Molecular epidemiological studies on trypanosomosis and piroplasmosis among livestock in southern Africa and central Asia

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Abbreviations

AAT	Animal African trypanosomosis
bp	Base pairs
BP	Bovine piroplasmosis
CATT	Card agglutination test for trypanosome
CDC	Center for Disease Control
CFT	Compliment fixation test
DDW	Double distilled water
DNA	Dioxyribonucleic acid
dNTPs	Dioxynucleotide triphosphates
ECF	East Coast fever
EDTA	Ethylene diaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
EP	Equine piroplasmosis
ICT	Immunochromatographic test
IFAT	Immunofluorescent antibody test
ITS	Internal transcribed spacer
IVM	Institute of Veterinary Medicine (Mongolia)
Kg	Kilogram
LAMP	Loop-mediated isothermal amplification
mg	Miligram
ml	Milliliter
OIE	Office International des Epizooties
PCR	Polymerase chain reaction
PCV	Packed cell volume

RBC	Red Blood Cell
TT	Tropical theileriosis
VBD	Vector borne disease
^{0}C	Degrees centigrade
µl	Microlliter
%	Percentage

General introduction

1. Introduction

Hemoprotozoan parasites, which include different species of *Theileria, Babesia* and *Trypanosoma*, often present a challenge to successful livestock farming (Sivakumar *et al*., 2012_a). Endemic parasites are a major source of economic loss in animal husbandry, especially in tropical areas and developing countries, but the extent of those losses has yet to be accurately specified, and knowledge about the economics of treatment of these diseases is inadequate, mostly because the damage functions, and in addition, the response functions to treatment are imperfectly known (Tisdell *et al*., 1999). Economic impacts of parasites of livestock may be divided into two groups - direct and indirect impacts. Direct impacts are those attributable purely to the presence of the parasite, e.g. reduced economic productivity of the livestock or economic loss due to mortality. Indirect impacts occur because some parasites, such as ticks, are also disease vectors, and the vector-borne diseases (VBDs), if transmitted to a livestock host, often result in much greater economic loss than the presence of the parasite. Furthermore, by weakening the host the presence of a parasite may make the animal much more susceptible to

infection and adverse impact from other diseases, or to environmental stresses such as food deficits (McCauley *et al*., 1984).

The relative significance of most VBDs in livestock is extremely difficult to quantify, because in most countries there is no formal reporting, poor diagnosis, and no surveillance or collated assessment of prevalence or economic impact. Even in highly regulated regions, such as Northern Europe, accurate statistics are elusive. However, certain VBDs do have a particularly significant impact on food production; for example; babesiosis, spread by ticks, is responsible for serious losses in cattle production in Latin America, Australia and Asia; it is also locally important in parts of Europe. The importance of babesiosis was such that an extensive control program was implemented in the United States to eradicate the tick vectors. This remains one of the very few examples of successful, sustained, area-wide vector eradication. It was successful because an area-wide approach was adopted using a variety of management tools with strict centralized coordination, underpinned by the budget of a developed nation. East Coast fever (ECF) is a particularly important tick-borne disease of cattle in Africa. In sub-Saharan Africa, trypanosomosis affects food production (IFAH report, 2014).

2. Trypanosomosis

Trypanosomes found in domestic animals are blood and sometimes tissue parasites of the order *Kinetoplasitida*, family *Trypanosomatidae*, and genus *Trypanosoma*. Animal trypanosomoses are nowadays a permanent constraint for livestock in Africa, Asia, and Latin America, but their geographical distribution is still evolving (Desquesnes *et al*., 2013). They are grouped into 2 sections: Stercoraria which develops in the insect's posterior digestive tract, for example *T. cruzi* , that is responsible for transmission of Chagas disease in humans affecting 15 million and threatening 100 million people in Latin America (Coura and Pereira, 2010) and Salivaria which develops in the anterior part of the insect's digestive tract, such as the main African livestock pathogenic trypanosomes, including the agents of sleeping sickness, a major human disease affecting around half a million people and threatening 60 million people in Africa (Rogers, 2009). African livestock trypanosomes are threatening 48 million cattle in an area of 10 million square kilometers in 37 African countries; they cause disease syndromes responsible for major production losses including death in the absence of treatment (Desquesnes *et al*., 2013).

2. 1. Transmission and lifecycle

The main pathogenic animal trypanosomes belong to three subgenera of the salivarian section, namely, *Nannomonas* (*T. congolense*), *Duttonella* (*T. vivax*), and *Trypanozoon* (*T. brucei* group). These parasites are mostly cyclically transmitted by the tsetse fly (*Glossina* spp.) in which procyclic forms undergo a cycle of transmissions and multiplications leading to infective metacyclic forms which may be inoculated by the tsetsefly with its saliva into a new host (Horare, 1972) (Figure. 1). This biological association restricts the trypanosomes' geographical distribution to that of the vector which is sub-Saharan Africa. However, in some instances, the geographical distribution of trypanosomes does not fit that of the tsetse flies, this is mainly due to other means of transmission. Direct vertical, oral, sexual and iatrogenic transmission may have an occasional impact, but the most important alternative way is mechanical transmission by biting flies (Desquesnes *et al*., 2013). This mechanical transmission does not involve a specific biological relationship between the parasite and the vector; rather the pathogens are simply sampled from one host, transported to another and inoculated together with the saliva of the biting insect, prior to the absorption of blood (Foil, 1989). This has therefore led to some African trypanosome species spreading not only outside the African tsetse belts, but also outside the continent (Horare, 1972). In the sub genus *Duttonella*, *T. vivax* is

mechanically transmitted by tabanids and *Stomoxys*, in both Africa (Desquesnes and Dia, 2003; Desquesnes and Dia, 2004) and Latin America (Jones and Davila, 2001; Desquesnes, 2004). Although *T. congolense* was suspected early to be mechanically transmitted, the relatively low parasitemia recorded in its main host (cattle) does not favor mechanical transmission which results in rare epidemiological evidence for such transmission in the field (Desquesnes and Dia, 2003). In the sub genus *Trypanozoon*, mechanical transmission of *T. brucei* was described through contamination by sucking flies and through serial biting action by biting insects especially *Stomoxys* (Mihok *et al*., 1995). *T. evansi*, in the same sub genus can no longer undergo its cycle in tsetse flies, thus its main transmission is through biting insects. For this reason, *T. evansi* spread outside the tsetse belts in Africa, towards the Middle East and Southern Asia and was exported to Latin America (Desquesnes *et al*., 2013).

T. evansi is thought to be derived from *T. b. brucei* (which is cyclically transmitted by tsetse flies), but it is no longer able to undergo its cycle in the tsetse fly due to the loss of maxicircles of kinetoplastic mitochondrial DNA which is required to undergo the procyclic form in tsetse flies (Lai *et al*., 2008) losing in consequence its ability to perform oxidative phosphorylation (Schnaufer *et al*., 2002) and thus “trapped” in its blood stream form. It is not known when this occurred, but it was even

suggested that it might have occurred in several instances (Lun and Desser, 1995; Lai *et al*., 2008). *T. evansi* also possesses single predominant minicircle sequence class (Li *et al*., 2006). A complete loss of the kinetoplastic DNA might even be possible and lead to akinetoplastic or diskinetoplastic *T. evansi*, which are observed in field stocks, but the use of some trypanocidal drugs may also enhance or induce diskinetoplastic forms (Chitambo *et al*., 1992; Schnaufar *et al*., 2002).

T. equiperdum is also thought to be derived from *T. brucei* by an alteration of maxicircle kinetoplastic DNA sequences (Li *et al*., 2006). *T. equiperdum* is a tissue parasite and is directly transmitted from equine to equine through coitus. The trypanosomes which are present in the seminal fluid and mucous membranes of the genitalia of the infected donor animal are transferred to the recipient animal (Wang, 1988). Trypanosomes are rarely observed in the blood stream of the host because they are normally localized in the capillaries of the mucous membranes of the urogenital tract. However, a few trypanosomes occasionally appear in the peripheral blood of animals with chronic infections. This could provide opportunity for blood sucking insects to mechanically transmit this parasite, although this is considered to be very rare (Brun *et al*., 1998). The fact that foals have been found to be infected with *T. equiperdum* may indicate that this parasite can also be directly transmitted through milk or from udder lesions (Brun *et al*., 1998). Distinction and even existence

of this parasite was always questioned since genetic differentiation is almost impossible, especially due to the absence of satisfying reference strains (Zablotskij *et al*., 2003; Claes *et al*., 2005); however, recently true *T. equiperdum* strain, IVM-t1 was isolated from the urethral mucosa of a dourine infected horse in Mongolia and successfully cultured (Suganuma *et al*., 2016) and also the distinction is still very clear when looking at the minicircle complexity, which is very high in *T. brucei* and scarce if any in *T. equiperdum* and *T. evansi*. This diversity is most likely linked to sexual recombination, which can only occur in *Glossina* (Gibson and Stevens, 1999).

Trypanosomes infect domestic and wild animals in Africa to cause Nagana in the tsetse belt. Wild life plays a major role as a reservoir for animal African trypanosomosis (AAT) because of its high tolerance to the various species of trypanosomes (Stevens *et al*., 1999; Taylor and Authie, 2004). However, Surra occurs in all countries where camels are present, from Senegal to Kenya, above the tsetse belt; it is found not only in Mauritania, Morocco, Algeria, Tunisia, Libya, Egypt, Sudan, Eritrea and Ethiopia but also in the northern parts of Mali, Burkina Faso, Niger, Nigeria, Chad, Somalia and Kenya (Desquesnes *et al*., 2013). In Africa, Surra is found under the Arabic name Debab (El debab in Algeria) which means fly, and also Mbori in Sudan, Guifar or Dioufar in Chad, Menchaca (which means emaciated despite sufficient food provision) in Touareg populations of Agadez area, Niger,

Yudleye or Yudle, which refers to an emaciated camel moving aimlessly or jolting forward, or even Dukhan or Salaf (or salef) in Somali or Tahaga and Su-auru (Atarhouch *et al*., 2003; Antoine-Moussiaux *et al*., 2007).

In Asia, trypanosomes in domestic animals mainly cause surra and dourine. *T. evansi* and *T. equiperdum* are continuously present eastwards, in the Arabian peninsula, including Jordan, Israel, Lebanon, Syria, Iraq and Turkey, it is present from Iran to Kazakhstan as well as in Afghanistan and Pakistan (Hassan *et al*., 2006; Wernery *et al*., 2001; Desquesnes *et al*., 2013). It is also present in India, China, Mongolia, Russia, Bhutan, Nepal, Myanmar, Laos, Vietnam, Cambodia, Thailand, Malaysia, Philippines, and Indonesia (Luckins, 1988; Reid, 2002).

2. 2. Pathogenesis and disease syndromes

Trypanosomes cause 3 major disease syndromes in domestic and wild animals; Nagana, a syndrome of AAT is tsetse fly transmitted and the most complex of all because of the number of trypanosome species involved but the main causative agents are *T. b. brucei* (sub-genus *Trypanozoon*), *T. congolense* (sub-genus *Nannomonas*) and *T. vivax* (sub-genus *Duttonella*) (Taylor and Authie, 2004). Nagana was first reported in Zululand by David Bruce in 1899 (Vickerman, 1997).

Domestic animals show a variety of pathogenic syndromes of Nagana ranging from acute to chronic depending on several factors such as animal species, breed, sex and age (Taylor and Authie, 2004; Simukoko *et al*., 2007$_{a,b}$; Courtin *et al*., 2008). Generally, infected animals show rather similar clinical symptoms including anorexia, lymph node enlargement, splenomegaly, anemia and death but the details especially the severity levels of the disease tend to be different. For example *T. congolense* infections in cattle are more severe than in horses whereas *T. b. brucei* infections though severely pathogenic in horses are mild in cattle (Taylor and Authie, 2004). Although Nagana is caused by many trypanosome species, *T. congolense* and *T. vivax* are the most detected in infected samples (Mugittu *et al*., 2001; Mahama *et al*., 2004; Thumbi *et al*., 2010).

Another syndrome is known as Surra and is caused by *T. evansi*. The word “Surra” comes from Hindi and means “rotten”, which refers to the state of the animal after chronic evolution of the disease (Desquesnes *et al*., 2013). *T. evansi* is the first mammalian trypanosome to be described in the World, in 1880 by Griffith Evans, in the blood of Indian equines and dromedaries. Its principal host is originally the camel but it is present in other domestic animals as well as in a large range of other hosts (Desquesnes *et al*., 2013). *T. evansi* is pathogenic to many domestic and wild animals but its pathogenicity varies according to the virulence of the strain, the host

species, unspecific factors affecting the animal such as other infections and general stress, and the local epizootiological conditions (Desquesnes *et al*, 2013). The clinical signs of Surra in most domestic and wild animals are characterized by fever, and anemia, followed by emaciation, edema, cachexia and enlargement of lymph nodes and spleen. Neurological symptoms occur late in the disease. Abortion may occur in late pregnancy. Acutely infected animals may die within weeks or months but chronic infections may continue for several years (Brun *et al*., 1998).

Dourine is the third syndrome and is caused by *T. equiperdum*. The clinical symptoms of dourine vary in their presentation. The earliest signs usually consist of swellings and local edema of the genital organs. Later, major clinical manifestations such as fever, anemia, neurological symptoms and abortion are observed especially in the late stages of the infection (Stephen, 1986). Plaques on the skin are characteristic of Dourine (Brun *et al*., 1998).

2. 3. Diagnosis and treatment

Diagnosis of trypanosomosis is through observation of clinical signs and confirmation through the laboratory. Parasite detection is the gold standard and it is a highly specific method. Parasite detection involves observation of the parasites through microscopy by preparation of wet or thick blood smears and buffy coat; this technique can be used to identify the morphological characteristics of the *Trypanosoma* species (Kronenberger and Miezan, 1988). Another parasite detection method is through molecular detection of the parasite by DNA amplification. Polymerase chain reaction (PCR) is a DNA amplification method that is very specific and sensitive that has changed the whole picture of diagnostics making it more reliable (Desquesnes *et al*., 2001). Its limitation is that PCR requires a thermal cycler to generate alternate temperatures for amplifying the target DNA sequence and therefore it is not a very useful method for field diagnosis, but it is very important for epidemiological studies (Aboulaila *et al*., 2010). Loop-mediated isothermal amplification (LAMP) is a novel DNA based identification technique that is based on a constant temperature; it has rapidity and high specificity (Notomi *et al*., 2000). LAMP has been applied in the diagnosis of trypanosomosis (Thekisoe *et al*., 2007; Thekisoe *et al*., 2010). Unlike PCR, LAMP does not require a thermal cycler but simple equipment such as a water bath.

Other laboratory diagnostic methods include; serological and antibody detection methods like ELISA (enzyme-linked immunosorbent assay) and card agglutination test for trypanosomes (CATT) (Olaho-Mukani *et al*., 1994; Nantulya, 1988; Buscher *et al*., 1999). Another test that has been recently developed is the TeGM6-4r based immunochromatographic test (ICT) for animal trypanosomosis. This test can detect the Asian as well as the AAT trypanosomes (Nguyen *et al*., 2015). It is a useful pen-side test for the local veterinarians' decision making in field conditions.

In most of sub-Saharan Africa, bovine trypanosomiasis continues to be controlled primarily by trypanocides. Only three compounds; isometamidium chloride, homidium (bromide and chloride) and diminazine aceturate, are available and have been used for over 40 years (Leach and Roberts, 1981).Isometamidium is principally used as a prophylactic drug and can provide up to six months' protection against tsetse challenge. Whilst homidium has limited prophylactic properties, it is primarily used as a therapeutic agent. Diminazine has only therapeutic properties (Holmes *et al*., 2004).

For the treatment of Surra and Dourine; suramin and quinapyramine are also used alongside diminazine and more recently cymelarsan was developed and proved effective (Brun *et al*., 1998).

3. Piroplasmosis

Piroplasmosis in domestic animals is mainly caused by *Babesia* and *Theileria* species. These main agents of animal piroplasmosis are vectored by ticks thus making tick borne diseases a major cause of concern to livestock producers. Tick-borne protozoan diseases (e.g., theileriosis and babesiosis) pose important problems for the health and management of domestic cattle in the tropics and subtropics (Jongejan and Uielenberg, 2004). The most widespread and malignant *Theileria* species is *Th. annulata*, causing tropical theileriosis, which occurs around the Mediterranean basin, in the Middle East, and in Southern Asia. The other malignant *Theileria* species is *Th. parva*, which occurs in East and Southern Africa and causes ECF. Bovine babesiosis is caused by *B. bovis* and *B. bigemina*, both of which occur worldwide in tropical and subtropical regions. *B. divergens* occurs in cattle in Europe and extends into North Africa (Bouattour and Darghouth, 1996). In addition, benign forms of theileriosis are caused by *Th. mutans, Th. velifera*, and *Th.*

taurotragi, which are mainly located in Africa, whereas parasites of the *Th.sergenti-buffeli-orientalis* group, referred to below as the *Th. orientalis* group, occur worldwide (Uilenberg, 2006).

3. 1. Transmission and lifecycle

Piroplasmosis is transmitted by ticks to the mammalian host but the infection establishment differs across the two genera (Duh *et al*., 2008). During the tick bite, sporozoites are injected into the host and directly infect the red blood cells in babesiosis whereas during theileriosis, the sporozoites do not readily infect the red blood cells but initially penetrate a lymphocyte or macrophage in which development of schizonts take place (Uilenberg, 2006). *Theileria* species infect a wide range of domestic and wild animals and are transmitted by ixodid ticks of the genera *Amblyoma, Haemaphysalis, Hyalomma* and *Rhipicephalus*. Most of these ticks are known for the large economic losses they cause to the agriculture industry due to disease outbreaks, mortalities, damage to hides and poor production in domestic animals (Bishop *et al*., 2004). The genus *Theileria* is distinguished by infection of leukocytes by sporozoites, maturation of schizonts into merozoites and subsequent infection of red blood cells to form piroplasms (Uielenberg, 2006). The life cycle of

Theileria includes secretion of infective sporozoites during tick feeding into the feeding site. Sporozoites then infect leukocytes and multiply by merogony, after which the merozoites are released, which invade the red blood cells thereby establishing the piroplasm stage (McKeever, 2009). During the next feeding cycle, larval or nymphal vector ticks ingest the piroplasms and the released parasites undergo syngamy in the tick gut, forming a zygote, the only diploid stage. The zygote divides into motile kinetes that infect the tick gut epithelial cells and migrate to the hemolymph and subsequently infect the salivary glands (McKeever, 2009). After moulting and commencement of feeding by the tick, sporogony results in the multiplication of sporozoites in the salivary gland acini before injection into the feeding site by nymphs or adult ticks (McKeever, 2009). The *Theileria* parasites can be classified into schizont “transforming” and “non-transforming” species. The non-transforming species are generally regarded as benign but still are able to cause disease as a result of anemia induced by the piroplasm stage (Sivakumar *et al*., 2014) (Figure. 3).

Babesia is vectored by ixodid ticks of mainly *Ixodes*, *Haemaphysalis, Boophilus, Rhipicephalus* and *Dermacentor* genera. During the life cycle of *Babesia*, sporozoites are injected into the host and directly infect the red blood cells. In the host, *Babesia* sporozoites develop into piroplasms inside infected erythrocytes

resulting into two sometimes four daughter cells that leave the host cells to infect other erythrocytes until the host dies or the host's immunity clears the parasites (Hunfield *et al*., 2008). The feeding tick ingests parasitized erythrocytes, inside the tick; *Babesia* zygotes multiply as "vermicules" which invade many of the tick's organs including the ovaries; *Babesia* species are readily passed to the next generation of ticks in the egg. *Theileria* parasites are only transmitted transstadially whereas *Babesia* species can be passed transovarially through several generations, although this varies with the species of *Babesia* and the tick species (Hunfield *et al*., 2008) (Figure. 2).

3. 2. Pathogenesis and disease syndromes

Piroplasmosis affects many domestic and wild animals but the most important disease syndromes are equine and bovine piroplasmosis. Equine piroplasmosis (EP) caused by *B. caballi* and *Th. equi* is considered an economically significant tick borne disease in horses. This disease is widely distributed in the world including most of the tropical and subtropical areas (Munkhjagal *et al*., 2013). Economic losses associated with EP are significant and include the cost of treatment, especially in acutely infected horses, abortions, loss of performance, death, and

restrictions in meeting international requirements related to exportation or participation in equestrian sporting events (Kerber *et al*., 1999). *B. caballi* and *Th. equi* share many of the same tick vectors, are usually present in the same geographic regions, and frequently co-infect horses. *B. caballi* and *Th. equi* are transmitted by more than 15 species of the tick genera *Dermacentor, Hyalomma*, and *Rhipicephalus* (Friedhoff *et al*., 1990; Stiller *et al*., 2002). Once infected with *Th. equi*, horses remain carriers for life, regardless of whether clinical signs resolve naturally or with drug treatment (Rothschild, 2013). Infection with *B. caballi* has been said to be self–limiting, lasting up to 4 years after infection. However, many horses that recover from *B. caballi* infection later relapse suggesting a temporary state in which organisms cannot be detected despite possible lifelong infection (Rothschild, 2013). Carrier horses represent a potential reservoir for maintaining and disseminating of parasites to ticks and horses. No evidence exists of transmission of EP by hematophagous dipterans such as tabanids (horse flies) or *Stomoxys calcitrans* (stable fly) (Rothschild, 2013). Chronically infected pregnant mares are at a risk of transplacental transmission, resulting in abortion, stillbirths, or birth of a sick foal that typically succumbs to the disease. *Th. equi* is most frequently involved in these transplacental transmission cases (De Waal, 1992; Lewis *et al*., 1999; Phipps and Otter, 2004). Transmission of parasites in semen has not been documented;

however, this may be possible if blood contamination of semen occurs (Metcalf, 2001). Few countries in the world are free from autochthonous infections with EP it is considered that only 10% of horses globally inhabit regions that are free of EP. Many areas currently free of EP are climatically suitable for appropriate tick vectors or already possess competent tick vectors (De Waal, 1992). The worldwide prevalence of EP is consistent with the worldwide distribution of its competent vectors (Rothschild, 2013). Because *Th. equi* and *B. caballi* share the same vectors in a given region, they are closely associated and endemic in tropical and sub-tropical climates. In most regions however, infections with *Th. equi* are more common than *B. caballi* (De Waal, 1992; Bruning, 1996). Outbreaks of overt clinical disease are uncommon in endemic areas, with the exception of India, despite the endemic status of implicated parasites (Kumar *et al*., 2003). In Africa, Morocco, Republic of South Africa, Madagascar and all other parts of the African continent are considered endemic for *Th. equi* and *B. caballi* (Zweygarth *et al*., 1997; De Waal *et al*., 1988; Rhalem *et al*., 2001). *Th. equi* and *B. caballi* are wide spread in the horse populations in Mongolia, China, and many parts of Asia (Avarzed *et al*., 1997; Battsetseg *et al*., $2002_{a,b}$; Boldbaatar *et al*., 2005). High infection rates are similarly reported in Korea (Friedhoff and Soule, 1996). High infection rates have also been

reported in the Middle East where *Th. equi* is said to have a higher sero-prevalence (Short *et al.,* 2012).

Incubation period of equine piroplasmosis associated with *Th. equi* is 12 to 19 days and approximately 10 to 30 days when caused by *B. caballi. Th. equi* tends to cause more severe disease than *B. caballi* (Rothschild, 2013). Equine piroplasmosis usually presents in acute, sub-acute or chronic forms. The peracute form is rare with only clinical observation being moribund and dead horses (Rothschild, 2013). The acute form is the most common and is characterized by fever exceeding 40ºC reduced appetite and malaise, elevated respiratory and pulse rates, congestion of mucous membranes, production of dark red urine, fecal balls that are smaller and drier than normal. Affected animals appear unthrifty; anemic and/or icteric (Rothschild, 2013). The sub-acute form is similar to the acute form but maybe accompanied by weight loss and intermittent fever, mucous membranes vary from pale pink to pink, or pale yellow to bright yellow; petechiae and/or ecchymoses may also be visible on the mucous membranes (Rothschild, 2013). Normal bowel movements may be slightly depressed and the animal may show mild signs of colic. The chronic cases usually present non specific clinical signs such as mild in-appetence, poor performance and drop in body mass (Rothschild, 2013).

Bovine piroplasmosis (BP) is caused by tick transmitted apicomplexan protozoan parasites of the *Babesia* and *Theileria* genera. BP causes direct economic losses such as mortality, reduction in milk and meat yield and indirectly through tick control measures (Gharbi *et al*., 2011). These infections are characterized by anemia, icterus, hemoglobinuria and death (Wagner *et al*., 2002; Vial and Gorenflot, 2006). Bovine babesiosis is caused by multiple *Babesia* species: *B. bigemina, B. bovis, B. divergens, B. major* and *B. ovata*. Two species; *B. bovis* and *B. bigemina* have a considerable impact on cattle health and productivity in tropical and subtropical countries (Iseki *et al*., 2010). Bovine *Theileria* species cause severe and mild infections in their hosts (Safieldin *et al*., 2011). Two of the *Theileria* species, *Th. parva* and *Th. annulata* cause lymphoproliferative disease with high mortality and morbidity in cattle known as ECF and tropical theileriosis (TT) respectively (Adham *et al*., 2009). In addition, benign forms of theileriosis are caused by *Th. mutans*, *Th. velifera,* and *Th, taurotragi*, which are mainly located in Africa, whereas parasites of the *Th. sergenti-buffeli-orientalis* group, often referred to as the *Th. orientalis* group, occurs worldwide (Gubbels *et al*., 1999).

Babesia and *Theileria* species are transmitted by ticks which become infected when they ingest parasites in the blood of infected cattle. The major vectors for *B. bigemina* are *Rhipicephalus microplus* and *R. annulatus*. The major vectors for *B.*

bovis are *R. microplus* and *R. annulatus*. Both *Th. parva* and *Th. annulata* are transmitted by ticks, the most important vector for *Th. parva* is *R. appendiculatus*, and *Th. annulata* is transmitted by *Hyalomma* spp. (Makala *et al*., 2003; Mans *et al*., 2014). The epidemiology of bovine piroplasmosis considers parasite and vector distribution, mortality and morbidity of disease outbreaks, disease outbreak risk assessment and disease control measures, socio-economic factors, climate change, host resistance and susceptibility (Gachohi *et al*., 2012). While many of these factors may be addressed by clinical differential diagnosis, vector mapping, vaccination strategies, and control policy, serological and molecular diagnosis remain central for confirmation of the parasite identity, distribution, disease surveillance, assessment of the vaccine and as a tool for informative control policies (Bakheit *et al*., 2004).

B. bovis is much more virulent than *B. bigemina*, with *B. bigemina*, pathogenic effects relate more directly to erythrocyte destruction. With the virulent strains of *B. bovis*, a hypotensive shock syndrome, combined with generalized non specific inflammation, coagulation disturbances and eythrocytic stages in capillaries contribute to the pathogenesis. The acute disease generally runs a course of about 1 week. The signs include fever (41ºC) which persists throughout and is accompanied by inappetence, increased respiratory rate, muscle tremors, jaundice, anemia and weight loss; hemoglobinemia and hemoglobinuria occur in the final stages. CNS

involvement can occur with *B. bovis* infections. Either constipation or diarrhea may be present. Late term pregnant cows may abort, and temporary infertility due to transient fever may be seen in bulls (Morrison, 2015).

In theileriosis, clinical signs vary according to the level of challenge, and they range from in-apparent to mild to severe and fatal. Typically fever occurs 7 to 10 days after parasites are introduced by feeding ticks and continues throughout the course of infection and may be 41ºC or more. Lymph node swelling becomes pronounced and generalized. Anorexia develops, the animal rapidly loses condition; lacrimation and nasal discharge may occur, terminally, dyspnea is common. Just before death, a sudden drop in temperature is usual and pulmonary exudates pours from the nostrils. Death usually occurs 18-24 days after infection. Anemia is not a major diagnostic sign as in babesiosis because there is minimal division of the parasites in the RBCs, and thus no massive RBC destruction (Morrison, 2015).

3. 3. Diagnosis and treatment

Diagnosis of piroplasmosis is through clinical signs, necropsy, and laboratory diagnosis. For laboratory diagnosis, thick and thin smears of blood from live animals, organ smears from necropsy and serum samples should be collected. Laboratory diagnostic procedures include; identification of the agent by microscopic examination and molecular based techniques based on species specific PCR, serological tests which include; indirect fluorescent antibody test (IFAT), ELISA and complement fixation test (CFT) (Morrison, 2015).

For the treatment of EP infections, the efficacy of drug treatment is highly variable and close monitoring of horses undergoing treatment is necessary to ensure success. Intramuscular administration of imidocarbdipropionate is considered the most effective treatment. For *B. caballi* diminazene aceturate is effective for treatment of acute disease when administered intramuscularly . *Th. equi* is resistant to most therapeutic agents, thus sterilization or even temporary clearance may not be accomplished even with persistent and repeated efforts (Rothschild, 2013). Anti-theilerial drugs have been used with variable success in the treatment of clinical signs of *Th. equi* infection but cannot completely eliminate the parasite (Kuttler *et al*., 1987).

The only drugs in common use for bovine babesiosis treatment are diminazene aceturate and imidocarbdipropionate. For treating cattle, diminazene is given intramuscularly at 3.5mg/kg, imidocarb is given at 1.2mg/kg subcutaneously. At 3mg/kg, imidocarb provides protection from babesiosis for 4 weeks and will also eliminate *B. bovis* and *B. bigemina* from carrier animals.

Treatment with parvaquone and its derivative buparvaquone is highly effective when administered in the early stages of clinical disease but is less effective in the advanced stages, in which there is extensive destruction of the lymphoid and hematopoietic tissues (Morrison, 2015).

4. Summary

Vector borne hemoprotozoan parasites continue to cause devastating diseases in developing countries crippling their agricultural and food security mechanisms thus threatening their livelihoods. Prevalence studies of these diseases will provide information to decision makers on the extent of the problem and thus provide insights into finding the mitigation measures for these problems. This study considers PCR based molecular epidemiological studies on two of the most devastating animal parasitic diseases in two developing countries with two

contrasting livestock systems in different continents and climatic zones; one in tropical Sub-Saharan Africa and another in temperate Central Asia.

5. Objective of this study

The study was aimed at:

1) Establishing PCR based prevalence of animal African trypanosomosis and selected piroplasm parasites of cattle and goats in Zambia.
2) Establishing PCR based prevalence of animal trypanosomosis and piroplasmosis among domestic animals in Mongolia.

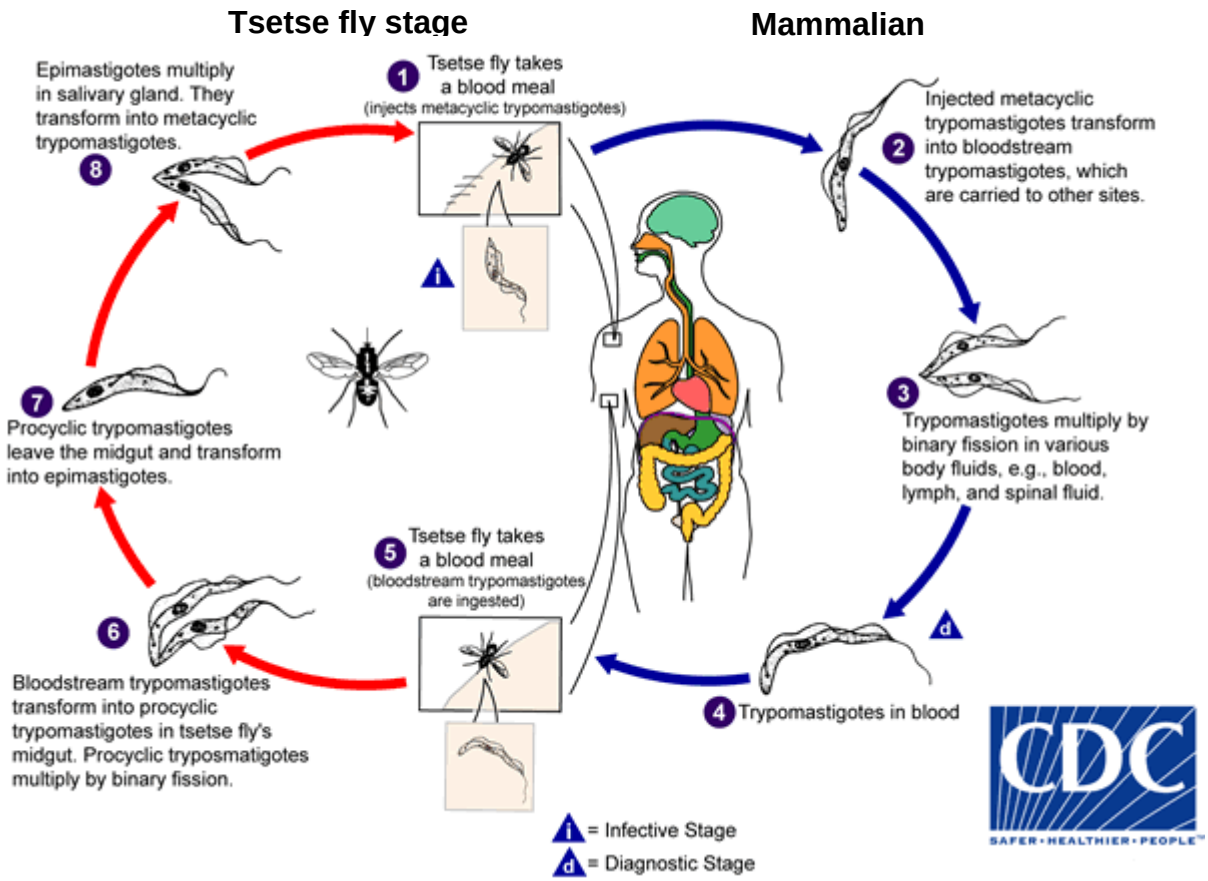

Figure 1: Generalized life cycle of tsetse transmitted trypanosomes (CDC)

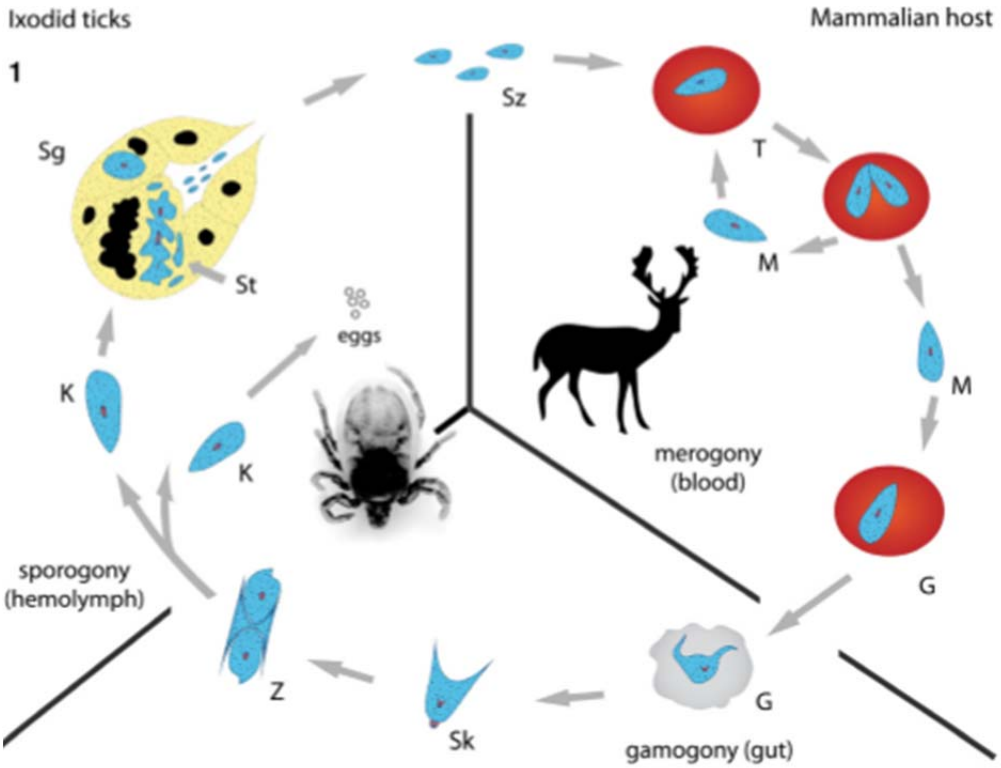

Figure 2: Generalized life cycle of *Babesia* species:

Sporozoites (Sz). Trophozoites (T). Gametocytes (G).‘‘Strahlenkorper” (Sk). Zygote (Z). Kinete (K). Eggs (E). Salivary glands (Sg). Sporoblast (St). (Hunfeld *et al*., 2008).

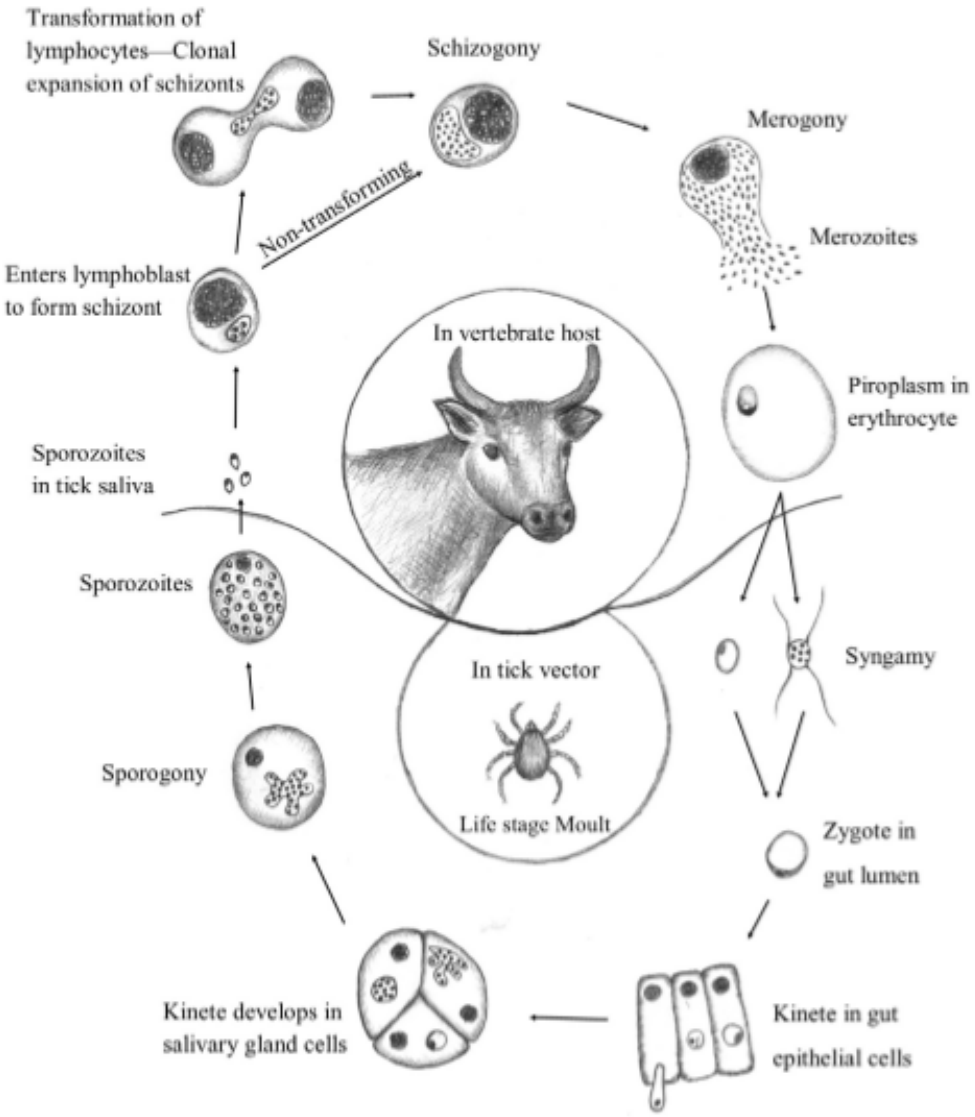

Figure 3: Generalized life cycle of *Theileria* species (Mans *et al*., 2014)

Chapter 1

A PCR based survey of animal African trypanosomosis and selected piroplasm parasites of cattle and goats in Zambia

1.1. Introduction

Amongst the five *Theileria* species and subspecies that are known to exist in Zambia, the most economically important are *Th. parva parva* and *Th. parva lawrencei* (Makala, *et al*., 2003). *B. bovis* and *B. bigemina* are recognized as being of economic importance to the Zambian cattle sector. *B. bigemina* is shown to be wide spread in Zambia, whereas *B. bovis* is mostly restricted to the eastern and north eastern parts of the country (Jongejan *et al*., 1988). Infections caused by *B. bigemina* are more extensive than those caused by *B. bovis* and this may be attributed to a wider vector range of *B. bigemina* (Makala *et al*., 2003).

In Zambia, trypanosomosis in cattle has more of an endemic nature and is characterized by high morbidity and rather low mortality. However the devastating effects of trypanosomosis on livestock production are very obvious in areas where susceptible livestock are introduced in a tsetse infested zone or vice versa. A good example being the plateau of eastern Zambia where devastating livestock

trypanosomosis epidemics occurred after game and tsetse spread south and east from the Luangwa valley onto the plateau (Van den Bossche and Delespaux, 2011). The reason for the epidemic nature of trypanosomosis is attributed to the diversity in trypanosome populations (Masumu *et al*., 2009). As a result of drug use and high mortality rates in untreated animals infected with virulent strains, the likelihood that virulent strains persist in susceptible livestock of the domestic transmission cycle is low. On the low plateau of eastern Zambia for example, only 20% of the trypanosome strains isolated from cattle had a virulent phenotype (Masumu *et al*., 2006). Moreover, laboratory experiments indicated that the presence of an infection with low virulent trypanosome strains confers protection against the deleterious effect of an infection with a virulent strain (Masumu *et al*., 2009).

This study aims at assessing the prevalence of trypanosomes and selected piroplasms of great importance to livestock farming in the areas of Chama, Monze and Mumbwa in Zambia. The present study will generate information that will give a glimpse of the nature of behavior and diseases associated with these parasites among the cattle and goats in these areas.

1. 2.Materials and methods

1. 2. 1. Study area and sample size

Sample collection was conducted in the Chama (Muchinga province), Mumbwa (Central province) and Monze (Southern province) districts in the dry season (May to October) of 2010 (Fig. 4). A total of 472 head of cattle from Chama (n=292), Mumbwa (n=96) and Monze (n=84) and 53 goats (all from Chama) were examined for the presence of animal African trypanosomes (*Trypanozoon*, *T. congolense*, and *T. vivax*), *B. bigemina* and *Th. parva* using PCRs.

1. 2. 2. Sample collection and DNA preparation

Blood samples were collected from cattle and goats whose owners consented to participate in the survey. Permission was obtained according to the standards of animal experimentation in Obihiro University of Agriculture and Veterinary Medicine (Approval No. 28-47), and department of Veterinary and Livestock Development, Zambia (Approval No. 212CO100011192). Approximately 5 ml of blood was drawn from the jugular vein of each animal using vacutainer tubes with EDTA-2Na (Terumo Co., Tokyo, Japan) and loaded into capillary tubes and the packed cell volume (PCV) values determined. These procedures were conducted in the field at the sampling site. In addition, the total DNA of each blood sample (1 ml) was isolated

using a DNA isolation kit for mammalian blood (Roche Diagnostics Mannheim, Germany) according to the manufacturer's suggested protocol. The resultant DNA was stored at -30ºC until use.

1. 2. 3. Detection of hemoparasites by PCR

This study used 2 different PCR techniques for trypanosome detection and species identification: (i) a KIN-PCR, which amplifies internal transcribed spacer 1 (ITS1), was used for *Trypanozoon* (*T. brucei brucei*, *T. b. rhodesiense*, *T. b. gambiense*, *T. evansi* and *T. equiperdum*) and *T. congolense*; and (ii) a TviCatL-PCR, which amplifies the highly conserved Cathepsin L-like gene among *T. vivax* isolates (McLaughlin *et al*., 1996; Cortez *et al*., 2009). A PCR that amplifies apical membrane antigen 1 (AMA 1) was used for *B. bigemina,* while a p104 gene PCR, which uses IL755 and IL3231 primers to amplify the p104 gene was used for *Th. parva* (Table 1) (Sivakumar *et al*., 2012_a; Skilton *et al*., 2002). All of the parasites were detected using single-step PCR methods. For the trypanosomes, the PCR reactions included 1µl of 10x reaction buffer, 0.3 µl 50mM magnesium chloride, 250 µM dNTPs and 0.1 µl of *Taq* DNA polymerase (all from Thermo Fisher Scientific Inc., Waltham, MA, USA), 1 µl of each of the forward and reverse primers and 5.1 µl of double distilled water (DDW); 0.5 µl of the DNA sample was added to the individual

PCR mixtures. PCRs were conducted on a Veriti™ Thermal cycler (Thermo Fisher Scientific). The PCR conditions have been described previously (Desquesnes *et al*., 2001). For the piroplasms, the PCR reactions included 1 µl of 10x reaction buffer, 200 µM dNTPs and 0.1 µl of DNA polymerase (AmpliTaq gold®) (all from Thermo Fisher Scientific), 0.5 µl each of the forward and reverse primers and 5.9 µl of DDW. One microliter of the DNA sample was added to the individual PCR mixtures. The PCRs were conducted on a Veriti™ thermal cycler. The PCR conditions used for *Babesia* and *Theileria* species have been described previously (Sivakumar *et al*., 2012_a).

1. 2. 4. Statistical analysis

The 95% confidence intervals determined using the Creative Research Systems software program (accessed at www.surveysystem.com). Chi-square and Fisher's exact tests were performed and the strengths of their associations were tested and their *p*-values determined using the GraphPadPrism software program (GraphPad Software Inc., La Jolla, CA, USA). Associations between the hematocrit values and the various parasitic infections were analyzed using a one-way analysis of variance, the strengths of the associations were tested using Tukey's mean difference and *p*-values determined using the GraphPadPrism software program.

1. 3. Results

1. 3. 1. Parasite prevalence by species

In the present study, 200 of the 525 (38.1%) samples were found to be positive for at least one hemoprotozoan parasite. *T. congolense*, *Trypanozoon*, *T. vivax*, *B. bigemina* and *Th. parva* were identified in cattle and goat samples collected from the three locations investigated in this study (Table 2). *T. vivax,* which was detected in 19.8% of the samples, was the most common parasite. The prevalence of *T. vivax* in goats (37.7%) was significantly higher than that in cattle (17.8%) (Table 3). The second most common parasite was *B. bigemina,* which was present in 16.1% of cattle. This was followed by *T. congolense* (1.9% and 5.7% in cattle and goats, respectively), then *Trypanozoon* (1.0% in cattle only) and lastly *Th. parva* (0.6% in cattle only). The only significant infection between species was *T. vivax* (Table 3). Twelve percent of the infected samples were mixed infections (Table 4). The most frequent mixed infections were *T. vivax/B. bigemina* (7.0%), followed by *T. congolense/T. vivax* (2.5%), then *T. congolense/B. bigemina* (1.0%) and *B. bigemina/Th. parva* (1.0%) and lastly *T. congolense/T. vivax/B. bigemina* (0.6% in cattle) (Table 4).

1. 3. 2. The prevalence rates according to location

Accordingly no trypanosome-positive samples were found in the samples from Monze and all the *Th. parva* positive samples were from Monze. The area with the highest prevalence in cattle was Mumbwa (69.8%) followed by Monze (44.0%) and Chama (25.0%) (Table 2). Although there was no significant difference in the *Trypanozoon* and *T. congolense* prevalence, there was a significant difference in the prevalence of *T. vivax, Th. parva* and *B. bigemina* positivity, with Mumbwa having a significantly higher prevalence of *T. vivax* (39.6%), Monze having the only *Th. parva* positive samples, and a significantly higher prevalence of *B. bigemina* (40.5%) (Table 2). Chama was the only area in which goat samples were collected; the rest only had cattle samples. *B. bigemina* was the only parasite found across all three areas, with the highest prevalence in Monze (40.5%) followed by Mumbwa (24.0%) and Chama (6.5%) (Table 2). In Chama and Mumbwa, *T*. *vivax* was the most prevalent trypanosome in cattle (15.8% and 39.6%, respectively) followed by *T. congolense* (1.4% and 5.2%) and finally *Trypanozoon* (1.4% and 1.0%) (Table 2).

1. 3. 3. Prevalence according to age and sex

There were no significant differences in the prevalence according to age and sex in cattle and goats; however, male cattle (39.5%) had a slightly higher

prevalence than female cattle (36.3%). In contrast female goats (62.5%) had a higher prevalence than male goats (38.5%). Adult animals (>2 years of age in cattle and >0.5 years of age in goats) of both species had a higher prevalence than juvenile animals (42.1% vs 26.8% in cattle, and 58.1% vs 54.5% in goats).

1. 3. 4. The relationship between PCV and infection

According to the hematocrit values, there was no significant difference between the positive and negative samples from goats (data not shown). In cattle, however, there was a significant difference between the mixed infections, the single infections and negative samples, with the mixed infection samples having a significantly lower hematocrit values (Figure. 5).

1. 4. Discussion

In this study, the prevalence of AAT and piroplasmosis in the three locations (Chama, Monze and Mumbwa) in Zambia was determined using PCR based methods. PCR based screening results suggested that *T. vivax* was the most prevalent parasite (Table 2). This can be attributed to the fact that this parasite, (unlike other tsetse-transmitted trypanosomes) can also be transmitted mechanically by other biting flies as has been shown in other studies outside the tsetse belts, especially in South America (Desquesnes and Dia, 2003; Kone *et al*., 2011). The second most common parasite was *B. bigemina,* which was also the only parasite that was found in all three locations (Table 2). This is in line with the results of earlier studies which reported that babesiosis caused by *B. bigemina* is widespread throughout Zambia (Jongejan *et al*., 1988; Makala *et al*., 2003). Widely distributed babesiosis caused by *B. bigemina* is therefore suggested to be a potential threat to cattle in the country. This could be attributed to the diversity of *B. bigemina* vectors, which include *Boophilus microplus*, *Bo. decoloratus* and *Rhipicephalus evartsi* (Makala *et al*., 2003) and the fact that the principle host tick, *Bo. decoloratus,* is also widely distributed throughout the country (Pegram *et al*., 1986). In addition, although babesiosis is recognized as an economically important disease, (unlike theileriosis and trypanosomosis) its control and eradication have not been a priority maybe

because of its chronic nature as compared to theileriosis which is acute and has dramatic effects. The control of babesiosisis mainly achieved through the treatment of the sick animals; however, it should be noted that tick control through acaricide use has also helped, even though it has mainly targeted theileriosis (Makala *et al*., 2003). *Th. parva*, was the least prevalent species (<1%), this prevalence is in agreement with the International Fund for Agricultural Development (IFAD) Zambia Country Programme evaluation report of 2014 (IFAD report, 2014). This can be attributable to the various efforts in tick control and vaccination against ECF that have been carried out by the Government of the Republic of Zambia and other donor agencies (Makala *et al*., 2003; Mubamba *et al*., 2011; IFAD report, 2014).The samples were collected from Chama, Monze and Mumbwa. Accordingly, no trypanosome positive samples were found from Monze (Table 2); this could be attributed to the eradication of tsetse flies from the Lake Kariba environs (Figure. 4).

Twelve percent of the positive samples were mixed infections (Table 4); there was no significant difference among the host species. However, in cattle there was a relationship between the hematocrit values and the presence of mixed infection (Figure. 5). Although there was no significance in the PCV values of the single infection and negative samples, there was a significant decrease in the PCV values of the mixed infection samples. The result suggested that mixed infection in cattle is

responsible for more cases of clinical disease. The development of anemia is one of the most common signs of disease caused by hemoparasites in susceptible cattle. The level of anemia or the PCV usually gives a reliable indication of the disease status and the productive performance of an infected animal (Trail *et al*., 1990; Trail *et al*., 1993). Determining the PCV of the negative animals helps to form a baseline for comparison of PCVs among the sampled animals because PCV is affected by confounding factors other than parasitic infections (Van den Bossche and Rowlands, 2001). While goats had a higher prevalence than cattle (Table 3), there was no significant difference in the PCV values of the infected and non-infected goats. This could be because they are more tolerant to these parasites than the cattle and because trypanosomosis in goats commonly follows a subclinical path, usually with low but persistent parasitemia (Gutierrez *et al*., 2006; Simukoko *et al*., $2007_{a,b}$). My results showed that goats can be an important reservoir for trypanosomes and treatment of goats when adopted can lead to reduction in trypanosomosis incidences in Zambia and other African countries.

In conclusion, I revealed the presence of high prevalence of hemoprotozoan parasites in cattle and goats in Zambia. This result suggested that the control of these parasite and vectors is important for the improvement of the agricultural sector in Zambia.

Table 1. The PCR primer sets

Target gene	Primer	Sequence (5'→ 3')	Specificity	Size (bp)	Reference
ITS1	KIN1	GCGTTCAAAGATTGGGCAAT	*T. congolense*	750**	McLaughlin *et al.*, 1996
	KIN2	CGCCCGAAAGTTCACC	*Trypanozoon*	540**	
CatL-like*	TviCatL1	GCCATCGCCAAGTACCTCGCCGA	*T. vivax*	177	Cortez *et al.,* 2009
	DTO 155	TTAAAGCTTCCACGAGTTCTTGATGATCCAGTA			
AMA-1	BI-AMA-F1	TACTGTGACGAGGACGGATC	*B. bigemina*	211	Sivakumar *et al.*, 2012_a
	BI-AMA-R1	CCTCAAAAGCAGATTCGAGT			
P104	IL3231	ATTTAAGGAACCTGACGTGACTGC	*Th. parva*	496	Skilton *et al.*, 2002
	IL755	TAAGATGCCGACTATTAATGACACC			

*_T. vivax_ cathepsin L-like cysteine protease

**KIN1 and KIN2 oligonucleotides are specific for both *T. congolense* (750bp band) and *Trypanozoon* (540bp band)

Table 2. The parasite prevalence at the three locations

Location	Host	N	P	%	*Trypanozoon*		*T. congolense*		*T. vivax*		*B. bigemina*		*Th. parva*	
					P	%	P	%	P	%	P	%	P	%
Chama	Cattle	292	73	25.0	4	1.4	4	1.4	46	15.8	19	6.5	0	0
	Goats	53	23	43.4	0	0	3	5.7	20	37.7	0	0	0	0
Monze	Cattle	84	37	44.0	0	0	0	0	0	0	34	40.5	3	3.6
Mumbwa	Cattle	96	67	69.8	1	1.0	5	5.2	38	39.6	23	24.0	0	0
Total		525	200	38.1	5	1.0	12	2.3	104	19.8	76	14.5	3	0.6
p-value					0.615		0.057		<0.0001*,[a]		<0.0001*, [b]		0.0004*, [c]	

*Statistically significant ($p<0.05$)

[a]The *T. vivax* infection rate was significantly higher in Mumbwa than in the other locations.

[b]The *B. bigemina* infection rate was significantly higher in Monze than in the other locations.

[c]*Th. parva* was only found in Monze.

N: Sample number, P: PCR positive sample number, %: PCR positive rate

Table 3. The Prevalence of parasites in each species

Species	N	P	%	*Trypanozoon*		*T. congolense*		*T. vivax*		*B. bigemina*		*Th. parva*	
				P	%	P	%	P	%	P	%	P	%
Cattle	472	177	37.5	5	1.1	9	1.9	84	17.8	76	16.1	3	0.6
Goats	53	23	43.4	0	0	3	5.7	20	37.7	0	0	0	0
Total	525	200	38.1	5	1.0	12	2.3	104	19.8	76	14.5	3	0.6
p-value				ND		0.111		0.025*, [a]		ND		ND	

*Statistically significant ($p<0.05$).

[a]The *T. vivax* infection rate was significantly higher in goats than in cattle

ND, not done; N, sample number; P, PCR positive number; %, PCR positive rate

Table 4. Mixed infections according to the species

Species	TP	MI	%	Tc/Tv		Tc/Bb		Tv/Bb		Bb/Tp		Tc/Tv/Bb	
				P	%	P	%	P	%	P	%	P	%
Cattle	177	22	12.4	3	1.7	2	1.1	14	7.9	2	1.1	1	0.6
Goats	23	2	8.7	2	8.7	0	0	0	0	0	0	0	0
Total	200	24	12.0	5	2.5	2	1.0	14	7.0	2	1.0	1	0.5

Tc/Tv, Tc/Bb, Tv/Bb, Bb/Tp and Tc/Tv/Bb indicate a mixed infection with the corresponding parasites.

TP, total number of PCR positive samples; MI, number of samples positive for mixed infection; P, PCR positive number for each parasite; %, PCR positive rate; Tc: *T. congolense*; Tv, *T. vivax*; Bb, *B. bigemina*; Tp, *Th. parva.*

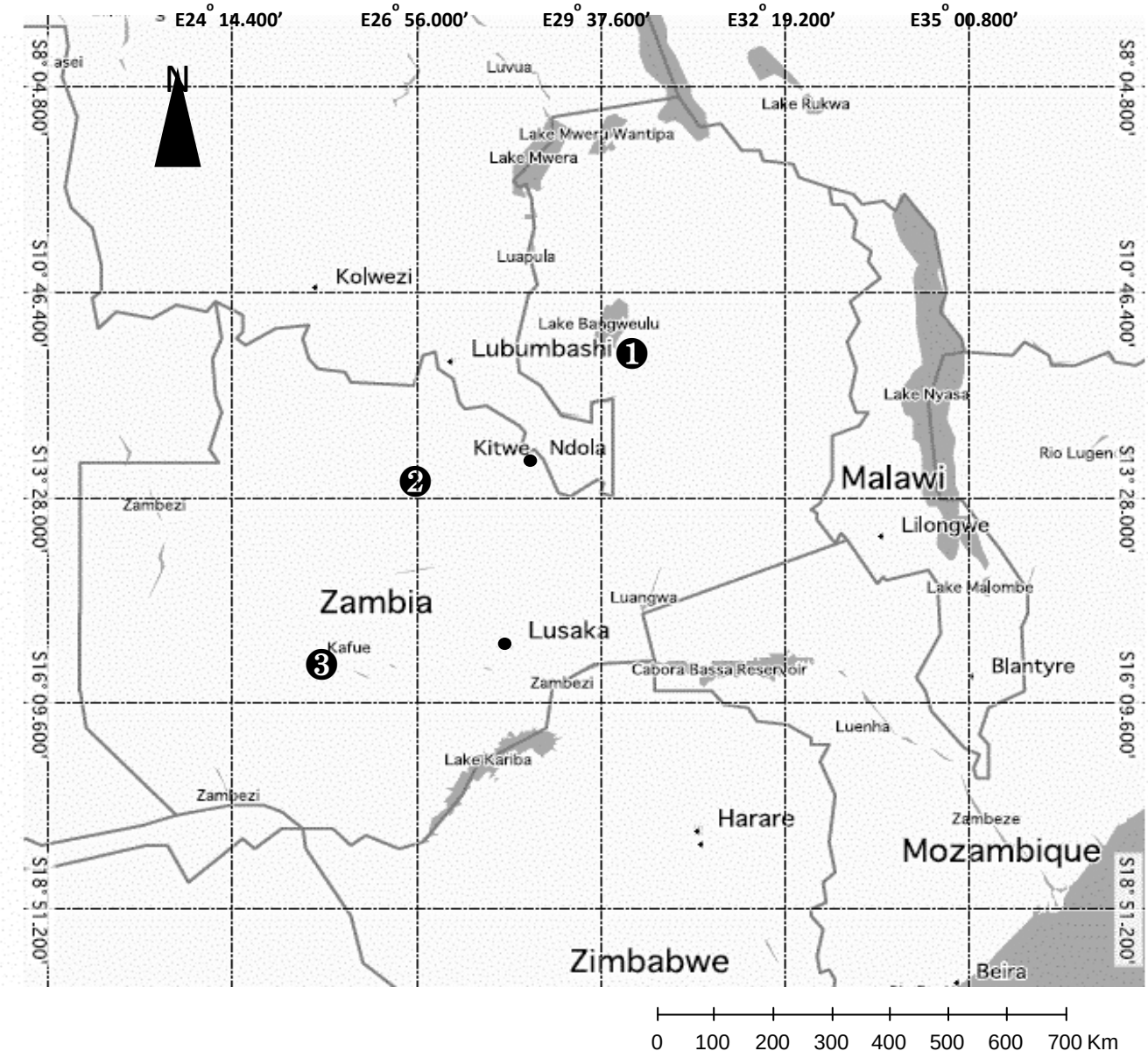

Fig 4: A map of Zambia showing the sampled areas, (http://www.freemap.jp)

❶Chama

❷Mumbwa

❸Monze

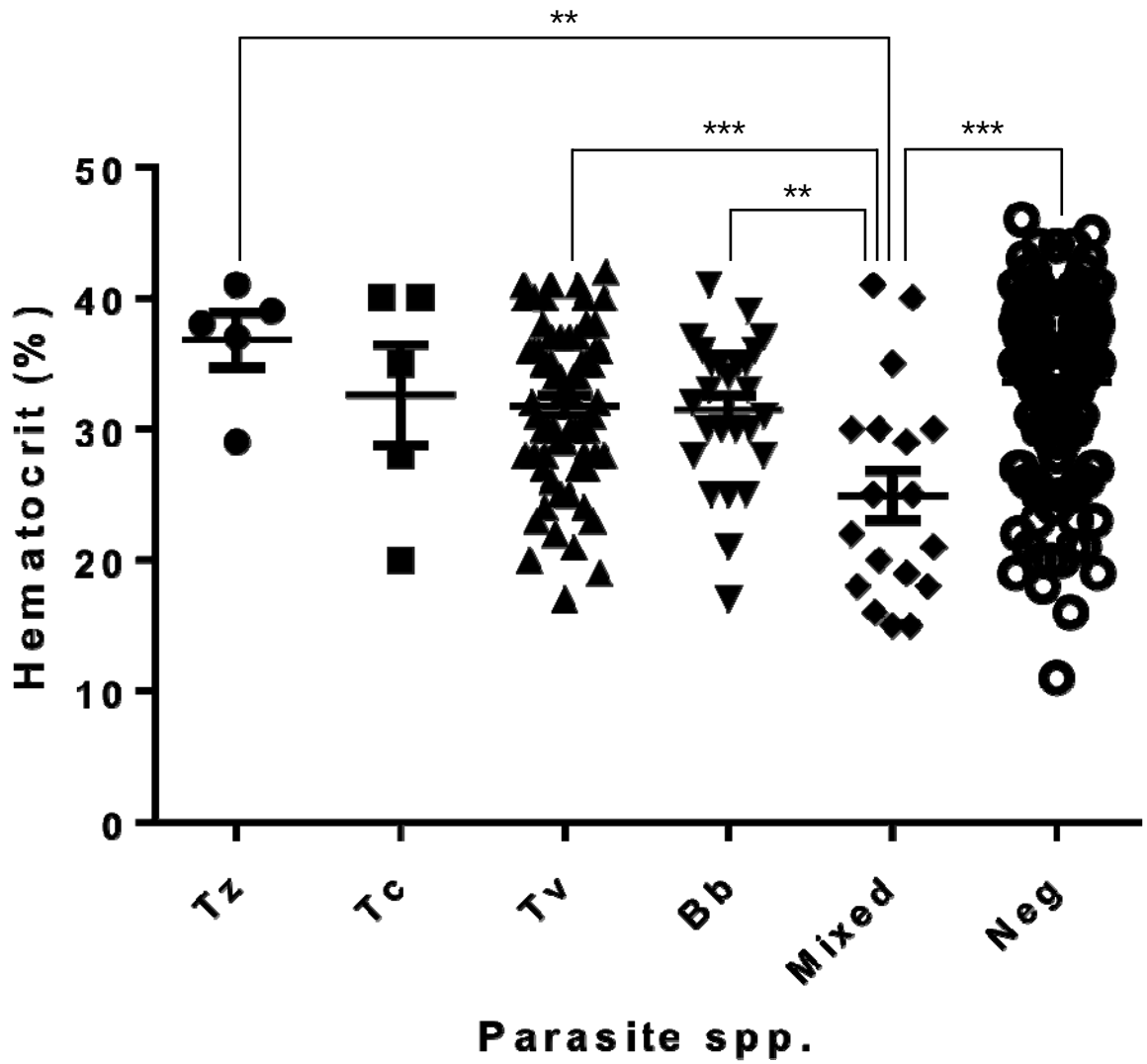

Fig 5: A graph showing the PCV values in cattle with the different parasite infections.

A comparison of the hematocrit values in cattle with mixed-infection, various parasitic infections and uninfected cattle. The bars and asterisks denote the levels of significance; (**, p<0.05; ***, p<0.0001).

Tz; *Trypanozoon,*Tc; *T. congolense*, Tv; *T. vivax*, Bb; *B. bigemina*, Mixed; mixed infections, Neg; negative for the screened parasites

Chapter 2

A PCR based survey of trypanosomosis and piroplasmosis among domestic animals in Mongolia

2.1. Introduction

Mongolia is the fifth largest country in Asia with a territory of 1.5 million km^2 and with one of the world's lowest population densities. The main pillar of the economy is the livestock sector based mainly on extensive livestock production with seasonal migration of mixed herds of sheep, goats, cattle, horses and camels between pastures (Clausen *et al*., 2003).

The livestock sector in Mongolia can be generally regarded as low-input and high risk, and the mentality of the herders is still geared towards increasing livestock numbers rather than intensification (World Bank, 2009). The Mongolian agricultural sector has been severely affected by various infectious diseases whose impact is currently a pertinent issue. Therefore understanding the epidemiology of these diseases is essential to avoid their devastating impacts on the industry (Altangerel *et al*., 2012).

Previous studies have shown the prevalence of equine piroplasmosis to be 51.2% (Munkhjargal *et al*., 2013) and bovine babesiosis 29% in Mongolia (Altangerel *et al*., 2012). However, there seems to be no reports regarding piroplasmosis prevalence in other domestic animal species. Previous reports have also shown the prevalence of trypanosomosis in Mongolia to be 6%-8% among equines (Clausen *et al*., 2003). However these reports never identified the causative species as it is difficult to distinguish between *T. evansi* and *T. equiperdum* using serological diagnostic techniques. On the other hand, *T. equiperdum* was isolated in the urethral tract of a horse showing dourine symptoms in Mongolia, characterized as a true *T. equiperdum* strain and named IVM-t1 strain (Suganuma *et al.,* 2016). Currently, there are no reports indicating trypanosomosis in other domestic animals in Mongolia. However, there have been reports of clinical symptoms of surra among domestic animals especially camels and horses in Bayan-olgiy and Hovd provinces by the local veterinarians.

This study therefore sought to find out the prevalence of trypanosomosis and piroplasmosis among various domestic animals in the two provinces of Bayan-olgiy and Hovd in Mongolia. This study will provide a baseline for further establishment of the prevalence of trypanosomosis and piroplasmosis among various domestic animals herded together under the extensive livestock system in Mongolia. This will

help in the establishment of solutions to mitigate the trypanosomosis and piroplasmosis infections in Mongolia.

2.2. Materials and methods

2.2.1. Study area and sample size

Sample collection was conducted in Bayan-olgiy and Hovd provinces in north western Mongolia in the summer (August) of 2014 (Fig. 6). A total of 1,058 blood samples were collected from various domestic animals as follows: 111 camels (5 from Bayan-olgiy and 106 from Hovd), 119 yaks (93 from Bayan-olgiy and 26 from Hovd), 255 goats (115 Bayan-olgiy and 140 Hovd), 258 sheep (119 Bayan-olgiy and 139 Hovd), 215 horses (105 Bayan-olgiy and 110 Hovd), 100 Cattle (17 Bayan-olgiy and 83 Hovd). These were then examined for trypanosomosis and babesiosis using PCR.

2.2.2. Sample collection and DNA preparation

Blood samples were collected from cattle, camels, yaks, horses, sheep and goats whose owners consented to participate in the survey (The standards of animal

experimentation in Obihiro University of Agriculture and Veterinary Medicine approval No. 28-45). Using vacutainer tubes with EDTA-2Na (Terumo Co., Tokyo, Japan), approximately 5ml of blood was drawn from the jugular vein of each animal, and in addition, the total DNA from each blood sample (1 ml) was isolated using phenol-chloroform DNA isolation protocol. The resultant DNA was stored at -30ºC until use.

2.2.3. Detection of trypanosomes and piroplasms by PCR

This study used KIN PCR technique for trypanosome detection and species identification (Table 1, in Chapter 1). For piroplasmosis, detection was by RLB primers that amplify the V-4 region of the 18S rRNA gene to detect *Theileria* and *Babesia* species giving 380 to 500 base pair products; RLB-F2: 5'-GACACAGGGAGGTAGTGACAAG-3' and RLB-R2: 5'-CTAAGAATTTCACCTCTGACAGT-3' (Georges *et al*., 2001). The parasites were detected using single-step PCR methods; the PCR reactions included 1µl of 10x reaction buffer, 0.3 µl 50mM magnesium chloride, 250 µM dNTPs and 0.1 µl of *Taq* DNA polymerase (all from Thermo Fisher Scientific Inc., MA,USA), 1µl of each of the forward and reverse primers and 5.1µl of DDW; 1µl of the DNA sample was added to the individual PCR mixtures. PCRs were conducted on a Veriti^{TM} Thermal cycler

(Thermo Fisher Scientific) under the following cycling conditions; initial denaturation step of 94ºC for 3 minutes followed by 35 cycles of 94ºC for 1 minute, annealing temperature of 60ºC for 1 minute and 72ºC for 1 minute and then final extension of 72ºCfor 7 minutes. The PCR conditions for piroplasmosis were carried out as described previously (Gubbels *et al*., 1999).

2.2.4. Statistical analysis

Chi-square and Fisher's exact tests were performed and the strengths of their associations were tested and their *p*-values determined using the GraphPadPrism software program (GraphPad Software Inc., La Jolla,CA, USA).

2.3. Results

2.3.1. Prevalence by species

In this study, 289 of the 1,058 sampled animals (27.3%) were found to be positive with at least one of the parasites. The prevalence of trypanosomosis was 21.3% (225 positive samples) and piroplasmosis 6.1% (64 positive samples all from horses). The highest prevalence of trypanosomosis was among the goats at 35.7%, followed by sheep at 26.4%, camels at 17.4%, horses at 15.8%, cattle at 7.0% and lastly yaks at 5.0%. The prevalence in small ruminants (sheep and goats) was significantly higher compared to the rest of the animals ($p<0.0001$) (small ruminants at 31% compared to others at 12.1%) (Table 5). Piroplasmosis was only found among the horses where the prevalence was 29.8%. Among the horses, the prevalence of piroplasmosis was significantly higher than that of trypanosomosis ($p<0.01$) and 11.2% of the total infections were mixed infections (Table 8).

2.3.2. Prevalence by sex

There was a significant difference in the prevalence of trypanosome infection between the sexes with males (25.6%) having a significantly higher prevalence than the females (19.8%) ($p<0.05$). All the animal species except cattle and yak which had only females being positive among the sampled animals, and camels which had a significantly higher female population among the sampled animals, had males with higher prevalence than females, with sheep having a significantly higher prevalence among the rams (37.5%) compared to the ewes (22.7%) ($p<0.05$) (Table 5). For the piroplasmosis, there was no significant difference in the prevalece though the mares had a higher prevalence than the stallions.

2.3.3. Prevalence by location

According to the 2 provinces, there was no significant difference in the prevalence rates in the trypanosome infections with Hovd having a higher prevalence rate at 22.4% than Bayan-olgiy at 19.8%. However sheep from Hovd province had a significantly higher prevalence rate (34.5%) than those from Bayan-olgiy (16.8%) ($p<0.01$) and the reverse was true for the horses with those from Bayan-olgiy having a significantly higher prevalence (29.5%) than those from Hovd

(2.7%) ($p<0.0001$) (Table 6). During the study, I found out that the sheep kept in Bayan-olgiy were mainly the Kazakh breed of sheep and when compared to the other breeds, the Kazakh sheep had a significantly lower prevalence of trypanosomosis at 17.3% versus 30.5% of the other breeds ($p<0.05$). For piroplasmosis, like in trypanosomosis, the horses from Bayan-olgiy (43.8%) had a significantly higher prevalence than those from Hovd (16.4%) ($p<0.0001$) (Table 8).

2.3.4. Prevalence by age

According to age there was a significant difference in the prevalence of trypanosomosis with adult animals having a significantly higher prevalence (22.1%) than young ones (9.0%) ($p<0.01$) (Table 7).

2.4. Discussion

This is the first study to report the prevalence of trypanosomosis among different species of domestic animals herded together in Mongolia. In this study, trypanosomosis in the 2 provinces was found among all the livestock species sampled (Table 5). This could be because of the extensive livestock system where all the animals are herded together, thus cross infection. It also could be because of the shortage of veterinary services and also the difficulty of organizing transport to the rural areas to provide these services (World Bank., 2009). This study also found that the prevalence among the small ruminants (sheep and goats) was significantly higher than the rest of the domestic animals. This is consistent with our previous study of African trypanosomosis in Zambia where a significantly higher prevalence among the goats was found (Chapter 1). This could be because the disease course in small ruminants is mainly sub-clinical and therefore not easily detected by the farmers for treatment (Gutierrez *et al*., 2006). It could also be because the general value of an individual small ruminant is not comparable to that of any of the large animals and so the attention paid to the small ruminants by the farmer is minimal compared to that paid to the large animals. This could mean that the small ruminants can easily act as reservoirs of trypanosomosis to the rest of the herd. Next to the small ruminants, camels (17.4%) and horses (15.8%) were found to have a higher

prevalence compared to the bovids (cattle 7.0% and yaks 5.0%) (Table 5). This could be because the horses and camels usually have little contact with the farmers or herdsmen as they are left to graze by themselves for many days whereas the bovids maintain daily contact with the farmer because of milking so infections in bovids are easily detected and dealt with. Horses could also have mixed trypanosome infections of Surra and Dourine. Dourine has previously been reported in Mongolia with a 6-8% prevalence rate (Clausen et al., 2003). *T. equiperdum* was also isolated from a horse with Dourine symptoms and characterized as true *T. equiperdum* strain (Suganuma *et al*., 2016). However, it is almost impossible to differentiate between *T. evansi* and *T. equiperdum* by PCR (Desquesnes *et al*., 2013).

Piroplasmosis was found only among the horses with a prevalence of 29% (Table 8). This agrees with previous reports, though they reported a higher prevalence of around 40% (Munkhjagal *et al*., 2013). This shows that equine piroplasmosis is a big problem in Mongolia and needs to be addressed seriously. This high prevalence could be due to the abundant presence of the vector ticks of equine piroplasmosis in Mongolia as previously reported (Byambaa *et al*., 1994).

According to sex, the males generally had a significantly higher prevalence rate of trypanosomosis than the females. This could be because the females are constantly in contact with the farmers and thus any slight discomfort can be detected especially during milking time and dealt with. On the contrary, male animals are usually detected when they have clinical disease or sometimes just found recumbent or dead. However, among the camels, yaks and cattle, the females had a higher prevalence. This could be attributed to the significantly higher numbers of female animals sampled compared to the males. Previous studies have shown that sex has an impact on the prevalence of equine piroplasmosis (Ruegg *et al*., 2007; Kouam *et al*. 2010). In this study, I found no significant difference in the prevalence between the sexes although females had a higher prevalence. This is in agreement with a previous study in Mongolia where no significant differences in the prevalence among the sexes was found but unlike this study, found that stallions had a higher prevalence (Munkhjagal *et al*., 2013).

According to age, the adults had a significantly higher trypanosomosis prevalence rate compared to the young animals. This could be due to the fact that very few young animals were among the sampled animals and actually for the sheep and goats no young animals were available for sampling. Also all the positive young animals were colts, all the other species' young were negative. This could be

because unlike the other domestic animals whose young ones are kept separately, the horses usually move with their young ones and so expose them to the same conditions where they can be parasitized. The other reason for the lower prevalence of the young animals could be the fact that compared to the adults, they produce less odor plumes due to their smaller body sizes and so less attractive to the vectors (Simukoko *et al*., 2007_a). With equine piroplasmosis, this study is in agreement with previous studies that found that age had an impact on the prevalence (Ruegg *et al*., 2007; Kouam *et al*. 2010). The infection in young horses was significantly higher than that in adult horses. This can be attributed to the relative higher resistance among the older horses compared to the younger ones. However, it also could be that fewer young horses were sampled.

According to the location, there was no significant difference regarding the prevalence in general though Hovd province had a slightly higher prevalence than Bayan-olgiy. However, considering the individual animal species, there was a significant difference in the prevalence among horses and sheep of the two provinces. Horses in Bayan-olgiy had a significantly higher prevalence of both trypanosomosis and piroplasmosis than those in Hovd province (Tables 5 and 8). This can be attributed to the differences in husbandry systems in the two areas, Bayan-olgiy is mainly high altitude hilly area and the livestock there are usually kept

in large herds of families, thus cross infection is more readily possible than in Hovd where horses are scattered in small grazing herds. The sheep in Bayan-olgiy had a significantly less prevalence of trypanosomosis than those of Hovd province. The fact that most of the sheep kept in Bayan-olgiy are Kazakh sheep breed could indicate a possible resistance of this breed to the trypanosome infections compared to the other breeds. However, there are no previous reports on this; but it is a point worth further investigation. It could also be due to the fact that livestock farmers in Bayan-olgiy keep large herds of animals therefore more commercial and thus take better care of their animals than those in Hovd who are small herders. The reports on animal husbandry practices in Mongolia indicate that farmers with small herds are more likely to neglect them than those with larger herds due to the relative economic importance (World Bank, 2009).

This study highlights the presence of trypanosomosis among many domestic animal species herded together in Mongolia. It also reveals the possible role of small ruminants as reservoirs of trypanosomosis. It furthermore shows that equine piroplasmosis is most cause for concern among all the other piroplasmoses in Bayan-olgiy and Hovd provinces in Mongolia.

Table 5. Trypanosomosis prevalence according to species and sex

Species	N	P	%	M			F		
				N	P	%	No.	P	%
Camel	111	19	17.4	36	5	13.9	75	14	18.7
Yak	119	6	5.0	3	0	0	116	6	7.6
Goat	255	91	35.7[a]	40	17	42.5	215	74	34.4
Sheep	258	68	26.4[a]	64	24	37.5[b]	194	44	22.7
Cattle	100	7	7.0	8	0	0	92	7	7.6
Horse	215	34	15.8	119	23	19.3	96	11	11.5
Total	1,058	225	21.3	270	69	25.6	788	156	19.8
p-value			<0.0001***		<0.05*				

N: number, P: PCR positive, %: PCR positive rate

[a]Positive rates among sheep and goats were significantly higher than other species $p<0.0001$

[b]Positive rates among rams were significantly higher than ewes, $p<0.05$

Statistically significant: *$p<0.05$, ***$p<0.0001$

Table 6. Trypanosomosis prevalence according to location

Location	Host	N	P	%
	Camel	5	0	0
	Yak	93	3	3.2
	Goat	115	36	31.3
Bayan-Olgiy	Sheep	119	20	16.8
	Cattle	17	0	0
	Horse	105	31	29.5[c]
	Total	**454**	**90**	**19.8**
	Camel	106	19	17.9
	Yak	26	3	11.5
	Goat	140	55	39.3
Hovd	Sheep	139	48	34.5[d]
	Cattle	83	7	8.4
	Horse	110	3	2.7
	Total	**604**	**135**	**22.4**
Grand Total		**1,058**	**225**	**21.3**
p-value				**0.59**

N: number, P: PCR positive, %: PCR positive rate

[c& d] Positive rates among horses in Bayan-olgiy were significantly higher than in Hovd province (p=0.0001[***]) and the reverse was true for sheep (p =0.002**)

Statistically significant: [**]*p*<0.01, [***]*p*<0.0001

Table 7. Trypanosomosis prevalence according to age

Species	N	P	%	Young			Adult		
				N	P	%	N	P	%
Camel	111	19	17.4	10	0	0	101	19	18.8
Yak	119	6	5	15	0	0	104	6	5.8
Goat	255	91	35.7	0	0	0	255	91	35.7
Sheep	258	68	26.4	0	0	0	258	68	26.4
Cattle	100	7	7	3	0	0	97	7	7.2
Horse	215	34	15.8	39	6	15.4	176	28	15.9
Total	1,058	225	21.3	67	6	9.0	991	219	22.1
p-value						0.009**			

N: number, P: PCR positive, %: PCR positive rate

Statistically significant: ** $p<0.01$

Table 8. Piroplasmosis and mixed infections in horses

		N	P	%	*p*-value	Mixed infections		
						Total[e]	Mixed	%
Location	Hovd	110	18	16.4	<0.0001***	21	1	4.8
	Bayan-olgiy	105	46	43.8		77	10	13
Sex	Male	119	32	26.9	0.37	55	4	7.3
	Female	96	32	33.3		43	7	16.3
Age	Adult	176	43	24.4	0.0008**	71	9	12.7
	young	39	21	53.8		27	2	7.4
	Total	215	64	29.8		98	11	11.2

N: number, P: PCR positive, %: PCR positive rate

[e]Total refers to total of trypanosomosis and piroplasmosis

Statistically significant: **$p<0.01$, ***$p<0.0001$

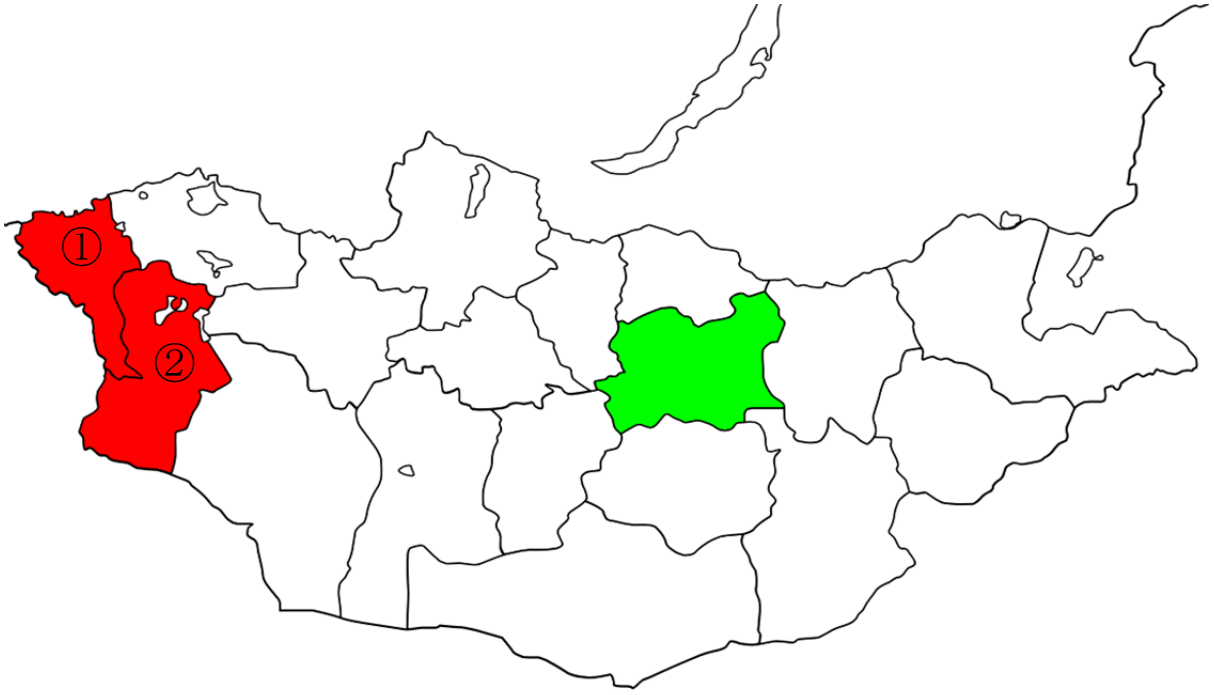

Fig 6: A map of Mongolia showing the sampled areas, (http://www.freemap.jp)

Red color area: Sampled area

Green color area: Capital city (Ulaanbaatar)

①Bayan-olgiy province

②Hovd province

General discussion

Vector borne hemo-protozoan diseases are very important livestock diseases in developing countries especially in sub-Saharan Africa and Central Asia. Trypanosomosis and piroplasmosis are a threat to the livestock industries of these countries. Vector control of these diseases (tsetse and other blood sucking flies in the case of trypanosomosis and ticks in case of piroplasmosis), is a sure way of controlling and even eradicating these diseases. However, these countries do not always have the budgetary resources to carry out these control measures. Epidemiological studies can help to effectively assess the status of these diseases and the information generated can always be used for efficient and cost effective planning for the eventual preventive and eradication measures.

In the study in Zambia, I detected several animal African trypanosomosis (AAT) agents namely; *T. congolense*, *T. vivax* and *Trypanozoon* in cattle and goats from 3 sampled districts of Chama, Mumbwa and Monze. *T. vivax* was the most prevalent of the three trypanosome species and this could be attributed to the fact that unlike the other AAT causative agents, it can also be transmitted by biting flies (Desquesnes and Dia, 2003; Kone *et al*., 2011). AAT prevalence was also found to be higher in goats than cattle; this was also the case with Mongolian trypanosomosis whose prevalence was significantly higher in small ruminants than in the other

domestic animals. This could be due to the resilience of small ruminants to trypanosomosis as compared to the larger animals; the disease in goats runs a sub-clinical path with a low but persistent parasitemia (Gutierrez *et al*., 2006; Simukoko *et al*., 2007$_{a,b}$). This situation compounded with the finding of the infected goats with normal PCV values shows that they can tolerate the disease well and therefore can act as reservoirs of trypanosomosis.

Both studies found that there was no significant difference in the infection with regards to sex; however, much as the same applied to the age in the Zambian study, there was a significant difference with regards to age in the trypanosomosis infections in Mongolian domestic animals with adults having a higher prevalence than young ones. The reason for the lower prevalence of the young animals could be the fact that compared to the adults, the produce less odor plumes due to their smaller body sizes and so less attractive to the vectors (Simukoko *et al*., 2007$_{a}$). The other reason could be that the young animals are less exposed to the vectors than the adults and thus lower infections. Also the fact that all the positive young animals were horses further lends credence to this notion because unlike the other domestic animals in Mongolia, horses usually stay with their young ones thus exposing them to the same condition as the adults.

This study also further confirmed that vector control is effective in the

eradication of trypanosomosis. This was evident in the Zambia study where Monze was found free of trypanosomosis due to the tsetse eradication that occurred around the lake Kariba area.

Regarding piroplasmosis, the study revealed that bovine babesiosis caused by *B. bigemina* was a serious problem to the Zambian cattle. This could be because *B. bigemina* has many vectors and its principle vector *Bo. decoloratus* is found all over the country (Makala *et al*., 2003). It could also be because unlike theileriosis, which is considered a priority disease (IFAD report, 2014), bovine babesiosis is neglected. This also manifested in the very low theileriosis prevalence found by the study. The study also found that equine piroplasmosis could be a big problem in Mongolia. This is in concordance with other studies carried out in Mongolia (Munkhjargal *et al*., 2013). This high prevalence could be due to the abundant presence of the vector ticks of equine piroplasmosis in Mongolia as previously reported (Byambaa *et al*., 1994). The study also showed that piroplasmosis affected the young horses significantly more than the adult horses. This agrees with previous studies that showed that age and sex can affect the prevalence of the infection (Ruegg *et al*., 2007; Kouam *et al*. 2010) although in my study, there was no significant difference in the prevalence among the sexes. There was a significant difference in the prevalence of piroplasmosis with regards to the sampled areas

where Bayan-olgiy had a higher prevalence than Hovd. This could be attributed to the differences in husbandry systems in the two areas, Bayan-olgiy is mainly high altitude hilly area and the livestock there are usually kept in large herds of families, thus cross infection is more readily possible than in Hovd where horses are scattered in small grazing herds.

Both studies found a considerable percentage of mixed trypanosomosis and piroplasmosis infections. In the Zambian study, the cattle with mixed infections had a significantly lower PCV compared to the single infections and the negative samples. This shows that mixed infections are the main cause of clinical disease.

Therefore vector borne protozoan disease are a considerable challenge to livestock sectors in both countries. Efficient prevention and control strategies of these will depend on the proper identification and quantification of these diseases.

General summary

Livestock plays a central role to the economies of developing countries and are usually a means of transitioning from poverty to wealth. Endemic parasites are a major source of economic loss in animal husbandry, especially in developing countries, but the extent of those losses has yet to be accurately specified, and knowledge about the economics of treatment of these diseases is inadequate, mostly because the damage functions, and in addition, the response functions to treatment are not yet very well known. The relative significance of most vector borne diseases in livestock is extremely difficult to quantify, because in most countries there is no formal reporting, poor diagnosis, and no surveillance or collated assessment of prevalence or economic impact. In sub-Saharan Africa and Central Asia, trypanosomosis and piroplasmosis are a very big threat to the livestock industry and epidemiological studies of these diseases can help to effectively assess their status for effective control. African livestock trypanosomes are threatening 48 million cattle in an area of 10 million square kilometers in 37 African countries; they cause disease syndromes responsible for major production losses including death in the absence of treatment. In Asia, trypanosomes in domestic animals mainly cause surra and dourine. *T. evansi* and *T. equiperdum* are continuously present eastwards, and throughout the whole of Asia. Piroplasmosis in domestic animals is mainly

caused by *Babesia* and *Theileria* species. These main agents of animal piroplasmosis are vectored by ticks thus making tick borne diseases a major cause of concern to livestock producers. Tick-borne protozoan diseases (e.g., theileriosis and babesiosis) pose important problems for the health and management of domestic cattle in developing countries. Piroplasmosis affects many domestic and wild animals but the most important disease syndromes are equine and bovine piroplasmosis. Vector borne hemoprotozoan parasites continue to cause devastating diseases in developing countries crippling their agricultural and food security mechanisms thus threatening their livelihoods. Prevalence studies of these diseases will provide information to decision makers on the extent of the problem and thus provide insights into finding the mitigation measures for these problems. This study considers PCR based molecular epidemiological studies on two of the most devastating animal parasitic diseases in two developing countries with two contrasting livestock systems in different continents and climatic zones; one in tropical Sub-Saharan Africa and another in temperate Central Asia.

In the Chapter 1, I examined the prevalence of trypanosomosis and selected piroplasms in cattle and goats in Zambia. The livestock sector in Zambia is characterized by small holder farmers with basically low input and low output. Productivity per animal is of minor importance and emphasis is put on the number of

animals. Livestock especially cattle are considered a symbol of family wealth. The animals are fed by grazing on open pastures and crop residues. In this study, 38.1% of all the animals sampled were positive for at least one of the parasites. This shows that these diseases are very important to the livestock sector and if not addressed they can lead to huge economic losses. The goats had a significantly higher prevalence of trypanosomosis compared to the cattle despite a relatively normal PCV ($p<0.05$). This shows that despite the infection with trypanosomosis, goats rarely show clinical disease or come down with mild symptoms that do not warrant the attention of the farmers. This then marks them out as reservoirs of trypanosomosis since they are usually left untreated. Bovine babesiosis was found to be in all the 3 sampled areas making it the most wide spread disease. In Zambia, farmers pay more attention to theileriosis because of its dramatic and acute nature and forget about babesiosis which is usually chronic and debilitating. This study shows that babesiosis is wide spread and also needs attention. According to the hematocrit values, the packed cell volume (PCV) (%) among the cattle with mixed infections was significantly lower than that of the other cattle. The presence of multiple parasite species and mixed infections among the Zambian cattle and goat populations is of both clinical and economic importance to livestock farming. The absence of trypanosomosis among the samples from Monze can be attributed to

tsetse eradication efforts that took place around Lake Kariba. This shows that the prevention and control of the vectors of these parasitic diseases can have a significant impact on the disease status, which can translate directly into the improvement of the livestock sector in Zambia.

In Chapter 2, the study focused on the prevalence of trypanosomosis and piroplasmosis in several domestic animal species in Mongolia. The Mongolian livestock industry depends on nomadic systems of husbandry where a variety of animals is herded together. It is characterized by low input and large herds grazing across pastures in the open country. Like the Zambian livestock system, it also emphasizes the numbers as compared to the productivity of the individual animal and adaptation to the environment is also considered an important factor. However, unlike Zambia, Mongolia's agricultural sector and livelihood heavily depends on livestock production. In this study, I found that trypanosomosis was prevalent in all the livestock species at 21.3% which raises a possibility of cross infection, with sheep and goats showing a significantly higher prevalence ($p<0.0001$) thus marking them out as possible reservoirs of trypanosomosis. Males also had a significantly higher prevalence rate than females ($p<0.05$), this is because the female animals get attention from the farmers during milking and thus it is easy to notice the abnormalities compared to the males which rarely interact with the farmers. Adult

animals had a significantly higher prevalence of trypanosomosis than young animals. This is because the young animals are usually kept apart from the adults and there is also less exposure and vector attraction to young animals because of their relatively smaller bodies which produce low amounts of odor plumes to attract vectors than the adults. The study also found that the sheep in Bayan-olgii province had a significantly lower prevalence than those in Hovd province (p=0.0017). The sheep in Bayan-olgiy are mainly Kazakh breed which are adapted to the mountainous terrain of Bayan-olgiy. However, no previous study has linked this breed to trypanosomosis resistance. The horses in Bayan-olgiy however had a significantly higher prevalence of trypanosomosis than those in Hovd province (p=0.0001). This could be attributed to the herd structure of the livestock in the two places whereby in the mountainous Bayan-olgiy, all the livestock graze in a compact group whereas in the plains of Hovd, the horses are usually scattered away from the rest of the herd thus decreasing the chance of cross infection. The study found piroplasmosis only among the equines at 29.8%, thus highlighting the importance of equine piroplasmosis in Mongolia. This study also highlighted the effect of husbandry system regarding a particular animal species to the disease prevalence.

The two chapters show that small ruminants could possibly be a reservoir for animal trypanosomosis in the two study areas. The study also highlights the

importance of vector control in control of vector borne diseases. This study shows that it is very important to know the prevalence of a disease because it highlights the various characteristic of the disease in the affected area thus making it easier to put in place effective and efficient intervention measures.

学位論文要旨

畜産業は、多くの発展途上国において重要な経済の一部門である。さらに、家畜は高い資産価値を有することから発展途上国の農民たちに対する、一種の社会的セーフティーネットとしての機能も併せ持つ。節足動物媒介性住血原虫病、中でもトリパノソーマ症とピロプラズマ症は家畜の生産性を著しく低下させることから、発展途上国の家畜産業において非常に大きな脅威であると考えられている。しかし、これらの疾患による家畜産業への被害は正確には推計されていない。そこで本研究では、南部アフリカ（ザンビア共和国）及び中央アジア（モンゴル国）においてPCR法を用いた分子疫学調査を行い、これらの地域における家畜のトリパノソーマとピロプラズマの感染率を明らかにすることを目的として研究を実施した。

第1章では、ザンビア共和国のウシとヤギにおけるトリパノソーマ症とピロプラズマ症の分子疫学調査を実施した。その結果、調査対象家畜の38.1%の家畜が少なくとも一種のトリパノソーマもしくはピロプラズマに感染していることが明らかとなった。ウシでは複数の住血原虫が混合感染すると、充填赤血球量（PCV）が有意に低下することが明らかとなった。一方、PCVが正常値であるにもかかわらず、ヤギはウシに比べて有意にトリパノソーマの感染率が高いことが明らかとなった。すなわち、多くのヤギが不顕性感染状態で存在し、トリパノソーマの感染源（レゼルボア）として重要であることが示唆された。また全ての調査地でバベシア陽性検体が検出されたことから、ザンビア国において家畜のバベシア症

が蔓延していることが明らかとなった。一方タイレリア感染陽性家畜は、調査個体の 0.6%であった。ザンビアでは一般的に急性劇症経過をたどる東海岸熱が住血原虫の中でも重要視されているが、同じピロプラズマ症であるバベシア症の対策も、家畜生産性を向上させるために重要であることが示唆された。以上より、住血原虫の高度な蔓延がザンビア共和国に広く認められ、その対策が家畜生産性を向上させるために重要であることが考えられた。さらにカリバ・ダム建設工事により、ツェツェバエが駆除された地域（Monze , モンザ）からはトリパノソーマ陽性の家畜が認められなかったことから、ベクターの駆除がトリパノソーマ症の予防に有効であることが示唆された。

第２章では、モンゴル国の主要家畜６種（ウシ、ウマ、ヤギ、ヒツジ、ヤク及びラクダ）におけるトリパノソーマ症とピロプラズマ症の分子疫学調査を実施した。同国の牧畜産業（遊牧）は、遊牧民が多種の家畜を同一の群で飼養する中央アジア諸国における一般的な様式であり、経済における農業部門の占める割合はザンビアに比べて大きい。本研究においてすべての家畜種に同属（*Trypanozoon* 亜属）のトリパノソーマの感染が認められたため、これらの家畜間でトリパノソーマが相互に感染し蔓延している可能性が示唆された。さらにヒツジとヤギで他種家畜に比べて有意にトリパノソーマの感染率が高かったことから、これらの小反芻獣がトリパノソーマのレゼルボアとして重要であることが示唆された。また小反芻獣のうちヒツジにおいて、バヤン・オルギー県に比べ、ホブド県で感染率が有意に高かった。これは、バヤン・オルギー県では、モンゴルの他の地域に比べより高地に順応した

カザフ種の飼養が一般的であるため、家畜種によるトリパノソーマに対する耐性の違いが認められたと考えられる。一方ウマではホブド県に比べ、バヤン・オルギー県で感染率が有意に高かった。これはホブド県ではより少ないウマを一群として飼養する遊牧民が多いため、個々のウマの状態を把握していることが考えられる。また、メスの感染率はオスに比べて有意に低く、また高齢家畜の感染率は若齢家畜に比べて有意に高かった。これらの結果は、メスの方がオスに比べて日常の搾乳によって飼育者の観察下に置かれる機会が多いこと、また若齢家畜は高齢家畜群から隔離されて飼養されるためベクターであるダニやアブに吸血される機会が少ないという牧畜形態が関連している可能性が示唆された。さらに、本研究においてピロプラズマ陽性家畜はウマのみであったため、モンゴル国においてウマピロプラズマ症は重要な家畜感染症であることが示唆された。以上より住血原虫病の感染率と多種の家畜を同一の群で飼養する中央アジア諸国に特徴的な牧畜形態との関連性が示唆されるとともに、モンゴル国の経済発展のためにさらなる住血原虫病対策が重要であることが強く示唆された。

本研究により南部アフリカおよび中央アジアの国において節足動物媒介性原虫病が蔓延していることが明らかとなった。特に小反芻獣が住血原虫病のレゼルボアになっている可能性が高いことから、今後本研究成果をもとにした効果的な節足動物媒介性原虫病対策の実施が期待される。

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References

Aboulaila, M., Yokoyama, N. and Igarashi, I. 2010. Development and evaluation of two nested PCR assays for the detection of *Babesia bovis* from cattle blood.*Vet. Parasitol*. **172**: 65-70.

Adham, F.K., Abd-el-Samie, E.M., Gabre, R.M. and El-Hussein, H. 2009. Detection of tick blood parasites in Egypt using PCR assay I- *Babesia bovis* and *Babesia bigemina*. *Parasitol. Res*. **105**: 721-730.

Altangerel, K., Sivakumar, T., Battsetseg, B., Battur, B., Ueno, A., Igarashi, I. and Yokoyama, N. 2012. Phylogenetic relationships of Mongolian *Babesia bovis* isolates based on the merozoite surface antigen (MSA)-1, MSA-2b and MSA-2c genes. *Vet. Parasitol*. **184**: 309-316.

Antoine-Moussiaux, N., Faye, B. and Vias, G.F. 2007. Touareg ethnoveterinary treatments of camel diseases in agadez area (Niger). *Trop. Anim. Health Prod*. **39**: 83-89.

Atarhouch, T., Rami, M., Bendahman, M.N. and Dakkak, A. 2003. Camel trypanosomosis in Morocco I: results of a first epidemiological survey. *Vet. Parasitol.* **111**: 277-286.

Avarzed, A., De Waal, D.T., Igarashi, I., Saito, A., Oyamada, T., Toyoda, Y. and Suzuki, N. 1997. Prevalence of equine piroplasmosis in central Mongolia. *Onderstepoort J. Vet. Res.* **64**: 141-145.

Bakheit, M.A., Schnittger, L., Salih, D.A.,Boguslawski, K.,Beyer, D., Fadl, M. and Ahmad J.S. 2004. Application of the recombinant *Theileria annulata* surface protein as an indirect ELISA for diagnosis of tropical theileriosis. *Parasitol. Res*. **92**: 299-302.

Battsetseg, B., Lucero, S., Xuan, X., Claveria, F. G., Inoue, N., Alhassan, A., Kanno, T., Igarashi, I., Nagasawa, H., Mikami, T. and Fujisaki, K. 2002_a. Detection of natural infection of *Boophilus microplus* with *Babesia equi* and *Babesia caballi* in Brazilian horses using nested polymerase chain reaction. *Vet. Parasitol.* **107**: 351-357.

Battsetseg, B., Lucero, S., Xuan, X., Claveria, F., Byambaa, B., Battur, B., Boldbaatar, D., Batsukh, Z., Khaliunaa, T., Battsetseg, G., Igarashi, I., Nagasawa, H. and Fujisaki, K. 2002_b. Detection of equine *Babesia* spp. gene fragments in *Dermacentor nuttali* Olenev1929 infesting Mongolian horses, and their amplification in egg and larval progenies. *J. Vet. Med. Sci*. **64**: 727-730.

Bishop, R., Musoke, A., Mozaria, S., Gardner, M. and Nene, V. 2004.*Theileria*: intracellular protozoan parasites of wild and domestic ruminants transmitted by ixodid ticks. *Parasitol*ogy **129**: S271-S283.

Boldbaatar, D., Xuan, X., Battsetseg, B., Igarashi, I., Battur, B., Batsukuh, Z., Bayambaa, B. and Fujisaki, K. 2005. Epidemiological study of equine piroplasmosis in Mongolia. *Vet. Parasitol*. **127**: 35-38.

Bouattour, A. and Darghouth, M.A. 1996. First report of *Babesia divergens* in Tunisia. *Vet. Parasitol***. 63**: 161-165.

Brun, R., Hecker, H. and Lun, Z.R. 1998. *Trypanosoma evansi* and *T. equiperdum*: distribution, biology, treatment and phylogenetic relationship (a review). *Vet. Parasitol.* **79**: 95-107.

Bruning, A. 1996.Equine piroplasmosis an update of diagnosis, treatment and prevention. *Br. Vet. J.* **152**: 139-151.

Büscher, P., Lejon, V., Magnus, E. and Van Meirvenne, N. 1999. Improved latex agglutination test for detection of antibodies in serum and cerebrospinal fluid of *Trypanosoma brucei gambiense* infected patients. *Acta Trop*. **73**, 11-20.

Byambaa, B., Dash, M. and Tarasevich, I.V. 1994. Newly diagnosed rickettsiosis. Essen Erdene press, Ulaanbaatar. pp. 10-13.

Chitambo, H., Arakawa, A. and Ono, T. 1992. *In vivo* assessment of drug sensitivity of African trypanosomes using akinetoplastic induction test. *Res. Vet. Sci*. **52**: 243-249.

Claes, F., Büscher, P., Touratier, L. and Goddeeris, B. M. 2005.*Trypanosoma equiperdum*: master of disguise or historical mistake? *Trends Parasitol*. **21:** 316-321.

Clausen P.H., Chuluun, S., Sodnomdarjaa, R., Greiner, M., Noeckler, K., Staak, C., Zessin, K.H. and Schein, E. 2003. A field study to estimate the prevalence of *Trypanosoma equiperdum* in Mongolian horses. *Vet. Parasitol*. **115**: 9-18.

Cortez, A.P., Rodrigues, A.C., Garcia, H.A., Neves, L., Batista, J.S., Bengaly, Z., Paiva, F. and Teixeira, M.M. 2009. Cathepsin L-like genes of *Trypanosoma vivax* from Africa and South America—characterization, relationships and diagnostic implications. *Mol. Cell. Probes* **23**: 44-51.

Coura, J.R. and Borges-Pereira. 2010. Chagas disease: 100 years after its discovery. A systemic review. *Acta Trop*. **115**: 5-13.

Courtin, D, Berthier, D., Thevenon, S., Dayo, G.K., Garcia, A. and Bucheton, B. 2008. Host genetics in African trypanosomiasis. *Infect. Genet. Evol*. **8**: 229-238.

Desquesnes, M., McLaughlin, G., Zoungrana, A. and Davila, A.M.R. 2001.Detection and identification of trypanosome of African livestock through a single PCR based on internal transcribed spacer 1 of rDNA. *Int. J. Parasitol*. **31**: 610-614.

Desquesnes, M. and Dia, M.L. 2003.*Trypanosoma vivax*: Mechanical transmission in cattle by one of the most common African tabanids, *Atylotus agrestis*. *Exp. Parasitol.* **103**: 35-43.

Desquesnes, M. and Dia, M. L. 2004. Mechanical transmission of *Trypanosoma vivax* in cattle by the African tabanids, *Atylotus fuscipes*. *Vet. Parasitol*. **119**: 9-19.

Desquesnes, M., Holzmuller, P., Lai, D.H., Dargantes, A., Lun, Z.R. and Jittaplapong, S. 2013. *Trypanosoma evansi* and surra: A review and perspectives on origin, history, distribution, taxonomy, morphology, hosts and pathogenic effects. *BioMed. Res. Intl*. 194176-194198.

De Waal, D.T., Van Heerden, J., Van den Berg, S.S., Stegmann, G.F. and Potgieter, F. T. 1988. Isolation of pure *Babesia equi* and *Babesia caballi* organisms in splenoctomized horses from endemic areas in South Africa. *Onderstepoort J. Vet. Res.* **55**: 33-35.

De Waal, D.T. 1992. Equine Piroplasmosis: a review. *Br. Vet. J*. **148**: 6-14.

Duh, D., Punda-Polic, V., Trilar, T. and Avsic-Zupanc, T. 2008. Molecular detection of *Theileria* sp. in ticks and naturally infected sheep. *Vet. Parasitol*. **151:** 327-331.

Foil, L.D. 1989.Tabanids as vectors of disease agents. *Parasitol. Today*. **5**: 88-96.

Friedhoff, K.T., Tenter, A.M. and Muller, I. 1990. Hemoparasites of equines: impact on international trade of horses. *Rev. Sci. Tech*. **9**: 1187-1194.

Friedhoff, K.T. and Soule, C. 1996. An account on equine babesiosis .*Rev. Sci. Tech*. **15**: 1191-1201.

Gachohi, J., Skilton, R., Hansen, F., Ngumi, P. and Kitala, P. 2012. Epidemiology of East Coast fever (*Theileria parva* infection) in Kenya: past, present and the future. *Parasit. Vectors*. **5**: 194.

Georges, K., Loria, G.R., Riili, S., Greco, A., Caracappa, S., Jongejan, F. and Sparagano, O. 2001. Detection of hemoparasites in cattle by reverse line blot hybridization with a note on the distribution of ticks in Sicily. *Vet. Parasitol*. **99**: 273-286.

Gharbi, M., Touay, A., Khayeche, M., Laarif, J., Jedidi, M., Sassi, L. and Darghouth, M.A. 2011. Ranking control options for at-risk dairy cattle in Tunisia using cost benefit analysis. *Rev. Sci. tech*. **30**: 763-778.

Gibson, W. and Stevens, J. 1999. Genetic exchange in the trypanosomatidae. *Adv. Parasitol.* **43**: 1-46.

Gubbels, J.M., de Vos, A.P., van der Weide, M., Viseras, J., Schouls, L.M., De Vries, E. and Jongejan, F. 1999. Simultaneous detection of bovine *Theileria* and *Babesia* species by reverse line blot hybridization. *J. Clin. Microbiol.* **37**: 1782-1789.

Gutierrez, C., Corbera, J.A., Morales, M. and Büscher, P. 2006. Trypanosomosis in goats - Current status. pp. 300-310. *In*: Blouin, E.F. and Maillard, J.C. Impact of Emerging Zoonotic Diseases on Animal Health. Blackwell Publishing, Oxford.

Hassan, M.U., Muhammad, G., Gutierrez, C., Iqbal, Z., Shakoor, A. and Jabbar, A. 2006. Prevalence of *Trypanosoma evansi* infection in equines and camels in the Punjab region, Pakistan. *Ann. N. Y. Acad. Sci*. **1081**: 322-324.

Holmes, P.H., Eisler, M.C. and Geerts, S. 2004. Current chemotherapy of animal trypanosomiasis. *In*: Maudlin, I., Holmes, P.H. and Miles, M.A. (Eds). The trypanosomiases. CABI Publishing, Wallingford, Oxfordshire, UK. pp 431-444.

Horare, C.A. 1972. The trypanosomes of mammals: A zoological monograph. Blackwell scientific Publications, Oxford, UK.

Hunfeld, K.P., Hildebrandt, A. and Gray, J.S. 2008. Babesiosis: Recent insights into an ancient disease*. Int. J. Parasitol*. **38**: 1219-1237.

IFAD report. 2014. Republic of Zambia Country Programme Evaluation Report. Report No. 3551-ZM.https://www.ifad.org/documents/10180/4d830f75-781c-4913-8236-dc901f586ef2.

IFAH report. 2014. The growing threat of vector-borne disease in humans and animals.http://healthforanimals.org/resources-and-events/resources/papers/143-ifah-white-paper-the-growing-threat-of-vector-borne-disease-in-humans-and-animals.html.

Iseki, H., Zhou, Z., Kim, C., Inpankaew, T., Sununta, C., Yokoyama, N., Xuan, X., Jittapalapong, S. and Igarashi, I., 2010. Seroprevalence of *Babesia* infections of dairy cows in Northern Thailand. *Vet. Parasitol*. **170**: 193-196.

Jones, T.W. and Davila, A.M.R. 2001. *Trypanosoma vivax*-out of Africa. *Trends Parasitol.* **17**: 99-101.

Jongejan, F., Perry, B.D., Moorhouse, P.D.S., Musisi, F.L., Pegram, R.G. and Snacken, M. 1988.Epidemiology of bovine babesiosis and anaplasmosi in Zambia*. Trop. Anim. Health.* Prod. **20**:234-242.

Jongejan, F. and Uilenberg, G. 2004. The global importance of ticks. *Parasitology* **129**: S3-S14.

Kerber, C.E., Ferriera, F. and Pereira, M.C. 1999. Control of equine piroplasmosis in Brazil. *Onderstepoort J. Vet. Res*. **66**: 123-127.

Koné, N., N'Goran, E.K., Sidibe, I., Kombassere, A.W. and Bouyer, J. 2011. Spatio-temporal distribution of tsetse and other biting flies in the Mouhoun River basin, Burkina Faso. *Med. Vet. Entomol.* **25**: 156-168.

Kouam, M.K., Kantzoura, V., Gajadhar, A.A., Theis, J.H., Paradopoulo, E. andTheodoropoulos, G. 2010. Seroprevalence of equine piroplasmosis and host related factors associated with infection in Greece. *Vet. Parasitol*. **169**: 273-278.

Kronenberger, P. and Miezan, B. 1988. *In vitro* culture of *Trypanosoma brucei gambiense* isolated from human celebro-spinal fluid. *Acta Trop*. **45**: 181-182.

Kumar, S., Gupta, A.K. and Pal, Y. 2003. *In vivo* therapeutic efficacy trial with artimisinine derivative, buparvaquone and imidocarb dipropionate against *Babesia equi* infection in donkeys. *J. Vet. Med. Sci*. **65**: 1171-1177.

Kuttler, K.L., Zaugg, J.L. and Gipson, C.A. 1987. Imidocarb and parvaquone in the treatment of piroplasmosis (*Babesia equi*) in equids. *Am. J. Vet. Res*. **48**: 1613-1616.

Lai, D.H., Hashimi, H., Lun, Z.R., Ayala, F.J. and Lukes, J. 2008. Adaptations of *Trypanosoma Brucei* to gradual loss of kinetoplast DNA: *Trypanosoma equiperdum* and *Trypanosoma evansi* are petite mutants of *T. brucei*. *Proc. Natl. Acad. Sci. USA.* **105:** 1999-2004.

Leach, T.M. and Roberts, C.J. 1981. Present status of chemotherapy and chemoprophylaxis of animal trypanosomiasis in the Eastern hemisphere. *Pharmacol. Ther*. **13**: 91-147.

Lewis, B.D., Penzhorn B.L. and Volkman, D.H. 1999. Could treatment of pregnant mares prevent abortions due to equine piroplasmosis? *J. S. Afr. Vet. Assoc*. **70**: 90-91.

Li, F.J., Lai, D.H., Lukes, J., Chen, X.D. and Lun, Z.R. 2006. Doubts about *Trypanosoma equiperdum* strains classed as *Trypanosoma brucei* or *Trypanosoma evansi*. *Trends Parasitol*. **22**: 55-56.

Luckins, A.G. 1988. *Trypanosoma evansi* in Asia. *Parasitol. Today*. **4**: 137-142.

Lun, Z.R. and Desser, S.S. 1995. Is the broad range of host and geographical distribution of *Trypanosoma evansi* attributable to the loss of maxi circle kinetoplast DNA? *Parasitol. Today*. **11**: 131-133.

Magona, J.W., Walubengo, J. and Odimin, J.T. 2008. Acute haemorrhagic syndrome of bovine trypanosomosis in Uganda. *Acta Trop.* **107**: 186-191.

Mahama, C.I., Desquesnes, M., Dia, M.L., Losson, B., De Deken, R. and Geerts, S. 2004. A cross-sectional epidemiological survey of bovine trypanosomosis and its vectors in the Savelungu and West Mamprusi districts of Northern Ghana. *Vet. Parasitol*. **122**: 1-13.

Makala, L.H., Mangani, P., Fujisaki, K. and Nagasawa, H. 2003. The current status of major tick borne diseases in Zambia. *Vet. Res.* **34**: 27-45.

Mans, B.J., Pienaar, R. and Latif, A.A. 2014. A review of *Theileria* diagnostics and epidemiology. *Intl. J. Parasitol. Parasites and Wildlife***.** **4**: 104-118.

Masumu, J., Marcotty, T., Geysen, D., Geerts, S., Vercruysse, J., Dorny, P. and Van den Bossche, P. 2006. Comparison of the virulence of *Trypanosoma congolense* strains isolated from cattle in a trypanosomiasis endemic area of eastern Zambia. *Int. J. Parasitol.* **36**: 497-501.

Masumu, J., Geysen, D. and Van den Bossche, P. 2009. Endemic type of animal trypanosomiasis is not associated with lower genotype variability of *Trypanosoma congolense* isolates circulating in livestock. *Res. Vet. Sci.* **87**: 265-269.

McCauley, R.H., Majid, A.A. and Tayer, A. 1984. Economic impact of evaluation of the production impact of bovine schistomiasis and vaccination in the Sudan. *Prev. Vet. Med.* **2**: 735-754.

McKeever, D.J. 2009. Bovine immunity - a driver for diversity in *Theileria* parasites? *Trends Parasitol.* **25**:269-276.

McLaughlin, G.L., Sesenyonga, S.S., Nanteza, E., Rubaire-Akiki, W.O., Hansen, R.D., Vodkin, M.H., Novak, R.J., Gordon, V.R., Montenegro-James, S., James, M., Aviles, H., Armijos, R., Santrich, C., Weigle, K., Saravia, N., Wozniak, E., Gaye, O., Mdachi, R., Shapiro, S.Z., Chang, K-P. and Kakoma, I. 1996. PCR-based detection and typing of parasites. pp. 261-287. *In*: Azcel, M.A and Alkan, M.Z. Parasitology for the 21st Century. CAB International, Wallingford Oxon.

Metcalf, E.S. 2001. The role of international transport of equine semen on disease transmission*. Anim. Reprod. Sci*. **68**: 229-237.

Mihok, S., Maramba, O., Munyoki, E. and Kagoiya, J. 1995. Mechanical transmission of *Trypanosoma* spp.by African *Stomoxyinae* (Diptera: Muscidae). *Trop. Med. Parasitol.* **46**: 103-105.

Morrison, W.I. 2015. Circulatory system blood parasites in: Khan, C. M. (Ed.). The Merck Veterinary Manual. Merck & Co. Inc. and Merial Ltd. http://www.merckvetmanual.com/mvm/circulatory_system/blood_parasites

Mubamba, C., Sitali, J. and Gurnmow, B. 2011. Trends of selected cattle diseases in eastern Zambia between 1988 and 2008. *Prev. Vet. Med.* **101**: 163-172.

Mugittu, K.N., Silayo, R.S., Majiwa, P.A.O., Kimbita, E.K., Mutayoba, B.M. and Masele, R. 2001. Application of PCR and DNA probes in the characterization of trypanosomes in the blood of cattle in farms in Morogoro, Tanzania. *Vet. Parasitol.* **94**: 177-189.

Munkhjargal, T., Sivakumar, T., Battsetseg, B., Nyamjargal, T., Aboulaila, M., Purevetseren, B., Bayarsaikhan, D., Byambaa, B., Terkawi, M.A., Yokoyama, N. and Igarashi, I. 2013. Prevalence and genetic diversity of equine piroplasms in Tov province, Mongolia. *Infect. Genet. Evol*. **16**: 178-185.

Nantulya, V.M. 1988. Immunodiagnosis of rhodesiense sleeping sickness: Detection of circulating trypanosomal antigens in sera and cerebrospinal fluid by enzyme immunoassay using a monoclonal antibody. *Bull. Soc. Pathol. Exot. Filiales*. **81**: 511-512.

Nguyen, T.T., Ruttayaporn, N., Goto, Y., Kawazu, S., Sakurai, T. and Inoue, N. 2015. A TeGM6-4r antigen based immunochromatographic test (ICT) for animal trypanosomosis. *Parasitol. Res*. **114**: 4319-4325.

Notomi, T., Okayama, H., Masubuchi, H., Yonekawa, T., Watanabe, K., Amino, N. and Hase, T. 2000. Loop-mediated isothermal amplification of DNA. Nucleic Acids *Res.* **28**: e63.

Olaho-Mukani, W., Nyang'ao, J.M.N., Ngaira, J.M., Omuse, J.K., Mbwabi, D., Tengekyon, K.M., Njenga, J.N. and Igweh, A.C. 1994. Immunoassay of circulating trypanosomal antigens in sleeping sickness patients undergoing treatment. *J. Immunoassay*. **15**: 69-77.

Pegram, R.G., Perry, B.D., Musisi, F.L. and Mwanaumo, B. 1986. Ecology and phenology of ticks in Zambia: Seasonal dynamics on cattle. *Exp. Appl. Acarol.* **2**: 25-45.

Phipps, L.P. and Otter, A. 2004. Transplacental transmission of *Theileria equi* in two foals born and reared in the United Kingdom. *Vet. Rec*. **154**: 406-408.

Reid, S.A. 2002. *Trypanosoma evansi* control and containment in Australasia. *Trends Parasitol*. **18**: 219-224.

Rhalem, A., Sahibi, H., Lasri, S., Johnson, W.C., Kappmeyer, L.S., Hamidouch, A., Knowles,D.P. and Goff, W.L. 2001. Validation of a competitive enzyme-linked immunosorbent assay for diagnosing *Babesia equi* infections of Moroccan origin and its use in determining the sero-prevalence of *T. equi* in Morocco. *J. Vet. Diagn. Invest.* **13**: 249-251.

Rogers, J. 2009. Human African trypanosomiasis, chemotherapy and CNS disease. *J. Nuroimmunol*. **211**: 16-22.

Rothschild, C.M. 2013. Equine piroplasmosis. *J. Equine Vet. Sci*. **33**: 497-508.

Ruegg, S.R., Torgerson, P., Deplazes, P. and Mathis, A. 2007. Age-dependent dynamics of *Theileria equi* and *Babesia caballi* infections in South west Mongolia based on IFAT and/or PCR data from domestic horses and ticks. *Parasitology* **134**: 939-947.

Safieldin, M., Abdel-Gadir, A. and Elmalik, K.H. 2011. Factors affecting prevalence of blood parasites in dairy cattle in Omdurman locality, Sudan. *J. Cell Anim. Biol*. **5**: 17-19.

Schnaufer, A., Domingo, G.J. and Stuart, K. 2002. Natural and induced diskinetoplastic Trypanosomatids: how to live without mitochondrial DNA. *Intl. J. Parasitol*. **32**: 1071-1084.

Short, M.A., Clerk, C.K., Harvey, J.W., Wenzlow, N., Hawkins, I. k., Allred, D.R., Knowles, D.P., Corn, J.L., Grause, J.F., Hennager, S.G., Kitchen, D.L. and Traub-Dargatz, J.L. 2012. Outbreak of equine piroplasmosis in Florida. *J. Am. Vet. Med. Assoc.* **240**: 588-595.

Simukoko, H., Marcotty, T., Phiri, I., Geysen, D., Vercruysse, J. and Van den Bossche, P. 2007_a. The comparative role of cattle, goats and pigs in the epidemiology of livestock trypanosomiasis on the plateau of eastern Zambia. *Vet. Parasitol.* **147**: 231-238.

Simukoko, H., Marcotty, T., Phiri, I., Vercruysse, J. and Van den Bossche, P. 2007_b. Heterogeneity in the trypanosomosis incidence in Zebu cattle of different ages and sex on the plateau of eastern Zambia. *Acta Trop.* **103**: 98-101.

Sivakumar, T., Kothalawala, H., Abeyratne, S.A.E., Vimalakumar, S.C., Meewewa, A.S., Hadirampela, D.T., Puvirajan, T., Sukumar, S., Kuleswarakumar, K., Chandrasiri, A.D.N., Igarashi, I. and Yokoyama, N. 2012_a. A PCR-based survey of selected *Babesia* and *Theileria* parasites in cattle in Sri Lanka. *Vet. Parasitol.* **190**: 263-267.

Sivakumar, T., Altangerel, K., Battsetseg, B., Battur, B., AbouLaila, M., Munkhjargal, T., Yoshinari, T., Yokoyama, N. and Igarashi, I. 2012_b. Genetic detection of *Babesia bigemina* from Mongolian cattle using apical membrane antigen-1 gene-based PCR assay. *Vet. Parasitol.* **187**: 17-22.

Sivakumar, T., Tattiyapong, M., Fukushi, S., Hayashida, K., Kothalawala, H., Silva, S.S., Vimalakumar, S.C., Kanagaratnam, R., Meewewa, A.S., Suthaharan, K., Puvirajan, T., de Silva, W.K., Igarashi, I. and Yokoyama, N. 2014. Genetic characterisation of *Babesia* and *Theileria* species in water buffaloes in Sri Lanka. *Vet. Parasitol*. **200**: 24-30.

Skilton, R.A., Bishop, R.P., Katende, J.M., Mwaura, S. and Morzaria, S.P. 2002. The persistence of *Theileria parva* infection in cattle immunized using two stocks

which differ in their ability to induce a carrier state: Analysis using a novel blood spot PCR assay. *Parasitology*. **124**: 265-276.

Stephen, L. 1986. Trypanosomiasis: A veterinary perspective. Pergamon Press, N.Y. USA.

Stevens, J.R., Noyes, H.A., Dover, G.A. and Gibson, W.C. 1999. The ancient and divergent origins of the human pathogenic trypanosomes, *Trypanosoma brucei* and *T. cruzi*. *Parasitol*ogy **110**: 107-116.

Stiller, D., Goff, W.L., Johnson, L.W. and Knowles, D.P. 2002. *Dermacentor variabilis* and *Boophilus microplus* (Acari: Ixodidae); experimental vectors of *Babesia equi* to equids. *J. Med. Entomol*. **39**: 667-670.

Suganuma, K., Narantsatsral, S., Battur, B., Yamasaki, S., Otgonsuren, D., Musinguzi, S.P., Davaasuren, B., Battsetseg, B. and Inoue, N. 2016. Isolation, cultivation and molecular characterization of a new *Trypanosoma equiperdum* strain in Mongolia. *Parasit. Vectors* **9**: 481.

Taylor, K. and Authie, E.M.L. 2004. Pathogenesis of animal trypanosomiasis. *In*: Maudlin, I., Holmes, P.H. and Miles, M.A. (Eds.). The trypanosomiases. CABI publishing, Wallingford, Oxfordshire, UK, pp. 331-353.

Thekisoe, O.M.M., Kuboki, N., Nambota, A., Fujisaki, K., Sugimoto, C., Igarashi, I., Yasuda, J. and Inoue, N. 2007. Species specific loop mediated isothermal

amplification (LAMP) for diagnosis of trypanosomosis. *Acta Trop*. **102**: 182-189.

Thekisoe, O.M.M., Rodriguez, C.V., Rivas, F., Coronel-Servian, A.M., Fukumoto, S., Sugimoto, C., Kawazu, S.-I. and Inoue, N. 2010. Detection of *Trypanosoma cruzi* and *T. rangeli* infections from *Rhodnius pallescens* bugs by loop-mediated isothermal amplification (LAMP). Am. *J. Trop. Med. Hyg*. **82**: 855-860.

Thumbi, S., Jung'a, J., Mosi, R. and McOdimba, F. 2010. Spatial distribution of African animal trypanosomiasis in Suba and Teso districts in western Kenya. *BMC Res.* Notes **3**: 6.

Tisdell, C.A., Harrison, S.R. and Ramsay, G.C. 1999. The economic impacts of endemic diseases and disease control programmes. *Rev. Sci. Tech*. **18**: 380-398.

Trail, J.C.M., Dieteren, G.D.M., Feron, A., Kakiese, O., Mulungo, M. and Pelo, M. 1990. Effect of trypanosome infection, control of parasitaemia and control of anaemia development on productivity of N'Dama cattle. *Acta Trop.* **48**: 37-45.

Trail, J.C.M., Dieteren, G.D.M., Murray, M., Ordner, G., Yangari, G., Collardelle, C., Sauveroche, B., Maille, J.C. and Viviani, P. 1993. Measurement of

trypanotolerance criteria and their effect on reproductive-performance of N'Dama cattle. *Vet. Parasitol.* **45**: 241-255.

Uilenberg, G. 2006. *Babesia*- A historical overview. *Vet. Parasitol*. **138**: 3-10.

Van den Bossche, P. and Rowlands, G.J. 2001. The relationship between the parasitological prevalence of trypanosomal infections in cattle and herd average packed cell volume. *Acta Trop.* **78**: 163-170.

Van den Bossche, P. and Delespaux, V. 2011. Options for the control of tsetse-transmitted livestock trypanosomosis. An epidemiological perspective. *Vet. Parasitol.* **181**: 37-42.

Vial, H.J. and Gorenflot, A. 2006. Chemotherapy against babesiosis. *Vet. Parasitol*. **138**: 147-160.

Vickerman, K. 1997. Landmarks in trypanosome research, *In*: Hide, G., Mottram, J.C., Coombs, G.H. and Holmes, P.H. (Eds) Trypanosomiasis and Leishimaniasis: Biology and control. CAB International, UK, pp. 1-37.

Wagner, G.G., Holman, P. and Waghela, S. 2002. Babesiosis and heartwater: threats without boundaries. *Vet. Clin. North Am. Food Anim. Pract*. **18**: 417-430.

Wang, Z.L. 1988. The similarities and the differences of the characteristics between *T. equiperdum* and *T. evansi*. *Bul. Vet. Col*. **8**: 300-303.

Wernery, U., Zacharia, R., Mumford, J. A. and Luckins, T. 2001. Preliminary evaluation of diagnostic tests using horses experimentally infected with *Trypanosome evansi*. *Vet. J*. **161:** 287-300.

World Bank, 2009. Sustainable Development Department, East Asia and Pacific region. Mongolia: Livestock sector study; synthesis report **1**: 50277 MN.

Zablotskij, V.T., Georgiu, C., De Waal, T., Clausen, P.H., Claes, F. and Touratier, L. 2003. The current challenges of dourine: difficulties in differentiating *Trypanosoma equiperdum* within sub-genus *Trypanozoon*. *Rev. Sci. Tech*. **22**: 1087-1096.

Zwygarth, E., Just, M.C. and De Waal, D.T. 1997. *In vitro* cultivation of *Babesia equi*: detection of carrier animals and isolation of parasites. *Onderstepoort J. Vet. Res*. **64**: 51-56.