Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Faculty of Veterinary Medicine and Animal Science
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Cross-sectional study of *Anaplasma spp.* in goats and sheep in Mongolia

A comparison between species in relationship to pasture conditions

Malin Jansson Lagerkvist

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Tvärsnittststudie av *Anaplasma spp.* bland får och getter I Mongoliet: En jämförelse mellan arter i relation till betesförhållanden

Malin Jansson Lagerkvist

Supervisor: *Jonas Johansson Wensman, Department of Clinical Sciences*
Assistant Supervisor: *Jonas Malmsten, Wildlife consultant Ltd, Vallentuna, Sweden*
Examiner: *Madeleine Tråvén, Department of Clinical Sciences*

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SUMMARY

Anaplasma ovis, the main aetiology behind of ovine and caprine anaplasmosis, is a vector-borne bacterium of the order *Rickettsiales*, capable of infecting erythrocytes of small ruminants. The infection is generally a subclinical or mild condition, but stress-factors as co-infections, vaccinations, or transports may aggravate the disease. Severe cases of *A. ovis* infection may involve anaemia, abortion, and mortality. The pathogen is widely distributed, and is endemic in several tropical and subtropical areas. In addition to goat and sheep, several species of wild ruminants are known to be susceptible to infection, but their importance as reservoirs in the epidemiology of *A. ovis* is yet uncertain.

The aim of this cross-sectional study was to investigate the prevalence of *A. ov*is in goats and sheep in Mongolia and to analyse the occurrences of changes in body condition, colours of mucous membranes and blood parameters due to infection. In addition, goats and sheep's tendency of developing clinical signs was compared between different pasture conditions, analysing whether their species-related pasture adoptions as browser respective grazers may contribute as a stress-factor in insufficient pasture. Although a third of Mongolian population relies on small ruminant-dominated livestock for subsistence, few previous studies of *A. ovis* have been undertaken. Three regions were selected for the study, based on their respective pasture conditions. Samples from 80 sheep and 88 goats were collected and analysed by microscopic examination of stained blood smears, polymerase chain reaction (PCR) for the 16s RNA gene of *Anaplasma* spp. (IVM Ulaanbaatar, Mongolia) and msp4 PCR (IVM Ulaanbaatar, Mongolia) specific for detection of *A. ovis*. In addition, haemoglobin (Hb), haematocrit (HCT), Body Condition Score (BCS) and FAMACHA© (i.e. method for detecting anaemia by grading the colour of the lower inner eyelid) was recorded for each animal.

The overall PCR-based prevalence of *Anaplasma* spp. in the study was 82.4 % with no significant difference between goats and sheep in any of the regions. The proportion of positive animals in South Gobi was significantly lower than in other regions. Typing of anaplasma subspecies was unsuccessful due to complications in the msp4 PCR, and *A. ovis* could therefore not be confirmed. There was a disagreement between the microscopic results and 16S rRNA PCR results, but all individuals with findings of more than eight inclusion-like structures during six minutes of microscopy were PCR-positive. Using this criterion, 34.2 % of PCR-positive individuals could be identified. Goats were generally over-represented compared to sheep regarding the occurrence of clinical parameters diverging from normal values. Neither low Hb-values nor low BCS occurred to a higher extent in anaplasma-positive animals but there was a significant correlation between the occurrence of pale mucous membranes (FAMACHA©scores below 3) and anaplasma-positivity among goats in the South Gobi. In addition, goats were slightly paler than sheep in general, suggesting that FAMACHA© scale might be less accurate for goats. An interesting finding was the high occurrence of Hb values below reference among anaplasma-negative goats with in South Gobi. The aetiology behind this is still unknown. Regional comparisons between the infected and non-infected population were limited by the low occurrence of PCR-negative individuals in two of the regions. For the same reason, assessments about pasture influence on disease development were not possible.

SAMMANFATTNING

Anaplasma ovis, den främsta etiologin bakom anaplasmos hos får och getter, är en vektorburen bakterie i ordningen *Rickettsiales* med förmågan att infektera erytrocyter hos små idisslare. Infektionen är oftast subklinisk till mild, men stressfaktorer som saminfektioner, vaccinationer eller transporter kan bidra till att sjukdomen förvärras, och allvarliga fall kan innebära anemi, aborter och dödsfall. *A. ovis* har en omfattande geografisk spridning och är endemisk i flera tropiska och subtropiska områden runtom i världen. Utöver getter och får har flertalet vilda idisslare visat sig vara mottagliga för infektionen, men huruvida de har en betydande roll som reservoarer i epidemiologin för *A. ovis*, är ännu inte klarlagt.

Syftet med denna tvärsnittsstudie var att undersöka förekomsten av *A. ovis* hos får och getter i Mongoliet, samt att utvärdera förändringar av fett- och muskelansättning, slemhinnefärg och blodparametrar till följd av anaplasmainfektion. Vidare jämfördes tendensen att utveckla kliniska symtom hos getter och får mellan olika betesförhållanden för att utvärdera om deras artspecifika betesanpassningar som buskätare- respektive gräsätare kan bidra som stressfaktor vid otillfredsställande beten. Trots att en tredjedel av Mongoliets befolkning försörjer sig på get- och fårdominerad boskapshållning har få tidigare studier av *A. ovis* genomförts i landet. Tre regioner valdes ut för studien baserat på deras respektive betesförhållanden. Prover samlades från 80 får och 88 getter och analyserades genom mikroskopering av färgade blodutstryk, polymeraskedjereaktion (PCR) för 16S rRNA genen i *Anaplasma* spp (IVM Ulaanbaatar, Mongolia) sampt msp4 PCR (IVM Ulaanbaatar, Mongolia), specifik för detektion av *A. ovis*. Därtill analyserades hemoglobin (Hb), hematokrit (Hk), Body condition score (BCS) och FAMACHA© (gradering av färgen på inre nedre ögonlocket för detektion av anemi) för samtliga provtagna individer.

Den totala PCR-baserade prevalensen av *Anaplasma* spp. var 82,4 %, där ingen signifikant skillnad kunde ses i förekomsten mellan får och getter i någon av regionerna. Andelen positiva djur var emellertid signifikant lägre i södra Gobi än i de övriga regionerna. Artbestämning av anaplasma-underarter misslyckades på grund av komplikationer i msp4-PCR:en och *A. ovis* kunde därmed ej med säkerhet fastställas som patogen bakom fynden i studien. Resultatet från mikroskoperingen skiljde sig något från 16S rRNA PCR-resultaten, men samtliga individer med fynd av fler än åtta inklusionskroppar under sex minuters mikroskopering var PCR-positiva. Om detta kriterium användes kunde 34,2 % av de PCR-positiva individerna identifieras genom mikroskopering. Getter var generellt överrepresenterade jämfört med får angående avvikelser från de kliniska parametrarnas normalvärden. Varken låga Hb-värden eller låga BCS förekom i större utsträckning hos anaplasma-positiva djur, men det fanns ett signifikant samband mellan bleka slemhinnor (FAMACHA© under 3) och anaplasma-positivitet bland getterna i South Gobi. Dessutom var getterna generellt något blekare än fåren vilket implicerar att FAMACHA©-skalan kan vara sämre anpassad för getter. Ett intressant fynd var den höga förekomsten av Hb-värden under referensintervall bland anaplasma-negativa getter i South Gobi. Etiologin bakom detta är fortfarande okänd. Regionala jämförelser mellan infekterade och icke-infekterade populationer begränsades av den låga förekomsten av PCR-negative individer i två av regionerna. Av samma skäl inskränktes bedömningen av betets inflytande över sjukdomsutvecklingen.

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ABBREVIATIONS

DNA	Deoxyribonucleic acid
PCR	Polymerase chain reaction
Hb	Haemoglobin
HCT	Haematocrit
TBD	Tick-borne diseases
Prepatent period	Peiod from infection to first laboratory appearance of a disease (i.e. detectable inclusion bodies during microscopic examination)
Subpatent	Bacteraemia below the microscopic threshold
Patent bacteraemia	Bacteria visible in the microscope
Incubation period	Period from infection to appearance of clinical symptoms
Recrudescence	Subpatent bacteraemia that becomes patent again
Normocytic	Red blood cells that are normal in size and usually also in haemoglobin content
Anisocytosis	The red blood cells are of unequal size
Normochromic	A red blood cell having normal color resulting from the presence of an adequate amount of haemoglobin
Polychromasia	Variation in the hemoglobin content of erythrocytes
Biological transmission	Vector-borne transmission of a pathogen, involving a biological process for the pathogen, e.g. a stage of development of in an intermediate host
Mechanical transmission	Transmitter of a pathogen where the tissue of the transmitter is not infected, the agent does not multiply

INTRODUCTION

Anaplasma ovis, the disease causing agent behind ovine and caprine anaplasmosis, was first described in 1912 (Bevan, 1912) and has since been found prevalent in many continents around the world. Today the arthropod-borne bacterium is known to be endemic in many tropical and subtropical areas (de la Fuente *et al.*, 2005b; Torina *et al.*, 2008a; Shompole *et al.*, 1989; Kocan *et al.*, 2003) but the magnitude of the economical-, welfare- and clinical consequences are still poorly understood. Though *A. ovis* infect and parasitise the erythrocytes of domestic and wild ruminants, the bacteria has long been regarded as a pathogen of minor relevance since it has been considered to cause predominantly subclinical disease with little apparent impact on the animals (Blood & Henderson, 1985). Today there is an on-going reassessment of the importance of the *A. ovis* based on upcoming knowledge about production losses, severe clinical cases, potential importance of co-infections and an increasing number of possible wild host species (Lu *et al.*, 1997; Stoltsz, 2004; de la Fuente *et al.*, 2007; Smith & Sherman, 2009; Yasini *et al.*, 2012; Li *et al.*, 2014).

Despite the increased interest of *A. ovis* during recent years, there are still few studies of the disease situation in Mongolia, a country where over a third of the population live from pastoral herding of livestock of which the majority are goats and sheep (Worden & Savada, 1989; Papageorgiou *et al.*, 2012)

Objectives

The idea of this study originated in observations made by an Australian veterinarian, who, during research in south Mongolia during 2012 to 2015, noticed that some of the goats in the area had rather pale mucous membranes. This observation, together with the sparse existing information of *A. ovis* in Mongolia, led to the main objective of this study; to investigate the prevalence of the bacterium in goats and sheep in Mongolia.

Specific objectives;

- Investigate and compare the prevalence of *A. ovis* in goats and sheep in three geographically separated regions of Mongolia by stained blood smears and PCRs.
- Investigate to what extent body condition, colours of mucous membranes and blood parameters are influenced by *A. ovis* infection.
- Compare the occurrence of clinical signs between infected goats and sheep under different pasture conditions to investigate whether their respective species specific pasture adoptions (where sheep are grazers and goats are predominantly browsers) affect their tendency to develop clinical sign in situations where the diversity of pasture is limited.

LITERATURE REWIEV

The genus of *Anaplasma*

The genus *Anaplasma* (order *Rickettsiales*, family *Anaplasmataceae*), includes species of gram-negative, obligate intracellular bacteria, that can affect both human and animal health (Shompole *et al.*, 1989; Dumler *et al.*, 2001; Razmi *et al.*, 2006; Torina & Caracappa, 2012; Noaman, 2013; Renneker *et al.*, 2013; Ybanez *et al.*, 2013).

In 2001 there was a significant reorganization within the order of *Rickettsiales* where the family *Anaplasmataceae* replaced the family *Ehrlichiaceae* and the classification within the genera was adjusted based on sequence analyses of 16SrRNA, groEL and surface protein genes (Dumler *et al.*, 2001). Today the genus *Anaplasma* contains the subspecies *A. platys*, *A. bovis*, *A. marginale*, *A.ovis*, *A. centrale*, and *A. phagocytohilum*.

These arthropod-borne bacteria all infect blood cells of eukaryotic hosts and parasitise exclusively within the membrane-bound intracytoplasmic vacuoles of the cells (Dumler *et al.*, 2001). The different subspecies have yet different cell and host preferences, where *A. platys* infect thrombocytes of canines, while *A. bovis* mainly parasitise within monocytes of ruminants (Sainz *et al.*, 1999; Goethert & Telford, 2003). *A. marginale*, *A. ovis* and *A. centrale* infect the erythrocytes of ruminants, and *A. phagocytophilum*, which attacks granulocytes, is pathogenic to several domestic and wild animals, as well as to humans (Rymaszewska & Grenda, 2008).

Table 1. *The charecteristic of pathogens of genus Anaplasma (modified from Rymaszewska & Grenda, 2008)*

Aetiological agent		**Disease**	**Infected organism or host**	**Infected cell**
before 2001	**after 2001**			
Ehrlichia bovis	*Anaplasma bovis*	Bovine anaplasmosis	Domestic ruminants, small mammals	monocytes
Anaplasma ovis	*Anaplasma ovis*	Ovine, caprine anaplasmosis	Domestic small ruminants & wild ruminants	erythrocytes
Anaplasma marginale	*Anaplasma marginale*	Bovine anaplasmosis	Domestic & wild ruminants	erythrocytes
Anaplasma centrale	*Anaplasma centrale*	Bovine anaplasmosis	Domestic & wild ruminants	erythrocytes
E. Equi, *E. Phagocytophila*, *Czynnik HGE*	*Anaplasma phagocytophilum (HGA agent)*	Human and animal graunlocytic anaplasmosis	Domestic & wild ruminants, domestic and wild horses, dogs, humans	granulocytes
E. Platys	*Anaplasma platys*	Canine cyclic thrombocytopenia	dogs	platelets

Diseases caused by anaplasma in small ruminants

The main diseases caused by anaplasma bacteria in small domestic ruminants are tick borne fever, caused by *A. phagocytophilum*, and ovine/caprine anaplasmosis, in which *A. ovis* is the most common disease-causing agent.

Tick borne fever

A. phagocytophilum is the the anaplasma subspecies which has been most widely studied during the years. This is partly due to the wide host spectra, including wild and domestic ruminants, equid, cats and dogs as well as humans (see review by Rymaszewska & Grenda, 2008). The disease caused by *A. phagocytophilum* has many names depending on the species of the host; Tick borne fever (TBF) in small ruminants, pasture fever in cattle, granulocytic anaplasmosis in horses and Human Granulocytic Anaplasmosis (HGA) in humans (Chen *et al.*, 1994). The bacteria infect granulocytes, foremost neutrophils but occasionally endothelial cells and macrophages which weakens the immune system (Papageorgiou *et al.*, 2012). TBF in small ruminants can either be subclinical or cause clinical signs such as fever, anorexia, abortions, weight loss and reduction in milk production (Rymaszewska & Grenda, 2008). For weakened or immune suppressed individuals the disease can be lethal and mortality as high as 24 % has been reported (Papageorgiou *et al.*, 2012).

Ovine and caprine anaplasmosis

A. ovis is considered to be the main agent of ovine and caprine anaplasmosis and the pathogen has been reported in both clinical and subclinical cases around the world (Stoltsz, 2004). However, there have been findings of other anaplasma infections in goats and sheep, which makes the aetiology of the disease a bit more complex. In 1979 a hitherto unrecognised anaplasma was reported from sheep in the Netherlands. The bacteria, much similar to *A. ovis*, was called *Anaplasma mesaeterum*, and in contrast to *A. ovis* it appears to be more pathogenic to sheep than to goats and non-infective to cattle (Uilenberg *et al.*, 1979).

Also *A. marginale*, a subspecies with high pathogenicity to cattle, has been proven capable of infecting goats and sheep (Kuttler, 1984). In experiments where splenectomised sheep were inoculated with *A. marginale*, the animals displayed a low level of bacteraemia and a moderate reduction in HCT but no signs of disease were detected (Kuttler, 1984; Razmi *et al.*, 2006). It is not yet assertive whether sheep infected with *A. marginale* can develop high enough bacteraemia to act as reservoirs and contribute to the spread of bacteria to cattle (Maas & Buening, 1981; Tavares-Marques *et al.*, 2010). However, the carrier state of *A. marginale* in sheep and goats is assumed to be relatively short and not capable of resulting in a persistent infection (Maas & Buening, 1981). *A. ovis* and A. *marginale* have many similarities regarding genetics as well as in microscopically appearance and potential host spectra (Splitter *et al.*, 1956; Kuttler, 1984; Lew *et al.*, 2003). Nevertheless, the pathogens do not seem to be immunologically similar enough to create cross-immunity during experimental attempts (Kuttler, 1984).

Anaplasma ovis

Pathogenesis and clinical signs

A. ovis infects and replicates within erythrocytes which are subsequently phagocytosised in the spleen and the bone marrow. The average prepatent period (i.e the time from infection to when inclusions are microscopically detectable) is about two weeks but may vary between 5 to 40 days (Splitter *et al.*, 1955, 1956; Neitz, 1968; Kuttler, 1981), depending on the quantity of infecting bacteria (Ryff *et al.*, 1964; Yasini *et al.*, 2012). From the point where infection is microscopically detectable, the bacteria continues to replicate in a pattern where they double every 24 hours (Splitter *et al.*, 1955), contributing to a further increase in bacteraemia in one to two weeks (Splitter *et al.*, 1955; 1956; Kuil & Folkers, 1967; Barry & Van Niekerk, 1990), before the process is arrested by the immune system. At the bacteraemia peak, 1 to 12 percent of the erythrocytes are infected with *A. ovis* in goats. The corresponding number for sheep is between 0.1 and 4 percent of the erythrocytes (Kuil & Folkers, 1967; Kuttler, 1981). In splenectomised animals, as many as 90 percent of the erythrocytes can be infected at the bacteraemia peak (Kuil & Folkers, 1967; Kuttler, 1981). The immune system responds to the increasing bacteraemia by eliminating the infected RBC through opsonisation and phagocytosis (i.e. extravascular haemolysis), leading to gradually declining levels of RBC, HCT and Hb (Yasini *et al.*, 2012). The lowest level of blood parameters (i.e the highest level of anaemia) occurs first a few days after the bacteraemia peak (Kuil & Folkers, 1967; Zwart & Buys, 1968), and this is when the clinical signs of an infected animal are most pronounced (Stoltsz, 2004).

Clinical signs observed in the acute phase of *A. ovis* infection are fever (steady elevated or fluctuating), pallor of mucous membranes, elevated heart and respiratory rates, depression and a marked decline in body weight (Stoltsz, 2004; Smith & Sherman, 2009; Yasini *et al.*, 2012; Neitz, 1968; Barry & Van Niekerk, 1990). Also rumen stasis and constipation might occur (Splitter *et al.*, 1956; Zwart & Buys, 1968; Barry & Van Niekerk, 1990). Abortions have been recorded in the acute phase of the disease (Smith & Sherman, 2009; Yasini *et al.*, 2012), mostly in animals that had developed severe anaemia and high fever (Smith & Sherman, 2009). Goats used for milking can get a marked decrease in milk yield that can persist for several weeks (Neitz, 1968; Smith & Sherman, 2009).

Both for goats and sheep, the infection is most often a subclinical or mild condition with slight weakness and passivity as the only observable signs (Splitter *et al.*, 1956; Zwart & Buys, 1968), but cases of moderate to severe clinical disease occurs especially for individuals with co-infections, malnutrition, pregnancy or other stressors (Stoltsz, 2004; Smith & Sherman, 2009). Acute disease outbreaks have also been described in association with stress factors, such as hot weather, movement of animals, vaccination, deworming, heavy tick infestation and long distance transportation (Khayyat & Gilder, 1947; Manickam, 1987; Friedhoff, 1997).

Early reports (Lestoquard, 1924: see Stoltsz, 2004) suggest that the severity of clinical signs to some extent is connected to the level of the maximum bacteraemia, but Haigh *et al.* (2008) found no correlation between the number of erythrocytes infected and the severity of clinical signs or disease history. On the contrary there are reports of severe anaemia even in animals with low maximum levels of bacteraemia (Splitter *et al.*, 1956; Mallick *et al.*, 1979). There are also findings of animals that show no obvious clinical signs during normal circumstances,

despite low HCT, but when they were herded or exercised, sudden signs of lethargy, severe respiratory distress and pulmonary edema became evident (Splitter *et al.*, 1956; Barry & Van Niekerk, 1990). Acute straining might even cause sudden death in infected animals (Stoltsz, 2004; Smith & Sherman, 2009).

The anaemia caused by *A. ovis* is regenerative, but it may take 1 to 3 weeks for the goats and sheep to develop a proper reticulocyte response (Yasini *et al.*, 2012). The erythrocytes are therefore initially normocytic and normochromic, but gradually, when the erythropoiesis is advancing, macrocytosis, anisocytosis and polychromasia are seen together with presence of Howell-Jolly bodies and basophilic stippling (Stoltsz, 2004; Yasini *et al.*, 2012). In cases with severe anaemia, the decrease in RBC is often greater than the amount of infected erythrocytes. This is due to an autoimmune response where the reticuloendothelial system starts to phagocytise uninfected as well as infected erythrocytes (Uilenberg *et al.*, 1979). In correspondence to the sinking level of RBC, the concentration of the oxygen-carrying Hb decreases (Splitter *et al.*, 1956; Ryff *et al.*, 1964; Zwart & Buys, 1968; Mallick *et al.*, 1979; Barry & Van Niekerk, 1990). This can, in severe cases, lead to hypoxia in various organs, which may be lethal.

The clinical phase of the infection normally lasts one to two weeks before the condition is reversed by increased erythropoiesis (Kuil & Folkers, 1967; Magonigle *et al.*, 1981). The recovery from clinical *A. ovis* infection is often slow, especially when access of nutrition is inadequate (Zaugg, 1987a; Yasini *et al.*, 2012), and the convalescence period may last from several weeks to a few months before the haematological parameters and clinical condition gradually return to normal (Zwart & Buys, 1968). Animals that recover from infection remain persistently infected (Neitz, 1939, 1968; Palmer *et al.*, 1998; Yasini *et al.*, 2012), with cyclic patterns of fluctuating bacteraemia and some longer periods of relatively constant bacteraemia levels (Palmer *et al.*, 1998). The spleen plays an important role in controlling the infection in these animals. In experiments, when the spleen has been removed in chronically infected animals, recrudescence pattern of fluctuating bacteraemia has been seen together with new manifestation of clinical disease (Splitter *et al.*, 1956; Kuil & Folkers, 1967; Kuttler, 1981).

Disease transmission

Bacteria of the genus *Anaplasma* are transmitted biologically by ticks, but also mechanically by biting insects, needles and other instruments (Shompole *et al.*, 1989; Haigh *et al.*, 2008). Most studies of anaplasma transmission have been focusing on tick species, though it has been suggested that the diversity of vector species for *A. ovis* may have been underestimated (de Silva & Fikrig, 1997; Hashemi-Fesharki, 1997; Uilenberg, 1997). Recently the pathogen was found in sheep keds *(Melophagus ovinus)*, suggesting that these lice may act as a reservoir for *A. ovis* (Hornok *et al.*, 2011). Several factors, as increased human travel, animal transport and environmental changes, may contribute further to new possibilities of vector distribution (Renneker *et al.*, 2013).

A frequently used simplification states that ticks of the genus *Dermacentor* are the vectors for *A. ovis* in the New World, while *Rhipicephalus bursa* and other ticks are the vectors in the Old World (Friedhoff, 1997). Several studies have been conducted on different tick species around the world to find out what pathogens they are carrying, and several families within *Ixodidae*

(hard ticks) have been proven capable of transmitting *A. ovis* (Bazartseren *et al.*, unpublished data; Lu *et al.*, 1997; Torina & Caracappa, 2012). During experimental transmission in China, both *Dermacentor nuttali*, *Rhipicephalus pumilo* and *Hyalomma asiaticum kozlovi* was capable of transmitting *A. ovis* between small ruminants (Lu *et al.*, 1997). PCR examination of collected ticks in Mongolia found *A. ovis* in *Dermacentor nuttali*, as well as in *Ixodes persulcatus* (Bazartseren *et al.*, unpublished data). In addition, *Dermacentor silvarum*, *Dermacentor marginatus*, *Dermacentor andersoni* and *Haemaphysalis sulcata* have been reported competent vectors (Torina & Caracappa, 2012)

Even though ticks can become persistently infected with A. ovis (Kocan *et al.*, 2010), there is no known occurrence of transovarial transmission (Stich, 1984). This makes the continued transmission of the disease dependent on reservoirs in nature, consisting of either mammalian or tick hosts with persistent infection (Kocan *et al.*, 2004).

The infection rate of *A. ovis* varies with season and increases in spring/summer when the tick burden increases (Lu *et al.*, 1997). The level of bacteraemia in the infected animals further affects the infection rate, where high levels of bacteraemia in for example immune suppressed animals increase the risk of transmission (Palmer *et al.*, 1998).

Intrauterine transmission of *A. ovis* has been reported in both goats (Barry & Van Niekerk, 1990) and sheep (Donatien *et al.*, 1934: see Stoltsz, 2004). In sheep, transplacental infection was observed during the second and third trimester, but no lesions were observed in the foetuses or lambs (Donatien *et al.*, 1934: see Stoltsz, 2004). In goats, a large proportion of the experimentally infected females aborted or reabsorbed their foetuses when they were exposed to repeated transport during the acute infection phase (Barry & Van Niekerk, 1990). Anaemia was recorded in the foetus *in utero* and *A. ovis* organisms were observed in one to 12 % of the RBC of foetus, live or stillborn kids (Barry & Van Niekerk, 1990).

Epidemiology

Geographic distribution

Ovine and caprine anaplasmosis is endemic in many tropical and subtropical areas in the world and has frequently been reported in temperate regions (de la Fuente *et al.*, 2005b; Hornok *et al.*, 2007; Liu *et al.*, 2011; Renneker *et al.*, 2013). *A. ovis* was first described in Zimbabwe in 1912 (Bevan, 1912) and has since been confirmed to occur in many parts of Africa, North America, Asia (including the Middle and Far East), and the southern and central parts of eastern and western Europe (Splitter *et al.*, 1955; Kuil & Folkers, 1967; Lu *et al.*, 1997; de la Fuente *et al.*, 2006; Hornok *et al.*, 2007; Chochlakis *et al.*, 2009; Papageorgiou *et al.*, 2012; Renneker *et al.*, 2013; Noaman & Bastani, 2016; Pereira *et al.*, 2016).

In the endemic areas, the prevalence rates of *A. ovis* vary considerably, both between and within countries. This variation may be influenced by the movement of livestock between non-endemic to endemic areas, as well as differences in measures of control (Neitz, 1968).

The fact that microscopic examination of blood smears and serological techniques fail to distinguish between *A. ovis* and *A. marginale* (Splitter *et al.*, 1956) leaves some uncertainty about the early findings of *A. ovis* when no other techniques were available. Besides, most of

the research done is cross-sectional studies of the prevalence of *A. ovis*, but little systematic surveillance exists in most countries and the present situation is still rather vague (Renneker *et al.*, 2013). The establishment of a specific PCR for the detection of *A. ovis* DNA (de la Fuente *et al*., 2002, 2007) has however contributed to a lot of new data the latest year.

Host occurrence

Both goats and sheep of all ages are susceptible to infection with *A. ovis* (Splitter *et al.*, 1956; Shompole *et al.*, 1989). The pathogen is generally more pathogenic for goats than sheep, and clinical signs are more frequently observed in goats (Splitter *et al.*, 1956; Zwart & Buys, 1968; Mallick *et al.*, 1979; Barry & Van Niekerk, 1990). The fact that the *A. ovis* is less pathogenic to sheep make them particularly important in the role as subclinical reservoir hosts in areas where both sheep and goats are kept together (Sinha & Pathak, 1966). No age-related difference in susceptibility to *A. ovis* has been shown for goats and sheep (Splitter *et al*., 1956), but older animals generally appear to suffer from greater reduction in HCT in the case of infection (Splitter *et al.*, 1956; Zwart & Buys, 1968).

Besides goats and sheep, also domesticated Mongolian reindeer *(Rangifer tarandus*) is known to be susceptible to *A. ovis* infection (Haigh *et al.*, 2008). The infected animals showed a high tendency to develop severe clinical disease with signs of fever, lethargy, pale mucous membranes and several cases of death (Haigh *et al.*, 2008).

Many attempts have been done to transmit *A. ovis* to cattle to find out whether they are susceptible to the pathogen and, in that case, if cattle can act as reservoirs for *A. ovis*. Several experiments to transmit *A. ovis* to splenectomised cattle have failed (Neitz, 1939; Splitter *et al.*, 1956; Magonigle *et al.*, 1981), suggesting that *A. ovis* is more host specific than *A. marginale*. However, a short term survival has been reported in splenectomised calves (Ryff *et al.*, 1964), and in one study the calves maintained infected in up to 262 days (Kuttler, 1981). After that time, no loss of virulence was observed in the pathogen, but attempts to recover the bacteria from the calf 17 days post infection and subinoculate it into splenectomised sheep failed (Kuttler, 1981).

In a study by Hornok *et al*. (2010) a bacteria much similar to *A. ovis*, (or alternatively a new *A. marginale* genotype closely related to *A. ovis*) was found by sequence analysis of *Linognathus vituli*, the assumed host-specific ectoparasite of cattle. Furthermore, *A. ovis* was found in ticks collected from cattle with anaplasmosis (Hornok *et al.*, 2012). These results support the suggestion that *A. ovis* may be infectious also to cattle.

A zoonotic potential has also been discussed for the pathogen after that an anaplasma with high sequence similarity to *A. ovis* was found in a human patient from Cyprus (Chochlakis *et al.*, 2010).

Wildlife reservoirs

Still today there is a lack of knowledge about the role of wild ruminants in the epidemiology of *A. ovis* (de la Fuente *et al.*, 2005a). White-tailed deer (*Odocoileus virginianus),* blesbok *(Damaliscus dorcas phillipsi),* elk *(Cervus elaphus),* mule deer *(Odocoileus hemionus),* bighorn sheep *(Ovis canadensis Canadensis)* and pronghorn antelope (*Antilocapra americana*) have all

been proven susceptible to experimental infection with *A. ovis* (Zaugg, 1987b, 1988; Neitz, 1939; Kreier & Ristic, 1963; Tibbitts *et al.*, 1992; Zaugg *et al.*, 1996), suggesting that these species could be potential reservoir hosts. The bighorn sheep developed severe clinical signs of icterus and anaemia as a result of the experimental *A. ovis* infection (Tibbitts *et al.*, 1992).

A. ovis has further been identified in naturally infected individuals of bighorn sheep and mule deer in North America (Goff *et al.*, 1993; Yabsley *et al*., 2006), common eland in Kenya (Ngeranwa *et al*., 1988) and Mongolian gazelle *(Procapra gutturosa)* in Northern China (Li *et al.*, 2014). These results corroborate the potential of the species to serve as wildlife reservoirs, but their importance in the epidemiology of *A. ovis* is not yet clarified.

Several of the species mentioned above has also been found to be potential reservoirs for *A. marginale* (Kuttler, 1984), which implicates an increased risk of potential co-infections with the two pathogens. For example white-tailed deer has been confirmed readily infected with both of the pathogens (Kreier & Ristic, 1963). Futhermore, both *A. bovis* and *A. phagocytophilum* were identified in addition to *A. ovis* during sequence analysis of the Mongolian gazelle (Li *et al.*, 2014).

Of what is known, most wild ruminants only get a subclinical to mild infection when naturally infected with *A. ovis*, but experimental splenectomy has demonstrated cases of clinical disease (Neitz, 1939; Kreier & Ristic, 1963). The wild reservoirs of *A. marginale* has so far been more frequently studied and most likely there are more species in the wild that could act as possible reservoirs for *A. ovis* than we know of today (Stoltsz, 2004).

Diagnostics of anaplasma infection

Microscopy

The diagnostics of *A. ovis* has for long been based mainly on microscopic examination of Giemsa-stained blood smears (Ndung'u *et al.*, 1995), because it is a relatively cheap analysis not demanding as advanced and expensive equipment as for serology and PCR. However, blood smear examination is a rather insensitive method requiring experienced personnel (Renneker *et al.*, 2013; Ybanez *et al.*, 2013), and more than 0.1 to 0.2 % of the erythrocytes need to be infected (Shompole *et al.*, 1989). For pre-symptomatic and persistently infected animals, where in general less than 0.1 % of the erythrocytes are infected, blood smear examination is not reliable and the inclusions cannot be differentiated from Howell-Jolly bodies (Shompole *et al.*, 1989; Ndung'u *et al.*, 1995; Noaman, 2013). Nevertheless, microscopic examination is a reasonably useful method for animal in the acute phase of disease, when the level of bacteraemia is high, if considered together with clinical signs and haematological parameters (Splitter *et al.*, 1956). However, in some cases the clinical signs are most pronounced when the level of anaemia is the greatest, i.e. after the bacteraemia has reached its peak and has started to decrease again due to phagocytosis of the erythrocytes (Splitter *et al.*, 1956). Furthermore, the process of erythropoiesis that is ongoing during this phase, contributes to an increased number of basophilic stippling and Howell-Jolly bodies in reticulocytes, which are difficult to distinguish from true anaplasma inclusion bodies (Stoltsz, 2004).

When visible, *A. ovis* can be seen as irregularly shaped, almost spherical granules, staining a deep purple inside the erythrocytes (Stoltsz, 2004). The inclusion bodies of *A. ovis* are indistinguishable from those of *A. marginale* (Splitter *et al.*, 1956). Studies have, however, found, that *A. ovis* inclusion bodies are located in the marginal of the erythrocytes in 60 to 65 % of the cases (Neitz, 1939; Splitter *et al.*, 1956), while *A. marginale* is found marginal in 90 % of the cases (Splitter *et al.*, 1956). The rest of the inclusions have been found either submarginal or central. Also the inclusion bodies of *A. mesaeterum* are morphologically identical to those of *A. ovis*, but for *A. mesaeterum* only less than 30 % of the inclusion bodies are located marginally (Stoltsz, 2004). Additionally, there is a high similarity between the anaplasma inclusions and other intra-erythrocyte structures like Heinz bodies, Howell-Jolly bodies, basophilic stippling in reticulocytes or staining artifacts (Noaman, 2013).

Molecular detection methods

Although anaplasmosis is one of the most common diseases of grazing animals worldwide, there has for long been a lack of rapid and effective tests able to discriminate between subspecies, especially *A. marginale* and *A.ovis* (Torina *et al.*, 2012). Several molecular methods have been used in the attempt to identify *A. ovis*, including PCR for 16S rRNA gene (Liu *et al.*, 2005) and major surface protein 4 (msp4) gene (de la Fuente *et al.*, 2007), reverse line blotting methods (Bekker *et al.*, 2002) and loop-mediated isothermal amplification (LAMP, Ma *et al.*, 2011).

The 16S rRNA is a component of the 30S small subunit of the ribosome and its gene has for long been useful for phylogenetic studies of bacteria, because of the highly conserved gene sequences in combination with hypervariable regions that enables species-specific identification (Coenye & Vandamme, 2003). Some of the anaplasma subspecies are also identifiable by the means of sequencing the 16S rRNA gene, but in the case of *A. ovis*, this gene only differs in two positions from *A. marginale* within the hyper variable region, which is too little to be able to design species-specific primers (Lew *et al.*, 2003; Liu *et al.*, 2005; de la Fuente *et al.*, 2007; Noaman, 2013).

Another gene of interest in anaplasma diagnostics is the gene coding for msp4. This is an outer membrane protein of the bacteria, which is known to play a crucial role in the interaction between anaplasma and the host cells (de la Fuente *et al.*, 2005d, 2006; Kocan *et al.*, 2004; Brayton *et al.*, 2006). The immune system of the host puts a high selective pressure on this protein which makes it likely to evolve more rapidly than other genes (de la Fuente *et al.*, 2005d, 2006; Kocan *et al.*, 2004; Brayton *et al.*, 2006). The protein has orthologs in all anaplasma species examined so far (de la Fuente *et al.*, 2005d), and has been used for phylogenetic studies of *A. marginale* and *A. phagocytophyphilum* (de la Fuente *et al.*, 2002a, 2005a,c). The sequence of msp4 for *A. ovis* has not been as extensively studied, but the reports so far indicate that it is less varying than in *A. marginale* och *A. phagocytophilum* even though there are various geographic and species genotypes (de la Fuente *et al.*, 2007).

Msp4 PCR has been more frequently used the latest years (de la Fuente *et al.*, 2005a,c,d; Torina *et al.*, 2008a; Hornok *et al.*, 2010, 2012), but the primers used has not been specific enough to differ between the *A. marginale* and *A. ovis* by only the means of PCR. Instead it demanded additional analysis, such as restriction enzyme analysis, Southern blot hybridization or

sequencing, which are all rather expensive and time-consuming methods (Torina *et al.*, 2012). Latest years, however, there have been successful attempts to identify hypervariable regions on the msp4 gene, which contains significant sequence differences between *A. ovis* and *A. marginale*, but which is conserved within the strains. By designing primers for those hypervariable regions, it has been possible to identify *A. ovis* by specific PCR assays (Torina *et al.*, 2012; Michelet *et al.*, 2014).

Another nucleic acid method that has been claimed to be successful to differentiate *A. ovis* and *A. marginale* is LAMP. In contrast to PCR, the LAMP reaction is an isothermal technique; i.e. it is performed at a constant temperature and does not require a thermocycler. By using primers designed for the msp4 gene, *A.ovis* was identified with a sensitivity of 95 % and with no cross-reactivity with *A. marginale* (Ma *et al.*, 2011).

Serology

Because of the difficulties with the microscopic examinations of *Anaplasma* spp., several serological approaches have been established during the years in the search for fast and sensitive detection of anaplasma (Noaman, 2013). Antibody titres in sheep and goats are highest during patent bacteraemia and lowest in persistently infected animals (Splitter *et al.*, 1956; Ryff *et al.*, 1964). The majority of the animals remain serologically positive after one year but there are reports about individuals that have converted back to seronegativity (Splitter *et al.*, 1956; Ryff *et al.*, 1964).

The major concern about serology is the occurrence of cross-reactivity between different anaplasma species (Shompole *et al.*, 1989; Noaman, 2013; Ybanez *et al.*, 2013). Competitive inhibition ELISA on the basis of major surface protein 5 (MSP5) has been used for identifying *A. marginale* in several studies, but it cross-reacts with antibodies to *A. centrale, A. ovis* and *A. phagocytophilum* (Palmer *et al.*, 1998). This cross-reaction is due to the fact that there is only one single gene encoding for the MSP5-protein and that gene is well conserved within the genus of *Anaplasma* (Visser *et al.*, 1992).

A complement fixation test for detection of antibodies to *A. marginale* is known to cross-react with *A. ovis* (Splitter *et al.*, 1956; Magonigle *et al.*, 1981; Kuttler, 1984). Nevertheless, the two bacteria are immunologically distinct enough for not providing cross-protective immunity in natural infection (Splitter *et al.*, 1956). For *A. ovis* and *A. mesaeterum* an incomplete cross-immunity has been observed, where goats firstly infected with *A. mesaeterum* later developed a lower level of bacteraemia when infected with *A. ovis* compared to goats without previous *A. mesaeterum* infection (Uilenberg *et al.*, 1979).

Anaplasma ovis in Mongolia

Livestock husbandry in Mongolia

Mongolia is located in northeast Asia, bordered by Russia and China. A population of almost 3 million people on 1,566,500 km^2 makes it one of the least densely populated countries in the world (Landguiden, 2012). The country consists of predominately three different landscape-types: desert, grassland, and forest steppe, with pockets of taiga (i.e. a biome characterised by

coniferous forests), situated in the north-central region of the country along the Siberian-Mongolian border (Papageorgiou *et al*., 2012). The country's high altitudes, large temperature fluctuations, long winters and low precipitation result in a short growing season and limited potential for agricultural development (MOFA, 2016). Nevertheless, the agricultural sector accounts for 34 % of the national GDP owing to the substantial livestock sector (Bazartseren *et al.*, unpublished data). Mongolia's livestock keeping is strongly characterised by its close ties to the traditional nomadic lifestyle, where the herders move their animals and their home several times each year to enable sufficient pasture. Still today, over a third of the Mongolians live as pastoral herders (Papageorgiou *et al*., 2012).

During the 20th century, the close relationship with Soviet Union dramatically influenced the Mongolian agriculture, leading to a collectivization of the farming where the state owned the animals of the families and the number of animals allowed per family member was limited (Jefferies, 2007). After the revolution in 1990, the agricultural cooperatives dissolved and farms were once again privatised (Bruun & Odgaard, 1996). At the same time, the fashion world's demand for cashmere wool peaked, leading to increased investments in goats in Mongolia, being the second largest producer of cashmere wool in the world in the beginning of the 1990's (Lecraw *et al.*, 2005). To keep up with the growing industry and large scale producers that pushed down the prices, the Mongolian herders started to increase their herd sizes (Lecraw *et al.*, 2005). This resulted in a dramatic increase of livestock, especially of goats in Mongolia, and in 2009 the total number had reached 44 million livestock in the country, where goats accounted for 44.7 % and sheep for 43.8 % (MOFA, 2016). The number of goats had then more than quadrupled since 1985 (Worden & Savada, 1989). The expanded herd sizes led to an increased livestock density, which likely contributes to an intensified infection pressure. The higher grazing pressure has also contributed to the on-going desertification (Lecraw *et al.*, 2005)

Reported anaplasma prevalence in Mongolia

For long, little has been known about the TBD in Mongolia, and still today there are few published reports on the situation of *A. ovis* in the country. Nonetheless, *Anaplasma* spp. have been more frequently reported in Chinese ticks, livestock and humans during the last years, which has increased the suspicions that the pathogen could be endemic in the region (Lu *et al.*, 1997; Chahan *et al.*, 2005; Liu *et al.*, 2005). An investigation of ovine anaplasmosis in Northwest China, close to the Mongolian border, took place during 1986 to 1991, revealing that *A. ovis* was widely distributed in goats and sheep in several of the counties (Lu *et al.*, 1997). At some places the morbidity of sheep and goats reached 40 to 50 %, and in one region the mortality due to *A. ovis* was as high as 17 % (Lu *et al.*, 1997)

After the first report of *A. phagocytophilum* among Mongolian humans (Walder *et al.*, 2006), the interest of anaplasma increased in the country. Though, the majority of the studies during the latest years have focused on *A. phagocytophilum* and TBD with pathogenicity to humans (Javkhlan *et al.*, 2014; Masuzawa *et al.*, 2014; Karnath *et al.*, 2015). When several reindeer suddenly died for the Tsaatan people in north-western Mongolia in 2004, and even more reindeer showed fever, lethargy and pale mucous membrane, an investigation started to evaluate the cause. Blood smears of clinically sick animals showed intra-erythrocytic inclusion bodies

resembling *Anaplasma* spp. and in an *Anaplasma ovis*-specific PCR, 80 % of the 66 samples tested positive (Haigh *et al.*, 2008).

A cross-sectional study of free-ranging livestock (cattle, sheep and goats) in northern Mongolia recorded an *A. ovis* prevalence of 61.9 % in Khuvsgul region, obtained by *A. ovis* specific PCR (Papageorgiou *et al.*, 2012). The single sample that was sequenced was identical to the sequence obtained from reindeer in 2008.

Additionally, unpublished data collected in three different Mongolian regions showed that 44.4 % of goats and 49.4 % of sheep were seropositive *for Anaplasma* spp. by IFA. Among the ticks collected from the livestock in the study, 51 % tested positive for *A. ovis* by PCR (Bazartseren *et al.*, unpublished data)

Anaemia in small ruminants

Anaemia, i.e. decreased amount of RBC or Hb, can have many possible causes in small ruminants and is the most common and significant haematological abnormality (Pugh & Baird, 2012). Normal variations in the number of RBC can occur for example during early lactation when HCT tends to decrease. Goats and sheep grazing on high altitude during longer periods can in contrast get elevated HCT and Hb concentrations (Pugh & Baird, 2012). A well-established method used for anaemia-investigation of small ruminants is the FAMACHA© eye colour chart by The Livestock Health and Production Group of the South African Veterinary Association. This is a five-point colour scale, which is compared to the conjunctiva of the animals.

Regenerative anaemia in sheep and goats

The majority of the anaemia in small ruminants is regenerative and caused either by blood loss or haemolysis of the erythrocytes (Pugh & Baird, 2012). Goats have in general a relatively mild regenerative response, even in severe cases of regenerative anaemia (Smith & Sherman, 2009).

Gastrointestinal parasites, primarily *Haemonchus contortus*, are the most common causes to blood loss in small ruminants but also ectoparasites are a possible aetiology (Pugh & Baird, 2012). Haemolysis on the other hand is often induced either by intra-erythrocytic parasites, toxins or chronic diseases (Pugh & Baird, 2012). Immune-mediated haemolysis (IMHA) is not common in goats and sheep, but could occur due to parasitaemia, antibiotic administration and in lambs or kids fed with bovine colostrum (Pugh & Baird, 2012).

Haemolysis is either intravascular (lysis of RBC within the blood vessels) or extravascular (removal of RBC by phagocytes, foremost in the liver and the spleen). Intravascular haemolysis in goats and sheep, with signs as haemoglobinemia and haemglobinuria, is often caused by bacterial toxins, copper toxicosis, or rapid reduction of plasma osmolarity (Pugh & Baird, 2012). *Clostridium perfringens* type A, *Clostridium haemolyticum*, and *Leptospira interrogans* are some of the bacteria capable of producing this type of toxins (Pugh & Baird, 2012). The most common cause of extravascular hemolysis is parasites/bacteria in the RBC, but opsonisation, or ingestion of toxic plants like kale and rapeseed, are other possible aetiologies (Pugh & Baird, 2012). An exceed intake of nitrates, nitrites and copper could also cause

extravascular haemolysis. The most commonly occurring parasites and bacteria within the RBC of small ruminants are *Anaplasma* spp., *Mycoplasma ovis*, and *Babesia* spp (Pugh & Baird, 2012). Extravascular hemolysis may result in icterus and dark urine (Pugh & Baird, 2012).

Non-regenerative anaemia in sheep and goats

Less common cause of anaemia in goats and sheep is a decline in the production of erythrocytes, leading to a non-regenerative anaemia. The most common aetiology is chronic disease which makes the body store the iron in the bone marrow in an unusable form, restraining the erythropoiesis. Iron deficiency as well as selenium- copper- and zinc deficiencies can also result in a mild non-regenerative anaemia (Pugh & Baird, 2012).

The fact that chronic disease can generate anaemia results in a long list of differential diagnosis when anaemia is detected. Conditions as pneumonia, foot rot and malnutrition can be enough to cause anaemia if they have been going on during a longer time (Pugh & Baird, 2012). Acute renal failure, which decreases the erythropoietin production in the kidneys, is another, less common cause to severe non-regenerative anaemia in small ruminants (Pugh & Baird, 2012).

Erythrocyte parameters of goats and sheep

The RBC in ruminants have a long lifespan, about 125 to 160 days (Pugh & Baird, 2012). The goat's erythrocytes are smaller, have a high osmotic fragility and are more prone to haemolysis than the RBC of the sheep (Pugh & Baird, 2012).

Table 2. *Normal erythrocyte parameters for sheep and goats (according to Pugh & Baird, 2012)*

	Sheep		***Goats***	
Measured Entity	**Range**	**Mean**	**Range**	**Mean**
Haematocrit (HCT): %	27-45	35	22-38	28
Haemoglobin (Hb): g/L	90-150	115	80-120	100

A study by Egbe-Nwiyi *et al.* (2000) concluded that erythrocyte parameters are not stable in goats and sheep; RBC, HCT and Hb all fluctuate during the lifespan of the animals. Age is the factor that seems to have impact on all three of the parameters, but for RBC count also sex showed a significant influence. In male goats, the RBC count was high at birth but decreased after three months and eventually became fluctuating. The female goats have a low RBC count the first six months after which it starts to increase and later become fluctuating. The male goats tend to have generally higher RBC counts than females. Among sheep on the other hand, females tend to have higher RBC values than males early in life. Both age and sex also seem to influence the HCT in sheep, while HCT in goats is only influenced by age. Hb is significantly influenced by age in both goats and sheep, increasing early in life, reaches a peak around 2 to 3 years of age after which it slowly decreases (Egbe-Nwiyi *et al.*, 2000).

Furthermore, RBC, HCT and Hb are higher in summer and autumn compared to winter and spring (Smith & Sherman, 2009). Pregnancy seems to have little effect on the RBC parameters,

but the first months of lactation can result in decreased level of HCT (Smith & Sherman, 2009). Stress and strenuous exercise, for example during handling, has proven to have a great influence of the RBC values in particularly goats (Gartner *et al.*, 1969). The increased levels of adrenaline make the spleen contract and release more RBC into the circulation

BCS-scoring of goats and sheep

Assessment of body condition score (BCS) is a hands-on method to estimate the deposition of fat and muscle in the animals. The BCS varies with nutritional and physiologic status and works as a general indicator of the condition of the animal (Smith & Sherman, 2009). Lack of suitable protein and lipid reserves affects the health as well as the milk production and wool quality of the animals.

Studies have described a positive correlation between BCS and HCT, suggesting that the BCS can be helpful in the search for anaemic animals (Yilmaz *et al.*, 2014). Furthermore, a high negative correlation has been found between BCS and FAMACHA©, showing that decreased BCS was significantly related to paler mucus membranes (Yilmaz *et al.*, 2014).

In sheep as well as for cattle, a lumbar system is used for BCS, where the size and shape of the fat and muscles covering the lumbar region, between the dorsal and transverse spinous processes, are evaluated (Pugh & Baird, 2012). This assessment is not suitable in goats since they (especially the milking goats) store the majority of their fat in the omentum and the perirenal tissues (Chilliard *et al.*, 1981: see Smith & Sherman, 2009). Not even obese goats store much of their fat subcutaneously which contributes to the risk of underestimation of the BCS if only the lumbar score was evaluated. Therefore, both lumbar and sternal scores should be evaluated to estimate the BCS in goats. The lumbar score, which is determined over the second to fifth lumbar vertebrae, better reflects the body protein of the goat, while the sternal score is a better measurement of the amount of adipose tissue (Morand-Fehr *et al.*, 1992). The final BCS of goats is an average of these two scores (Smith & Sherman, 2009).

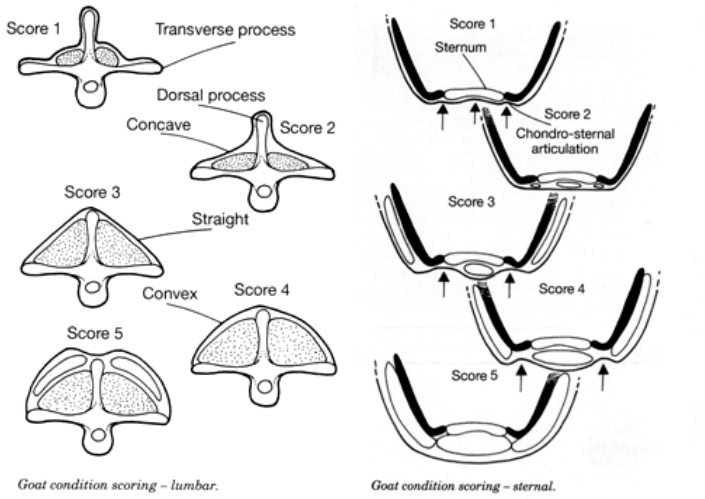


Figure 1. *The body condition scoring system used for goats in the study* (Harwood, 2016).

Both sheep and goats are scored on a scale from 1.0 to 5.0, where 1.0 represents emaciation and 5.0 represents extreme obesity (Pugh & Baird, 2012). The ideal BCS for goats and sheep varies between 2.5 and 4.0 depending on where the animal is in the reproductive and production cycles (Pugh & Baird, 2012; Yilmaz *et al.*, 2014). The BCS charts used for goats in this study can be seen in figure 1.

Pasture influences on infections in goats and sheep

There are considerable differences in the feeding behaviour of sheep and goats, which could influence the health of the animals. Sheep are roughage grazers, mainly feeding at ground level (Smith & Sherman, 2009), and prefers the higher-quality portions of the plant (Pugh & Baird, 2012). Goats, on the other hand, can use a wide variety of different plant material, such as flowers, fruits, leaves, bushes, twigs, and different kinds of grass to fulfil their nutritional requirements (Pugh & Baird, 2012). The upper lip of the goat lacks the dividing philtrum that is present in sheep, and the lips and tongues of goats are very muscular and exhibit a high degree of mobility. This allows great selectivity and favours the grasping and tearing of browse (Smith & Sherman, 2009; Pugh & Baird, 2012). Goats are active foragers that tend to select the highly digestible portions of grasses and are characterised as an intermediate between true grazers and true browsers (Smith & Sherman, 2009). When goats get the chance to choose forage in a pasture with a wide variety of plants, between 50 to 80 per cent of the feed is made out of browse (Pugh & Baird, 2012)

The flexible feeding behaviour of goats has made it a popular livestock in many areas around the world where the pasture is very sparse and poor. Their incredulous capacity to feed on marginal plant growth makes them less sensitive to overstocking, which in turn contribute as a risk factor to the on-going desertification in many countries around the world (Smith & Sherman, 2009).

In surveys where sheep and goats have been allowed to choose their natural pasture behaviour, sheep has shown to generally carry a higher burden of intestinal worms (Le Riche *et al.*, 1973). This is due to the fact that sheep, which are grazing close to the ground, are more exposed to parasites compared to goats that eat from bushes and tall weed at higher levels above the ground (Smith & Sherman, 2009). Goats in this situation develop less immunity to intestinal parasites and are therefore highly susceptible if they are forced to feed on flat and homogenous pasture (Pugh and Baird, 2012). Goats managed in exclusively grazing pastures during prolonged might have equal or greater risk of nematode parasitism compared to sheep (Le Jambre & Royal, 1976).

MATERIAL AND METHODS

Sampling areas and study population

Sample size and inclusion criteria

This cross-sectional study was conducted on 88 heads of goats and 80 heads of sheep in three different areas in Mongolia. In each of the three regions, three nomadic herders were visited, making a total of nine herders. The herders in the study were chosen opportunistic with help from a local contact person with a good knowledge of the selected area. Road conditions, distance from the base camp and the possibility of the herder to devote time and gather the animals, where factors that were taken into account of the selection. An inclusion criterion for participation in the study was that the herder should have mixed herds of goats and sheep with at the least 10 of each species.

The intention was to sample 10 goats and 10 sheep in each herd. To enable this, the goats and sheep were herded into corrals where they were opportunistically individually caught for examination and blood collection. Lactating females with one lamb/kid this season were preferably chosen to achieve a uniform sample group.

Pasture characteristics in different regions

The sampling regions (South Gobi, Bayan Unjuul, and Khuvsgul) were chosen due to type of pasture and vegetation. South Gobi held the dry conditions of a half-desert/steppe on an altitude of 2 200 meters, with a pasture consisting mostly of rocks, low grass and few bushes. The sampling area in Bayan Unjuul, 1 400 meters above sea level, included grassland and steppe pasture with higher grass, more bushes and more frequent precipitation. Khuvsgul was characterised by forest steppe with close reach to streams and thereby a greener area with richer pasture on an altitude of 1 300 meters. GPS coordinates for all sampling sites were recorded using a hand held GPS receiver (Garmin eTrex 10, Garmin, Olathe, USA), and their location is seen in Figure 2.

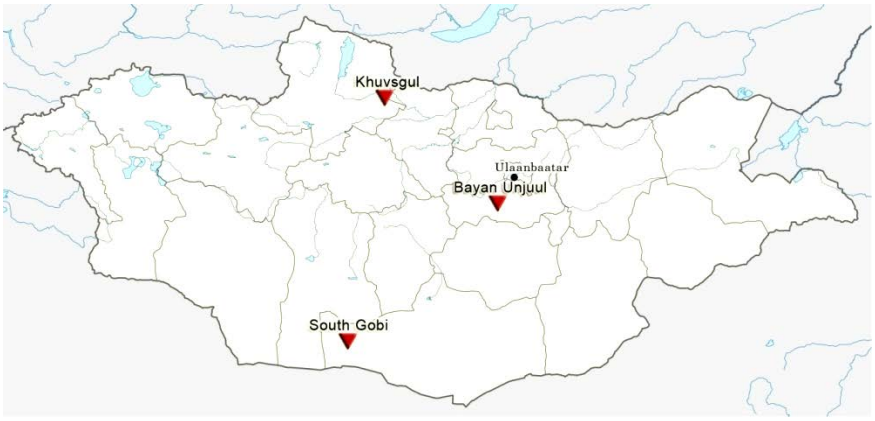


Figure 2. *Location of the three sampling areas; South Gobi, Bayan Unjuul and Khuvsgul*.

Questionnaire

To collect data about herd composition and pasture circumstances a short questionnaire in Mongolian was filled out by the herder prior to the sampling (Appendix 1). The questionnaire was translated into English with help of an interpreter. A majority of the questions were designed with closed questions but for numerical answers, open questions were used. Regarding pasture descriptions, 12 choices of characteristics were available where the herder was free to choose an unlimited number to best describe his/her pasture.

Sampling

Body condition score

Body condition score (BCS) was estimated according to Harwood (2016) for goats and Gård & djurhälsan (2016) for sheep. The scoring stretched from number 1 to 5 in which half numbers were allowed as a score. Since this part of the data collection was included in two studies, both authors did a freestanding, blinded scoring. The final BCS was calculated by the mean value of the two estimations. In this study, BCS scores at or below 2.5 were regarded as 'divergent' values.

FAMACHA© scoring

A FAMACHA© eye colour chart is a validated method for grading anaemia in small ruminants (Kaplan *et al.*, 2004). The mucosa of the lower eyelid of the animal was compared with the laminated eye colour chart calibrated into 5 grades where 1 = red (non-anaemic) and 5 = white (severely anaemic). The data collection was included in two studies, and therefore both authors did a blinded individual scoring. The final FAMACHA© score was then calculated by the mean of the two estimations. In this study, FAMACHA© scores above 3 were regarded as 'divergent' values.

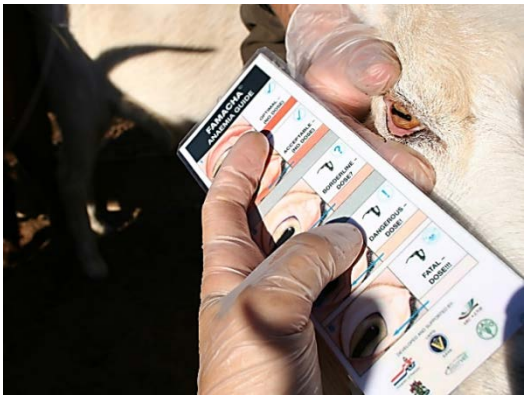


Figure 3. *FAMACHA© card was compared with the colour of the lower inner eyelid of the animal, with a 5 grade scale from1 = red (non-anaemic) to 5 = white (severely anaemic).*

Blood Sampling, storage and preparation

Blood samples were obtained from each animal by intravenous jugular puncture with Vacutainer® needle system (Becton, Dickinson & Co, Franklin Lakes, USA) 18 gauge. Two 4 ml blood tubes where collected from each animal, one additive-free and one containing EDTA. All samples kept stored in a cooling box until blood parameters where analysed, which was

done maximum 12 hours after sampling. Due to the lack of good long-term cooling possibilities, 0.5 to 1ml EDTA-blood from each animal was transferred onto numbered 3MM filter papers (Sartorius, Göttingen, Germany) within one hour after sampling. The same day as sample collection, two thin blood smears were prepared from the EDTA blood of each individual. Both of the blood smears were air dried and fixed in methanol and one of the smears from each individual was additionally stained with Hemacolor® (Merck KGaA, Darmstadt, Germany) according to the manufacturer's instructions. Additionally, within the same day of sampling both Hb and HCT was measured on the anti-coagulated blood with the means of HemoCue® Hb 201+ (Merck, Darmstadt, Germany) respective Compur M1100 Minicentrifuge (Bayer Diagnostic + Electronic Gmbh, Munchen, Germany). Finally, sera were separated from the additive-free tubes and stored in-20 °C for future purposes.

Analyses

Microscopy

The stained blood smears were examined at 100 times magnification on a NikonYS 100 microscope (Nikon, Bangkok, Thailand). Every smear was studied during six minutes for findings of spherical deep purple inclusions in the periphery of the RBC. Only intact, non-overlapping, clearly outlined erythrocytes were evaluated and the microscopic examination was performed blinded from the haematological parameters.

DNA Extraction

DNA was extracted from the dried blood spots on the filterpapers. For each sample, a 1.5x1.5cm was cut into four smaller triangles to make sure that the paper would reach the bottom of the Eppendorf tube and consequently be covered by the extraction buffers. A PCR-template Preparation kit (Roche Diagnostics GmbH, Penzberg, Germany) was used according to the manufacturer's instructions, and the DNA of each sample was eluted in 200µl elution buffer. The extracted DNA samples were analysed in a Nanodrop (Thermo Fisher Scientific Inc, Wilmington, USA) to control the presence of DNA and then kept stored at -20°C until PCR-analysis.

Polymerase Chain Reaction

In total, three PCR-analyses were performed during the study. The first one, involving primers for the 16S rRNA gene of *Anaplasma* spp./*Ehrlichia* spp., was carried out of all samples selected for DNA-extraction. A second PCR, with primers for a region on the msp4-gene in common for *A. marginale, A. ovis and A. centrale,* was performed on the samples that came out positive in the first PCR. Finally, a third PCR, using a primer for a hypervariable region on msp4 that is specific *for A. ovis,* was performed on the samples that were positive in previous PCRs. The nucleotide sequences of the used primers are listed in Table 3.

Table 3. *Nucleotide sequences of primers used in the present study*

Pathogen	Target gene	Oligonucleotide sequences (5′-3′)	Amplicon size (bp)	Reference
Anaplasma spp./ *Ehrlichia* spp.	16S rRNA	Forward: GGTACCYACAGAAGAAGTCC Reverse: TAGCACTCATCGTTTACAGC	345	(Parola *et al.*, 2000)
A. marginale/ A. centrale/ A. ovis	msp4	Forward: GGGAGCTCCTATGAATTACAGAGAATTGTTTAC Reverse: CCGGATCCTTAGCTGAACAGGAATCTTGC	854	(de la Fuente *et al.*, 2002a)
A. ovis	msp4	Forward: TCATTCGACATGCGTGAGTCA Reverse: TTTGCTGGCGCACTCACATC	92	Michelet *et al.*, 2014

16S rRNA

PCR amplifications were performed in a total volume of 10 µl, containing 2 µl template and 8 µl mastermix. The mastermix included 0.5 U Taq Polymerase (TaKaRa), 500nM of each primer (Sigma-Aldrich Sweden AB, Sweden), 250 µM of dNTP:s (TaKaRa), 1X PCR-buffer (10xPCR buffer,TakaRa), and distilled water. In all amplifications, positive controls (containing confirmed genomic DNA of *A. ovis*) and negative control (containing sterile water) was included.

PCR amplifications were carried out in a thermocycler (Amplicon ThermoEx 500 ver 1.2) under the following conditions: 5 min initial denaturation at 95°C, 40 cycles of 1 min at 95°C (denaturation step), 1 min at 53°C (annealing step) and 1 min at 72°C (extension step), followed by a final extension of 7 min at 72°C. PCR-products were analysed by gel electrophoresis (1.5 % agarose gel in 1X TBE buffer) and visualised by ethidium bromide and UV-illuminator. A 1000bp DNA ladder (TaKaRa) was use as a molecular-weight size marker.

Msp4 for A. marginale/A. centrale/A. ovis and msp4 specific for A. ovis

Amplifications of msp4 were performed in a total volume of 25 µl, containing 2 µl template and 23 µl mastermix; 0.2 U Taq Polymerase (TaKaRa), 40 nM of each primer (Sigma-Aldrich Sweden AB, Sweden), 200µM of dNTP:s (TaKaRa), 2.5 µl PCR-buffer (10xPCR buffer,TakaRa) and distilled water. Positive controls (containing confirmed genomic DNA of *A. ovis*) were included in the first two attempts. PCR amplifications were carried out in a thermocycler (Ampicon ThermoEx 500) under the following conditions: 30 sec of initial denaturation at 95°C, 40 cycles of 30 sec at 95°C (denaturing step), 1 min at 60°C (annealing step) and 1 min s at 72°C (extension step) and a final extension of 10 min at 72°C. Visualisation of the PCR-products was conducted as described for 16S rRNA PCR.

Statistical analyses

To accomplish comparisons between different prevalences, either Pearson's $\chi 2$-test or Fisher's exact test was applied. Fisher's exact test was used when comparing small sample sizes and when the contingency table contained a value below five (i.e. when the use of Pearson's $\chi 2$-test is dissuaded). For comparison between mean values, Student's t-test was applied in the case of two sample groups whereas 1-way ANOVA test was used when comparing means of three groups. Exact binomial 95 % confidence intervals (CI) were defined for the proportions and a significance level was set at $\alpha = 0.05$.

Analyses were performed in Microsoft Office Excel 2007, using the XLSTAT statistical analysis software.

RESULTS

Sampling areas and study population

Sample size

The intention of sampling 10 goats and 10 sheep in each herd was partly limited, resulting in final sample group of 168 individuals; 88 (52.4 %) cashmere goats and 80 (47.6 %) fat-tailed sheep. Out of the goats, 97.7 % were females, all of them lactating. The corresponding number for the sheep was 88.8 % females but less than half of them were lactating. Of collected samples, 34.5 % (58/168) was from South Gobi, 29.7 % (50/168) from Bayan Unjuul and 35.7 % (60/168) from Khuvsgul. The detailed distribution of sampled individuals from the different regions is shown in Table 4.

Table 4. *Distribution of sampled goats and sheep corresponding to sampling areas*

		Sampling region										
		South Gobi			**Bayan Unjuul**			**Khuvsgul**			**Total**	**%**
		1	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**		
Goats (Cashmere)	Female	10	10	10	10	10	6	10	10	10	86	97.7
	Male	0	0	0	0	0	2	0	0	0	2	2.3
Sheep (Fat-tailed)	Female	8	9	10	1	7	6	10	10	10	71	88.8
	Male	0	1	0	1	3	4	0	0	0	9	11.3
Total		18	20	20	12	20	18	20	20	20		
			58			50			60		168	

Farm characteristics

All herdsmen included in this study shared a traditional nomadic way of farming. The grazing system was exclusively free range grazing, where goats and sheep were held together as one herd. Out of the 9 herdsmen in the study, 7 shared pasture with neighbouring herders this season. The mean herd size (total number of goats and sheep) in the study was 545 animals (range 250- 1 030). The animal possession total of each herder is shown in Figure 4.

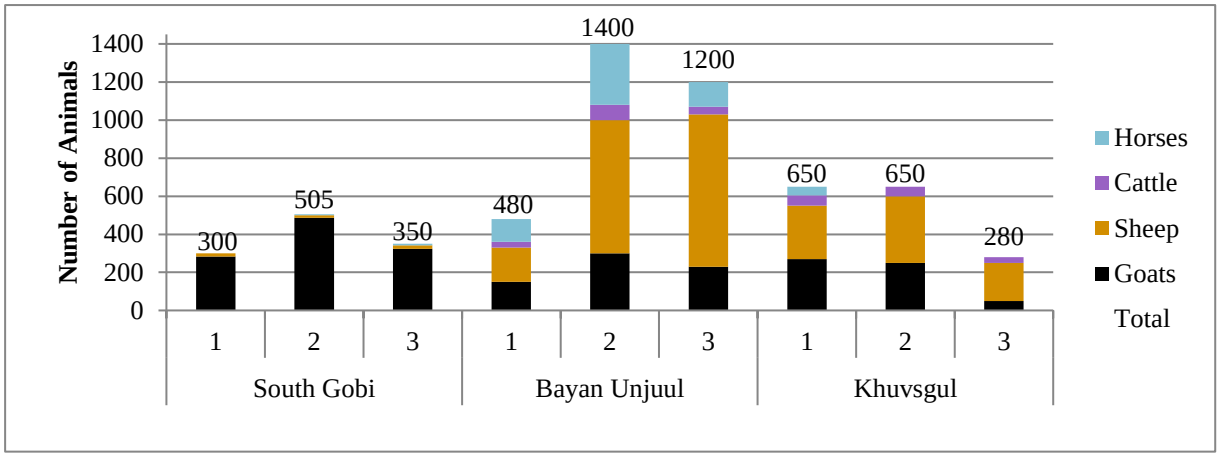
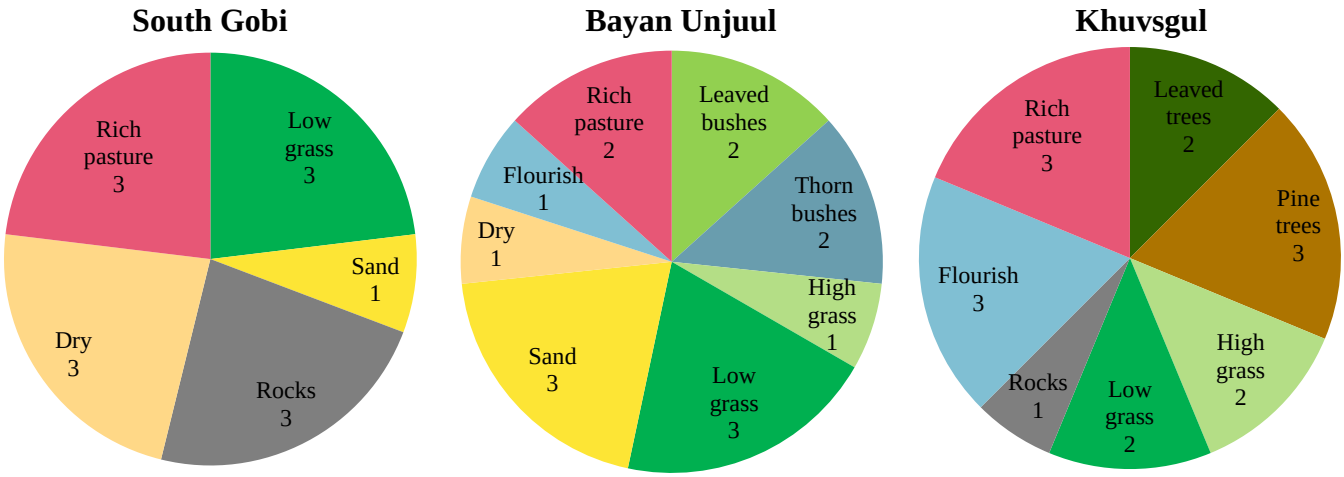


Figure 4. *Herd sizes and animal distribution of the three herders included from each region.*

Pasture characteristics in different regions

All herders included in the study (three in each region), filled in a questionnaire about pasture conditions where they were free to choose an unlimited number out of 12 characteristics (leaved trees, pine trees, leaved bushes, thorn bushes, high grass, low grass, sand, rocks, dry, flourish, rich and poor) to best describe their pastures. An overview of the answers of the herders is shown in Figure 5. In addition to ’rocks’, ‘dry’ and ’low grass’, all herders in South Gobi also filled out ‘rich’ in the questionnaire. When asked, they explained that this was, despite the drought, the greenest year of the area for the last 30 years.



were free to choose an unlimited number out of 12 characteristics (leaved trees, Pine trees, leaved bushes, thorn bushes, high grass, low grass, sand, rocks, dry, flourish, rich, poor) to best describe their pastures. Numbers in the figure indicate how many of the 3 herders in the region who chose that characteristic.

FAMACHA, BCS and Blood parameters

Mean values of Hb, HCT, FAMACHA and BCS of goats and sheep in the study are shown in Table 5. Both for goats and sheep, the mean values of Hb differed significantly between regions, where the lowest mean values were found in South Gobi respective Bayan Unjuul. There were no animals in the study with HCT values below range (data not shown), and regarding mean values of HCT, BCS and FAMACHA in sheep; there were no significant regional differences. For goats, mean HCT was significantly higher in Bayan Unjuul than other regions and mean BCS was significantly lower in South Gobi. The total mean value of BCS did not differ between goats and sheep but the FAMACHA mean value was significantly higher in goats. None of the sheep and goats had HCT values below normal range.

Table 5. *Regional and overall mean values (with standard deviation) of the clinical parameters measured in the study (haemoglobin, haematocrit, FAMACHA and BCS) for goats and sheep*

		Normal Range	South Gobi	Bayan Unjuul	Khuvsgul	Total mean	P-value*
Mean Hb value (g/L)	**Goats**	80-120	85.7 (± 8.9)	99.5 (± 9.8)	95.8 (± 10.8)	93.5 (± 11.4)	< 0.001
	Sheep	90-150	117.6 (± 7.8)	115.5 (± 7.2)	123.4 (± 14.0)	119.2 (± 10.9)	0.0217
Mean HCT value (%)	**Goats**	22-38	31.0 (± 5.2)	34.7 (± 4.7)	31.6 (± 4.8)	32.4 (± 5.1)	0.0128
	Sheep	27-45	37.1 (± 3.9)	37.7 (± 3.8)	38.9 (± 6.4)	38.0 (± 5.0)	> 0.05
Mean FAMACHA score	**Goats**	-	3.3 (± 0.6)	3.1 (± 0.5)	3.3 (± 0.6)	3.2 (± 0.6)	> 0.05
	Sheep	-	2.1 (± 0.6)	2.3 (± 0.6)	2.2 (± 0.5)	2.2 (± 0.6)	> 0.05
Mean BCS score	**Goats**	-	2.5 (± 0.4)	2.9 (± 0.4)	3.0 (± 0.4)	2.8 (± 0.5)	< 0.001
	Sheep	-	2.7 (± 0.6)	2.9 (± 0.4)	2.8 (± 0.4)	2.8 (± 0.5)	> 0.05

* 1-way ANOVA tests were applied

Detection of anaplasma

Microscopy

Microscopic examination, searching for inclusions within the erythrocytes, was performed on all of the 168 samples (Table 6). Overall occurrence of samples with at least one 'anaplasma-like inclusions' was 86.3 % where there was no significant difference between the proportion of goats and sheep with findings. The total percentage of goats and sheep with anaplasma-like inclusions was significantly (P = 0.004) lower in Khuvsgul compared to South Gobi and Bayan Unjuul. The proportions of inclusion-positive sheep did not differ significantly (P > 0.05) between the regions but for the goats was the occurrence of inclusion-like findings was significantly (P = 0.025) lower in Khuvsgul compared to South Gobi and Bayan Unjuul.

Table 6. *Percentage of goats and sheep with peripheral inclusions present during microscopical examination.*

	South Gobi Positive/Tested (%)	Bayan Unjuul Positive/Tested (%)	Khuvsgul Positive/Tested (%)	Total Positive/Tested (%)
Sheep	24/28 (85.7)	22/22 (100.0)	26/30 (86.7)	72/80 (90.0)
Goats	25 /30 (83.3)	27/28 (96.4)	21/30 (70.0)	73/88 (83.0)
Total	49/58 (84.5)	49/50 (98.0)	47/60 (78.3)	145/168 (86.3)

A generally higher number of inclusions were found in each animal in Bayan Unjuul, where the mean numbers of inclusions were 10.9 per individual, compared to 4.4 and 3.4 inclusions in South Gobi and Khuvsgul respectively (data not shown).

PCR

16S rRNA for Anaplasma spp./*Ehrlichia* spp.

The PCR analysis on the 16S rRNA gene, was performed on 56 % (94/168) of the sampled individuals out of which 45.7 % (43/94) where sheep and 54.3 % (51/94) goats. The selection of animals for PCR was firstly based on two criteria; either animals with haematology parameters below the normal range or findings of more than 16 inclusion bodies during the six minutes of microscopic examination. Totally, 21 of the 168 sampled animals met any of these criteria; nine with Hb values below the normal range, nine with more than 16 inclusions and three animals with the occurrence of both above mentioned criteria. None of the sampled animals had HCT values below normal range. Additionally, 73 individuals (35 sheep and 39 goats) were chosen by random selection and included in the PCR. The distributions of animals between the selection criteria, and the proportion of individuals positive for *Anaplasma* spp./*Ehrlichia* spp. in each group is visualised in Table 7.

Table 7. *Outcome in 16S rRNA PCR for the groups; animals with Hb-values below reference, animals with findings of more than 16 inclusions in six minutes of microscopically examination and those with both of above mentioned parameters. Additionally a group chosen by random selection was included*

	Sheep Positive/Tested (%)	**Goats** Positive/Tested (%)	**Total** Positive/Tested (%)
Hb below reference	1/1 (100.0)	3/8 (37.5)	4/9 (44.4)
Findings of > 16 inclusions/6min	8/8 (100.0)	1/1 (100.0)	9/9 (100.0)
Both inclusions and blood parameters	0/0	3/3 (100.0)	3/3 (100.0)
Random selection	28/34 (82.4)	32/39 (82.1)	60/73 (82.2)
Total	37/43 (86.0)	39/51 (76.5)	76/94 (80.9)

In the PCR for the 16S rRNA gene totally 80.9 % (76/94) of the samples were positive. The proportion of positives in the in the random selected group was 82.2 %. There was no significant (P = 1.000) difference between the percentage of positive of sheep and goats, neither in the overall results, nor in the group of random selected animals. The group selected for Hb-values below range held a significantly (P = 0.021) lower proportion of positives than the group of random selected animals. In the group with findings of more than 16 inclusions, all animals were positive on the PCR, but this was not significantly (P = 0.343) higher than the proportion of positive animals in the group chosen by random selection.

Msp4 PCR for A. marginale/A. centrale/A. ovis and specific Msp4 PCR for A. ovis

All samples positive in 16S rRNA PCR were included in the second PCR, amplifying a sequence on the msp4-gene common for *A. marginale, A. centrale* and *A. ovis*. All samples came out as negative, including the positive control, despite several attempts. The same was the case for the *A. ovis* specific msp4 PCR.

Regional- and animal species differences in positivity for *Anaplasma* spp.

Following statistics, describing the occurrence of anaplasma-positivity in the different species and regions, is based on the results of the 16S rRNA PCR including only the animals chosen by random selection.

Out of the total number of PCR-positive individuals, 46.7 % (28/60) were sheep and 53.3 % (32/60) were goats. The proportion of positive animals did not differ significantly ($P > 0.05$) between goats and sheep in any of the regions (percentages shown in Table 8). There was however a significant ($P < 0.001$) difference in the total occurence of PCR-positive animals between the regions, where the proportion of positive animals in South Gobi was significantly lower than in Bayan Unjuul and Khuvsgul.

Table 8. *Occurrence of PCR positivity for 16S rRNA among the randomly selected sheep and goats in the different sampling regions*

	South Gobi Positive/Tested (%)	**Bayan Unjuul Positive/Tested (%)**	**Khuvsgul Positive/Tested (%)**	**Total Positive/Tested (%)**
Sheep	5/10 (50.0)	11/11 (100.0)	13/14 (92.9)	29/35 (82.9)
Goats	3/9 (33.3)	14/15 (93.3)	15/15 (100.0)	32/39 (82.1)
Total	8/19 (42.1)	25/26 (96.2)	28/29 (96.6)	61/74 (82.4)

Correlation between PCR-results and divergent values of BCS, FAMACHA© and haematology parameters

Difference between species

'Divergent' clinical parameters in this study refers to Hb-values below range for respective species (Table 2), FAMACHA© scores above 3 and BCS-score at, or below 2.5. Overall, goats were markedly over-represented compared to sheep regarding occurrence of deviating clinical parameters (Figure 6). Hb values below reference occurred in 7.1 % (12/168) of all sampled animals, and were significantly ($P = 0.005$) more observed in goats 12.5 % (11/88) than in sheep 1.3 % (1/80). Among the PCR-analysed animals, there was a significantly ($P = 0.034$) higher proportion of individuals with Hb-values below reference in the negative group; 27.8 % (5/18) compared to the positives; 9.2 % (7/76).

Regarding divergent FAMACHA© scores, there were significant ($P < 0.01$) more goats (36.4 %; 32/88) than sheep (2.5 %; 2/80) with FAMACHA© scores above 3. Looking at goats included in the PCR, there was no significant ($P = 0.323$) over-representation of divergent FAMACHA© scores among the PCR-positive individuals (43.6 %; 17/39) compared to the PCR-negative (25 %; 3/12).

For BCS, the occurrence of scores at, or below 2.5, was not significantly (P = 0.749) higher in goats (36.4 %; 32/88) than in sheep (33.8 %; 27/80). BCS at or below 2.5 occurred in a larger proportion (P = 0.051) among PCR negative individuals (55.6 %; 10/18) than PCR positive PCR-positive (28.9 %; 22/76). The relationship between species, clinical parameters, and PCR-results is shown in Figure 6.

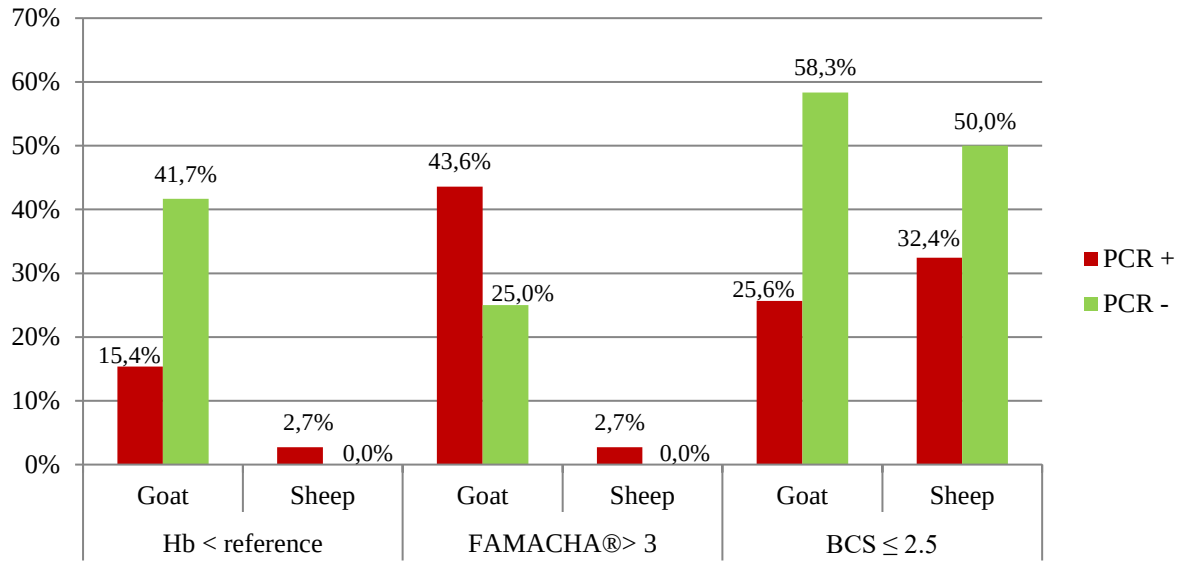


Figure 6. *Comparison of the occurrence of deviating clinical parameters (Hb below reference, FAMACHA > 3 and BCS ≤ 2.5) among the PCR-positive and PCR-negative sheep and goats.*

Differences between regions

In the following text, describing divergent clinical parameters in relation to PCR-positivity on regional level, statistical analyses of the PCR-negative population in Bayan Unjuul and Khuvsgul were not obtainable considering the existence of only one PCR-negative in each of these regions.

For goats, Hb-values below reference occurred to a significantly (P = 0.032) higher extent in South Gobi region (26.7 %) compared to Bayan Unjuul (3.6 %) and Khuvsgul (6.7 %) (Figure 7a). The presence of Hb-values below reference was not higher (P = 1.000) in PCR-positive individuals than PCR-negatives in South Gobi, neither for goats nor sheep.

For FAMACHA, there were no significant (P = 0.583) regional differences between the proportion of animals with scores below 3 for, neither for sheep nor goats. In South Gobi, the only region where it is possible to make comparisons between PCR-positive and PCR-negative individuals, there was a significantly (P < 0.050) higher occurrence of FAMACHA© scores below 3 in the PCR-positive goats (85.7 %) compared to the PCR-negative (27.3 %) (data not shown). In the same region, none of the sheep, PCR-positive or negative, had divergent FAMACHA values.

The occurrence of BCS ≤ 2.5 in goats differed significantly (P = 0.002) between the regions. In South Gobi, 60.0 % (18/30) of the goats scored ≤ 2.5 whereas in Bayan Unjuul and Khuvsgul the corresponding numbers were 32.1 % (9/28) and 16.7 % (5/30) respectively. There was no significant difference (P =1.000) in the occurrence of BCS ≤ 2.5 between the PCR-positive and PCR negative individuals in South Gobi, neither for goats nor sheep. Regarding sheep in the study, there were no regional differences (P > 0.05) in the occurrences of any of the three clinical divergences discussed (7b). The occurrences of divergent Hb-, FAMACHA-, and BCS values in relation to positivity for 16S rRNA is visualised in Figure 7a for goats and 7b for sheep.

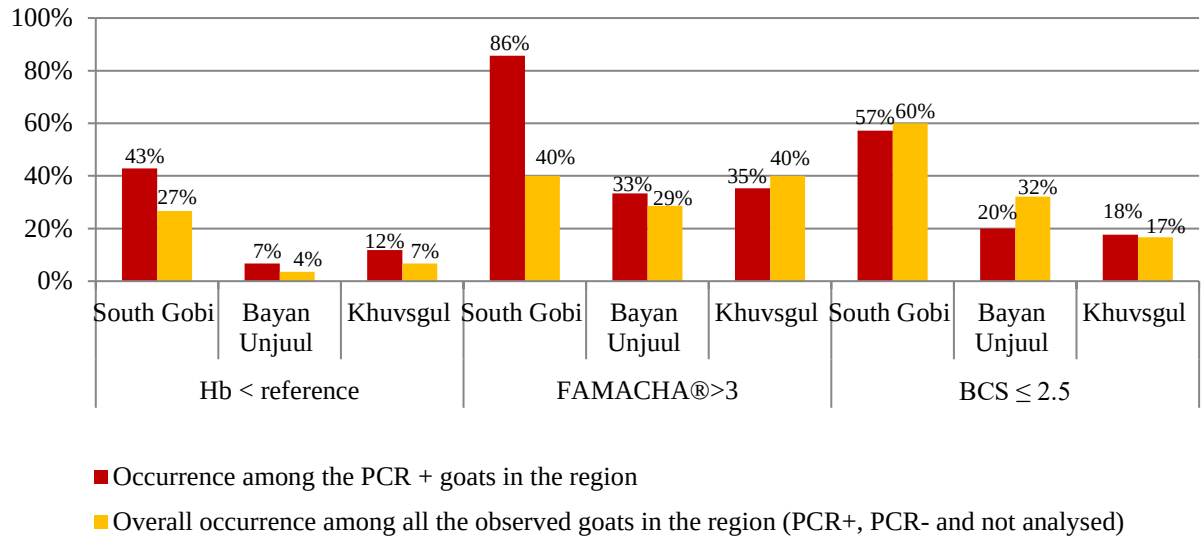


Figure 7a. *The occurrence of divergent values in the clinical parameters Hb, FAMACHA and BCS in 16S rRNA PCR-positive goats compared to all goats sampled.*

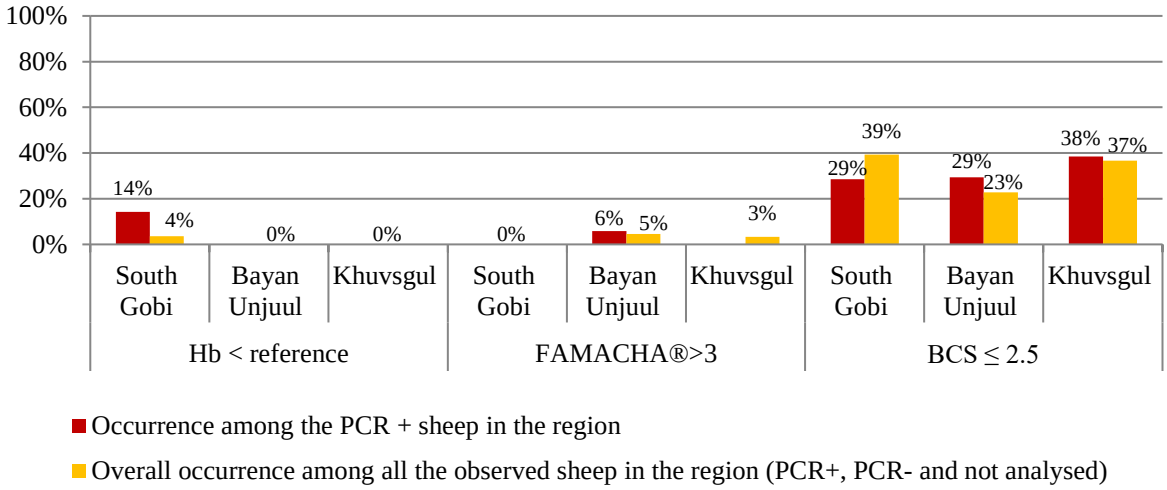


Figure 7b. *The occurrence of divergent values in the clinical parameters Hb, FAMACHA and BCS in 16S rRNA PCR-positive sheep compared to all sheep sampled.*

Comparison between microscopic results and PCR-results

The total anaplasma prevalences found by microscopy (86.3 %) and by PCR of randomly selected individuals (82.4 %) were close by numbers but there was a significant difference between the regionally results. The microscopically obtained prevalence of South Gobi (85.5 %), was significantly ($P < 0.001$) higher than the PCR-based prevalence (42.1%) and in Khuvsgul, the microscopically obtained prevalence (78.3 %) was significantly ($P = 0.031$) lower compared to the PCR results (96.6 %). The prevalences of Bayan Unjuul, 98.0 % and 96.2 %, respectively, did not differ significantly ($P = 1.000$) between the two methods.

For 10.5 % (8/76) of the PCR-positive individuals, no inclusions were found during six minutes of microscopy. Among the PCR-negative animals, 83.3 % (15/18) had one or more structure that was perceived as inclusion bodies during the microscopy.

There was a significant ($P = 0.022$) difference in the mean quantity of findings between the PCR-positive and PCR-negative individuals. In PCR-negatives, on average 2.9 inclusion-like structures was found in six minutes. For PCR-positive animals, the corresponding number was 8.1 inclusion-like structures. Among the animals that were analysed with PCR, all individuals (26/26) that had findings of more than 8 inclusions tested positive for *Anaplasma* spp./*Ehrlichia* spp. However, this only applied for 34.2 % (26/76) of all the PCR-positive individuals.

DISCUSSION

The aim of this study was to investigate the prevalence of *A. ovis* in goats and sheep in Mongolia, and to evaluate the impact of the infection on measurable clinical parameters as BCS, FAMACHA© and blood values. Furthermore, the relation between the sufficiency of the pasture and the tendency among infected animals to present clinical signs was investigated.

As many as 82 % of goats and sheep in this study are positive for *Anaplasma* spp./*Ehrlichia* spp. and no significant differences in prevalence is observed between the species. Regarding the clinical parameters observed, FAMACHA© is the only factor showing a higher occurrence of divergences in infected individuals. Neither Hb-values nor BCS diverges from normal to a higher extent in anaplasma-positive individuals. Assessments of the impact of pasture conditions on the development of clinical disease are limited by the low prevalence of non-infected individuals in the study, making it difficult to estimate the significance of findings in the infected population.

The results obtained, both by microscopic examination (86.3 %) and PCR (82.4 %) indicate a remarkably high occurrence of *Anaplasma* spp./*Ehrlichia* spp. among goats and sheep in Mongolia. Since there was a disagreement between the results of the two methods, especially on regional level, emphasis were put on the PCR-results for further evaluations of clinical signs and species comparisons. This was concluded based on the low sensitivity of microscopic examination of persistent infected individuals together with the low experience of the microscopic examiner.

However, due to the inconclusive msp4 PCR, differentiation of anaplasma subspecies was unsuccessful, and the study thereby fails in the intention to investigate the prevalence of *A. ovis* in the country. The reason behind the unsuccessful msp4 PCR is still uncertain. The positive control for *A. ovis* came out negative together with the samples, indicating a shortcoming in the implementation of the method. There was a question about the liability of the polymerase but another one was not obtainable at the time. In the last attempts, there was a deficiency of positive control, making it difficult to conclude whether it still was the method that failed or if the negative outcome was a result of insufficiency of bacterial DNA in the samples. Since msp4 PCR is less sensitive than 16S rRNA PCR, and demands four to five copies of msp4/ng DNA (Torina *et al.*, 2008b), it is possible that the amount of bacterial DNA in the samples were too low to be detected in second and third PCR. The local veterinarians and laboratory workers (Lkhagvatseren Sukhbaatar, September 2016), stated that *A. ovis* is the dominating anaplasma agent in Mongolian goats and sheep and therefore the most probable agent behind the high anaplasma prevalence found in the study.

Regarding the prevalence of *Anaplasma* spp./*Ehrlichia* spp. in this study, there is no significant difference between goats and sheep in any of the regions, suggesting that the susceptibility to infection is similar in the two species. There is however a significant difference between regions, where the overall prevalence of South Gobi (42.1 %) is markedly lower compared to Bayan Unjuul (96.2 %) and Khuvsgul (96.6 %). The most probable cause of the regionally differences is the distribution of tick species in Mongolia, where *A. ovis* predominantly is found in *Dermacentor nuttali* and *Ixodes persulcatus* (Bazartseren *et al.*, unpublished data). *I.*

persulcatus is most abundant in the forested taiga region and *D. nuttalli* in the forested steppe, while the dominating ticks in the Mongolian desert are *Rhipicephalus pumilio* and *Hyalomma asiaticum* (Dash, 1988). However, both of the latter have also been reported capable of transmitting *A. ovis*, at least experimentally (Lu *et al*., 1997). The tick distribution and their infection rate of *A. ovis* in Mongolia remains uncertain however, as there are few recent publications about the subject and no tick data was collected in this study.

The highest prevalence of *Anaplasma* spp./*Ehrlichia* spp. is found in Khuvsgul, where as many as 92.9 % of the sheep and 100 % of the goats tested positive. The corresponding results for *A. ovis* in Khuvsgul during 2007 to 2008 was 44-64 % in goats and 40-88 % in sheep (Papageorgiou *et al.,* 2012). Due to the non-specification of subspecies in the present study, it is impossible to know whether the high anaplasma prevalences obtained, is due to increased *A. ovis* occurrence, an add-on effect by other anaplasma subspecies or simply a result of false positivity, which have been suggested to occur in 16S rRNA PCR (Torina *et al.*, 2008b). Also the inclusion criterion for the sample population (lactating females), might be contributing to higher prevalences, according to the findings of Papageorgiou *et al.* (2012) where *A. ovis* was more abundant in females than in males. Similar results are not observed in the present study, where all of the PCR-analysed males (seven individuals) are positive for *Anaplasma* spp./*Ehrlichia* spp., but the sampling group is too small to make estimations about the population at large. Moreover, the season of sampling (August and September) should be take into account regarding the prevalence result, as the infection rate of *A. ovis* is known to increase during spring/summer (Lu *et al.,* 1997). The exceptional verdure and greenness that characterised this years' pasture in Mongolia might additionally be of impact regarding the tick prevalence. Finally, there is always the risk of selection bias in the use of opportunistic capture of animals, where individuals that are weakened somehow might be less able to elude capture. However, no signs of weakness or exercise exhaustion were observed during handling. About the accuracy of the 16S rRNA PCR, there is a possibility that PCR inhibitors or degradation of DNA during suboptimal sample storage could contribute to false negative results.

Regarding the impact of anaplasma infection on BCS, FAMACHA and blood parameters, the assessments are partially limited by the high prevalence of anaplasma obtained. Only 18 of 94 individals tested negative for anaplasma, out of which 16 originated from South Gobi. This makes statistical comparisons between infected and non-infected individuals in Bayan Unjuul and Khuvsgul impossible, leaving the significance of findings in positive individuals unknown. In South Gobi however, where comparisons between infected and non-infected individuals are possible, there is a significantly higher presentation of pale mucous membranes in infected goats (85.7 %) compared to non-infected (27.3 %). This suggests that mucous membrane assessment may be a useful complement when trying to identify goats infected with anaplasma. In contrast, none of the sheep in South Gobi, regardless of infection-status, show signs of pale mucous membranes. Neither BCS ≤ 2.5, nor Hb-values below reference values occur to a higher extent in the infected goats and sheep in South Gobi. On the contrary, the prevalence of anaplasma is significantly lower in the animals selected for PCR, based on their low Hb-values, than in randomly selected individuals (Table 7). This indicates that there might be another background to the rather high occurrence of Hb values below range (to be discussed later).

The third aim of the study was to investigate whether the pasture sufficiency as regards the pasture adoptions of the species, influenced their tendency to develop a clinical presentation of anaplasma infection. The hypothesis was that deprivation of browse in South Gobi, and to some extent in Bayan Unjuul, could act as a stress-factor for goats, contributing to an increased occurrence of clinical presentation in infected animals. Similarly, the sheep would have a higher tendency of developing clinical signs in regions as South Gobi where the pasture is sparse and it is difficult to maintain adequate nutrition. However, the fact that the study took place in the end of summer, in what happened to be the greenest year in three decades, made the pasture not fully so sparse that was intended for the study. To evaluate the pasture's impact on infected animals, the clinical signs that were found significant for PCR-positive individuals in the previous objective, were to be compared between regions. This assessment is limited by the low occurrence of PCR-negative individuals. As it is unfeasible to conclude which divergences that are significant for infected individuals in Bayan Unjuul and Khuvsgul, comparisons between the regions are unworkable. The fact that infected goats to a higher extent than infected sheep presents pale mucous membranes in South Gobi is however an interesting finding, regarding the premise that goats in general are superior to sheep in regions with sparse pasture conditions. On the other hand, this might be an example of what has been stated previously about anaplasma infection; that goats are more sensitive and to a higher extent show clinical signs when infected (Splitter *et al.*, 1956; Zwart & Buys, 1968; Mallick *et al.*, 1979; Barry & Van Niekerk, 1990).

Although FAMACHA© is the only parameter in the study that shows a significant correlation with anaplasma infection, there are other interesting observations regarding the results of the studied parameters. Regardless of infection-status, goats in all regions are highly over-represented, both with FAMACHA© score above three and Hb below reference, compared to sheep. As a matter of fact, the mean FAMACHA© score for goats are higher than three in all sampling regions, and on average one unit higher than for sheep (3.2 respective 2.2). These species differences might have several possible causes. For FAMACHA© it can be a result of natural variation where goats may have slightly paler mucous membranes than sheep. If that is the case, it would indicate that the FAMACHA© method is less accurate in goats than in sheep, something that previous has been suggested by (Kaplan *et al.*, 2004). Also the inventors of the FAMACHA© method mentioned in a later report that the range of colours in the conjunctivae in goats might be smaller than in sheep, which could make the FAMACHA© system more difficult to apply (Van Wyk & Bath, 2002). Furthermore, it has been suggested that small breed differences in conjunctivae colour might limit the applicability of the FAMACHA© method. Such differences has been reported for sheep (Moors & Gauly, 2009) while another study, including various breeds of sheep and goats, found no significant differences in applicability (Burke *et al.*, 2007).

The higher occurrence of Hb-values below reference in goats could be due to an age difference between goats and sheep in the study, where goats (which to a higher extent are used for milking in Mongolia) might have had a higher mean age than the sheep. Normal ranges given by textbooks are often based on average values for each species, but the Hb in the individual animal is not constant for sheep and goats. It increases early in life, reaches a peak around 2 to 3 years of age, and then slowly decreases (Egbe-Nwiyi *et al.*, 2000). Unfortunately, no age data was

obtainable for the animals in the study to enable further investigation of this hypothesis. Another possible cause to the observed species differences in Hb and FAMACHA© could be the occurrence of other pathogens or deficiencies, that in this case affects the goats more than the sheep. This, in turn could be related to the higher usage of goats for milking, putting them at higher risk for negative energy-balance and immunosuppression under sparse pasture conditions. Nearly all goats in the study where lactating females, but due to low presence of milking sheep, almost half of the sheep were either non-lactating females, or rams. This might contribute to a bias, but if this circumstance would be the dominating underlying cause behind the observed species differences, a higher occurrence of divergent values would be expected also for sheep in Khuvsgul region, where all sheep included were lactating females. However, none of the sheep in Khuvsgul had divergent Hb values, and FAMACHA© scores above three were less abundant than in Bayan Unjuul where the majority of the sheep were non-lactating females (Figure 7b).

A further observation of interest is that 27 % of goats in South Gobi (out of which the majority were PCR negative for *Anaplasma* spp.) presented Hb-values below reference. This is not only a markedly high number compared to sheep in the region, but also significantly higher than goats in other regions (Figure 7a). The same finding is visualised in the regional Hb mean values, where the goats in South Gobi on average measure 85.7 grams Hb per litre. This is considerably lower than the set mean value for goats (100 g/L, Pugh & Baird, 2012), and significantly lower than in both Bayan Unjuul (99.5 g/L) and Khuvsgul (95.8 g/L, Table 5). No similar divergence is seen for sheep, where all regional mean values are equal with, or higher than the set mean value for goats of 115 g/L (Pugh & Baird, 2012). The aetiology behind these findings remains unknown. An assessment of *Haemonchus contortus*, undertaken on the same study population in Mongolia, found that nematode prevalence was surprisingly low both for goats and sheep in the South Gobi and did not explain the distinctly high occurrence of low Hb-values and high FAMACHA© scores that was found in the region (Ek-Terlecki, Unpublished data).

This study is an additional contribution to the disease mapping of ovine and caprine anaplasmosis. Although a third of Mongolian population relies on small ruminant-dominated livestock for subsistence, few studies of ovine and caprine anaplasmosis have previous been undertaken in the country. The low occurrence of clinical divergences detected in this study, together with the high prevalence (particularly in a region where *A. ovis* previous been confirmed) indicates that it might be a considerable high number of subclinical infected animals in Mongolia. This is far from irrelevant, regarding the fact that animals persistently infected with *A. ovis* may suddenly aggravate if subjected to stress factors as transportations, vaccinations, co-infections or hard weather conditions (Khayyat & Gilder, 1947; Manickam, 1987; Friedhoff, 1997). This fact implicates that apparently healthy animals might become occasional ill due to ordinary events, something that could easily pass unrecognised to both farmers and veterinarians. What is more, recent studies suggest that *A. ovis* might be contributing to gradual, often overlooked, production reductions in persistent infected individuals (Torina & Caracappa, 2012). This indicates that A. *ovis* might be capable of causing extensive losses in small ruminant farming, which could be of major importance in countries like Mongolia, where farmers completely rely on their livestock for sustenance. Furthermore,

in many countries with non-functional banking system, livestock is not only the major source of meat, milk and wool, but are also the main form of capital safekeeping (Okaiyeto *et al.*, 2008).

Altogether, this contributes to a disease profile of *A. ovis*, which seems to be far more unpredictable and complex than what for long has been assumed, particularly considering the increasing number of known wild reservoirs and possible vectors for the pathogen. The most pressing (and challenging) matter for further studies is to disambiguate the holistic picture of *A. ovis* and thereby enable a full assessment of the overall clinical and socioeconomic importance of the pathogen.

CONCLUSION

The occurrence of anaplasmosis was found to be high in Mongolian goats and sheep, with prevalences ranging between 33.3 % and 96.6 % in different regions of the country. Goats and sheep appear to be equally susceptible to infection, with no significant differences in prevalence in any of the regions. The majority of infected animals did not show any obvious divergences in either blood parameters, BCS or FAMACHA©, making it difficult to distinguish the carriers. In South Gobi, however, an increased occurrence of high FAMACHA-scores was seen in association with anaplasma infection, suggesting that examination of mucous membranes might be a useful, but yet insufficient tool in detection of infected individuals. The results of this study indicate that the mucous membrane of goats are in general slightly paler than those of sheep, suggesting that the well-established FAMACHA© scale might be less accurate for goats.

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APPENDIX 1

QUESTIONNARIE (HERDER)

Location: _____ Herd number: _____ Date: _____

1. **How many animals do you herd in this neaby area, approximately?**

   Number:

2. **How many of them are:**

   Goats? Sheep? Cows?

   Horses? Others? (Species and numbers)

3. **Do you share the pasture with other herders during this season?**

   Yes No

4. **How many off-springs (on average) do your:**

   Goats have?

   Ewes have?

5. **Choose between the wordsbelow that best describe the pasture during the last month?**

Leaved trees	Pine trees	Sand
Leaved bushes	Thorn bushes	Rocks
High grass	Low grass	Dry
Rich pasture	Poor pasture	Flourish

Do you consent that we shave a little bit of wool/fur in the neck area prior to blood sampling?

Yes No

Thank you for your cooperation!