *Tabanus taeniola*, *Atylotus agrestis*, *Ancala africana* and *Philoliche zonata*, and these are widespread and commonly seen during the wet season (Dirie et al., 1989).

2.3.4 Distribution of *T. evansi*

The prevalence of surra in camels has been studied in several countries including Kenya (Njiru et al., 2004); Ethiopia (Zelege and Bekele, 2001); Chad (Delafosse and Doutoum, 2004); Mauritania (Dia et al., 1997); Jordan (Al-Rawashdeh et al., 2000); Saudi Arabia (Al-Qarawi et al., 2004); Sudan (Elamin et al., 1998); India (Pathak et al., 1993); and Morocco (Rami et al., 2003). This parasite spread from its original African home to other parts of the world following the movement of infected animals, especially camels. In the north of the tsetse belt, *T. evansi* is distributed from Africa through the Arabian Peninsula into Iran, Mongolia and Indonesia (Desquesnes et al., 2009).

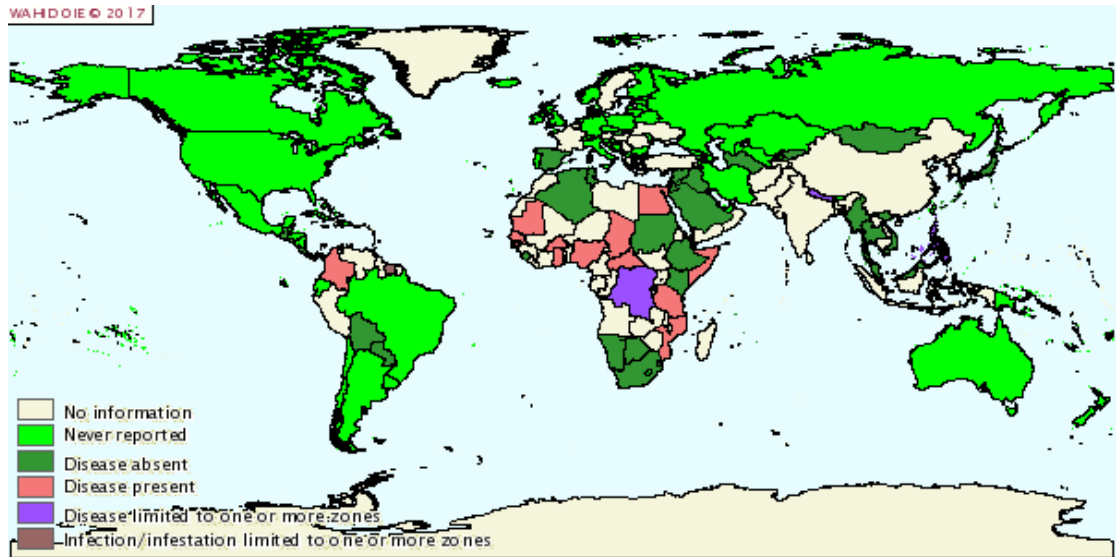


Figure: 2.2 Map indicating global distribution of surra for the period January – Jun 2016 (OIE, 2017)

In India, *T. evansi* is present in arid and semiarid regions, and has spread to places where ecological conditions are favourable for the breeding of biting flies (Pathak et al., 1993). Importation of livestock from India was thought to be the cause for the spread of the disease throughout Asia and South East Asia (Luckins, 1988; Pathak et al., 1993).

In Europe, surra was first reported in France in 2006 in sheep and camels in the Aveyron region and in Spain in 2008 in the Alicante province. Both outbreaks were the result of importation of camels from the Canary Islands (Gutierrez et al., 2010). Similarly, the disease spread to South America through animal movements from west Africa (Desquesnes et al., 2009). These reports suggest a strong association between animal movements and the spread of *T. evansi*. Epidemic outbreaks of surra were reported in Mauritius in the early 1900s, resulting in large losses of equines.

The parasite spread through the importation of camels into Australia 1907, although it was subsequently eradicated. The infection entered North America in 1909 and Bulgaria in 1939 (Desquesnes et al., 2008). However, there is little information on the prevalence and economic importance of *T. evansi* in endemic regions where migratory or pastoral systems are adopted. The prevalence of surra in camels in Kenya has been reported to be higher in adult camels compared to immature camels and calves (Njiru et al., 2004). In Ethiopia, a higher prevalence of *T. evansi* has been observed during and immediately after the rainy seasons and this could be associated with favourable weather conditions for the tabanids (Zelege and Bekele, 2001). It has also been suggested that the occurrence of surra is influenced by ecological and temperate climatic conditions, abundance of biting flies and high animal density (Hagos et al., 2009). Cases of surra are reported throughout the year and it is endemic in the three ecological zones of Somaliland, however further studies are required to determine the determinants of infection.

2.3.5 Pathology and pathogenesis

The main pathological feature of infection with *T. evansi* in camels is haemolytic and haemophagocytic anaemia (Enwezor and Sackey, 2005; Hagos et al., 2009). Anaemia is a major component of the pathology of surra, with parasitaemia resulting in removal of a large number of red blood cells (RBC) from the circulation leading to a decreased packed cell volume (PCV) (as low as 10%) (Eyob and Matios, 2013). These pathological events occur during the initial stages of infection and probably continue to activate the mononuclear phagocytic system in the spleen, liver, lungs, lymph nodes and bone marrow (Enwezor and Sackey, 2005).

As the infection of surra extends, anoxia is likely to occur as a result of tissue and vascular damage. This is followed by the release of large quantities of cytoplasmic and mitochondrial enzymes, especially aspartate alanine transferase (AST) and alanine transferase (ALT) into the serum, which results in further cellular and tissue damage (Enwezor and Sackey, 2005). The release of these enzymes probably results from host damage caused by infection with *T. evansi* (Enwezor and Sackey, 2005). In addition, *T. evansi* suppresses the immune system predisposing the infected animal to other infections and potentially death. Camel trypanosomosis induced hypothyroidism with resultant emaciation and reduced body condition score also contributes to the economic loss from this disease (Al-Qarawi et al., 2004).

There is evidence that *T. evansi* belongs to the *brucei* group of trypanosomes, which have a known preference for connective tissues of a host (Enwezor and Sackey, 2005). *Trypanosoma evansi* has the ability to invade tissues and can lead to tissue anoxia and vascular damage (Enwezor and Sackey, 2005). However, post-mortem reports of infected camels also include reports of multiple necrotic foci in the liver and spleen and hyperplasia of lymphoid tissue (Enwezor and Sackey, 2005). Infected camels may develop anoxia with consequent development of cerebral anoxia (Eyob and Matios, 2013). The oedema in the lower part of the abdomen appears to result from a significant decrease in the albumin level and resulting changes in the osmotic pressure of the blood (Enwezor and Sackey, 2005). The immune response to infection is the most marked features of the pathogenesis, particularly through increased production of large amounts of IgM (Boyd et al., 1996; Boyd et al., 1985).

The level of IgM in both naturally and experimentally-infected animals may increase by up to five times of pre-infection levels, and may remain high despite drug treatment (Boid et al., 1985). The disease appears more virulent and has significant negative effects on the reproductive performance of dromedary bulls (Al-Qarawi et al., 2004).

2.4 Surra in pastoral communities

Surra has been present in Somalia for many centuries and its presence was reported by British scientists during the colonial era (1890 -1900). These scientists identified a disease that was transmitted by biting flies (Dirie et al., 1989) and the disease (surra) has been recognised by Somali pastoralists in camels from most regions of the country at least since the early 1930's (A. Artan, personal communication). Not surprisingly Somali herders can recognise the clinically features of surra and recognise the two forms of the disease: a chronic form (Dhukan or Salaf) which is associated with horse fly (Duqsi Faras) other than tsetse flies; and an acute form (Gandi) transmitted by tsetse flies (Dirie et al., 1989). Pastoralists use smoke as a fly repellent and sometimes graze their camels at night to avoid the flies (Dirie and Abdurahman, 2003). In north eastern Kenya camel herders also manage their livestock to minimize contact with vectors that transmit trypanosomosis through the use of experience and knowledge acquired over many generations (Dirie and Abdurahman, 2003). Although camels are comparatively hardy animals and able to survive feed shortages in the arid and semi-arid lowlands, surra can have a significant impact as poorly nourished animals are affected more severely than well fed animals (Lemecha. et al., 2008).

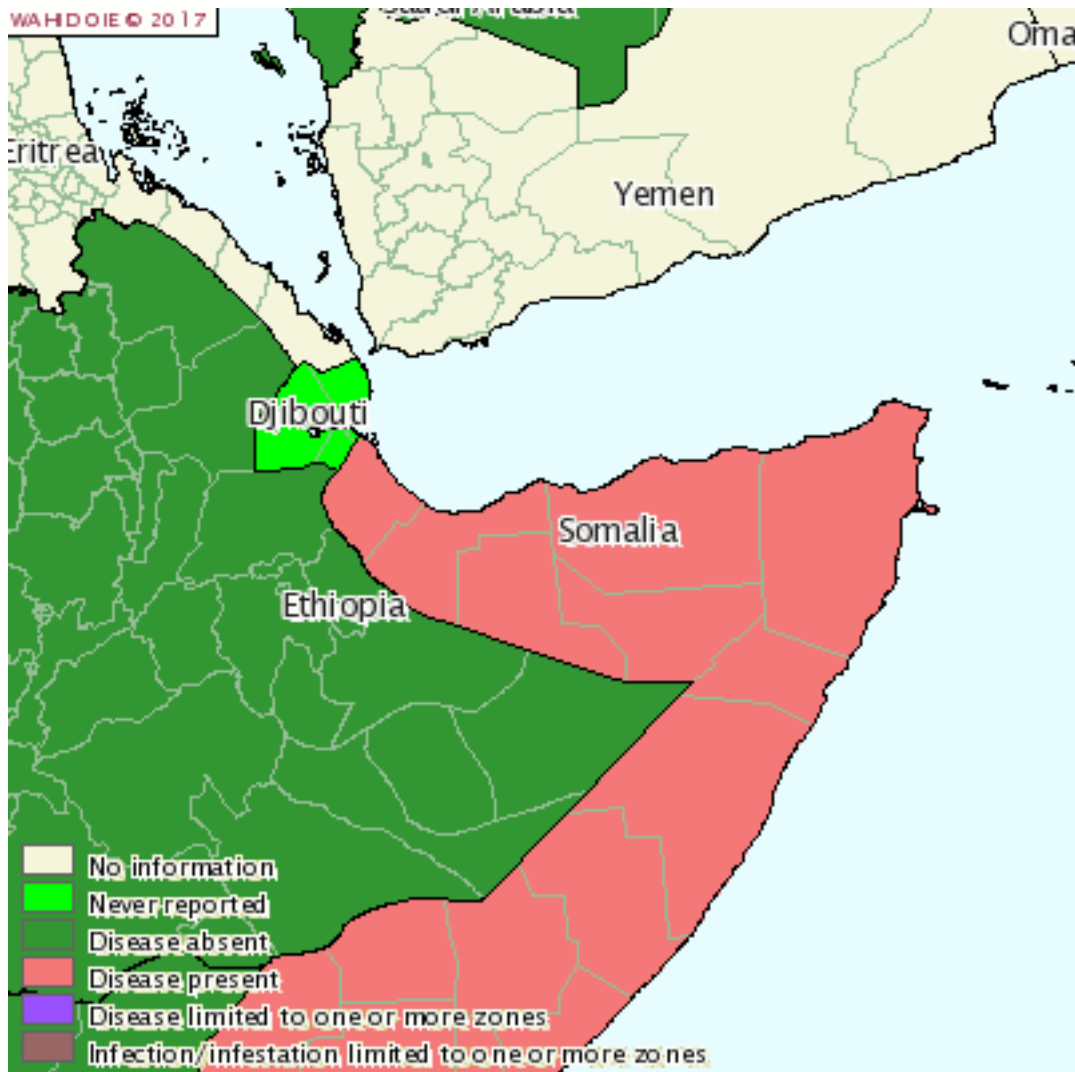


Figure: 2. 3 Map indicating the distribution of surra in Somalia January - Jun 2016 (OIE, 2017)

2.5 The influence of seasonal animal movementson the transmission of *T. evansi*

Livestock production in remote areas usually involves a migratory system and the livestock system is characterised by low inputs, poor management and in accessibility to veterinary services.

Consequently the pastoralist is under constant threat from animal diseases, as well as subject to the vagaries of a harsh environment. *Trypanosoma evansi* has been reported throughout the country in camel rearing areas, however the coastal plain (*Gubanzone*) is the key ecological zone for the transmission of *T. evansi* infection (Mohamed Dirie, personal Communication) because a large population of camels move to this area during the rainy season.

Primarily the presence or absence of water, rather than the availability of food governs livestock movement. As a result animal movements occur both during the rainy and dry seasons, and the movements follow a clear pattern with livestock spending six months during the dry period on the coastal plains and six months during the rainy period in the *Ogo* or the highlands (the *Golis* ranges) surrounding the *Guban* (Sommerlatte and Umar, 2000). Consequently theseasonal movement of livestock is an important feature of the Somali pastoralist with considerable internal and cross-border movement occurring at well-defined periods in Somalia. Animals normally stationed along the coast move inland at the start of the rains and back to the coast again in December (FAO, 2004). Pastoralists in the *Haud* plateau, along the western border of the country, also move into Ethiopia and Kenya at the beginning of the main rainy season in April and return to Somalia at the start of the dry season in December (FAO, 2004). During drought periods these pastoralists move from *Haud* areas (higher plateau) to the coastal regions for salt grazing. The coastal plains are an important grazing area for a substantial number of livestock during the November/December rains.

Camels are grazed on the *Suaeda* within a short distance of the sea. At this time these camels produce a high milk yield of good quality (Sommerlatte and Umar, 2000). In the northern parts of the country there is also some limited movement to the coastal areas from the mountains at the time of the winter rains with the return trek taking place as these rains subside (Sommerlatte and Umar, 2000). The risk of epidemic disease potentially threatens all pastoralists. However, poor pastoralists are more vulnerable to this risk, as by living in remote areas they often lack access to veterinary services and do not have the means to purchase drugs for disease prevention and treatment of diseased animals (Rass, 2006).

2.6 Socio-economic importance of livestock in Somalia and Somaliland

Livestock and their products are the principal source of income and food for millions of pastoralists in the Horn of Africa. Somalia has a traditional livestock sector based on nomadic pastoralism and a growing private sector led export industry (Anonymous, 2009; FAO, 2004). The livestock sector dominates the economy, creating about 60% of Somalia's job opportunities and generating approximately 40% of Somalia's GDP and 80% of the country's foreign currency earnings (Anonymous, 2009; FAO, 2004; Soumare, 2006). The export data, collected by the FAO-managed Food Security and Nutrition Analysis Unit (FSNAU), indicates that Somalia exported 4.6 million goats and sheep, 340,000 cattle and 77,000 camels in 2014, worth an estimated \$360 million (Anonymous, 2013). Somalia has the largest dromedary camel population in the world, estimated at over six million head (Dirie et al., 1989; Farah et al., 2007). Camels are regarded as the most valued domestic animal reared by the Somali pastoralists and play a central role in providing meat, milk, wool and hides and determining the wealth and

social status of pastoralists. They are used for riding, transport and there is a large market for trade in live camels (Dirie and Abdurahman, 2003; Farah et al., 2007). Sales of surplus milk, livestock and livestock products are key sources of income for pastoralists (FAO, 2004).

Camels are mainly dairy animals in Somalia, although there are no data on the production output. However Somalia is reported to have the highest level of milk and meat production in the world in terms of international prices (INT) and metric tonnes (MT)(Figures 2.4 and 2.5), estimated to be worth US\$434,806 in 2011 (FAO, 2011). Livestock production and marketing has traditionally been at the heart of Somaliland culture and the Somaliland economy. In 1997 it was estimated that 2.8 million live animals were exported from Berbera port in Somaliland resulting in the largest number of animals exported by a single port in the world that year(Holleman, 2002). Therefore the livestock sector and their associated exports play a major part in the country's food security, and growth of the livestock sector is critical for the country (Anonymous, 2009).

Camel exports showed the highest increase in recent years from 2,810,482 head in 2010 to over 3,505,050 in the following year, because of the lifting of export trade bans by importing countries due to suspicions of outbreaks of trade limiting animal diseases (Table 2.1). This resulted in the opening of the King Saudi Arabia (KSA) market in 2009. In 2012, over 87% of all camel exports went to KSA. At present approximately 100,000 head are exported annually accounting for 16% of the total export livestock revenue. However, the export trade faces a number of constraints and the sector remains vulnerable to external restrictions and negative impacts (Anonymous, 2013).

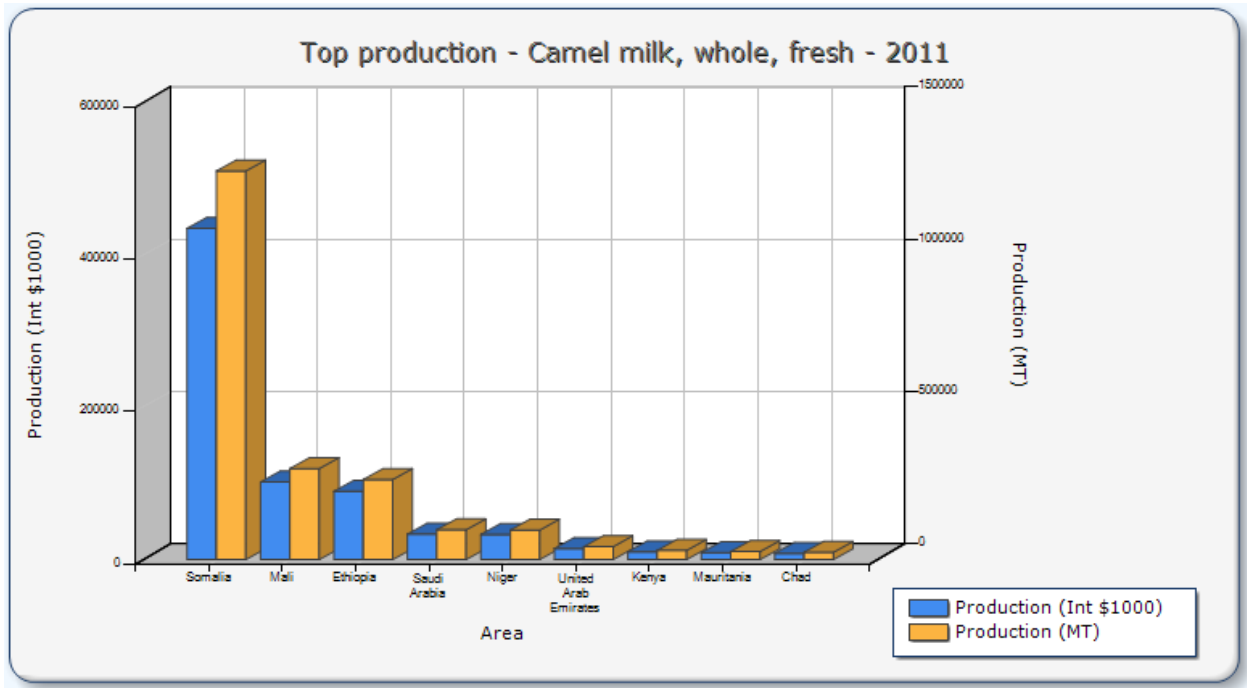


Figure: 2.4 Production of camel milk by different countries in 2011(FAO, 2011)

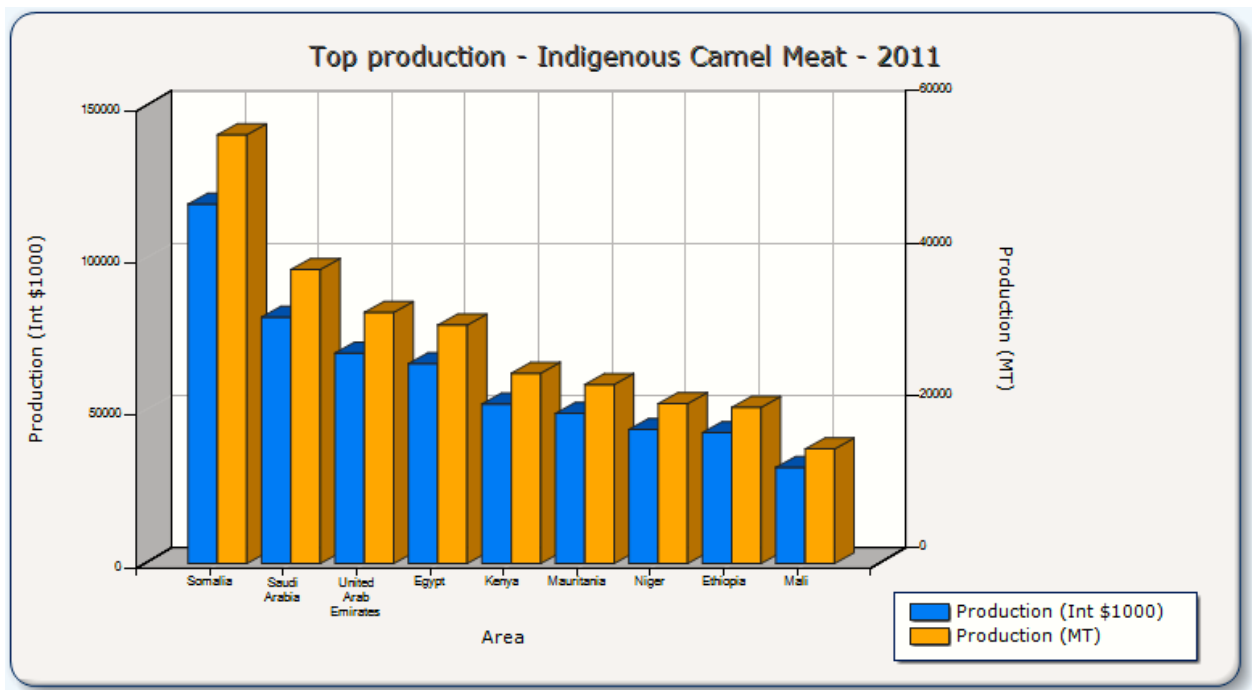


Figure: 2.5 Production of camel meat by different countries in the world in 2011 (FAO, 2011)

Somaliland exports approximately two million livestock to Saudi Arabia and other Gulf countries each year (Table 2.1) and the contribution of the livestock sector to the country's economy is significant (Table 2.2) with foreign exchange earnings being used to purchase food for import into Somaliland (Negassa et al., 2008). Furthermore the government obtains revenue through taxes on livestock exports and livestock related products (Table 2.3).

Table 2.1 Number of livestock exported from 2010 to 2015 through Berbera Port, Somaliland (Source: Ministry of Livestock)

Year	Goat/Sheep	Cattle	Camel	Total
2010	2,584,810	133,021	92,651	2,810,482
2011	2,663,270	120,111	96,097	2,879,478
2012	3,220,394	158,770	125,886	3,505,050
2013	2,875,494	189,103	75,898	3,140,495
2014	3,112,018	251,381	63,208	3,426,607
2015	3,244,481	158,444	60,003	3,462,928

Table 2.2 Livestock prices at local and international markets (Personal communication with different livestock dealers)

Country	Sheep/Goats	Cattle	Camels
Somalia	US\$ 50 - 60	US\$ 300 – 400	US\$ 400 – 500
Yemen	US\$ 70 - 80	\$ 500	N/A
Saudi Arabia	US\$ 120 -140	US\$ 600 – 700	US\$ 700
Saudi Arabia	US\$ 120 -140	US\$ 600 – 700	US\$ 700
Egypt	N/A	N/A	US\$ 600

Table 2.3 Tax per head on animals exported from Somalia (Ahmed Matan, personal communication)

Animal Species	US Dollars
Camels	18
Sheep and Goats	3.6
Cattle	12

2.7 Economic impact of surra

In the Horn of Africa, surra is one of the most important diseases that reduces camel productivity, particularly through reduced milk and meat production of adult camels (Boyd et al., 1985). Economic losses due to surra result in significant social impact to producers, although the actual losses are difficult to estimate (Mochabo et al., 2006; Reid, 2002). This is particularly important in the pastoral communities of east Africa where camels have important social and economic significance. The losses attributed to infection with *T. evansi* can be through reduced productivity, deaths, abortions and the cost of treatment and preventive measures (Derakhshanfar et al., 2010; Reid, 2002). Further costs of infection include reduced ability of draught animals to work and the adverse effects on the trade of live animals (Mochabo et al., 2006; Reid, 2002). Although the economic impact of surra is believed to be significant in Somaliland, data are lacking to confirm the losses through morbidity, mortality and reduced productivity. The most important direct losses of the disease include morbidity of up to 30% and mortality of approximately 3% (Adeiza et al., 2010).

Infected camels with surra are also often sold for slaughter at lower prices to avoid treatment costs (Yusuf, personal, communication). Livestock trade is vital to Somali pastoralists and export bans on Somali livestock imposed by countries in the Arabian Peninsula have resulted in a significant negative impact on the Somalia economy (FAO, 2004; Soumare, 2006).

Pastoralists particularly suffer from the effects of the disease as their nutritional and socio-economic well-being is entirely dependent on their livestock and any disease that reduces production will potentially trigger a shortage of food and potentially social conflict within the region. Furthermore a survey reported that livestock production in Somalia has a great impact on food security and poverty, as animal exports are an important source of foreign exchange, as well as being needed for food and the basic necessities of life (Negassa et al., 2008).

2.8 Diagnosis

The diagnosis of *T. evansi* is usually difficult due to the fact that the clinical signs of disease are not pathognomonic, and parasitaemia fluctuates and hence diagnostic tests are required to confirm the presence of the parasite (Herrera et al., 2004). Direct parasitological diagnosis may be achieved through examination of wet blood films, thick and thin blood smears and by the inoculation of laboratory animals. These parasitological techniques detect current infections, however the level of parasitaemia fluctuates, particularly during the chronic stages of infection, and may not be at a detectable level (Njiru et al., 2004; Verloo et al., 2000).

Several serological tests have been developed to detect *T. evansi*. These include a direct agglutination test (CATT/*T. evansi*), ELISA/*T. evansi*, an indirect agglutination test LATEX/*T. evansi* and Suratex (Ngaira et al., 2003; Verloo et al., 2000). These tests generally require advanced laboratory facilities, however the CATT/*T. evansi* and Suratex have been evaluated in the field (Ngaira et al., 2003). A polymerase chain reaction (PCR) has also been developed and has been used for confirming the presence of *T. evansi* in camels in Kenya and Egypt (Abdel-Rady, 2008; Njiru et al., 2004). A number of serological tests have been developed to detect antigen of or antibody to *T. evansi* such as the enzyme-linked immunosorbent assay (Ab-ELISA), Card Agglutination Test (CATT/*T. evansi*) and Suratex[®] for antigen detection in camels (Abdel-Rady, 2008) and cattle (Reid and Copeman, 2003; Reid et al., 2001).

However, most of the available diagnostic tests have a low sensitivity for routine surveillance purposes (Reid and Copeman, 2003; Verloo et al., 2000). Comparative studies of serological tests and parasitological techniques have been carried out in many countries. In Kenya, the overall prevalence of surra from camels was 5.3% using the Micro-Haematocrit Centrifugation Technique (MHCT) and 45.9% using the CATT/*T. evansi* (Njiru et al., 2004). Similarly, in Egypt of 193 samples from camels, 6.2% were positive on the MHCT, 43.5% on a CATT/*T. evansi* and 56% to a PCR (Abdel-Rady, 2008). Nevertheless, the serological tests were useful in the detection of *T. evansi* and for studying the sero-epidemiology of trypanosomosis, but they have not been able to distinguish past infection from current infection (Abdel-Rady, 2008; Njiru et al., 2004).

2.8.1 Parasitological tests

The diagnosis of *T. evansi* is often made by using parasitological tests because they are easy, rapid and economic to perform (Abdel-Rady, 2008). These techniques are mainly conducted through the detection of the parasite directly in wet blood films, MHCT or buffy coats and/or inoculation of camel' blood into mice or rats (Abdel-Rady, 2008; Dirie et al., 1989; Enwezor and Sackey, 2005; Njiru et al., 2004). However, these techniques are not very sensitive and, due to the intermittent nature of parasitaemia, may fail to detect infection (Pathak et al., 1997; Singh et al., 2004). When parasitaemia is high level, parasitological tests are generally efficient in detecting *T. evansi* in infected animals (Davison et al., 1999; Ijaz et al., 1998; Nantulya, 1990). The prevalence of *T. evansi* was reported to be 1.7% based on blood smears of camels from central Somalia (Baumann and Zessin, 1992), with 1% of camels tested in Somaliland positive on the MHCT (Dirie et al., 1989) and 12% positive in the Sahil region of Somaliland based on blood smears (Mohamed, 2007).

2.8.1.1 Examination of Blood Smears

The direct examination of blood smears through preparation of wet blood films and thick and thin blood smears is a simple and inexpensive technique to detect blood parasites. These techniques are used as an adjunct to the standardised MHCT and serological tests. They have been evaluated for the diagnosis of surra in naturally infected camels, although they have limited sensitivity. Examination of 108 camels clinically suspected of *T. evansi* infection in India, found that 45.4 and 38.9% were positive on wet and thick blood smears, respectively, compared with the mouse

inoculation test and the CATT/*T. evansi* test which detected 87.0 and 72.7%, respectively (Pathak et al., 1997). In another study of naturally infected camels, 4.14% were classified as positive by wet blood film examination and 4.6% by thin blood smear examination (Singh et al., 2004). Wet blood and Giemsa-stained thin smears have been used for the diagnosis of surra in Somalia. Of 720 camels for slaughter, 18.5% were positive by these tests (Dirie et al., 1989).

2.8.1.2 Micro-Haematocrit Centrifugation Technique (MHCT)

The MHCT technique has been standardized through the use of micro-haematocrit tubes and special centrifuges to measure the PCV, which indicates the presence and degree of anaemia. This technique is cheap, fast and easy to perform under field conditions and the sensitivity is higher than by direct examination methods. The MHCT has a reported detection sensitivity of ~ 85 trypanosomes ml^{-1} blood and this can be improved approximately tenfold when using direct examination of the buffy coat (Reid, 2002). In Kenya, the overall prevalence of surra from camels was found to be 5.3% when using the MHCT and 45.9% when using the CATT/*T. evansi* (Njiru et al., 2004). Similarly, in Egypt, of 193 camels tested 6.2 and 43.5% were positive on the MHCT and CATT/*T. evansi*, respectively (Abdel-Rady, 2008).

2.8.1.3 Animal inoculation

A number of animal species, including rats and mice, have been commonly inoculated and used to confirm the presence of *T. evansi*. The mouse inoculation (MI) test is generally accepted as the most sensitive method for the isolation of the parasite from infected hosts. This method is the only gold-standard diagnostic parasitological test for animal trypanosomosis (Ijaz et al., 1998; Reid et al., 2001) and its sensitivity can also be improved approximately 10-fold through the use of buffy coat rather than whole blood inoculum (Reid et al., 2001). Such a procedure was shown to be capable of detecting as few as 1.25 *T. evansi*/ml blood by using two or more mice per sample rather than one (Reid et al., 2001). However, this test is impractical for a large scale epidemiological survey due to the high cost of the rodents and it also requires many days to obtain results (Pathak et al., 1997). Therefore, this technique is suitable only when highly sensitive detection is required and the MI test has been used in selected camels displaying clinical signs characteristic of surra (Njiru et al., 2004).

2.8.2 Serological and antigenic tests

Diagnosis of animal trypanosomosis by conventional parasitological methods is often difficult since trypanosomes cannot always be detected in the peripheral blood. Serological methods are often used to detect immune responses of the host to the infection. Therefore, different immunodiagnostic tests have been developed for *T. evansi*, namely the card agglutination test for trypanosomosis CATT/*T. evansi* (Bajyana Songa and Hamers, 1988), the latex agglutination test, LATEX/*T. evansi* (Verloo et al., 2001), an indirect agglutination test, ELISA/*T. evansi* (Verloo et al., 2001) and immune

trypanolysis (Van Meirvenne et al., 1995). In addition, serological diagnosis in camels has been made with indirect immunofluorescent antibody tests (IFAT) and the complement fixation test (CFT) (Luckins et al., 1979). The main drawback of these tests is the inability to differentiate current from past infections (Luckins, 1988).

More recently developed TeGM6-4r Antigen-Based Immunochromatographic Test (ICT) for Animal Trypanosomosis suggested potential of a field diagnostic test. This test was evaluated in *T. evansi* antibody detection in water buffaloes (Nguyen et al., 2015; Nguyen et al., 2014).

2.8.2.1 Card agglutination test for *T. evansi*

The CATT/*T.evansi* was developed from an early *T. evansi* serotype (Rode Trypanozoon antigen TypeRo Tat 1/2) isolated in 1982 from an Indonesian buffalo by the Laboratory of Serology, Institute of Tropical Medicine, Antwerp, Belgium (Bajyana Songa and Hamers, 1988). It is the most commonly used test for antibody detection and can be useful in studying the sero-epidemiology of surra under field conditions (Hilali et al., 2004; Verloo et al., 2000). The reagent consists of formaldehyde fixed, Coomassie-stained, freeze-dried trypanosomes of *T. evansi* variable antigen type (VAT) Ro Tat ½ (Bajyana Songa and Hamers, 1988).

The entire trypanosome cell membrane within the host is covered by a homogenous single coat of variant surface glycoprotein (VSG)(Pays et al., 2004). This VSG is responsible for exploiting the VAT of an individual trypanosome.

Trypanosomes manage to survive for long periods in their host by exploiting the high immunogenicity and this allows these trypanosomes to escape the antibody response and repopulate the host, resulting in the development of long-lasting chronic infection (Pays et al., 2004). It has been reported that a small number of isolates of *T. evansi* from camels in Kenya did not possess the Ro Tat 1.2 VSG gene and animals infected by these isolates were negative when tested using the CATT (Ngaira et al., 2004; Ngaira et al., 2005).

More recently VAT RoTat 1.2 *T. evansi* antigen has been isolated from camels in Kenya after being first isolated in 1980 (Njiru et al., 2006). However, this new isolate of *T. evansi* strain type B has been detected in only one camel which does not express Ro Tat 1.2 VSG (Njiru et al., 2006). This Ro Tat 1.2 VAT is highly immunogenic and elicits specific antibodies with agglutinating and lytic activities (Van Meirvenne et al., 1995).

The CATT has been validated for diagnosing trypanosomosis in camels (Dia et al., 1997; Ngaira et al., 2003), cattle (Reid and Copeman, 2003) and buffalo (Davison et al., 1999). However, its usefulness is limited by a low sensitivity but it is highly specific (Reid and Copeman, 2003). The CATT had the highest reported specificity of 100% and was suggested to be the best test to correctly classify truly infected buffaloes in Indonesia (Davison et al., 1999). Although this is a useful test to detect early and late induced antibodies, it is not capable of differentiating past from present infection (Ngaira et al., 2004).

The sensitivity and specificity of the CATT/ *T. evansi* has also been evaluated in camels (Dia et al., 1997; Ngaira et al., 2003). The sensitivity of the test in Kenya has been reported to be 66.5 and 68.6% and 86.95% in Mauritania and the specificity can be up to 100% (Dia et al., 1997; Ngaira et al., 2003; Ngaira et al., 2004). The CATT/*T.evansi* at ¼ serum dilution has a high sensitivity (Reid and Copeman, 2003), with a reported sensitivity and specificity of 83 and 96%, respectively, when the test was used to test Indonesian cattle infected with *T. evansi* and uninfected Australian cattle, respectively (Reid and Copeman, 2003). Surveys that have used the CATT/*T. evansi* or micro ELISA tests have found a higher prevalence than studies using parasitological tests (Baumann and Zessin, 1992; Dia, 2006).

2.8.2.2 Latex agglutination test or Suratex

The latex agglutination test has been developed to detect trypanosome-circulating antigens and can be used in the field (Ngaira et al., 2003). This test is a suspension of latex particles that have been sensitised with a monoclonal antibody against trypanosome antigen and converted into a test kit by Brentec Diagnostics, Nairobi (Ngaira et al., 2003). It has been reported that Suratex detects circulating antigen in the blood as early as 3 days post-infection(pi) in horses due to the increasing numbers of parasites in the blood (Wernery et al., 2001). Suratex detected 10 of 12 camels infected with *T. evansi* by day 7 (pi), compared to only 7 animals detected by buffy coat. Of 60 camels infected with *T. evansi* 53 (88.3%) were positive to this test (Nantulya, 1994). It has also been reported that antigens were detectable 2 to 3 weeks after infection in camels (Olaho-Mukani et al., 1996).

The detection of antigen in the blood is an important indication of infection and therefore it is also ideal for the evaluation of the efficacy of chemotherapeutic agents (Olaho-Mukani et al., 1996).

The reported sensitivity of Suratex in camels varies from 58.8 (Ngaira et al., 2003) to 88% (Olaho-Mukani et al., 1996), however a specificity of 100% has been reported (Ngaira et al., 2003). The strength of this test is its ability to detect infection only 3 days after infection in horses. It has been suggested that it is suitable for detecting the early phase of infection (Wernery et al., 2001).

However in contrast in camels it has been suggested that antigen may not be detected during the early stage of infection, since trypanosomes are destroyed by the immune system (Olaho-Mukani et al., 1996).

2.8.2.3 Enzyme-linked immunosorbent assay (ELISA)

The introduction of the ELISA was a major breakthrough in the field of immune-diagnosis. The test was first used for the diagnosis of *T. rhodesiense* infections in humans and subsequently has been used for the diagnosis of *T. evansi* in animals (Nantulya, 1990). This test has generated significant interest for the detection of *T. evansi* infected camels because of its ability to measure specific antibodies and antigens in the blood of infected animals (Molina et al., 2000). The antigen-ELISA (Ag-ELISA) and antibody-ELISA (Ab-ELISA) have been mostly employed to detect infected animals, but the tests have inherent weaknesses that limit their sensitivity and specificity (Singh et al., 2004). However, a rigorous evaluation of an IgG-specific antibody

detection ELISA (IgG ELISA) and two monoclonal-based antigen-ELISAs demonstrated that the Ab-ELISA was more efficient than the two Ag-ELISA for detecting *T. evansi* infection in buffalo in Indonesia (Davison et al., 1999). In that study the sensitivity of the IgGAb-ELISA was higher (89%) than the two Ag-ELISAs (71 and 81%) (Davison et al., 1999). Others have also reported a higher sensitivity of the Ab-ELISA compared with the Ag-ELISA in horses (Ramírez-Iglesias et al., 2011) and camels (Olaho-Mukani et al., 1993).

Ag-ELISAs have been developed using polyclonal or monoclonal antibodies to capture *T. evansi* antigens in serum, but have not yet reached a satisfactory level to be recommended for routine diagnostic use (Reid and Copeman, 2003). Additionally, in the first days of infection or when the parasitaemia is cryptic, the circulating antigens are below the detection level of the test (Ramírez-Iglesias et al., 2011). This test has been reported to lack sensitivity and specificity when used to detect *T. evansi* infection in buffalo in Indonesia (Reid and Copeman, 2003). Attempts have been made to develop and improve the Ab-ELISA detection for *T. evansi* by incorporating three fractions of antigen purified from a detergent extract of *T. evansi* (Reid and Copeman, 2003). The Ab-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 a specificity of 99% when used to test sera from non-infected Australian cattle (Reid and Copeman, 2003).

The Ab-ELISA has previously been used in central regions of Somalia to detect antibody to *T. evansi* and 56% of camels demonstrated antibody responses (Baumann

and Zessin, 1992). The Ab-ELISA has been reported to be the most practical and appropriate tool for determining antibody levels following treatment for *T. evansi* (Monzon et al., 2003). It has also been recommended in clinical or epidemiological studies and in disease control trials (Rami et al., 2003). More recently surra was detected in camels from France using an Ab-ELISA and after the first treatment, parasites and DNA disappeared in all animals with antibody levels decreasing until the animals became seronegative on the test 3 to 4 months later (Desquesnes et al., 2009).

2.8.2.4 Trypanosomal DNA Detection, DNA Probe and PCR

The polymerase chain reaction (PCR) is a highly sensitive and specific molecular diagnostic technique to detect and identify trypanosomes at the species level (Desquesnes et al., 2001; Holland et al., 2001). Several applications of PCR-based techniques have been developed for the detection of DNA from trypanosomes in host blood and vectors (Desquesnes et al., 2001; Masiga et al., 1992). Detection of trypanosomes and characterisation by molecular methods relies on species-specific primers. Such primers, suitable for the *Trypanozoon* subgenus, have been developed and used to characterise these parasites in epidemiological studies (Desquesnes and Dávila, 2002; Thumbi et al., 2008). One pair of primers is needed to detect each subgenus, species or type (Desquesnes et al., 2001). Isolates of *T. evansi* strains can be divided into type A and type B, the type A is the most abundant and found in Africa, Asia and South America. Occurrence of type B minicircle is not common and has been isolated from camels in Kenya and Ethiopia (Birhanu et al., 2015; Njiru et al., 2006).

The PCR allows the detection of a single specific DNA sequence and using satellite DNA is sensitive enough to detect only a few trypanosomes. Satellite DNA is the preferred technique used for the detection and identification of trypanosomes belonging to the *Trypanozoon* subgenus (*T. brucei* spp., *T. evansi* and *T. equiperdum*) (Desquesnes et al., 2001).

Kinetoplastid mini-circle DNA sequences have been designed to target the development of specific primers for detection of *T. evansi* and these PCR primers for *T. evansi* do not amplify the mini-circle sequence from *T. brucei* spp. (Artama et al., 1992; Desquesnes and Dávila, 2002). The specific primers have proven to be highly sensitive, but they require multiple-copy genes to give higher sensitivity and the internal transcribed spacer (ITS) region of ribosomal DNA presents the advantages of being a multi-copy locus (Thumbi et al., 2008). Primers ITS1 CF and ITS1 BR, previously designed to amplify the internal transcribed spacer (ITS1) of rDNA, have been evaluated for use in a universal diagnostic test for detection of pathogenic trypanosomes (Njiru et al., 2005). PCR amplification and sequence analysis of part of the ribosomal RNA gene, including 18S, ITS1, 5.8S and ITS2 regions, have been achieved for the detection of *T. evansi* from dromedary camels (Amer et al., 2011).

PCR-based tools have been found to differentiate isolates of *T. evansi* and are useful in studies of the parasite population (Desquesnes and Dávila, 2002). The PCR has been used in surveys to determine the prevalence of *T. evansi* in buffalo in Vietnam (Holland et al., 2001) and camels in Kenya (Njiru et al., 2004).

This technique has also been used to monitor and evaluate the efficiency of chemotherapy for controlling trypanosomosis (Njiru et al., 2004; Singh et al., 2004) with the test failing to detect *T. evansi* in previously infected buffalo 24 hours after they had been treated with diminazene (Holland et al., 2001). The test has been reported to have a sensitivity of 78% in experimentally-infected buffalo (Holland et al., 2001) and between 97 and 100% in naturally-infected camels (Njiru et al., 2004; Singh et al., 2004).

2.8.2.5 Loop-mediated isothermal amplification (LAMP)

Loop-mediated isothermal amplification (LAMP) is a novel method for DNA amplification that can be performed in the field or in a basic laboratory. The test is simple, easy to perform and results can be seen through a colour change within one hour under isothermal conditions (Njiru et al., 2010; Thekisoe et al., 2007). It is also rapid with amplification achieved within 20 – 25 min at 63°C (Njiru et al., 2010). The LAMP technique has been successfully developed and applied to detect human and African trypanosomes (Njiru et al., 2010). It has been suggested that this technique can be used in surra endemic countries as an alternative to PCR diagnostic methods, particularly in regions/laboratories with financial restrictions (Njiru et al., 2010; Thekisoe et al., 2007).

2.9 Prevention and control of surra

No effective vaccine is available against *T. evansi*, and consequently chemotherapy and chemoprophylaxis are relied upon (Anene et al., 2001). Chemotherapy has relied upon the use of the salts of three compounds: diminazene, an aromatic diamidine; homidium,

a phenanthridine; and isometamidium, a phenanthridine–aromatic amidine. Of these diminazeneaceturate is the most commonly used therapeutic agent while isometamidium chloride is most commonly used as a prophylactic agent against trypanosomes, although not *T. evansi* (Luckins, 2000). In addition, quinapyramine, suramine and recently melarsen oxide (Cymelarsan[®]) are generally used for therapy and prophylaxis of *T. evansi* in camels (Anene et al., 2001; Enwezor and Sackey, 2005). However the therapeutic and prophylactic use of trypanocides against trypanosomes is threatened by drug resistance and toxicity (Anene et al., 2001).

Suramine and quinapyramine have been in use since the 1920s and 1950s and melarsen oxide was introduced in 1985. This is an arsenical drug, related to melarsen (which is used in human sleeping sickness) and it was introduced for the treatment of surra in camels and other *brucei*-group trypanosome infections because of the development of drug resistance to the previously used drugs (Anene et al., 2001). It has also been reported to be efficient against *T. evansi* that are resistant to quinapyramine and suramine and *T. brucei* resistant to diminazene acetate (Payne et al., 1994). Cymelarsan[®] is dispensed as a dry powder, is highly soluble in water and is only registered for use against *T. evansi* in camels. Melarsomine is administered as a single dose, preferably intramuscularly, and although it has no prophylactic action, it does clear the parasitaemia from infected camels. Treatment of *T. evansi* infected camels with melarsomine has been shown to decrease the seroprevalence from 58 to 19% within a year (Enwezor and Sackey, 2005; Rami et al., 2003). However, relapses in camels after treatment have been reported

(Anene et al., 2001) which may be due to the survival of drug resistant trypanosomes or the result of parasites escaping the drug's actions (Monzon et al., 2003).

Cymelarsan[®] has also been shown to be effective against surra in camels in Ethiopia at a dose of 0.3 and 1.25 Mg/Kg body weight (Zellek et al., 1989). Trials with this drug in camels in Kenya experimentally infected with *T. evansi* have been effective therapeutically at a dose range of 0.2 to 1.2 mg/kg body weight (Nyang'ao et al., 1995; Otsyula et al., 1992). In Somalia, Cymelarsan[®] has been used in treatment campaigns against surra in camels and this drug has been shown to be effective when administered as a single intramuscular dose (Ahmed Artan, Personal Communication).

Understanding the epidemiology of *T. evansi* in camels, in particular its prevalence and distribution, is critical when developing control programmes and evaluating the impact of the disease to livestock and the community. In the following chapter a study is undertaken to determine the prevalence and distribution of infection with *T. evansi* in camels in Somaliland.

CHAPTER THREE

Prevalence and distribution of *Trypanosoma evansi* in camels in Somaliland

3.1 Introduction

As outlined previously trypanosomosis is the most important parasitic disease of camels in Somaliland and is caused by infection with *T. evansi* transmitted mechanically by haematophagous biting flies, especially tabanids (Dirie et al., 1989). These biting flies require specific ecological conditions, including suitable vegetation and water points, to survive (Dirie et al., 1989). In Africa the epidemiology of *T. evansi* is of particular interest because the disease has a specific spatial distribution being endemic in arid and semi-arid countries of the Horn of Africa (Dirie et al., 1989; Enwezor and Sackey, 2005; Njiru et al., 2004). Furthermore there are differences in the disease's occurrence throughout the year with disease appearing more commonly during the rainy seasons when it can be associated with a high number of mortalities in infected camels (Dirie et al., 1989).

There are considerable differences in the severity of clinical signs induced by *T. evansi* in different geographical areas and these are dependent upon the virulence of the infecting strain and the susceptibility of the host (Herrera et al., 2004). A thorough understanding of the epidemiology of a disease is required before effective disease control programmes can be implemented. However, prior to the study reported in this chapter there had not been a systematic survey conducted in Somaliland to determine the prevalence and distribution of *T. evansi*, even though trypanosomosis in camels in

Somalia had first been investigated in the early 1980's (Dirie et al., 1989). This study was undertaken to determine the seroprevalence and distribution of infection with trypanosomes in camels in Somaliland.

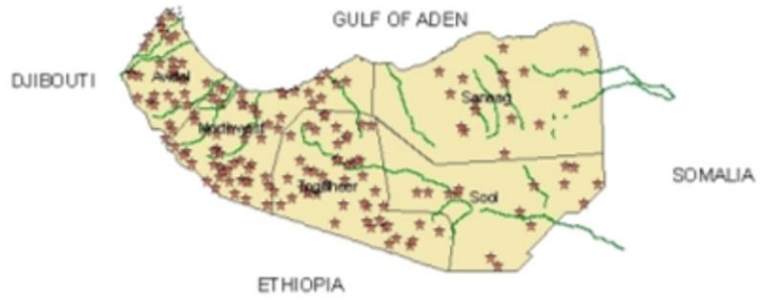
3.2 Materials and methods

3.2.1 Sampling sites

The study was conducted in the northern regions of Somalia-Somaliland between 2011 and 2012. A total of 144 sites were sampled in 18 districts of the six regions in Somaliland (Table 3.1). The sampling sites had an estimated area of 137,600 km² with a coastline of 850 km and were located between latitudes 8°00' and 11°27' north and longitudes 42°30' and 49°00' east (Figure 3.1). In this area rainfall is irregular, ranging from 100 to 300mm per annum, with an average temperature between 25°C and 28°C (77°F and 82°F). Temperatures fall as low as 0°C (32°F) in the mountains of the north and reach as high as 47°C (117°F) on the coast. In this region the vegetation includes a mixture of trees and shrubs with open or continuous structure, grasses with the occasional tree or bush/shrub in the grassland area. The plants include *Acacia* species (bush land), *Suaeda* species (Shrubland), *Balanites* bush land and *Panicum* grasslands (Sommerlatte and Umar, 2000). Rearing of different species of animals together is a traditional practice of the nomadic pastoralists found in this region with the camel playing a major economic and social role in the household.

As outlined in Chapter 1, pastoralists from Somaliland recognise three ecological zones in their country (*Guban*, *Ogo* and *Haud*) (Figure 3.2).

Study Area of Camel Trypanosomosis



Legend

- * Random coordinate sites
- Seasonal rivers
- Somaliland



Figure: 3.1 Geographic coordinates for the sites sampled in Somaliland

3.2.2 Study design and sampling

A cross sectional survey was conducted using a two-stage random sampling design, drawing the required number of animals from the nearest herd to each point, the primary sampling units being villages or herds, settlement, water point or grazing area and the secondary sampling units being animals (Cameron, 1999). The computer package Survey Toolbox (www.ausvet.com.au/) was used to calculate the total number of primary sampling sites/locations and an average of 18 animals were required to be sampled per village/herd. The random points selected for sampling were generated by using Geographic Information System (GIS) software (ArcGIS 9.2). The longitude and latitude coordinates of sampling sites were geo-referenced then the actual sampling sites were the village, watering point, market or grazing settlement closest to the selected point where animals could be found (Soumare et al., 2007). A Geographical Positioning System (GPS) navigation system was used to identify the target sites for sample collection within the specified 10 Km radius from the selected site (Appendix 2).

In addition, when using this sampling procedure, it is probable that quite a high proportion of selected sites/locations would have no herds or animals within the specified radius of 10 Km (James, 1998). In order to cope with this scenario, 50 “spare” sampling sites (above the needed number of primary sampling units) were generated. The total number of spare sampling sites represented 30% of the total number of target sampling sites assigned to each region (Appendix 3). A total of 2,575 camels owned by nomadic pastoralists were selected for sampling (Table 3.1).

Table 3. 1 Location of camels sampled from six regions of Somaliland (2010 to 2011)

Region	Number of sites sampled	Number of samples collected
Sanaag	16	288
Sool	15	271
Togdheer	49	878
Sahil	19	381
Waqoyi Galbed	21	339
Awdal	24	418
Total	144	2575

Blood samples (10 ml) were collected from the jugular vein into plain vacutainers from the selected animals. Each animal was sampled once and data were collected using a semi-structured questionnaire (Appendix 1). This questionnaire collected information about the presence of clinical signs observed by the owners, and data on the characteristics of the camels (including location, age, gender, herd size, body condition, and illness). The body condition of the camels was visually assessed and rated as poor, fair or good (1 to 3). Sampling had been approved by the Murdoch University Animal Ethics Committee (R2408/11).

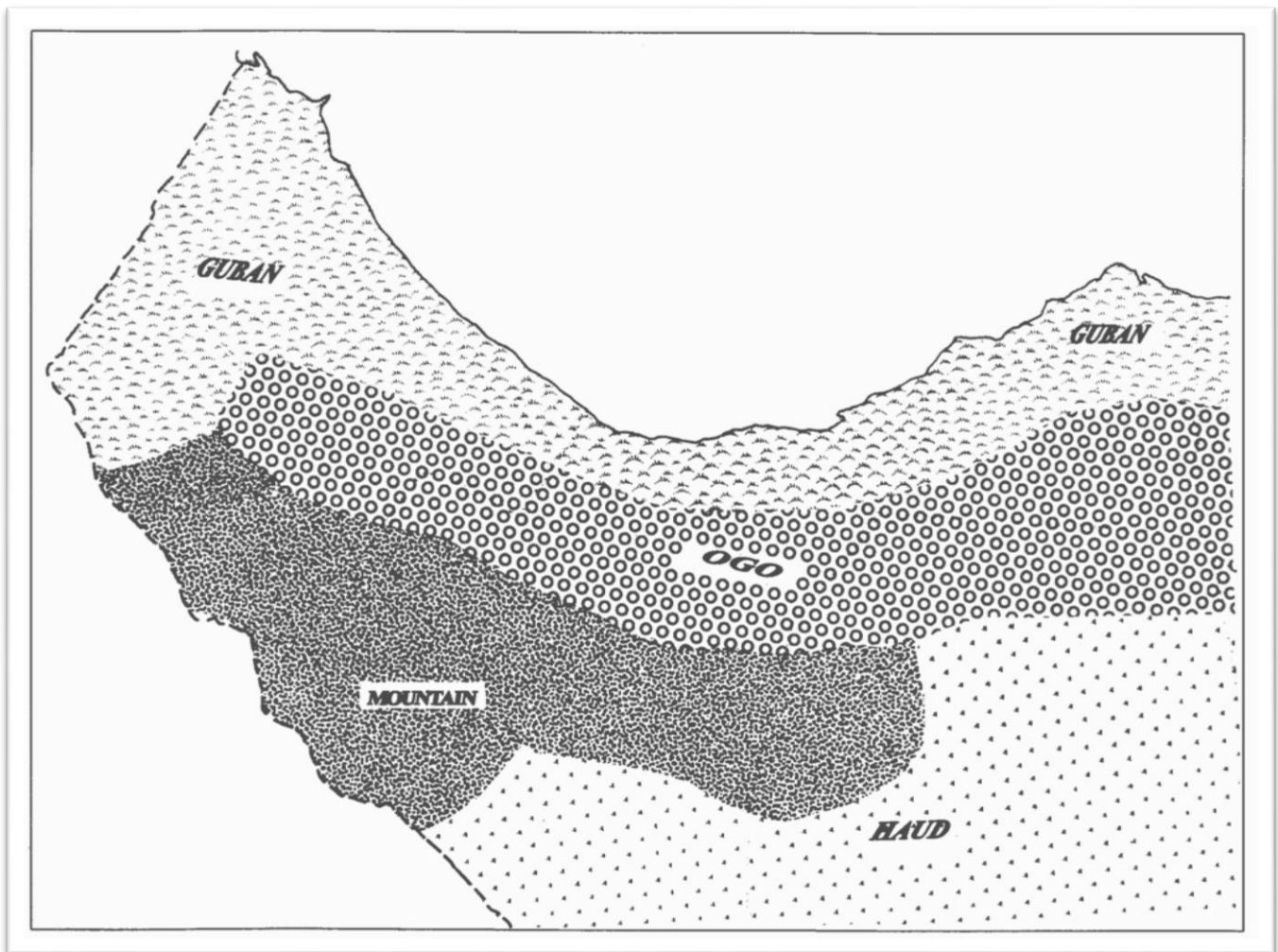


Figure: 3.2 Ecological zones in Somaliland (Sommerlatte and Umar, 2000)

3.2.3 Serological test

3.2.3.1 Card agglutination test (CATT/*T.evansi*)

The card agglutination test for *T. evansi* (Institute for Tropical Medicine, Antwerp, Belgium) was used to determine the seroprevalence in this study. Sera were tested to detect anti-trypanosome antibodies as per the manufacturer's instructions.

Briefly, 45 µl of freshly resuspended antigen was placed into a circle on a plastic card and mixed with 25 µl of diluted serum (1:4 dilution) using a stirring rod. The card was gently agitated with a card rotator for 5 minutes then examined visually for the presence of agglutination. A positive reaction was indicated by the presence of blue agglutination (Bajyana Songa and Hamers, 1988). The real prevalence of infection was estimated (Rogan and Gladen, 1978) using the published sensitivity and specificity of the CATT at a 1:4 serum dilution of 68.6 and 100%, respectively (Ngaira et al., 2003). This real seroprevalence was then used to estimate the impact of the disease on camels.

3.2.4 Statistical analyses

Data analyses were carried out using a statistical software program (SPSS for Windows, Version 15.01, SPSS Inc. Chicago, USA). The potential risk factors for seropositivity were assessed at the animal level by calculating odds ratios and their 95% confidence intervals. Statistical differences were assessed using the chi-squared (χ^2) test for independence. The 95% confidence intervals for the seroprevalence were calculated using the exact binomial method.

3.3 Results

3.2.1 Seroprevalence test analysis

A total of 2575 camels owned by nomadic pastoralists were tested for infection with *T. evansi* using the CATT/*T. evansi*. The overall animal level apparent (test) prevalence in Somaliland was 26.4% (95% CI: 24.8, 28.2). After adjusting for the sensitivity and

specificity of the test, a real prevalence of 38.2% (95% CI: 36.0, 40.0) was calculated for animals. The prevalence was highest in the Sahil region (37.3%) followed by Togdheer (28.4%), Sanaag (27.4%) and Sool (26.2%). Camels reared in the Sahil region (OR = 2.8; 95% CI: 2.0, 4.0) or in the Togdheer region (OR = 1.9; 95% CI: 1.4, 2.6) were more likely to be infected than camels reared in the Awdal region (Table 3.2).

Overall there was a significant difference in the seroprevalence between the regions (Table 3.3) ($\chi^2 = 35.2$, $df = 1$, $P < 0.001$) with the prevalence being significantly higher in Sahil than in Waqoyi Galbed and Awdal, respectively. The clinical signs reported in camels by the pastoralists included a rough coat, emaciation, loss of hair from the tail, a characteristic fetid urine smell, disappearance of the hump, severe muscle atrophy of the rear quarters, abdominal oedema, corneal opacity, diarrhoea and sexual excitement.

3.3.2 Distribution of surra

The seroprevalence of *T. evansi* infection in camels was determined in each of the 18 districts studied in Somaliland (Figure 3.3). There was a higher prevalence in camels from the eastern districts (29.8%; 95% CI: 27.7, 31.9) compared with the western districts (18.5%; 95% CI: 15.8, 21.4). Camels from the eastern districts were 1.9 times (95% CI: 1.5, 2.3) more likely to be infected than camels from the western districts ($\chi^2 = 34.9$, $df = 1$, $P < 0.001$) (Figure 3.3). Herds in the coastal area were also more likely to be infected (37.0%; 95% CI: 32.6, 41.5) than herds from the inland areas (26.9%; 95% CI: 24.5, 29.3).

Table 3.2 Seroprevalence of *T. evansi* infections in regions in Somaliland (2011 to 2012)

Region	Number of camels tested	CATT/ <i>T. evansi</i>			OR	95% CI	Chi-square value (χ^2)	p-Value
		Number positive	Test prevalence%	95% CI				
Sanaag	288	79	27.4	22.3, 32.9	1.8	1.2, 2.6	9.1	0.00
Sool	271	71	26.2	21.3, 31.7	1.7	1.1, 2.5	7.0	0.01
Togdheer	878	249	28.4	25.2, 31.1	1.9	1.4, 2.6	15.5	0.00
Sahil	381	142	37.3	31.6, 42.2	2.8	2.0, 4.0	35.2	0.00
Waqoyi								
Galbed	339	59	17.4	13.7, 21.8	1.0	-	-	-
Total	2575	681	26.4	24.8, 28.2				

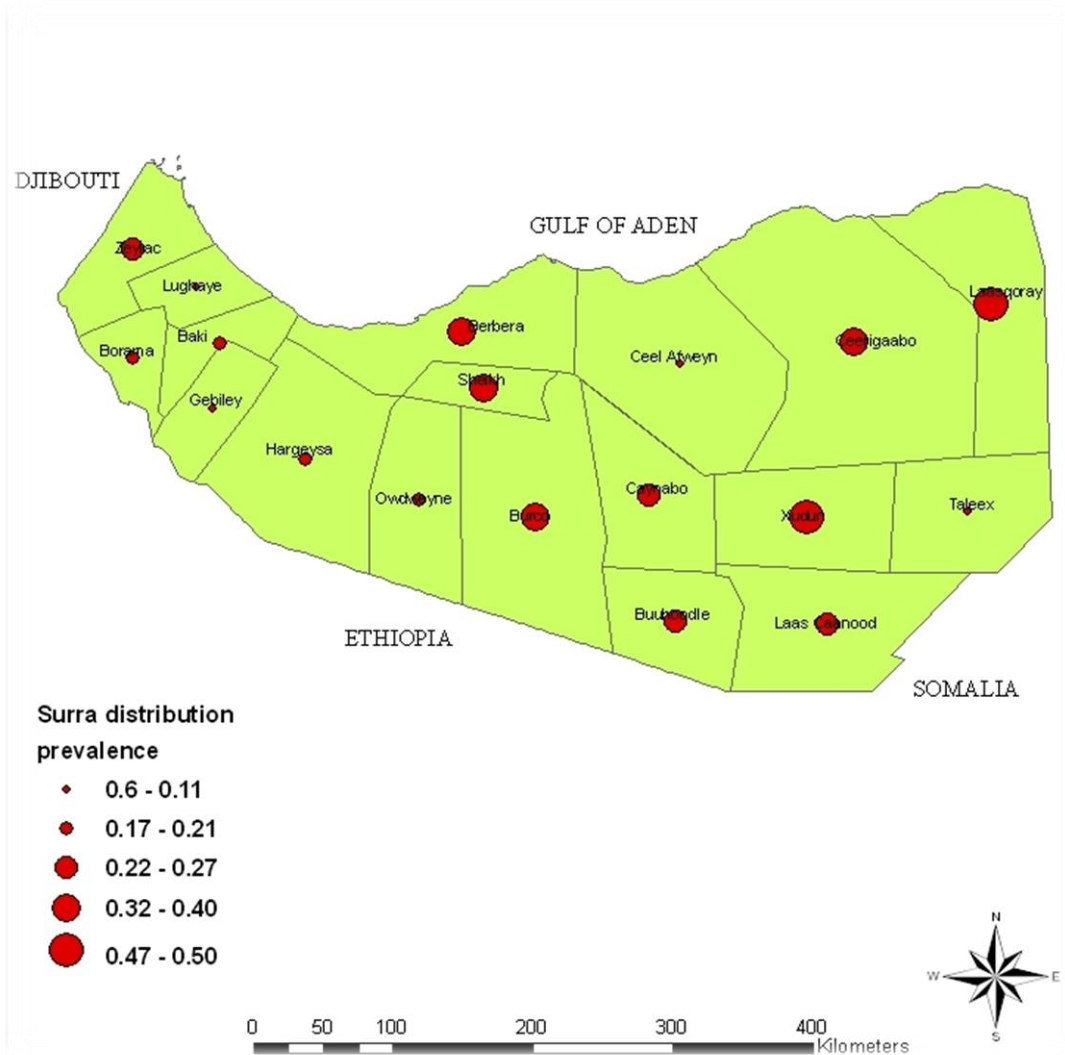


Figure: 3.3 Geographical distribution of seroprevalence

3.3.3 Ecological zones

In Table 3.3 the seroprevalence of *T. evansi* infection in the three ecological zones is summarised. The higher seroprevalence was found in camels originating from the *Guban* zone (36.8%; 95% CI: 33.6, 40.2) and *Haud* zone (27.4%; 95% CI: 24.5, 30.4) compared to the *Ogo* zone (15.8%; 95% CI: 13.5, 18.3). Camels from the *Guban* (OR =

3.1; 95% CI: 2.5, 3.9) and *Haud* zones (OR = 2.0; 95% CI: 1.6, 2.6) were more likely to be infected than camels reared in the *Ogo* zone (Table 3.3) ($\chi^2= 97.7$, df = 1,P<0.001 and $\chi^2=35.0$, df= 1,P<0.001, respectively).

Table 3.3 Seroprevalence of *T. evansi* infection in ecological zones

Ecological zone	Number tested	Number positive	Prevalence			
			(%)	95% CI	OR	95% CI
<i>Guban</i>	820	302	36.8	33.5, 40.2	3.1	2.5, 3.9
<i>Haud</i>	880	241	27.5	24.4, 30.5	2.0	1.6, 2.6
<i>Ogo</i>	875	138	15.8	13.4, 18.4	1.0	-
Total	2575	681	26.4	24.8, 28.2		

3.3.4 Age and gender

Infection with *T. evansi* was observed in camels of all ages and genders. Adult camels (26.8%) were slightly more likely to be CATT/*T. evansi*-positive (OR = 1.2; 95% CI: 0.6, 2.4) than calves (22.9%), however this difference was not significant ($\chi^2=0.36$, df= 1, P = 0.55)(Table 3.4).

Table 3.4 Effect of age-class on the seroprevalence of *T. evansi* using the CATT/*T. evansi*

Age class	Number tested	Number positive	Prevalence			
			(%)	95% CI	OR	95% CI
Adults	1921	515	26.8	24.8, 28.9	1.2	0.6, 2.4
Young adults	606	155	25.6	22.1, 29.2	1.2	0.6, 2.3
Calves	48	11	22.9	12.0, 37.3	1.0	-
Total	2575	681	26.4	24.8, 28.2		

The seroprevalence in females (26.7%) was similar to that of males (25.9%) (OR = 1.0; 95% CI: 0.9, 1.3) ($\chi^2=0.15$, df= 1, P = 0.70).

3.3.5 Body condition score

The influence of infection with *T. evansi* on body condition score is summarised in Table 3.5. The seroprevalence varied significantly between body condition score groups ($\chi^2=60.6$, df=2, P<0.001) with the highest seroprevalence in camels of poor body condition (39.5%) and the lowest in camels of good condition score (18.4%). Animals in poor body condition were almost three times more likely to be positive than animals in good body condition (OR = 2.9; 95% CI: 2.2, 3.8).

Table 3.5 The seroprevalence to surra in camels with different body condition scores

Body score	Number of camels tested	Number positive	Seroprevalence (%)	95% CI	OR	95% CI	p-Value
Poor	702	277	39.5	35.8, 43.2	2.9	2.2, 3.8	0.00
Fair	1374	312	22.7	20.5, 25.0	1.3	1.0,1.7	0.05
Good	499	92	18.4	15.1, 22.1	1.0	-	
Total	2575	681	26.4	24.8, 28.2			

3.4 Discussion

The present study is the first epidemiological study on *T. evansi* using the CATT/*T. evansi* serological test in camels in Somaliland. This study demonstrated that *T. evansi* is widespread in Somaliland with an animal-level seroprevalence of 38.2%. This would indicate that *T. evansi* is potentially having a major impact on the productivity of camels in the region and hence the livelihood of the nomadic pastoralists, as has been reported elsewhere (Baumann and Zessin, 1992; Lemecha. et al., 2008). The seroprevalence found in this study was higher than that reported in camels from neighbouring countries 20.6% (Zelege and Bekele, 2001) and 24.9% (Hagos et al., 2009) in Ethiopia, 32.2% (Ngaira et al., 2003) in Kenya and 28.80% (ElSaid et al., 1998) in Sudan. In contrast the seroprevalence previously reported in central Somalia was higher (56.4%) than that reported in the current study, although in the earlier study the micro ELISA test was used (Baumann and Zessin, 1992). It is possible these differences could be due to differences in the epidemiology of *T. evansi* infection in the different regions or ecological zones or due to differences in the accuracy of the diagnostic assays used. The micro ELISA test has a higher sensitivity than the CATT/*T. evansi*. In contrast, the CATT is highly specific (Reid and Copeman, 2003).

In this study conducted during the rainy season a higher seroprevalence was found in the eastern regions including Sahil (37.3%), Togdheer (28.4%), Sanaag (27.4%) and Sool (26.2%) compared to the regions of Waqoyi Galbed (17.4%) and Awdal (19.4%). These geographical differences are likely to be due to increasing numbers of biting flies in specific locations during the rainy season and increased movements of camels at this

time (Mohamed, 2007). The higher prevalence reported in herds from the northern region of Somalia (Somaliland) has been linked to the presence of tabanids in this region (Dirie et al., 1989; Mohamed, 2007). The high-risk zones are well defined in Somali and pastoralists would appear to be aware of the means for the transmission of surra, however they are not always able to avoid the risk of *T. evansi* infection. Dirie et al. (1989) reported that cases of surra occurred throughout the year and could be found in most regions of Somalia. In the present study more infected herds were found in the coastal area (37.0%) compared to herds from the inland area (26.8%). This difference is again likely to be an indication of different risk factors for camel trypanosomosis in different ecological zones, with the coastal region having suitable environmental conditions for the tabanids facilitating the transmission of surra. A higher prevalence was observed in camels from the *Guban* zone (36.8%) compared to the *Haud* (27.4%) and *Ogo* (15.8%) zones. The movement of camels has the potential to distribute infection between zones. Most infected camels in the *Guban* zone (41.4%) originated from the Sahil region. The higher prevalence in the *Guban* zone is likely to be associated with a combination of factors, including a suitable ecological zone for the survival of the biting flies, sampling during the rainy season when biting flies are abundant and animal movements.

In Ethiopia, the risk of *T. evansi* infection is associated with the presence of permanent and temporary waterways and the plant canopy (Hagos et al., 2009). In that study the highest seroprevalence of surra in the Dello-Mena district was linked to ecological conditions favourable for the survival and multiplication of the biting flies. Furthermore, Hagos et al. (2009) observed that camels in the riverine areas were more

likely to be infected than herds from the non-riverine areas. In Somalia, Baumann and Zessin (1992) reported that infection with *T. evansi* was highest in the riverine zone during the rainy season. The risk of infection has been shown to be influenced by climatic and ecological conditions, and disease is prominent during the wet season in areas where favourable conditions are present for the biting flies (Hagos et al., 2009). Prior to the study reported in this chapter surra had not been documented as an important disease in Somaliland since an outbreak in 1983 in camels in the Daragodle area of the Sahil region. That outbreak resulted in significant mortality (up to 30%) in camels (Dirie et al., 1989).

However the current study clearly identified a high level of infection with *T. evansi* in camels originating from the eastern regions of Somaliland. The *Guban* zone is characterised by low and irregular rainfall, sandy and well-drained soils, high daily temperatures and sub-surface ground water (Sommerlatte and Umar, 2000). The vegetation of the *Guban* zone is well adapted to an arid climate and the zone is an important grazing area for camels and goats during November and December when the rains occur. Camels graze on *Suaeda* spp. within short distances of the sea (Sommerlatte and Umar, 2000). In this region a nomadic system of livestock production is adopted involving livestock migrations in seasonal or annual cycles. These migrations are adopted to minimise the impact of local shortages in water and fodder. However herd movements into the high-risk areas for surra will likely result in increased disease and greater opportunities for the transmission of the parasite from infected to susceptible animals.

In Sudan, herds owned by nomads had a higher level of infection with *T. evansi* than

did herds raised under an agro-pastoralist system (Elamin et al., 1998). Nomadic herds have increased opportunities for disease transmission through contact with other herds at watering and grazing areas, as has been reported in Chad (Delafosse and Doutoum, 2004). A study in Mauritania highlighted that camels migrating to the south had a higher prevalence than those kept in the north (Dia et al., 1997). Those differences were due to southern districts providing favourable conditions for the transmission of surra through biting flies. The clinical signs observed in this study were similar to those reported by others (Baumann and Zessin, 1992; Dirie et al., 1989; Mohamed, 2007) and included rough coats, emaciation, loss of hair from the tail region, a characteristic fetid urine smell, disappearance of the hump, severe muscle atrophy of the rear quarters and abdominal oedema. Similar clinical signs have also been reported in the neighboring countries of Kenya (Njiru et al., 2004) and Ethiopia (Hagos et al., 2009).

In the current study although the seroprevalence in female camels was slightly higher than that of males, the difference was not significant. In camel rearing areas in Pakistan a higher prevalence of *T. evansi* infection was also reported in females, although again the difference was not significant (Bhutto et al., 2010). This observed difference was attributed to the stress on females during pregnancy and lactation, which had the potential to decrease the animals' resistance to infection with *T. evansi*. Similarly in Mauritania a higher prevalence has been reported in female camels (Dia et al., 1997).

In the current study there was no significant difference in the seroprevalence in adults, young adults and calves, although adult camels did have a higher seroprevalence than calves. This increase with age could be attributed to increased opportunities to exposure to infected tabanids. Furthermore, (Elamin et al., 1998) also reported that flies

preferentially fed on adults rather than calves, and this could also explain the slightly higher prevalence in adult camels. Several other studies have similarly reported an increase in seroprevalence with age (Dia et al., 1997; Njiru et al., 2004; Tadesse et al., 2012).

The seroprevalence was highest in camels with a poor body condition. This may be due to the camels being in the chronic stages of surra when loss of body condition is a common presenting clinical sign of trypanosomosis. Most of the camel herders reported that disappearance of the hump, a “tucked up” abdomen and severe muscle atrophy of the rear and front quarters were characteristic of the final stages of trypanosomosis in camels. Furthermore infection can result in elevation of body temperature and camels may present with complicated clinical signs and secondary infections due to immunosuppression (Enwezor and Sackey, 2005; Njiru et al., 2004) and infected animals may be more vulnerable to concurrent infection with other pathogens.

This is the first study to describe the seroprevalence of infection with *T. evansi* in camels in Somaliland. Infection was widespread in camel herds, particularly in the *Guban* and *Haud* ecological zones. It was evident that trypanosomosis in camels is a significant problem in herds with the potential to significantly reduce the productivity of livestock and hence reduce the income of the nomadic herders and pastoralists. There is a need for the establishment of cost-effective programmes for controlling surra in Somaliland. In the following chapter the results of a study designed to quantify the economic benefits of different disease control programmes in camels in Somaliland are reported.

CHAPTER FOUR

Modelling the potential benefits of different strategies to control infection with *Trypanosoma evansi* in camels in Somaliland

4.1 Introduction

Surra is one of the important protozoan diseases affecting camel productivity, with up to 30% morbidity and 3% mortality being reported (Njiru et al., 2002). The disease is of significant concern to Somali pastoralists due to the high number of camels raised, their importance in providing meat and milk to Somalis, their role in providing financial support to over 55% of the human population and their social and cultural significance to the community (Dirie and Abdurahman, 2003; Farah et al., 2007; Negassa et al., 2008). Furthermore they play an essential role as beasts of burden transporting households, milk and water (Farah et al., 2004). Although the disease can result insignificant losses for pastoralists, the only widespread intervention measure available is the use of chemotherapeutic agents (Anene et al., 2001; Baumann and Zessin, 1992; Enwezor and Sackey, 2005). However the therapeutic and prophylactic use of trypanocides is threatened by the potential development of drug resistance or toxicity from overuse (Anene et al., 2001).

A National Tsetse and Trypanosomosis Control Project (NTTCP), funded by the European Union, was established in the 1980s in Somalia, however following the collapse of the military regime in 1991 no control measures to reduce the losses from trypanosomosis have been implemented. Since then the Veterinary Service Department

(VSD) in Somaliland has relied upon disease reporting surveillance without the concurrent implementation of disease control strategies. It is likely that the number of cases reported to the VSD only reflects outbreaks where the mortality is high. The actual figures may be significantly higher than those reported because of poor surveillance and under-reporting by pastoralists. For effective involvement of pastoralists in a disease control programme, they need to be aware of the financial benefits from adopting control measures.

Economic analysis is an important element in the development of control programmes to determine the potential benefits arising from such programmes. An important component of this analysis is simulation of animal production (Perry and Randolph, 1999). Analytical models for the transmission of surra and vector and disease dynamics can be constructed to evaluate different disease control strategies (Dobson et al., 2009). In this chapter the results obtained from a dynamic infectious disease model are reported. This model was adapted from one developed for buffalo (Dobson et al., 2009) and was used to simulate and estimate the economic benefits of four different control options against surra in camels.

4.2 Materials and methods

4.2.1 Susceptible-infectious-subclinical (SIC) disease model for surra

This model was developed for surra endemic countries and contains deterministic and stochastic components. The susceptible-infectious-subclinical (SIC) model has been described in detail for models of *T. evansi* and its control and economic impact on

small-holder livestock owners in the Philippines (Dobson et al., 2009). By making some assumptions, models of the disease outcome in camels can be constructed in order to determine criteria for successful disease control by mass and targeted chemotherapy. The model framework allows the input of parameters in order to calculate the cost-benefit of different interventions and the potential benefit of surra control strategies in Somaliland.

The 2011 and 2012 seroprevalence data for *T. evansi* infection in camels in Somaliland was modelled using a SIC model framework as displayed in Figure 4.1. This model illustrates two populations of infected camels: the ‘Innate Resistant’ and ‘Innate Susceptible’ cohorts. Therefore, in each infected cohort there are three kinds of nodes (*o*, *i* and *c*) indicating uninfected-susceptible, infectious and subclinically-infected animals, respectively. In the SIC model, the transition between the three disease states depends on vectors, the disease prevalence, the host resilience and treatment (Dobson et al., 2009).

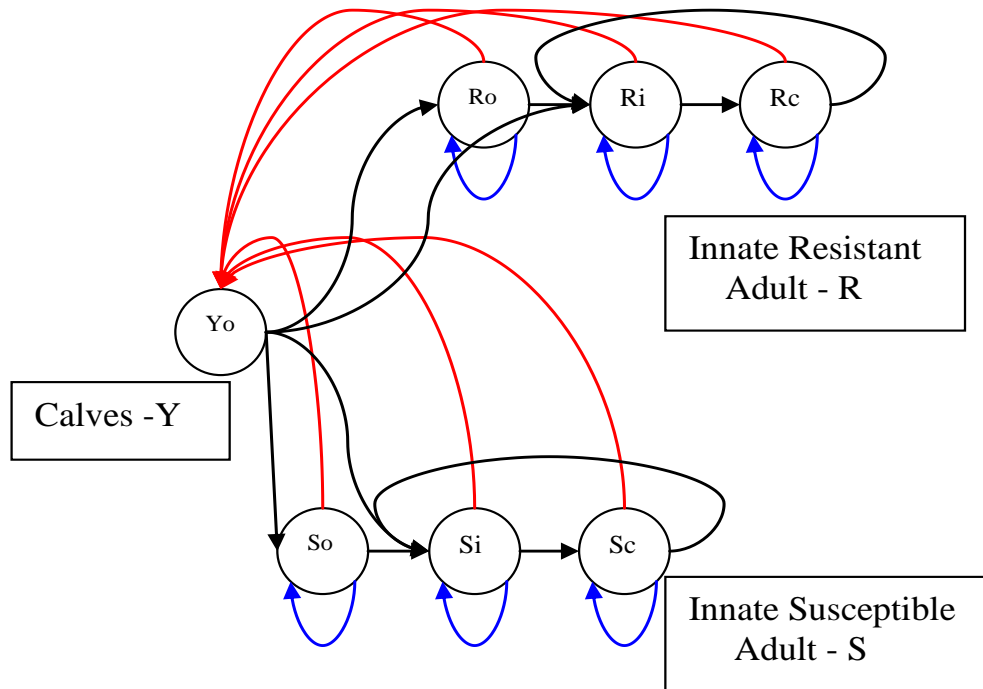


Figure: 4.1 Susceptible -infectious-subclinical (SIC) model framework of *T. evansi* infection (Dobson et al., 2009)

4.2.1.1 SIC transition states model

In Table 4.1 the transition states *o*, *i* and *c* are described. No transition state occurs from the two infected states (infectious - *i* and subclinical - *c*) to the uninfected-susceptible state (*o*), which is a common transition state for bacterial and viral diseases models such as the susceptible-infectious-resistant-susceptible (SIRS) model (Zheng and Wang, 2009). In Figure 4.1 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 (Dobson et al., 2009).

Table 4.1 Parameters and formulas used in the SIC model for *T. evansi* in camels

Cohort	Change	=	Uninfected * proportion	- losses	+ gains
Uninfected	ΔS_o	=	$((1 - Innate) * Y_o) * (1 - V * S \text{ to } I)$	$- V * S \text{ to } I * S_o$	
Uninfected	ΔR_o	=	$(Innate * Y_o) * (1 - V * R \text{ to } I)$	$- V * R \text{ to } I * R_o$	
Clinical	ΔS_i	=	$((1 - Innate) * Y_o + S_o) * (V * S \text{ to } I)$	$- S_i * I \text{ to } C$	$+ S_c * C \text{ to } I$
Clinical	ΔR_i	=	$(Innate * Y_o + R_o) * (V * R \text{ to } I)$	$- R_i * I \text{ to } C$	$+ R_c * C \text{ to } I$
Sub-clinical	ΔS_c	=		$- S_c * C \text{ to } I$	$+ S_i * I \text{ to } C$
Sub-clinical	ΔR_c	=		$- R_c * C \text{ to } I$	$+ R_i * I \text{ to } C$

[‡]Cohort size is represented by S_o , R_o , S_i , R_i , S_c , R_c & Y_o (calves), where S and R stand for innate susceptible and resistant adults, respectively

The SIC model allows the infected population that have been successfully treated in a time-step to be moved to the appropriate uninfected group (S_o or R_o) and therefore the simulation model time-step was set at one month (Dobson et al., 2009). There was no direct transition to a subclinical cohort without first becoming infectious (clinically infected). The main parameters of the model were either quantified or estimated as summarised in Table 4.2.

Table 4.2 Input parameters and symbols used for the SIC model to assess the four control regimens

Parameter (<i>symbol</i>)	Input value	Range	Assumptions
Innate	0.1	0 - 1	Proportion with innate resistance
<i>S to I</i>	0.8	0 - 1	Infection success for uninfected innate susceptible host
<i>R to I</i>	0.2	0 - 1	Infection success for uninfected innate resistant host
<i>I to C</i>	0.9	0 - 1	Transition proportion from infectious to subclinical
<i>C to I</i>	0.6	0 - 1	Transition proportion from subclinical to infectious
# Sex	0.8	0 - 1	Female proportion of the population
<i>Birth rates</i>			
μ_{ro}	0.49	0-40	Uninfected resistant-Ro female
μ_{so}	0.49	0-40	Uninfected normal-So female
μ_i	0.10	0-40	Infectious Si & Ri
μ_c	0.10	0-40	Sub-clinical infected Sc&Rc
<i>Death rates</i>			
λ_y	0.12	0-1	Calf uninfected Yo
λ_{ro}	0.12	0-1	Uninfected resistant Ro
λ_{so}	0.12	0-1	Uninfected normal So
λ_i	0.20	0-1	Infected Si & Ri
λ_c	0.15	0-1	Sub-clinical infected Sc&Rc
<i>Drug treatment</i>			
<i>Treat I</i>	0.90	0 - 1	Proportion of infectious animals treated
<i>Treat C</i>	0.15	0 - 1	Proportion of sub-clinical animals treated
<i>Treat Y</i>	0.04	0 - 1	Proportion of uninfected animals treated
^a Efficacy	0.99	0 - 1	Proportion of treated animals rendered uninfected

Parameter (<i>symbol</i>)	Input value	Range	Assumptions
^a Drug	12	0 - 12	Number of drug treatments/year
Cost/Benefit parameters			
Labour and cost per test	\$5		
Drug and labour cost per treatment	\$12		
Sale or purchase	\$1000		Price of replacement host
Infection dynamics			
^a maxMonth	5	1 - 12	Month in which the maximum fly intensity occurs
^a V _i	1 to 9	0 - 10	Arbitrary measure of vector
SCinfect	0.15	0 - 1	Proportion of subclinical animals that are infectious
V=V _i *I/N			Vector transmission
^a N= S _o +R _o +S _i + R _i +S _c +R _c +Y _o			Total camel population
^a I = S _i +R _i + SCinfect* (S _c +R _c)			Number of infectious animals
^a Parameters were not subjected to Monte Carlo simulation			

4.2.1.2 Mechanical transmission

Trypanosoma evansi is mechanically transmitted between camels by members of the *Tabanidae* (Dirie et al., 1989). The efficiency of transmission is dependent upon the interval between two successive feeds, a subsequent attempt to bite animals and the density of

biting flies (Desquesnes et al., 2009; Enwezor and Sackey, 2005). The transition details in the SIC model are displayed in Tables 4.1 and 4.2. Most of the variables involved in the model are dependent upon factors linked to the vector, parasite or host. In this simulation model tabanid intensity or attack rate was assumed to be seasonal and randomly was set to range from 0 to 10 to represent vector intensity (V_i) (Dobson et al., 2009). Three parameters of fly intensity ($maxMonth$, max and min) were used in the simulation, which fix the month of maximum fly intensity due to the mechanical transmission of surra. 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 and fly density (Dobson et al., 2009).

In this study the maximum fly intensity was assumed to occur in April, May and June (4th, 5th, 6th months) (Mohamed, 2011). The minimum level of fly intensity was assumed to occur six months after the maximum level, with *Vector Intensity* (V_i). A seasonal fluctuation between the maximum and minimum levels of V_i is displayed in Figure 4.2. The parameters for the innate susceptible to infection and innate resistant to infection (S to I and R to I) are described as the probability of successful transmission from one animal to another through a bite from a fly carrying the parasite for the innate susceptible and resistant cohorts, respectively. The proportion of uninfected camels that become infected in a particular month was calculated as $V * S$ and I and $V * R$ to I for the innate susceptible and resistant hosts, respectively.

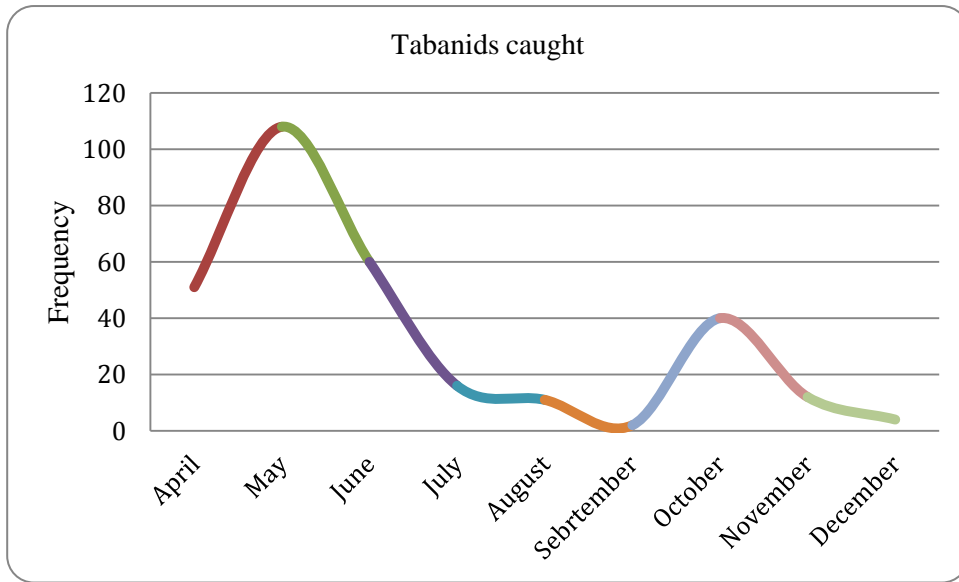


Figure: 4.2 The number of Tabanids caught on camels in open fields over the year (Mohamed, 2011)

The SIC state transition from susceptible to infectious was determined by the two equations displayed in Tables 4.1 and 4.2. This is similar to the ‘frequency-dependent transmission’ defined for host-micro-parasite relationship models (Begon et al., 2002). The probability of infectious animals within the camel population is assumed to represent I/N , where N is the total number of host. Therefore, the probability of infectious animals is assumed to be a proportion not a 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 (Dobson et al., 2009). To estimate transmission success the proportion of infectious animals (I/N) was further amplified or decreased by V_i to yield V (i.e., $V = V_i * I/N$), which was used in the model (Table 4.2).

4.2.2 Evaluation of the SIC model for surra control regimens in Somaliland

A simple infectious disease model was used to assess the success of different control regimens for infection of camels with *T. evansi* in Somaliland. The primary purpose of this model was to quantify the economic benefits of different control regimens with respect to the impact of surra on productivity. Monte Carlo simulation was undertaken to assess the different treatments. Random variables and Monte Carlo simulations were generated by using the add-in program to Excel (Microsoft Inc., USA), Pop Tools version 3.2.5 (CSIRO, Australia) (Hood et al., 2009). Using Monte Carlo methods enables an assessment of the relative importance of the parameters to be made (Dobson et al., 2009). The period of simulation was 15 years though the results of the models focused on the first five years in order to estimate the benefit of the control regimens. The four treatment regimens considered were: (1) Biannual treatment of all camels; (2) Monthly targeted treatment of clinically sick camels; (3) Biannual targeted treatment of seropositive camels; and (4) no treatment of camels.

The model input parameters, drug efficacy and the proportion of infectious animals and the proportion of sub-clinical animals treated for the four different control simulations are summarised in Table 4.3. For regimens 1 to 3 the drug efficacy was assumed to be 99% (i.e., for Cymelarsan[®]) shown to be effective against surra in camels in Ethiopia (Zellek et al., 1989). For regimen 1, biannual treatment of infectious camels (*I*), subclinical camels (*C*) and uninfected camels (*So*) was each set at 100%. In regimen 2, the monthly targeted

treatment of all clinically sick camels the values were set at 0.90 (*I*), 0.1 (*C*) and 0.04 (*So*). For regimen 3, the biannual targeted treatment of seropositive camels, the efficacy of treatment for seropositive animals was dependent upon the sensitivity and specificity of the diagnostic test used. Therefore, the proportion of infectious (*I*), sub-clinical (*C*) and uninfected camels (*So*) treated was set at 0.83, 0.83 and 0.04, respectively. It was assumed that the CATT had a sensitivity and specificity of 83 and 96%, respectively (Reid and Copeman, 2003).

To simulate these regimens, additional assumptions were made. When estimating the total number of drug treatments required for regimens 1 to 3, drug treatment was stopped when no new clinical cases occurred in the host. The proportion of camels successfully treated in a particular time step was removed from the infected groups and returned to uninfected groups. If the number of drug treatments/year were set at 0.5, 1 or 2 then a simulation treatment would be planned once every 24, 12 or 6 months, respectively. The annual birth and death rates were converted to monthly rates. The proportion of births, deaths, chemotherapy and transition rates were treated as random variables for 500 iterations using the Monte Carlo simulation method to estimate the mean model results and their 95% confidence limits (CL). Random selection for the parameters given in or as described above was generated using the Pert (using “expert opinion”) distribution, the most likely value being that given in (Table 4.2).

Table 4.3 Model input parameters used for four different control options for surra in camels

Regimen	Drug (Cymelarsan) efficacy	Proportion infectious treated	Proportion sub-clinical treated	Proportion uninfected treated
(1) Biannual treatment of all camels	0.99	1.00	1.00	1.00
(2) Monthly targeted treatment of clinically sick camels	0.99	0.90	0.10	0.04
(3) Biannual targeted treatment of seropositive camels	0.99	0.83	0.83	0.04
(4) No treatment of camels	-	-	-	-

4.2.3 Cost-benefit analysis

Cost-benefit analysis is one of the most common economic methods to determine the profitability of an intervention program. It is typically carried out to compare and contrast different strategies to determine the benefit of action or value over an extended period of time (Dijkhuizen et al., 1995; Rushton et al., 1999). Cost-benefit analysis of different control regimens that will run for a number of years can be evaluated to study control strategies for surra. The Monte Carlo simulation enabled an assessment of the total benefit for all herds for the treated and untreated control options. The net-benefit for each drug regimen was obtained by subtracting the cost-benefit result for the untreated simulation (regimen 4) from the cost-benefit result for the drug treatment regimens (1 to 3). This was done to simplify the cost-benefit analysis by ignoring common costs and benefits (Dobson et al., 2009). The cost parameters of the disease were fixed in the model, as the impact of the disease on productivity was the primary concern of the study rather than accounting for the influence of fluctuations in market prices (Dobson et al., 2009).

In the simulation T , M and R represented animal *Treatment*, *Maintenance* and *Replacement* costs, respectively, and D , C and E represented the benefits from animal *Draught-power*, *Consumption* and *Export*, respectively (Dobson et al., 2009). If B_t and B_u are the benefits from the *treated* and *untreated* regimens, respectively, then the net-benefit for a treatment regimen ($B_t - B_u$) is given by:

$$Bt - Bu = Dt - Du + Ct - Cu + Et - Eu - Tt - Mt + Mu - Rt + Ru.$$

Which reduces to: $Bt - Bu = Et - Eu - Tt - Rt + Ru$,

if it is assumed that under both regimens: (a) draught-power requirements are sufficient; (b) consumption of animals is similar; and (c) maintenance costs are similar. Thus, the simplified cost-benefit analysis for each regimen needed 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 only the net-benefit for each treatment regimen is estimated (Dobson et al., 2009).

The mean benefit (mean of the benefit of the camels) in Somaliland Shilling or US\$ was determined for each drug treatment regimen and the untreated hosts (i.e., $\{\sum B_i\}/1$ where B_i was the mean benefit/year for host i and $i=1$). The total benefit for a treatment regimen was estimated using the following formula (Dobson et al., 2009)

Total benefit = $\sum N_i * B_i$; where N_i was the number of animals of host type i and $i=1$

The total financial losses in camels in the study area due to infection with *T. evansi* were estimated by subtracting the total benefits (sum of the benefit for all animals in a village) for the untreated (regimen 4) from the total benefit obtained with the most effective treatment regimens (1, 2 or 3).

4.3 Results

4.3.1 Surra/camel SIC model for control regimens

This model contained deterministic and stochastic components by using the most likely values of parameters required for surra control regimens from 500 random simulations in Tables 4.2 and 4.3. The results of the deterministic model, the mean results from the drug treatments used in the first five-year period and the lower and upper 95% confidence limits (CL) are displayed in Table 4.4. The results showed that regimen 2 (the monthly-targeted treatment of clinically sick camels) and regimen 3 (biannual targeted treatment of seropositive camels) required significantly fewer treatments than the treatment of all camels biannually. However, the results indicated that the lower confidence limits for regimens 2 and 3 were less than 300 treatments, whilst the value for regimen 1 (biannual treatment of all camels) was greater than 700.

Table 4.4 The results of the deterministic model and the mean (total number of treatment regimens 1-3) of drug treatments used in the first five years for three different regimens 1 to 3

Number of drug treatments	Model	Mean	95% CL
(1) Biannual treatment of all camels	311	776	(765, 786)
(2) Monthly targeted treatment of clinically sick camels	311	338	(249, 473)
(3) Biannual targeted treatment of seropositive camels	290	284	(136, 450)

The net benefit from the three drug treatment regimens with Cymelarsan[®] was obtained by subtracting the cost/benefit result for the untreated simulation (regimen 4) from the cost/benefit result for the drug treatment regimens (1, 2 and 3). The model's prediction of the mean seroprevalence of surra for regimens 1, 2, 3 and 4 was 7.4, 6.4, 6.7 and 72.2%, respectively (Figure 4.3).

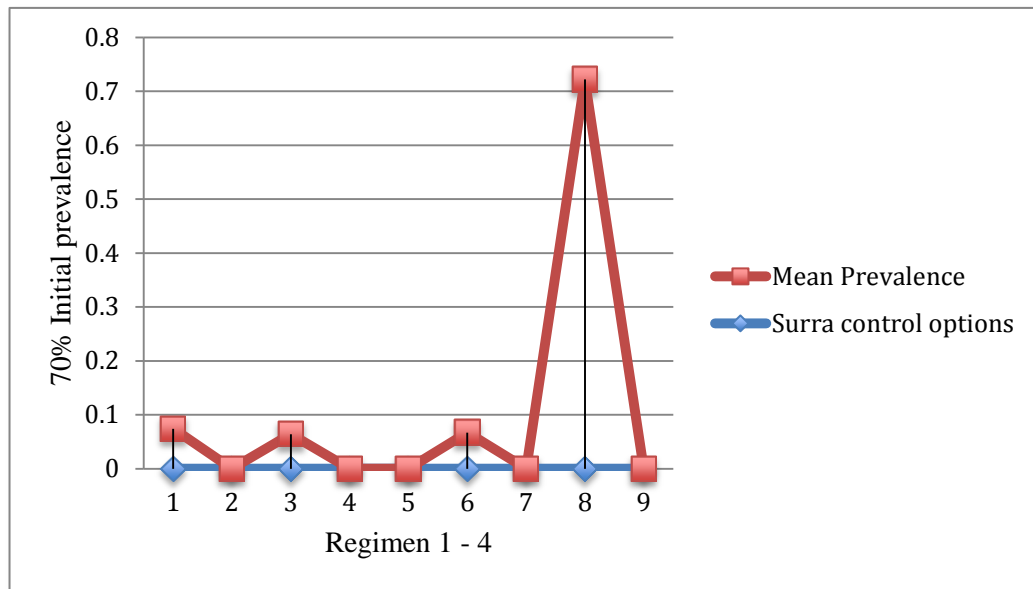


Figure: 4.3 The mean seroprevalence predicted during the first five years of treatments

4.3.2 Cost benefit analysis

The results for drug treatment regimens 1, 2, 3 and 4, assuming 99% drug efficacy with Cymelarsan[®], were 212,400,000, 255,600,000, 172,200,000 and 82,200,000 Shillings (assuming 600,000 shillings is approximately US \$100) per animal per year, respectively.

The mean-benefit for each drug regimen and 95% confidence limits is given in Table 4.5. The mean benefit for regimens 1, 2 and 3 increased 158, 211 and 109%, respectively compared with the untreated camels. Regimen 2, the monthly-targeted treatment of clinically sick camels, had a mean benefit up to 2.1 times greater than that for untreated camels. For regimens 1 and 3, the benefit was 1.6 and 1.1 times greater than that for the untreated regimen, respectively.

The results indicate that the total benefit or loss for a village (average of 80 camels) for a five year period was highest for the monthly-targeted treatment of clinically sick camels (102.3 billion shillings per annum - US \$170,577) followed by biannual treatment of all camels (US\$ 141,431) compared with 32.9 billion (US \$54,972) for untreated camels (Table 4.5). The mean benefit per animal per year was calculated at US\$354, US\$426 and US\$287, respectively compared with US\$137 for untreated camels (Table 4.5). Consequently the model predicted that the total net benefit loss to a village (80 camels) that was not applying the recommended effective surra control strategy was 69.4 billion shilling per annum (US \$115,605). In terms of the value to the camel this was estimated to be US \$217, \$289 and \$150 per animal/year.

Table 4.5 Mean annual benefit per animal and the total mean benefit per village (average of 80 camels) for five years simulation

Treatment group	Mean benefit per animal (\$)	95%CL	Mean benefit per village of 80 camels for five years (\$)	95%CL
(1) Biannual treatment of all camels	354	(211, 509)	141,431	(84,401; 203,690)
(2) Monthly targeted treatment of clinically sick camels	426	(321, 543)	170,577	(128,327; 217,018)
(3) Biannual targeted treatment of all seropositive camels	287	(126, 454)	114,625	(50,483; 181,679)
(4) Untreated camels	137	(53, 239)	54,972	(21,228; 95,709)

4.4 Discussion

The introduction of disease control strategies provides benefits that increase over time as the productivity of the herds improve. Consequently it usually takes several years for livestock farmers to gain the full benefits of implementing a new control strategy (Kristjanson et al., 1999); however estimating economic losses from parasitic disease can be difficult resulting in evaluating different disease control measures challenging. There is an increasing number of integration techniques for economic analysis and simulation models of animal health and production dynamics within a systems framework, and these techniques have been used to evaluate the financial implications of the direct and indirect effects of various management options (Perry and Randolph, 1999).

In this chapter economic models were developed and applied to study the impact of trypanosomosis in camels in Somaliland. The potential benefits to farmers through the control of surra were assessed through an SIC model (Dobson et al., 2009). This model has the advantage that parameters can be easily adjusted to evaluate changes in the potential control program. The SIC model outlined in this chapter was used to predict losses due to the disease and the net-benefits from different control regimens. Such a model can also be used in other animal species and importantly helps evaluate different disease control strategies to enable selection of the most appropriate cost-effective control measure for the disease in Somaliland.

Mathematical models to determine the epidemiology and control of parasitic disease have been developed to study the economic benefits of different disease control strategies (Habtemariam et al., 1988). A dynamic mathematical model has previously been developed to understand the development and potential reversal of trypanocide resistance (Grace, 2006). Studies on the mechanical transmission of trypanosomes in Africa (Desquesnes et al., 2009) have also been used to measure the costs of animal trypanosomosis in Africa (Kristjanson et al., 1999). The strength of these studies has been their ability to evaluate different disease control strategies and the potential economic benefits from the control of this disease. Similarly, the SIC model reported in this chapter estimated the cost of the disease in an endemic area and it is clear that this disease has the potential to have a

significant impact on the livelihood and social well-being of pastoral communities. Camels play a major economic role in the community in Somaliland through the production of milk, meat and offspring, and are the cornerstone of the social organization of the pastoral society. This is particularly relevant given that approximately 55% of the population in Somaliland are nomadic pastoralists who live in the extensive dry areas of the country (FAO, 2004).

In the SIC model reported in this chapter, the financial losses arising from reduced weight and milk yield associated with *T. evansi* infection were not evaluated (Dobson et al., 2009). As outlined previously, the cost parameters were fixed in the model, as the effect of the disease on productivity was the primary concern of the study. However, taking into consideration the above factors, the total cost of the disease is likely to be well in excess of US \$115,605 (69.4 billion shilling) per five years (80 camels) in the villages. This study reported the financial losses attributable to *T. evansi* infection in Somaliland, and the costs that potentially could be saved as a result of implementing an appropriate disease control strategy.

The estimates arising from the modelling reported in this chapter only form part of a complete economic analysis of the disease. However the results can provide a better overall understanding of the impact of the disease in camel herds and can contribute to estimating the value of the losses from surra (Dijkhuizen et al., 1995). The losses in an individual camel herd can be considerable and these losses can be used to compare the benefits of implementing different disease control strategies. Most estimates of the economic losses

arising from infection with *T. evansi* are based on direct losses from the disease's effect on livestock mortality and morbidity. Studies in Indonesia estimated the annual economic losses due to morbidity and mortality in all livestock at US\$28 million and reduced weight gain by 7.6 kg over three months in individual cattle fattened for beef production(Reid, 2002). In the Philippines estimated deaths from *T. evansi* infection has been valued at US\$1.1 million over a nine year period (Reid, 2002).The estimated financial losses to an individual village in the Philippines from infection of buffalo owned by smallholder farmers was US\$158,000 per annum(Dobson et al., 2009). In another study in horses in Pantanal, Brazil the estimated annual losses from surra to ranchers were considerably higher at US\$2.4 million (Seidl et al., 1998).

In the present study the model predicted that the biannual treatment of all camels and monthly-targeted treatment of clinically sick camels using a highly efficacious drug (Cymelarsan[®]) were the best treatment options for controlling infection of camels with *T. evansi* in Somalia/Somaliland and parts of East and North Africa. Of the drugs available in the region only melarsomine hydrochloride (Cymelarsan[®]) is a dry powder, highly soluble in water and is only registered for use against *T. evansi* in camels.

The model suggested that if villages containing 80 camels used an effective curative and preventive treatment, a mean benefit of US\$141,431biannual treatment of all camels and US\$170,577monthly-targeted treatment of clinically sick camels would eventuate for five years. This financial benefit was associated with a concurrent reduction in disease prevalence over a five year period. Similar to the findings of the current study, another

study using an infectious disease model for small-holder livestock in Philippines showed that 75% fewer treatments were required if clinically sick animals were specifically targeted for treatment, although it took a long time to reach a low prevalence (Dobson et al., 2009).

A significant challenge in the control of surra in the current environment is a lack of a proper surveillance and diagnostic capability in pastoral communities. Implementation of this strategy would require undertaking training and increased awareness of camel herders on the economic benefits of controlling the disease and the costs associated with the disease. This option would have the advantages of enhanced disease surveillance and reporting, and would allow improved herd management through monthly monitoring for the clinical signs of *T. evansi* infection. Although it is apparent that herders could not totally avoid the risk of infection in their camels, the implementation of effective chemotherapy, in combination with consistent monthly disease reporting, could lower the infection rate within the region and therefore lead to increased productivity and herd fertility. The mean results of monthly-targeted treatment of clinically sick camels (Regimen 2) and biannual targeted treatment of seropositive camels (Regimen 3) overall required fewer treatments than did the treatment of all camels biannually (Regimen 1).

The results indicate that regimens 2 and 3 had lower overall confidence limits (lower 95% CI <300; and upper values <500). In comparison regimen 1, which involved treating all camels biannually, had lower confidence limits which were greater than 700. For Regimen 1, the mean results from drug treatments over the first five-year period showed significantly

higher results compared to regimens 2 and 3 (Table 4.3). The main disadvantage in this regimen is the indiscriminate use of drug therapy that could increase the risk of drug resistance developing. Drug resistance of trypanosomes has increased dramatically in recent years and there is evidence to suggest widespread resistance of *T. evansi* in Africa to drugs (Afewerk et al., 2000; Assefa and Abebe, 2001; El Rayah et al., 1999). Therefore, any widespread treatment to control surra has the potential to promote the development of resistance and result in significant financial implications through the loss of efficacious drug treatments.

In conclusion, the control of *T. evansi* depends mainly on chemotherapy and therefore implementing the biannual treatment of all camels and monthly-targeted treatment of all clinically sick camels are recommended to reduce the significant mortality and production losses associated with the disease. These options would be suitable approaches for controlling surra in Somaliland, as they increase benefits, require fewer treatments and therefore have lower costs than alternative treatments. It is important that for control programs to be adopted, pastoralists must understand the impact of the disease on their animal's productivity and the financial losses arising from the disease. The results of this study provide a valuable indication of the benefits of different surra control strategies and highlight the need for implementation of adequate control measures. These assessments require a more thorough economic assessment of the impact of the disease, and the total losses from the disease need to be calculated to indicate the extent of the problem. In the following chapter the losses associated with reduced productivity and the financial benefit in adopting different control strategies are evaluated.

CHAPTER FIVE

Estimating the economic impact of *T. evansi* on pastoral camel production in Somaliland

5.1 Introduction

The traditional livestock sector in Somalia is based on nomadic pastoralism where sheep, goats and camels are herded in large numbers. However the level of animal productivity is generally low due to disease and poor management (FAO, 2004). Surra and sarcoptic mange are reported to be the two most important diseases of camels in the central and northern rangelands of Somalia (Baumann and Zessin, 1992). Pastoralists mainly keep camels under a subsistence production system and these animals are a source of prestige and have cultural importance, as well as being an important source of income (Dirie and Abdurahman, 2003). Approximately 60% of the dromedary camel population in the world is found in Somalia, Sudan, Kenya and Ethiopia with Somalia having the largest population, estimated at over 6 million head (Farah et al., 2007).

Camel milk is one of the main components of the pastoralists diet and contributes up to 30% of the annual caloric intake (Baumann and Zessin, 1992). The camels produce more milk and lactate for longer than do any other milking animal raised under similar harsh environmental conditions (Pasha et al., 2013). The daily milk yield ranges between 3 to 10 kg in a lactation period of 12 to 18 months and this year-long milk production makes the animal the most valuable of all livestock species in Somali pastoral societies.

There is the potential for individual camels to produce more than 15 litres of milk a day during the peak of their lactation (Farah et al., 2007). Camels represent a significant business in Somalia, generating an annual income of US\$250 million through exports (FAO, 2012). Although the economic impact of surra is believed to be significant in Somaliland, data are lacking to confirm the exact losses. There is limited information on the losses associated with reduced milk yield and meat production due to the lack of epidemiological data. As well as the direct losses incurred through reduced productivity and deaths, camels infected with surra are often sold for slaughter at lower prices to avoid treatment costs if their production performance decreases substantially (Yusuf Mohamed, personal communication). Several studies in Kenya and Ethiopia have suggested considerable economic losses through the disease's impact on growth, production, productivity and reproduction (Njiru et al., 2004; Zeleke and Bekele, 2001).

Based on data from Chapter Four the financial benefits of various control options for surra, including a no control strategy, were calculated to quantify the benefit in controlling the disease. Previously there has been no attempt to assess the economic impact of surra in camels reared under the Somaliland pastoral system. Such information would be useful for estimating the economic loss of the disease and to evaluate the financial profitability of a control strategy for the disease. Data from the cross-sectional seroprevalence survey and the economic impact of *T. evansi* on camels was used to estimate the losses associated with

reduced productivity and the financial benefit in adopting control strategies against the disease.

5.2 Materials and Methods

5.2.1 Description of the Study Area

The area of study in this chapter was described in detail in Chapter Three.

5.2.2 Study Design

A questionnaire survey was conducted from 2011 to 2012 in different ecological zones used for rearing camels in Somaliland. A total of 40 participants who owned camels and were familiar with their husbandry were selected using a purposive sampling technique in order to collect data on the economic impact of surra on camels raised under a nomadic pastoralist system. The impact of surra on milk production and the export market was investigated. Financial modelling was based on estimates of the average values for a range of production parameters generated through a questionnaire (Table 5.1).

5.2.3 Assessment of the economic loss from surra

Camels are very reliable milk producers during dry seasons, particularly in drought years when milk from cattle, sheep and goats is scarce. At such times camels can contribute up to 50% of the nutrient intake of the pastoralists (Farah, 2004). Camel meat is also an important by-product, mainly as a source of income and export of live camels, and the slaughter of males and unproductive females at local butcherries is very common in Somalia. However, it is difficult to evaluate the economic contribution made by camels as

most of their products are traded in the informal market sector. A large proportion of a herd (70 to 80%) is female. This high number is needed to satisfy the large milk requirement of the nomadic community (Farah et al., 2007). Loss of production is the most significant economic impact of animal disease (Perry and Randolph, 1999). In this study the economic loss due to infection with *T. evansi* on milk and meat products per year was determined for camels from data collected by interviewing camel herders and through field data collected from the seroprevalence survey. This study only examined productive male and female camels between the ages of 5 and 20 years old. Females are sexually mature at the age of 4 to 5 years and can remain fertile up to the age of 25 years (Farah, 2004).

Of the 2575 camels belonging to 144 herds sampled in Chapter 3, 2159 camels were selected for estimating economic impact of surra on camel. These animals were productive male and female camels since a representative of sample of the estimating economic impact of *T. evansi* on production was required in Somaliland. The division of these animals into groups is summarised in Figure 5.1. On the basis of these figures, the effect of infection with *T. evansi* on the milk and meat yield was investigated. The total value of the live camel export market, the total value of milk produced per camel each year, the total loss per year if animals were not treated, the total value per year if animals were treated and the total production losses were evaluated. The effects of surra on production were calculated by multiplying the total number of clinically affected camels in each category by the reduced value of the affected camels.

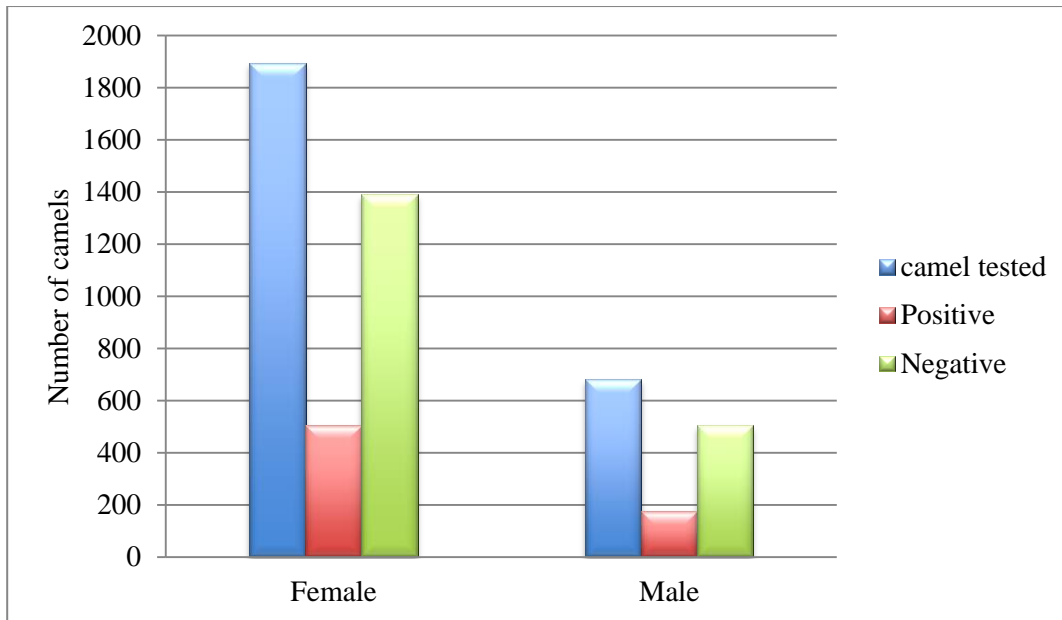


Figure: 5.1 Camels included in the study to determine the impact of *T. evansi* on camel production

The annual loss from milk and meat products were the key parameters assessed as these had previously been identified as key outputs of camels in Somaliland (Farah et al., 2004). Data on the overall annual milk production from a healthy camel and the losses associated with surra and the retail market price of camel milk were collected (Table 5.2). In the present study, the average milk yield of camels with and without disease were estimated at 2 and 6 litres per day. The local unit price of camel milk was recorded as US\$0.50. The average market price of a camel exported to the UAE was estimated at US\$820 (FAO, 2012). The cost of treatment due to the disease was estimated at US\$10 per head (M.F.

Dirie, personal communication) and the total herd cost was estimated by multiplying this value by the number of animals in the herd with clinical disease.

5.2.4 Direct financial costs and benefits of *T. evansi* treatment strategies

In this study, the cost of the disease was estimated by quantifying the direct and indirect losses due to the disease. The total cost (C) of a disease is thus the sum of the production losses (L), both direct and indirect, and the control expenditures incurred (E):with $C = L + E$ (Rushton et al., 1999). The losses due to surra included the reduction in the revenue value of milk and export products and losses from the animal's resultant poor body condition.

Table 5.1 Definition of effect, population size, production loss and benefits of treatment against surra

Effect	Population size	Loss of production	Benefit of treatment
Loss of milk yield	Number of females lactating	Loss of milk yield per camel	Price of milk yield
Loss of live animal exports	Number of males exported	Loss of value per camel exported	Price of camel exported

5.2.5 Risk of *T. evansi* infection

The annual risk of infection with the disease was determined as the probability that any herders would have an outbreak in a given year. Among the parasitological methods used to detect *T. evansi* in the blood stream, the MHCT is recognised as the most sensitive test. Examination of the blood samples with MHCT in the field showed that 7.2% of the camels

were newly infected with *T. evansi* in central Somalia (Baumann and Zessin, 1992). Similar studies have reported that 5% of camels from herds examined in Sool and the northwest regions of Somalia were infected with *T. evansi* (Dirie et al., 1989).

Most serological tests can effectively complement parasitological methods to improve diagnosis and determine disease prevalence. Nevertheless, surra has been found to be endemic in Somalia, with an estimated prevalence of 56% when using an ELISA (Baumann and Zessin, 1992). It has also been reported that higher levels of infections are present in the long wet season in the riverine zones (Baumann and Zessin, 1992). Most importantly surra generally is chronic and infected animals survive for up to 3 to 4 years, resulting in heavy production losses (Ngaira et al., 2003).

Taking all of this into consideration, a conventional estimate of the risk of infection with *T. evansi* in camels from Somalia is between 1.7 and 56.2% (Baumann and Zessin, 1992). In the present study, a seroprevalence of 38.2% (data reported in Chapter Three) was used to investigate the financial impact of surra on camel production (Table 5.2).

Table 5.2 Components of the estimated benefit-cost analysis for evaluating treatment strategies for surra

Parameter	Value	Range	Units	Source
Herd risk of infection	49.2	1.7 - 56.2	%	(Baumann and Zessin, 1992)
Expected animal losses	20	1.0 - 30.2	%	(Dirie et al., 1989)
Herd size	80	30 - 100	#	Field data
Female herd size	75	70 - 80	#	(Farah et al., 2004)
Value of a female camel	900	600 - 1200	US\$	Field data from 2011 - 2013
Value of a male camel	820	500 - 1000	US\$	(FAO, 2012)
Treatment cost per camel	10.1	10.0 - 10.5	US\$	Cymelarsan [®] (personal communication)
Cost of diagnosing disease per camel	1	-	US\$	(Soumare, 2006)
Average milk production per day	6	5 - 15	Litres	(Farah et al., 2004)
Reduction in milk production	27	-	%	Field data from 2011 - 2012
Reduction in value of camels	26	-	%	Field data from 2011 - 2012

5.2.6 Components of the treatment strategies to control infection with *T. evansi*

The components of the different control strategies for surra discussed in Chapter Four were evaluated through calculating the benefit-cost ratio in Somaliland (Table 5.3). The anticipated financial impacts of adopting the treatment strategies are dependent upon their costs and benefits and the probability of an outbreak. The strategy of no treatment or doing nothing was compared to the other treatment strategies.

Table 5.3 Control strategies against *Trypanosoma evansi* evaluated in camels from Somaliland

Strategy	Description	Number of camels treated
No treatment	No camels are treated and no control strategy is adopted	-
Biannual treatment of all camels in the herd	Camels are treated during two consecutive wet seasons (99% assumed to be treated)	2137
Monthly treatment of clinically sick camels	Camels with clinical signs are treated	565
Biannual treatment of seropositive camels	Camels that are seropositive are treated in two consecutive wet seasons	816

For the biannual treatment strategy of all camels it was assumed that 99% of the camels received treatment. For the monthly-targeted treatment of all clinically sick camels it was assumed that 26.5% were treated in a given year (Table 5.3). Biannual treatment of seropositive camels was assumed to involve treatment of 38.2% of the camels – the number of camels infected with surra in a given year. This assumed a 68% sensitivity and 100% specificity for the CAT test (Ngaira et al., 2003).

A benefit-cost analysis was evaluated to determine the financial profitability of implementing different control strategies against surra.

5.2.7 Data Analysis

A spreadsheet was constructed in Excel 2011 (Microsoft) to quantify the benefits and costs of controlling *T. evansi* under a variety of strategies. Losses due to the production effects and prevalence of *T. evansi* were estimated from the seroprevalence survey data conducted

over two years in Somaliland and special emphasis was given to milk production and the market value of exported camels (Chapter Three). These data were used to estimate the economic value of milk loss each year and losses associated with reduced exports in the number of camels.

The total cost of treatment was calculated by multiplying the cost of treatment, including the cost of implementation, by the number of animals treated. The cost components of treatment included the unit cost of the drugs, the number of camels dosed, the number of times camels were dosed per year and the cost of veterinary services and transport. Costs of treatment also included diagnosis and treatment of clinically affected camels. Benefits of the treatment components were estimated using gains from the disease control strategies and revenue obtained from the production. Similarly, the annual production loss due to disease prevalence was estimated.

The benefit cost ratio (B/C) was then calculated (Ramsay et al., 1999). A sensitivity analysis was conducted to determine the sensitivity of the results to the parameter estimates used in the study.

5.3 Results

This study analysed data from all females (1609) of which 27% (431) were lactating and 550 males, of which 144 (26%) were exported. The results indicate that the majority (88%) of respondents recognised the importance and impact of surra on camel production,

particularly household income and the consequent social impact of the disease. Most respondents (80%) reported that surra could impact the fertility of their herds. Most respondents (94%) also attributed biting flies to be responsible for causing surra. In addition, herders recognised the different risk between different ecological zones with a substantial increase in the risk of infection when biting flies were present in these areas.

All the interviewed camel herders could identify biting flies responsible for surra and understood the role the vector played in the transmission of *T. evansi*. The economic losses from trypanosomosis in this study included a reduction in body condition by 39.5% for camels in the study area. However the economic study indicated that the greatest losses were associated with decreased milk yield and carcass weight. A reduction in milk yield of 27% and reduced export market value of 26% was reported in this study.

5.3.1 Economic loss assessment

Estimating the economic loss resulting from surra is not easy due to limited information on the impact of the disease on the population. The impact of surra has previously been restricted to losses from mortality and the costs in controlling the disease (Reid, 2002). In the present study, losses through reduced milk yield and export marketing of males were analysed. In Table 5.4 the benefits of the no treatment strategies are reported. On the basis of the surveyed herders the annual benefits of the no treatment and curative treatment strategy (when no surra was present) were US\$1,808,225.00 and US\$2,212,855.00, respectively in the studied population.

Table 5.4 Comparison of the revenue generated when surra was present or absent

Category	Population size	Camels affected if disease present	Revenue if surra is present (US\$)	Revenue if surra was not present (US\$)
Female	1609	431	1,447,225.00	1,761,855.00
Male	550	144	361,000.00	451,000.00
Total	2159	575	1,808,255.00	2,212,855.00

The no treatment strategy gives an indication of the amount of revenue herders surveyed obtain if nothing was done to control the disease. The financial loss was calculated by subtracting the total value of sick camels if no treatment was administered from the total value of camels when treatment was administered.

The expected amount of revenue the herders studied in Somaliland could lose per year in the studied area was estimated at US\$404,630 (Table 5.5). The results show that the largest loss was associated with decreased milk yield (US\$314,630). The revenue obtained from the market value of exported animals was also reduced by US\$90,000 if no treatment was administered (Table 5.5).

Table 5.5 Economic effects of surra on camel production and the benefits of treatment

Category	Value of reduced production (US\$)	Average loss per head (US\$)	Benefit of treatment (US\$)	Average benefit per head if affected animals are treated (US\$)
Female	314,630.00	195.54	310,320.00	720
Male	90,000.00	163.64	88,560.00	615
Total	404,630.00	359.18	398,880.00	1335

The benefits of treatment were calculated by subtracting the total cost of treatment administered to the herds from the benefits obtained through increased production due to control of the disease (Table 5.5). Overall the benefit in treating camels in the study area was US\$398,880 (for 2159 camels). Most of this (US\$310,540) was due to improved milk production in treated animals (average of \$720 per head). The remaining \$88,560 was obtained from the increased value of exported camels (US\$615 - Table 5.5).

The average loss per animal estimated for reduced production and body condition loss in the study area of Somaliland was US\$195.54 and US\$163.64. Extrapolating the current findings to the total camel population at risk of acquiring surra in the endemic region of Somaliland (with approximately 2 million head), a loss in the magnitude of US\$223,164,000 would eventuate from surra. This loss is composed of reduced milk production (US\$164,253,600) and loss of body condition (US\$58,910,400).

5.3.2 Benefit/cost ratios of treatment strategies

The results of calculating benefit/cost ratios for different treatment strategies are presented in Table 5.6. The B/C ratios of the three treatment strategies are all greater than one, indicating there is financial benefit in adopting any of the three control strategies in Somaliland. The most economical viable treatment of *T. evansi* in Somaliland was the biannual treatment of seropositive camels (Table 5.6).

Table 5.6 Benefit-cost ratios of different treatment strategies for *T. evansi* in camels

Treatment options for surra	Number treated	Treatment cost (US\$)	Benefit/cost ratio
Biannual treatment of all camels	4275	76,656.10	5.00
Monthly treatment of all clinically sick camels	6784	93,800.70	4.08
Biannual treatment of all seropositive camels	1633	39,844.91	9.61

5.4 Discussion

The results presented in this chapter provide valuable information on the economic impact of trypanosomosis in camels raised under an extensive pastoral system in Somaliland. A simple spread sheet was used to estimate the economic losses from reduced milk yield and meat production resulting from infection of camels with *T. evansi* and to compare the economic viability of different disease control strategies. This study highlighted the impact surra had on milk yield and meat production if no treatment was undertaken. Furthermore most respondents (80%) reported that surra affected the fertility of their female camels resulting in increased calf mortality, although this was not measured in the current study.

Another study undertaken in the central regions of Somalia also reported the detrimental effects of trypanosomosis on the fertility of camels and high mortalities in calves during their first year of life (Baumann and Zessin, 1992). In addition, an extended inter-calving interval of 34 months was reported (Baumann and Zessin, 1992). Others have reported an inter-calving interval of 24 months in camels raised under a pastoral production system

(Farah, 2004). A major constraint to production of camels is the high mortality of calves in the first three months of age (Farah, 2004). However, it was uncertain if this is due to trypanosomosis, a reflection of the poor management system adopted or the presence of other infectious disease(s). Nevertheless, it is important to assess the economic impact of reduced production and disease in camels raised under pastoralist management systems.

The study reported in this and previous chapters provided measurements of losses associated with surra in these situations. In this study, 39.5% of carcasses were of poor quality. This could be the result of the chronic form of infection with *T. evansi*. Loss of body condition, progressive emaciation and reduced draught power are characteristic findings of camels affected with surra (Njiru et al., 2004; Swai et al., 2011). These presentations would also be associated with reduced milk and growth rate and poor carcass quality.

Surra causes high mortality, low milk and meat production and reduced draught output and manure production (Desquesnes et al., 2013). In the present study, the results indicated that surra led to a 27% reduction in milk yield and a reduction of 26% in meat production. Infection of camels with *T. evansi* is usually chronic, and therefore the economic impact of the disease on production is more likely in the areas where the disease is endemic. This study found that the financial loss attributable to surra in the endemic area, if no control programme was adopted, was US\$404,630. As was expected, the financial loss associated with reduced milk yield in lactating camels was greater than the reduced income obtained

from camels exported for meat. These findings agree with those reported by others on the impact of surra on camel production (Njiru et al., 2004; Zeleke and Bekele, 2001).

Several studies have highlighted the reduced meat production and milk yield of infected cattle in the Philippines (Reid, 2002). In that study cattle infected with *T. evansi* produced significantly less milk than did non-infected cattle and milk production increased after treatment with isometamidium (Pholpark et al., 1999). This highlights the significant negative impacts when no disease control programmes are implemented. The annual risk of infection of camels with *T. evansi* has the potential to result in significant annual financial losses through morbidity and mortality. Dobson et al. (2009) reported that failure to adopt effective treatment strategies for surra in an endemic area by small-holder livestock owners in the Philippines could result in a loss of US\$160,000 per year.

This study only investigated benefits from controlling the disease on milk and meat production, and did not study costs associated with mortality, reduced reproductive performance and lower draught potential or the costs of replacing camels. Consequently it is likely that the losses associated with the disease are even higher than that reported here. The financial losses due to *T. evansi* infection in horses in the Brazilian Pantanal region has been estimated to cost ranchers US\$2.4 million annually due to the mortality of over 6,000 horses (Seidl et al., 1998). Similarly, in Indonesia the annual loss from surra was estimated at US\$28 million (Reid, 2002). These losses can be greatly reduced if an effective control strategy is adopted (Seidl et al., 1998).

In this study adopting an annual treatment strategy resulted in a substantial benefit of US\$2.2 million annually to the surveyed herders, and over \$220 million to the national herd. This highlights the significance of surra on animal health and production in Somaliland.

In the current study all three treatment options evaluated were economically beneficial strategies, however the biannual treatment of seropositive camels in the herds was the best financial option. This regimen relies on the treatment of seropositive animals twice a year allowing the camels to be treated in the two wet seasons. In the wet seasons it is likely that the risk of infection increases as most infections are reported during this time (Baumann and Zessin, 1992). Treatment recommendations and strategies for chemotherapeutic control depend on information about the risk of trypanosomosis and the disease's prevalence (Abdel-Rady, 2008). However, mass treatments with chemotherapeutic drugs and overdosing have led to increasing resistance in Africa and potential toxicity (Anene et al., 2001; Salifu et al., 2010). The highest cost of treatment in this study was the monthly-targeted treatment of all clinically sick camels. An important advantage of this treatment strategy is that all sick animals receive a treatment. However, in the current study, the biannual treatment of seropositive camels was more economically efficient.

Pastoral camel production provides a positive impact on the economic, social and environmental well-being of humans who live in harsh ecological zones. The adverse effects of *T. evansi* on animal health and production have been investigated in many parts

of the world. Most studies suggest that economic losses are due to deaths, reduced productivity and the cost of chemotherapeutic interventions (Derakhshanfar et al., 2010; Reid, 2002). The evaluation of economic losses of trypanosomosis in camels raised under extensive pastoral systems is difficult because of the absence of individual animal records and the challenges of collecting epidemiological data in the field. This is mainly due to the management practices of nomadic pastoralists and the remote areas camel herders operate in. However, improving animal health and productivity is becoming critically important in this livestock industry. Furthermore, considering strategic approaches to the control of surra can play a major role in achieving efficient and economically viable production. Losses from trypanosomosis can be reduced greatly if appropriate interventions are adopted in all camel rearing areas. A control strategy for *T. evansi* depends mainly on the use of curative and prophylactic drugs and consideration of other factors, including animal movement and herd management.

Surra is transmitted by biting flies and knowing the distribution of these is important in understanding the epidemiology of the disease, including its distribution, and developing effective control programs. In the next chapter the results of an observational study conducted to identify the potential vectors for surra in camels and to establish their activity patterns and preferred landing site on camels is outlined.

CHAPTER SIX

The alighting and activity patterns of potential vectors of *T. evansi* on camels in Somaliland

6.1 Introduction

The family *Tabanidae* contains at least 137 genera and 4154 species of insects (Barros and Foil, 2007; Foil, 1989). Tabanids are commonly called horse flies and act as mechanical vectors for a wide variety of infectious diseases and agents in humans as well as domesticated and wild animals, including equine infectious anaemia virus, *Anaplasma marginale*, *T. evansi* and *T. vivax* (Barros and Foil, 2007; Foil, 1989). Tabanids are blood-feeding insects whose mouthparts can transfer agents during feeding. They tend to return to the same host instead of moving to another host following interruption of their feeding (Foil, 1989). A large range of biting flies can transmit *T. evansi*, facilitating transmission and distribution of the parasite to susceptible animals (Barros and Foil, 2007).

Different tabanids have varying host-orientated behaviours in response to the same set of environmental conditions (Foil, 1989). In Sudan at least 74 species of tabanids have been reported to be capable of mechanically transmitting animal trypanosomosis and their attacks on cattle are as much as 10 to 50 times the levels typically found in temperate environments (Mohamed-Ahmed and Mihok, 2009). Transmission of surra has been observed in areas where tsetse fly is absent in Somalia (Dirie et al., 1989) with tabanids

existing in the riverine region where they are capable of spreading infection (Baumann and Zessin, 1992; Dirie et al., 1989). Several species in the genus *Philoliche* are involved in the mechanical transmission of surra in Somalia (Dirie et al., 1989). This genus, which belongs to the subfamily Pangoniinae, is a relatively large fly that represents the horse flies found in sub-Saharan Africa. Female *Philoliche* can cause a painful bite when they feed on camels (Morita, 2008).

In the Brazilian Pantanal tabanids have been incriminated as the agents of non-tsetse transmitted trypanosomosis and equine infectious anaemia (Barros and Foil, 2007). These tabanids may vary in their daily activity patterns with many flies active during the early and late hours of the day (Gibson and Torr, 1999). The daily activity patterns and landing sites of tabanids have been investigated in many parts of the world to understand the relationship between the host and vector. This information can be critical in the development of better control strategies for the vector and consequently reduced disease transmission.

In this chapter the potential vectors for surra in camels in Somaliland are described for the first time to establish their preferred site of landing on camels and their activity patterns. Determining the alighting sites of vectors is useful for improving the effectiveness of traps for monitoring and/or controlling these biting flies (Mihok, 2002), and hence potentially reducing the occurrence of surra.

6.2 Materials and Methods

6.2.1 Study area

The study was conducted on grazing land in the Sheikh District of Somaliland. A pair of Nzi and biconical traps was assembled in three sites, one site was at low elevation (~1000 m above sea level) at the bottom of the Golis ranges in Sheikh district, and the other two sites were at higher elevations (~1450 m) but approximately 15 km apart on the highlands of the Golis ranges. This area is known for camel rearing, has a history of surra (Chapter 3) and also has reports of several species of biting flies occurring during the wet seasons. At each site the two traps were placed approximately 100 metres apart. Logistical constraints prevented setting of traps in all regions of Somaliland previously sampled (Chapter 3).

6.2.2 Study design

Tabanids were caught during a four week period in the wet season (May to June 2011) using Nzi and biconical traps which had been imported from Kenya and also with hand nets. The Nzi trap was sewn using Sunbrella® Pacific blue and black coloured fabrics developed in Kenya for trapping biting flies (Mihok, 2002). Biconical traps are traps designed for catching tsetse flies and contain blue cotton or synthetic fabric on the lower cone and white nylon mosquito net on the upper cone (Figure 6.1). The inner part has four compartments with segments of black cloth (Mohammed et al., 2010). The traps were baited with the same attractant (camel urine) to attract biting flies. The urine was collected

from one of the Sheikh Technical Veterinary School's female camels. A plastic bag containing the camel urine was then placed in each trap. Insects caught in the trap were removed at weekly intervals. Hand nets were also used to catch flies from camels, particularly when the flies were feeding. Flies were individually pinned as dried specimens in insect boxes and transferred to the STVS Laboratory. The biting flies were identified in the laboratory using an Olympus SZX12 stereomicroscope at 10X magnification.

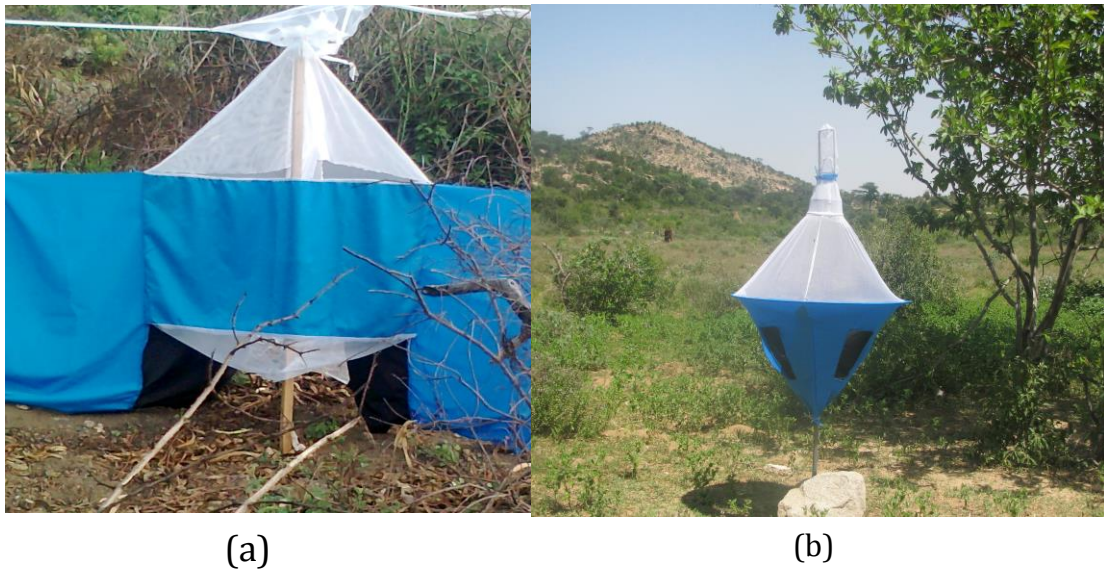


Figure: 6.1 Photo of Nzi (a) and biconical traps for fly collection (b)

6.2.3 Field observations on the daily activity and alighting pattern of tabanids

Tabanids have different activity patterns throughout the day being active during the early and late hours of the day but calm at night. A combination of climatic and biotic factors

increases the tabanid attacks and feeding at different times of the day (Gibson and Torr, 1999). A study on the feeding activity and landing positions of tabanids was conducted on camels in the Sanaag and Sahil regions of Somaliland. These locations were selected because *T. evansi* had been identified in camels in villages from these regions (Mohamed, 2007). The study was conducted daily over 15 days with each daily observation period lasting two to four hours between 08:00 and 18:00. The species of tabanid landing on the camels, the landing site on the body and the activity of the flies during the day were recorded. The alighting sites on the body were categorised as upper body (including head, neck and hump), lower body (including the lower flank and belly) and legs (including forequarters and hindquarters). The defensive behaviours adopted by the camels to the flies were also recorded. This included head tosses and licks (using the head to dislodge flies), tail flicks, skin twitching and feet stomping.

6.2.4 Wing length measurement

The incidence of transmission of surra is related to the size of the biting flies and the number of biting flies around the host (Desquesnes et al., 2013). Measurement of the wing length is important because it provides information on body size and an estimate of the fecundity of the insect (Leprince and Foil, 1993). The wings of adult flies were placed on a slide and the wing length measured from the costa to the branch of the R4 vein. Photographs of the wings were taken with an Olympus DP 10 Camera attached to an Olympus SZX12 stereomicroscope.

6.2.5 Data analysis

Data were entered into and analysed with Microsoft Excel (2011 version) to quantify the main vectors for camel trypanosomosis in the study area. The efficiency of the trapping technique, the daily activity patterns and alighting positions of flies on the different regions of hosts were analysed using chi-squared tests for independence and odd ratios and their 95% confidence intervals. The mean and standard deviation of the number of alighting biting flies were recorded and the size of the *Philoliche* and *Tabanus* genera were measured as these represented the two most common flies observed and therefore were considered the most important in the transmission of surra. Meteorological data (temperature, rainfall, humidity and wind speed) were provided by the Somalia Water and Land Information Management (FAO, 2016). These data were used for analysing weather effects on fly activity through calculating Pearson's correlation coefficients (SPSS for Windows, Version 15.01, SPSS Inc. Chicago, USA).

6.3 Results

A total of 159 biting flies belonging to the family *Pangoniinae* and *Tabanidae* were caught with more flies belonging to the subfamily *Philoliche* and *Tabanus*. Overall the horse flies collected during the fieldwork belonged to three genera; *Philoliche* which accounted for 84% (n=133) of flies, *Tabanus* 13% (n=20) and *Haematopota* 4% (n=6). Of the 159 horse flies caught more were caught in the hand net (n=127, 80%) than the Nzi and biconical traps (n=32, 20%). Of these latter flies 66% were caught in Nzi traps and 34% in the biconical traps. Most (87%) of the biting flies trapped with the hand nets (110/127) and

72% of the flies trapped with Nzi and biconical traps (23/32) belonged to the genus *Philoliche* (Figure 6.1). There was no significant difference between the number of *Philoliche*, *Tabanus* and *Haematopota* flies collected by traps and hand nets ($\chi^2 = 4.07$, df=2, P=0.13).

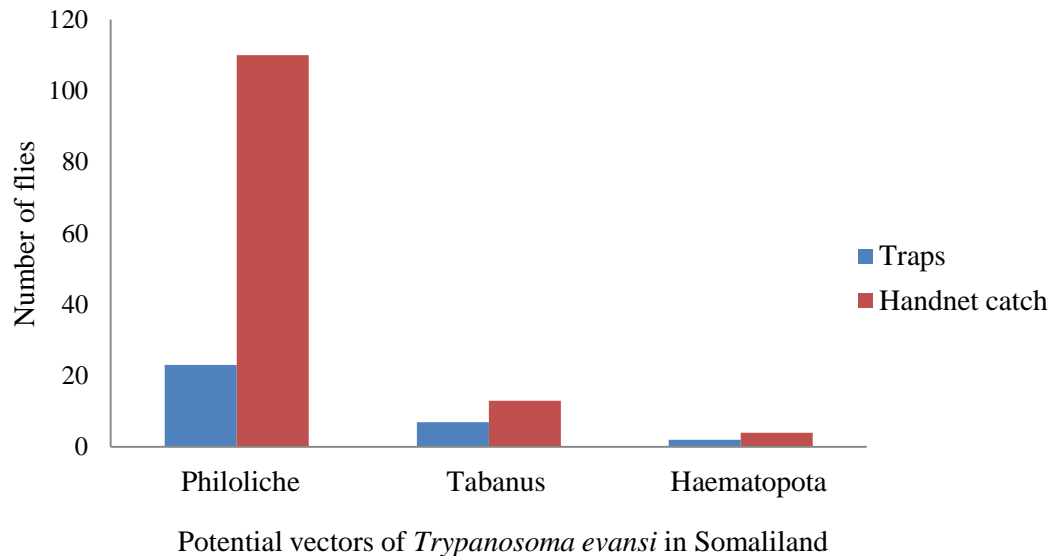


Figure: 6.2 Distribution of Tabanids caught using traps and hand nets during the field observations

6.3.1 Daily activity and alighting pattern of tabanids on camels

The daily activity and the alighting pattern on various parts of the camel's body by the flies are summarised in Tables 6.1 and 6.2. The highest activity of biting flies was observed around midday and in the morning. Horse flies landed mostly on the lower body and belly of camels with an average of 0.87 ± 0.34 flies belonging to the *Philoliche* species alighting on these sites. The legs and tail region was the second most common alighting site for

Philoliche spp. (0.56 ± 0.50) and the lowest frequented site was on the upper body (the head, neck and hump) of camels (0.44 ± 0.50).

Table 6.1 Activity pattern of Tabanids feeding on camels during the day in the field

	Odds ratio	Lower 95% CI	Upper 95% CI
Morning (8 – 11)	1.88	0.76	4.65
Midday (11 – 2)	5.79	1.98	16.93
Afternoon (2 – 5)	1.0 (referent)	-	-

The activity of the biting flies was significantly different between the times of the day ($\chi^2=11.24$, $df=2$, $P=0.0036$). The peak activity of the flies was around midday (OR = 5.8; 95% CI: 1.98, 16.93 compared with the evening). The activity in the morning was comparable to that of the evening (Table 6.1).

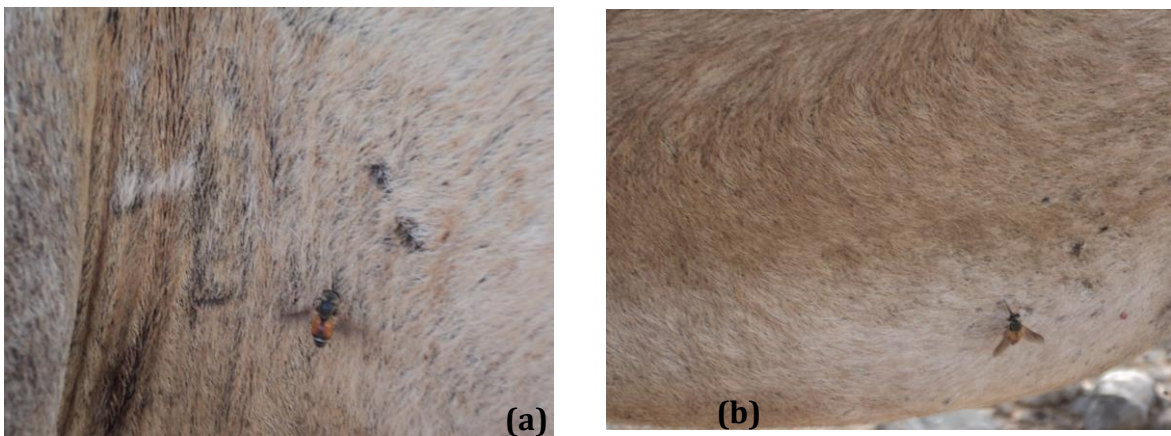


Figure: 6.3 *Philoliche* species feeding on the lower flank (a) and belly of a camel (b)

Philoliche species were also the most common biting fly attaching to the lower body and belly of the camels, however the sample size was too small to show a statistical significant difference overall between the *Philoliche* species and other biting flies attached to the lower body and belly (Figure. 6.3).

Table 6.2 Alighting sites of horse flies on camels during the field study

Landing site	Odds ratio	Lower 95% CI	Upper 95% CI
Lower body	8.80	2.84	27.23
Legs and tail	1.67	0.69	4.08
Upper body	1.0 (referent)	-	-

There was a significant difference between the alighting sites of biting flies on camels ($\chi^2=16.68$, $df=2$, $P=0.00024$) with the lower body and belly being nine times (OR = 8.80; 95% CI: 2.84, 27.23) more likely to be targeted by biting flies than the upper body (the head, neck and hump) of camels. There was no difference between the targeting of the legs and tail and the upper body (Table 6.2).

The camel's defensive behaviours observed against alighting flies included head tosses and licks, tail flicks and skin twitching and foot stomping. No further information on the frequency of the defensive behaviors adopted by the camels was available because of insufficient time to enumerate the defensive behaviours.

6.3.2 Wing length measurement

The largest of the horse flies caught in the study area belonged to the *Philoliche* genus (Figures 6.4 a and b), with a mean wing length measurement of 15.63 ± 3.17 (n= 133).

This genus was the most widely distributed and abundant biting fly in the study area. The adult flies were black (Figure 6.4a) or brown (Figure 6.4c) in colour with brownish wings and a blackish abdomen. The next largest flies belonged to the genus *Tabanus* (Figures 6.4 d and e) and these had a blackish abdomen and orange-brown wings, which were 7.10 ± 2.13 mm long (n=20).

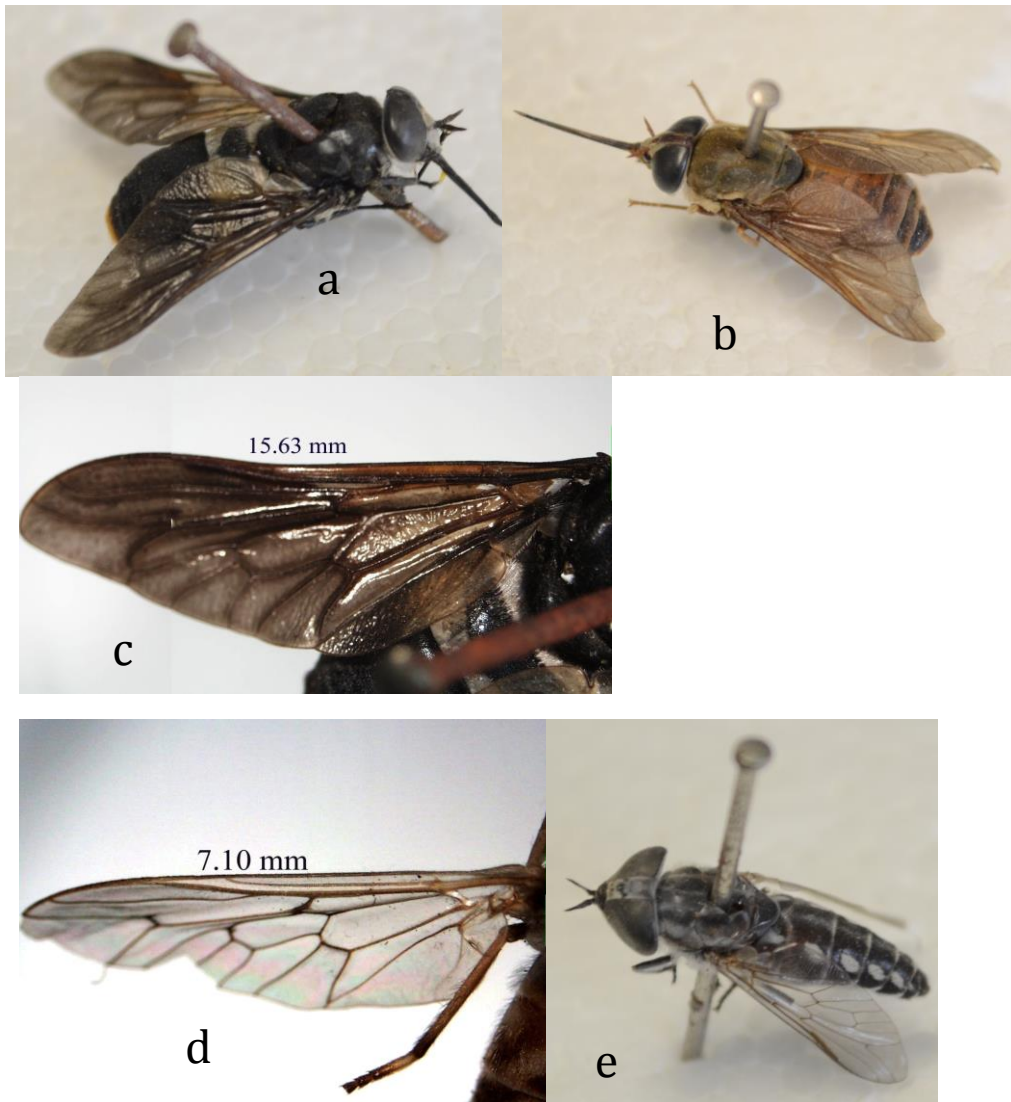


Figure: 6.4 *Philoliche* (a, b and c) and *Tabanus* genus (d and e) - the potential vectors for transmission of surra on camels in Somaliland

Table 6.3 Weather variables and flies collected over the four weeks

	Number of Flies	Minimum temperature (°C)	Maximum temperature (°C)	Average temperature (°C)	Relative Humidity (%)	Wind Speed (km/h)	Rainfall
1st week	50	22	40	32.5	51.6	2.9	3.2
2nd week	38	23	37	31.2	54.6	2.9	0.4
3th week	35	25	38	30.6	57.3	3.6	0
4th week	36	25	37	30.3	49.2	3.6	0

6.3.3 Effect of weather variables on total weekly catch

The total weekly catch of biting flies and weather variables were analysed. A table of the weather variables and their values over the four-week-collection period is presented in Table 6.3.

A strong positive correlation was found between the average temperature and rainfall and the number of flies (with coefficients $r = 0.96$ and 0.99 , respectively). Both humidity and wind showed a negative correlation with the number of flies, although these values were not significant (Table 6.4).

Table 6. 4 Values of the mean flies caught and correlation coefficient of different weather variables

	Mean number of flies caught	r	p-value
Temperature	31.1	0.9625	0.0375
Rain	0.9	0.9971	0.0029
Relative humidity (%)	53.2	-0.3173	0.6827
Wind speed (km/h)	3.2	-0.7065	0.2935

6.4 Discussion

The current study demonstrated that three genera of tabanids were present in the survey area and these have the potential to transmit trypanosomosis between camels in Somaliland. In this study the *Philoliche* spp. had the highest abundance (84%) compared to *Tabanus* spp. (13%) and *Haematopota* spp. (4%). In other studies 69% of flies have been identified as *Tabanus*, 18% *Philoliche* and 13% *Haematopota* spp. (Mohamed, 2007; Mohamed, 2011).

The genus *Philoliche* has been implicated in the mechanical transmission of surra between camels and is likely to be the key genus involved in the transmission of surra within the region (Mohamed-Ahmed and Mihok, 2009). According to previous studies there are approximately a dozen species belonging to the genera *Philoliche*, *Tabanus* and *Haematopota* (Dirie et al., 1989). A disadvantage of the present study was that flies were not identified to the species level. Identification to the species level requires an entomology expert which unfortunately was not available in this study, and this expertise should be sourced for future studies. The number of tabanids observed feeding on camels would support the observation that these flies frequently bite susceptible hosts and, because of their size and the form of their mouthparts, they would facilitate mechanical transmission of trypanosomes during feeding (Foil, 1989).

There have been no recent studies conducted indicating the effectiveness of vectors for the mechanical transmission of camel trypanosomosis. The results of the current study indicated that the largest horse flies caught belonged to the *Philoliche* genus which had a wing span of, on average, 15.63 ± 3.17 mm. Although in the current study, there was no significant difference in the numbers of flies belonging to the different genera collected by traps and hand nets, hand nets were more successful in catching *Philoliche* than *Tabanus* or *Haematopota* species. This may be associated with the larger size of members of the *Philoliche* genus.

The results of the present study suggest that *Philoliche* and *Tabanus* spp. have the potential to be more effective vectors because of their size and abundance, and potentially they may be more resistant to the wind and heat of the wet season than *Haematopota* spp. The *Philoliche* and *Tabanus* spp. are more numerous at the start of the rainy season, and are frequently observed around shady trees and flowers along the coastal plains. It has been demonstrated that the transmission of infection is linked to both the size of biting flies and their abundance (Desquesnes et al., 2013). In a study on camel trypanosomosis and its vectors in Somalia the spread of transmission of *T. evansi* has been linked to the distribution of *P. zonata* and *P. magretti* (Dirie et al., 1989) and for this reason *P. zonata* was incriminated in the surra outbreak in 1983. This may also explain the high prevalence of *T. evansi* infection found in camel herds in this region in the current study (Chapter Three).

Tabanids have been reported to increase in number early in the wet season (Ahmed et al., 2005). Similarly, a study on trypanosomosis and vectors has reported an increase in the vector population during the rainy seasons (April-June) and (September-October) in the coastal area of the Sahil region of Somaliland. These insects not only have the potential to transmit trypanosomosis but also cause distress to camels from their bites, interfering with their grazing activities and hence resulting in reduced productivity (Mohamed, 2011).

Other studies have reported that smaller tabanids move between hosts less frequently and ingest a smaller blood meal than larger tabanids (Foil, 1989). For example, only 2.8% of *Chrysops* species transferred from one host to another, while of the larger horse flies observed, 7.1% of the *T. sulcifrons* and 12.3% of the *T. petiolatus* transferred to new hosts during feeding (Foil, 1989).

In the present study few biting flies were caught and this may have been due to a late start to the rainy season and it lasting for a shorter duration than normal. The late onset of rains would have affected the development of the fly population resulting in an overall smaller number available for capture. In Somalia, winds usually develop in summer towards the end of the rainy season (June), which can have a negative effect on the fly population. In the current study there was a negative, although not significant, correlation between wind speed and the number of flies caught/trapped.

Given the short duration of the present study, we can attribute this decrease mainly to the prevailing weather conditions and the late onset of rains. Although Burnett and Hays (1974) and Dale and Axtell (1975) reported that different weather factors strongly influenced the daily activity of horse flies. In the current study more flies were caught when the temperature was more than 31°C. Similarly another study (Tamás Herczeg et al., 2014) observed that all trap types caught horse flies when temperatures were above 31°C. However some vectors have been reported to survive throughout the year in Sudan and Nigeria, and these have the potential to maintain the disease, although the effect of wind and temperature during the dry seasons dramatically reduced their numbers (Mohamed-Ahmed and Mihok, 2009).

In the present study significantly more biting flies were observed during the middle part of the day compared with the morning or afternoon. These findings were similar to previous studies conducted in Somalia (Dirie et al., 1989) and those reported by Gibson and Torr (1999) who found that *P. (Stenophora) zonata* Walker had peak activity at this time.

Out of 32 biting flies caught in traps during the four week study period, two-thirds were trapped with the Nzi traps and one third with the biconical traps. This may have been due to the wind blowing from the start of June, which could affect the ability of the traps to capture flies. Differences in the trap designs may also lead to differences in the number of tabanids caught. The performance of traps could also be affected by other factors including location, host abundance and changes in the weather of the day and the physiological status

of the biting fly (Mohammed et al., 2010). The present study suggests that the lower tabanid density in the Sheikh district might be due to climatic and vegetation related factors within the region. For example the presence of local vegetation can alter trap catches. The effect of trap site could not be examined separately; therefore, its role in the differences between the traps cannot be discounted.

Use of hand nets was more successful in catching flies with 80% of all flies caught through this method. This indicates that collection of biting flies in grazing areas should be targeted towards catching flies directly off animals as reported by Gibson and Torr (1999).

Similar results have been reported in studies in Sudan, where Tabanids, such as *Philoliche* spp. and *Tabanus* spp. were difficult to catch in traps (Mihok, 2002; Mohamed-Ahmed and Mihok, 2009).

Tabanid species are more responsive to moving targets than to stationary ones. For example, more *P. (Stenophora) zonata* Walker were caught by a hand net from moving oxen than from stationary oxen (Gibson and Torr, 1999). Vector mobility and frequency of blood feeding attempts are important to the success of mechanical transmission of surra. However, there have been reports that most horse fly species pursue a single host until they are engorged (Foil, 1989). They expose animals to the parasite by flying between animals after feeding on an infected animal. In this study tabanids landed on various parts of the camel's body, with the lower body and belly of camels being preferred alighting sites compared to the upper body (the head, neck and hump) (OR = 8.80; 95% CI: 2.84, 27.23).

Tabanids have preferred feeding/landing sites on hosts and have been shown to be highly selective and species-specific for landing sites (Hollander and Wright, 1980; Mohamed-Ahmed and Mihok, 2009; Muzari et al., 2010; Phelps and Holloway, 1990).

However, several studies suggested that surface cues of the host, such as movement, play a role in the selection of a suitable landing site (van Hennekeler, 2007). The preferred landing site for some tabanids on horses is on the belly and it has been proposed that this location could be important for high engorgement success on the host (Muzari et al., 2010). At this site it is harder for the host to dislodge the tabanids, allowing them to complete their feeding with less disturbance through the host's defensive behaviours than with other sites (Davies, 1990; Foil, 1989; Muzari et al., 2010). These sites are also softer than the upper body of the camels.

The present study did not enumerate the actual number of flies alighting on the camel's body as the study was designed to understand the preferential biting site selected. Numerous authors have, however, observed more horse flies landing at the anterior end of grazing cattle rather than at the posterior end, presumably because of the protective effect of tail swishing (Mohamed-Ahmed and Mihok, 2009). Others have shown that the largest tabanids more often attack the legs, belly, flanks and back region of cattle compared to the smaller tabanids (Mohamed-Ahmed and Mihok, 2009). In Zimbabwe, the preferred biting site for tabanid species was reported to be the lower portion of the host (Phelps and Holloway, 1990). The attraction of tabanids and the sites at which they alight on animals

appear to be influenced by fly size rather than infectivity and height of the host (Mohamed-Ahmed and Mihok, 2009; van Hennekeler, 2007). Identifying the preferred alighting sites of vectors and the species and genera involved are important when developing control options for vector borne diseases such as trypanosomosis.

The disturbance in feeding, associated irritation and blood loss can result in significant reduction in productivity particularly during the rainy seasons when flies are more prevalent (Mohamed-Ahmed and Mihok, 2009). As highlighted in Chapter Three, the distribution of surra varies between districts. It is likely that camels from areas with a high number of biting flies are more likely to be exposed to *T. evansi*, particularly when the reservoir animals and susceptible animals graze together. This study also has shown that the movement of the camels for grazing and watering points during the wet and dry seasons potentially allows contact between susceptible and infected animals.

The risk of infection could be reduced if appropriate interventions were implemented, including animal movement control and adoption of vector control strategies. Vector control can include the use of repellents such as smoke. Smoke through a smouldering fire is a traditional method used by pastoralists in Somalia and other countries to control the vectors. The vectors can also be caught using traps, especially in the rainy seasons. In the current study the Nzi trap was found to be the most efficient trap to catch flies and these traps could be used to reduce the fly challenge. Reducing the number of flies would provide relief to camels allowing them to exploit and graze the green and abundant pasture present

at this time potentially resulting in increased milk-yield for their owners. This trap is suitable provided there is minimal wind and with regular observation and maintenance the Nzi trap has the potential to catch a large number of tabanid flies. However traps may not be practical in open pasture environs due to the high cost of implementing such a strategy and the likelihood that flies would enter control regions from adjacent regions (Desquesnes et al., 2013).

It is recommended that further studies should be undertaken in other areas and longitudinal studies conducted in different months of the year. It is also important to carry out further studies on the relationship between potential vectors and the ecological zones to understand the dynamics for disease transmission in the pastoral production system. This information would help in developing sustainable vector control programmes to reduce trypanosomosis in camels. Insecticide treatment of animals has been adopted to control vectors feeding on cattle in Sudan (Mohamed-Ahmed and Mihok, 2009) and a similar strategy could be developed for use in Somaliland. Furthermore a regular and sustained surveillance system should be developed to monitor the efficiency of any disease control strategies implemented.

CHAPTER SEVEN

General Discussion

The research outlined in this thesis was undertaken to determine: the epidemiology of *T. evansi*; the impact of the parasite on camels; and the potential vectors responsible for transmission of trypanosomosis in Somaliland. Prior to this study there was limited information on the extent and impact of surra on camels in pastoral communities and further knowledge was needed to evaluate potential disease control strategies. Effective control programs will improve livestock productivity and production in rural areas resulting in socio-economic benefits for both livestock owners and the general community, especially given the important role of camels to the Somali community (Farah et al., 2004).

In this study the results of the CATT/*T. evansi* demonstrated that *T. evansi* is widespread in the surveyed regions of Somaliland and the disease is a major threat to the productivity of camels and consequently the livelihood of Somali pastoralists. The seroprevalence in this study was 38.2%, which was less than that reported in central regions of Somalia (56.4%) in an earlier study (Baumann and Zessin, 1992). This difference could be due to the different diagnostic procedures used (the latter study used the micro ELISA test to confirm disease). The CATT/*T. evansi* has been shown to have a high specificity in Kenya,

although it was reported to lack sensitivity (Njiru et al., 2004). It is also possible the results differed because of different antigen type expression. Although the CATT/*T. evansi* and the Ab-ELISA have similar sensitivities, the ELISA is reportedly a better assay (Reid and Copeman, 2003) as it can detect more seropositive animals (Atarhouch et al., 2003). However, the CATT has been critically evaluated and validated for the detection of surra in many parts of the world in both camels (Dia et al., 1997; Ngaira et al., 2003; Pathak et al., 1997) and water buffalo (Davison et al., 1999; Hilali et al., 2004; Verloo et al., 2000).

The results of the current survey confirm that the prevalence of surra is higher in Somaliland than in other African countries, including Ethiopia (Hagos et al., 2009; Zeleke and Bekele, 2001), Kenya (Ngaira et al., 2003; Njiru et al., 2004) and Sudan (ElSaid et al., 1998). The seroprevalence of *T. evansi* varied between regions in this study in Somaliland with a higher prevalence in the eastern districts where the environmental conditions are likely to be more favourable for disease transmission. In this ecological zone the low altitude and high temperatures, which prevail during the daytime, are more conducive to the survival and distribution of the key vectors (tabanids) associated with the disease. Infection with *T. evansi* was more common in camels originating from the coastal plains (*Guban zones*) in Sahil, Sanaag, Waqoyi Galbed and Awdal regions with 41.4% of camels from Sahil being infected. This finding is in agreement with those from studies in other arid and semi-arid regions with increased prevalence in regions with conditions which favour the vector (Atarhouch et al., 2003; Njiru et al., 2004).

The study also highlighted that transmission of *T. evansi* was likely to be facilitated by the

traditional herd movements adopted by herders who move camels into the high-risk areas for surra in response to shortages of water and forage associated with dry times. Similarly movement of livestock is important for the spread of surra in transhumant systems in eastern Chad and as the length of migration increases, the risk of disease transmission also increases (Delafosse and Doutoum, 2004). Other studies have reported that herds owned by agro-pastoralists had a lower prevalence than camels owned by nomads. This could be due to agro-pastoralists having access to pastures/grazing of better nutrition and better management practices through the regular use of trypanocidal drugs (Elamin et al., 1998). Similarly, in Mauritania trypanosomosis was higher in camels which regularly migrated than in non-migratory herds (Dia et al., 1997). It is likely that the epidemiological pattern of trypanosomosis in camels in sub-Saharan Africa is influenced primarily by the livestock production system adopted, as well as the ecological zones present fostering the survival of the vectors.

The seroprevalence in this study increased with age indicating that young camels are potentially less susceptible to infection as reported by others (Dia et al., 1997; Elamin et al., 1998; Njiru et al., 2004). Similarly it has been reported that young cattle are less attractant to infection with *T. congolense* than are adults (Elamin et al., 1998). It is also likely that young animals have less potential for exposure to biting flies than do older animals.

The seroprevalence of infection was higher in camels with a poor body condition. It is likely that camels on a poor nutritional plane and those with concurrent infections are at greater risk of infection with trypanosomes. In Tanzania the prevalence has also been

reported to be higher in camels with a poor body condition (Swai et al., 2011). Similarly in high-risk areas of Mindanao, Philippines, infection of buffalo cows was more commonly associated with emaciation and anaemia (Dargantes et al., 2009). This highlights the need to develop a treatment and control strategy that is implemented in conjunction with other disease or managerial improvements. It is, however, possible that the poor body condition was as a result of infection with trypanosomes rather than a cause of the infection. Furthermore infection with parasitic disease can interfere with immunity, and animals exposed to stress from overwork, food shortage and poor quality water could result in animals being more susceptible to other infectious agents (Desquesnes et al., 2013; Dia et al., 1997; Enwezor and Sackey, 2005).

The results of the model reported in this thesis highlighted the economic benefits from controlling the disease and emphasised the financial losses attributable to infection with *T. evansi* in Somaliland. The total net benefit loss for a village of 80 camels that was not applying any surra control strategies was predicted at US \$87,239 per annum. Other studies have demonstrated that different disease control strategies can result in different financial benefits and have highlighted the financial advantages of selecting the most viable control measure (Habtemariam et al., 1988; Kristjanson et al., 1999). In the current study monthly-targeted treatment of clinically sick camels was found to be the most efficient, practical and economical treatment option to control infection in camels in a high-risk area. Although biannual treatment of all camels was predicted to provide similar financial returns to monthly treatments, it would likely promote the development of drug resistance from the

widespread use of the therapeutic agents. Biannual treatment would also require more implementation costs associated with the need to conduct treatment campaigns and surveys for enhanced surveillance. If treatments were specifically targeted at seropositive camels the number of camels requiring treatment would be less. However, this strategy would require improving the diagnostic capability in the field and a sustained and effective surveillance system. To improve the effectiveness of the surveillance system for infection with *T. evansi*, there is a need for a highly sensitive and specific test to be developed that is robust, inexpensive and capable of being used in the field.

Infection of camels with *T. evansi* has the potential to result in significant financial losses through reduced milk yield in lactating camels and decreased value of animals. Therefore, the Somaliland government and international funding agencies should give priority to supporting control efforts for the disease. The implementation of sustainable surveillance activities and control strategies could significantly reduce the financial losses described in this study. Any control programme implemented must be dependent on understanding and improving the pastoralist management practices, identifying the high-risk areas and evaluating the cost-effectiveness of disease surveillance and different intervention options. Disease mitigation measures should be implemented in areas with the highest prevalence where the need for disease control is greatest.

The risk of infection in camels could be reduced if appropriate interventions were implemented; including establishment of surveillance in the *Guban* zone and adoption of vector control strategies, and further research is needed in evaluating different disease

control methods and their financial benefit.

The present study demonstrated that three genera (*Philoliche*, *Tabanus* and *Haematopota*) of tabanids were potential vectors transmitting trypanosomosis in camels in Somaliland. Comparison of these tabanids trapped with a hand net and with Nzi and biconical traps indicated that there were no significant differences between their potential for disease transmission. In addition, high numbers of *Philoliche* spp. trapped in the vicinity of camels should give an indication as to the potential of these flies for disease transmission. However, vector incrimination studies are required to ensure that the species of biting flies that consistently attack hosts are capable of mechanically transmitting infection.

This study provided information relevant to the risk of transmission of *T. evansi* by tabanids and their daily activity patterns throughout the day. The highest level of activity of biting flies was observed around the middle of the day. This finding is in agreement with previous studies in Somalia (Dirie et al., 1989) and is well documented by Gibson and Torr (1999), who reported that *Philoliche* had peak activity at midday. Targeting a vector control program at these high activity times is recommended.

This study demonstrated that the lower body and belly of camels were the preferred alighting sites of tabanids, where it is difficult for the host to dislodge them (van Hennekeler, 2007), compared to the legs and upper body. Information on the preferential alighting and feeding sites of tabanids on camels is important when developing effective control measures. This would help recognise the vectors preferred biting/landing sites and

hence where application of insecticides should be focussed in order to ensure maximum efficacy.

The present study has demonstrated the distribution of surra in Somaliland, identified the risk factors for disease, quantified the financial losses associated with the disease and identified a viable control program. Control of the disease requires prioritising resources to areas and times when the risk of transmission is highest to have the greatest economic benefit. It is concluded that surra is a serious disease of camels in Somaliland, impacting on the health and productivity of these animals. Control of the disease would result in significant economic benefits for both the livestock herders and the general community in Somaliland.

The key findings of this thesis are that the coastal plain areas extending across the *Guban* in Somaliland pose a substantially higher risk of surra incursion than other areas of the country. It was demonstrated that in the absence of appropriate control measures, there is a risk that animals originating in areas of high risk could introduce the parasite to other areas resulting in outbreaks and substantial losses of income. Losses due to the disease, in conjunction with the current husbandry practices adopted, would eventually reduce livestock productivity and the viability of the pastoral system. Presently, there is little provision of animal health services by the Government to combat endemic diseases or to improve livestock productivity, with such services considered to be the responsibility of the individual livestock owners/herders. There should be a particular focus on surveillance

activities to detect disease so that effective control measures for surra can be developed. The improvement of animal health and production should follow implementation of disease control strategies. Although this is the first epidemiological study of surra in Somaliland, it is recommended that further studies are conducted to assess the knowledge of camel herders' on the disease and its transmission. There is a need to establish preventive measures in the camel herds which are cost-effective, efficacious and sustainable in Somaliland.

The efforts to improve livestock production and reduce disease, particularly camel trypanosomosis (surra), should rank highly in the countries Government and international community efforts to combat poverty and improve the livelihood of pastoralists. There is a need for clear State policies on the funding for the implementation, maintenance and upgrade of the national animal health system. Funds could be provided by the Somaliland government or from International development fund agencies for these improvements. Development aid agencies can also be potential sources of funds for animal health and improving Government livestock institutions. These support structures will help develop the local export industry and improve productivity of livestock leading to improvement of the countries economy and well-being of the pastoralist community.

Future Directions

As reported this is the first significant epidemiological study of trypanosomosis in camels in Somaliland. The sensitivity of diagnostic techniques is a major factor hampering the control of this disease. Further studies are required to clarify the relationship between vectors and the disease's prevalence and incidence, and vector incrimination studies should be conducted in high risk areas for surra. There is a need to study the species of biting flies, such as *Philoliche* and *Tabanus*, which are incriminated in the transmission of *T. evansi*. The ecology of these vectors should also be investigated in order to identify potential methods of controlling these biting flies. The use of GIS mapping of high risk areas in the *Guban* zone and the link between surra ecological zones are key to understanding spatial patterns of *T. evansi* distribution and identifying areas where there is a high risk of disease transmission.

Finally it is recommended a change of Government policy is adopted for the livestock sector of Somaliland. The government should adopt a more proactive attitude towards the development of the livestock industry. This should include a long term vision and plan for the development and implementation of strategic disease control programs which should include control methods for surra.

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APPENDICES

Appendix 1 Questionnaire for recording epidemiological data at village level

QUESTIONNAIRE ON SURRA IN SOMALILAND

Date-----/-----2012-----QUESTIONNAIRE...

Trypanosomosis affects camel leading to the disease Surra. It leads to fever, reduced growth rate, reduced milk production, general loss of condition of affected animals and can be fatal.

All the information we collect from you and your animals will be kept strictly confidential. No information that might identify you or your animals will be used in any publication from the research without your permission.

SECTION I- GENERAL INFORMATION

Name of the interviewer

Date	Region	District

Name of location	GPS Coordinates

Name of respondent	Sex	Age

SECTION II EPIDEMIOLOGICAL INFORMATION

Q1. What symptoms have you observed in your camels consistent with surra?

A.....B.....C.....
D.....E.....F.....

Q2. Which disease can you easily confuse with surra?

Q3. Do you consider surra is an important disease for camels?

Yes

No

If yes what factors make surra important?

Q4. Would a diagnostic test be useful in management of surra?

Yes

No

Q5. In which season/s have you noticed surra is more common in your camels?

Seasons	Camels
Jiilaal/winter	
Gu/autumn	
Xagaa/ summer	
Dayr/spring	

Q6. Why do you think is occurs during this season?

Q7. Please indicate how the disease is transmitted between animals?

Q8. Mention the types of flies you find on your camels?

Q9. Are there biting flies in your area?

Yes

No

Q10. Which season have you noticed the biting flies?

Wet

Dry

Q11. If you have noticed biting flies when during the day are there more?

Morning

Midday

Afternoon

Q12. During the seasonal movement have you moved your camels into the area of the biting flies?

Yes

No

Q13. How often camels have been introduced into the area of biting flies?

Weekly

Monthly

Yearly

Q14. What routes do the camels take during the seasonal movement to the grazing/watering?

Q15. How long are herds away during the seasonal movement?

3 months

6 months

12 months

Q16. Are there any deaths associated with surra?

Q17. How do you treat camels suspected of surra?

Q18. What problems do you encounter with the treatment?

Q19. Do you use any treatment or control measures for surra?

Yes No

If yes what do you do?

Q20. Do the sick animals usually recover when treated?

Yes No

Q21. If yes how long do they usually take to recover?

Days Weeks Months Years

Q22. What do you usually do with an infected camel with surra?

Treatment Sold

Q23. How much is the cost of treating the camel and what is the benefit gained?

Q24. In what ways does surra affect your household and community?

Q25. How many camels have you sold (in the last year) because they were infected with surra?

Q26. Does surra affect the price of camels in the local and export market?

Yes No

Q27. What is the price of a camel suspected of surra?

Surra

No surra

If young camel what is the price?

Surra

No Surra

Q28. Does surra affect the amount of milk produced?

Yes

No

Q29. How many liters of milk are produced per day by a camel if it has surra and if it doesn't have surra?

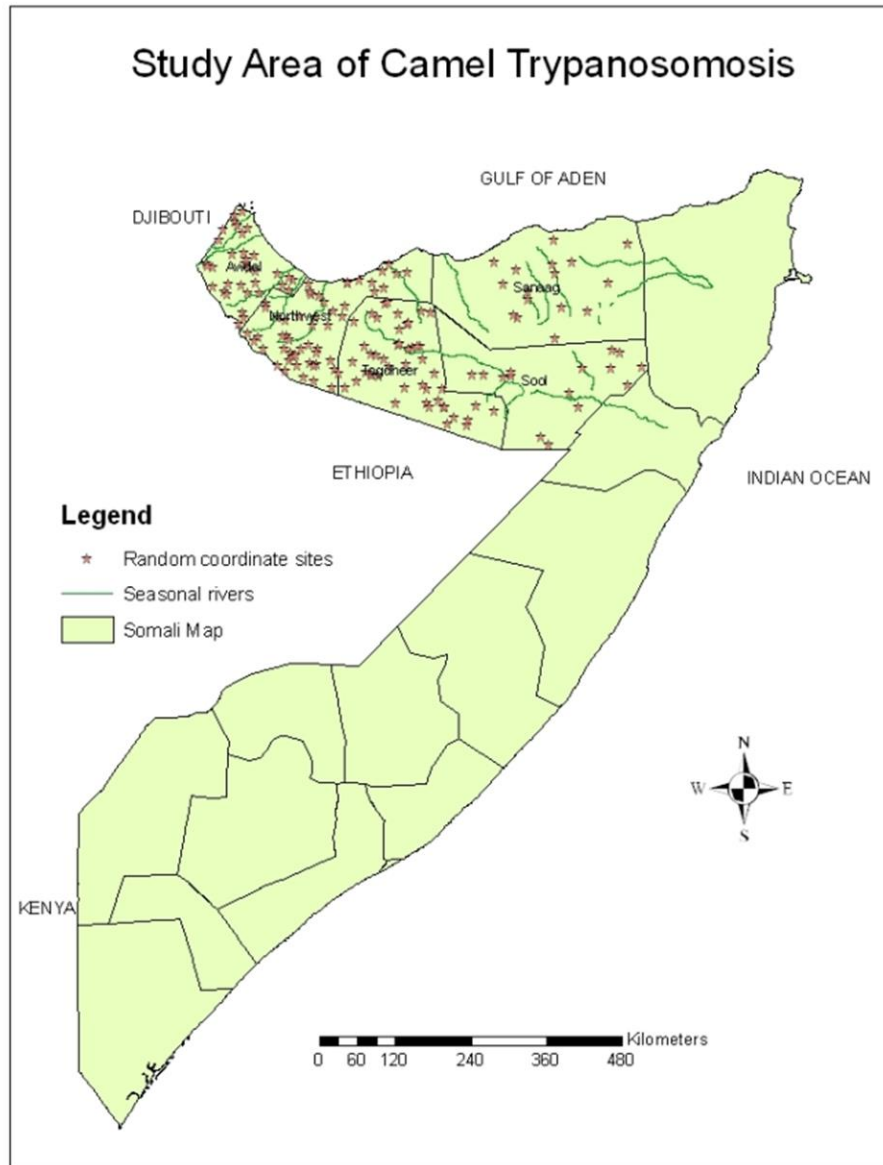
Surra

No Surra

Q30. Does surra affect the calving?

Yes

No



Appendix 2 – Selected points for sampling camels in Somaliland

List of Target Coordinates (Appendex3)

SITE ID	Latitude	Longitude	Region
1	43.58155	10.22107	Awdal
2	42.92451	10.59852	Awdal
3	43.42865	10.58428	Awdal
4	43.20154	10.77198	Awdal
5	43.33393	9.95637	Awdal
6	43.13099	10.34867	Awdal
7	43.32761	10.34239	Awdal
8	43.29073	9.78125	Awdal
9	43.36898	9.88397	Awdal
10	42.82685	10.65000	Awdal
11	43.52306	10.54725	Awdal
12	44.05598	10.41638	Awdal
13	43.08498	10.24825	Awdal
14	43.83091	10.50834	Awdal
15	43.38078	10.65071	Awdal
16	43.50346	10.76929	Awdal
17	43.39387	10.70732	Awdal
18	43.34016	11.08077	Awdal
19	43.12690	10.21542	Awdal
20	43.99884	10.45305	Awdal
21	42.91337	10.31223	Awdal
22	43.36536	10.80436	Awdal
23	43.34965	11.38989	Awdal
24	43.22441	11.33935	Awdal
25	43.01300	10.98780	Awdal
26	43.53151	10.37623	Awdal
27	43.41753	10.23420	Awdal
28	43.23407	11.24317	Awdal
29	43.05892	11.12821	Awdal
30	42.84375	10.61649	Awdal
31	43.90883	10.33697	Awdal
32	43.99640	10.26346	Awdal
33	43.41155	11.14410	Awdal
34	43.26658	11.18583	Awdal

Spare Coordinate

SITE ID	Latitude	Longitude	Region
1	43.85479	10.44484	Awdal
2	43.43053	10.66321	Awdal
3	43.11959	11.22246	Awdal
4	43.20443	10.45660	Awdal
5	43.58301	10.89866	Awdal
6	42.83488	10.75487	Awdal
7	43.21033	11.33976	Awdal
8	44.07839	10.42343	Awdal
9	43.38848	10.77565	Awdal
10	43.42329	10.09413	Awdal

List of Target Coordinates

SITE ID	Latitude	Longitude	Region
1	44.76809	9.90065	Northwest
2	45.22694	10.24626	Northwest
3	44.35099	8.97118	Northwest
4	43.66991	10.08576	Northwest
5	44.55950	9.77444	Northwest
6	43.66918	10.01679	Northwest
7	43.96644	9.62574	Northwest
8	44.50712	10.10149	Northwest
9	44.14420	9.89043	Northwest
10	43.95740	9.83978	Northwest
11	44.20513	9.03284	Northwest
12	44.62036	8.88497	Northwest
13	43.56518	9.59299	Northwest
14	44.84276	10.37458	Northwest
15	44.38206	9.88401	Northwest
16	43.83522	9.87857	Northwest
17	43.83114	9.20440	Northwest

18	44.00195	9.26525	Northwest
19	44.02033	9.38616	Northwest
20	44.29267	9.23427	Northwest
21	44.41841	9.43498	Northwest
22	44.43558	10.19231	Northwest
23	45.18105	10.38103	Northwest
24	44.17564	9.43888	Northwest
25	43.94061	9.12362	Northwest
26	44.30951	10.21341	Northwest
27	44.12343	9.20554	Northwest
28	43.99725	9.57372	Northwest
29	44.64009	9.96951	Northwest
30	44.80588	10.05235	Northwest
31	43.41451	9.66585	Northwest
32	45.54110	10.49814	Northwest
33	44.65206	9.14789	Northwest
34	45.35400	10.30581	Northwest
35	44.29032	10.36003	Northwest
36	45.67520	10.52836	Northwest
37	44.38325	9.20886	Northwest
38	43.63639	9.43845	Northwest
39	44.39340	9.37691	Northwest
40	44.05154	9.33143	Northwest
41	45.41641	10.63883	Northwest
42	44.99341	10.41135	Northwest
43	44.29815	10.24357	Northwest
44	44.34771	9.20306	Northwest
45	45.32707	10.54972	Northwest
46	43.51572	9.55301	Northwest
47	44.33868	9.77298	Northwest
48	44.83598	10.38334	Northwest
49	44.10459	9.35294	Northwest
50	43.91900	9.61754	Northwest
51	43.92971	9.43163	Northwest
52	44.60165	9.26337	Northwest
53	44.27438	9.49083	Northwest

Spare Coordinate

SITE ID	Latitude	Longitude	Region
1	44.55650	9.27542	Northwest
2	43.45691	9.57354	Northwest
3	43.86810	9.92495	Northwest
4	43.53531	9.41891	Northwest
5	45.48863	10.33214	Northwest
6	45.55701	10.45915	Northwest
7	44.32230	10.03380	Northwest
8	45.28815	10.50575	Northwest
9	43.53734	9.87533	Northwest
10	45.87854	10.30762	Northwest
11	44.51128	8.90663	Northwest
12	44.59951	10.08868	Northwest
13	45.84189	10.47752	Northwest
14	45.30848	10.37244	Northwest
15	44.05248	9.39134	Northwest
16	45.73941	10.16089	Northwest

List of Target Coordinates

SITE ID	Latitude	Longitude	Region
1	45.15887	9.35609	Togdheer
2	45.88582	9.96429	Togdheer
3	45.17967	9.06828	Togdheer
4	45.75278	9.45069	Togdheer
5	46.65441	8.63314	Togdheer
6	45.64465	8.89159	Togdheer
7	45.68421	9.77696	Togdheer
8	45.85742	9.47879	Togdheer
9	44.96180	8.98141	Togdheer
10	46.01609	9.95206	Togdheer
11	46.18205	8.87028	Togdheer
12	45.70309	9.43566	Togdheer
13	46.07099	9.08005	Togdheer
14	45.66329	9.22253	Togdheer

15	45.69270	9.81162	Togdheer
16	45.52076	8.66287	Togdheer
17	45.28363	9.04510	Togdheer
18	45.24808	9.33788	Togdheer
19	46.34684	8.45568	Togdheer
20	45.89732	9.28021	Togdheer
21	45.35863	9.28091	Togdheer
22	44.80562	8.88761	Togdheer
23	44.89906	9.24444	Togdheer
24	44.71337	9.07989	Togdheer
25	46.24620	8.37220	Togdheer
26	46.19039	8.60683	Togdheer
27	46.90708	8.55266	Togdheer
28	45.95525	8.66376	Togdheer
29	45.54330	9.48620	Togdheer
30	46.00092	8.59828	Togdheer
31	45.56649	9.71315	Togdheer
32	45.15784	9.06384	Togdheer
33	45.37706	10.07335	Togdheer
34	45.82100	9.47449	Togdheer
35	45.96096	8.86951	Togdheer
36	46.52216	8.35138	Togdheer
37	45.09693	9.10738	Togdheer
38	46.22454	8.61000	Togdheer
39	45.56427	9.50473	Togdheer
40	46.12423	8.69813	Togdheer
41	46.55380	8.42020	Togdheer
42	45.26284	9.88420	Togdheer
43	45.40140	10.09859	Togdheer
44	45.41843	9.17344	Togdheer
45	44.93490	9.81455	Togdheer
46	45.55143	9.92950	Togdheer
47	45.17689	9.93787	Togdheer
48	45.90980	8.92099	Togdheer
49	45.07008	9.48205	Togdheer

Spare Coordinate

SITE ID	Latitude	Longitude	Region
1	45.53143	8.68627	Togdheer
2	45.87348	10.03127	Togdheer
3	46.07245	9.08289	Togdheer
4	45.93106	9.09161	Togdheer
5	45.03301	8.66151	Togdheer
6	45.10339	9.24254	Togdheer
7	46.45932	8.72603	Togdheer
8	46.51797	8.47735	Togdheer
9	45.51155	8.94087	Togdheer
10	46.71216	8.27284	Togdheer
11	44.77268	9.39751	Togdheer
12	45.15821	8.80568	Togdheer
13	45.87249	9.65458	Togdheer
14	45.84856	9.02678	Togdheer
15	45.46596	9.51984	Togdheer

List of Target Coordinates

SITE ID	Latitude	Longitude	Region
1	47.14998	9.04062	Sool
2	49.01790	9.17795	Sool
3	48.82183	8.92050	Sool
4	47.58449	8.18811	Sool
5	47.68208	8.08094	Sool
6	48.11874	8.61086	Sool
7	46.77071	9.06588	Sool
8	48.57513	9.16611	Sool
9	48.16989	9.15217	Sool
10	47.04808	9.03700	Sool
11	47.16400	9.10487	Sool
12	48.59229	9.41524	Sool
13	48.70242	9.38940	Sool
14	47.98259	8.80208	Sool
15	46.60893	9.05896	Sool

Spare Coordinate

SITE ID	Latitude	Longitude	Region
1	47.19398	8.89653	Sool
2	46.27709	9.34170	Sool
3	48.22195	9.23132	Sool
4	47.30655	8.22657	Sool
5	46.91149	9.01512	Sool

List of Target Coordinates

SITE ID	Latitude	Longitude	Region
1	48.23511	9.96597	Sanaag
2	46.90811	10.66536	Sanaag
3	47.39490	10.13853	Sanaag
4	47.87708	10.03079	Sanaag
5	47.78349	10.51320	Sanaag
6	47.75940	10.97676	Sanaag
7	47.78402	9.58290	Sanaag
8	48.03016	10.66501	Sanaag
9	48.82239	10.93256	Sanaag
10	48.54464	10.37504	Sanaag
11	47.24142	9.87095	Sanaag
12	47.05275	10.35900	Sanaag
13	47.23133	10.56133	Sanaag
14	47.20029	9.92273	Sanaag
15	47.39382	10.24916	Sanaag
16	47.74604	10.62671	Sanaag

Spare Coordinate

SITE ID	Latitude	Longitude	Region
1	46.51849	9.76375	Sanaag
2	48.61654	9.78472	Sanaag
3	47.58374	10.15642	Sanaag
4	48.24230	9.81197	Sanaag
5	48.59847	10.65823	Sanaag