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Assessment of nematode parasitism and clinical parameters in goats and sheep in Mongolia

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Rundmaskparasitism och kliniska parametrar hos får och getter i Mongoliet

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SUMMARY

Haemonchus contortus is one of the most pathogenic gastrointestinal parasites of small ruminants in the world and causes major losses and animal welfare issues in the animal husbandry. In Mongolia, over a third of the population lives as pastoral herders and relies largely on animal husbandry for food, and livestock production plays an important role in the national economy. The aim of this study was to compare the occurrence of *H. contortus* in goats and sheep in different environments; desert, grassland and forest steppe. This was done by investigating the occurrence of trichostrongylid eggs and the variations in the clinical parameters in the different locations and host species. Species identification of *H. contortus* was unsuccessful due to complications in the PCR analysis. The study was conducted on 88 heads of goats and 80 heads of sheep from 3 different locations in Mongolia; South Gobi, Bayan Unjuul and Khuvsgul. Faecal and blood samples were collected and FAMACHA© and BCS were estimated for every individual. The faeces were analysed for trichostrongylid eggs (FEC), and blood samples were analysed for haemoglobin and PCV.

The FEC of trichostrongylid eggs showed that 98.2 % of the sampled animals indicated no or low worm burdens, with a mean FEC of 68.8 (± 108.3 SD) for sheep and 67.6 (± 108.8 SD) EPG for goats. Mean FEC of trichostrongylid eggs was significantly lower in the desert region (South Gobi), which is in accordance with previous research in Mongolia. In contrast to previous research, no correlation was found between FEC of trichostrongylid eggs and FAMACHA©. There were host species differences for FAMACHA©, haemoglobin values below reference level and in animals with BCS ≤2.5. Goats scored higher on mean FAMACHA© and had a higher occurrence of animals below reference level for FAMACHA©, haemoglobin and BCS. Differences in locations were found in goats for FAMACHA© >3, haemoglobin values below reference level and BCS. Goats in South Gobi had a high occurrence of values that deviate from the normal range for FAMACHA© and haemoglobin, and with lower mean BCS, differing significantly from goats in other locations. In line with previous studies, there was a significant negative correlations between haemoglobin values and FAMACHA© (r = -0.03) as well as between PCV and FAMACHA© (r = -0.58) in this study. However, no correlation was found between FAMACHA© and BCS in contrast to a previous study.

Species differences were found between common clinical parameters such as FAMACHA©, haemoglobin values and BCS. South Gobi appears to be a less beneficial location for goats, as the clinical parameters have a higher occurrence of values outside the normal range there. However, the causal relationships between the studied clinical parameters and parasitism could not be verified and require further research.

SAMMANFATTNING

Haemonchus contortus är en av de mest patogena gastrointestinala parasiterna hos små idisslare och ger stora förluster och djurhälsoproblem världen över. Över en tredjedel av Mongoliets befolkning lever som nomader och är beroende av djurproduktionen som föda, och djurproduktion spelar en stor roll för landets ekonomi. Målet med denna studie var att jämföra förekomsten av *H. contortus* hos får och getter i olika betesförhållanden; öken, stäpp samt stäpp med skogsinslag. Detta genomfördes genom att undersöka förekomsten av trichostrongylida ägg samt variationer av kliniska parametrar mellan lokalerna samt mellan får och getter. Identifiering av *H.contortus* misslyckades på grund av komplikationer i PCR analysen. I studien undersöktes totalt 88 getter och 80 får från tre olika delar av Mongoliet; South Gobi, Bayan Unjuul och Khuvsgul. För varje individ togs ett träckprov och blodprov, samt en värdering av FAMACHA© och BCS. Träckproven analyserades med avseende på trichostrongylida ägg (FEC) och blodprover analyserades med avseende på hemoglobin och hematokrit.

I studien visade sig 98.2 % av djuren ha en obefintlig till låg nivå av FEC och den genomsnittliga FEC var genomgående lägre än förväntad. FEC var signifikant lägre i South Gobi, vilket överensstämmer med tidigare forskning i Mongoliet. Till skillnad från tidigare studier sågs ingen korrelation mellan FEC och FAMACHA©.

Skillnader mellan får och getter sågs för FAMACHA©, hemoglobinvärden under referensintervall, samt hos individer med BCS $\leq$ 2.5. Getter hade generellt ett högre FAMACHA© -värde, samt hade fler individer utanför referensvärde för FAMACHA©, hemoglobin och BCS. Skillnader mellan lokaler sågs hos getter med FAMACHA© > 3, haemoglobinvärden under referensintervall samt BCS. Getter i South Gobi skilde sig signifikant mot getter i de andra lokalerna då de hade lägre BCS samt fler individer utanför referensintervall för både FAMACHA© och hemoglobin. I studien sågs en signifikant negativ korrelation i studien mellan både hemoglobin och FAMACHA©, samt mellan hematokrit och FAMACHA© vilket överensstämmer med tidigare forskning. Ingen korrelation sågs dock mellan FAMACHA© och BCS till skillnad från en tidigare studie.

Artskillnader sågs mellan flera kliniska parametrar som analyserades och detta skulle kunna påverka hur getter utvärderas i det kliniska arbetet. I South Gobi hade getter flera avvikande parametrar vilket gör att lokalens egenskaper hos framstår som mindre lämpade för getter. Sambanden mellan dessa är ännu inte kända och fortsatt forskning behövs.

CONTENT

ABBREVIATIONS

BCS	body condition score
EPG	trichostrongylid eggs per gram faeces
FEC	faecal egg count of trichostrongylid eggs
L3	infective third stage larva
PCR	polymerase chain reaction
PCV	packed cell volume
RBC	red blood cell

INTRODUCTION

Haemonchus contortus is one of the most pathogenic gastrointestinal parasites of small ruminants in the world. The nematode causes major losses and animal welfare issues in the animal husbandry because of reduced weight gain and even death to the animals because of its blood sucking activities (Radostits *et al.*, 2007). *H. contortus* is well studied and causes much concerns among livestock farmers worldwide, not only due to its pathogenicity but also as a consequence of its rapid adaptations and attainment of anthelmintic resistance (Besier *et al.*, 2016b).

In Mongolia, over a third of the population lives as pastoral herders and rely largely on animal husbandry for food (LPP, LIFE Network, IUCN–WISP and FAO, 2010; Papageorgiou *et al.*, 2013), and livestock production plays an important role in the national economy (Lecraw *et al.*, 2005; LPP, LIFE Network, IUCN–WISP and FAO, 2010).

The main objective of this study was to compare the occurrence of *H. contortus* in goats and sheep in different environments; e.g. desert, grassland and forest steppe. The aim was to investigate the levels of *H. contortus* infection and to relate these to clinical parameters in goats and sheeo from different environments. The hypothesis was that there is less shedding of trichostrongylid eggs in the desert because of the ecology of *H. contortus*, and that goats will be less affected in their clinical parameters in locations where they are able to browse. Because of being preferably browsers, goats will likely be disadvantaged in locations where the pasture give the goats less opportunities to browse.

Specific research objectives:

- Investigate and compare shedding of trichostrongylid eggs in sheep and goats in different pasture environments based on faecal egg counts (FEC).
- Species identification of *H. contortus* by molecular markers.
- Investigate the correlation between anemia and FEC.
- Investigate and compare clinical parameters linked to anemia and animal health in sheep and goats from different environments.
- Correlate clinical findings with *H. contortus*.

LITERATURE REVIEW

Livestock husbandry in Mongolia

Mongolia is located in northeast Asia, bordering to Russia and China. With a population of less than 3 million people dispersed on 1 566 500 km^2, it is one of the least densely populated countries in the world (*Landguiden*, 2012). In general, three different biomes dominate; i.e. desert, grassland and forest steppe. However, in the north-central region of the country along the Siberian-Mongolian border, taiga is the dominant biome (Papageorgiou *et al.*, 2013). The country's high altitudes, large temperature fluctuations, long winters and low precipitation results in a short growing season and limited potential for agriculture (*MOFA*, 2016). Nevertheless, the agricultural sector accounts for 34 % of the country's GDP owing to the substantial livestock sector (Bazartseren *et al.*, unpublished data), which contributes with 85 % of the total agricultural production (LPP, LIFE Network, IUCN–WISP and FAO, 2010).

Husbandry of livestock in Mongolia is characterized by its strong ties to the traditional nomadic lifestyle, and herders move their animals and their home throughout the year to enable sufficient pasture. Mongolians rely largely on animal husbandry for food and over a third of the Mongolians live as pastoral herders (LPP, LIFE Network, IUCN–WISP and FAO, 2010; Papageorgiou *et al.*, 2013).

During the 20th century, the close relationship with the Soviet Union dramatically influenced Mongolian agriculture, leading to a collectivization of the farming and limitations on livestock numbers. After the revolution in 1990, agriculture became less regulated, the agricultural cooperatives dissolved and farms were privatized (Bruun & Odgaard, 1996). At the same time, the fashion world's demand for cashmere peaked (LPP, LIFE Network, IUCN–WISP and FAO, 2010), leading to increased investments in goats in Mongolia, being the second largest producer of cashmere wool in the world in the beginning of the 90's (Lecraw *et al.*, 2005). This has resulted in dramatic increase in the number of goats and larger herd sizes (Lecraw *et al.*, 2005). The amount of goats had in 2009 more than quadrupled since 1985 (Worden & Savada, 1989; *MOFA*, 2016). The expanded herd sizes have led to an increased livestock density, thus intensifying the potential for transmission of various pathogens. The higher grazing pressure has also contributed to the ongoing desertification (Lecraw *et al.*, 2005).

Anemia in small ruminants

Anemia, i.e. decreased amount of red blood cells (RBCs) or haemoglobin, can have many possible causes in small ruminants. Among abnormalities in red blood cell counts of goats and sheep, anemia is the most common. However, there is also normal variation of RBC counts and during early lactation PCV tends to decrease. Goats and sheep grazing on high altitude during longer periods can in contrast get elevated PCV and haemoglobin levels. (Pugh & Baird, 2012)

The most common type of anemia found in small ruminants is regenerative and caused either by loss of blood or haemolysis of the erythrocytes (Pugh & Baird, 2012). Goats do in general have a relatively mild regenerative response, and even in severe cases of regenerative anemia, only a mild to moderate reticulocyte response is usually shown (Smith & Sherman, 2009). The abomasal parasite *H. contortus,* is one of the most common causes responsible for blood loss in small ruminants, but also external parasites such as blood sucking lice and selected protozoans may contribute. Haemolysis, on the other hand, is often induced either by intraerythrocytic parasites, toxins or chronic diseases. The most commonly occurring parasites within the RBC of small ruminants are *Anaplasma* spp., *Mycoplasma* *ovis*, and *Babesia* spp. The most commonly occurring parasites within the RBC of small ruminants are *Anaplasma* spp., *Mycoplasma ovis,* and *Babesia* spp. (Pugh & Baird, 2012).

Non-regenerative anemia is a less common type of anemia in goats and sheep and is most commonly caused by chronic diseases. Conditions such as pneumonia, foot rot and malnutrition may also cause anemia. Furthermore, mineral deficiencies such as in iron, selenium, copper, and zinc can result in a mild non-regenerative anemia. Acute renal failure, is another, less common cause to severe non-regenerative anemia in small ruminants. (Pugh & Baird, 2012)

Blood parameters

RBC in ruminants have a lifespan of about 125 to 160 days. The goat's erythrocytes are smaller, have a higher osmotic fragility and are more prone to haemolysis than those of the sheep (Pugh & Baird, 2012). The standard distribution of erythrocyte parameters can be seen in Table 1.

Table 1. *Erythrocyte parameters for sheep and goats (Pugh & Baird, 2012)*

	Sheep		***Goats***	
	Range	**Mean**	**Range**	**Mean**
Packed Cell Volume (%)	27-45	35	22-38	28
Haemoglobin (g/L)	90-150	115	80-120	100

Egbe-Nwiyi *et al.* (2010) found that the erythrocyte parameters RBC, PCV and haemoglobin fluctuate substantially both in goats and sheep. Age impacts all three parameters, but for RBC, also sex had a significant influence. Male goats tend to have higher RBC values than females. In sheep, females generally have higher RBC values than males early in life. Both age and sex influence the PCV in sheep, while PCV in goats is mainly influenced by age. Haemoglobin concentration is significantly influenced by age in both goats and sheep. It increases early in life, reaches a peak at around 2-3 years of age and thereafter slowly decreases (Egbe-Nwiyi *et al.*, 2010).

Furthermore, the RBC, PCV and haemoglobin proved to be higher in summer and autumn compared to winter and spring. Pregnancy has little effect on red blood cell parameters, but the first months of lactation can result in a decreased level of PCV (Smith & Sherman, 2009). Stress and strenuous exercise, for example during handling, has a great influence on RBC values in goats and can result in elevated levels (Gartner *et al.*, 1969).

FAMACHA©

FAMACHA© eye colour chart is a system developed and validated in South Africa for grading anemia in sheep and goats by comparing the colour of the conjunctiva with a colour chart in a 1-5 scale from; red (1), red-pink (2), pink (3), pink-white (4) or white (5), which is correlated with PCV values of sheep. The purpose of this method is to be an on-farm tool for rapid identification of anemic animals in the management of haemonchosis and thereby reduce the use of anthelmintics by selective targeted treatment of individuals in need or at risk, i.e. only the most anemic animals (Vatta *et al.*, 2001; Van Wyk & Bath, 2002).

FAMACHA© has been thoroughly validated for goats and sheep by Kaplan *et al.* (2004), who found significant positive correlations both between PCV and FAMACHA© eye scores and FEC and

FAMACHA© eye scores. The FAMACHA© method does however appear to be less accurate in goats than in sheep (Kaplan *et al.*, 2004).

Haemonchus contortus

H. contortus is a blood sucking nematode of the abomasum and it is regarded as one of the most pathogenic parasites of small ruminants. It causes major losses and animal welfare issues in the animal husbandry because of weight loss, reduced weight gain and even death to the animals (Radostits *et al.*, 2007). The parasite is well studied and causes much concerns among farmers worldwide, not only due to its pathogenicity but also as a consequence of its rapid adaptations and attainment of anthelmintic resistance (Besier et al., 2016b).

H. contortus belongs to the nematode family Trichostrongylidae (Urquhart et al., 1996) and is one of the major gastrointestinal nematodes of clinical importance in sheep and goats together with *Teladorsagia circumcincta*, *Trichostrongylus* spp. (predominantly *Trichostrongylus colubriformis*), *Cooperia curticei* and *Oesophagostomum* spp. (Smith & Sherman, 2009).

Life cycle and epidemiology

Like other trichostrongylids, *Haemonchus* spp. has a direct life cycle which is divided in to a parasitic phase inside the host and a free-living phase in the external environment. Eggs are shed from mature adult female worms to the pasture via the faeces of the host. The eggs then develop into 1st, 2nd and 3rd stage larvae, where the 3rd stage larvae infect animals grazing on pasture. The larvae then move to the predilection site in the abomasum and moult two more times before reaching its adult phase, which feeds on blood from the mucosa (Urquhart *et al.*, 1996). A single female adult worm can shed up to 10 000 eggs per day (Radostits *et al.*, 2007). The infectious 3rd stage larvae are resilient and can survive on pasture for several months depending on temperature and moisture (O'Connor *et al.*, 2006).

H. contortus is present globally where small ruminants are grazed, with a particularly high prevalence and concern in tropical and sub-tropical climates (Waller, 1997; O'Connor *et al.*, 2006; Radostits *et al.*, 2007). This can be explained by the parasite's requirement and tolerance for warm temperatures and strict need for moisture, but being more sensitive for low temperatures (O'Connor *et al.*, 2006). It is however also present in colder regions on the northern hemisphere (Waller & Chandrawathani, 2005). Because of the widespread prevalence of the parasite, the potential for haemonchosis outbreaks, regardless of climate zones is of great concern (Besier *et al.*, 2016b). In arid and desert regions, *H. contortus* may be of less importance as lack of moisture is a critical limitation for the survival of the external stages. The parasite may still be present, but usually only in smaller numbers and rarely leading to haemonchosis. Periods of heavy precipitation may however lead to favourable conditions for larval development on the pasture and to heavy worm burdens (Besier *et al.*, 2016b) when large numbers of infective larvae rapidly can accumulate (Radostits *et al.*, 2007). O´Connor (2006) reviewed that a monthly precipitation of minimum 50 mm was required for haemonchosis outbreaks according to bioclimatographs developed by early researchers.

Hypobiosis, or arrested development, is a mechanism that allows the 4th stage larvae to survive in the gut mucosa of the host during periods of unfavourable conditions. This mechanism is considered a requirement for the survival of the pathogen for example during extreme cold winters (Waller *et al.*, 2004). Hypobiosis is reported to have led to outbreaks of haemonchosis in the spring because of the resumption of development of overwintered larvae inside the host in spring (Sargison *et al.*, 2007).

Haemonchosis is more common in young animals, but adult animals may also be severely affected (Radostits *et al.*, 2007). A difference has been seen in worm burden between adult goats and sheep and where sheep seems to become more resistant towards *H. contortus* with increasing age than goats when sharing the same grazing pasture (Le Jambre & Royal, 1976; Radostits *et al.*, 2007).

Symptoms and diagnosis

The most obvious and well known clinical sign of *H. contortus* infection in small ruminants is anemia and other effects related to blood loss from the blood-feeding activities of the parasite. Sudden deaths, submandibular oedema and lethargy are also common signs related to the pathology (Urquhart *et al.*, 1996). The clinical effects of haemonchosis depends in the worm burden and the animal's ability to compensate for the loss of blood constituents (Radostits *et al.*, 2007; Besier *et al.*, 2016a).

Haemonchosis usually occurs in an acute form with a varying rate of onset and mortality. The major clinical signs are pale mucus membranes, weakness, and oedema because of rapid blood loss and anemia (Besier *et al.*, 2016a). In a chronic form of haemonchosis, reduced weight gain, lethargy and a mild anemia are due to a sustained small number of worms (Radostits *et al.*, 2007; Besier *et al.*, 2016a). Animals in poor body condition may be clinically affected by smaller worm burdens that otherwise wouldn't harm healthy animals (Radostits *et al.*, 2007).

Post mortem examination and visual inspection of the abomasum is a rapid confirmation in an outbreak of haemonchosis, and burdens with several thousands of adult worms is then a strong indicator of acute haemonchosis. Outbreaks tend to coincide with rainy seasons or after rainfall and this epidemiological data also play an important role to the diagnostics (Besier et al., 2016a).

Fecal egg count is a well-known method related to parasite management in small ruminants, using a quantitative flotation technique such as the McMaster method for enumeration of trichostrongylid eggs in host faeces. A generally accepted guideline is that 0-500 of trichostrongylid eggs per gram faeces (EPG) represents a low parasite burden, 500-2,000 EPG a moderate burden, and more than 2,000 trichostrongylid EPG is considered as a heavy burden (Smith & Sherman, 2009). Contreas *et al.* (1976) *see:* Smith & Sherman (2009) found that goats with FEC of trichostrongylid eggs between 650-4,100 EPG was associated with a 10-30 % mortality associated with *H. contortus.*

Because FEC of trichostrongylid eggs is a quantitative analysis but without the ability to verify the nematode species, direct correlations between egg counts and the severity if infection do not necessarily exist (Pugh & Baird, 2012). For identification of nematodes in the live host, morphology of third stage larvae by microscope was for a long time been standard procedure, but molecular markers have during the last decades enabled species determination that requires less experience and are less time consuming (Schnieder *et al.*, 1999).

However, studies have shown a correlation between FEC of trichostrongylid eggs and worm burden, making FEC a good estimate for affected animals (LeJambre *et al.*, 1971; Roberts & Swan, 1981; Bisset *et al.*, 2001; Rodríguez *et al.*, 2015). Considering anemia to be the main pathologic effect of *H. contortus*, the burden for the animal will be a function of the amount of erythrocyte loss (LeJambre *et al.*, 1971).

FEC of trichostrongylid eggs and PCV have been correlated with the number of *Haemonchus* spp. worms present in the abomasum of sheep (LeJambre *et al.*, 1971) and as stated earlier the correlation between FEC, PCV, Hb and FAMACHA© has been observed in several studies (Bisset *et al.*, 2001; Kaplan *et al.*, 2004; Rodríguez *et al.*, 2015).

Haemonchus in Mongolia

The knowledge on the helminth fauna in Mongolia is limited, and among the few studies that have been conducted, these are published mainly in Mongolian or Russian languages (Sharhuu & Sharkhuu, 2004).

Sharkhuu (2001) examined the prevalence and intensity of helminths of goats from all provinces in Mongolia, divided into the geographic zones: Gobi, steppe and forest steppe. A total of 39 species of helminths were found through post-mortem examinations. *H. contortus* was found in low numbers in the Gobi, and higher numbers in the steppe and forest steppe zones, though the exact prevalence of *H. contortus* is not presented. Nematode infections such as with *Teladorsagia*, *Marshallagia* and *Nematodirus* were also common in all of the three sampled zones. Furthermore, the study found that the prevalence and intensity of the helminth fauna between sheep and goats were slightly different depending on the geographic zones, and the incidence of goat helminths varied with age, season and geographic zone. Namjil (1967), translated by Lkhagvatseren *et al.* (2015), recorded a prevalence of 91.6 % small ruminants infected with *Haemonchus* spp. The sampled area for this study however not described.

A study by Lkhagvatseren *et al.* (2015) showed that 120 naturally small ruminants were infected with trichostrongylid parasites and they had a mean EPG of 303 (min: 50; max: 1250) sampled in a steppe zone in Mongolia before they were treated with one of the anthelminthic groups ivermectin, albendazole or fenbendazole.

The role of pasture for small ruminants

Goats and sheep have different feeding behaviours, playing an important role for the health of the animals. Sheep are grazers, mainly feeding at ground level and prefer the higher-quality parts of the plants, whereas goats are semi-browsers and feed on a wide variety of different plant material (Smith & Sherman, 2009; Pugh & Baird, 2012), preferring to include at least 50-80 % of browse in their diet (Walker *et al.*, 1994). The flexible feeding behaviour of goats has made them an important livestock especially in areas where the pasture is sparse and poor. The ability to feed on marginal plant growth means that they are less sensitive to overstocking and can therefore be kept in larger numbers (Smith & Sherman, 2009). The heavy grazing and browsing pressure can however increase both contamination and exposure of internal parasites on the pasture (Pugh *et al.*, 1998), as well as contribute to desertification (Smith & Sherman, 2009).

In studies where sheep and goats were allowed to choose their pasture, sheep generally carried a higher amount of intestinal worms (Smith & Sherman, 2009) and shed more helminth eggs than goats (Kanyari, 1993). This is considered to be a consequence of their feeding behaviours, i.e. browsing versus grazing, as grazing behaviour increases the exposure of the animal to infective larvae on the pasture (Radostits *et al.*, 2007). Goats therefore develop less immunity to intestinal parasites and are highly susceptible if they are forced to feed on low and homogenous pasture (Le Jambre & Royal, 1976; Pugh & Baird, 2012). Goats exclusively managed in grazing pastures during a prolonged time have equal or greater risk of nematode parasitism compared to sheep (Le Jambre & Royal, 1976).

Plants that contain high levels of condensed tannin have been found to reduce FEC and to a lesser extent worm numbers in goats and sheep. Many of these plants can be included in the goats' browsing diets and can hence reduce their parasite loads (Pugh & Baird, 2012).

The characteristics of the pasture have to be considered when studying the exposure of sheep and goats to nematode parasitism. The landscape and fauna influence not only the nutritional contents and the parasite community, but also the extent to which the host animals get exposed to parasites and their susceptibility to infestation. (Smith & Sherman, 2009)

Body Condition Score of goats and sheep

Assessment of the Body Condition Score (BCS) is a hands-on method to estimate the deposition of fat and muscle in the animals without any need for advanced tools. The BCS varies with nutritional and physiological status and works as a synoptic indicator of the general condition of the animal (Smith & Sherman, 2009).

A positive correlation has been found between BCS and PCV, suggesting that BCS can be helpful in detecting anemic animals. A high negative correlation was found between BCS and FAMACHA©, showing that decreased BCS was significantly correlated with pale mucus membranes (Yilmaz *et al.*, 2014).

In sheep, a lumbar score is used and the size and shape of the lumbar fat and muscles are evaluated (Pugh & Baird, 2012). This assessment is not suitable for goats as they store the majority of the fat reserves in the omentum and the perirenal tissues (Harwood, 2006; Chilliard *et al.*, 1981 *see*: Smith & Sherman, 2009). Also obese goats store little fat subcutaneously, which contributes to the risk of underestimating the BCS if only the lumbar score is evaluated. Therefore, both lumbar and sternal scores should be evaluated when estimating the BCS in goats. The lumbar score, which is assessed over the second to fifth lumbar vertebrae, better reflects the body protein of the goat while the sternal score better reflects the amount of adipose tissue (Morand-Fehr *et al.*, 1992; Harwood, 2006). The final BCS of goats is an average of these two scores (Smith & Sherman, 2009).

Both sheep and goats are scored on a scale of 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 the animal's reproductive status (Pugh & Baird, 2012; Yilmaz *et al.*, 2014).

MATERIAL AND METHODS

Sampling areas and study populations

The study was conducted on 88 heads of goats and 80 heads of sheep from 3 different locations in Mongolia; South Gobi, Bayan Unjuul and Khuvsgul. The locations can be seen in figure 1. The sampling locations were chosen based on the type of pasture and vegetation in order to provide a broad representation of pasture conditions in Mongolia. South Gobi holds the dry conditions of a half-desert/steppe and a low yearly precipitation. The pasture consists mostly of rocks, low grass and few bushes. The sampling area in Bayan Unjuul included grassland and steppe pasture with higher grass, more bushes and more frequent precipitation. Khuvsgul was chosen due to forest steppe with close reach to steams and springs and thereby cover a greener area with richer pasture.

Figure 1. *Map of regions sampled in Mongolia, with red triangles representing South Gobi, Bayan Unjuul and Khuvsgul.*

In each region 3 nomadic herders were visited (making a total of 9 herders) with the intention of sampling 10 goats and 10 sheep in each herd. The herders in the study were selected by a local contact person with a good knowledge of the chosen area. The conditions given for selection was that the herder would have at least 10 sheep and 10 goats. Road conditions, distance from the base camp and the herders' possibilities to devote time and gather the animals where factors that were taken into account for the selection

Table 2. *Distribution of sampled goats and sheep in the different regions of Mongolia*

Region	Herd	Latitude	Longitude	Landscape
South Gobi	1	43° 12.502'	100° 29.528'	Half desert
	2	43° 14.470'	100° 43.835'	Half desert
	3	43° 12.044'	100° 38.188'	Half desert
Bayan Unjuul	4	46° 59.106'	106° 12.190'	Grassland, steppe
	5	46° 59.715'	106° 11.835'	Grassland, steppe
	6	46° 59.556'	106° 12.075'	Grassland,steppe

Khuvsgul	7	49° 43.977'	101° 55.050'	Forest steppe
	8	49° 36.571'	101° 57.659'	Forest steppe
	9	49° 54.827'	102° 05.638'	Forest steppe

The purpose of sampling both goats and sheep was to compare the occurrence of *H. contortus* between host species in different pasture conditions. The grazing systems were predominantly free range grazing, where herders moved their animals during the day.

Sampling

Questionnaire

During the sampling session, a questionnaire (see Appendix 1) in Mongolian was filled out by the herder. The questionnaire was then translated into English with the help of an interpreter. A majority of the questions were designed with closed questions but for numerical answers and use of prophylaxis open questions were used.

Faecal samples

Faecal content was collected rectally from each animal and stored individually in a cooling box until analysis.

FAMACHA scoring

A FAMACHA© eye colour chart is a validated method for grading anemia in small ruminants (Kaplan *et al.*, 2004). The mucosa of the lower eyelid of the animal was compared with the laminated eye colour chart spanning 5 grades where 1 = red (non-anemic) and 5 = white (severely anemic) according to the FAMACHA© method (Malan *et al.*, 2001; Van Wyk & Bath, 2002). 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.

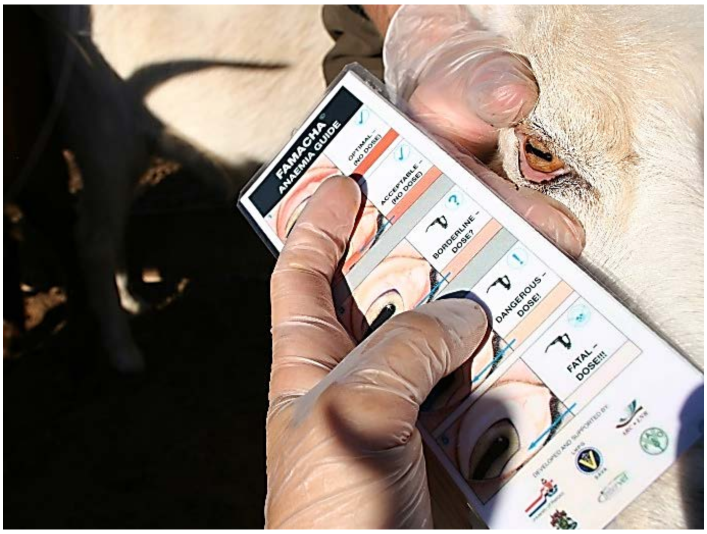

Figure 2: *A FAMACHA© card was compared with the colour of the lower inner eyelid of the animal, with a 5 grading scale from 1 = red (non-anemic) to 5 = white (severely anemic).*

Blood samples

Blood samples were collected from each animal by intravenous jugular puncture into two 4 ml vacutainer tubes, one with EDTA as an additive and one for whole blood. All the blood samples kept stored in a cooling box until further use. Additionally, sera were extracted from the whole blood tubes and stored in -20°C for future purposes.

Body Condition Score

Body Condition Score (BCS) was estimated according to Harwood (2006) for goats and "Informationsbroschyr om hullbedömning av får" (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 blinded individual scoring. The final BCS was calculated by the mean of the two estimations. The BCS charts used for goats in this study can be seen in figure 3.

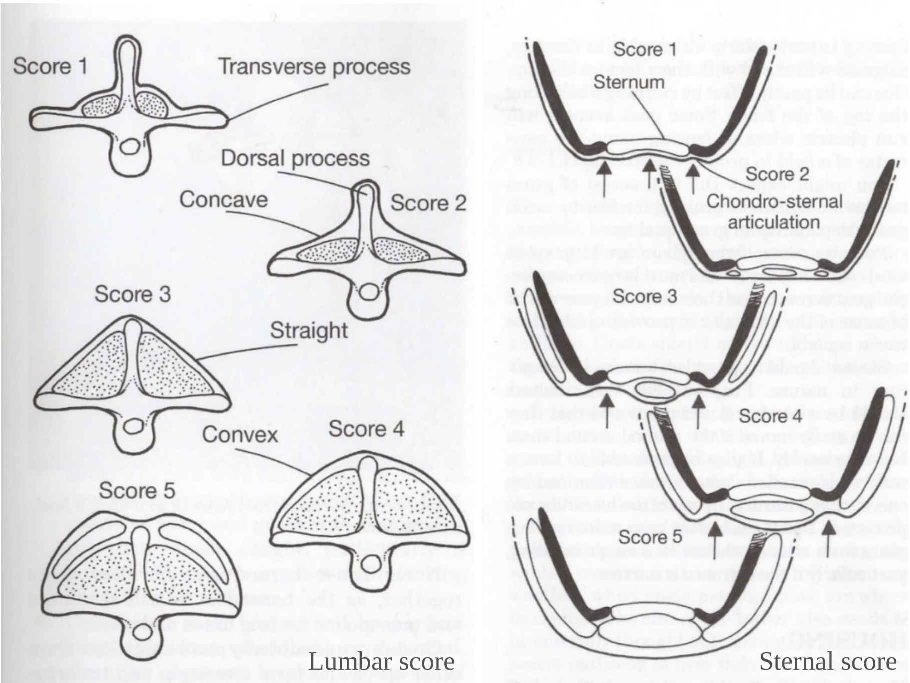

Figure 3. *BCS scoring used for goats in the study. Lumbar condition scoring (left) and sternal conditions scoring (right). Used with permission from Harwood* (2006).

Analyses

Fecal egg counts

A modified McMaster technique (Zajac & Conboy, 2012) for flotation was performed on the faeces 3-16 hrs after sample collection. Two grams of faeces were weighed with a scale with 0.1 gram incitement, thereafter, 28 mL of saturated sodium chloride (NaCl) solution was added. The faecal solution was then run through a tea strainer after it had been soaked for 5 minutes, and immediately filled a McMaster chamber with a plastic pipette. The McMaster chamber was then let to stand for at least 5 minutes so the eggs would be able to float to the surface before trichostrongylid eggs were counted in a microscope run by solar light at 100x magnification.

The number of trichostrongylid eggs per gram (EPG) faeces was calculated by multiplying the total number of trichostrongylid eggs found in the two chambers by a factor of 50 meaning that each trichostrongylid egg found in the McMaster chamber represents 50 eggs per gram faeces. (Zajac & Conboy, 2012)

Blood samples

Within the same day of sample collection (2-6 hrs) haemoglobin and PCV (packed cell volume) was measured from the EDTA-blood with HemoCue® Hb 201+ analyzer and Bayer® Comphur M 1100 Mini centrifuge respectively.

Larvae culture and DNA Extraction

Individual faecal samples were put into plastic containers with fenestrated lids and the faeces was broken up and stirred. The samples were first kept under field conditions for 3-7 days (with the daily sample temperature ranging from +9.5°C to +32°C) and at night kept warm by human warmth. A small amount of water was added to moisten the faecal sample if needed. The faeces were then incubated for 3-7 additional days in a controlled incubator at +25°C making the total incubation time at least 10 days for all samples.

Larvae were collected by adding water to the samples and a petri dish on top and then turned upside down before filling the petri dish with water. The larvae then migrated out into the water and could be harvested after 3-6 hours with the help of a plastic pipette.

DNA was extracted from cultivated larvae's using NucleoSpin® Tissue kit (Clontech Laboratories, Inc.) according to the manufacturer's instructions. Briefly, at least 5-10 L3 were collected and subsequently pre-lysed in 180 µL lysis buffer and 25 µL proteinase K overnight. After adding 200 µL of binding buffer and incubation 10 min at 70°C to lyse the sample, 210 µL 96 % ethanol was added and then vortexed to adjust DNA binding conditions. The complete volume was then loaded in NucleoSpin Tissue Column and centrifuged at 11 000 x g, then washed with 500 µL washing-buffer and dried by centrifugation. The DNA was finally eluted by using 100 µL elution buffer and centrifuged at 11 000 x g.

Polymerase Chain Reaction

The samples were screened for the presence of *H. contortus* targeting the ITS-2 (internal transcribed spacer region 2) of the ribosomal RNA gene, using species specific primers (Wimmer *et al.*, 2004). The primer sequences used was HcFor 5´ GTTACAATTTCATAACATCACGT 3´ and HcRev 5´ TTTACAGTTTGCAGAACTTA 3´.

PCR amplifications were performed in a 25 µl final volume containing PCR buffer, 0,4 µM of each primer, 200 µM of each dNTP (dGTP, dCTP, dATP and dTTP), 2mM of $MgCl_2$, and 1 U AmpliTaq Gold polymerase. Thermocycling conditions were 94°C for 10 min followed by 40 cycles of 94°C in 30 s, 50°C in 30 s and 72°C in 30 s followed by 72°C for 10 min and 4°C until finish.

PCR product was expected to be 325 bp.

Statistical analyses

The statistical analyses were conducted in RStudio© 2009-2016 (version 0.99.903), an open source interface for the programming language R. An α-level of 0.05 was used for the critical values. Results were considered significant when $p<0.05$ and the critical values were reached or exceeded by relevant statistics for the different tests.

One-way ANOVA analysis was used when testing the differences in haemoglobin since the data was normally distributed. Kruskal-Wallis one-way analysis of variance was used when testing the differences in FAMACHA©, BCS and EPG between sites and species. When the test results were significant, post hoc Wilcoxon rank-sum tests were performed for the above mentioned parameters except haemoglobin. The mean and standard deviation was calculated for all groups in Excel 2013.

To analyse the prevalence of animals with values outside the normal variance for haemoglobin (Table 1), FAMACHA© (> 3) and BCS (≤ 2.5), logistic regressions were run on the binomial variances where 1 = outside normal variance and 0 = within normal variance. Generalized linear models were used when conducting the logistic regressions as they account for differences in sample sizes. Chi^2 tests were then performed on the logistic regressions.

Spearman's rank correlations were performed to identify covariance between two variables. The covariance between FAMACHA© and haemoglobin as well as between FAMACHA© and EPG was tested.

RESULTS

Sampling areas and study population

A total number of 168 animals were sampled for the study. Totally 88 (52.4 %) of them were cashmere goats and 80 (47.6 %) were 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 the 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 can be seen in Table 3.

Table 3. *Distribution of species and gender of sampled animals in the different locations*

		Sampling region										
		South Gobi			**Bayan Unjuul**			**Khuvsgul**			**Total**	**%**
Herd number		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**		
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 of the visited herders 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 herders in the study, 7 shared pasture with neighbouring herders. The mean herd (refer to the total number of goats and sheep) size in the study was 545 animals (range 250- 1 030). All herders in Bayan Unjuul (3/3) and Khuvsgul (3/3) kept cows. Of these, the mean cow herd was 47.5 (± 18.9 SD) cows (range 30-55). No herder in South Gobi kept cows. Horses were kept by 6 of the 9 herders and the mean was 104.7 (± 118.4 SD) horses per herder (range 5- 320).

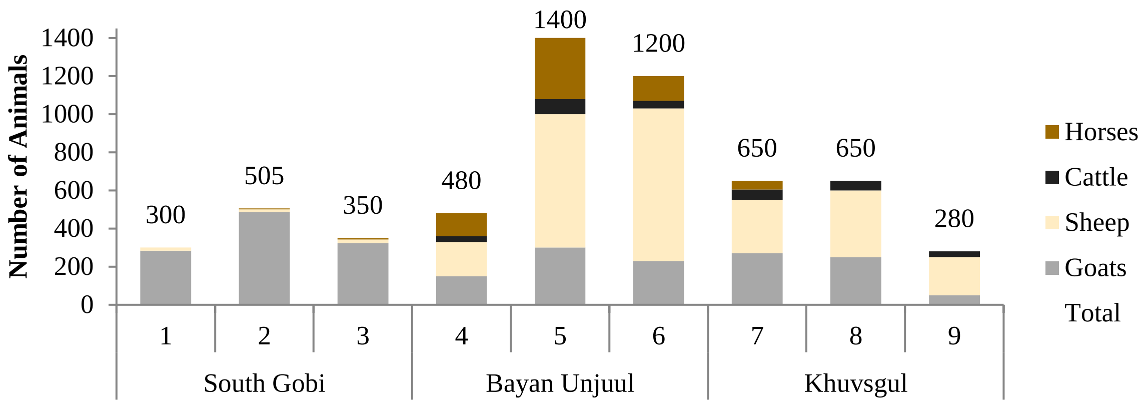

Figure 4. *Herd (1-9) sizes and animal distribution of the herders in the study.*

Pasture characteristics in different regions

According to the questionnaire, all (3/3) herders in South Gobi chose “low grass”, “dry” and “rocks” as a description for their pastures and one (1/3) additionally chose “sand”. In Byan Unjuul, all (3/3) herders chose “low grass” and “sand”. Two (2/3) also filled out “leaved bushes” and “thorn bushes” and one (1/3) chose “High grass”, “dry” and “flourish”. Khuvsgul was described as “flourish” by all (3/3) herders. Two (2/3) added “low grass” and “high grass” and one (1/3) herder filled out “rocks”. For the description “rich pasture”, all of the herders in South Gobi (3/3) and Khuvgul (3/3) agreed with this description, and two (2/3) herders in Bayan Unjuul did as well. When asked, the herders in South Gobi explained that this was the greenest year of the area for the last 30 years. The herders’ description of their pastures can be seen in Figure 5

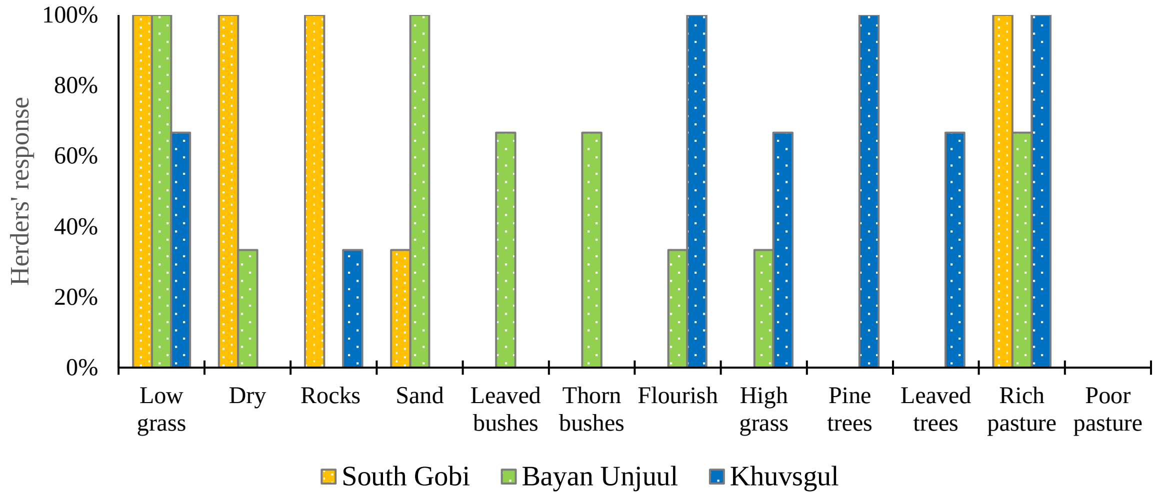

Figure 5. *Distribution of the three herders’ pasture descriptions in each sampling region. The herders were free to choose an unlimited number of the 12 characteristics in a questionnaire to best describe their pastures.*

EPG counts

The mean FEC of trichostrongylid eggs was for sheep 68.8 (± 108.3 SD) and for goats 67.6 (± 108.3 SD) EPG. The highest mean values were measured in Khuvsgul for sheep (143.3 ± 136.9 SD) and for goats (123.3 ± 160.7 SD). Out of the sampled animals, 50 % (84/168) were measured with trichostrongylid FEC = 0 EPG and 48.8 % (82/168) had a low burden between 50 and 450 EPG (Table 4). Only two animals (1.2 %) had a FEC ≥ 500 EPG and both were goats sampled in Khuvsgul.

Table 4. *Distribution of trichostrongylid EPG between locations and species*

	Location							
FEC	**South Gobi**		**Bayan Unjuul**		**Khuvsgul**			
Trichostrongylid eggs	*Sheep*	*Goats*	*Sheep*	*Goats*	*Sheep*	*Goats*	**Total**	**%**
No 0 EPG	28	25	10	6	7	8	**84**	50
Low 50-450 EPG	0	5	11	23	23	20	**82**	48.8
Moderate 500-750 EPG	0	0	0	0	0	2	**2**	1.2
							168	100

There was a significant difference in trichostrongylid EPG values between the different locations both for sheep ($P < 0.0001$) and goats ($P < 0.0001$), with South Gobi scoring the lowest numbers and Khuvsgul the highest (Fig 6). However, there were no significant difference in EPG between goats and sheep, within locations ($P > 0.05$).

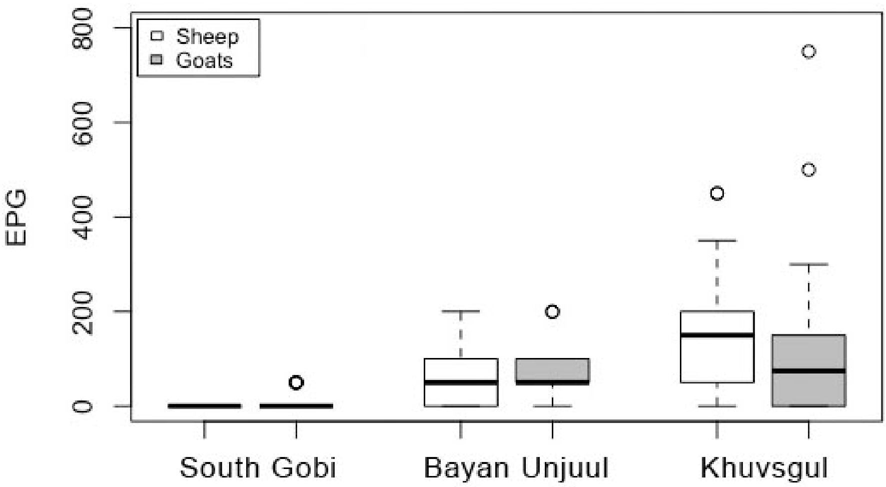

Figure 6. *Distribution of EPG of trichostrongylid eggs between sheep and goats, and sampling regions. There was a significant difference between locations for goats (P < 0.0001) and for sheep (P < 0.0001), as well as between locations (P < 0.0001).*

FAMACHA© score

The mean FAMACHA© scores were for sheep (3.2 ± 0.6 SD) and goats (2.2 ± 0.6 SD). There was a significant difference between sheep and goats ($P < 0.0001$, Fig. 7) regardless of location, and also a significant difference between sheep and goats within each of the three locations ($P < 0.001$) South Gobi, Bayan Unjuul and Khuvsgul. However, there were no significant differences for either goats or sheep between locations ($P > 0.45$).

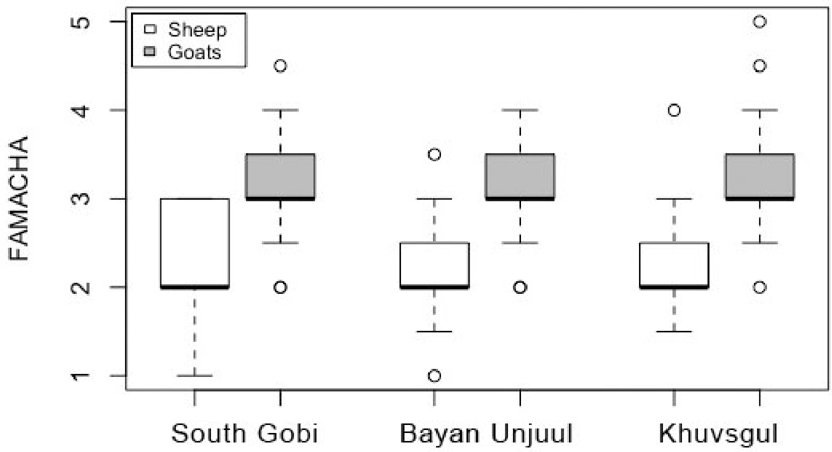

Figure 7. *Distribution of FAMACHA© between sheep and goats, and sampling regions. There was a significant difference for FAMACHA© between sheep and goats (P << 0.0001) and within each location (P < 0.001), where the goats in general scored lower values.*

When comparing all the animals with FAMACHA© > 3, there was a significant difference (P < 0.0001, Fig. 8) between goats and sheep, with more goats scoring above 3. Out of the sampled goats in each location, 40 % were above the given range of FAMACHA© for South Gobi and Khuvsgul, and 29 % in Bayan Unjuul. The corresponding percentages for sheep were 0 % in South Gobi, 5 % in Bayan Unjuul and 3 % in Khuvsgul. There was also a significant difference between sheep and goats within the locations South Gobi (P < 0.0001) and Khuvsgul (P < 0.001)

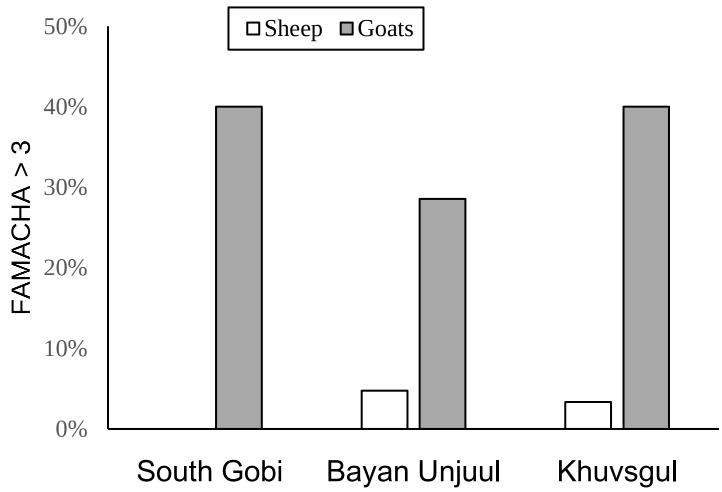

Figure 8. *Distribution of FAMACHA© > 3 between sheep and goats, and sampling regions. There was a statistical difference (P << 0.0001) between sheep and goats regardless of location, and a statistical difference between sheep and goats in South Gobi (P < 0.0001) and Khuvsgul (P < 0.001).*

Haemoglobin

The mean haemoglobin level was for sheep 119.2 (± 10.9 SD) g/L and for goats 93.5 (± 11.4 SD) g/L. There was a significant difference for goats (P < 0.0001) and sheep (P < 0.022) between locations, as well as a significant difference (P < 0.023) for animals between locations (Fig. 9). South Gobi was the location with the lowest mean Hb levels for goats, Khuvsgul was the location with the highest mean Hb levels for sheep.

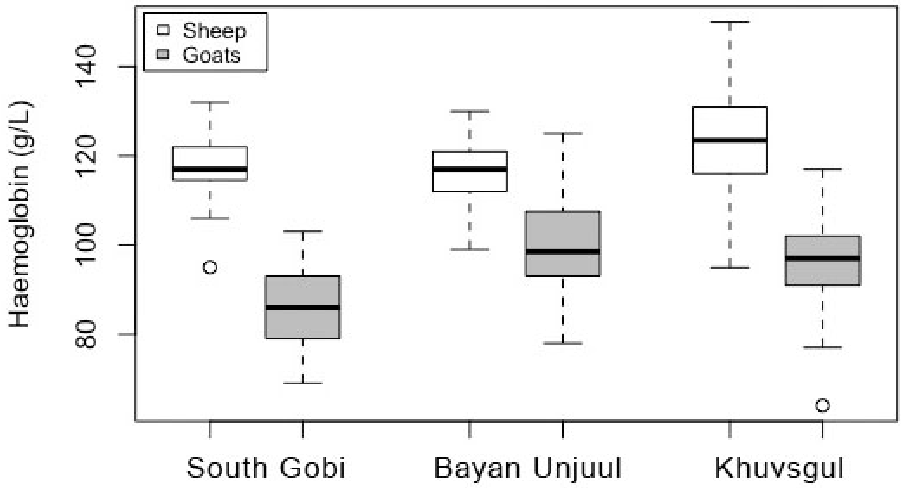

Figure 9. *Distribution of Hb between sheep and goats, and sampling regions. There was a significant difference for goats (P < 0.0001) and for sheep (P < 0.022) between locations.*

For Hb levels below the optimal range for each species, 44 % of the goats in South Gobi, 11 % in Bayan Unjuul and 7 % in Khuvsgul were measured below the given range for goats. No sheep was measured with a Hb-level below the range for sheep. There was a significant difference (P < 0.01, Fig. 10) for goats between locations, and between sheep and goats within South Gobi (P < 0.001). No significant difference was shown between sheep and goats in Bayan Unjuul (P > 0.55) or Khuvsgul (P > 0.09).

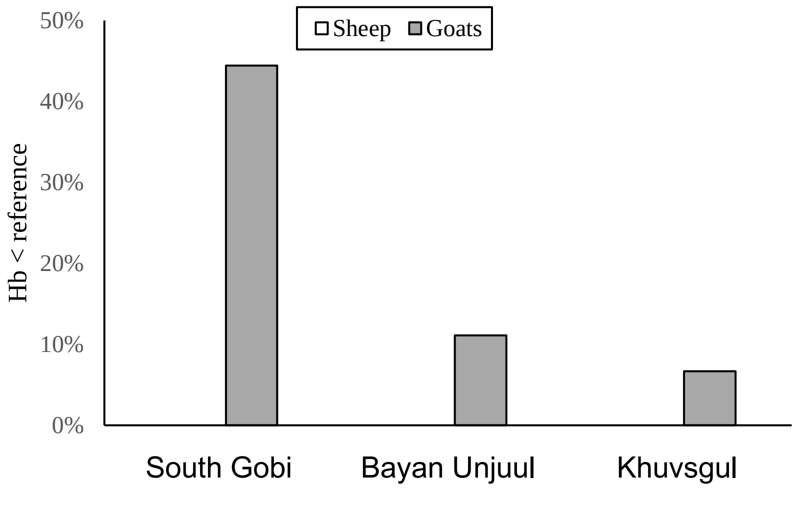

Figure 10. *Distribution of Hb below optimal range, for sheep and goats, and sampling regions. There was a significant difference (P < 0.01) for goats between locations, and between sheep and goats within South Gobi (P < 0.001). No significant difference was shown between sheep and goats in Bayan Unjuul (P > 0.55) or Khuvsgul (P > 0.09).*

PCV

The mean PCV for the sheep was 38.0 % (± 5.0) and for the goats 32.4 % (± 5.1). There was a significant difference (P > 0.02) for goats between locations, but no difference (P > 0.05) for sheep (Fig. 11). No animals were considered below the lower optimal range for the species.

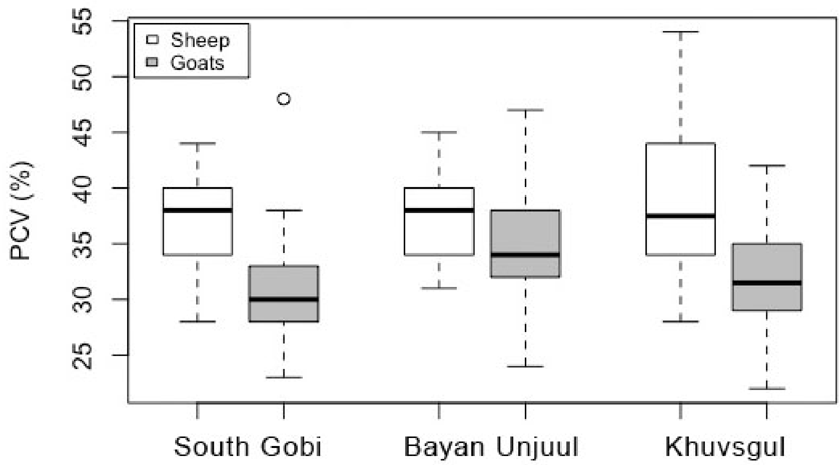

Figure 11. *Distribution of PCV between sheep and goats, and sampling regions. There was a significant difference for goats (P < 0.02), but not for sheep (P > 0.05) between locations.*

Body condition score

The mean BCS values were nearly identical for sheep and goats (2.8 ± 0.5 SD). There was a significant difference of BCS for goats (P < 0.0001, Fig. 12) between locations, where the goats in South Gobi scored the lowest values. There were no significant differences between sheep and goats, within the location, or without taking the location in consideration.

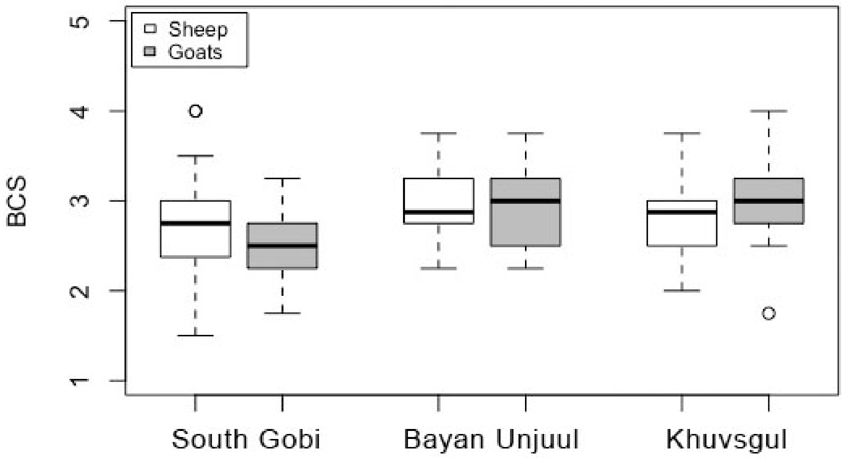

Figure 12. *Distribution of BCS between sheep and goats, and sampling region. There was a difference (P < 0.0001) for BCS of goats between locations, where the goats in South Gobi scored lower values.*

When comparing the animals with BCS ≤ 2.5, there was a significant difference (P < 0.0017, Fig. 13) for goats between locations. Out of the sampled goats, 60 % in South Gobi were at or below the given lower range of BCS, 32 % in Bayan Unjuul, and 17 % in Khuvsgul. The corresponding relations for sheep were 37 % in South Gobi and Khuvsgul, and 24 % in Bayan Unjuul. There was a significant difference (P < 0.015) between locations regardless of species, where South Gobi had the highest proportion of animals below the criteria for BCS. For sheep between locations, there was no such significant difference (P > 0.48) between locations.

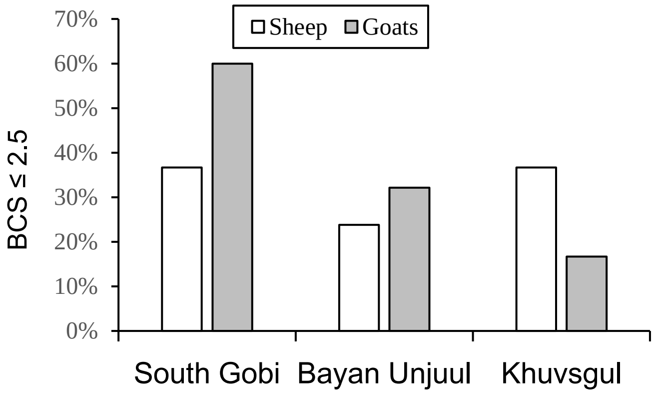

Figure 13. *Distribution of BCS ≤ 2.5 between sheep and goats, and sampling regions. There was a statistical difference (P < 0.0017) for goats between locations, where the goats in South Gobi scored lower values.*

Correlations

There was a significant negative correlation (P < 0.0001, Fig. 14) between haemoglobin values and FAMACHA© score (r = -0.03) as well as a significant negative correlation (P < 0.0001, Fig. 15) between PCV and FAMACHA© score (r = -0.58). No significant correlation was found between EPG and FAMACHA©, or between BCS and FAMACHA© (P > 0.05). A FAMACHA© score of 4 had a variation of Hb between 106- 100 g/L for goats, and 112 g/L for one sheep. A FAMACHA© score 3.5 had a variation of Hb between 69- 117 g/L for goats, and responded to 112 g/L for one sheep.

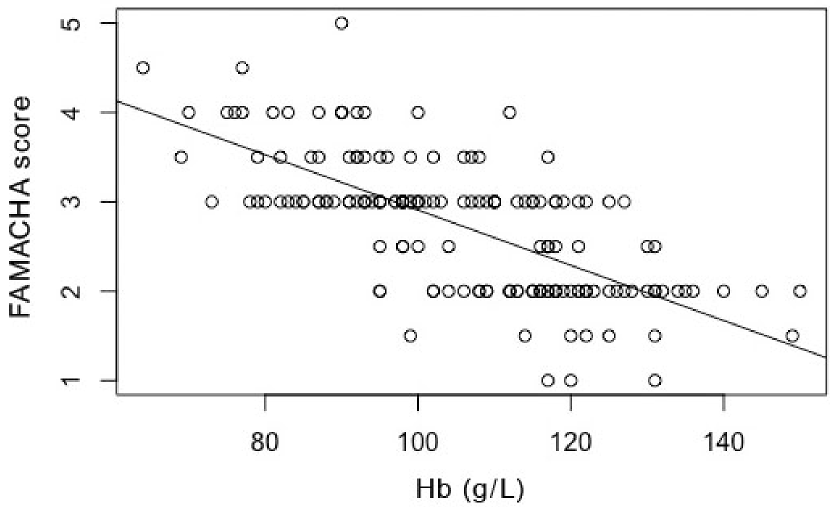

Figure 14. *Correlation between haemoglobin and FAMACHA©. A significant negative correlation (P < 0.0001) was found (r = -0.03).*

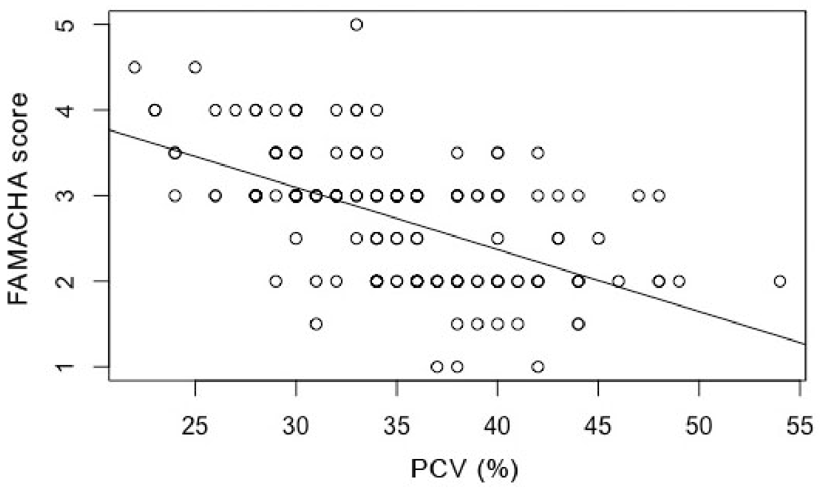

Figure 15. *Correlation between PCV and FAMACHA©. A significant negative correlation (P < 0.0001) was found (r = -0.58).*

PCR

Due to laboratory errors in the analyses, there have not yet been any results from the PCR.

DISCUSSION

This study investigates the occurrence of trichostrongylid eggs in goats and sheep in Mongolia in different types of pasture, and show the variations in relation to clinical parameters such as FAMACHA, haemoglobin, PCV and BCS between different locations and host species.

Half of the sampled animals had a trichostrongylid FEC of 0 EPG and a no or low burden was found in nearly all animals (Table 4). The mean FEC of trichostrongylid eggs was lower than expected throughout the study, with a maximum value of only 750 EPG. FEC was significantly lower in South Gobi (Fig. 6), which supports the hypothesis and is in accordance with previous research in Mongolia (Sharkhuu, 2001). The lower FEC in South Gobi is also supported by research about *H. contortus*, which has shown that the parasite is of less importance in arid climates and deserts (Besier *et al.*, 2016b). In Khuvsgul, the mean FEC was higher in spite of the colder climate, but the critical limitations for moisture were likely fulfilled as the climate in Khuvsgul is more temperate. The higher FEC in Khuvsgul is in accordance with previous research, which has shown that this parasite can survive also in colder climates as long as the moisture requirements are fulfilled (Troell *et al.*, 2005; Waller & Chandrawathani, 2005).

The high occurrence of animals with an FEC of 0 EPG can be related to a number of different factors. The precipitation is sparse in Mongolia and, as mentioned before, this limits the possibilities for larvae development. A less plausible reason for the absence of eggs is that *H. contortus* was arrested because of unfavourable conditions. However, the herders recalled that the months prior to sampling had been the greenest summer in 30 years, which would make this explanation less likely. In the questionnaires, the question about vaccination or deworming was not properly filled out due to miscommunication. Therefore, the FEC results cannot be put with any confidence in relation to the usage of anthelminthics. In Mongolia, deworming is commonly done once a year in spring when the goats are combed for their cashmere (personal communication). This means that the deworming likely should have taken place 4-6 months before the sampling.

In the study, there were no statistical differences in FEC of trichostrongylid eggs between the total amount of sheep and goats, and no difference between the species within the regions. This means the hypothesis that goats would gain from living in areas with more bushes and thus have a lower occurrence of FEC could not be confirmed. This contrasts the results in a study made by Le Jambre and Royal (1976). The overall small variations in FEC in this study mean that it is difficult to draw any conclusions.

In the study, there was no correlation between FEC of trichostrongylid eggs and FAMACHA©, in contrast to previous research (Kaplan *et al.*, 2004). This can likely be explained by the low FEC in this study, which means that clinical symptoms were unlikely to be found. Rodriguez *et al.* (2015) found that a mean FEC of 1758 EPG was considered to be indicative of anaemia in sheep, corresponding to a mean PCV value of 23 % and a mean haemoglobin value of 63 g/L. The single highest FEC in this study was found in a goat that had 750 EPG, which means that it was considerably higher than the average EPG among goats (67.6). It is below the EPG that Rodriguez *et al.* (2015) found to be indicative of anaemia in sheep and is within the moderate burden category. The same goat had a haemoglobin value of 99 g/L, which is above the mean haemoglobin value for sheep with anaemia (Rodríguez *et al.*, 2015).

Unfortunately, molecular differentiation of *H. contortus* was unsuccessful due to an uncertain laboratory failure with the PCR analysis and it could not be repeated due to time constraints. Although the DNA extraction was performed according to the manufacturer's prescriptions, several errors related to laboratory skills may have occurred. Larvae had been observed microscopically in all samples where DNA was extracted, but likely the number of larvae in the substrate was too low. Positive controls were detected but the research objective regarding *H. contortus* have not been fulfilled with any confidence.

When investigating the animals below the reference range of haemoglobin, a significant difference between sheep and goats was found in South Gobi, with more goats being below the reference haemoglobin range (Fig. 9, Fig. 10). These findings may be explained by host species differences, other pathogens or nutritional deficiencies. Egbe-Nwiyi (2010) found that haemoglobin levels in small ruminants vary with age, and age differences between the sampled sheep and goats can therefore affect the data. Unfortunately, the data on age distributions in this study was insufficient and the relationship between haemoglobin and age could therefore not be investigated. It is also plausible that the reference range that was used for goats in this study was not appropriate for the sampled animals, due to breed variations. Not much research has been conducted on the Mongolian goat breeds and the reference levels are thus not adapted specifically for them. Some goats can thus have been falsely recorded as being below the reference range. The high occurrence of goats with low haemoglobin values in South Gobi can however not be explained by an inadequate haemoglobin reference range. It is therefore more likely caused by one or more of the other suggested aetiologies, such as species differences, other pathogens or nutritional deficiencies.

A significant difference in FAMACHA© values between sheep and goats was found, with goats scoring higher (paler) than sheep. Furthermore, significantly more goats had a FAMACHA© scoring above 3 (Fig. 7, Fig. 8). Also this may be explained by factors such as host species differences, other pathogens that affect the goats more severely or nutritional deficiencies among the goats. The FAMACHA© scoring system was developed primarily for sheep but has also been widely used in goats. However, Kaplan *et al.* (2004) suggested that the scoring system may be less accurate for goats than sheep. An explanation for this can be that goats may have paler mucus membranes in general.

The mean BCS values were nearly identical for both sheep and goats. There was a significant difference in BCS for goats between locations, where the goats in South Gobi scored the lowest values. Furthermore, when comparing the animals with BCS ≤ 2.5, there was a significant difference for goats between locations (Fig. 12, Fig. 13). South Gobi had the highest amount of goats with low BCS (≤ 2.5). In South Gobi, no herders kept cows and therefore used goats for milk, whereas the sheep were not milked. Lactating females may have a lower BCS score because of the higher energy demand caused by milk production for human use. The occurrence of low BCS values in South Gobi coincide with the occurrence of haemoglobin values below reference range, which were also found in goats in South Gobi. Thus, the low BCS values may be explained by similar aetiology as the low haemoglobin values, e.g. nutritional deficiencies, a pathogen that is more prominent in this location or less beneficial pasture conditions in the areas where goats had low BCS.

In this study, no correlation was found between FAMACHA© and BCS as FAMACHA© varied between species and BCS varied between locations (Fig. 14, Fig. 15). This is contrasted by a study performed by Yilmaz *et al*. (2014). Since the two scoring systems are developed to measure different parameters that are not necessarily interlinked, a correlation cannot be assumed. Thinness is not inherently related to all kinds of anaemia and not all anaemic animals have a low BCS. However, a poor body condition can increase the animal's susceptibility to infections and other deficiencies and some pathogens can cause both poor body condition and anaemia, explaining the correlation in Yilmaz et al. (2014). This study was not able to find a correlation between the two, indicating that the aetiology behind the two parameters was not shared.

There was a significant negative correlation between haemoglobin and FAMACHA© as well as between PCV and FAMACHA© in this study. This was expected and is supported by several studies (Vatta *et al.*, 2001; Van Wyk & Bath, 2002; Kaplan *et al.*, 2004; Burke *et al.*, 2007). FAMACHA© is inherently linked to the red blood cell parameters and the correlations support the notion that FAMACHA© is a suitable and non-invasive method for clinical and hands-on estimation of anaemia in small ruminants.

Jansson Lagerkvist (2017) investigated the occurrence of *Anaplasma ovis* as a potential cause of anaemia, with the same sample population as in this study. The study found that *Anaplasma* spp. did not overall explain the low clinical values of FAMACHA©, haemoglobin, PCV or BCS. For the goats in South Gobi a FAMACHA© > 3 was correlated with the prescence of *Anaplasma* spp. but this could however not be confirmed since there was no correlation between haemoglobin and the of *Anaplasma* spp. Hence, *Anaplasma* spp. can likely be excluded as a causing agens in this study. The prevalence of *Anaplasma* spp. was however surprisingly high (Jansson Lagerkvist, 2017).

Potential sources of error

The study is based on a relatively low number of samples, which means that it does not necessarily give a correct representation of the populations. Furthermore, the selection process of individuals within the populations were selected relatively arbitrarily. Efforts were made to fulfil pre-set criteria for the selection, but these had to be adjusted after the animals present. The sheep were not lactating to as high extent as the goats, which skewed the representation within the samples. Sometimes, the sample sizes are inconsistent, due to temporal restrictions and miscommunications.

Lack of practice with the different measurement methods before the initiation of the study is a considerable source of error in this study. Measures were taken to decrease this error, such as acquiring the help of a trained parasitologist when FEC of trichostrongylid eggs. Furthermore, the BCS and FAMACHA© were estimated in collaboration with Jansson Lagerkvist (2017) with a blinded method, thereby decreasing the risk for individual misjudgements. Because the FAMACHA© colour chart is determined by visual examination by an observer, the light setting at the time of sampling is of importance and may also interfere with the result, but no such conclusions have been made for this study. A digital measurement of the eye colour of the mucus membranes would limit this error. The used BCS scoring methods had not been developed for the breeds that were sampled in this study, potentially giving rise to erroneous estimations. Measuring BCS in goats is generally considered to be difficult, making faulty estimations yet more likely when insufficient practice was had on beforehand.

The sampling locations were chosen due to their diverged pasture conditions. However, sampling was performed after a summer that had been the greenest in 30 years, and therefore the pasture was not as sparse as intended and the differences may have been evened out.

Because of limited storage possibilities for faecal samples and cultivations, the temperatures fluctuated more than desirable and may thus have had a negative impact on the cultures. The harvesting of larvae was however to some extent successful as the majority of the cultivated samples with FEC >50 had eggs that hatched to become larvae.

The animals in South Gobi and Bayan Unjuul were taken to waterholes every day since other sources for water was limited. The ground around the water holes during this time, when a large herd of small ruminants was around, was very wet making transmission pathways of especially gastro- intestinal nematodes between animals possible. Water samples from these sources would be of further interests.

The size of this study is limited, which has implications for the reliability of the drawn conclusions. To improve the reliability, further research with larger numbers would be beneficial.

CONCLUSION

The study showed a no to low worm burden of small ruminants in the sampled locations, with the mean FEC of trichostrongylid eggs being lower than expected throughout the study. Species differences were found between common clinical parameters such as FAMACHA© and haemoglobin values. Goats scored higher on mean FAMACHA© and had a higher occurrence of animals below reference for FAMACHA© and haemoglobin. In line with previous studies, there was a significant negative correlation between haemoglobin and FAMACHA© as well as between PCV and FAMACHA©. In contrast to previous research, no correlation was found between FEC and FAMACHA© nor between FAMACHA© and BCS.

Differences between locations were found in goats for haemoglobin levels, haemoglobin values below reference level, BCS and FEC of trichostrongylid eggs. FEC of trichostrongylid eggs was significantly lower in South Gobi, which supports the hypothesis and is in accordance with previous research in Mongolia. South Gobi appears to be a less beneficial location for goats, as the clinical parameters have a higher occurrence of values outside the normal range. Goats in South Gobi thus differ significantly from goats in other locations, indicating that there may be underlying factors in this location affecting the goats. The causations are not yet known and further research would thus be valuable.

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APPENDIX I

QUESTIONNARIE

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. **Are you deworming/vaccinating your animals?**

   Yes No

   If yes, how often?

   Against what disease(s):

6. **Choose between the words below 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 if needed? Yes No

Thank you for your cooperation!