Sudan University of Science and Technology

College of Graduate Studies

Prevalence and Risk factors of Theilerioses in cattle In Omdurman localities-Khartoum State-Sudan

دراسة عن معدل انتشار مرض الثايليريا وعوامل الخطر في الابقار في محليات امدرمان ولاية الخرطوم- السودان

Thesis Submitted in Partial Fulfillment of the Requirements for Master
Degree of Preventive Veterinary Medicine (MPVM)

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Dedication

To my

Helpful family

With respect and love

Acknowledgment

Thanks first and last to Allah, who made this work possible then; I would like to express my great appreciation to my supervisor Prof. Siham E. Suliman, Faculty of Veterinary Medicine, Sudan University of Science and Technology for guidance and encouragement.

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Abbreviation

TBDS	Tick Borne Diseases
CSESP	Committee on Systematic and Evolution of the Society of Protozoologists
IFA	Indirect Flourescent Antibody
ELIZA	Enzyme Linked Immunosorbent Assay
PCR	Polymerase Chain Reaction
DDT	Dichlorodiphenyletrichloroethane
SPSS	Statistical Package for the Social Science
PS	Production System
PT	Production Type
SR	Separate Room

Abstract

This study was conducted from February to march 2019 in Omdurman localities [Omdurman, Umbada and Karrary] in Khartoum state to investigate the prevalence rate of bovine Thieleriosis and associated risk factors of the disease. A total of 104 blood smear samples were taken from suspected cattle with disease and Gimsa's staining technique was used. Also a questionnaire was filled from 15 farm owners including management and environment risk factors associated with the disease. The results showed that the prevelance of Theileriases in Omdurman localities was 29.8%. the univariate chi-square analysis result showed that there was no relationship between ages, breed and production system and Thieleriosis [$P \geq 0.05$], the result also revealed there was no effect of fencing situation of the farm, the floor moisture, separation of rooms according to ages [$P \geq 0.05$] then the multivariate analysis reveals that sex of cattle and application of tick control measure is associated with Thieleriosis prevelance [$P \leq 0.25$]. The study concludes that bovine Thieleriosis prevalent in Omdurman localities and application of tick control will definitely decrease the disease rate.

الملخص

تمت هذه الدراسة في فترة من فبراير الى مارس 2019م في محليات امدرمان وامبدة وكرري في ولاية الخرطوم للتقصي حول معدل انتشار مرض الثايليريا في الابقاروالعوامل المؤثرة على المرض. تم جمع 104 عينة مسحة دموية من حيوانات مشتبه اصابتها وتم فحصها باستخدام صبغة جيمسا. تم ملء عدد 15 استبيان من اصحاب المزارع ويحتوي على العوامل الادارية والبيئية المتعلقة بالمرض. أوضحت النتائج ان معدل انتشار مرض الثيليريا في محليات ام درمان كان (29.8%). أظهر التحليل الاولي للبيانات باستخدام مربع كاي انه لا توجد علاقة بين الاصابة بالثايليريا وعمر الابقار وسلالة الابقار ونظام الانتاج [$P \geq 0.05$] وأوضحت النتائج ايضا انه لايوجد تأثير لحالة السياج في الحظيرة ورطوبة الأرضية وتقسيم الحظائر حسب العمر [$P \geq 0.05$] ثم في المرحلة الثانية من التحليل تبين أن جنس الأبقار وتطبيق اجراءات مكافحة القراد في الحظيرة والحيوان يؤثران في معدل انتشار مرض الثايليريا [$P \leq 0.25$]. استخلصت الدراسة مرض الثايليريا في الابقار منتشر في محليات امدرمان وأن تطبيق اجراءات مكافحة القراد تقلل من معدر انتشار المرض.

Introduction

Theilerioses is one of the Trans-boundaries Disease (TBDs) of cattle, which are economically important protozoan disease in tropical and subtropical regions; they are transmitted by ixoded ticks and have complex life cycle in both vertebrate and invertebrate hosts (OIE, 2018).

The disease occurs in wild zone of Africa, southern Europe and large part of Asia (El Hussien *et al*., 2012). The endemic areas of Theileriosis stretch out from the Mediterranean coastal regions in Southern Europe, Northern Africa including Mauritania, and surroundings of the Nile into Central Sudan and Middle East to south of Caucasus splitting round the Himalaya (Dolan, 1989).

The *Theileria* species infect a wide range of both domestic and wild animals and are transmitted by ixodid ticks of the genera *Amblyomma*, *Haemaphysalis*, *Hyalomma* and *Rhipicephalus* (FAO, 1983).

T.annulata infections are mainly restricted to the southern and Mediterranean areas and the infection can occur in cattle, yaks, water buffalo and camels (OIE, 2018).

Earlear studies based on Giemsa stained blood and lymph node biopsy smear have shown high prevalence and wide spread nature of *T.annulata* infection in northern Sudan (El Hussien *et al.*, 2012). *T.annulata* is transmitted in Sudan mainly by tick species *Hayalomma anatolicum* which is abundant in central, northern Sudan and western Sudan (Abakar *et al.*, 2017). Outbreaks and individual cases in Sudan are mainly seen in exotic bread animals or crosses; the disease is rarely seen in local breeds except when they are stressed, example in pregnancy and lactation period or under nutritional stress.

Previous studies based on serological diagnosis showed that *Theileria annulata* infection is prevalent in Khartoum state (Elhussien *et al.*, 1991; Salih and Hassan, 2003; Ali *et al.,* 2006) with certain risk factors considered as age, geographic areas, management systems, breeds and tick infestation burden. These studies showed that a high percentage of animals examined were healthy carrier of *T.annulata*.

The disease is believed to be enzootically stable, but this stability is currently upset by large scale introduction of exotic cattle and upgrading programmes of local types, and by movement of susceptible indigenous manimals from non-endemic to endemic areas (Salih and Hassan, 2003). Tropical theileriosis has been reported to cause considerable damage to livestock development in the Sudan. For this economic evaluation is very useful in animal health management, including prevention measures based on financial data and records and data analysis (Lopes *et al.,* 2015).

Objective of this study is:

- To determine prevalence of theileriosis in cattle in Omdurman localities.
- To Study the risk factors associated with theileriosis in Omdurman.

Chapter One

Literature Review

1.1 Origin and Classification of *Theileria* species

The *Theileria* are protozoan parasites infecting wild and domestic animals throughout much of the world. They have a schizogonous reproductive cycle, usually in lymphocytes of the vertebrate host, and a piroplasm stage in erythrocytes (Dolan, 1989).

African buffalo acts as a reservoir host for *Theileria* infection to domestic animals. Young *et al*. (1973, 1978) showed that African buffalo acts as an important source of bovine theileriosis in many areas. They found that the carrier state of buffalo theileriosis might extend up to two years in the absence of re-infection.

Ultrastructural studies and description of sexual forms of *Theileria* and *Babesia* led to the following classification according to the revision of the Committee on Systematic and Evolution of the Society of Protozoologists (CSESP) which was published by Levine *et al.* (1980)

Phylum Apicomplexa

Class Sporozoea

Subclass Piroplasmia

Order Piroplasmida

Family Theileridae

Genus *Theileria*

The species and subspecies are identified on the basis of their immunological, biochemical and molecular biological differences in addition to their morphology, host and tick specificities (Uilenberg, 1981).

1.2 Species of *Theileria*

The criteria used to distinguish between these species are based on the morphological, epidemiological, serological and infectivity characterization, host and vector specificities, drug sensitivity, biological and molecular differences (Salih and Hassan, 2003). The most important species affecting cattle are *T. parva* and *T. annulata*, which cause widespread death in tropical and subtropical areas of the Old World including Africa, south of Caribbean and Island (OIE, 2018).

Table 1: *Theileria* parasite of cattle (Salih and Hassan, 2003)

***Theileria* species**	**Vector**	**Disease**
T.annulata	*Hyalomma* species	Tropical Theileriosis
T.parva	*Rhipicephalus appendiculatus*	East coast fever
T.mutans	*Ambylomma* species	Weakly pathogenic
T.taurotragi	*Rhipicephalus appendiculatus*	Weakly pathogenic
T.velifera	*Ambylomma* species	Non pathogenic
T.buffeli	*Haemophysalis* species	Weakly pathogenic

1.3 Life cycle of *Theileria* species

The life cycle of *Theileria* parasite involves two components, the vertebrate host and the vector tick.

1.3.1 Life cycle of *Theileria* invertebrate host

The bovine host is infected by inoculation of sporozoites by infected tick during feeding and sporozoite invades leukocytes, both monocytes and B-cells with high efficiency.

After entery to the traphozoite which after nuclear division develops into the macrochizont, inducing the host cell to become large lymphoplastoid cells which divide in synchrony with the macrochizonts.

The parasite induces lymphoproliferation, a large population of parasitized cells develop in the infected animal. These further develop into microchizonts and ultimately merozoites which are released from the lymphocytes, the merozoites invade erythrocytes and develop into piroplasms, this stage completing the life cycle within the bovine host (Tait and Hall, 1990).

Melhorn and schien (1984) mentioned that some very small merozoites change into ovoid forms only these ovoid forms are able to develop within the gut of feeding tick

1.3.2 Life cycle of *Theileria* in tick vector

The clean tick (larva or nymph) feeds on an infected vertebrate host,Piroplasms-infected erythrocytes are ingested in the gut. Sexual reproduction of the parasite begins with the release of the piroplasms in the gut of the tick resulting in the fusion of macro and microgametes (gametogony). The fusion of two gametes forms a zygote, which is the only diploid stage in the parasite life cycle. Subsequently, the parasite invades the epithelial cell of the tick gut and undergoes differentiation into motile kinetes which migrate through the haemolymph till they reach and invade the tick salivary glands (Mehlhorn and Schein, 1984).

The sporogony stage begins when a kinete invades the salivary gland acini and become rounded (sporont in shape). The sporont then differentiates into a sporoplast, when the tick moults into the next stage (nymph or adult), and starts feeding on a vertebrate host, the sporoplasts become mature and form sporozoites (infective stage). The sporozoites are injected into the host found in the saliva of the tick during the feeding process (Mehlhorn and Schein, 1984).

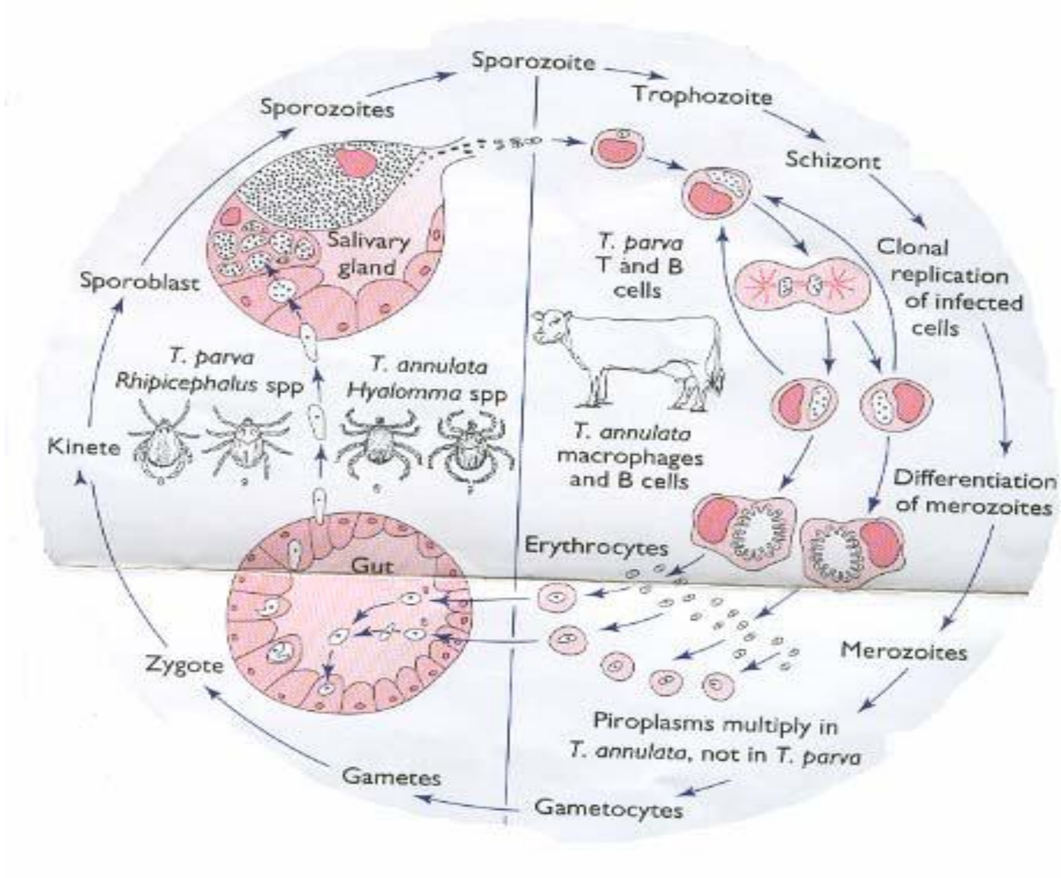

Fig. 1: The life cycle of *T. annulata* (Adopted from Preston and Jongejan, 1999).

1.4 Diagnosis of theieriosis

1.4.1 Clinical examination

Theileriosis is mostly subclinical in buffaloes but very pathogenic in cattle with associated clinical symptoms as swelling of the superficial lymph nodes (parotid, prescapular and prefemoral lympf nodes), followed by fever up to 42°C, lethargy after some days and anaroxia and lacrimation, often constipation followed by diarrhea. Dysnea in the terminal stage, progressive anemia,icterus,brown urine and hemorrhagic diarrhea present in terminal stage followed by recumbence then death 15-25 days after infection (OIE, 2018).

1.4.2 Postmortem and Histopathological examination

Oryan *et al.*, (2012) described the main postmortem finding of bovine tropical thieleriosis as pale mucus membrane and petechial and eccymotic hemorrhage in mucosal and serosal surface together with lymphoadenopathy. The liver was fraiable , yellowish and larger than normal . haemorage and punched out ulcer were observed in abomasal mucus membrane. The main histopathological lesions were characterized by hyperplasia of lymphoid cells at the haemopoietic centres in lymph nodes. Infiltration by lymphocytes and macrophages was observed in intermysial (heart) and periportal (liver) areas and in interstitial spaces in kidneys.

1.4.3 Laboratory diagnosis

1.4.3.1 Microscopic examination

Diagnosis is usually achieved by finding *Theileria* parasites in Geimsa-stained blood smears and lymph node needle biobsy smears, but species specific diagnosis is difficult as most thielerial proplasms are morphologically identical except for *T.velifera*. Schizonts are not always present in the superficial lymph nodes during the disease course (OIE, 2009).

Impression smear from lungs, spleen, kidney and lymph node, air dried and fixed in methanol for demonstration of schizonts, but the parasite appear shrunken and the cytoplasm is barely visible (OIE, 2018). Also serum used for antibody detection (OIE, 2009). Piroplasm of most species of *Theileria* may persist for months or years in recovered animals and may be detected intermittently in subsequent examination. However negative results of microscopic examination of blood films do not exclude latent infection.

1.4.3.2 Serological examination

1.4.3.2.1 The indirect fluorescent antibody test

The most widely used diagnostic test for *Theileria* species, its robust and easy, provide adequate sensitivity and specificity if used in defined field for detection of prior infection with *T.parva* and *T.annullata* under experimental situation and in defined epidemiological environment where only one thielerial species is present. The IFA test has limited for large scale serological surveys due to its reduced specificity in field situation where several *Thieleria* species can exist (OIE, 2018).

1.4.3.2.2 Enzyme linked Immunosorbent Assay[ELIZA]

Serological test based on ELIZA have been developed for the detection of antibodies to *T.annulata*, but tests used for *T.parva* and *T.mutans* are indirect ELIZAs based on parasite specific antigens, these ELIZAs provide higher sensitivity than IFA test (over 95%) (OIE, 2018).

1.4.3.3 Molecular techniques

The first PCR application for *T.annulata* diagnosis in bovine host was based on the Thams-1gene combining PCR with southern blot hybridization enabling detection during the carrier state. It was also possible to detect *T.annulata* in Hy*alomma* ticks using the same techniques (d'oliveira *et al.,* 1997).

1.5 ick infestation in Sudan

In the Sudan, tick fauna comprises over 70 species prevalent in diverse ecological zones (Abaker, 2017). Also Salih and Hassan (2003) mentioned 11 genera of ticks and 63 ticks' species infesting variety of animals.

Ticks infesting livestock in the Sudan are mainly *Hyalomma anatolicum anatolicum, H. dromedarii, H. marginatum rufipes, H. impressum, H.impeltatum, H.truncatum, Rhipicephalus evertsi evertsi, R. sanguineus* *group, R. simus group, R.appendiculatus, Boophilus decoloratus, B.annulatus, Amblyomma lepidum* and *A.*variegatum.

Previous study of tropical theileriosis in Khartoum state demonstrated that the disease is highly prevalent among all breeds and age groups of cattle and this is proportional with the high relative prevalence of tick *Hyalomma anatolicum anatolicum*. Also the distribution of the disease concides with the distribution of the vector species and it seems that in some areas where the vector ticks are present, but the disease has not been reported. Transmission of the disease by ticks takes place after period of feeding which is necessary for the development of effective form of the parasite (Mohammed, 1992).

1.6 Economic important of theilerosis

In Khartoum state *T. annulata* is one of the major impediments to development of cattle industry, the disease cause severe losses in milk production and weight gain, abortion and reduced fertility all in exotic breeds of cattle and crosses. Direct losses involve the cost of chemotherapy with antithielerial drugs and chemical acaricides. Gamal and ElHussien (2003) reported that 1.5million Sudanese dinars (6000 US dollars) were lost in one farm due to an outbreak of tropical thieleriosis, these losses increased with the economic inflation in Sudan and the uncontrolled increase in drug prices.

1.7 *Theileria annulata* (Tropical Theileriosis)

1.7.1 Host range

Thieleria Annulata infects cattle, yaks, water buffalo (*Bubalis bubalis*) and camels (OIE,2018) causing tropical theileriosis, also attempts to infect sheep and goats with *T.annulata* produce only macro and microschizonts in the lymphocytes and piroplasm developed (FAO,1983).

1.7.2 Vector Specificity

Hyalomma anatolicum anatolicum and *H. detritum* are the most important vectors of *T.annulata*, also several tick species of the genus *Hyalomma* are capable to transmit the infection experimentally. *H. excavatum, H. dromedari, H. marginatum rufips, H. marginatum marginatum.* Conversely *H. truncatum* plays no role as vector in nature (Kirvar et al., 2000).

In Sudan *H. anatolicum* is the most important efficient vector of *T. annulata* (Elhussien *et al.*, 2012).

1.7.3 Distribution

T. annulata is endemic in the area of Mediterranean coastal region and northern Africa including Mauritania and surroundings of the Nile into central Sudan and middle east to south of Caucasus (Pakistan and India)and North of Caucasus(Afganistan,southern Russia and north eastern China) (Dolan,1989).

In the Sudan *T. annulata* is endemic in northern Sudan (FAO, 1983). Antibodies were detected in southern Sudan (Morzaria *et al.*, 1981).

In related study, Elhussien *et al*. (1991) reported that 37% of examined animal showed *Theileria* in north Sudan. Mohammed (1992) conducted a survey in Khartoum state and found that the prevalence is 43% at Soba and 41.3% in Kuku, Salih and Hassan (2003) conducted a cross sectional survey in Sudan and revealed that 13.8% of surveyed cattle had piroplasm, while seropositivity 33.3% and 35.7% by using ELIZA and IFA test respectively.

1.7.4 Pathogenicity

Theileria appears in the red blood cells during the acute state of disease and doesn't multiply after invading the erythrocytes. After inoculation into the blood stream of susceptible animal, the parasites enter lymphoid cells of the spleen, lymph nodes and liver, were asexual multiplication or schizogony takes place. The schizonts in the lymphocytes are called Koch's blue bodies and are of diagnostic value. They are of 2types, macrochizont and microchizont. The particles of the latter breaks away from the host cell and invade the erythrocytes. These erythrocyte forms can be ingested by a vector tick and enable the infection to be passed on to the next bovine host (Siegmund and Fraser, 1973).

1.7.5 Chemotherapy

Tropical theileriosis is treated with naphthoquinine derivative, but the treatment with these agents does not completely eradicate thielerial infections and lead to development of carrier states in their host (OIE, 2018). Chemotherapy has approved satisfactory only when animals are treated in very early stages of the disease. Many drugs have been used in cattle; oxytetracycline and chlorotetracycline will inhibit further development of schizonts, primmaquinone phosphate cause degeneration of the erythrocyte forms when administered during the incubation period only (Siegmund and Fraser, 1973).

Synthetic naphthquinine (Clexon, Parvaxon) are effective against schizonts and Buprvaquone (Butalex) is effective against both scizonts and piroplasm stage (Salih and Hassan, 2003).

1.8 Control Measures

The most practical and widely used method for controling of theleriosis is the chemical control of tick with acaricides (OIE, 2018). But this method is not sustainable, expensive, cause environmental damage and development of acaricide resistance.

1.8.1 Tick control

Different methods are being used, depending on number of animal and price of drugs (Salih *et al.,* 2012).In Sudan for large herds of cattle they use machine spray and for single animals and small herds hand spray or pour on system is used (Almubarak,2017).

In situation were indigenous cattle are maintained in endemic stability for *T.annulata* intensive tick control is unnecessary, uneconomical and even harmful (d'oliveiran, 1997). Where in situation of exogenous cattle are reared which are highly susceptible to *Theileria* infections intensive short interval treatment for complete tick determination is recommended (Salih and Hassan, 2003).

An integrated control strategy of ticks in cattle based on the following measures is recommended and studied by several researchers like Muhammad *et al.* (2008) and Elhaj *et al.*(2016).

1.8.1.1 Housing in tick proof building

Cattle shed should be tick proof especially for the housing of exotic and cross bred cattle, as they are more susceptible to tick infestation than native cattle, there should be no cracks and crevices in the buildings, heaps of bricks woods or trash may also provide breeding places to ticks in animal sheds and should therefore be removed regularly (Muhammad *et al.*, 2008).

1.8.1.2 Slow burning of waste near the walls of the animals shed

Since the female ticks generally lay their eggs in cracks and crevices in the walls of the animals shed, scraping of the farm waste against the wall of the of the unoccupied paddocks and its slow burning over a period of one or two days is quite effective in reducing the tick burden on the animals. All common sense precaution should be exercised while doing this practice and it should be periodically repeated (Muhammad *et al.*, 2008).

1.8.1.3 Separate housing of cattle

Cattle in particular those with exotic blood when mixing species or ages or breeds of animals, cattle suffer from heavy tick infestation therefore cattle should be housed separately (Muhammad et al., 2008)..

1.8.1.4 Quarantine

Newly purchased animals should not be mixed right away with the already existing stock on the farm if ticks are present on the bodies of new arrivals, they should be treated with acaricides so that they are free from tick before adding them to the existing herd (Muhammad et al., 2008)..

1.8.1.5 Rotational Grazing

If cattle are placed on divided pastures when the ticks are producing few or no progeny and then alternated at monthly intervals the tick population can be controlled with markedly lower number of acaricidal treatment. Rotaion is not effective for the control of multihost ixoded ticks (Hyalomma *anatolicum anatolicum*) because of the long survival periods of the unfed nymphs and adults (Muhammad *et al.*, 2008).

1.8.1.6 Use of acaricides

Periodic application of acaricides is the most widely used method it may be directed against the free living stages of ticks in the environment or parasitic stages on host. Acaricides can be applied by dipping, washes, spraying, pour on, spot-on or by injection. Insectecides ear tags are commercially available in some countries (Muhammad *et al.,* 2008). The most chemical used are synthetic Pyrethroids as Permethrine ,Deltamethrine and Stomoxyne which they are 10 times stronger than DDT and 5 times stronger than Organic phosphorus compounds (Almubarak,2017).

1.8.1.7 Biological control

Ticks have numerous natural enemies, predators including Birds, Rodents Shrews, Ants and Spiders. Rising poultry in cattle barns greatly reduce tick burden on the infested herds but it's associated with hazard of the infectious disease like salmonellosis (Muhammad *et al.*, 2008).

1.8.2 Vaccinaton

Reliable vaccines of known efficacy have been developed for *T.parva* and *T.annulata*. The vaccine is prepared from schizonts infected cell lines that have been isolated from cattle and attenuated during in vitro culture, the vaccine is used in Israel, Iran, Turkey, Spain, India, Northern Africa, Central Asia and the People's Republic of China(OIE, 2018).

The use of vaccine has been limited due to concern about introduction of vaccine derived parasites into the field tick population and some attenuated cell lines have lost the ability to differentiate to erythrocytic merozoites when inoculated to cattle and in one hyalomma nymphs fed on vaccinated cattle didn't b ecome infected, all these made the individual countries to develop vaccines from local isolates (OIE, 2018).

1.9 Production system in Sudan

1.9.1 Nomadic

Livestock mainly camels and sheep with some goats are raised entirely on natural rangelands. Households move with their animals and have no permanent base on which to grow crops; they spend the rainy season in the northern, semi-desert zone and during the dry season move further south into the savannah. Income is derived from sale of animal's meat and milk in form of white cheese (Babo and Ahmed, 1977).

1.9.2 Transhumant

In the transhumant agro pastoral system, households depend mainly on livest ock, mostly cattle with some sheep and goats, although there is some cropping. In western Sudan households migrate north during the rainy season and return the savannah during the dry season. In the central and eastern states, migration is towards the Nile during rainy season and back during dry season (Babo and Ahmed, 1977).

1.9.3 Sedentary

The sedentary system exists where there is rain fed, enable farming in settled village. Some livestock mainly small ruminants are kept, but the animals are less important than the crops, Sorghum, Sesame and Cotton are grown on clay soils and Millets and Groundnuts on sandy soils (Babo and Ahmed, 1977).

1.9.4 Migratory agro Pastoral

This is found in Southern Sudan where livestocks are raised in traditional rain fed agricultural systems in settled villages. Livestocks are moved away from the Nile in the period of flooding and back when the floods recede (Babo and Ahmed, 1977).

1.9.5 Other systems

Other animal's production systems include ranching, feedlots operations and peri-urban backyards livestock production. Ranching is a recent trend in Sudan; animals are raised for meat on natural rangelands in western Sudan, in Kordofan and Darfur and in Butana in Kassala State. Poor range managements within the ranch is a major constraints. Feedlots have existed for over 30 years, animals mainly beef cattle are brought on the hoof from western Sudan and fattened in Khartoum State on Sorghum grain, oil seeds cakes and roughage, with gain up to 1Kg/day in cattle and 0.35kg in sheep.

Near and within urban areas goats and poultry fed on households waste (Babo and Ahmed, 1977).

Chapter two

Materials and Methods

2.1 Study Area

Omdurman district contains three localities and these were Omdurman, Umbada and Karrary from where the sample was taken extending in 8208km^2 on the west bank of White Nile and River Nile the climate is semi-arid , cool and dry in winter and characterized by low humidity extending from November to February, from April to July a hot dry weather prevails and wet rainy season is during the mid of July to mid of September, in the rainy season there is increase in relative humidity extending till January (Annual Report, 2017).

There is 178.697 cattle in Omdurman 51,917 are females producing 17.2%of total milk production in Khartoum State and also a good attribution in meat production(Annual Report, 2017). There is multiple type of production and management system for cattle investment were cross production constituted the majority of cattle population distributed in small herds managed by different owners in projects or individual manners.

2.2 Study Animal

The study subjects include cattle of different age, breeds and both sexes that kept under traditional or intensive management systems. Clinically suspected cattle were studied during February-March 2019.

2.3 Study Design and sample size

Cross sectional type of study was used for prevalence determination and sample size was set according to the relative formula for 95% confidence interval (Thrusfield, 2007)

$n = 1,96^2 \; Pexp(1\text{-}Pexp) / d^2$

Where

n = required sample size

Pexp = expected prevalence

d = desired absolute precision

The expected prevalence was obtained 7.3% from Abakar *et al.*, (2017).

The sample size calculated was 104 samples.

2.4. Clinical examination of suspected animal

The cattle were examined for clinical signs and symptoms after taking history from owners. Animal with fever or enlarged lymph node, change in color of urine or feces, breathing difficulties, change in feeding behavior, corneal opacity and emaciation were examined and sampled (Jagtap *et al.*, 2015).

2.5. Blood films

Thin blood smears [104 smears] were made from ear vein puncture, after cleaning the exposed skin with 70% Ethanol, the marginal ear vein was punctured with sterile needle (Salih and Hassan, 2003). A drop of blood was taken on clean glass microscopic slide, spread by another slide at an acute angle, air dried and fixed with absolute methanol for 2 minutes. The slide was labeled indicating the location, date and animal number were kept in slide box and transferred to the laboratory.

2.6 Gimsa's stain procedure

In laboratory, fixed smear stained with 10% Gimsa's solution for 30 minutes, then washed with distilled water and air dried and examined under oil immersion lens for presence of piroplasms.

2.7 Questionnaire

Questionnaire was filled in farm before taking the sample, originally written in English, it was framed around three aspects**:**

1. General information about the herd.
2. Management system of the herd.
3. Economic effect of thieleriosis and all TBDs.

The risk factors included in the 3 aspect of questionnaire for herd is production system and production type and for management systems of the herds, questions about the housing of the farm if fenced with solid wall or cracked wall which include wood or iron materials, the floor moisture of the farm if wet or dry, the roof availability and the arrangement of the room in the farm, lastly the application of the control and prevention measures in farm. For the economic effect aspect, questions included about losses due to thieleriosis and all TBDs if there is deaths, aborted fetus, reduction in milk yield or weight gain and other losses. Age, sex and breed of cattle also included as risk factors and collected in the sampling forms.

2.8 Data Analysis

Data set entered into excel spread sheets and coded, statistical analysis carried out with the computer application SPSS version 16 using pearson's chi-square and logistic regression.

Chapter three
Result

3.1 Clinical cases of thieleriosis:

Among the 104 animals investigated in Omdurman area 9 animals showed typical clinical signs. The main observed clinical signs were general weakness, pale mucus membrane; diarrhea and enlargement of superficial lymph nodes were also seen.

3.2 Prevalence of thieleriosis in Omdurman:

Prevalence of thieleriosis encountered in Omdurman locality was 29.8%, 31out of 104 cattle were found infected with *Thieleria* (Table 1).

Table 1: prevelance of thileriosis in omdurman

Result	Frequency	Percentage
Positive	31	29.8%
Negative	73	70.8%
Total	104	100%

Table 2: summary of risk factors associated with Thieleriosis in suspected cattle [n=104] in Omdurman localities – Khartoum state:

Groups		Examined	Positive	prevalence	χ^2	p- value
Age	Young	25	9	8.7%	0.603	0.43
	Adult	79	22	21.2%		
Sex	Female	79	26	25%	1.51	0.21
	Male	25	5	4.8%		
Breed	Cross	81	26	25%	0.919	0.44
	Local	23	5	4.8%		
PS	Sedentary	99	30	28.8%	0.241	0.62
	Transhumant	5	1	1%		
PT	Dairy	83	27	26%	1.45	0.22
	Feedlot	21	4	3.8%		
Fenced	Cracked	36	9	8.7%	0.608	0.43
	Un cracked	68	22	21.2		
Floor	Wet	9	2	1.9%	0.271	0.60
	Dry	95	29	27.9%		
Roof	No	50	18	17.3%	1.765	0.18
	Yes	54	13	12.5%		
SR	NO	88	27	26%	0.209	0.64
	YES	16	4	3.8%		
Control	No	80	26	25%	1.201	0.27
	Yes	24	5	4.8%		

3.3 Univariate analysis

3.3.1 Age:

As shown in table 2 the adult cattle, 22 out of 79 were positive and the prevelance was 21.2%.While 9 out of 25 in young cattle were positive and the infection rate 8.7%. There was no significant association between the age and the disease [X^2=0.603].

3.3.2 Sex:

The result [Table 2] show female cattle examined, 26 out of 79 were positive and the prevalence was 25%.While in male cattle 5 out of 25 were positive and the prevalence was 4.8%. The significance tested with logistic regression for $p \leq 0.25$ [Table 3].

3.3.3 Breed:

Crosses cattle examined 26 out of 81 were positive [Table 2] and the prevalence was 25% while local cattle examined 5 out of 23 were positive. There was no significant association between the breed of cattle and the disease [X^2=0.919].

3.3.4 Production system (PS):

The majority of examined cattle were sedentary 99 cattle, 30 positive and prevalence was 28.8%[Table 2]. Transhumant cattle examined 5, 1 positive and prevalence was 1%. There was no significant association between the production system and the disease [X^2=0.241].

3.3.5 Production type (PT):

As shown in table 2 the dairy cattle examined was 27 out of 83 were positive and prevalence was 26%. While feedlot cattle were 4 out of 21 positive and the prevalence was 2.8%. The significance tested with logistic regression for $p \leq 0.25$ [Table 3].

3.3.6 Fencing of the farm:

As shown in the result [Table 2] there 68 cattle were living in farmed with un- cracked fence, 22 positive and the infection rate was 21.2% while 36 examined cattle lived in farm with cracked fenced, 9 positive and the prevalence was 3.8%. There was no significant association between fencing of the farm and the disease [X^2=0.608].

3.3.7 Floor situation of the farm:

Cattle live in dry floor were 95, 29 positive and infection rate was 27.9% [Table 2], while cattle in wet floor were 9 cattle, 2 positive and the prevalence was 1.9%. There was no significant association between farm floor situation and the disease [X^2=0.271].

3.3.8 Roof availability:

In table 2 the cattle shaded under roof were 54 cattle, 13 positive and the infection rate was 12.5%. While the examined un-shaded were 50 cattle, 18 positive and the infection rate was 17.3 The significance tested with logistic regression for $p \leq 0.25$ [Table 3].

3.3.9 Separation of rooms in farm:

Cattle farmed in mixed area were 27 out 88 positive and the prevalence was 26%. While examined cattle [Table 2] lived in separate room for each age group was 4 out of 16 positive and the infection rate was 3.8%. There was no significant association between separation of rooms and the disease [X^2=0.209].

3.3.10 Application of control measure:

Control measure applied in 24 cattle 5 positive and the prevalence was 4.8%. While 80 cattle with no control measure applied 26 were positive and the prevalence was 25%[Table 2]. The significance tested with logistic regression for $p \leq 0.25$ [Table 3].

Table 3: Logistic Regression of risk factors associated with Thieleriosis in suspected cattle in Omdurman localities

Risk factor	B	S.E.	Wald	df	Sig.	Exp(B)	95.0% C.I.for EXP(B)	
							Lower	Upper
sex	.629	.585	1.156	1	.282	1.876	.596	5.905
PT	-.533	.722	.544	1	.461	.587	.142	2.418
Roof	-.326	.501	.422	1	.516	.722	.271	1.927
Control	-.842	.580	2.106	1	.147	.431	.138	1.343

*****$p \geq 0.25$

3.4 Multivariate analysis:

Each risk factor had a P value ≤ 0.25 estimated as significantly associated and tested by logistic regression to determine the highly associated risk factor.

Sex, production type, presence of roof and application of control measure were the covariate tested by logistic model.

The goodness of fit test for logistic regression model lead to reject the null hypothesis and accept the alternative that the fitted model is correct (table 4: hosmer and lemshow test).

There was no significant association between production types, presence of roof in cowshed on the prevalence of Thieleriosis [P ≥ 0.25]. Sex of cattle and application of tick control measure were significantly associated with the prevalence of Thieleriosis in cattle [P ≤ 0.25].

Chapter four

Discussion

Thieleriosis is one of the blood parasitic disease that obviously result in reduced productivity of cattle specially for cross breed holders, those specially raised for increasing production of milk. In the study result the overall prevalence in Omdurman localities was 29.8% which is higher than Saifieldin (2005) who revealed 7%, 5.2% and 6.32% in dry cold, dry hot and wet hot seasons, respectively. Ali (2006) reported that the infection rate was 16.6% of bovine thieleriosis in Khartoum state.

In the study results the prevalence in age group was 8.7% in young cattle and 21.1% in adult respectively. Concerning prevalence in breeds cross breed were 25%. The same result was calculated for sex of examined animals that the females had the highest prevalence 25% and the males had 4.8%. The multivariate analysis declared that sex of cattle affect the presence of Thieleriosis. Saleem *et al*.(2014)reported that females are more infected than males suggesting that females are less resistant to infection than males because of the regular decrease in their immune system(pregnancy and lactation) .

Cattle of local breeds are generally more resistant than foreign animal, other studies attributed the difference insensitivity to tropical thieleriosis between Holestein and local breeds to a genetic difference leading to high production level of pro-inflammatory cytokines in Holestein cattle (Ayadi *et al*., 2016).

Adult females cattle from cross breed is sedentary in most of Omdurman farm for milk production purpose and most of the surveyed farms was dairy farms this is the cause of high prevelance in the female adult cross breed cattle in Omdurman locality.

Concerning production system, in Khartoum state specially Omdurman the main if not the only production system is sedentary; these consequences into a higher prevelance of thieleriosis, also animals spend whole life days inside the cowshed which lead to more exposure to tick infestation.

Production type in surveyed cattle in Omdurman area divided into dairy and feedlot farms, represented 83(26%) and 21(3.8%) cattle respectively, revealing dairy cattle is more susceptible to theileriosis and in other side the feedlots are exposed to better veterinary services and had a shorter life span than dairy cattle in Al-Rodwan project was attributed to the fact that most of the farm in this area were infested with tick (Saifeldin, 2005).

All farms are categorized as cracked or un-cracked built with mud or block stones, wood parts, trees parts and iron slides, all constitute a suitable environment for ticks, the study revealed that there is no effect of farm fencing on the prevelance of thieleriosis, this finding is disagreed with the result of previous researcher (Saifeldin, 2005), but agreed with (Ayadi *et al*, 2016). Cracks in cowshed walls are favorable to egg-laying tick vector whereas un-cracked wall prevent nymph and adult tick to hide. Elimination of cracks, smoothing of inner surface, also using organized wood parts or iron parts with no cracks without addition of fabrics or cartons or plastic bags, will reduce the incidence of tropical thieleriosis.

Variation in prevelance rate between wet and dry farm floor, but it's a consequences of the farm surveyed which is 1.9% in wet and 27.9% in dry farm , this could be attributed to tick challenges throughout the years. This finding agreed with Rodgers *et al*. (2007) who found the statistically significant relationship between relatives humidity, exposure duration and nymph survival increase the knowledge of the ability of the tick to withstand desiccation. Also tick survived exposure to dry conditions as long as they were returned to air daily it seems likely that typical summer conditions will not lead to abrupt change .This justification is also suitable for the result of high prevalence of farm with no roof (17.3%) verse farm with roof (12.5%).

In sedentary system farm with separate rooms for calves and adults, calves are confined, fed alone and not permitted to fed with adults this limits their exposure to ticks. The only time calves between 0-12 months is mixed with their mothers is at time of milking when they are used to stimulate let-down of milk. This practice keeps calves highly susceptible to TBDs especially at the point at which their maternal antibodies decline (Muhanguzi *et al.*, 2014).

Results of this study suggested that the regular use of acaricides suppressed the prevalence of thieleriosis this result strengthens by Pvalue result $P \leq 0.25$. The combined use of oral and injectable ivermectin and the use of permitherine to reduce tick abundance in environment reduced the disease rate this result agreed with Cauvin *et al.,* (2018) that acaricides do have measurable success at reducing the burden of TBDs on farmed white tailed deer during summer months.

In Animal, health economic consequences are often uncertain, but it affect production and productivity, but doing some actions like controlling the disease before it spreads preparing for economic loss before starting treatment in the herd or individually and using alternative treatment. All these actions should be done to prevent such losses.

In this study questions was asked to the owners if there is a difference noticed in milk yield and weight gained in tick infested animal, all the answers was consent of noticed change in productivity, questions concerning economic losses was simple and solely for milk losses and weight gain losses requiring no analysis , because the impact are multidimensional and not always well understood , despite the complexity of calculating the cost of disease, estimation is needed to guide resource allocation at the farm, national and regional level.

Conclusions and Recommendation

Conclusions

The study conclusion that Thieleriosis in Omdurman localities was higher spread in Females cattle (25%) more than male (4.8%) . application of acaricides and antithielerial drugs and tick control managements decrease the disease rate.

Recommendation

- Further tick control is needed to reduce tick population and result of TBDs on farms.
- There is a greater need to understand how the use of acaricides on farm and animal individually because owner misuse these drugs leading to resistant of vectors.
- It will be of great interest to study the economic impact of the disease and the benefits of the tick control and practicing good management on the herds.

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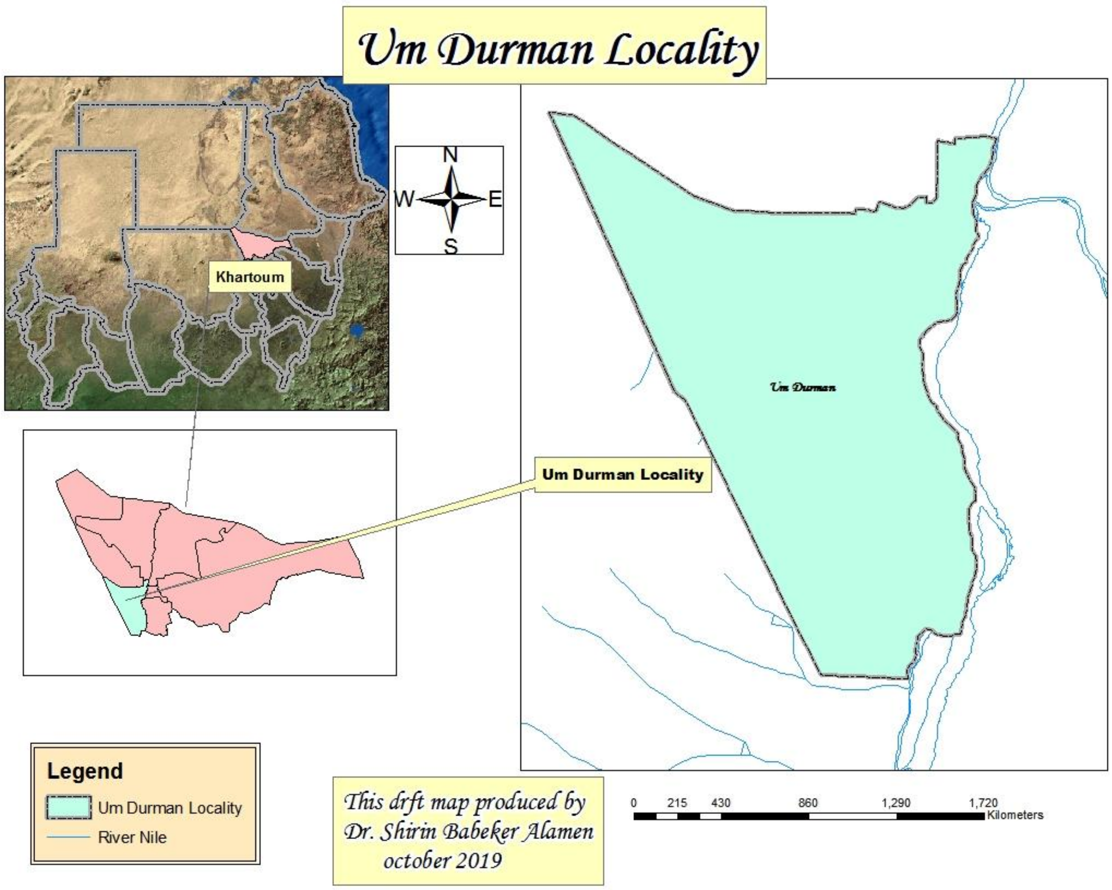
Appendices

Appendix 1

Figure 2: Map of Omdurman locality (Study area)



Appendix 2

Sample form:

Form Number …….. Herd No ……… Location ……………………………………………

Owner Name ………………………………………. Phone Number ………………………

Serial No.	Breed	sex	Age	Result

Date………………

Appendix 3

Questionnaire:

Questionnaire Serial Number ……..	Herd No ………
Owner Name ………………………………………..	Phone Number ……
Location ………………………………………………………………	

General information

Production system:

- Sedentary
- Transhumant

Production type:

- Dairy farm
- Feedlot

Management information

Housing

1. Fence
- Un- ckraced
- Cracked

2. Floor
- Wet
- Dry

3. Roof
- Yes
- No

4. Separate room for age groups
- Yes
- No

Control and prevention measure

- Yes
- No

Economic effect

Losses due to TBDs

1. Death …………………………………..
2. Abortion ……………………………….
3. Reduce milk yields ……………………….
4. Reduce weight gain ……………………..
5. Selling ……………………..

Appendix 4

Cross tabs and chi- square tables :

Age group

Crosstab

			Result		Total
			positive	Negative	
Age Group	Calve	Count	9	16	25
		% of Total	8.7%	15.4%	24.0%
	Adult	Count	22	57	79
		% of Total	21.2%	54.8%	76.0%
Total		Count	31	73	104
		% of Total	29.8%	70.2%	100.0%

Chi-Square Tests

	Value	Df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.603[a]	1	.437		
Continuity Correction[b]	.276	1	.599		
Likelihood Ratio	.589	1	.443		
Fisher's Exact Test				.459	.295
Linear-by-Linear Association	.597	1	.440		
N of Valid Cases[b]	104				

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 7.45.

b. Computed only for a 2x2 table

Sex

Crosstab

			Result		Total
			Positive	negative	
Sex	Male	Count	5	20	25
		% of Total	4.8%	19.2%	24.0%
	female	Count	26	53	79
		% of Total	25.0%	51.0%	76.0%
Total		Count	31	73	104
		% of Total	29.8%	70.2%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	1.513[a]	1	.219		
Continuity Correction[b]	.959	1	.327		
Likelihood Ratio	1.598	1	.206		
Fisher's Exact Test				.316	.164
N of Valid Cases[b]	104				

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 7.45.

b. Computed only for a 2x2 table

Breed

Crosstab

			Result		Total
			Positive	negative	
breed	cross	Count	26	55	81
		% of Total	25.0%	52.9%	77.9%
	local	Count	5	18	23
		% of Total	4.8%	17.3%	22.1%
Total		Count	31	73	104
		% of Total	29.8%	70.2%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.919[a]	1	.338		
Continuity Correction[b]	.490	1	.484		
Likelihood Ratio	.961	1	.327		
Fisher's Exact Test				.442	.246
N of Valid Cases[b]	104				

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 6.86.

b. Computed only for a 2x2 table

Production system

Crosstab

			result		Total
			positive	negative	
production system	sedentry	Count	30	69	99
		% of Total	28.8%	66.3%	95.2%
	transhumant	Count	1	4	5
		% of Total	1.0%	3.8%	4.8%
Total		Count	31	73	104
		% of Total	29.8%	70.2%	100.0%

Chi-Square Tests

	Value	Df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.241[a]	1	.623		
Continuity Correction[b]	.000	1	1.000		
Likelihood Ratio	.260	1	.610		
Fisher's Exact Test				1.000	.530
Linear-by-Linear Association	.239	1	.625		
N of Valid Cases[b]	104				

a. 2 cells (50.0%) have expected count less than 5. The minimum expected count is 1.49.

b. Computed only for a 2x2 table

Production type

Crosstab

			Result		Total
			positive	negative	
production type	dairy	Count	27	56	83
		% of Total	26.0%	53.8%	79.8%
	feedlots	Count	4	17	21
		% of Total	3.8%	16.3%	20.2%
Total		Count	31	73	104
		% of Total	29.8%	70.2%	100.0%

Chi-Square Tests

	Value	Df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	1.456[a]	1	.228		
Continuity Correction[b]	.883	1	.347		
Likelihood Ratio	1.556	1	.212		
Fisher's Exact Test				.292	.175
Linear-by-Linear Association	1.442	1	.230		
N of Valid Cases[b]	104				

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 6.26.

b. Computed only for a 2x2 table

Fencing

fenced * result Crosstabulation

			Result		Total
			Positive	Negative	
fenced	Cracked	Count	9	27	36
		% within result	29.0%	37.0%	34.6%
		% of Total	8.7%	26.0%	34.6%
	Uncracked	Count	22	46	68
		% within result	71.0%	63.0%	65.4%
		% of Total	21.2%	44.2%	65.4%
Total		Count	31	73	104
		% within result	100.0%	100.0%	100.0%
		% of Total	29.8%	70.2%	100.0%

Chi-Square Tests

	Value	Df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.608[a]	1	.435		
Continuity Correction[b]	.308	1	.579		
Likelihood Ratio	.619	1	.432		
Fisher's Exact Test				.504	.292
Linear-by-Linear Association	.602	1	.438		
N of Valid Cases[b]	104				

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 10.73.

b. Computed only for a 2x2 table

Floor

Crosstab

			Result		Total
			Positive	negative	
floor	wet	Count	2	7	9
		% of Total	1.9%	6.7%	8.7%
	dry	Count	29	66	95
		% of Total	27.9%	63.5%	91.3%
Total		Count	31	73	104
		% of Total	29.8%	70.2%	100.0%

Chi-Square Tests

	Value	Df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.271[a]	1	.603		
Continuity Correction[b]	.019	1	.889		
Likelihood Ratio	.285	1	.593		
Fisher's Exact Test				.721	.463
Linear-by-Linear Association	.268	1	.604		
N of Valid Cases[b]	104				

a. 1 cells (25.0%) have expected count less than 5. The minimum expected count is 2.68.

b. Computed only for a 2x2 table

Roof

Crosstab

			result		Total
			Positive	negative	
Roof	no	Count	18	32	50
		% of Total	17.3%	30.8%	48.1%
	yes	Count	13	41	54
		% of Total	12.5%	39.4%	51.9%
Total		Count	31	73	104
		% of Total	29.8%	70.2%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	1.765[a]	1	.184		
Continuity Correction[b]	1.241	1	.265		
Likelihood Ratio	1.769	1	.184		
Fisher's Exact Test				.204	.133
Linear-by-Linear Association	1.748	1	.186		
N of Valid Cases[b]	104				

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 14.90.

b. Computed only for a 2x2 table

Separate room

Crosstab

			Result		Total
			Positive	negative	
seperate rooms	no	Count	27	61	88
		% of Total	26.0%	58.7%	84.6%
	yes	Count	4	12	16
		% of Total	3.8%	11.5%	15.4%
Total		Count	31	73	104
		% of Total	29.8%	70.2%	100.0%

Chi-Square Tests

	Value	Df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.209[a]	1	.648		
Continuity Correction[b]	.026	1	.873		
Likelihood Ratio	.215	1	.643		
Fisher's Exact Test				.772	.448
Linear-by-Linear Association	.207	1	.649		
N of Valid Cases[b]	104				

a. 1 cells (25.0%) have expected count less than 5. The minimum expected count is 4.77.

b. Computed only for a 2x2 table

Control

Crosstab

			Result		Total
			positive	negative	
Control	no	Count	26	54	80
		% of Total	25.0%	51.9%	76.9%
	yes	Count	5	19	24
		% of Total	4.8%	18.3%	23.1%
Total		Count	31	73	104
		% of Total	29.8%	70.2%	100.0%

Chi-Square Tests

	Value	Df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	1.201[a]	1	.273		
Continuity Correction[b]	.708	1	.400		
Likelihood Ratio	1.263	1	.261		
Fisher's Exact Test				.319	.202
Linear-by-Linear Association	1.189	1	.275		
N of Valid Cases[b]	104				

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 7.15.

b. Computed only for a 2x2 table

Appendix 5

Logistic regression analysis

Variables in the Equation

		B	S.E.	Wald	df	Sig.	Exp(B)	95.0% C.I.for EXP(B)	
								Lower	Upper
Step 1[a]	sex(1)	.629	.585	1.156	1	.282	1.876	.596	5.905
	PT(1)	-.533	.722	.544	1	.461	.587	.142	2.418
	Roof(1)	-.326	.501	.422	1	.516	.722	.271	1.927
	Control(1)	-.842	.580	2.106	1	.147	.431	.138	1.343
	Constant	1.990	.826	5.809	1	.016	7.313		

a. Variable(s) entered on step 1: sex, PT, Roof, Control.

Appendix 6

Hosmer and lemshow test

Step	Chi-square	df	Sig.
1	3.022	6	.806