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**A One Health approach to investigating the health  
and prevalence of zoonotic pathogens in snow  
leopards, sympatric wildlife, domestic animals and  
humans in the South Gobi Desert in Mongolia**

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Cairns, March 2018

A thesis submitted for the Degree of Doctor of Philosophy  
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## Acknowledgements

I would like to thank my advisory panel Lee, Lee, Nick and Jenny for their support and patience through this whole process. Especially guiding me with the writing and editing of chapters and manuscripts of which I was terribly out of practice.

To my family, Andrew, Jeremy, Rhys, Penny, Freya and Gaby, for their support and patience during this time. Dealing with me being away for weeks on end while doing field and lab work overseas and at times not being able to do the “normal” mother things while away (although good excuse for Andrew to finally get a cleaner in!). Thanks to my son Jeremy for coming with me as my field assistant for five long weeks, with no showers, electricity, internet, inside toilet and bland, monotonous diet. However, I think he was quite pleased at the thought of probably being the only Australian teenager to find a wild snow leopard cub in its den.

To my friends Kristen, Juliet, Karen and Mandy for always being there to pick up/drop off kids and help Andrew out when I was in the field. Organize time out for dinner and movies and just for listening despite not knowing what I was going on about when I was talking work, all from different walks of life.

To my other special friend Aditi, who sadly passed away during the course of my PhD, but was a constant source of inspiration with her positive outlook on life and sense of humour. For when things were getting me down or super stressed I would just look at her and my problems appeared very small.

To my co-workers at Ulysses vet surgery, especially Demian for putting up with me disappearing for weeks on end and yet still employing me, thanks so much.

A very big thank you to Orjan Johansson for providing me with the opportunity to see/touch/sample my first wild snow leopard Aztai. Despite it being after midnight sub zero temperatures and the constant wind that cut through you, making your eyes water and nose drip, I would not have swapped places with even David Attenborough himself at that point in time. Then for all the subsequent captures of these stunning, amazing animals that I was privileged to be a part of. Thanks also for the many hours and kilometres traversing the study site by motorbike and seeing this amazing country, while looking at kill sites and for dropped collars and potential rodent

trapping areas. For the friendship and support and always replying to questions so quickly via email once home, despite the time difference!

A very big thankyou also to Gustaf Samelius for his friendship and all his help sampling the rodents, despite the assertions that welding gloves were a bit of an overkill to hold a 20gm rodent! For always being positive and supportive in the field and helping to make my PhD so very memorable. Thank you too for helping make my time in Sweden while doing lab work enjoyable too and forcing me to take some days off to sightsee while I was there.

I am glad I had the opportunity to carry out my lab work in Sweden. Everyone at the Uppsala Biomedical Centre was so friendly and helpful. Thank you especially to Tanja Strand for organizing my time while I was there and providing me with tech support Per Ericksson, Olivia Borg and Tove Hoffman for all the laboratory work that I was completely ignorant about when I first started. I still miss the traditional 10 am "Fika". Thank you too for your friendship Tanja while I was there and the other members of the lab, Michelle, Olivia, Clara, who readily accepted me into their social group. Still have the cinnamon bun recipe.

To the other members of the One Health group Lee Skerratt, Lee Berger, Laura Grogan Laura Brannelly, Rebecca Webb, Rick Speare, Diana Mendez, Jon Kolby, Gerardo Martin, Alex Roberts, Tiffany Kosch, Marcia Bianchi, Ben Scheele, Tory Llewelyn, Amy Shima, Jenny Laycock, Sherryl Robertson, Siara Claytor and AliciaMaclaine, thanks for your support and suggestion to improve my project.

## Statement of the Contribution of Others

Nature of Assistance	Contribution	Names, Titles
Intellectual support	Proposal writing	Lee F. Skerratt <sup>1</sup> and Lee Berger <sup>1</sup>
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	Editorial Assistance	Lee Skerratt, Lee Berger and Gustaf Samelius <sup>2</sup> Katharine Fowler
Financial support	Field Research	Snow Leopard Trust
	Laboratory Analyses and Bench Fees	Snow Leopard Trust, Åke Lundkvist <sup>7</sup> and Johan Michaux <sup>8</sup>
	Stipend Grants	Australian Postgraduate Award Winifred Violet Scott Foundation Award Snow Leopard Network Grant
Data collection		<i>Research Assistance:</i> Gustaf Samelius, Orjan Johansson, Nadia Mijiddorj, Lkhagvasumberel Tomorsukh <sup>6</sup> , Volunteers –Mattia Colombo, Jeremy Krockenberger, Gary Koehler
Logistical Support		<i>Field research:</i> Snow Leopard Trust- Charudutt Mishra <sup>4</sup> , Gustaf Samelius, Orjan Johansson <sup>2,5</sup> Snow Leopard Conservation foundation- Nadia Mijiddorj <sup>6</sup> Bayarjargal Agvaarnsteren <sup>6</sup> , Purevjav Lkhagvajav <sup>6</sup>
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## Abstract

The endangered Snow leopard (*Panthera uncia*) inhabits the high mountain regions through central Asia and is subjected to numerous threats including poaching for traditional Chinese medicine, retribution killing for preying on domestic stock, and habitat fragmentation. However the occurrence and impact of disease on snow leopard populations is unknown. As emerging infectious diseases of wildlife can be an insidious yet important cause of population decline due to mortality or reproductive failure, my study aimed initially to gain knowledge of pathogens circulating among wild and domestic hosts in this region. I used a broad One Health approach to survey a range of species to collect data on disease occurrence that would be useful in improving human and livestock health, as well as snow leopard conservation.

This study is set in the Tost Mountains of the South Gobi Desert of Mongolia and was prompted due to the unexplained deaths of four snow leopards detected within a short timeframe during an ecological study by members of the Snow Leopard Trust. However, investigating disease occurrence in remote, rare and endangered species is a challenge due to inaccessibility of sites, difficulty of capture, and processing samples without facilities.

A One Health approach uses multidisciplinary expertise such as ecological, medical and veterinary, to understand host, pathogen and environmental disease factors. This approach is especially useful for diseases that transfer between people, domestic animals and wildlife. As snow leopards are a rare and elusive species, my surveys were aimed at assessing pathogens circulating in snow leopards as well as in sympatric wild and domestic animals. I collected samples from the following hosts: snow leopards – the target species; rodents which are ubiquitous over the study area and are a suitable sentinel species; ibex which are a native ungulate and natural prey species of the snow leopard; domestic goats which are also a prey species of the snow leopard; free-ranging domestic dogs which interact with the goats. The local indigenous people interact with all these species including snow leopards, mostly via retribution killing. Water samples were also collected from waterholes and wells, which are communal meeting places as drinking sources for all species, hence



enabling pathogen exchange. Samples collected included blood samples, faecal samples or rectal swabs and ectoparasites if present. These samples were transported to laboratories in Sweden and Belgium where I conducted diagnostic assays for zoonotic pathogens that are present in other regions of Mongolia and impact the health of humans and animals. I used enzyme-linked immune assay (ELISA), polymerase chain reaction (PCR) and next-generation sequencing (NGS) for pathogens including *Coxiella burnetii*, *Toxoplasma gondii*, *Leptospira* spp., *Brucella* spp., *Yersinia pestis* and tick borne encephalitis virus. Serovars of *Leptospira* were elucidated using microscopic agglutination tests (MAT). The dog blood samples were also tested for canine distemper virus. Ticks, faeces, rectal swabs and water were tested for bacteria, *Echinococcus*, *Giardia* and *Cryptosporidium* using PCR and NGS.

Health records for humans and animals in the region were not available so, in addition to testing animal samples, I used questionnaire surveys to obtain information on perceptions of the herders concerning health of their families, their domestic animals and wildlife. Questions also assessed preventative health management and treatments used.

Over three field trips I caught and sampled twenty snow leopards, 177 rodents (8 species), 41 dogs and 270 goats. I also sampled 11 waterholes/wells, and preserved 18 ticks, hundreds of fleas and collected faecal samples from ibex.

Most animals that were sampled and examined clinically appeared in good health, but the serosurvey revealed a moderate to high level of exposure to serious pathogens: *C. burnetii*, *T. gondii* and *Leptospira* spp. There were no published reports of human infections with these pathogens in the study area, which is likely due to a lack of testing.

Snow leopards had the highest prevalence of *C. burnetii* antibodies (25%), followed by rodents (16%), dogs (10%) and goats (9.5%). Goats had the highest prevalence of *T. gondii* antibodies (90%), dogs (66%), snow leopards (20%) and rodents (16%). Rodents had the highest prevalence of *Leptospira* spp. (34%), followed by snow leopards (20%) and dogs (5%). Serovars *interrogans* Australis was identified in the rodents and snow leopards and *interrogans* Ictohaemorrhagiae was identified in the

rodents and dogs. Other serovars were also present from the results of the ELISA but did not match those listed in the MAT panel, so could not be identified. Goats were not tested for infection with leptospirosis. *Brucella* was not identified in the goats even though it occurs at high prevalence in stock in the rest of Mongolia where it is a large health and economic concern. In rodents, the zoonotic Puumala and Seoul hantavirus were identified for the first time in Mongolia. Analysis of data from rodents showed the pathogens detected (*C. burnetii*, *T.gondii*, Hanta virus and *Leptospira* spp.) differed significantly in prevalence, with a strong year effect driven mainly by *Leptospira*, which increased in prevalence across the three year study period. *Toxoplasma gondii* differed slightly in prevalence among rodent species. There was no significant difference in prevalence of interaction of pathogens among years or rodent species.

Poor health was detected in goats with 10 out of the 14 goats tested via haematology and biochemistry being anaemic with haematocrits less than 20%. Haematology and biochemistry values for the other animal species appeared normal. I established haematology and biochemistry reference tables for two rodent species - red-cheeked ground squirrels and jerboas.

Water samples were negative for serious pathogens. Fleas were negative for *Yersinia pestis*. However, ticks were positive for several genera of potential zoonoses, including *Anaplasma*, *Bacillus*, *Coxiella*, *Clostridia*, *Francisella*, *Rickettsia*, *Staphylococcus*, *Streptococcus* and *Yersinia*. Faecal samples were also positive for genera of potentially zoonotic bacteria including those listed above plus *Bacteroides*, *Bordetella*, *Campylobacter* and *Enterococcus*.

Results from the two questionnaire surveys revealed the main reported illness in people were colds and flu. However, the local doctor also reported hepatitis as common. She also said that the local people contracted brucellosis whereas I did not identify this pathogen in their livestock. The herders thought their main loss of stock was from predation, with wolves identified as the main predator and snow leopards as the second. Other causes of stock loss perceived as important were adverse climatic conditions such as drought or severe winters while infectious disease was not a concern. Results from these surveys also highlighted gaps in health care for humans and livestock, especially around vaccination and parasite treatments.

In summary, I found that snow leopards and other wild and domestic animals within the study area tested positive for previous exposure to several important zoonotic pathogens. These pathogens were likely circulating among species via contamination of pasture and via predation and have potential to cause illness and reproductive loss. However, I detected no adverse effects on the health of the animals due to infection with these pathogens, and observed no related mortality or illness during my field trips. Hence the deaths of the four snow leopards that were the impetus for my study have not been explained, and monitoring and surveillance of this population should continue.

My findings on wildlife and domestic animal pathogens have relative importance to improving productivity of livestock and the health of the nomadic herders. I recommend improving the health of goats through vaccination and anti-parasite programmes, which will improve their fecundity and survival and thus increase herder income. These programmes will also have flow-on effects to improve the health of the native ungulates that share the grazing areas by decreasing the risk of pathogen transfer between them and also to the snow leopards that prey on them. Demonstrating the importance of herd health may also help mitigate herder wildlife conflict as increased productivity could decrease the perceived importance of predation on herd numbers.

*Coxiella burnetii* and *Leptospires* spp are a likely cause of illness in people, despite the lack of reported diagnoses. As rodents had a moderate prevalence of all pathogens tested and inhabit the gers of the local people, it is important to raise awareness of the risk of pathogen transfer to people via rodent excrement contaminating stored food and eating utensils. Risk of human exposure to pathogens during goat slaughter can also be reduced via improved hygiene practices.

By identifying pathogens with broad host ranges in a variety of species in this remote mountainous region, my study provides the basis for understanding health risks to wildlife, domestic animals and humans. Consideration of likely transmission routes for pathogens between species can inform current recommendations to improve health, productivity and hence conservation, of the endangered snow leopard – The Ghost of the Mountain.

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## Chapter. 1 Introduction

The Snow Leopard (*Panthera uncia*) is a rare and endangered apex predator distributed throughout the mountainous regions of Asia. Major threats to its survival include habitat destruction from mining, depredation of native ungulates, their prey, due to competition with domestic stock, retribution killing from herders due to consumption of domestic stock and poaching for pelts and body parts for traditional medicine (Ikeda 2004, Jackson and Wangchuk 2004, Bagchi and Mishra 2006, Wegge et al. 2012, Lyngdoh et al. 2014). The growing effects of climate change also pose serious threats to the existence of these vulnerable populations (Munson et al. 2008, Sheehy et al. 2008).

Disease is a potential, yet an unknown threat to this iconic species. Despite the growing number of studies on basic ecology, life history strategies and predator prey interactions, there have been no studies on disease in wild snow leopards and any subsequent population impacts as the pressures on these fragile populations increase (Bagchi and Mishra 2006, Anwar et al. 2011, Sharma 2013, Ale et al. 2014, Lyngdoh et al. 2014, Johansson et al. 2016).

Mongolia is a landlocked nation between Russia and China and home to a diverse but poorly studied variety of endangered wildlife including the snow leopard (Akiner 1991, Ebright, Altantsetseg, & Oyungerel 2003). The Snow Leopard Trust –a non-profit organisation that I am a member of, works in five countries that together contain over 75% of the worlds' population of wild snow leopards. Mongolia is one of these countries, providing a key habitat area for the threatened snow leopard, which until recently has been little studied in this country. Snow leopards in Mongolia are distributed across regions inhabited by nomadic herders and their livestock. Direct interactions between people and leopards occur when leopards prey on their stock with subsequent retribution killing. Retribution killing has been identified as a significant threat to conservation of the snow leopard in this region (Jackson 2015). However, there are other more subtle threats to the conservation of the snow leopard that have yet to be investigated. Disease is one such threat to snow leopards that has not been investigated as having an impact on their population and hence conservation. A detailed investigation into the pathogens present within their habitat

is required to determine if there are any direct effects on the health of snow leopards that could be a threat to their conservation. As well as direct illness there are also indirect effects such as the poor health of native prey and domestic stock where pathogens may cycle between predator and prey. Illness in the ungulates could reduce prey availability with pathogens cycling within the system. This leads to the concept of a One Health approach.

The One Health approach utilises multiple disciplines drawing on knowledge from natural, social and health sciences and the humanities. It applies the findings to benefit people, domestic animals and wildlife (Zinsstag et al. 2011). The health of herders, domestic livestock and other wildlife in the area has been poorly documented or not at all. Hence, my study sought to assess the prevalence and impact of diseases with broad host ranges that may spread between humans, domestic and wild animals and affect the health of these host groups. Disease ultimately could impact on the survival of wild snow leopards either directly or indirectly. For this reason, known important zoonotic pathogens were targeted for study as they can have broad host ranges and severe impacts.

These recommendations should enable improved health in all sectors, human, agricultural and environmental, by decreasing the diseases that may have an impact on snow leopards and other members of the environment they live with. Flow on benefits should increase livestock productivity and wildlife conservation concerning wild snow leopards.

## **1.1 Snow Leopard Conservation and Disease**

Snow leopard conservation, as for so many endangered enigmatic species, is faced with many challenges. Snow leopards are called the “Ghost of the Mountains” due to their cryptic and elusive nature and the challenging terrain they inhabit. These factors have contributed significantly to the difficulty in studying them in the wild. Snow leopards have survived for thousands of years despite threats from hunting and poaching for pelts. Snow leopard pelts are highly prized in the illegal wildlife trade. Thirteen fresh skins were found in a Chinese border town and another 15 skins on the north-west border of Mongolia and these ones that are discovered represent only a fraction of those illegally traded (Wingard and Zahler, 2006). In traditional Chinese

medicine, snow leopard bones are also highly sort after providing another illegal market (Thiele 2003, Wingard and Zahler 2006, Jackson 2012). Ancient folklore counteracts this by stating that snow leopards remove people's sins from past lives, so that if a snow leopard is killed, those sins will transfer to the killer. This legend may have reduced hunting in old times, but if livestock are preyed upon, there is still retribution killing by herdsman (Champion 2015).

The wild population is reported to be declining with an estimated 3000-7000 individuals remaining in the wild and approximately only 4500 of them reproductive, stretching across their home range of the central mountains of Asia (IUCN, 2013). Several studies have trialed various population census techniques including camera trapping and non-invasive methods such as scat collections and genotyping in Nepal, China and Mongolia (Janecka et al. 2008, Karmacharya et al. 2011, Sharma et al. 2014). However, none give accurate representations of the total population, only subsets at the locations where the studies were performed.

Firstly, I examined the literature for reports of snow leopards affected by disease. The reports found were all restricted to zoo-based animals, however this does not mean that these diseases do not occur in wild snow leopards, they have just not been looked for. The reports on captive animals documented the common feline viruses, tuberculosis, several types of tumours, ocular colobomas, parasites, papilloma virus specific to snow leopards and several zoonoses, including toxoplasma (Schmidt et al. 1984, Munson and Worley 1991, Maity et al. 1994, Mitsouras et al. 2011). Canine distemper virus has been reported in captive snow leopards and other wild felids (Fix et al. 1989, Appel et al. 1994, Myers et al. 1997, Gilbert et al. 2014). There are reports of African lions and tigers having contracted canine distemper virus from both wild and domestic dogs (Roelke-Parker et al. 1996, Cleaveland et al. 2000). It is, therefore, possible for pathogens to pass the species boundary and cross to snow leopards.

Secondly, the literature was examined to determine which pathogens are known to occur in my study area of the South Gobi Desert in Mongolia and the potential pathogen transmission pathways among snow leopards, its natural prey, other wildlife and the traditional indigenous nomadic community that co-exists in the region. There was no available information in the literature as to diseases occurring within



the study area. Therefore, the current state of knowledge of zoonotic diseases and emerging infectious diseases in Mongolia was examined and extrapolated as a guide and basis for my study.

## **1.2 Zoonoses and Emerging Infectious Diseases**

In all countries, zoonoses are a major concern to public health (Katara & Kuma, 2010, Mocellin and Woggin, 2008). The World Health Organisation (WHO) defines zoonoses as “diseases and infections that are naturally transmitted between vertebrate animals and humans. A zoonotic agent may be a bacterium, a virus, a fungus or other communicable disease agent”.

As well as the newly emerging zoonotic diseases, there is always the threat of previously known zoonoses recurring under favourable environmental conditions. These include diseases such as anthrax, rabies, plague, leptospirosis and toxoplasmosis, which can occur in a variety of hosts including wildlife and domestic animals (Thompson 2013). Most of these, for example, plague, are considered as diseases of a bygone era but retain the zoonotic potential to fatally infect humans as shown by outbreaks of bubonic plague in Kyrgyzstan and pneumonic plague in Madagascar in 2013.

According to the WAHID (World Animal Health Information Database, 2013), there have been recent outbreaks of these diseases across many continents. Any database relies on diseases being recorded, so it is likely that there have also been other outbreaks that have not been reported and recorded. With the sporadic emergence of these zoonoses under changing environments, including changing climate, changing population dynamics and densities (human, domestic animal and wildlife populations), preparation should be made for the emergence of these diseases in previously unaffected areas. This makes the ability to be able to predict the risk of infection important at a range of ecological levels (Hampson 2011).

There are regulatory bodies in place for disease surveillance among human populations, such as the WHO and in the trade of domestic animals led by the World Animal Health Organisation (OIE). However, in some countries, there is little

regulation on the trade of wildlife for bush meat markets and the exotic pet trade, therefore increasing the risk of disease transfer with unknown consequences.

Emerging infectious diseases (EID) are generally defined as: those diseases that have not been identified previously; a pathogen that was in one species and then crossed the species boundary to appear in another; a disease that was restricted to one geographic area to appear in another distinct and pathogens with increasing virulence and incidence (Hulme et al. 2014, Williams et al. 2002, Jones et al. 2008, Katare and Kumar 2010, Mackey and Liang 2012, Funk et al. 2013, Sorci et al. 2013, Grogan et al. 2014, Tompkins et al. 2015). Emerging infectious diseases are reportedly having profound effects globally on economics and public health (Jones et al. 2008). Worldwide, zoonoses are one of the principal causes of illness and death from EID (Arricau-Bouvery and Rodolakis 2005, Chomel et al. 2007, Katare and Kumar 2010). The majority (71.8%), of these zoonotic EID, originate from wildlife (Jones et al. 2008). A changing climate is likely to further fragment and impact populations of disease reservoirs and vectors and thus drivers of disease emergence (Wright et al. 2005). Understanding the relationship between the threat of emerging diseases and the possible evolution of those diseases that already exist globally, has become an important part of maximizing conservation outcomes in a complex and changing landscape.

Emerging infectious diseases and their effect on host species is influenced via a number of factors or drivers (Woolhouse & Gowtage-Sequeria, 2005). Tompkins et al. (2015) reviewed current literature to determine that there were eight drivers of EID, several of which were not mutually exclusive. These included human movement, animal movement by sales at markets, international trade and illegal poaching of both wildlife and domestic animals. They also included the movement of insect vectors carrying pathogens. Insect movement can be influenced by climate change allowing insect range extensions and causing further spread of pathogens (Gray et al, 2008). Nutritional and habitat stress, through animal population density, can cause immune compromise of genetically fragile species further endangering them to the threat of invading pathogens (Lafferty & Holt, 2003). Funk et al. (2013) devised a model to predict whether a disease was truly an emerging disease and the temporal time frame of when it emerged and which drivers were present at that time. If drivers

of EIDs can be identified and controlled this should aid in diverting the threat or at least slowing the emergence to determine appropriate control measures to stop the spread of disease.

### **1.3 The One Health Concept**

The concept of One Health to investigate emerging diseases exemplifies a greater understanding of the world in which we live. The world is not composed of individual sections but a plethora of intertwined entities, where one influences the other (Karesh and Cook 2009). A One Health approach looks at utilizing multiple disciplines working locally, nationally and globally to obtain optimal health for people, animals and the environment, (One World, One Health Symposium, 2004). There have been few One Health studies in rural and remote communities where there is a clear need for such work, when humans and animals interact closely, relying on each other for their existence. Domestic animals, wildlife and humans share many common pathogens, with these pathogens being able to readily move from animals to humans, such as anthrax, plague, rabies and leptospirosis and some from humans to animals, including gorillas, pigs, dogs and cats (Rwego et al. 2008, Dehove 2010, Zeibeida et al. 2011). To date, most of the studies performed have looked at pathogen transfer between domestic animals and humans without considering the role of the environment or wildlife either as reservoirs for pathogens or part of the pathogen transmission pathway (Kaplan et al. 2009, Dehove 2010, Dockrell 2012). There are an increasing number of studies in the literature on One Health approaches to looking at the movement of pathogens between diverse hosts (Rabinowitz et al. 2009, Karesh and Cook 2009, Dehove 2010, Monath et al. 2010, Ahmed et al. 2010, Sherman 2010, Batsukh et al. 2012, Dantas-Torres et al. 2012, Dockrell 2012, Rostal et al. 2013, Thompson 2013, Aenishaenslin et al. 2014, Buttke et al. 2014). All health workers, medical, veterinary and environmental need to be aware of and work together under a One Health framework to better address the ever-growing number of zoonotic diseases being recognized, both new and re-emerging. With intensification of current farming methods to provide food sources for the ever-growing global population, combined with greater urbanisation, trade and travel and subsequent greater contact between humans, domestic animals and

wildlife, the risk of spread of infections between humans and animals will increase (Dockrall 2012).

#### **1.4 Herd Health Programs in Mongolia**

The management of herd health in Mongolia has changed in recent years. During the Russian era of control of Mongolia vaccination programmes were under government control and were quite well regulated, after independence resources were not available to continue these practices (Akiner 1991, Milne 1991, Miller 1995). With the decrease in vaccination programmes there was a subsequent rise in brucellosis reported after 1990 in many species of domesticated animals, including goats, sheep, camels and horses (Mocellin and Foggin 2008). As of 2001 there have been no animal vaccination programmes in Mongolia (Ebright, Altantsetseg, & Oyungerel 2003). This has resulted in the recurrence of several disease outbreaks of brucellosis (Ebright, Altantsetseg, & Oyungerel 2003). Occasional cases of tularemia have been reported and animal cases of anthrax involving cattle, sheep and goats are reported sporadically (Ebright, Altantsetseg, & Oyungerel 2003). An animal vaccine for anthrax is available but has not been routinely administered (Ebright, Altantsetseg, & Oyungerel 2003). Foot-and-mouth disease caused by aphthovirus of the family Picornaviridae was thought to have been eradicated in Mongolia by 1973 but reappeared along the Chinese border in 2000 (Ebright, Altantsetseg, & Oyungerel 2003). By 2001 it had spread to livestock near Ulaanbaatar, which led to interventions including livestock vaccination, with the outbreak under control by 2001 (Ebright, Altantsetseg, & Oyungerel 2003).

Rabies, although not directly a herd health issue, is still an endemic problem in Mongolia among dogs and wolves, with the occasional human case reported (Boldbaatar et al. 2010). Rabies is an issue for herders, especially when their dogs encounter infected wildlife, which then poses the risk of spread of the pathogen from wildlife to dogs to stock (Boldbaatar et al. 2010). There is a locally produced, goat brain derived vaccine available but it is not routinely administered (Boldbaatar et al. 2010).

## **1.5 Current Disease Surveillance in Mongolia**

With the help of WHO a One Health approach has been implemented in Mongolia, Healthy Animal-Healthy Food-Healthy People, (Batsukh et al. 2012) and guided by the Asia Pacific Strategy for Emerging Diseases (APSED). A multisectorial panel, utilizing experts from the veterinary, public health, environment and emergency management sector, was established in March 2010. From this co-ordinated approach over 20 bacterial and viral zoonotic diseases and 18 parasitic zoonoses were identified in Mongolia, with the most prevalent including anthrax, rabies, tularaemia, echinococcosis, brucellosis, leptospirosis and plague (Ebright, Altantsetseg, & Oyungerel 2003, WHAD 2013). Endemic zoonoses, as mentioned above, such as rabies, anthrax, plague and other vector-borne diseases occur regularly and presumably are under-reported due to a lack of public awareness. The actual surveillance techniques are not reported in the One Health Document, but monthly reports from local health and veterinary workers are provided to the government once a month (Batsukh et al. 2012).

## **1.6 Knowledge Gaps**

From examination of the literature, there appears to be many deficiencies in the knowledge publicly available about snow leopard health, human and domestic animal health and other wildlife health and disease issues in the South Gobi Desert of Mongolia. I was told by Dulamjav Jamsransuren, one of the authors of the One Health document and who works at the National Centre for Zoonotic Disease, that there are some local reports but that these are difficult to obtain and are all in Mongolian. The following is from a personal communication with Dulamjav Jamsransuren "I have just informed you to the vaccination programme in this country is only for human. Those vaccination programmes are nationwide, but there is only against anthrax vaccination in your research area and/or in Gurvantes and recording and evaluating system the implementation of the counting injures and vaccination efficacy results are very weak, I would say. Why against anthrax only is because there are no active natural foci of plague and tick borne encephalitis and livestock anthrax and even spore-infected soil having being enlarged in Mongolia. For against rabies, in Mongolia, affected people due to bitten by rabies suspected livestock and

wild animals are treated with post exposure vaccination usually. In this country, against zoonotic diseases vaccination among human population and in order to preparedness activities not so strong enough, don't you think so?"

## **1.7 Rationale for and Scope of the Research**

This research forms an adjunct to an ongoing ecological project on snow leopards of the Tost Mountains in the South Gobi Desert of Mongolia. Initially, I was involved in a voluntary capacity in the ecological project in 2011. While conducting the ecological study, the need to investigate disease threats to the snow leopards in this region arose when four snow leopards were found dead within a few months of each other and in different locations within the study area. One of these was bleeding from its nose and anus, which raised the alarm for anthrax which is known to occur in the region. However anthrax was never confirmed and the cause of death was not established as the vet involved was not equipped with personal protective gear and could not conduct a post mortem. Just prior to this, a wildlife biologist of large felids conducted a post-mortem on a mountain lion carcass and died soon after from plague caused by *Yersinia pestis* (Eisen et al. 2008). This was the impetus for investigating diseases affecting snow leopards and the other inhabitants of the region. The Snow Leopard Trust asked me to come back in 2012 and run a pilot disease study, which then formed the basis of this PhD project.

This lack of knowledge and resources for health issues in the South Gobi Desert of Mongolia puts into context the problems faced not only by the snow leopard, but other wildlife, domestic animals and people in this environment. The direction of my project and the pathogens I chose for testing were guided by this lack of data. I decided to concentrate on zoonotic diseases that could occur in this region which would affect all inhabitants of the study area including snow leopards and which would therefore fill major gaps in the knowledge base. Due to the small sample size of snow leopards available for sampling a One Health approach was adopted when designing this study. I utilised resources and expertise from synergistic fields of study, bringing together researchers in human and veterinary medicine with ecologists, to simultaneously investigate pathogens of humans, production animals

and wildlife. This method can help identify which hosts are key pathogen reservoirs enabling targeted interventions to reduce pathogen transmission.

Snow leopards were the primary target species for disease surveillance. All other hosts were selected based on their ability to be part of a potential food chain for snow leopards and their potential significance in a pathogen transmission cycle, their conservation status and whether they would complement the other selected species. Numerous other wildlife inhabit the area such as wolves, foxes, argali sheep, birds and mustelids but consideration was taken as to which were the most available for capture and sampling. Rodents, which are often disease reservoirs and inhabit all areas of the study area, from the open steppes, to the rocky canyons to the herders' Gers (dwellings), were selected for disease surveillance. The other wildlife species considered included Ibex (*Capra ibex*), a native ungulate to the area and a native prey species of the snow leopard but difficulties with capture prevented sampling apart from collecting faeces. Cashmere goats are the most common livestock in the area and were chosen as their grazing overlaps with native ungulates and on occasion they are a prey source for snow leopards. This predation causes herder/wildlife conflict leading to retribution killing of the snow leopards by the herders. Domestic dogs were chosen as the second representative of domestic animals as they roam over the entire area and are in contact with all the other species examined in the study, either directly or indirectly. Water holes and wells were chosen for sampling as a representative of an environmental source for pathogens. They are the communal meeting place of wild and domestic animals and facilitate opportunities for pathogen exchange. Finally, the nomadic herders and their families, who reside in the area, were interviewed as the last link in a possible chain of pathogen transmission. The herders were given questionnaire surveys as an efficient way to collect preliminary data on their health and gauge their attitudes to the health of their animals and that of the surrounding wildlife. Other domestic stock such as horses, camels and some sheep were also owned by the herders in the region but in far smaller numbers than the goats. It was beyond the scope of this study to sample all species physically so the aim of the survey was to also try and gain some information about these other species in the region with respect to disease affects on them or predation affecting their survival.



**Figure 1.1 Possible interaction and transmission pathways for pathogens between snow leopards and other environmental constituents.**

The pathogens selected to test for comprised *Coxiella burnetii*, *Toxoplasma gondii*, *Leptospira*, canine distemper virus, Hantavirus, *Giardia*, *Cryptosporidium* and *Echinococcus*. These pathogens were selected based on their known occurrence in other parts of Mongolia, their zoonotic potential and their economic importance. Initially, tick borne encephalitis was selected as well, but as so few ticks were collected and preliminary results were negative it was decided not to continue testing for this pathogen. Rabies and anthrax are two other serious zoonoses present within the area. However, it was decided not to test for these. Animals rarely survive rabies infection, so it is highly unlikely to have obtained any positive survey results. Anthrax is also highly fatal to its host. It can be an environmental contaminant as spores but does not sporulate at environmental temperatures less than 9-12°C (Beyer and Turnbull 2009). Therefore, it was again decided that the chances of detecting this pathogen were very limited given the environmental temperature extremes (-40°C to +40°C) in this part of Mongolia.



There are no reports available as to the health of wild snow leopards or the impacts of disease on wild populations. There is nothing in the literature on diseases of Ibex in Mongolia. There is only limited information on disease and vaccination programmes on domestic ungulates in the country and none in the study area. Many of the pathogens I tested for are known to have broad host ranges but their effects on snow leopards are unknown (Galdan et al. 2010, Hampson et al. 2011, Song et al. 2014, Tompkins et al. 2015). The role of interspecies transmission and reservoir species for the pathogens I selected for testing have not been determined for these host species communities. Information on diseases circulating through this network of wildlife, domestic animals and humans is necessary for targeted interventions to reduce disease impacts.

Therefore, this project aims to identify and assess the effects of infectious zoonotic pathogens on the endangered snow leopard, other wildlife, domestic animals and humans in a remote, rural area of Mongolia. My study will contribute to the limited literature available on what is known about the pathogens identified and the animal species they infect in the Gobi Desert. It will also be the first holistic study that targets all potential hosts in the one community in Mongolia.

## **1.8 Research Aims**

- (1) Using a One Health approach, this project aims to identify zoonotic pathogens that may impact the endangered snow leopard
- (2) Following this One Health approach aim to identify zoonotic pathogens in sympatric wildlife, domestic livestock and humans in a remote, rural area of Mongolia
- (3) To investigate links between pathogens and health status of the species present in the region
- (4) Examine overlap and transmission pathways for movement of these pathogens between species
- (5) Assess perceptions and attitudes of the local people to wildlife, domestic animal and human health, disease and management via questionnaire surveys

- (6) To identify critical gaps in the knowledge about diseases that are re-emerging or newly emerging in this region, that can be a threat to the snow leopard and the environment in which it lives.
- (7) Formulate management plans and intervention sites to lead to improved disease control for all species

These recommendations should enable improved health in all sectors, human, agricultural and environmental, by decreasing the diseases that may have an impact on snow leopards and other members of the environment they live with. Flow on benefits should increase livestock productivity and wildlife conservation concerning wild snow leopards.

## 1.9 Sections of the Study

**Chapter 1** is an introductory chapter and provides the background and rationale for the thesis. The review of the literature is brief here as each subsequent chapter reviews the pertinent literature for that part of the thesis. This is because each chapter is written as a manuscript for submission to a journal for publication.

**Chapter 2** investigates the general health of the wild snow leopards. Snow leopards were trapped in conjunction with an ecological study run by the Snow Leopard Trust. General physical health was examined, physiological measurements recorded and samples comprising of blood, rectal smears and external parasites were collected and analysed for *Coxiella burnetii*, *Toxoplasma gondii* and *Leptospira* spp. Next generation sequencing and polymerase chain reaction were utilised to screen the rectal smears, any faeces collected and external parasites for *Giardia*, *Cryptosporidium* and *Echinococcus*. Potential impacts of these pathogens on the health of snow leopards were assessed.

**Chapter 3** reports on the health of the rodents trapped during the study. Rodents, being ubiquitous over the study area, were used as sentinel species for pathogens. They are intermediate hosts for many of the pathogens of interest especially *T.gondii* and *Leptospira* spp. The rodents were also tested for hantavirus, which is a serious zoonotic pathogen and has not been recorded in Mongolia before. I also generated haematological and biochemical reference ranges for species that had not previously

been recorded. As for the snow leopards, the rodents had a physical health assessment before collection of samples. Samples included, blood, faeces and external parasites.

**Chapter 4** describes a taxonomic study that was developed during my PhD project when I discovered there were numerous rodent species over the study area, which had not been adequately described previously. This study used DNA analyses and morphological measurements to characterise species to improve the accuracy of identification. Detailed observations and photographs of the different rodents' habitats were recorded as each species occupies a different ecological niche within the study area. There was also the potential for different species to carry different pathogens, which was examined when the results of the pathogen analyses were available.

**Chapter 5** assesses the health of domestic animals owned by the herders, focusing on goats and dogs. Sampling natural and artificial water sources as an environmental source of pathogens as part of the One Health approach was also incorporated into this chapter. The same sample types as for the snow leopards and rodents were collected and tested for the same zoonotic pathogens; dogs were also tested for canine distemper virus. Other large felids, such as lions, have contracted canine distemper virus from domestic and/or wild canids, with detrimental results for their health (Roelke-Parker et al. 1996). In captivity, snow leopards have been reported to contract the disease (Fix et al. 1989), so I wanted to determine if the virus was prevalent and a potential threat to the wild snow leopards.

**Chapter 6** describes the results of the questionnaire surveys that were presented to the local people with questions regarding their health and that of their animals and the wildlife that they came in contact with on a daily basis. I also wanted to understand their perceptions as to the effects of predation on their herds and whether they thought disease was an issue.

**Chapter 7** is a summary chapter, linking the previous chapters together in a cohesive story. It shows how using the One Health approach and sampling not only the target species, snow leopards, but the other species they interact with, domestic and wild animals, the environment they all inhabit and questioning the people who live there,

provides a more holistic picture of disease threat to all. To some extent this approach overcomes the limited number of snow leopards available for sampling. In this chapter, I also put forward recommendations for disease management and future research based on the outcomes of the study.

# **Chapter. 2 Zoonotic Infections of *Panthera uncia* - a Serosurvey of Wild Snow Leopards in the South Gobi Desert of Mongolia**

## **2.1 General Introduction and Premise of Chapter 2**

Chapter 2 is the first research chapter of my thesis. The main thrust of my study was to determine if the population of endangered snow leopards in the Tost region of the South Gobi Desert of Mongolia is under threat from infectious disease. The conservation of this vulnerable species in this region is subject to other threats, such as hunting, poaching, retribution killing and habitat destruction. In order to plan an effective conservation strategy all known stressors need to be identified so that intervention can be targeted and with accurate and beneficial impact.

As sample sizes of the threatened snow leopard population in this region were low, a more holistic approach was utilised to address the problem of detecting possible pathogens. A One Health approach was chosen to maximise resources, so not only were snow leopards sampled but also other inhabitants and environmental samples of the area were selected. For this reason, we tested for zoonotic pathogens that have broad host ranges and cause severe illness, to obtain an indication of the disease risks to snow leopards and other hosts in the region.

Snow leopards were being trapped and sedated in a concurrent, long-term ecological study monitoring home ranges and basic life history strategies. To maximize resources these snow leopards had blood samples, faecal samples and external parasites collected while under sedation for placement of radio collars. Blood serum samples were tested for *Coxiella burnetii*, *Toxoplasma gondii* and *Leptospira*. External parasites and faecal samples were analyzed via Next Generation Sequencing and Polymerase Chain Reaction for bacteria and parasites.

This chapter has been submitted to the Journal of Infection, Ecology and Epidemiology in December 2017 for review and publication.

*Article title: Zoonotic infections of Panthera uncia -a serosurvey of wild snow leopards in the South Gobi Desert of Mongolia*

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## **2.2 Abstract**

Snow leopards, *Panthera uncia*, are a threatened apex predator, scattered across the mountainous rangelands of Central and South Asia. Infectious disease threats to wild individuals or populations have not been investigated. Between 2008 and 2015 twenty individual snow leopards in the South Gobi Desert of Mongolia were captured and immobilised for health screening and placement of radio-tracking collars. Clinical assessment included general physical exam, measurement of body temperature, heart rate, respiratory rate and body weight. Blood samples, faeces and external parasites were collected for pathogen analyses using enzyme-linked immunosorbent

assay (ELISA), microscopic agglutination test (MAT) and next-generation sequencing (NGS) techniques. The animals had no clinical signs of disease, however, serum antibodies to significant zoonotic pathogens including *Coxiella burnetii*, (25%), *Leptospira* spp., (20%) and *Toxoplasma gondii* (20%) were detected. Faeces were positive for bacterial genera including *Staphylococcus* spp., *Streptococcus* spp. and *Stenotrophomonas* spp. however, negative for *Giardia*, *Cryptosporidium* and *Echinococcus*. The ticks collected from snow leopards contained potentially zoonotic bacteria from the genera *Bacillus*, *Bacteroides*, *Campylobacter*, *Coxiella*, *Rickettsia*, *Staphylococcus* and *Streptococcus*. The zoonotic pathogens that were identified in this study although in the short-term did not appear to cause illness in the snow leopards, have caused illness in other wild felids. Therefore surveillance needs to be implemented to monitor for longer-term disease impacts on this snow leopard population.

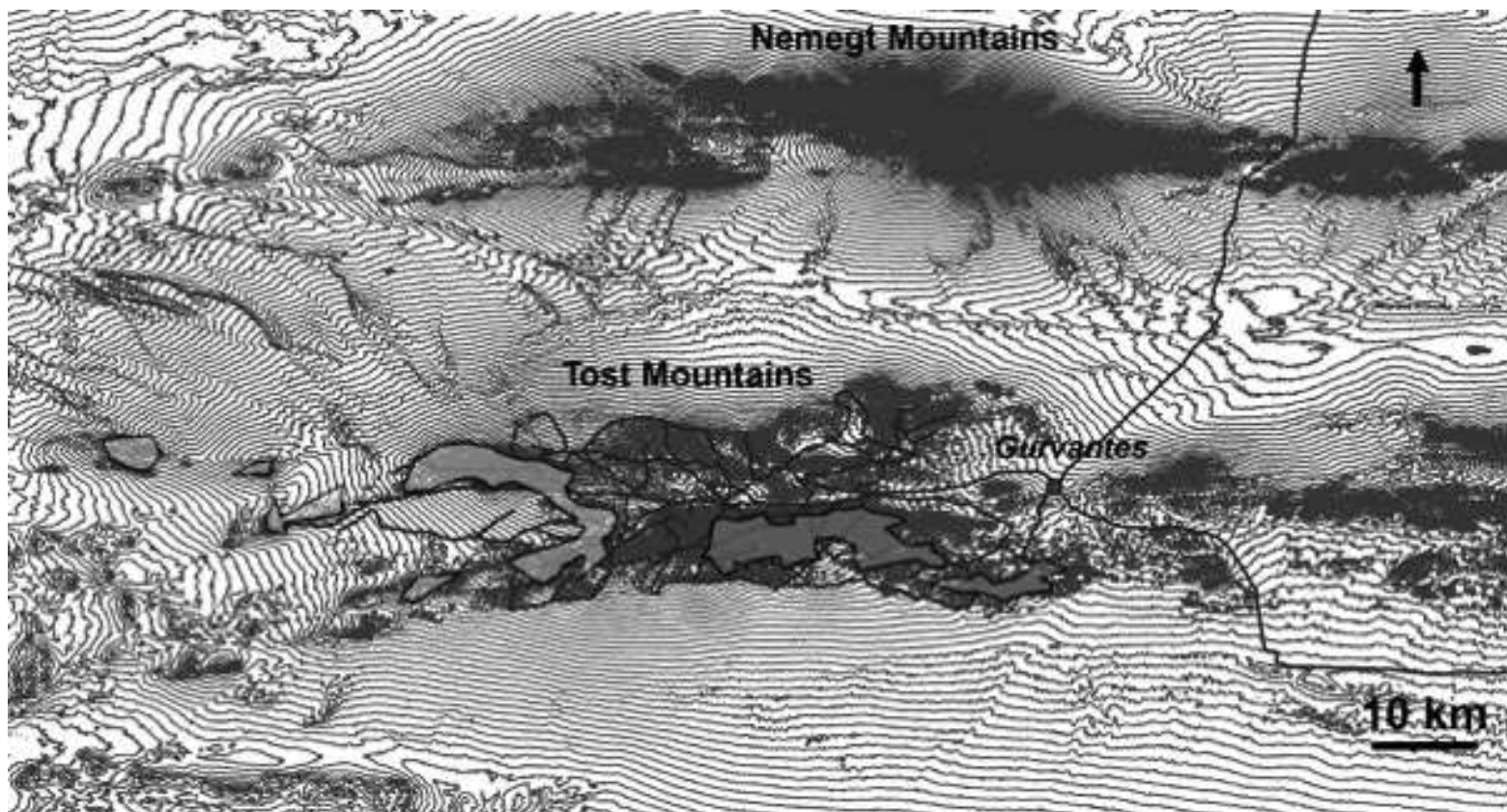
### **2.3 Introduction**

While the overall decrease in biodiversity is often attributed to environmental changes such as land clearing, habitat destruction, feral pests and climate change, emerging infectious disease can also act as a primary or contributory cause (Munson et al. 2008, Smith et al. 2009, Cook & Karesh 2002, Woolhouse 2005, McFarlane et al. 2012, Tompkins et al. 2015). Pathogens can particularly impact endangered and threatened species where populations are already depleted and genetic diversity may be low (Sorci et al. 2013). The rarity of endangered species makes them difficult to sample systematically, but constant surveillance and collection of baseline health data with ongoing monitoring will aid in determining the impacts of disease on surviving populations.

Snow leopards (*Panthera uncia*) are a rare and threatened species, occurring along the high mountains of South and Central Asia including the Himalayas in the south, through the Pamirs, Tien Shan and Altay in the north. The population of reproductive snow leopards is believed to be fewer than 4500 and thought to have declined by approximately 20% during the 16 years prior to 2008 with further decline predicted (Jackson et al. 2008). However during 2017, a review of population studies across their range suggested the decline to be 10% and their current status be altered to

vulnerable rather than endangered (IUCN 2018). There appears to be a large amount of controversy around this decision. In 2008, Snow Leopard Trust and Snow Leopard Conservation Fund initiated an ongoing, long-term ecological study of snow leopards in the South Gobi Province of Mongolia. During 2011, the field team recorded four snow leopard carcasses on separate occasions in the study area. Three were in the Tost Mountains and one in the Nemegt Mountain range, an adjacent range to the north (Figure 2.1). Thorough necropsies could not be performed, so the causes of mortality were not established.





**Figure 2.1** Map of the study area showing the Tost Mountain Range and the adjacent Nemegt Mountain Range. The pale grey shaded areas are the home ranges of the two dead collared snow leopards and where they were found.

There were no signs of trauma or starvation therefore other potential causes of death included infectious disease. Two of the dead snow leopards were radio-collared territorial males with no overlap in their home ranges, suggesting independent causes of death.

Despite the range of investigations into threats to snow leopards, none have addressed the prevalence or impacts of disease in the wild. All prior reports on diseases in snow leopards are restricted to zoo animals and include common feline viruses such as feline parvovirus, calicivirus, feline infectious peritonitis, feline immune deficiency virus, canine distemper virus, as well as papillomavirus specific to snow leopards (Schmidt et al. 1984, Munson and Worley 1991, Maity et al. 1994, Mitsouras et al. 2011). Non-viral diseases included veno-occlusive disease, ocular colobomas and Tyzzer's disease (Schmidt et al. 1984, Fix et al. 1989, Munson and Worley 1991, Barnett and Lewis 2002, Thiele 2003, Wingard and Zahler 2006, Jackson et al. 2008). Several zoonotic diseases such as anthrax, rabies, leptospirosis, tuberculosis and *Toxascaris* infection have also been recorded (Schmidt et al. 1984, Maity et al. 1994, Helman et al. 1998, Dobson et al. 2013).

Threatened species are not likely to support infectious agents unique to that species because densities are low and intra-species interactions are in many cases infrequent (Millaín Javier et al. 2009). As snow leopard numbers are low identifying pathogens specific to felids and their effects would be challenging and limited. However, pathogens with reservoir hosts, especially zoonotic pathogens, can impact species at low abundance (Power and Mitchell, 2004).

The most prevalent zoonotic infections reported in Central Asian Mountain livestock are rabies, anthrax, plague, leptospirosis, Q fever, brucellosis, toxoplasmosis and echinococcosis (Ebright, et al. 2003, Tomley and Shirley 2009, Boldbaatar et al. 2010). Endemic zoonoses are often under-reported due to a lack of public awareness and public health services and so the disease threat may be greater than what is reflected in the literature (McFadden and Muellner 2013). Snow leopards live alongside nomadic herders and their stock throughout their range (Bagchi and Mishra 2006), which may be sources of infection to snow leopards and vice versa. This study aimed to investigate important zoonotic diseases that may impact on the conservation of snow leopards in Mongolia. Due to the low numbers of snow

leopards available for sampling, combined with the possibility of reservoir hosts for disease and the closeness of the nomadic herders to all components of their environment, I decided to target zoonotic pathogens that could circulate between different host species and hence also impact the health of snow leopards. The zoonotic pathogens selected to sample for were based on prior occurrence in Mongolia, pathogenicity and potential to infect snow leopards and economic loss for the herders. These pathogens included *Coxiella burnetii*, *Toxoplasma gondii*, *Leptospira*, *Echinococcus*, *Cryptosporidium* and *Giardia*. Anthrax and rabies, two of the most severe zoonoses are known to occur within the study area were not tested for as I was looking at prior exposure not active infection. Therefore the potential of identifying positive results for those two pathogens would have been extremely unlikely (Leung et al. 2007, Hartinger et al. 2000).

## **2.4 Methods**

### **2.4.1 Study area**

This study was conducted in the Tost Mountains (43° N, 100° E) in the Gobi Desert in southern Mongolia from 2012 to 2015 (Figure 2.2). The Tost Mountains cover an area of approximately 1700 km<sup>2</sup> and the population of snow leopards, estimated annually, were between 10-14 adults during our study (Sharma et al. 2014). The area is also home to approximately 90 herder families, their goats (*Capra aegagrus hircus*), sheep (*Ovis aries*), horses (*Equus caballus*), camels (*Camelus bactrianus*) and domestic dogs (*Canis lupus familiaris*). Other wild species in the area include the grey wolf (*Canis lupus*), corsac fox (*Vulpes corsac*), red fox (*Vulpes vulpes*), pallas cat (*Felis manul*), Eurasian lynx (*Lynx lynx*), Eurasian wildcat (*Felis silvestris*) and several members of the mustelid family (Nyamsuren et al. 2014, Sharma et al. 2014). Ungulates include the Siberian ibex (*Capra sibirica*) and argali sheep (*Ovis ammon*), which are a major component of the snow leopard's diet, along with livestock (Johansson et al. 2015).



**Figure 2.2** Location of the study area in the South Gobi Desert of Mongolia. Insert shows the topography of the region with trapping areas delineated in red. Grey lines are small roads and tracks that traverse the area (Johansson et al. 2014).

#### **2.4.2 Handling and measurements**

Twenty individual snow leopards were captured and sampled in conjunction with an ongoing radio telemetry study (see Johansson et al. 2013 for detailed capture methods) over a five-year period. Captures were performed under permits from The Mongolian Ministry of Environment and Green Development. Snow leopards were darted with a combination of medetomidine and tiletamine-zolazepam at a dose rate range of  $0.02 \pm 0.04$  mg/kg body mass medetomidine and  $2.17 \pm 0.45$  mg/kg tiletamine-zolazepam (Johansson et al. 2013). (Plate 2.1).



**Plate 2.1 Just darted snow leopard (*Panthera uncia*).**

Sedation lasted approximately one hour, which permitted fitting of the radio-collar for monitoring snow leopards' movements for 12 to 18 months, physical examination and collection of samples. Atipamezole hydrochloride (Antisedan vet 5 mg/ mL, Orion Pharma Animal Health) reverses the effects of medetomidine and was administered intramuscularly to aid a smooth recovery from the sedation when handling was completed (Johansson et al. 2013). Thorough clinical examinations ascertained the general health and body condition of the snow leopards.

Due to lack of a standard method for measuring body condition in wild snow leopards and limited descriptions for other wild felids (Marker and Dickman 2003) I developed a consistent technique for measuring body condition. This technique was based on the amount of muscle over the shoulders and hips and whether ribs, scapular spine and iliac crest were visible and could be palpated. If scapular spine and iliac crest of hip bones were prominent, then the body condition was 'poor'; if bony prominences were difficult to palpate the body condition was rated 'good'. In between these two extremes, body condition was scored as 'moderate'. Pelage was examined for thickness, signs of rubbing, alopecia and wounds. External wounds were recorded. Eyes were examined for any sign of injury or defects. The oral cavity, including tongue and gingiva, were inspected for inflammation, ulcers or other lesions. Teeth were also examined as one of the indicators of age, or if broken or infected (Plate

2.2). Age estimation was based on body mass and tooth wear and colour, with animals developing a darker cream tooth colour as they age.



**Plate 2.2 Teeth of a mature male snow leopard.  
Note worn incisors, canines and premolar. I-incisor, C-Canine, P- Premolar**

Scarring of the face in males was also used as an approximate indicator of age as younger males (<two-three years), have few or no scars. Presumably older males obtain facial scars from territorial or resource fights. Females that had not apparently reproduced (< three years of age) typically had nipples that were lighter in colour and smaller than those of known reproductive females.

Body weight, tail length and sex were also recorded. During the course of the study several animals developed from subadults to adults so physiological measurements were repeated. Heart rate, respiratory rate and body temperature were monitored at 10-minute intervals during the sedation, to collect physiological data and as a routine measure of physiological stability while sedated. Many of the captures took place at night at low ambient temperatures, so body temperature was closely monitored while the animal was sedated to ensure it did not drop below physiologically accepted levels (West et al. 2007). A pulse oximeter was attached to one ear to measure oxygen saturation. Samples collected for health screening included blood, rectal swabs and external parasites.

### 2.4.3 Blood collection and storage

Twenty millilitres of blood was collected from the cephalic vein. Two milliliters aliquots were placed into three separate, two millilitre blood serum separating tubes (Interpath services) for later serology analyses. Five millilitres of blood was placed into tubes with ethanol for DNA analyses and 1.0 ml into lithium heparin tubes for haematology and biochemistry. The remainder of the blood sample was placed on Nobuto strips, which hold 0.1 ml of whole blood or 0.04 ml of serum (Toyo Roshi Kaisha, Ltd., Tokyo, Japan; distributor Advantec MFS Inc.) and Whatman FTA cards (GE Healthcare UK Limited, Little Chalfont, Buckinghamshire, UK). The advantage of both the Nobuto strips and Whatman FTA cards are they can be stored at room temperature indefinitely. They were air dried and stored in paper envelopes.



#### Plate 2.3 Taking a blood sample from a snow leopard.

Thin blood smears were made in the field then fixed in Diff Kwik fixative at camp (Fronine Laboratory Supplies, Riverstone, NSW Australia). The smears were stained with Diff Kwik (eosin and methylene blue) and microscopically examined at 40 x and 100 x (oil) magnification for cellular abnormalities and haemoparasites once back in the laboratory.

Serum tubes stood overnight to separate cells and serum. Serum was decanted into sterile cryovials and held at  $-18^{\circ}\text{C}$  until transport back to the National Veterinary

Institute in Uppsala, Sweden for storage at -80° C until testing, six to twelve months later.

#### **2.4.4 External parasites and faecal samples**

External parasites were collected during physical exam of the pelage and placed in ethanol for later testing for bacterial pathogens. Rectal swabs were placed in charcoal carrier medium for bacterial analyses (Copan Italia S.P.A Perotti, Brescia, Italy). These were stored at -18° C. Faeces collected opportunistically in the field were placed in paper bags and kept dry for DNA analyses for bacteria and intestinal parasites.

#### **2.4.5 Laboratory analyses**

Snow leopard serum samples were tested for the presence of antibodies against *Toxoplasma gondii* and *Coxiella burnetii* and *Leptospira* spp. utilising Enzyme-Linked-Immuno-Assay (ELISA). If positive results were obtained for *Leptospira* spp., the samples were then tested using Microscopic agglutination test (MAT) against a panel of serovars. Next-Generation Sequencing (NGS) was used to screen faeces and ticks for bacteria (



Table 2.2). Faecal samples were also screened for intestinal parasites including *Echinococcus*, *Giardia* and *Cryptosporidium* using Polymerase Chain Reaction (PCR) and NGS at the Universiteit of Liege in Belgium.

#### 2.4.5.1 Elution of sera from Nobuto strips

Serum was removed from the Nobuto strips following the manufacturer's instructions (Toyo Roshi Kaisha. Ltd, 2007, Japan). The strips were cut into small pieces and placed in one ml Eppendorf tubes. Serum was eluted and diluted to 10% by adding 200200  $\mu$  l or 400  $\mu$ l phosphate buffer solution (PBS) depending on whether one or both sides of the strip were saturated with blood. After incubating for an hour to allow the serum to enter the PBS, the serum in the Eppendorf tubes was heat inactivated at 60 °C for one hour, centrifuged and filter paper removed from the tubes. The resultant sera were stored at -80 °C until analysis.

#### 2.4.5.2 *Toxoplasma gondii*

Antibodies against *T. gondii* were detected using a commercially available ABNOVA IgG antibody ELISA kit (ABNOVA, Taipei City 114 Taiwan). As this was a human kit, the methods were modified accordingly. The enzyme conjugate in the kit was changed to an Alkaline phosphatase-conjugated affinipure Goat Anti-Cat IgG (H+L). The methods also called for a 1:40 dilution of the test sample, but as the samples were already diluted 1:10, they only required an additional four times dilution. All ELISA plates were read on a Multiskan FC microplate photometer, Thermo Scientific machine at 450 nm. Results were calculated by methods outlined in the kit guide.

#### 2.4.5.3 *Leptospira* spp.

*Leptospira* antibodies were first identified using a commercially available ELISA kit *Leptospira* IgG (LS-IgG) ELISA kit, (MBS036971, Mybiosource, San Diego, California, USA). As this was a rodent test kit the conjugating enzyme was replaced with an alkaline phosphatase-conjugated AffiniPur Goat Anti-Cat IgG (H+L) (Jackson ImmunoResearch Laboratories, INC.). *Leptospira* serovars were then identified using the Microscopic Agglutination Test- MAT (Faine et al. 1999). The MAT panel the samples were tested against consisted of serovars: Australis, Hebdomadis, Icterohaemorrhagiae, Pomona, Canicola and Grippotyphosa. These

serovars were chosen as they had previously been reported to occur in other regions of Mongolia (Odontsetseg et al. 2005a, Odontsetseg et al. 2005b). The antigens used were live cultures of referenced strains. All sera that gave a positive reaction at a 1:100 dilution were further titrated in serial two-fold dilutions to titre endpoint that is 50% agglutination. A titre  $\geq 100$  was deemed positive to exposure to leptospires.

#### *2.4.5.4 Coxiella burnetii*

Antibodies against *C. burnetii* were detected using an Innovative Diagnostics Q Fever Indirect Multi-species ELISA kit. The ELISA was performed following the manufacturer's instructions (Idvet, 310, Grabels. France). The plate results were read at 450 nm.

Positive or negative results were calculated using:

$$\text{S/P\%} = \frac{\text{OD}_{\text{sample}} - \text{OD}_{\text{NC}}}{\text{OD}_{\text{PC}} - \text{OD}_{\text{NC}}} \times 100$$

Where S is the sample, PC is the positive control, NC negative control and OD is the optical density recorded.

If  $\text{S/P\%} \leq 40\%$  the result is negative, if  $40\% \leq \text{S/P\%} \leq 50\%$  result is doubtful,

If  $50\% \geq \text{SP\%} \leq 80\%$  result is positive, if  $\text{SP\%} \geq 80\%$  result is strongly positive.

#### **2.4.6 Ticks and faeces**

The ticks collected from the snow leopards and faeces that were collected opportunistically from the field, were analyzed for bacteria using NGS at the Génétique de la conservation Chemin de la Vallée. These analyses detect all bacterial genera present, as well as endosymbionts or opportunists (Gillet et al. 2015). NGS also verified whether faeces belonged to snow leopards. Total DNA was extracted from faecal samples. Each sample was manually mixed with a sterilized scalpel. Three treatments were performed to allow a better harvest of gram-positive bacterial DNA. First, samples were immersed for 1 hour at 37 °C in an enzymatic lysis buffer consisting of 20 mM Tris·Cl, pH 8.0, 2 mM sodium EDTA, 1.2% Triton® X-100 and 20 mg/ml of lysozyme as described in the Dneasy™ Tissue Kit handbook. Second, samples were submitted to three freeze-thaw cycles (Zhou, Bruns and Tiedje 1996). Finally, 25 µl of proteinase K and 200 µl of buffer A was added to the sample before an overnight shaking incubation at 56 °C. 200 µl of this mix was then introduced in a QIAcube (Qiagen®, Hilden) following the manufacturer's protocol for purification of total DNA from animal tissues. After the extraction step, we performed an Illumina amplicon sequencing following a modified Miseq protocols (16S Metagenomic Sequencing Library Preparation). Total genomic DNA from faeces was subjected to PCR amplification targeting a ~142-bp fragment of the 16S rRNA variable regions 5 and 6 (V5-V6) using the primer pair 784F-1061R originally designed by (Andersson 2008) coupled with the Illumina overhand adapters. One extraction negative control was added to every batch of 24 samples and two additional negative controls were added for the PCR steps. A mock community

sample (HM-783D, BEI resources) containing genomic DNA from 20 bacterial strains at concentrations ranging between 0.6 and 1400 pg/μl was also added in triplicate to confirm the reliability of our method. Purified products were quantified using Quanti-iT™ PicoGreen® dsDNA Assay Kit following the manufacturer protocol on a fluorimeter (FilterMax F3, Molecular Devices). Quantified products were then pooled in equimolarity and sent to the GIGA Genomics platform (Ulg) for sequencing on an ILLUMINA MiSeq V2 benchtop sequencer (Ghosh et al. 2000, Chaya and Parija 2014).

*Giardia*, *Echinococcus*, *Cryptosporidium* and other internal parasites were screened for in faecal samples following the protocols proposed by (Ghosh et al. 2000) and (Chaya and Parija 2014) as outlined above.

## **2.5 Results**

### **2.5.1 Health and physiological data**

All of the captured snow leopards appeared in good physical condition and healthy except for one 32 kg female (ID F9) that was scored as poor to moderate body condition. Her body weight was lower than recorded averages for females (Table 2.1) and bony prominences (scapular spine, ribs and iliac crest) were easily palpable. From her tooth wear, she was determined to be an older animal. Despite the poor to moderate condition score, she was negative for antibodies to the tested pathogens and was reproducing. She was later detected in camera trap records together with two cubs and survived at least 15 months before her collar dropped off after which she could no longer be monitored.

No ocular lesions or eyelid deformities were present in the animals examined. Oral cavities were clear of ulcers, other visible lesions or gingivitis. Physiological parameters measured from the snow leopards combining data from two new snow leopards with those reported by Johansson et al. 2013 and 2015 are presented in Table 2.1.

Table 2.1 Summary of physiological measurements of captured snow leopards during this study. (Mean  $\pm$ SD).

<b>Sex</b>	<b>Body weight (kg) (Range)</b>	<b>Heart rate (beats per minute) (Range)</b>	<b>Body temperature (°C) (Range)</b>	<b>Respiratory rate (breaths per minute) (Range)</b>
Male (adult) N=10	43.1 $\pm$ 2.3 (40.7-45)	97.5 $\pm$ 10.6 (87-110)	37.3 $\pm$ 1.1 (36.9-39.2)	27 $\pm$ 4.2 (24-36)
Female (adult) N=8	36.0 $\pm$ 2.9 (32-41.5)	107 $\pm$ 5.6 (89-113)	38.4 $\pm$ 0.14 (37.2-39.1)	27 $\pm$ 4.2 (24-36)
Male (subadult) N=4	34.8 $\pm$ 2.9 (34-39)	98 $\pm$ 1.4 (93-101)	37.25 $\pm$ 1.1 (36.9-39.3)	21 $\pm$ 1.1 (20-22)
Female (subadult) N=3	28.3 $\pm$ 2.8 (25-30)	108 $\pm$ 23.3 (100-122)	38. 38.1 $\pm$ 0. (37.9-39)	25.5 $\pm$ 2.12 (23-50)

N is the number of animals in each category.

For detailed individual snow leopard measurements from the same study see Johansson et al. 2013.

### **2.5.2 Known survival times of snow leopards after collaring at first capture**

Known survival times for snow leopards after the first capture and sample collection depended on how long collars stayed on. This time-frame ranged from four to 58 months with an average of 20.15 months. Thirteen collars dropped off snow leopards, some before their programmed date. Five of the collared snow leopards had died by the end of this study. Four of these were suspected to have been killed by people and their collars destroyed. The remaining individual died from unknown causes. The four dead animals that initiated this study were not included in this total.

### **2.5.3 Haematology**

All cellular components in the blood smears, (red blood cells, white blood cells and platelets) from each snow leopard, were normal in appearance when viewed under the microscope at 40 x magnification and 100 x (oil immersion). Extra- or intra-cellular haemoparasites were not observed.

#### **2.5.4 Serology**

Four of 20 snow leopards (20 %) were seropositive for *T. gondii* (M1, M9, M11 and F7). Five of the 20 snow leopards (25%) (M4, M10, F1, F7 and F8) were seropositive for *Coxiella burnetii* and four of 20 snow leopards (20%), (F5, M6, M11, M7) were seropositive for *Leptospira*. *Leptospira interrogans* sv. Australis was identified in two snow leopards in 2013 (F5, M7) but the other two samples were not positive in MAT.

#### **2.5.5 Faecal analyses**

Seventeen genera of bacteria were identified in all snow leopard faecal samples tested. The three genera of concern for potentially causing illness were *Staphylococcus*, *Streptococcus* and *Stenotrophomonas*. Parasites (*Giardia*, *Echinococcus* and *Cryptosporidium*), were not identified by PCR and Sanger sequencing. However, DNA recovery was low and may not have been amplified by the PCR.

#### **2.5.6 Tick analyses**

Four ticks were collected, each from a different snow leopard (F8, M4, M7, M1). One hundred and sixteen genera of bacteria were identified from the ticks in total, with the potentially significant zoonotic bacteria (that is those zoonotic bacteria that can cause disease) listed in

Table 2.2. (Igbenosa et al. 2012, Kandi 2016, Wexter, 2007, Wendelboe & Van Rie, 2006, Trubiano et al. 2016, Bernard 2012, Roussett et al. 2009, Winn, 1996. Parkins & Floto, 2015, Dantes Torres, 2012, Kloos, 1980, Kohler 2007.

Table 2.2 Bacterial genera identified in ticks collected from wild snow leopards in South Gobi Mongolia.

Bacteria genera	Snow leopard ID			
	M1	M4	M7	F8
<i>Aeromonas</i>	+	-	-	-
<i>Bacillus</i>	+	+	+	+
<i>Bacteroides</i>	+	+	+	-
<i>Bordetella</i>	+	-	-	-
<i>Clostridium</i>	+	+	+	+
<i>Corynebacterium</i>	+	+	-	+
<i>Coxiella</i>	-	+	+	+
<i>Legionella</i>	+	+	+	-
<i>Pandorea</i>	+	+	+	+
<i>Rickettsia</i>	+	+	+	+
<i>Staphylococcus</i>	+	+	+	+
<i>Streptococcus</i>	+	+	+	+

(+ denotes bacteria presence, - denotes bacteria absence)

## 2.6 Discussion

The majority of snow leopards within my study in the Tost Mountains in Mongolia appeared to be clinically healthy on physical exam. Only one female had below optimum body condition but was later observed in camera trap photos together with two cubs. The energetic cost of feeding the cubs could account for her decreased body condition. Ibex numbers were reported to be lower by the local herders due to the previous harsh winter decreasing vegetation availability for the ibex. A decrease in prey density, perhaps exacerbated by the need to provide for the cubs may have contributed to her lower body condition.

We collected novel physiological data on heart rate, respiratory rate and body temperature for wild (sedated) snow leopards (Table 2.1). This reference data is useful for future health assessments of wild populations and also for monitoring captive animals undergoing similar examinations. Due to the low number (23) of wild



snow leopards for which we had physiological data, which was collected over five years of intense fieldwork it was deemed important to add the data obtained from the two new animals to those published by Johansson et al. (2013).

In captive snow leopards, the presence of ocular coloboma (defects affecting many regions of the eye) has been reported on several occasions with the causative aetiology unknown (Schäffer et al. 1987, Barnett and Lewis 2002). This condition was not observed in any of the 20 wild snow leopards sampled or four additional cubs that were also examined (Esson et al. 2019).

I detected antibodies to *T. gondii*, *L. interrogans* serovar Australis and *C. burnetii* in wild snow leopards from the Tost Mountains of Mongolia. These pathogens have not been identified in wild snow leopards and are all serious zoonotic infections (Afonso et al. 2006, Hide et al. 2009, Tenter et al. 2000, Hill and Dubey 2002, Pappas et al. 2008), although the impacts on snow leopards are unknown. All snow leopards in this study appeared healthy, with data from radio telemetry showing that the individuals that were seropositive for these pathogens survived for at least 12 to 24 months after collection of samples. This finding implies that at least some of the individuals survive infection without apparent adverse effects in the long term. However, we cannot rule out unobserved negative impacts from these or other pathogens especially if the health of animals is reduced for other reasons. It could also be that some animals succumbed to the effects of infection prior to the sampling period.

These pathogens may have sub-lethal effects on reproduction that are difficult to quantify. Infections in wild animals may be exacerbated by complex co-factors that reduce health. For example, immune suppression due to stressors such as nutritional compromise, wounds from fighting followed by secondary bacterial infection, other traumas and extreme environmental conditions (Munson et al. 2008). An example is canine distemper virus causing a fatal outbreak in lions (Munson et al. 2008). Lion deaths were associated with a heavy co-infection of the haemoparasite *Babesia* combined with the immunosuppressive effects of CDV. In that study, there were increased ticks, vectors of *Babesia*, on ungulate prey due to an extreme drought followed by heavy rains (Munson et al. 2008).

If the snow leopard populations decrease in the region, there will be a higher risk of inbreeding depression as has been observed in declining tiger populations (Kenney et al. 2014). An increase in inbreeding depression can impact the immune system response to pathogens, as was the case in an isolated population of lions (*Panthera leo*), which lead to an increase in susceptibility for Bovine tuberculosis (Trinkel et al. 2011). Inbreeding depression also lead to a decrease of the variability of the major histocompatibility complex genes as seen in the endangered European mink (Becker et al. 2009, Hedrick and Garcia-Dorado 2016). A decrease in immune response of the snow leopards in this population would increase the risk of mortality linked to infectious pathogens they maybe exposed to in the region, not necessarily the pathogens tested for. This is an extinction vortex, where a species is subjected to multiple stressors (associated with hunting, poaching, habitat destruction extreme weather conditions), which will accelerate mortality rates because of the impact of a lower resistance of the immune system to disease and a lower adaptive potentiality to each stressor (Brook et al. 2008, Blomqvist et al. 2010).

Therefore there is a need for continual, long-term health surveillance of snow leopards, including autopsies of dead animals to determine disease impacts. If dead snow leopards are found in the future, protocols need to be in place so that appropriate samples can be collected to determine the cause of death. Due to the risk of exposure to highly virulent zoonoses, personnel trained in sample collection along with a necropsy kit complete with personal protection gear for sampling needs to be readily available.

The other issue is where did these pathogens originate? Are snow leopards dead-end hosts or are they part of a transmission cycle and act as reservoirs of infection? Rodents, dogs and goats were positive for exposure to these pathogens in concurrent studies in the region (Esson et al. unpublished data). The snow leopards also overlapped with scavengers, such as raptors and foxes at kill sites, allowing indirect interactions with other potential hosts and the pathogens they shed. These indirect interactions could include sharing common environmental resources that are contaminated with pathogens such as water bodies and being parasitised by common arthropods that act as vectors for pathogens. Again this warrants long-term studies on this population of snow leopards to elucidate the source(s) of the pathogens and others that may be present.

Felids are the definitive hosts for *T. gondii*, an obligate intracellular protozoan with a complex life cycle (Riemann and Franti 1978, Tenter et al. 2000, Hill and Dubey 2002). Broadly, transmission occurs when oocysts are shed in the faeces by the definitive host and then consumed by an intermediate host, which can be a rodent or another mammal (Tenter et al. 2000). Antibodies to *T. gondii* occurred in 20% of the snow leopards tested, including males and females and across all years of the study. The cub (F8) of F7 was negative to *T. gondii*, suggesting that F7 did not have an active infection during pregnancy or the pathogen did not cross the placenta in this case. *Toxoplasma gondii* can be transmitted horizontally via an intermediate host or vertically (Dubey 2014). F7 may also have acquired the infection after giving birth. These results indicate that snow leopards in this region may be acting as a reservoir host for this parasite. *Toxoplasma gondii* was identified in Pallas cats (*Otocolobus manul*) in 2005 (Brown et al. 2005) in an area north of the study area and has only recently been identified in humans in Mongolia (Batsukh et al. 2012). However, no other reports could be identified in the literature of *T. gondii* in other species in Mongolia, so the prevalence of this parasite across the country is unclear. The snow leopards did not appear to be impacted negatively by this parasite, however, negative effects have been observed to occur in other wild felids from infection with *T. gondii*. These include the death of a juvenile bobcat (*Pardus lynx*) from acute toxoplasmosis, a juvenile cheetah (*Acinonyx jubatus*) a Siberian tiger (*Panthera tigris altaica*), two lions (*Panthera leo*) all that died from acute disseminated toxoplasmosis (Brown et al. 2005, Millán et al. 2009, Llyoyd and Stidworthy 2007, Dorny and Fransen 1989, Ocholi et al. 1989). Pallas cats that also inhabit the same area as snow leopards, are extremely susceptible to infection to *T. gondii* (Brown et al. 2005). This pathogen therefore can have detrimental effects on members of the definitive host family (Dubey and Carpenter, 1993) so it would be prudent to continue long-term monitoring of the snow leopards to determine if negative impacts do occur. The snow leopards may also act as a reservoir for this pathogen for contamination by intermediate hosts. Toxoplasmosis is a severe zoonosis, causing neurological problems in the foetus and adults of its intermediate hosts and spill-over hosts including humans (Afonso et al. 2006, Hide et al. 2009). This would pose a public health risk to the nomadic people of the region.

*Coxiella burnetii*, the aetiological agent of Q fever, is common in Mongolian domestic animals (Tenter et al. 2000) but has not been reported in wildlife in this region. Ruminants are the main carriers of this pathogen and shed it in their milk, blood, placenta and faeces (Molia et al. 2004, Meredith et al. 2015, Torina et al. 2007). However, ticks can act as vectors and other mammals such as rodents can carry *C. burnetii* (Baca and Paretsky 1983, Rousset et al. 2009, Dantas-Torres 2012). The main mode of transmission and infection of this pathogen is via inhalation of contaminated aerosols and contaminated fomites (Willeberg et al. 1980, Moila et al. 2004, Torina et al. 2007). Ruminants are considered the primary reservoir for humans and in the study area it would be unusual that *C. burnetii* infection would not be identified in wildlife species from areas with a consolidated human/domestic animal/wildlife interface (Candela 2017). Antibodies to this pathogen were detected in five of 20 snow leopards, M4, M10, F1, F7 and F8) including both males and females. This is the first time *C. burnetii* has been recorded in this species in the wild. Although very few species of wild felids have been tested, it has been detected in the European wildcat (Candela et al. 2017, Rousset et al. 2009). These studies provide an overview of the level of infection in free-roaming felines and highlight their potential zoonotic risk to humans. Sixteen wild caught pallas cats near the Russian-Mongolian border were reported to be negative for *C. burnetii* in 2010. However, *C. burnetii* has been detected in other felid species in captivity, such as in acinical lions in a zoo (Torina et al. 2007). Felids, including domestic cats, have been identified as potential reservoir hosts for the bacteria and a source of infection for humans and other animals (Meerburg and Reusken 2011, Morand et al. 2015, Meredith et al. 2015, Shapiro et al. 2015). The positive results of *C. burnetii* antibodies in the snow leopards suggests they may function as a part of the epidemiological cycle of *C. burnetii* as domestic cats have been reported to in other regions (Shapiro et al. 2015). Assessment of the role of wild and domestic hosts as potential reservoirs of misdiagnosed zoonoses, such as Q fever by *C. burnetii*, is an important public health issue today both for wildlife conservation and management of disease in human–livestock–wildlife interface (Cooper et al. 2013, Candela 2017). *Coxiella burnetii* causes abortions mainly in ruminants however its pathogenicity in cats has not been established to date (Woldehiwet 2004). F8 is the cub of F7 suggesting vertical transmission or common and continual exposure of this pathogen. Continued monitoring of these animals is necessary to determine if there is any effect on

reproductive rate in these animals. As DNA was collected from all animals, parentage of future captures can be determined to see if there has been a possible effect of *C. burnetii* on future generations. The snow leopards may have been exposed to the pathogen when preying on domestic goats, ibex or rodents that also inhabit the area. These species also tested positive for exposure to this pathogen in concurrent studies (Esson et al. in prep 2017, Chapter 2 and 4). *Coxiella burnetii* is extremely resistant to environmental conditions being able to withstand cold temperatures and UV light, so could remain viable in the Mongolian environment (Vanderburg et al. 2014). Vaccinating domestic stock would help control transmission among livestock and to native ungulates such as ibex and Argali sheep by reducing the deposition of pathogens onto shared grazing regions. Vaccinating against *C. burnetii* would potentially help mitigate the risk of spillover to carnivorous wildlife, including snow leopards. Ticks from three of the snow leopards tested positive for unidentified species of *Coxiella* but were not from those snow leopards that were seropositive for *C. burnetii*. It is possible that the hosts did not have time to develop antibodies prior to sampling during the study. The role of ticks in the transmission of *C. burnetii* is unknown (Dantas-Torres 2012).

Four snow leopards tested positive for *Leptospira* antibodies via ELISA, two of which were positive for *L. interrogans* Australis via MAT. Mean agglutination test is considered the “gold standard” for identification of leptospira serovars (Bharti, 2003); *Leptospira interrogans* Australis is considered one of the pathogenic serovars of the genus *Leptospira* (Bharti et al. 2003). *Leptospira interrogans* Australis has not previously been identified in any Mongolian species so the finding of previous exposure in snow leopards is important from a public health point of view. The identification of this pathogen indicates there is a potential reservoir in the area that the snow leopards and other wildlife, domestic animals and people can be exposed to. Different *Leptospira* serovars to Australis have been reported in other wild felid species, but the effects are still unclear (Ullmann et al. 2012). Two of the snow leopards that were positive for *T. gondii* were also positive for *Leptospira* showing there has been exposure to multiple pathogens. No clinical signs of infection were observed (e.g. pyrexia, jaundice), similar to results from captive neotropical felids in Brazil. Here an ocelot (*Leopardus pardalis*) and a female marguay (*Leopardus wiedii*) tested positive to two different serovars of *Leptospira* without showing clinical signs

of disease (Ullmann et al. 2012, Canon-Franco et al. 2013). *Leptospira* spp. are spirochaete bacteria that reside in the kidney tubules, with rodents being a significant reservoir for the bacteria (Holt et al. 2006), however other mammals including cats have been reported as reservoirs of the pathogen (Everard et al. 1979, Azócar-Aedo. 2014, Schuller et al. 2015). *Leptospira* are readily transmitted to humans and is a zoonosis of global importance (Pappas et al. 2008). *Leptospira interrogans* Hardjo was the most common serovar in cattle and horses reported in two other provinces of Mongolia (Arricau Bouvery et al. 2003, Odontsetseg et al. 2005b) and ten other serovars were identified in companion dogs in other provinces of Mongolia (Odontsetseg et al. 2005c). Wild and domestic carnivores in Spain with *Leptospira* antibodies commonly had interstitial glomerular nephritis upon necropsy, despite no clinical signs of disease initially (Millán et al. 2009). It was suggested that these carnivores were terminal hosts, unable to transmit the disease (Millán et al. 2009). Leptospirosis could therefore potentially shorten the lifespan of the positive animals in our study if sufficient renal damage had occurred (Rodriguez et al. 2014, Schuller et al. 2015).

Numerous bacteria were detected in faeces although none are known to be significant pathogens under routine conditions and numbers (Madigan et al. 2015). *Streptococcus* spp. and *Staphylococcus* spp. were the most common pathogens. These may have been contaminants as *Staphylococcus* spp. normally resides on the skin and *Streptococcus* spp. occurs in the respiratory and intestinal systems rather than as inherent components in faeces. Each region of the intestine has its own unique bacterial flora, thus faecal elements do not usually contain the same bacteria as the proximal intestines (Madigan et al. 2015).

Bacterial genera, both zoonotic and non-zoonotic, were identified in the ticks collected from the snow leopards. The genera of major concern comprised *Bacillus* spp., which can include *B. anthracis* that causes anthrax and *B. pilliformis* that causes Tyzzer's disease. Tyzzer's disease has previously been described in captive snow leopards and caused fatal infections (Schmidt et al. 1984). Other genera identified were *Coxiella* spp., *Rickettsia* spp. that include species causing anaplasmosis and ehrlichiosis, both include intracellular bacteria that infect and destroy white blood cells, cat scratch fever, plus *Staphylococcus* spp. and *Streptococcus* spp. (Pfaffle et al. 2013, Ramirez-Hernandez et al. 2013, Madigan et

al. 2015). For the majority of these pathogens, there are no previous reports of occurrence in snow leopards. However, based on these results it would be prudent to test for the presence of these bacteria in future health-screening studies on this and other snow leopard populations. In my results, those snow leopards that tested positive for *Coxiella* antibodies showed no clinical signs of illness. The presence of *Coxiella* in ticks may, however, indicate a mechanism of transmission of the pathogen between snow leopards and other hosts as it has in other species (Cooper et al. 2013, Dante-Torres et al. 2012).

Faecal samples were negative for *Giardia*, *Cryptosporidium* and *Echinococcus*. Confidence in these negative results is low due to the harsh environmental conditions the faecal samples were exposed to before collection. DNA from these parasites may not have been preserved. *Echinococcus* has only recently been described to occur in the red fox, corsac fox and wolves in Mongolia from 10 provinces distant to the study area (Ito and Yanagida 2013). Two species of *Echinococcus* were described in the ten provinces- *E. multilocularis* and *E. canadensis* (Ito and Yanagida 2013). *Echinococcus* is a genus of tapeworm belonging to the Family Taenidae. The definitive hosts are carnivores with ungulates, rodents and other small mammals being intermediate hosts (Thompson 2013). This parasite is introduced to wildlife by interaction with domestic animals such as dogs (Thompson 2013).

Similarly, *Cryptosporidium* and *Giardia* have been found in other provinces in Central Mongolia. *Cryptosporidium* occurred in cattle but not in sympatric sheep or goats (Burenbaatar et al. 2008). *Giardia* has only been reported in people to date in Mongolia (Ebright, Altantsetseg, & Oyungerel 2003). It is possible that these pathogens are an under-reported cause of illness due to lack of investigation and identification rather than lack of occurrence. *Giardia* and *Cryptosporidium* are ubiquitous environmental parasitic contaminants of mainly water-ways soil or untreated faecal waste, causing severe diarrhoea and nutritional issues in their hosts, infecting humans, domestic animals and wildlife (Savioli et al. 2006). These parasites are included in the WHO Neglected Diseases Initiative. Therefore, their actual occurrence and effects in the Tost region should be determined by ongoing surveys.

## 2.7 Conclusions

Disease threats (both infectious and non-infectious) to endangered species tend to be overlooked in light of more apparent threats such as habitat destruction and hunting. This study is the first to detect exposure of wild snow leopards to zoonotic pathogens. Potential sources of the three pathogens identified that is *Coxiella burnetii*, *Toxoplasma gondii* and *Leptospira interrogans Australis*, include snow leopard prey such as wild and domestic ungulates and overlap with scavengers, such as raptors and foxes at kill sites, allowing indirect interactions with other hosts and the pathogens they shed. These indirect interactions could include sharing common environmental resources that are contaminated with pathogens such as water bodies and being parasitised by common arthropods that act as vectors for pathogens.

There was no evidence of adverse impacts of the zoonotic pathogens on the health and reproduction of the snow leopards in the Tost Mountains. Identification of these pathogens was based on antibody identification from prior exposure and not an active infection. Hence, we do not think they were the cause/s of the mortalities of snow leopards observed in 2011. With rare species, such as the snow leopard, there is a distinct need to continue long-term monitoring of their health to generate comprehensive baseline knowledge of their pathogens and disease threats. Monitoring the survival and condition of animals over time can be achieved through radio-collaring, continual collection and testing of samples as per this study and camera trapping. Only through continual monitoring, including disease surveillance, can we start to understand the threats to this endangered species.

### Acknowledgements

We thank the volunteers who helped with the fieldwork on this project and the Snow Leopard Conservation Foundation, Snow Leopard Network and Winnifred Violet Scott Foundation for funding. The Whitley Fund for Nature has been a long-term supporter of snow leopard research and conservation and we thank them and Fondation Segre for supporting part of this research. We thank all the staff of the Snow Leopard Conservation Foundation, for their help, support and local knowledge. Many thanks also to Per Eriksson, Olivia Borg, Tove Hoffman, Jennifer Vernier-Carlsson for their help and expertise with the laboratory work. Thank you to Zebra vet and Nicolette Newell for supplying and donating veterinary consumable goods and



the Abaxis Istat machine and cartridges. Finally a big thankyou to Gustaf Samelius for his patience and comments on this manuscript.

# **Chapter. 3 Pathogens of Rodents in the South Gobi Desert of Mongolia**

## **3.1 General Introduction and Premise of Chapter 3**

In keeping with the One Health approach to this study, rodents were chosen as a second representative wildlife species to sample for the presence of pathogens in the region that may be transferable to the snow leopards. Rodents are ubiquitous over the study area occupying many environmental niches that overlap with the other study species. They are well-known reservoirs and carriers of zoonotic pathogens so were therefore selected as the ideal sentinel species to survey for pathogens. The rodents were trapped in different areas each year of the study, so that a broad region could be surveyed. Blood, faeces, tissue samples and ectoparasites were collected in keeping with the samples collected from the snow leopards. These were tested for the same suite of pathogens as the snow leopards, to determine the possibility of overlap or exchange of pathogens. There were several different species of rodents occurring in the region and occupying different habitats, so it was possible that each rodent species may have been a reservoir for various pathogens. The laboratory analyses of the rodent samples were the same as those for the snow leopard samples for much of the testing, therefore the method descriptions are somewhat repetitive between chapters. However, the thesis has been kept in this format as each chapter has been submitted to a different journal as a manuscript for publication.

This Chapter has been submitted to the Journal of Ecohealth March 2018 and undergoing review.

*Article title: Pathogens of Rodents and their Ectoparasites in the South Gobi Desert of Mongolia*

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### **3.2 Abstract**

Rodents are known to host numerous zoonotic pathogens and are important in the transmission of zoonotic disease world-wide. We conducted the first survey of rodents and their ectoparasites for zoonotic pathogens in the Tost Mountains of the South Gobi Desert of Mongolia. The area is sparsely populated with nomadic herders, their domestic animals, the iconic snow leopard and other species of wildlife. During 2012, 2013 and 2015, we collected blood, faeces, fleas and ticks from 142 rodents, belonging to seven species and the genus *Alticola* where specimens from the latter could not be identified to species. Samples were tested via serology, real time (RT)-PCR and next generation sequencing. Antibodies to zoonotic pathogens were identified from six rodent species, in all years, with prevalences ranging between 12.8% to 52.0% for *Leptospira* spp., 4.0% to 21.2% for *Toxoplasma gondii*, 10.0 % to 21.2% for *Coxiella burnetii*, 0% to 5.7% for Puumala hantavirus and 1.0%

total for Seoul hantavirus. Ticks contained genera of potentially zoonotic bacteria, including *Rickettsia*, *Yersinia*, *Fransicella*, *Bacillus*, *Mycobacteria*, *Coxiella* and *Bordetella*. Rodent faeces contained a number of the same genera of zoonotic bacteria to those in the ticks and a number of different genera. Fleas were negative for *Y. pestis* by RT-PCR. The rodents showed no clinical signs of disease. This study showed that rodents carried pathogens that pose a significant disease risks to the indigenous nomadic herders, their livestock and other wildlife. We suggest targeted disease interventions to reduce transmission between these hosts in this mountainous desert region and that rodents can be used as sentinels for disease studies in this environment.

Keywords: Rodents, pathogens, zoonoses, Mongolia

### **3.3 Introduction**

Zoonotic diseases constitute the majority of emerging infectious disease, with most of them originating in wildlife (Chomel et al. 2007). Rodents are well known for their ubiquitous distribution and can transmit over 60 zoonotic pathogens, which can have severe effects on human health and health of other animals (Meerburg et al. 2009, Luis et al. 2013). Zoonotic and livestock pathogens transmitted by rodents include those that cause plague, hantavirus infections, leptospirosis, Q fever and toxoplasmosis. Hence rodents make an excellent sentinel species when aiming to detect multiple environmental pathogens.

In many countries, regardless of the level of industrialization, rodents are carriers of disease that have been classified as old, re-emerging and new (Singleton and Krebs 2003). Many of the pathogens that are considered to be emerging, such as hantaviruses, have likely caused infections in the past but have only recently been identified due to improved disease surveillance methods (Singleton and Krebs 2003, Adjemian et al. 2008, Schmidt et al. 2014).

Ectoparasites such as ticks, fleas, mites and lice parasitizing rodents are also vectors for pathogens causing zoonotic disease, including tick-borne encephalitis, plague babesiosis, ehrlichiosis and lyme-disease (Dantas-Torres 2012, Frye et al. 2015, Gutierrez et al. 2015). Although rodents are transmitters of plague, their fleas are the

main reservoir for *Y. pestis* (Bitam et al. 2010, Riehm et al. 2011, Richard et al. 2015).

In the mountains of Central Asia, livestock herding is the primary occupation. In countries such as Mongolia, with agriculture supplying 40% of the employment with a third of the population being nomadic or semi-nomadic herders, living in close contact with their animals, wildlife and the environment (Mishra et al. 2003, Batsukh et al. 2012). The proximity of people and animals provides ample opportunity for the exchange of pathogens between all members of the ecosystem in a complex cycle (Foggin et al. 2000, Batsukh et al. 2012). This cohesive type of environment is exemplified in the South Gobi region of southern Mongolia. Zoonotic diseases carried by rodents are therefore a significant concern in this region where they can impact the lives and livelihoods of the herders (Foggin et al. 2000, Odontsetseg et al. 2005b, Riehm et al. 2011).

Zoonotic diseases reported in Mongolia to date include brucellosis, leptospirosis, rabies, anthrax, plague and toxoplasmosis (Foggin et al. 2000, Odontsetseg et al. 2005b, Riehm et al. 2011, Batsukh et al. 2012). Pathogens carried by rodent species in this area or any other part of the Gobi Desert have not been identified or catalogued previously. Other wildlife species in the area, which can act as modes of pathogen transfer via interactions with rodents, include the iconic snow leopard (*Panthera uncia*), wolves (*Canis lupus*), red foxes (*Vulpes vulpes*), ibex (*Capra ibex*), argali (*Ovis ammon*), tolai hare (*Lepus tolai*.) and several mustelid and bird species. Approximately 90 herder families and their domestic animals such as goats, sheep, camels, horses and dogs also live in the area. Mining and tourism are increasing in the area and could be affected by zoonotic pathogens by affecting mining workers and tourists.

*Coxiella burnetii*, *Toxoplasma gondii*, *Leptospira* spp. and hantavirus infections, are important zoonotic diseases that can be transferred by rodents and can have a substantial economic impact on livestock herding, health of people and other wildlife (Adjemian et al. 2008, Yong-Zhen Zhang et al. 2009, Schmidt et al. 2014, Vanderburg et al. 2014).

*Coxiella burnetii* – the causative organism of Q fever, is a gram-negative bacterium, which commonly affects small ungulates such as goats and sheep and can cause abortion in these species, although the effects can be extremely varied (Woldehiwet 2004). Rodents have been identified as reservoirs of *C. burnetii* and provide a pathway for pathogen transmission from rodents to their predators but also other organisms such as livestock and people (Webster et al. 1995). In people, the pathogen can cause acute febrile conditions with flu-like symptoms as well as pneumonia, endocarditis and hepatitis (Vanderburg et al. 2014).

*Toxoplasma gondii* is a ubiquitous protozoal parasite (including marine environments) with the definitive host being members of the felid family (Tenter et al. 2000). Intermediate hosts encompass a wide host range and cause a spectrum and severity of symptoms (Dubey 2004, McAllister 2005). It is a parasite of extreme Neurological effects such as altered behaviour and loss of predator responses have been noted in rodents (Bech-Nielsen 2012) - enabling the parasite's life cycle to be completed when intermediate hosts are consumed by the definitive host (Dubey 1996, McAllister 2005, Afonso et al. 2006). Humans who thus live in close association with rodents (intermediate host) and cats (definitive host) can readily be exposed to infection by the faecal-oral route if in contact with cat faeces if the cat has consumed a rodent carrying the tissue cyst. The most severe effects on humans are neurological signs, brain damage and abortion of foetuses (Webster 2001, Macpherson 2005, McAllister 2005). However, it is also thought to modify behaviour in adults and is associated with schizophrenia (McAllister 2005, Bech-Nielsen 2012). Toxoplasmosis has only recently been reported to occur in humans in Mongolia, with no specific information available on prevalence or distribution (Batsukh et al. 2012).

Leptospirosis is not pathogenic to rodents but can be fatal to people, dogs and other animals, occurring worldwide (Bharti et al. 2003). It was thought to be mainly a pathogen of moist tropical areas but is appearing in desert regions, by residing in kidney tubules of host species that inhabit the desert regions (Holt et al. 2006). There are no reported human cases of leptospirosis in Mongolia but this is likely due to lack of testing (Victoriano et al. 2009). Symptoms of leptospirosis in people include fever, headaches, eye aches and if severe, jaundice due to liver and kidney damage (Romero-Vivas et al. 2013), Vanasco et al. 2003). In dogs, leptospirosis can cause jaundice, renal disease, fever, uveitis, pulmonary haemorrhage and death (Sykes et

al. 2011). Small ruminants such as goats and sheep can become carriers of leptospirosis, with the bacteria also causing abortion and reduced fecundity in these species (Lilenbaum et al. 2009).

Rodents are the main reservoir of hantaviruses with each species carrying a specific strain of the virus (Vapalahti et al. 2003). Hantavirus is a serious zoonotic pathogen that comes in two forms – a pulmonary form that mainly occurs in the Western hemisphere and a haemorrhagic renal form occurring primarily in Asia and Europe (Vapalahti et al. 2003, Yong-Zhen Zhang et al. 2009). Thousands of human cases occur annually worldwide (Kallio et al. 2009). It is a notifiable disease in most countries and is mainly transmitted from rodents to people via inhalation of aerosolized excrements and saliva (Yong-Zhen Zhang et al. 2009). Transmission can occur indirectly as the virus can survive for a long time in the environment (Kallio et al. 2006).

This study aimed to survey rodents and their ectoparasites in the South Gobi Desert of southern Mongolia for zoonotic pathogens and thereby increase knowledge of the potential pathogens that can pass from rodents to human, domestic animals and biodiversity in remote mountain desert ecosystems. Such knowledge is important both for improving the health and livelihoods of local herders, while concurrently facilitating conservation efforts of wildlife species. By identifying pathogens present in the system and possible pathogen transmission pathway(s), targeted interventions can limit spread between hosts and thus aid in the control of these diseases.

## **3.4 Methods**

### **3.4.1 Study area**

The study was conducted in the Tost Mountains in the Gobi Desert (43° N, 100°E) located in the province of Umnogovi in Mongolia in May and June in 2012 and 2013 and in March and April in 2015 (Plate 3.1). The area varies from open and dry with sparse vegetation consisting of mountain shrubs and mountain grasslands to rocky steppe regions and craggy mountains. The temperature ranges between -40°C in winter and +40°C in summer and the altitude ranges between 1,600 and 2,500 m above sea level. The study area is approximately 1 700 km<sup>2</sup> in size, with 23 native resident species of rodents reported (Batsaikhan et al. 2010).



**Plate 3.1 The Tost Mountains in the South Gobi Desert of Mongolia.**

### **3.4.2 Rodent trapping and field-collections**

Rodent trapping was carried out in three 4-weeks sessions (in May and June 2012, June 2013 and March and April 2015, respectively) and in three subsections of the Tost Mountains (Plate 3.1). Trapping was performed under ethics approval A1919 from James Cook University, Australia and approval from the Ministry of the Environment, Mongolia.



Two sizes of Sherman/Elliot live-traps (SFG Folding Trap 5.08 x 6.35 x16.51 cm and XLK Folding Trap 7.62x 9.525 x30.48 cm) were used. Bait comprised a mixture of rolled oats, honey and peanut butter. Trap placement was set opportunistically based on signs of small rodent activity. Eight trap lines were set in each year, with 10 traps on each line, approximately 20m apart covering a variety of habitats (**Plate 3.2**). These habitats included the flat steppe areas, rocky gullies and shallow gullies with the red bush *Bulis* (*Amygdalus pedunculata*). Several traps were also set within gers (local dwellings) with the owners' permission. Traps were set late in the afternoon and checked early the next morning (**Plate 3.2**). Trapped animals were carried to camp for processing unless trap lines were more than 30 minutes from the camp in which case animals were processed immediately in the field.

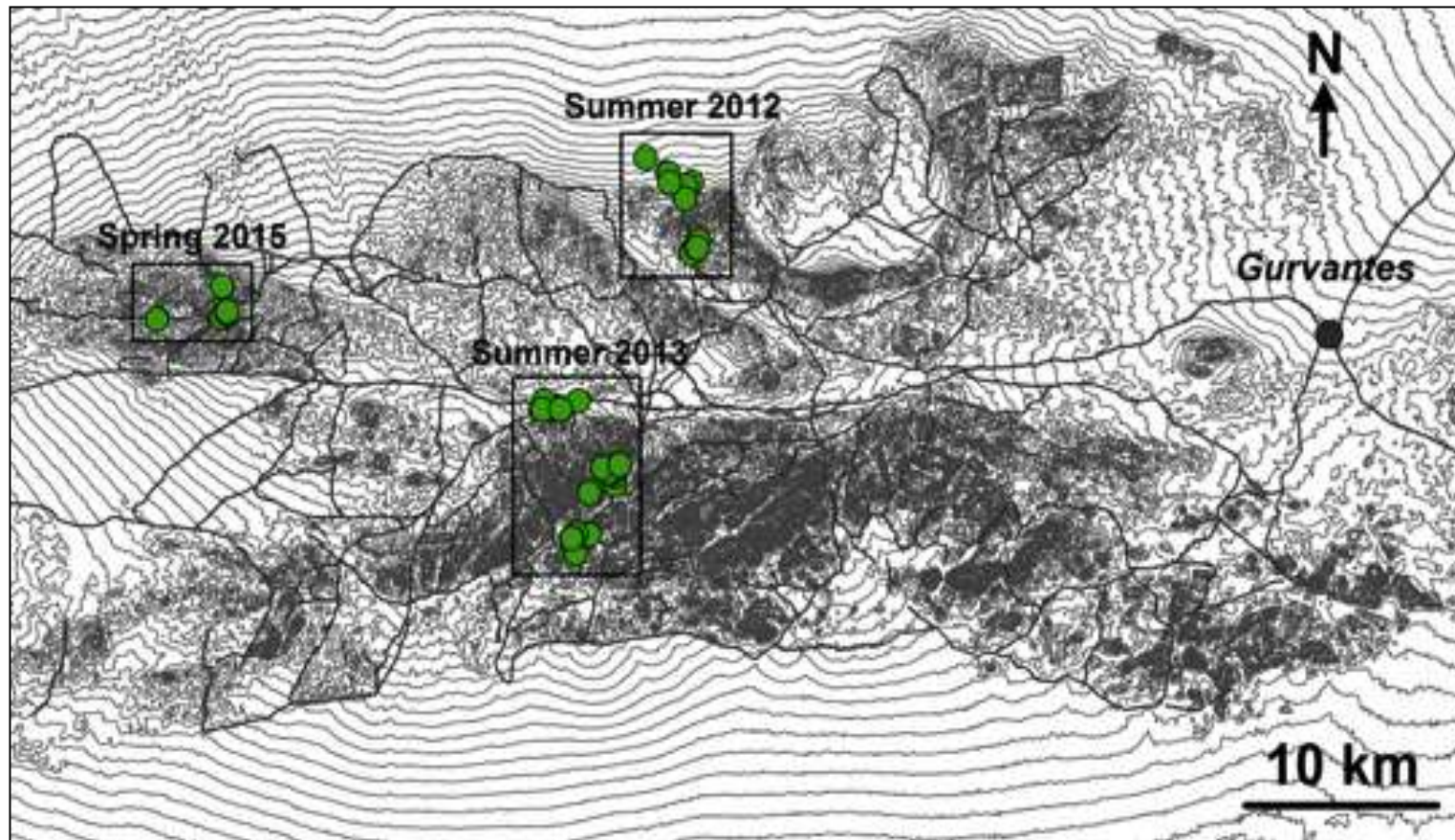


Figure 3.1 Map of study area in southern Mongolia, where green circles show rodent trapping sites in each year. Dark lines show small roads that traverse the study area.

### 3.4.3 Handling and measurements of rodents and their parasites



**Plate 3.2 Folding Elliot trap, camouflaged from road.**

Animals were removed from the traps and placed in calico bags. A Pesola spring balance was used to weigh the animals while in the bag. Animals were restrained by hand and examined for external wounds, parasites and sex determined before clinical parameters were measured.



**Plate 3.3 4 LW Scientific Zip spin solar centrifuge.**

Blood samples were collected and one ear was notched before rodents were released at their point of capture (see details of blood sampling below). Rodents' ears were notched to (1) assure that rodents were not sampled twice (i.e. I did not sample animals that had a notch in the ear) and (2) use for genetic identification of species (see Chapter 4 for detail). Ear notches were stored in 70% ethanol. Details of phenotypic characteristics and body measurements are presented in Chapter 4. (Esson et al. submitted). Fleas and ticks that were collected from the rodents were also stored in 70% ethanol.

### **3.4.3 Blood, faeces and flea collection**

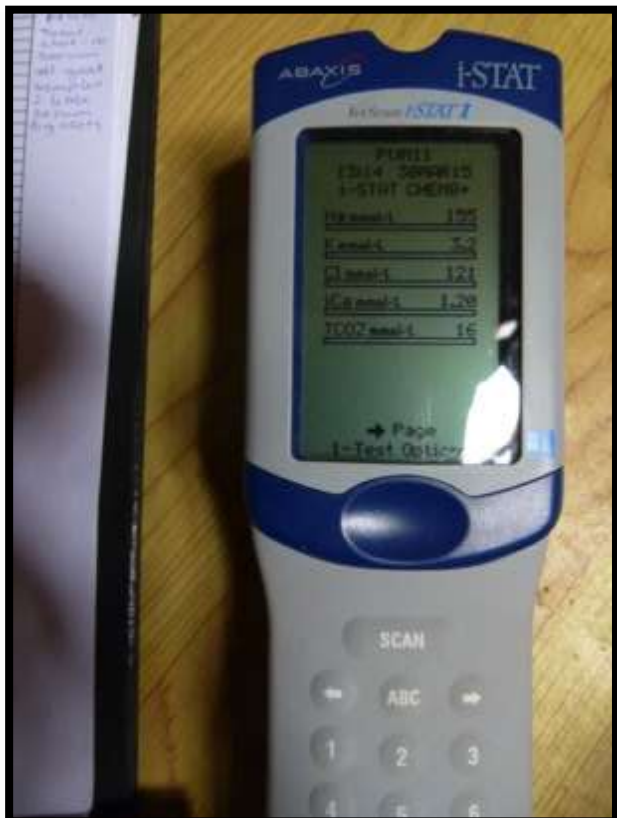
Small rodents (< 60 gms) were bled via cardiac puncture whereas larger species (>60 g) were bled via cardiac, cephalic or tail veins. Cardiac puncture was performed under local anaesthetic, with the rodents being restrained by hand. Once the sample had been collected the rodent was given fluids and glucose subcutaneously. Blood was not collected from rodents that weighed less than 20 g as large enough volumes for testing could not be obtained. Between 0.1 ml and 1.0 ml of blood was collected per animal. The volume of the majority of blood samples from the rodents were less than 0.5 ml and were stored on Advantec Nobuto filter paper strips (Toyo Roshi Kaisha, Ltd. Tokyo Japan 2007). Each strip holds a maximum of 100 µl of whole blood or 40 µl of sera. Strips were air-dried and then placed in paper envelopes and stored at room temperature until analyzed. Larger blood samples were placed in 1.0 ml or 0.5 ml serum separation tubes and spun using an LW Scientific 800 T26 7345 Zip spin solar powered centrifuge (Plate 3.3). Serum was stored in sterile cryovials at -20.0 °C until transport to the laboratory where it was then stored at -80 °C. The limited volume of blood from the smaller rodents (20 g) was only adequate for either serum analyses or haematology and biochemistry analyses. The priority was given to serum analyses, as the main aim of the study was to detect pathogen exposure. Additional blood was placed in lithium heparin tubes for haematology and biochemical analyses. Thin blood smears were made directly in the field and air-dried before placing in fixative back in the camp. They were then stored in plastic slide containers for transport back to the laboratory.

Faeces were collected from the trap or the holding bag and placed in RNA Later (Sigma-Aldrich Pty Ltd Castle Hill NSW 1765 Australia) or air dried and stored in paper envelopes at room temperature until analyzed.

#### 3.4.3.1 Species identification of rodents

Species were identified using a combination of morphological features as described in the literature (Nyamsuren et al, 2014) and DNA extracted from tissue and faecal samples. The sequences were sorted using a bioinformatic script (see André et al. 2017) with slight modifications. These sequences were compared with sequences available in BOLD databases (Ratnasingham & Hebert, 2007). Sequences that had a unique best-hit with an identity score greater than or equal to 98% were considered to be positive matches and allowed identification of the species producing the faeces.

#### 3.4.4 Haematology and biochemistry



The blood from the lithium heparin tubes was analyzed in the field with a hand-held Abaxis I-Stat Analyser (REM Systems Pty, Ltd, North Ryde, Australia) for haematology and biochemistry. Two cartridges were used per sample, with each cartridge requiring only two drops of blood (

Plate 3.4).

**Plate 3.4 Handheld Abaxis Istat analyser for measuring haematological and biochemical parameters in blood samples. (REM Systems Pty, Ltd, North Ryde, Australia)**

Blood smears were air dried and placed in Diff Kwik fixative in the field for transport. These were stained using haematoxylin and eosin Diff Kwik stains once back at the laboratory, then examined microscopically at 40 x and 100 x magnification for any red and white cell abnormalities and haemoparasites.

**3.4.4.1 Elution of sera from Nobuto strips**

Nobuto strips were cut into small pieces and placed in Eppendorf tubes with 200µl phosphate buffer solution (PBS) if only one side of the strip was saturated with blood or 400µl of PBS if both sides of the strip were saturated with blood. Nobuto strips sat in the solution for one hour for the serum to elute. The whole nobuto strip holds 40µl of serum, therefore, once eluted the serum is diluted 1:10. The tubes were heat-inactivated at 60° C for one hour, centrifuged and the filter papers removed. Remaining serum was then stored at -80°C until analyzed.

**3.4.5 Pathogen identification using ELISA, NGS and RT-PCR**

Rodent sera, faeces and/or ectoparasites were tested for *Coxiella burnetii*, *Leptospira* spp., *Yersinia pestis*, *Toxoplasma gondii*, Hantavirus, *Echinococcus*, *Giardia* and *Cryptosporidium*.

**3.4.5.1 *Coxiella burnetii***

Serum was screened for antibodies to *C. burnetii* using an Innovative Diagnostics Q Fever Indirect Multi-species enzyme-linked immunosorbent assay ELISA kit (Idvet, 310, rue Louis Pasteur – Grabels-France). The ELISA was performed on the eluted serum samples following the manufacturers' protocol. The plate results were read at 450nm on a Thermo Scientific Multiskan FC plate reader (Thermo Scientific, Vantaa, Finland). Positive or negative results were calculated using:

$$\text{S/P}\% = \frac{\text{OD}_{\text{sample}} - \text{OD}_{\text{NC}}}{\text{OD}_{\text{PC}} - \text{OD}_{\text{NC}}} \times 100$$

Where S is the sample, PC is the positive control, NC negative control and OD is the optical density recorded.

If  $\text{S/P}\% \leq 40\%$  the result is negative, if  $40\% \leq \text{S/P}\% < 50\%$  result is doubtful,

If  $50\% \leq \text{S/P}\% < 80\%$  result is positive, if  $\text{S/P}\% \geq 80\%$  result is strongly positive.

#### 3.4.5.2 *Toxoplasma gondii*

Antibodies to *Toxoplasma gondii* were detected using an ABNOVA IgG antibody ELISA kit (ABNOVA, Taipei City 114, Taiwan). This was a human kit, so was modified by replacing the enzyme conjugate with the appropriate rodent conjugate. The species of rodents were unknown at the time of sample analyses, so test samples were run to determine which enzyme conjugate to use (anti-rat, anti-hamster or anti-mouse) depending on the lineage of the rodent being tested. The plate results were read at 450 nm on a Thermo Scientific Multiskan FC plate reader (Thermo Scientific, Vantaa, Finland). A positive result was recorded when the optical density was greater than the critical cut-off value.

#### 3.4.5.3 *Leptospira spp.*

Serum was screened for *Leptospira* antibodies using a qualitative rat *Leptospira* IgG (LS-IgG) ELISA kit, (MBS036971, Mybiosource, San Diego, California, USA) which uses a double antigen sandwich ELISA. Plates were pre-coated with rat *Leptospira* antigen, *L. icterohaemorrhagiae* and *L. grippityphosa* and the appropriate horseradish peroxidase (HRP) conjugated antigen before adding test samples. The plates were incubated at 37°C for one hour, then washed. HRP substrates were added and incubated for 15 minutes before stopping the reaction. The plate results were read at 450 nm on a Thermo Scientific Multiskan FC plate reader as above. Positive results were obtained if the value recorded for the optical density (OD) of the sample was greater than the critical cut off value. The critical cut off value was the average of the OD of the negative control well plus 0.15 as instructed in the kit methods.

Positive samples were sent to the Swedish Veterinary Institute for Microscopic Agglutination Tests (MAT, Faine et al. 1999) and tested against the following serovars: Australis, Hebdomadis, Icterohaemorrhagiae, Pomona, Canicola and Grippotyphosa. These serovars were selected as they had previously been reported to occur in other regions of Mongolia (Odontsetseg et al. 2005a, Odontsetseg et al. 2005b). The antigens used were live cultures of referenced strains. All sera that gave a positive reaction at a 1:100 dilution were further titrated in serial two-fold dilutions to titre endpoint that is 50% agglutination. A titre  $\geq 100$  was deemed positive to exposure to leptospire.

#### *3.4.5.4 Hantavirus*

Rodent serum samples were tested for antibodies against Puumala (PUUV) and Seoul (SEOV) hantaviruses. A PUUV IgG ELISA was run using the protocol earlier described by Verner-Carlsson et al. (2014). Serum samples were diluted to 1:300 in ELISA buffer (EB) and incubated for one hour at 37°C. Alkaline phosphatase (ALP)-conjugated antibody (anti-rat, anti-mouse and anti-hamster, Jackson ImmunoResearch) diluted in EB were added and incubated for one hour at 37°C. P-nitrophenyl phosphate (PnPP) in diethanolamine was used as substrate and incubated for 15 minutes at 37°C. The absorbance was read at 405 nm. Due to the small amount of rodent serum available single, rather than duplicate samples were analysed. All positive samples were retested.

Serum was tested for antibodies to SEOV using an IgG monoclonal antibody (MAB)-capture ELISA technique as described earlier (Verner-Carlsson et al. 2015): An anti-hantavirus nucleocapsid Mab in coating buffer was added to plates and incubated overnight at 4°C. Plates were washed and 200  $\mu$ l per well of blocking buffer was added and incubated for one hour at 37°C. 100  $\mu$ l/well of SEOV antigen diluted 1:300 in EB was placed in the top half of the plate, the lower half of the plate had 100  $\mu$ l/well of EB added as negative control. Plates were incubated again for 1 hour at 37°C. Positive and negative controls were added to the first 4 wells, with serum samples to the remaining wells on the plate and incubated for 1 hour at 37°C. One hundred microlitres of the appropriate ALP-conjugated antibody as described above for PUUV analysis, diluted in EB, was added to each well incubated for 1 hour at



37°C. One hundred microlitres/well of pNPP substrate was added and incubated at room temperature for 20 minutes before reading at 405nm.

### **3.4.6 Flea analyses**

Fleas were tested for *Yersinia pestis* using an EZ1 machine from Qiagen with a DNA tissue kit (48). A minimum of five fleas were placed in Eppendorf tubes and crushed with a pestle. Fleas from different rodents had to be pooled as not all rodents had five fleas or more. The crushed fleas then were lysed in 2 ml Nuclisens lysis buffer (Biomérieux) for an hour. Two hundred microlitres of the resultant solution was placed in the DNA extraction machine where each extraction panel consisted of six samples with 5 µl of seal herpes virions added to one sample as an internal extraction control. One hundred microlitres of DNA was eluted from each sample, which was then run by RT-PCR with a specific probe for *Y. pestis*.

### **3.4.7 Faecal samples and ticks**

Total DNA was extracted from faecal samples and rectal smears that were stored in RNAlater. Next Generation Sequencing (NGS) was used to test for bacterial genera. These analyses detect all bacterial genera present, as well as endosymbionts or opportunists (Chaya and Parija 2014). Initially each sample was manually mixed with a sterilized scalpel to commence the DNA extraction process. Three treatments were performed to allow a better harvest of gram-positive bacterial DNA. First, samples were immersed for 1 hour at 37°C in an enzymatic lysis buffer consisting of 20 mM Tris·Cl, pH 8.0, 2 mM sodium EDTA, 1.2% Triton® X-100 and 20 mg/ml of lysozyme as described in the Dneasy™ Tissue Kit handbook. Second, samples were submitted to three freeze-thaw cycles (Zhou, Bruns and Tiedje 1996). Finally, 25 µl of proteinase K and 200 µl of buffer A was added to the sample before an overnight shaking incubation at 56°C. 200 µl of this mix was then introduced in a QIAcube (Qiagen®, Hilden) following the manufacturer's protocol for purification of total DNA from animal tissues. After the extraction step, an Illumina amplicon sequencing following a modified Miseq protocol was performed (16S Metagenomic Sequencing Library Preparation). Total genomic DNA from faeces were subjected to PCR amplification targeting a ~142-bp fragment of the 16S rRNA variable regions 5 and 6 (V5-V6) using the primer pair 784F-1061R originally designed by (Andersson 2008) coupled with the Illumina overhand adapters. One extraction negative control was

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added to every batch of 24 samples and two additional negative controls were added for the PCR steps. A mock community sample (HM-783D, BEI resources) containing genomic DNA from 20 bacterial strains at concentrations ranging between 0.6 and 1400 pg/μl was also added in triplicate to confirm the reliability of our method. Purified products were quantified using Quant-iT™ PicoGreen® dsDNA Assay Kit following the manufacturer protocol on a fluorimeter (FilterMax F3, Molecular Devices). Quantified products were then pooled in equimolarity and sent to the GIGA Genomics platform (Ulg) for sequencing on an ILLUMINA MiSeq V2 benchtop sequencer.

Faecal samples were screened for *Giardia*, *Echinococcus* and *Cryptosporidium* following the protocols proposed by (Ghosh et al. 2000) and (Chaya and Parija 2014).

Ticks were also screened for bacterial genera using the same method as for the faecal samples. Six ticks from six hosts from two species of rodent, red-cheeked ground squirrel and long-tailed dwarf hamster were tested.

#### 3.4.7.1 Statistics

General linear models (using binomial logistic regression; family= binary, link=logit) in the glm and car packages in program R (R-Development Core Team 2014) were used to examine if pathogen prevalence differed among pathogens, years of the study and host rodent species. That is, the dependent variable in each analysis was the proportion of individuals testing positive for a pathogen, weighted by the number of individuals used to calculate the proportion. An initial overall analysis used pathogen, rodent species, and year as the explanatory variables, as well as all 2-way interactions between those three variables. Then each pathogen species was analyzed separately, testing the effects of rodent species, year, and their interaction (Appendix 1).

## **3.5 Results**

### **3.5.1 Rodent trapping and field-collections**

I trapped a total of 177 rodents and obtained samples from 142 of these individuals from seven different species and one genus between May 2012 and April 2015. The species were *Spermophilus erythrogegens*- Red-cheeked ground squirrel (n=22), *Allactaga sibirica* - Siberian jerboa (n=17), *Meriones meridianus*- midday gerbil (n=27), *Cricetulus longicaudatus*- longtailed dwarf hamster (n=60), *C. migratorius* - grey dwarf hamster (n=30), *C. kamensis* - Kam hamster (n=4), *Ochotona pallasi* - Pallas's pika (n=4) or Mongolian pika and *Alticola spp.* - Voles (n=12) could not be identified further than genus.

### **3.5.2 Physical condition**

The rodents appeared in good physical health. Only one juvenile ground squirrel with a heavy flea burden appeared lethargic and one hamster was missing an ear. No other external wounds were observed. Approximately 26% of the females captured were either pregnant or lactating at the time of capture. No adult male red-cheeked ground squirrels were caught with only one juvenile male red-cheeked ground squirrel caught. Details of phenotypic characteristics and body measurements are included in a separate paper (Esson et al. submitted, Chapter 4).

### **3.5.3 Haematology and biochemistry**

The haematology and biochemistry results are presented in Table 3.1. One jerboa was slightly anaemic with a haematocrit of 24% ( $41.6\% \pm 9.7$ ) and a long-tailed dwarf hamster had high creatinine levels, but both animals were negative for the pathogen antibodies tested for.

Blood smear cell morphology looked normal (n=142 smears from seven different rodent species), although the occasional anisocytotic cell was seen in 11 smears but these may have been artifacts of sample preparation. Heinz bodies were present within the red blood cells of a jerboa but they were not associated with signs of illness. Based on the blood results, rodents within the study appeared to be in good health.



Table 3.1 Haematology and biochemistry values for rodents captured in the Tost Mountains in Mongolia from 2012 to 2015.

<b>Rodent species</b> <b>Sample size</b>	<b>Na</b> <b>mmol/L</b>	<b>K</b> <b>mmol/L</b>	<b>Cl mmol/L</b>	<b>iCa</b>	<b>Tco2</b>	<b>Glu</b> <b>mmol/L</b>	<b>Urea</b> <b>mmol/L</b>	<b>Crea</b> <b>umol/L</b>	<b>Hct</b> <b>%PCV</b>	<b>Hb* g/dL</b>	<b>AnGap</b> <b>mmol/L</b>
Red Cheeked Ground Squirrel N=5	138.4±3.8	4.7±1.6	106.8±2.6	1±0.1	23.2±2.2	8.3±1.7	12±2.6	32.8±4	43.4±6.3	14.8±2.2	14.4±2.6
Jerboa N=5	150.6±2.3	4.7±2.3	120.6±4	1±0.1	15±7.2	9.2±3.6	8.9±0.9	22.8±5.2	41.6±9.7	14.2±3.3	20.8±5.5
Middy Gerbil N=5	154±4.3	4.9±2.8	125.4±7.1	1.2±0.1	12.6±5.1	9.5±3.7	11.1±4.1		42.2±5	14.3±1.5	
Long-tailed Dwarf hamster N=4	143.3	6.9	128.3	1.1	11.3	7.1	14.6		42.3	14.4	21.5
Pika N=2	129	4.2	97	1.08	12	12	5.6	<18	39	13.3	26

Values are means plus/minus standard deviation. N =number of animals sampled.

Sodium –Na, Potassium- K, Chloride- Cl, Calcium –Ca, Carbon Dioxide within the body –TCO<sub>2</sub>, Glucose –Glu, Urea, Cre-Creatinine, Haematocrit –Hct, Haemoglobin-Hb, Anion Gap-AnGap

### 3.5.4 Pathogens detected

I detected serum antibodies via serological assay to *Coxiella burnetii*, *T. gondii*, *Leptospira* spp and Hantavirus, in the red-cheeked ground squirrels, jerboas, midday gerbils, long-tailed dwarf hamsters, grey dwarf hamsters and pikas between 2012 and 2015 (Table 3.2, Figure 3.2). No antibodies to the pathogens tested for were identified in the Kam dwarf hamster or the vole species. In 2012 antibodies to *C. burnetii* were detected in three species of rodents - a midday gerbil, two long-tailed dwarf hamsters and two grey dwarf hamsters. In 2013 *C. burnetii* was detected in the same three species it was detected in, in 2012 plus ground squirrels, jerboas and pikas. In 2015 no ground squirrels or jerboas were trapped and only one gerbil was captured with *C. burnetii* detected only in one long-tail dwarf hamster and one grey hamster (Table 3.2). Antibodies to *T. gondii* were identified in three species of rodents in 2012– two ground squirrels, one jerboa and one grey hamster. In 2013 it was detected in all six species tested and in 2015 it was only detected in one pika (Table 3.2).

*Leptospira* antibodies were detected in four of the rodent species in 2012 – two ground squirrels, one midday gerbil, two long-tailed dwarf hamsters and one grey hamster. In 2013 antibodies were detected in all six rodent species from all trapping sites. In 2015 it was identified in three species, ten long-tailed dwarf hamsters, two grey hamsters and one pika (Table 3.2). *Leptospira* serovars identified by MAT were *L. interrogans* Australis in one pika (R 42) with a 1:100 titre from 2013 and *L. interrogans* Icterohaemorrhagiae in one midday gerbil (R53) with a 1:100 titre and one jerboa (R76), with a 1:400 titre both from 2013.

Puumala hantavirus (PUUV)- reactive antibodies were only detected in the long-tailed dwarf hamster in 2012 and the ground squirrel, midday gerbil and jerboa in 2013. It was not detected in 2015. Seoul hantavirus (SEOV) – reactive antibodies were only identified in one ground squirrel in 2013 (Table 3.2).

All fleas collected from 71 rodents were negative for *Yersinia pestis* by RT-PCR, therefore, the rodent sera were not tested for *Y. pestis*.

Table 3.2 Seroprevalence of *C. burnetii* in six rodent species captured in the Tost Mountains in southern Mongolia from 2012 to 2015.

<b><i>Coxiella burnetii</i></b>					
<b>Rodent species</b>	<b>Year</b>	<b>Prevalence</b>	<b>Sample size</b>	<b>*LCL</b>	<b>*UCL</b>
Ground squirrel	2012	0	9	0	0.34
	2013	0.44	9	0.14	0.79
	2015	-	0	-	-
Midday Gerbil	2012	0.17	6	0.004	0.64
	2013	0.25	16	0.07	0.52
	2015	0	1	0	0.98
Jerboa	2012	0	5	0	0.52
	2013	0.29	7	0.04	0.71
	2015	-	0	-	-
*LT dwarf hamster	2012	0.18	11	0.023	0.518
	2013	0.11	19	0.012	0.331
	2015	0.91	11	0.002	0.413
Grey hamster	2012	0.13	8	0.003	0.527
	2013	0.08	13	0.002	0.360
	2015	0.14	7	0.004	0.579
Pika	2012	-	0	-	-
	2013	0.50	2	0.013	0.987
	2015	0	1	0	0.980

The small numbers of voles and Kam hamsters tested were negative to antibodies.

\* LT dwarf hamster- Long-tailed dwarf hamster, LC- Lower confidence limits

Table 3.3 Seroprevalence of *T.gondii* in six rodent species captured in the Tost Mountains in southern Mongolia from 2012 to 2015.

<b><i>Toxoplasma gondii</i></b>					
<b>Rodent species</b>	<b>Year</b>	<b>Prevalence</b>	<b>Sample size</b>	<b>*LCL</b>	<b>*UCL</b>
Ground squirrel	2012	0.22	9	0.028	0.600
	2013	0.33	9	0.075	0.701
	2015	-	0	-	-
Midday Gerbil	2012	0	6	0.00	0.336
	2013	0.12	17	0.015	0.364
	2015	0	1	0.00	0.975
Jerboa	2012	0.20	5	0.005	0.716
	2013	0.22	9	0.028	0.60
	2015	-	0	-	-
*LT dwarf hamster	2012	0	11	0.00	0.285
	2013	0.12	17	0.015	0.364
	2015	0	16	0.00	0.206
Grey hamster	2012	0.13	8	0.003	0.527
	2013	0.33	12	0.099	0.651
	2015	0	7	0.00	0.410
Pika	2012	-	0	-	-
	2013	0.50	2	0.013	0.987
	2015	1.00	1	0.025	1.00

The small numbers of voles and Kam hamsters tested were negative to antibodies.

\* LT dwarf hamster- Long-tailed dwarf hamster, LC- Lower confidence limits



Table 3.4 Seroprevalence of *Leptospira* spp. in six rodent species captured in the Tost Mountains in southern Mongolia from 2012 to 2015.

<b><i>Leptospira</i> spp.</b>					
<b>Rodent species</b>	<b>Year</b>	<b>Prevalence</b>	<b>Sample size</b>	<b>*LCL</b>	<b>*UCL</b>
Ground Squirrel	2012	0.20	10	0.025	0.556
	2013	0.22	9	0.028	0.600
	2015	-	0	-	-
Midday Gerbil	2012	0.17	6	0.004	0.641
	2013	0.39	18	0.173	0.643
	2015	0	1	0	0.975
Jerboa	2012	0	15	0.00	0.218
	2013	0.60	10	0.262	0.878
	2015	-	0	-	-
*LT dwarf hamster	2012	0.25	8	0.32	0.65
	2013	0.35	17	0.142	.647
	2015	0.63	16	0.354	0.848
Grey hamster	2012	0.13	8	0.003	0.527
	2013	0.57	14	0.289	0.823
	2015	0.29	7	0.37	0.71
Pika	2012	-	0	-	-
	2013	0.50	2	0.013	0.987
	2015	1.00	1	0.025	1.00

The small numbers of voles and Kam hamsters tested were negative to antibodies.  
 \* LT dwarf hamster- Long-tailed dwarf hamster, LC- Lower confidence limits

Table 3.5 Seroprevalence of Puumala (PUUV) hantavirus in six rodent species captured in the Tost Mountains in southern Mongolia from 2012 to 2015.

<b>Puumala (PUUV) hantavirus</b>					
<b>Rodent species</b>	<b>Year</b>	<b>Prevalence</b>	<b>Sample size</b>	<b>*LCL</b>	<b>*UCL</b>
Ground Squirrel	2012	0	9	0.00	0.336
	2013	0.11	9	0.003	0.482
	2015	-	0	-	-
Midday Gerbil	2012	0.17	6	0.004	0.641
	2013	0.10	20	0.025	0.556
	2015	0	1	0	0.975
Jerboa	2012	0	15	0	0.218
	2013	0.10	10	0.003	0.445
	2015	-	0	-	-
*LT dwarf hamster	2012	0.17	6	0.004	0.641
	2013	0	17	0	0.195
	2015	0	16	0	0.206
Grey hamster	2012	0	8	0	0.369
	2013	0	12	0	0.265
	2015	0	7	0	0.41
Pika	2012	-	0	-	-
	2013	0	2	0	0.842
	2015	0	1	0	0.975

The small numbers of voles and Kam hamsters tested were negative to antibodies.

\* LT dwarf hamster- Long-tailed dwarf hamster, LC- Lower confidence limits

Table 3.6 Seroprevalence of Seoul (SEOV) hantavirus in six rodent species captured in the Tost Mountains in southern Mongolia from 2012 to 2015.

<b>Seoul (SEOV) hantavirus</b>					
<b>Rodent species</b>	<b>Year</b>	<b>Prevalence</b>	<b>Sample size</b>	<b>*LCL</b>	<b>*UCL</b>
Ground Squirrel	2012	0	9	0.00	0.336
	2013	0.11	9	0.003	0.482
	2015	-	0	-	-
Midday Gerbil	2012	0	6	0	0.459
	2013	0	20	0	0.168
	2015	0	1	0	0.975
Jerboa	2012	0	15	0	0.218
	2013	0	10	0	0.308
	2015	0	1	0	0.975
*LT dwarf hamster	2012	0	6	0	0.459
	2013	0	17	0	0.195
	2015	0	16	0	0.206
Grey hamster	2012	0	8	0	0.369
	2013	0	12	0	0.265
	2015	0	7	0	0.41
Pika	2012	-	0	-	-
	2013	0	2	0	0.842
	2015	0	1	0	0.975

The small numbers of voles and Kam hamsters tested were negative to antibodies.

\* LT dwarf hamster- Long-tailed dwarf hamster, LC- Lower confidence limits

UC-Upper confidence limits

### 3.5.5 Pathogen prevalences

The prevalence of antibodies differed between *C. burnetii*, *T. gondii*, *Leptospira* spp. and *PUUV* ( $\chi^2_3 = 51.03$ ,  $P < 0.001$ ) and among years ( $\chi^2_2 = 13.7$ ,  $P < 0.001$ ) but not among host rodent species ( $\chi^2_5 = 4.72$ ,  $P=0.45$ ). Nor was there evidence for interactions among pathogen, year and host rodent species (Table 3.).

In the absence of significant host effects or significant interaction terms, we pooled results to look at overall pathogen prevalence across the three years (Figure 3.2).

**In the absence of significant host effects or significant interaction terms, we pooled results to look at overall pathogen prevalence across the study site and overall trends between years**

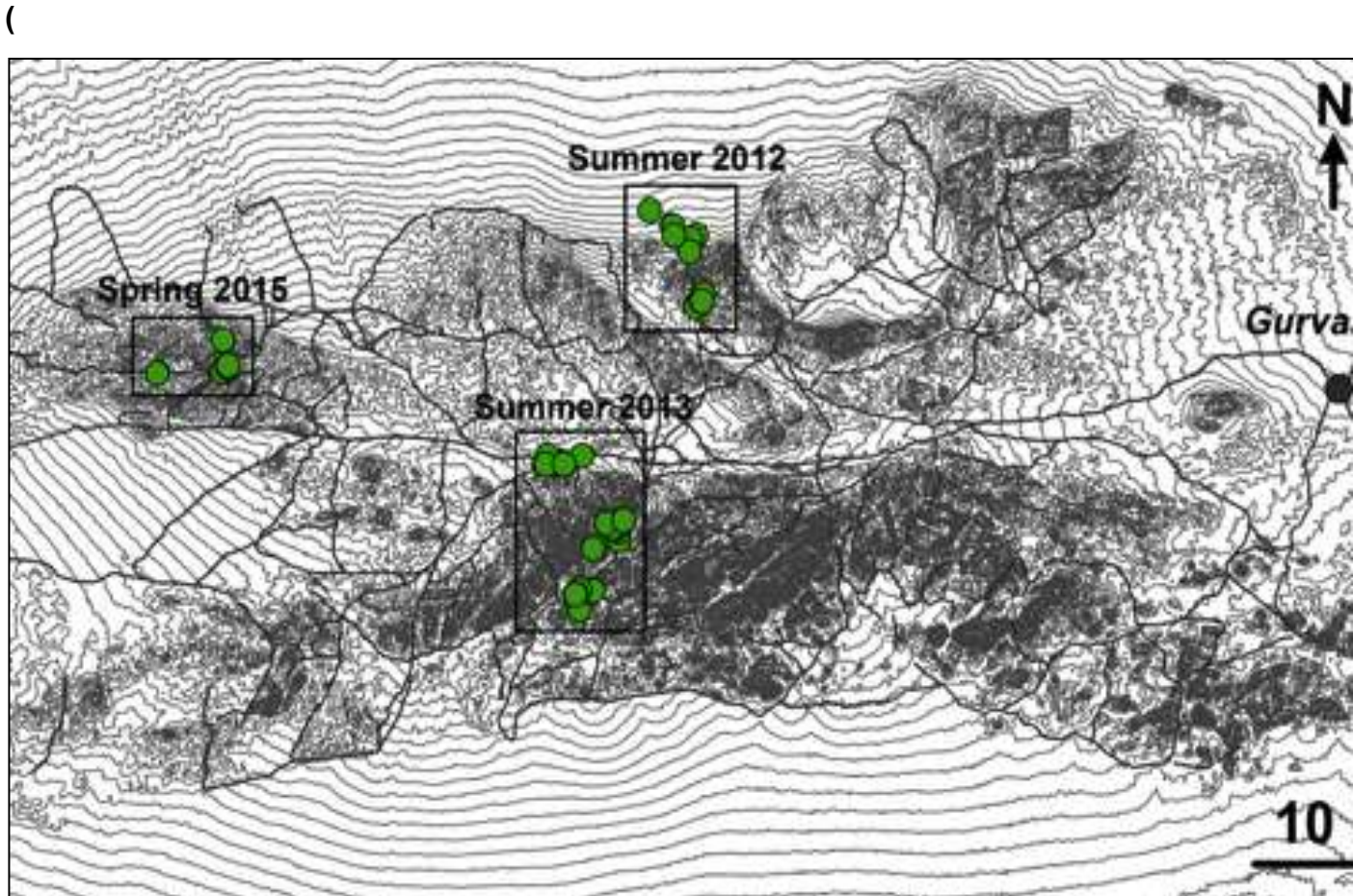


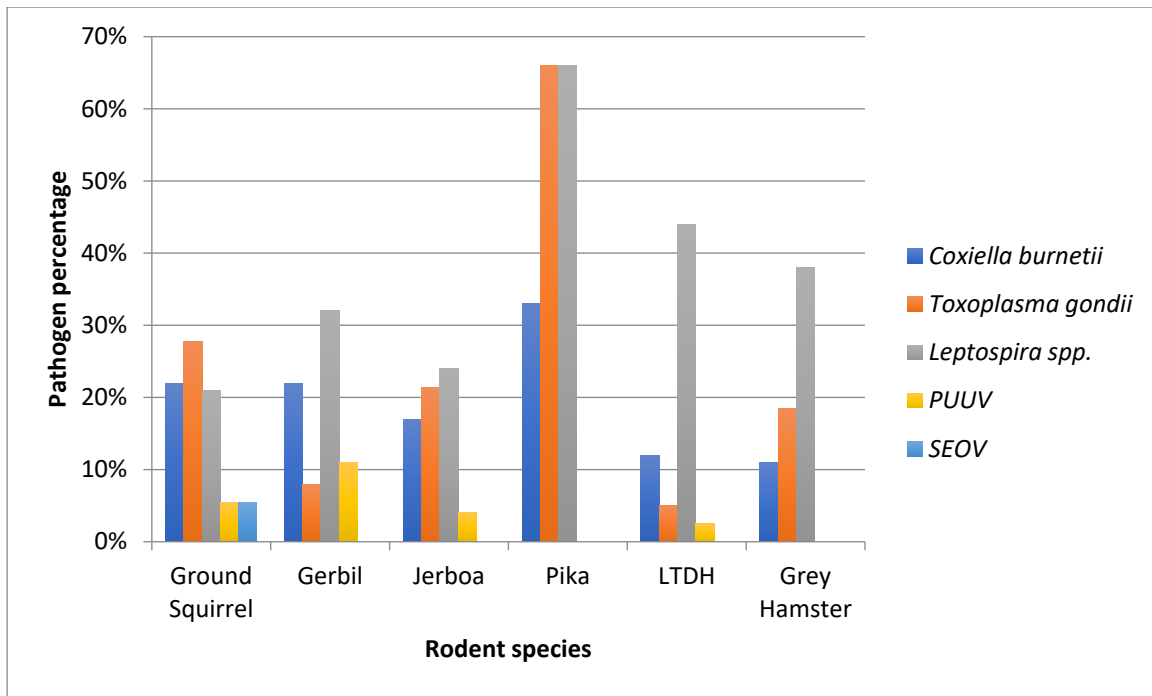
Figure 3.1, Figure 3.2). However, sample sizes of the different rodent species are small, so is it possible that more data would identify differences between hosts and differences in temporal trends among pathogens.

Table 3.7 Analyses of Deviance for logistic regression (Type II tests).

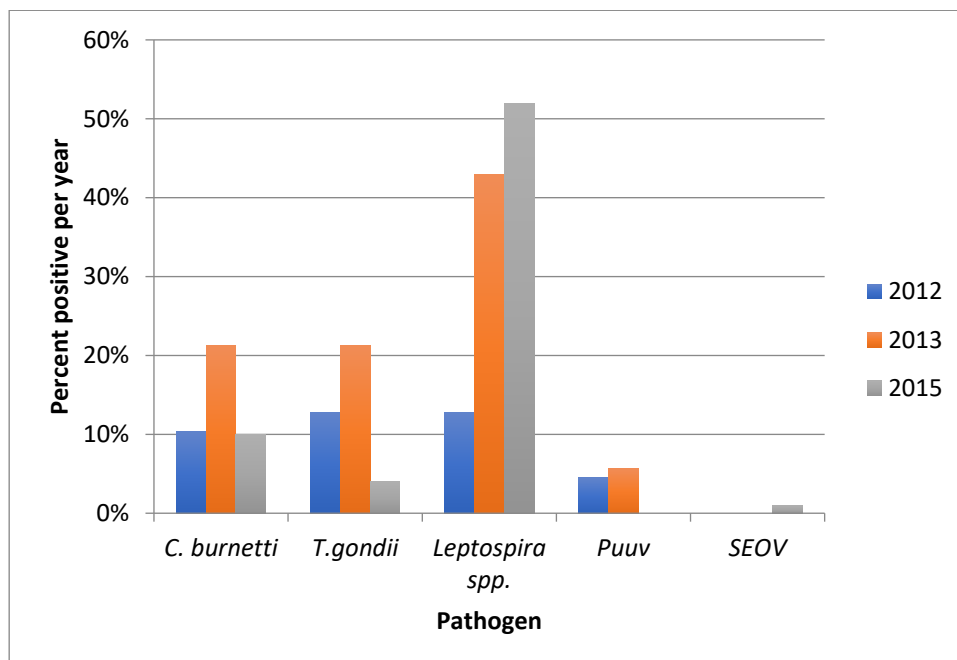
Variable	LR $\chi^2$ square	Degrees of freedom	P value
Pathogen	51.029	3	4.822e-11 ***
Rodent	4.722	5	0.450733
Year	13.700	2	0.001059 **
Pathogen: rodent	13.756	15	0.544081

Pathogen: year	7.638	6	0.265869
Rodent: year	11.818	7	0.106715

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**Figure 3.2 Seroprevalence of pathogens in six rodent species where the prevalence is pooled for all years of study from 2012 to 2015. LTDH = Long-tailed dwarf hamster. The small numbers of voles and Kam hamsters tested were negative.**



**Figure 3.3 Seroprevalence of *Coxiella burnetii*, *Toxoplasma gondii*, *Leptospira spp.*, PUUV and SEOV (hantavirus), antibodies for all rodent species combined across the three years of the study.**

### 3.5.6 Annual and host rodent species variation for individual pathogen prevalence

Analysing the prevalence of each pathogen separately and considering the effects of year and host rodent species showed no significant difference among host species for prevalence of *C. burnetii* ( $\chi^2_5 = 9.2$ ,  $P = 0.83$ ), or year ( $\chi^2_2 = 2.4$ ,  $P = 0.31$ ) or interaction of rodent and year ( $\chi^2_7 = 9.2$ ,  $P = 0.23$ ). For *T. gondii* there was significant difference in prevalence among rodent hosts ( $\chi^2_5 = 11.9$ ,  $P = 0.04$ ), and no significant difference among years ( $\chi^2_2 = 5.0$ ,  $P = 0.08$ ) or evidence of an interaction ( $\chi^2_7 = 6.3$ ,  $P = 0.51$ ). For *Leptospira* spp. there was no significant difference among host species for the prevalence of *Leptospira* spp. ( $\chi^2_5 = 1.6$ ,  $P = 0.91$ ), a strongly significant year effect ( $\chi^2_2 = 13.7$ ,  $P = 0.002$ ), a very weak suggestion year effect may vary between species ( $\chi^2_7 = 13.6$ ,  $P = 0.06$ ). For Puumala hantavirus there were no significant effects of rodent species ( $\chi^2_5 = 3.9$ ,  $P = 0.55$ ) or year ( $\chi^2_2 = 1.3$ ,  $P = 0.52$ ) or interaction between host species and year ( $\chi^2_7 = 6.3$ ,  $P = 0.52$ ). The inability to detect year-to-year changes in pathogens other than *Leptospira* is likely to be influenced by their lower prevalence. (See Appendix 2 for single pathogen analyses).

### 3.5.7 Next-generation sequencing of rodent ticks and faeces

NGS analyses resulted in identification of 216 genera of bacteria from 24 ticks and 250 genera of bacteria from 39 rodent faeces. Individual rodents did not shed the same zoonotic bacteria in their faeces as was present in the ticks they harboured. Of the 216 genera of bacteria found in ticks, 21 were potentially zoonotic (Table 3.). Of the 250 genera of bacteria found in the rodent faeces 17 were potentially zoonotic (Table 3.). Parasites, *Giardia*, *Cryptosporidium*, *Echinococcus* and other endoparasites were not identified from the faecal samples.

Table 3.7 Genera of only the zoonotic bacteria identified in ticks collected from rodents and in rodent faeces where the genera were determined by using NGS methods.

<b>Genus of bacteria</b>	<b>Prevalence in ticks collected from rodents</b>		<b>Prevalence in rodent faeces</b>	
<i>Aeromonas</i>	0%	0/8	11%	1/9
<i>Bacillus</i>	12%	1/8	11%	1/9
<i>Bacteroides</i>	38%	3/8	11%	1/9
<i>Bartonella</i>	12%	1/8	11%	1/9
<i>Batrachyspira</i>	0%	0/8	11%	1/9
<i>Bordetella</i>	12%	1/8	0%	0/9
<i>Burkholderia</i>	100%	8/8	11%	1/9
<i>Campylobacter</i>	0%	0/8	22%	2/9
<i>Clostridia</i>	100%	8/8	11%	1/9
<i>Corynebacterium</i>	100%	8/8	0%	0/9
<i>Coxiella</i>	75%	6/8	0%	0/9
<i>Enterococcus</i>	12%	1/8	11%	1/9
<i>Escherichia/Shigella</i>	50%	4/8	11%	1/9
<i>Francisella</i>	12%	1/8	11%	1/9
<i>Helicobacter</i>	12%	1/8	11%	1/9
<i>Legionella</i>	50%	4/8	11%	1/9
<i>Mycobacterium</i>	25%	2/8	0%	0/9
<i>Mycoplasma</i>	0%	0/8	11%	1/9
<i>Pandoraea</i>	100%	8/8	0%	0/9
<i>Pseudomonas</i>	0%	0/8	11%	1/8
<i>Rhodococcus</i>	38%	3/8	0%	0/9
<i>Rickettsia</i>	100%	8/8	11%	1/9
<i>Staphylococcus</i>	100%	8/8	0%	0/9
<i>Streptococcus</i>	63%	5/8	0%	0/9
<i>Treponema</i>	12%	1/8	11%	1/9
<i>Yersinia</i>	12%	1/8	0%	0/9



### 3.6 Discussion

I found that rodents in the Tost Mountains of Mongolia had been exposed to significant bacterial, protozoal and viral zoonotic pathogens. Ticks collected from the rodents contained many genera of potentially zoonotic bacteria. Important zoonotic pathogens identified by serology included *Coxiella burnetii*, *Toxoplasma gondii*, *Leptospira* spp. and hantavirus. Overall, *Leptospira* and *T. gondii* occurred at higher prevalences in the rodents sampled than *C. burnetii*, PUUV and SOEV hantavirus. Several of the rodent species were seropositive for exposure to more than one pathogen but did not show any clinical signs of ill health at the time of capture. However, sample sizes of the different rodent species are small, so it is possible that more data would identify differences between hosts and differences in temporal trends among pathogens. Apart from *T.gondii* the seroprevalence of pathogens did not vary significantly among species. However, there was a strong year effect driven largely by *Leptospira* and weakly by *T.gondii*. The inability to detect year-to-year changes in pathogens other than *Leptospira* is likely to be influenced by their lower prevalence.

Rodents were trapped from three different areas over the study site suggesting these pathogens are widely distributed in the study area and pose a constant risk to humans and other animals. One tick from a ground squirrel was positive for *Yersinia* sp., but could not be identified to species level. *Yersinia pestis* (plague) is mainly transmitted by rodent fleas (Riehm et al. 2011) but was not detected in any of the fleas collected from the rodents in this study. However, *Y. pestis* has been identified in jerboas and gerbils in other regions of Mongolia adjacent to the study area (Riehm et al. 2011). Therefore it is prudent to maintain surveillance for this bacterium, as it is highly pathogenic in humans and other animals (Riehm et al. 2011). These results suggest that rodents are important as sentinel species for zoonoses for this region as they have been found to be in other areas of the world (Adjemian et al. 2008, Rabinowitz et al. 2009, Achazi et al. 2011).

### 3.6.1 Pathogens detected

#### 3.6.1.1 *Coxiella burnetii*

The prevalence of *C. burnetii* antibodies (the cause of Q fever) was relatively uniform across rodent species and years. Although there looks like an apparent increase in *C. burnetii* in 2013 this was not substantiated statistically. There maybe a number of reasons for temporal variation in prevalence of *C. burnetii* such as climatic variation, although the bacteria are resistant to harsh environmental conditions lasting two weeks in aerosols and more than 20 days in contaminated soil (Enright et al. 1971, Arricau-Bouvery and Rodolakis 2005, Evstigneeva et al. 2007 Woldehiwet, 2004.). The exact amount of time *C. burnetii* remained viable in the soil could not be determined from the current literature. As the rodents were sampled from a relatively large area, it suggests that *C. burnetii*, poses a significant risk to both domestic animals and wildlife in the area and may represent a larger threat to the health of animals and people in the desert areas of Central Asia than previously known despite the significant health and economic impacts the infection can cause (Vanderburg et al. 2014).

Molecular epidemiology using nucleotide sequencing would help elucidate if these were the same or different strains of the bacteria and hence if pathogen transmission was occurring between species or there was a common environmental reservoir. Molecular epidemiology was used to examine the Rabies virus in Mongolia to determine its genetic diversity and identify what strains it resembled. With this technique, it was identified that the same strains of rabies occurred in different host species (Boldbaatar, 2010). Infection of native ungulates by *C. burnetii* could have serious direct as well as indirect effects on the native carnivores such as snow leopards and wolves. Predators may become directly infected from ingesting prey (Woldehiwet 2004) or be indirectly affected by reduction in wild prey abundance caused by *C. burnetii*. A reduction in wild prey, in turn, could lead to increased predation of domestic livestock thereby exacerbating herder/wildlife conflict. *C. burnetii* appears to be an under appreciated zoonosis not having been identified in the Tost region before, despite the significant health and economic impacts the

infection can cause. *C. burnetii* requires further investigation into its significance and impact in this region.

### 3.6.1.2 *Toxoplasma gondii*

*Toxoplasma gondii* antibodies were detected in all rodent species tested, with limited variation in seroprevalence among years. The prevalence of *T. gondii* varied among rodent species and was highest in pikas, ground squirrels and grey hamsters. However samples sizes were small so repeat sampling is needed to validate this trend. Felids (the definitive host) in the area include snow leopards (*Panthera uncia*), European lynx (*Lynx lynx*) and Pallas's cats (*Otocolobus manul*). A study on Pallas's cats and their prey (45 rodents) in the province north of the study site conducted in 2005 found that 2 out of 15 Pallas's cats were seropositive with all rodents testing negative for *T. gondii* as compared to 39% of the rodents testing positive in this study (Brown et al. 2005). Pallas cats are susceptible to *T. gondii* in captivity so the low prevalence of antibodies to *T. gondii* in wild Pallas cats found by Brown et al. (2005) could be a reflection of either low prevalence in the province north of my study area or high mortality of Pallas cats in the study by Brown et al. (2005). As we captured rodents that were positive for antibodies to *T. gondii*, inside local dwellings, it is essential to collect information regarding human exposure in our study area to understand the potential risk of *T. gondii* for local people.

### 3.6.1.3 *Leptospira* spp.

*Leptospira* spp. were present in six of the rodent species tested and the prevalence of *Leptospira* spp. changed during the study period. It is unknown as to why there was a strong year effect on *Leptospira* spp. but as *Leptospira* is mainly a water borne pathogen the variation may have been related to variation in precipitation patterns (Adler and de la Peña Moctezuma 2009). Increase in *Leptospira* spp. was found with increased rainfall in outdoor kept swine (Boqvist 2012). To date, there are no reports of leptospirosis in rodents or other wildlife available in Mongolia. However, positive serological analyses for *Leptospira* spp. have been reported for various domestic species including cattle, sheep, goats and dogs from different areas in Mongolia (Odontsetseg et al. 2005a, Odontsetseg et al. 2005c, Pappas et al. 2008, Anan'ina et al. 2011). The serovars identified in these studies, *L. interrogans* hardjo

(Odontsetseg et al. 2005b) and *L. cynopteri* (Odontsetseg et al. 2005a) were different to the serovar identified in my study. Leptospirosis is generally not pathogenic to rodents but can be fatal to people, dogs and other animals and occurs worldwide (Bharti et al. 2003). Pet and laboratory hamster species however, appear to be more susceptible to leptospirosis compared with other rodents and can show clinical illness (Ko et al. 2009). The hamsters I caught appeared healthy and it is possible the serovars I identified were less pathogenic to both the hamsters and to the other rodents or that wild hamsters are more tolerant to *Leptospira* spp. than are captive animals. Serovar *icterohaemorrhagiae*, which I identified in one midday gerbil and one jerboa, is reportedly the most frequent form of *Leptospira* infection in humans (Picardeau 2013). There are no reported human cases of leptospirosis in Mongolia but this is likely due to lack of testing (Victoriano et al. 2009). Symptoms of leptospirosis include fever, headaches, eye aches and if severe, jaundice due to liver and kidney damage (Romero-Vivas et al. 2013). Gerbils were frequently caught inside gers, thus posing a potential health risk to the local people, through contamination of drinking water, food and utensils with urine.

#### 3.6.1.4 *Hantavirus*

I detected antibodies to PUUV hantavirus in four species of rodents whereas SEOV hantavirus was detected in only one ground squirrel. Rodents are the main reservoir of hantavirus with each species carrying a specific viral strain, which usually results in subclinical infections (Gavrilovskaya et al. 1990, Meyer and Schmaljohn 2000). Although we obtained positive reactions by the Puumala (PUUV) ELISA and one by the Seoul (SEOV) ELISA there was the possibility they were cross-reactions to other strains of hantaviruses (Kallio et al. 2006). Gerbils were trapped inside gers and were one of the species that tested positive for antibodies for PUUV, therefore posing a potential serious human health risk to the residents via aerosolized rodent excrement and saliva.

Rodent infections are an important driver of human hantavirus outbreaks and human hantavirus cases fluctuated seasonally in concordance with the abundance of voles in Finland (Kallio et al. 2009). In Inner Mongolia, a province in China, south to the study area, haemorrhagic fever with renal syndrome is a significant public health risk from Hantaan virus (another strain of Hantavirus) and SEOV in the central and

western areas (Yong-Zhen Zhang et al. 2009). There are no reports of hantavirus infection in people living in the study area even though we had positive reactions to antibody testing in four of the rodent species. More frequent sampling of the rodents in the study area and use of PCR to test tissue samples would enable a greater understanding of the epidemiology and diversity of strains of hantavirus in the Gobi Desert.

### **3.6.2 Haematology and biochemistry**

The haematology and biochemistry results from the rodents in this study mostly fell within reference ranges reported for other rodents (McClure 1999). However, most reference values are from pets or laboratory animals whereas my results are from the wild that may have different tolerance to pathogens than do laboratory bred animals. The information on haematology and biochemistry in this study are also the first available for the Siberian jerboa and the red-cheeked ground squirrel regardless of whether the animals are captive or wild. Baseline haematology and biochemistry values are important reference points for studies on physiological health. Even though many of the rodents had seroconverted for several pathogens, they showed no physiological deviation from the reference ranges in previous reports (McClure 1999), this combined with normal blood smears and cell counts indicated that the rodents were healthy and no significant inflammatory responses or illnesses were occurring. Therefore it is suggested that they serve as important reference values for healthy rodents in the wild. We do not know if the rodents that tested positive to previous exposure to these zoonotic pathogens are reservoirs for the pathogen as well. What the results do show is that the pathogens have been present at some time and are also possibly in the environment, so rodents need to be treated as potential sources of zoonotic pathogens.

### **3.6.3 Ticks**

Many of the genera of bacteria identified in the ticks collected from the rodents contained potentially zoonotic species. These genera included *Bacillus*, *Corynebacterium*, *Coxiella*, *Francisella*, *Legionella*, *Mycobacteria*, *Rickettsia*, *Treponema* and *Yersinia*. However, only a few bacteria use ticks specifically as vectors, including *Coxiella burnetii* and *Francisella tularensis*, *F. tularensis* causes

tularemia, a highly infectious zoonotic illness that causes fever and ulcers (Dantas-Torres 2012). Maintenance hosts for the ticks and in some cases the bacteria are usually rodents or lagomorphs and spillover hosts can be any mammal (O'Stedt and Anders 2007). Several species of *Rickettsia* are tick commensals transmitted by ticks and give rise to different forms of typhus and spotted fever. Ticks and fleas, especially from marmots and other rodents are also vectors for *Yersinia* spp. including *Yersinia pestis*, which causes plague (Gage and Kosoy 2005, Vandamm et al. 2014). Further work is required to determine if the *Yersinia* detected in this study was *Y. pestis*, which would indicate a public health risk. In Mongolia a number of endemic plague regions have been identified including the Umnogovi province where I worked but to the east of the study area (Riehm et al. 2011). Plague was identified in this adjacent province in 2002 and 2006, with gerbils (*Meriones* spp.) and jerboas (*Allactaga* spp.) identified as the main reservoir species (Riehm et al. 2011). Having both these rodent species in my study area, *Y. pestis* could be a potential threat in the Tost region even if not identified in my study. Transmission of *Y. pestis* appears to result from a complex interaction between the specific species of flea hosting the pathogen, host density and environmental conditions, especially temperature (Gage and Kosoy 2005). It is prudent to maintain surveillance for this bacterium as it is highly pathogenic in humans and therefore poses a threat to the local herders (Riehm et al. 2011).

### **3.6.4 Faecal analyses**

Seventeen genera of zoonotic bacteria and a total of 237 genera of bacteria were detected in the rodent faeces in my study, many of which were the same as those genera identified in the ticks collected from the rodents. The potentially zoonotic genera in faeces included *Bacillus*, *Bacteroides*, *Bartonella*, *Campylobacter*, *Clostridia*, *Enterococcus*, *Escherichia/Shigella*, *Francisella*, *Helicobacter*, *Legionella*, *Mycoplasma*, *Rickettsia* and *Treponema*. These genera all contain serious zoonotic bacteria that can cause illness in both animals and humans, again reinforcing the need for adequate hygiene measures and the removal of rodents from gers (Sadowsky, 2011). *Cryptosporidium*, *Giardia* and *Echinococcus* were not identified in the faeces, however, the amount of DNA recovered may have been too low to give a positive reading due to the small quantities of DNA in faecal samples or there may

have been low burdens of the parasites. These zoonotic parasites have also been found in other rodent species in other countries and are a significant cause of gastrointestinal illness in people and other animals (Gholipoury, 2016). Bacterial communities in rodent faeces change according to diet (Kohl 2014) so it may be difficult to determine relationships between bacteria in ticks and faeces from the same animal. As individual rodents did not shed the same bacteria in their faeces as was present in the ticks they harboured, this suggests bacteria in ticks and faeces from the same animal are independent of each other.

### **3.7 Summary**

In this study I showed that the rodents and their ectoparasites in the mountains of the South Gobi Desert of Mongolia carried antibodies against significant zoonotic pathogens; *Coxiella burnetii*, *Toxoplasma gondii*, *Leptospira* spp., PUUV and SEOV hantavirus. These pathogens caused no apparent illness in the rodents but most have serious zoonotic potential, especially *Leptospira* spp (which occurred at a significantly higher prevalence than the other pathogens) and hantaviruses. Seven species and one genus of rodents were identified, occupying a variety of habitats over the study area. Six species of rodent had individuals positive for exposure to all of the pathogens identified except for SEOV hantavirus which was found only in one juvenile ground squirrel. The rodents interacted with sympatric wildlife, domestic animals and humans that also inhabited the region, demonstrating that there is potential for pathogen exchange between all (see Chapters 2 and 5). The rodents may pose a significant risk to human health with many of the smaller rodents, such as the gerbils and hamsters, inhabiting gers (local dwellings) resulting in potentially high exposure of people to these pathogens. Recommendations for risk reduction, such as removal of rodents from inside the gers and the appropriate storage of food and utensils to reduce potential contamination, will be disseminated to public health officials and inhabitants of the region.

Educational programs need to raise awareness of symptoms of these diseases and encourage people to seek medical help if these symptoms occur. Knowledge of species differences is important for disease surveillance, risk assessment and control. For example, it enables identification of high-risk areas based on rodent

presence and can lead to targeted rodent control. Rodents are easy to trap and are important sentinels for monitoring the presence of zoonotic pathogens and other emerging pathogens that could impact public health, animal welfare and production and wildlife conservation in this developing region.

### **Acknowledgements**

Funding was provided by the Snow Leopard Network and the Winnifred Violet Scott Foundation, David Shepherd Wildlife Foundation, Helsinki Zoo, Partnership Funding by Fondation Segré managed by Whitley Fund for Nature. The Abaxis Company (Australia) generously lent the Abaxis I-Stat machine and donated the cartridges and Zebra Vet Australia donated veterinary consumables.

A big thanks to Purevjav Lkhagvajav and Bayarjargal Agvaansteren for logistical support. We are very grateful to the local herders for their co-operation in trapping rodents inside their gers, to Mattia Columbo, Jeremy Krockenberger and Gary Koehler for helping with the fieldwork and Per Eriksson, Jenny Verner-Carlsson, Olivia Borg and Johan Lindh for their expertise in laboratory diagnostics.



## 3.8 Appendices

### 3.8.1 Appendix 1

Table 3.8 Haematology and biochemistry of individual rodents trapped during this study in the Tost region of Mongolia.

Rodent	Na mmol	K mmol	Cl mmol	iCa	Tco2	Glu mmol/L	Urea mmol/L	Crea umol/L	Hct %PCV	Hb* g/dL	AnGap mmol/L
111 Ground Squirrel	143	2.5	105	1.07	20	9.6	12.3	26	42	14.3	22
96 Ground Squirrel	135	5.4	104	0.95	23	6.1	16.4	35	46	15.6	15
69 Ground Squirrel	137	4.5	109	0.98	26	10.3	10.2	33	33	11.2	8
68 Ground Squirrel	135	3.9	106	1	23	6.4	10.3	35	47	16	10
10 Ground Squirrel	142	7.4	110	1.16	24	9	10.7	35	49	16.7	17
Mean	138.4	4.7	106.8	1	23.2	8.3	12	32.8	43.4	14.8	14.4
SD	3.8	1.6	2.6	0.1	2.2	1.7	2.6	4	6.3	2.2	2.6
103 Jerboa	153	4.4	118	1.08	25	4.1	9.1	29	50	17.3	15

97 Jerboa	151	8.4	127	0.99	9	7.8	9.6	19	24	8.2	24
83 Jerboa	149	3	118	0.93	18	13.7	7.7	25	40	13.6	18
86 Jerboa	148	2.6	118	0.83	16	11	8.3	18	43	14.6	17
62 Jerboa	153	5.1	122	1.17	7	9.4	9.9	<18	51	17.3	30
Mean	150.6	4.7	120.6	1	15	9.2	8.9	22.8	41.6	14.2	20.8
SD	2.3	2.3	4	0.1	7.2	3.6	0.9	5.2	9.7	3.3	5.5
95 Midday Gerbil	156	2.6	119	1.04	15	4.2	5.9	<18	43	14.6	25
53 Midday Gerbil	156	8.1	129	1.14	9	14.3	14.3	<18	36	12.2	28
48 Midday Gerbil	158	4.1	123	1.21	19	9.4	8.4	28	48	16.3	21
29 Midday Gerbil	153	>9.0	136	1.41	6	8.5	15.7	32	44	15	<>
5 Midday Gerbil	147	>9.0	120	1.03	14	11.3	11.4	<10	40	13.6	<>
Mean	154	4.9	125.4	1.2	12.6	9.5	11.1		42.2	14.3	
SD	4.3	2.8	7.1	0.2	5.1	3.7	4.1		5	1.5	
25 Long tailed Dwarf hamster	146	8.5	126	0.9	12	9.4	18.2	<18	42	14.3	17

Long tailed dwarf hamster	137	5.9	141	0.91	12	6	10.7	<18	41	13.9	<>
19 Long-tailed Dwarf hamster	147	6.4	118	1.26	10	6	15	****	44	15	26
Mean	143.3	6.9	128.3	1.1	11.3	7.1	14.6		42.3	14.4	21.5
Pika	129	4.2	97	1.08	12	12	5.6	<18	39	13.3	26

Table 3.9 Istat results of rodent haematology and biochemistry from rodents sampled in the Tost region of Mongolia.

Rodent	Na mmol	K mmol	Cl mmol	iCa	Tco2	Glu mmol/L	Urea mmol/L	Crea umol/L	Hct %PCV	Hb* g/dL	AnGap mmol/L
111	143	2.5	105	1.07	20	9.6	12.3	26	42	14.3	22
103	153	4.4	118	1.08	25	4.1	9.1	29	50	17.3	15
97	151	8.4	127	0.99	9	7.8	9.6	19	24	8.2	24
96	135	5.4	104	0.95	23	6.1	16.4	35	46	15.6	15
95	156	2.6	119	1.04	15	4.2	5.9	<18	43	14.6	25
83	149	3	118	0.93	18	13.7	7.7	25	40	13.6	18
76	148	2.6	118	0.83	16	11	8.3	18	43	14.6	17
69	137	4.5	109	0.98	26	10.3	10.2	33	33	11.2	8
68	135	3.9	106	1	23	6.4	10.3	35	47	16	10
62	153	5.1	122	1.17	7	9.4	9.9	<18	51	17.3	30
53	156	8.1	129	1.14	9	14.3	14.3	<18	36	12.2	28
48	158	4.1	123	1.21	19	9.4	8.4	28	48	16.3	21
29	153	>9.0	136	1.41	6	8.5	15.7	32	44	15	<>
25	146	8.5	126	0.9	12	9.4	18.2	<18	42	14.3	17
19	147	6.4	118	1.26	10	6	15	****	44	15	26

---

10	142	7.4	110	1.16	24	9	10.7	35	49	16.7	17
5	147	>9.0	120	1.03	14	11.3	11.4	<10	40	13.6	<>
Pika	129	4.2	97	1.08	12	12	5.6	<18	39	13.3	26
Vole	137	5.9	141	0.91	12	6	10.7	<18	41	13.9	<>

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### 3.8.2 Appendix 2- Statistical analyses

#### Coxiella

1. Logistic regression looking at joint effects of year and rodent species (using glm and car packages in R)

#### view(coxiella)

	rodent	Year	pathogen	positive	sample	p.pos
1	Grnd squirrel	2012	Coxiella	0	9	0.00000000
2	Grnd squirrel	2013	Coxiella	4	9	0.44444444
3	Grnd squirrel	2015	Coxiella	0	0	NA
4	M gerbil	2012	Coxiella	1	6	0.16666667
5	M gerbil	2013	Coxiella	4	16	0.25000000
6	M gerbil	2015	Coxiella	0	1	0.00000000
7	Jerboa	2012	Coxiella	0	5	0.00000000
8	Jerboa	2013	Coxiella	2	7	0.28571429
9	Jerboa	2015	Coxiella	0	0	NA
10	LD Hamster	2012	Coxiella	2	11	0.18181818
11	LD Hamster	2013	Coxiella	2	19	0.10526316
12	LD Hamster	2015	Coxiella	1	11	0.09090909
13	G Hamster	2012	Coxiella	1	8	0.12500000
14	G Hamster	2013	Coxiella	1	13	0.07692308
15	G Hamster	2015	Coxiella	1	7	0.14285714
16	Pika	2012	Coxiella	0	0	NA
17	Pika	2013	Coxiella	1	2	0.50000000
18	Pika	2015	Coxiella	0	1	0.00000000

```
cox.glm = glm(p.pos ~ rodent*Year, family=binomial(), weights=sample, data=coxiella)
```

```
Anova(cox.glm)
```

```
Analysis of Deviance Table (Type II tests)
```

```
Response: p.pos
```

```
LR Chisq Df Pr(>Chisq)
rodent    2.1173 5  0.8327
Year      2.3695 2  0.3058
rodent:Year 9.2347 7  0.2362
```

2

No significant effects (ie no significant difference in prevalence between species or years, and no evidence that annual differences in prevalence differ between hosts).

Toxoplasma

	rodent	Year	pathogen	positive	sample	p.pos
1	Grnd squirrel	2012	Toxoplasma	2	9	0.2222222
2	Grnd squirrel	2013	Toxoplasma	3	9	0.3333333
3	Grnd squirrel	2015	Toxoplasma	0	0	NA
4	M gerbil	2012	Toxoplasma	0	6	0.0000000
5	M gerbil	2013	Toxoplasma	2	17	0.1176471
6	M gerbil	2015	Toxoplasma	0	1	0.0000000
7	Jerboa	2012	Toxoplasma	1	5	0.2000000
8	Jerboa	2013	Toxoplasma	2	9	0.2222222
9	Jerboa	2015	Toxoplasma	0	0	NA
10	LD Hamster	2012	Toxoplasma	0	11	0.0000000
11	LD Hamster	2013	Toxoplasma	2	17	0.1176471
12	LD Hamster	2015	Toxoplasma	0	16	0.0000000
13	G Hamster	2012	Toxoplasma	1	8	0.1250000
14	G Hamster	2013	Toxoplasma	4	12	0.3333333
15	G Hamster	2015	Toxoplasma	0	7	0.0000000
16	Pika	2012	Toxoplasma	0	0	NA
17	Pika	2013	Toxoplasma	1	2	0.5000000
18	Pika	2015	Toxoplasma	1	1	1.0000000

```
tox.glm = glm(p.pos ~ rodent*Year, family=binomial(), weights=sample, data=tox)
```

```
Anova(tox.glm)
```

```
Analysis of Deviance Table (Type II tests)
```

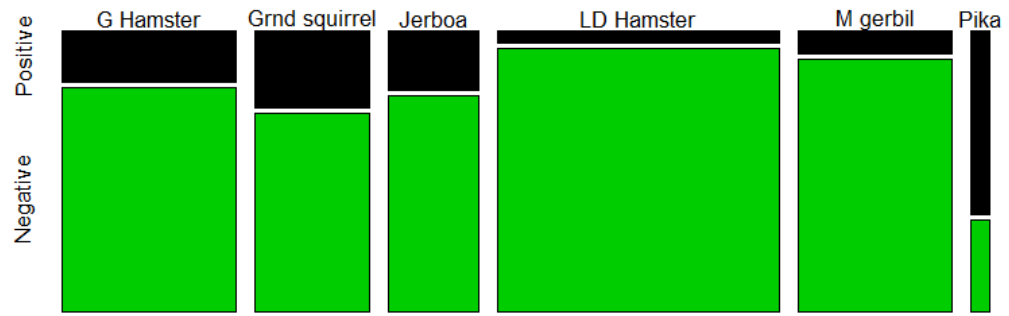
```
Response: p.pos
```

```
LR Chisq Df Pr(>Chisq)
rodent    11.8813 5  0.03645 *
Year      5.0321 2  0.08078 .
rodent:Year 6.2822 7  0.50721
```

Significant difference in prevalence among rodent hosts, hint of change between years, no evidence of an interaction.

Host differences (NB bar widths proportional to sample size)

### Proportions positive for Toxoplasma





## Leptospira

	rodent	Year	pathogen	positive	sample	p.pos
1	Grnd squirrel	2012	Leptospira	2	10	0.2000000
2	Grnd squirrel	2013	Leptospira	2	9	0.2222222
3	Grnd squirrel	2015	Leptospira	0	0	NA
4	M gerbil	2012	Leptospira	1	6	0.1666667
5	M gerbil	2013	Leptospira	7	18	0.3888889
6	M gerbil	2015	Leptospira	0	1	0.0000000
7	Jerboa	2012	Leptospira	0	15	0.0000000
8	Jerboa	2013	Leptospira	6	10	0.6000000
9	Jerboa	2015	Leptospira	0	0	NA
10	LD Hamster	2012	Leptospira	2	8	0.2500000
11	LD Hamster	2013	Leptospira	6	17	0.3529412
12	LD Hamster	2015	Leptospira	10	16	0.6250000
13	G Hamster	2012	Leptospira	1	8	0.1250000
14	G Hamster	2013	Leptospira	8	14	0.5714286
15	G Hamster	2015	Leptospira	2	7	0.2857143
16	Pika	2012	Leptospira	0	0	NA
17	Pika	2013	Leptospira	1	2	0.5000000
18	Pika	2015	Leptospira	1	1	1.0000000

**lep.glm = glm(p.pos ~ rodent\*Year, family=binomial(), weights=sample, data=lepto)**

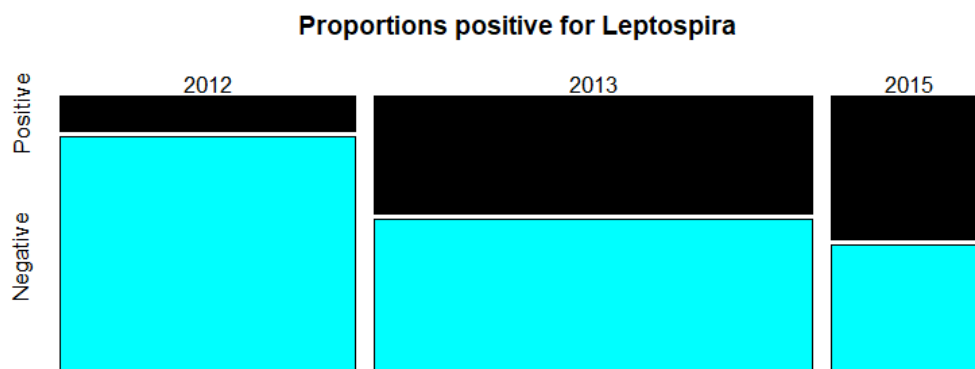
**Anova(lep.glm)**

Analysis of Deviance Table (Type II tests)

Response: p.pos

	LR	Chisq	Df	Pr(>Chisq)
rodent	1.5553	5	0.906597	
Year	12.4272	2	0.002002 **	
rodent:Year	13.5624	7	0.059535 .	

Strongly significant year effect, weak suggestion that year effect may vary between rodent species.



PUUV

	rodent	Year	pathogen	positive	sample	p.pos
1	Grnd squirrel	2012	PUUV	0	9	0.0000000
2	Grnd squirrel	2013	PUUV	1	9	0.1111111
3	Grnd squirrel	2015	PUUV	0	0	NA
4	M gerbil	2012	PUUV	1	6	0.1666667
5	M gerbil	2013	PUUV	2	20	0.1000000
6	M gerbil	2015	PUUV	0	1	0.0000000
7	Jerboa	2012	PUUV	0	15	0.0000000
8	Jerboa	2013	PUUV	1	10	0.1000000
9	Jerboa	2015	PUUV	0	0	NA
10	LD Hamster	2012	PUUV	1	6	0.1666667
11	LD Hamster	2013	PUUV	0	17	0.0000000
12	LD Hamster	2015	PUUV	0	16	0.0000000
13	G Hamster	2012	PUUV	0	8	0.0000000
14	G Hamster	2013	PUUV	0	12	0.0000000
15	G Hamster	2015	PUUV	0	7	0.0000000
16	Pika	2012	PUUV	0	0	NA
17	Pika	2013	PUUV	0	2	0.0000000
18	Pika	2015	PUUV	0	1	0.0000000

```
puuv.glm = glm(p.pos ~ rodent*Year, family=binomial(), weights=sample, data=puuv)
```

```
Anova(puuv.glm)
```

Analysis of Deviance Table (Type II tests)

Response: p.pos

	LR	Chisq	Df	Pr(>Chisq)
rodent	3.9941	5	0.5503	
Year	1.3172	2	0.5176	
rodent:Year	6.3449	7	0.5001	

No significant effects

PATHOGEN PREVALENCES

```
overall = glm(p.pos ~ (pathogen+rodent+Year)^2, family=binomial(), weights=sample, data=path)
```

```
Anova(overall)
```

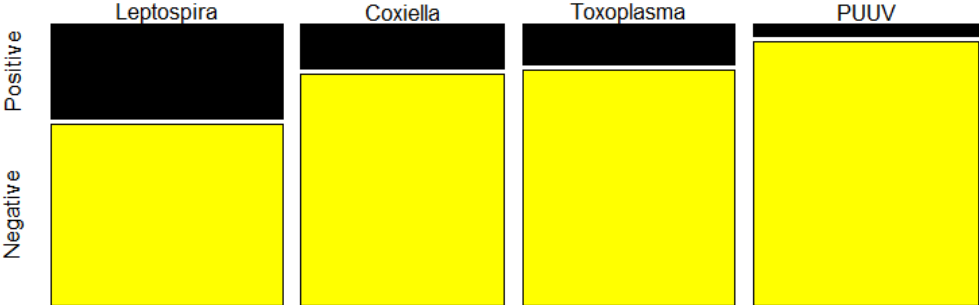
Analysis of Deviance Table (Type II tests)

Response: p.pos

	LR	Chisq	Df	Pr(>Chisq)
pathogen	51.029	3	4.822e-11 ***	
rodent	4.722	5	0.450733	
Year	13.700	2	0.001059 **	
pathogen:rodent	13.756	15	0.544081	
pathogen:Year	7.638	6	0.265869	
rodent:Year	11.818	7	0.106715	

Looking at the whole data set, there are very strong differences overall in the prevalence of different pathogens, plus a quite strong year effect (driven largely by Leptospira, but there is not much evidence that patterns of pathogen prevalence differ among rodent species, or between years).

**Proportions positive for each pathogen**



## **Chapter. 4 Rodent Species of the South Gobi Desert in Mongolia**

### **4.1 General Introduction and Premise of Chapter 4**

As the study progressed, it became obvious there were numerous rodent species over the study site with each species occupying a different environmental niche. This chapter therefore, is concerned with the species identification of the rodents captured. The accurate identification of the rodent species is necessary for the rodent pathogen chapter, as different rodent species may have carried different pathogens. This was important in looking at possible transmission pathways for pathogens to the snow leopards, as rodents are ubiquitous over the study area. Each rodent species habitat overlapped with the other animal species sampled in this study, providing an opportunity for pathogen transmission between the rodents and the other animals including people. The pikas, hamsters and voles were found more in the rocky areas overlapping with the snow leopards and ibex. Ground squirrels and Jerboas were found in the steppe areas where the goats grazed and dogs wandered. Gerbils, jerboas and hamsters were also found inside peoples' homes. This information will be important concerning public health if species and pathogen links can be attributed to those rodents that were trapped inside the local people's gers.

Rodents were trapped as per Chapter 3. All rodents were weighed and body measurements recorded. Distinctive physical characteristics such as pelage colour, number of toes, presence or absence of hair on foot-pads or tails were also recorded. Ear notches were collected to identify animals if recaptured, with this tissue sample being stored in RNAlater for DNA analyses for species identification. If tissue samples could not be collected faecal samples were collected as well and DNA was extracted from those.

As there is a dearth of general knowledge pertaining to the basic life history and ecology of many of these rodent species, the information collected in this study will contribute significantly to the little knowledge available.

This Chapter will be submitted to the Journal of Conservation Biology

*Article: Rodent species of the South Gobi Desert in Mongolia*

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## **4.2 Abstract**

The South Gobi Desert of Mongolia is a remote and rugged environment that is home to numerous rodent species. The population status, distribution, habitat preferences and basic biology of most species however, are poorly understood. I live-captured 177 rodents from seven species and one species that could only be identified to genera) during three four-week trapping sessions in the Tost Mountains over a three-year period (May/June 2012, May/June 2013, March/April 2015), to identify species presence, habitat occupied and basic morphometric, physiological and reproductive data. DNA analyses from tissue samples and morphological characteristics were used to identify the following species: red-cheeked ground squirrel *Spermophilus erythrogenys*, Siberian jerboa *Allactaga sibirica*, the midday gerbil *Meriones meridianus*, long-tailed dwarf hamster *Cricetulus longicaudatus*, grey dwarf hamster *Cricetulus migratorius*, the Kam dwarf hamster *Cricetulus kamensis*, Mongolian pika *Ochotona pallasii*, several vole species belonging to the genus *Alticola*, that could not be identified to species. This is the first identification of the Kam hamster in Mongolia. This study provides new and essential baseline information on the rodent species of the South Gobi Desert that will contribute to the development of conservation plans for the region.

### **4.3 Introduction**

Rodents are endemic to every continent of the world except Antarctica and are the largest group of mammals worldwide with nearly 2300 species (Amori et al. 2008). They range in size from the dwarf three-toed jerboa, (*Salpingotulus michaelis*) whose adults weigh 3.75 g (Holden and Musser 2005) to the capybara (*Hydrochoerus hydrochaeris*), which weigh 35-66 kg (Vucetich and Dozo 2005).

Rodents are significant components of the majority of terrestrial systems as they are important prey for other species and are notorious carriers of diseases (Singleton et al. 2003, Achazi et al. 2011). They are also often seen as sentinels for environmental health (Singleton and Krebs 2003, Achazi et al. 2011, Meerburg and Reusken 2011, Lu et al. 2012, Schmidt et al. 2014, Morand et al. 2015). In Mongolia 63 species of rodents have been recorded with 24 of those occurring in the Gobi Desert (Batsaikhan 2010, Nyamsuren et al. 2014). However, little is known about rodent species in Mongolia and especially within mountainous areas of the Gobi Desert. Recent work in the area has involved health investigations of rodents, resulting in detection of zoonotic pathogens and establishing baseline haematology and biochemistry parameters (Esson et al. submitted, Chapter 3). I used genetic analyses and morphological identification to produce a more accurate species list for the area. Morphometric, physiological and reproductive data were collected and I strived to describe the habitat in which each species occurred.

### **4.4 Methods**

#### **4.4.1 Study area**

The Gobi Desert is a remote region in Southern Mongolia and Northern China. The study was conducted in the Tost Mountains in the South Gobi Desert (43<sup>0</sup>N, 100<sup>0</sup>E) located in the province of Umnogovi in Mongolia. The study site covers an area of 1700 Km<sup>2</sup>. Field-work field was conducted in May/June 2012, 2013 and in March/April 2015 (Plate 4.1).



**Plate 4.1 Tost Mountains in the Gobi Desert of Mongolia.**

#### **4.4.2 Trapping**

Rodent trapping was carried out for four weeks each year between May/June 2012, May/June 2013 and March/April 2015. Trapping was performed under ethics approval A1919 from James Cook University, Australia and approval from the Ministry of the Environment, Mongolia. Two sizes of Sherman/Elliott traps (5.08 x 6.35 x 16.51 cm and XLK Folding Trap 7.62x 9.525 x 30.48 cm) were used to maximize rodent species capture success

We used a mixture of rolled oats, honey and peanut butter as bait. Eight trap-lines were set in each year, with 10 traps in each line, approximately 20m apart covering a variety of habitats. These included the flat, open steppe areas, rocky gullies and shallow gullies with the red bush Buils (*Amygdalus pedunculata*). Several traps were also set within gers with the owners' permission. Traps were set late in the afternoon and checked early the next morning.

#### **4.4.3 Physical examination and morphometrics**

Each rodent was removed from the trap and placed in a preweighed calico bag and weighed with a Pesola spring balance. Body measurements were taken using vernier

calipers for the smaller species and a tape measure for the larger species such as the ground squirrels. Measurements were as follows (Plate 4.2): Head width (HW) is the distance between left and right zygomatic arches; Head length (HL) = length from the occipital crest to the tip of the nose; Body length (BL) = the length from the occipital crest to the tail base using vernier calipers; Tail length (TL) = length from the tail base to the tip of the tail; Hind foot length (HFL) = length from the heel to the tip of the toes, excluding the nail length; Leg length (LL) = length from the stifle to the ankle; Ear length (EL) = length from the ear base to the tip of the ear; Testis width (TW) = distance across the middle of both testis; Testis length (TL) = the length of the scrotal sac for males (Table 4.1). Sex of animals, pregnancy and lactation were noted, where the sex of animals was based on external genitalia and pregnancy was based on obvious swelling of the abdomen and development of mammary glands. Any other distinguishing physical characteristics were also noted, such as coat colour, hair on feet and injuries. External parasites such as fleas and ticks were collected and placed in 70% ethanol. The left ear was notched if a male was caught and right ear if a female, allowing for identification of repeat captures. The ear tissue was placed in ethanol for DNA extraction for species identification. The ground squirrels' ears were too small for notching, so permanent marker symbols were placed on their head. Faecal samples were collected into ethanol for genetic species identification if tissue samples were not available.



Plate 4.2a. Measuring foot length.



Plate.4.2b. Measuring head width.

#### **Plate 4.2 Measuring foot length, head width of rodents.**

#### **4.4.4 Species identification of rodents**

Species were identified using a combination of morphological features as described in the literature and DNA extracted from tissue and faecal samples. We performed



genetic identification of rodent species based on genetic markers following Gillet et al. (2015). Briefly, DNA was extracted from ear samples using the DNeasy extraction Kit (Qiagen Inc., Hilden, Germany). An illumina amplicon sequencing was then performed following a modified Miseq protocol (Metagenomic Sequencing Library Preparation). Total genomic DNA of the samples were subjected to PCR amplification targeting a ~133-bp fragment of the cytochrome oxydase I gene (COI) using a modified forward primer LepF1 (Hebert et al. 2004) and a modified reverse primer EPT-long-univR (Hajibabaei et al. 2011). Purified products were quantified using Quant-iT™ PicoGreen® dsDNA Assay Kit on a fluorimeter (FilterMax F3, Molecular Devices). Quantified products were pooled in equimolarity and then sent them to the GIGA Genomics platform (ULg) for sequencing on an ILLUMINA MiSeq V2 benchtop sequencer. Raw sequences were processed using a script that consisted of a mix of the Fastx-toolkit ([http://hannonlab.cshl.edu/fastx\\_toolkit](http://hannonlab.cshl.edu/fastx_toolkit)) and the Usearch function (Edgar 2010). Processed sequences were then compared with published sequences in the BOLD databases (Ratnasingham and Hebert 2007) where we considered sequences that had identity scores of  $\geq 98\%$  to be positive matches.

#### **4.4.5 Statistical analyses**

Body weight measurements between sexes of the same species were compared by Students T test, to determine if there was sexual dimorphism.

### **4.5 Results**

#### **4.5.1 Species trapped**

Over 5040 trap-nights 177 rodents were trapped and processed. Seven species and species from one genus of rodent were identified by NGS, either from tissue samples or faecal samples. These were *Spermophilus erythrognys*- red cheeked ground squirrel, *Allactaga sibirica* –Siberian jerboa, *Meriones meridianus*- midday gerbil, *Cricetulus longicaudatus*- long-tailed dwarf hamster, *Cricetulus migratorius*- grey dwarf hamster, *Cricetulus kamensis* – Kam dwarf hamster, *Ochotona pallasii* -pika and *Alticola* spp. – Voles could not be identified further than genus. Three specimens

could not be identified, as they did not match any known descriptions either morphologically or by DNA. (See Plate 4.3)



**Plate 4.3 Unidentified rodent species.**

The rodent species pictured in plate 4.3 was captured three times and remains unidentified – it did not match any description in the guidebook or DNA identification. It was significantly bigger than the other hamster/vole species, furrier and had a reddish/tan tinge to the fur tip.

**4.5.2 Habitat**

The red-cheeked ground squirrels were captured in the open steppe areas with little rock cover and also on the low hillsides with their burrows dug on an angle into the ground. There was generally a pile of dirt around the entrance to the burrow from the excavating. The jerboas were captured in the open flat steppe areas but in areas with harder, rockier soil and occasional small bushes. The jerboas' burrows appeared to go straight down (Plate 4.4, Plate 4.5).

During the 2013 field, trip a flash flood occurred covering the area where burrows of ground squirrels and jerboas occurred but rodent populations appeared physically

unaffected. We reset traps once the water had subsided and animals were caught the next day,

Gerbils were captured along small sandy gullies, with their burrows dug in the root system of *Amygdalus pedunculata*. There were often caches of nuts from the bushes around the entrance to the burrows (Plate 4.6). The pikas were captured in rocky outcrops/boulder piles with a network burrow system with numerous entrances and exits. The entrances to the burrows were often marked by piles of faecal stacks and stores of nesting material (Plate 4.7). An exception was a solitary pika trapped in 2015, with a burrow under a red bush, distant from any colony. Hamsters and voles were trapped in the steep rocky gullies and recaptures were common (Plate 4.9).

Most species appeared to be nocturnal or crepuscular, no traps were set off during the day although young ground squirrels were observed playing during the day.



**Plate 4.4 Ground squirrel habitat.**



**Plate 4.5 Jerboa habitat.**



**Plate 4.6 Gerbil habitat.**



**Plate 4.7 Pika habitat.**



**Plate 4.8 Nest of plant material at entrance to pika burrow.**



**Plate 4.9 Hamster and vole habitat.**

### **4.5.3 Physical assessment**

Many of the females of all species were pregnant or lactating (26%) at the time of capture. Approximately equal numbers of both sexes for each species were trapped, except for the ground squirrels where no adult males were trapped. Only one juvenile male ground squirrel was caught late in the season. In 2015 we trapped earlier (March to April) to determine if males came out early for mating but no ground squirrels were seen of either sex.

Sexual dimorphism was observed between the male and female long-tailed dwarf hamsters when their body weights were compared by students T-Test ( $P=0.05$ ,  $t=3.9$ ). There was no statistically significant sexual dimorphism between the remaining rodent species when weights were compared. Jerboas  $P=0.05$ ,  $t=0.21$ , gerbils  $P=0.05$ ,  $t=1.97$ . With the remaining species-: red-cheeked ground squirrels, grey hamsters, Kam dwarf hamsters, pikas and the voles, the numbers were too small to get significant results with the T-test.



**Plate 4.10 Variation in tail tip colouration in gerbils.**



**Plate 4.11 Grey fur under tan surface colouration on gerbil tail.**

Table 4.1 Mean +/- Standard Deviation and range of physical measurements of rodents captured in the Tost Mountains of Mongolia between 2012 and 2015.

Species	Weight g	HL mm	HW mm	CR m	TL mm	FL mm	LL mm	EL mm
Ground Squirrel <i>Spermophilus erythrognys</i> N=19	270±29 230-330	48.9±3.7 44-58.5	29.5±3.3 22-33	166±15 135-188	53±5.3 44-60	36± 34-39	43± 36-48	
Jerboa <i>Allactaga sibirica</i> N=16	92±6.8 85-100	38±2.4 34-42	23±2.9 20-29	116±8.6 105-130	181±14 145-200	64±2.7 58-68	58±5 42-62	32±5 25-35
Gerbil <i>Meriones meridianus</i> N=23	58±8. 50-70	38±2.7 34-41	20±2.1 17-21	99±11.4 70-116	99±14 70-116	29±2.1 26-31	35±2.9 33-40	12±22 9-16
Long-tailed Dwarf Hamster <i>Cricetulus longicaudatus</i> N=67	22.6±5 15-30	27.3±2.9 23-31	13.8±1.9 12-15	76.8±13.6 55-110	30±5 21-39	16±1.5 11-18	23.7±3.6 16-30	13±1.7 11-16
Vole <i>Alticola sp.</i> N=5	44±5.5 40-50	32.8±3.6 25-33	16.8±1.8 15-19	98.8±5.7 92-107	22.4±2.1 20-25	17.2±2.2 15-20	29.4±5.8 24-39	12.6±1.7 11-15
Kam Hamster <i>Cricetulus kamensis</i> N=5	28±4 20-30	27±4 20-30	15±2 12-18	71±8.4 58-81	22.6±3.7 19-27	15±2 12-17	22±2.6 19-26	12.4±0.8 11-13

N=number of animals sampled, HL-head length, HW- head width, CR-crown rump, TL-tail length, FL-Foot length, LL-leg length, EL-ear length.

## 4.6 Discussion

We trapped at least eight of the 24 species of rodents described for the Tost region (Batsaikhan 2010). Morphological characteristics that we observed in each rodent species differed from those characteristics reported by Batsaikhan (2010). Moreover, we found that pelage colour, tail tassels and presence of hair on hind feet pads were unreliable descriptors for identification as they varied between individuals within species. The only reliable source of species identification was by genetic analyses which illustrates the benefit of genetic approaches when studying rare and elusive species (Esson et al. 2017). Trapping, handling and sample collection appeared to have little impact on pregnant females as juveniles, especially ground squirrels, were observed in burrows where females had been trapped and handled.

Establishing information on the assembly of rodent species in mountain deserts is important, as rodents are significant prey species and carriers of disease (Singleton et al. 2003, Achazi et al. 2011). For example, Esson et al. (unpublished data, Chapter 3) identified rodents in this regions had been exposed to serious zoonotic pathogens such as *Toxoplasma gondii* (16.3%), *Coxiella burnetii* (16%), Puumala hantavirus (6%) and *Leptospira interrogans* (34%) serovars Australis and *Icterohaemorrhagiae*. These zoonoses pose a threat to sympatric wildlife, the local people and their livestock. The fact that all species except for the ground squirrels and pikas were trapped inside gers means a potential health risk for the local people.

### 4.6.1 Species identification

#### 4.6.1.1 Ground Squirrels

DNA analyses identified the ground squirrels we trapped as red-cheeked ground squirrels however their body measurements were consistent with descriptions of the Alashan ground squirrel- *Spermophilus alashanicus*, by Batsaikhan (2010). The red-cheeked ground squirrels in my study had body weights ranging from 230-330 g compared to 350 g reported in (Batsaikhan 2010) and crown rump measurements of 135-188 mm compared with 235-260 mm. Our measurements were much closer to that of the Alashan ground squirrel by (Batsaikhan 2010) with a body weight up to 280g and body length 180-230 mm. The ground squirrels I trapped were also darker



in colouration than reported for the red-cheeked ground squirrel in (Batsaikhan 2010). Batsaikhan did not provide information on sex and age, sample sizes and capture location, all of which may have influenced differences in results between studies.

Unexpectedly, we did not trap any mature male ground squirrels. Female ground squirrels from other regions in Mongolia are reported to live with their young until the young disperse, while juveniles and males are solitary (Nyamsuren et al. 2014). Male arctic ground squirrels may disperse straight after mating in the burrows or disperse as soon as they emerge from hibernation (Karels and Boonstra 2000, Gillis 2002). The literature did not suggest male ground squirrels exhibit semelparity as some male dasyurids do (Boonstra, 2005), but they do undergo stress responses due to mating that may cause them to die post-mating (Boonstra 2001, Gillis 2002). It is possible the male ground squirrels in Mongolia die underground after mating or disperse. However, further work on their life history is needed. Juveniles were seen to emerge in late June.

#### 4.6.1.2 *Jerboas*

The Siberian jerboa is one of 10 species of jerboa that are reported to occur in the Gobi Desert (Nyamsuren et al. 2014). Our measurements differed to Batsaikhan (2010), particularly in body length (105-125mm, vs 125 -180) weight (85-100g vs 85-170g) and ear length (23-35mm vs 36-55mm). These small discrepancies could be due to variation between individuals within species, geographic variation, or different reference points for taking measurements. As sample sizes, sex and age were not given in (Batsaikhan 2010), the significance of the differences could not be determined.

#### 4.6.1.3 *Gerbils*

Gerbils were identified genetically as midday gerbils *Meriones meridianus* but phenotypically had characteristics of both the midday gerbil and the Mongolian gerbil, which is also reported in the area (Batsaikhan 2010). Midday gerbils are not described as having black hairs to the tail tips or a tassel, (Batsaikhan 2010), whereas we found specimens both with and without black tail tassles. All animals had

hairy foot-pads. Our body measurements were similar to those previously described (Batsaikhan 2010).

#### 4.6.1.4 Hamsters

Long-tailed dwarf hamsters, grey dwarf hamsters and the voles were all difficult to distinguish based on physical characteristics, although the voles on average were larger. There were large variations in coat colour, tail length and hair cover and presence of hair on foot-pads of the hamsters and voles. It is possible that there are hybrids among these species and further genetic sequencing could be used to determine relatedness. However, characteristic traits for the species were apparent with the long-tailed dwarf hamsters (nick-named “lion mice”) being very vocal and aggressive while being handled. Voles had very loose skin and could easily rotate while being held but were not as aggressive as the hamsters. In 2015 we trapped in a different area and found many of the long-tailed dwarf hamsters had definite bends or “kinks” in the distal third of their tails. It is possible that there are hybrids among those species – and similarly to above more work is needed on genetic sequencing to examine this possibility further.

The grey hamster is listed as data deficient and near threatened in the IUCN Mongolian Red list of animals (Clark 2006); therefore, the information gained in this study will contribute to the knowledge of the biology of the grey hamster. We also trapped hamsters that looked like the desert hamster *Phogopus roborovskii*, but their DNA matched either the grey dwarf hamster or the long-tailed dwarf hamster. Surprisingly we did not trap any *Mus musculus* (House mouse), which are reportedly ubiquitous over the whole of Mongolia (Nyamsuren et al. 2014).



**Plate 4.12 Dwarf grey hamster.**



**Plate 4.13 Desert hamster.**

A dwarf grey hamster that we trapped (Plate 4.12) compared with a photo of a desert hamster (Plate 4.13) from the guidebook (Nyamsuren et al. 2014). They have a similar appearance, but the grey dwarf hamster has furrier ears.

#### 4.6.1.5 *Cricetulus kamensis*

Also known as the Kam hamster, *Cricetulus kamensis* has not been recorded in this area before, with the nearest report in China approximately 500kms away (Kang et al. 2016, Esson et al. 2017).

#### 4.6.1.6 *Pikas*

We trapped one of two species of pikas listed for the region, *Ochotona pallasii* (Mongolian pika) by (Batsaikhan 2010). All pikas observed (except one), lived in family groups in boulder fields with numerous entrances and exits to their burrow system. We caught only four individual pikas, which differed greatly in physical measurements to each other, likely due to differences in age of the animals captured. The pikas had four toes on the hind feet, which were covered in tan coloured fur, whereas Batsaikhan (2010) describes them with five toes and pads that are covered in grey hair.

#### 4.6.2 Habitat

The habitats used by the different species were similar to habitats described for these species where they occurred in other regions (Gillis 2002, Liu et al. 2007, Nyamsuren et al. 2014). However, where we captured jerboas and gerbils, their burrows appeared to be in areas with less grass cover than previously described.

### 4.7 Summary and Recommendations

We trapped and identified seven species and one genera of rodent in the study area in the Tost Mountains of Mongolia. Each species occupied a different area with only minor overlaps between the red-cheeked ground squirrels and the Siberian jerboas. Phenotypic descriptions and morphometric measurements were not sufficient to identify and distinguish between closely related species such as the grey dwarf hamster, desert hamster and the long-tailed dwarf hamster.

DNA analyses of tissue or faecal samples were required to accurately identify specimens to species. The discrepancies in many of the morphological measurements between and within species in my study and those in (Batsaikhan

2010) emphasize the need for further collection of physical and physiological data on species in this region.

Diet analyses and environmental niche monitoring will also be important in furthering our knowledge of the ecology of these species and the ecosystem at large. The underground burrow system could be investigated with optic fibres to see how the animals escaped the floodwaters. Seasonal variation in burrow temperature, humidity and oxygen concentration could be monitored.

The lack of male ground squirrels observed during the three field trip periods requires further investigation on the ecology of male ground squirrels. T

The widespread occurrence of zoonoses such as *Leptospira* spp, *Toxoplasma gondii*, *Coxiella burnetii* and hantavirus (Esson et al. submitted, Chapter 3) highlights the role of rodents in disease transmission between humans, domestic and wild animals within this region.

The results of this study contribute significantly to the knowledge on identification and distribution of the rodents of the Tost region.

### **Acknowledgements**

Thank you to the volunteers that helped with trapping: Mattia Colombo, Jeremy Krockenberger and Gary Koehler. We also thank Tserennadmid Mijiddorj, Bayarjargal Agvaansteren and Purvejav Lkhagvajav for helping with trapping and permits. Thanks to Orjan Johansson for local knowledge of where to place the traps.

# **Chapter. 5 Investigating Zoonotic Pathogens in Domestic Goats, Dogs and Communal Water Sources in the South Gobi Desert of Mongolia**

## **5.1 General Introduction and Premise of Chapter 5**

This chapter is concerned with the sampling of goats, dogs and water sources representing the domestic and environmental sections of the One Health study into snow leopard health. Goats share grazing areas with the wild ungulates, the ibex and argali sheep, which are natural components of the snow leopards diet. Overlap of grazing and contamination of pasture with faeces provides an opportunity for pathogen transfer between domestic stock and wildlife including snow leopards. Snow leopards opportunistically prey on the goats as well as the native ungulates, therefore providing two modes of possible pathogen transfer.

The dogs are in constant contact with the goats and overlap with the native ungulates as well. Dogs eat placentas from the goats and any offcuts that the herders throw to them after slaughtering of goats. They scavenge on carcasses of other wildlife including those from kill sites of the snow leopards. Although it was not observed it was hypothesized that the dogs caught rodents opportunistically. Hares were also present in the study area, which dogs may have caught. There was also the possibility of interacting with other wild canids such as foxes and wolves that were observed, both personally and on camera trap photos.

The methodology in the chapter will largely be a repeat of those described in the previous chapters. There are some modifications that have been made to the analyses due to species differences.

Water sources included natural springs and man-made wells. Water sources were an ideal environmental representative to sample, providing the perfect conditions for pathogen exchange between species. Camera traps were placed at many of these springs and depicted the numerous species that utilized these water sources. Faeces from a variety of animals, including camels and birds, that were not part of the sampling study for logistic reasons, were observed in these waterways, providing a source of contamination. People were also seen on the camera trap photos drinking

from the springs. Snow leopards were seen numerous times on the camera trap photos verifying their interaction with all other species of the study via the water sources.

The Chapter has been written as per the previous chapters in a format for a stand-alone publication. It will be submitted for publication in Ecohealth.

*Article: Investigating zoonotic pathogens in domestic goats, dogs and communal water sources in the South Gobi Desert of Mongolia*

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Keywords: One Health, Dogs, Goats, Mongolia

## **5.2 Abstract**

Pathogens can transmit between wildlife, livestock, indigenous people and the environment affecting the health of all. The South Gobi Desert is home to over 90 herding families, their stock and sympatric wildlife including the iconic Snow Leopard (*Panthera uncia*), but there have been no disease studies in this region. To survey for pathogens, I sampled blood, faeces and ectoparasites from 270 domestic goats, 41 dogs and 11 wells and springs distributed over the study area. The study was conducted over three field seasons of four weeks duration each, from May 2012 until

April 2015. Samples collected were analyzed using serology, (Enzyme-Linked Immunosorbent Assay ELISA), Polymerase Chain Reaction (PCR) and Next Generation Sequencing (NGS), to detect antibodies to zoonotic pathogens. Goats were seropositive for *Coxiella burnetii* (11.4% in 2015, 5.1% in 2013 and 13.5% in 2012) and *Toxoplasma gondii* (83% in 2015, 94% in 2013 and 93% in 2012) but were seronegative for *Brucella* spp. Several goats were anaemic and some presented with coughs. Dogs were seropositive for *C. burnetii* (10.5%), *T. gondii* (66%) and *Leptospira* spp. (5.3%) including serovar Icterohaemorrhagiae. All dogs were seronegative for canine distemper virus. *Taenia hydatigena* was detected in dog rectal smears by PCR. Ticks from the goats carried numerous genera of zoonotic bacteria including *Anaplasma* sp., *Bacillus* sp., *Coxiella* sp, *Rickettsia* sp. and *Mycobacterium* sp. Parasites and pathogens were not detected in the water samples. The results indicated the goats, dogs and their external parasites have been exposed to several highly pathogenic zoonoses. These pathogens could cause significant illness in the local herders and decrease their livestock productivity. Pathogens could also cause illness in sympatric wildlife. Implementation of preventative vaccination programmes and worming protocols should improve herder health, goat health and hence productivity. Flow on effects will aid in minimizing transmission of pathogens to other domestic animals and surrounding wildlife. Interventions should be assessed by ongoing monitoring and health checks of the dogs and goats on a yearly basis.

### **5.3 Introduction**

Zoonoses are common in developing rural communities where subsistence living is typical, with people and animals living in close association with each other (Zinsstag et al. 2006). Co-operation between medical and veterinary disciplines is beneficial when looking at zoonotic diseases and the possible pathogen pathways for infection between different species and people (Zinsstag et al. 2011). The detection of reservoir and spillover host species is key knowledge for controlling epidemics. However, health studies of domestic animals, people and wildlife, in remote and rural areas can be challenging due to inadequate facilities and resources, as well as difficulties accessing sites and storage and transport of diagnostic samples (Mocellin & Foggin 2007, Pers Obs). For these reasons, sufficient knowledge for effective health prevention programmes for zoonoses is often lacking.



The South Gobi Desert in Mongolia is a remote and rugged environment, home to nomadic herders and their flocks since the days of Ghengis Khan. Pastoralism contributes about 20% of Mongolia's GDP and 40% of the employment (Batsukh et al. 2012). Approximately 30% of the human population is nomadic, raising sheep, goats, cattle, camels and horses, despite the recent drift to more urban lifestyles (Bedunah and Schmidt 2004). The major zoonoses reported to affect stock, people and wildlife in Mongolia include brucellosis, anthrax, rabies, plague, tularemia, leptospirosis, Q fever, echinococcosis and vector-borne diseases such as rickettsiosis and bacillosis (Batsukh et al. 2012). However, due to the difficulties mentioned above and a subsequent lack of public awareness and reporting, the incidence and health impacts on the people, their livestock and sympatric wildlife are unclear (Mocellin and Foggin 2008, Batsukh et al. 2013). This is especially true for my study area in the South Gobi Desert where no government or local reports on zoonoses were available.

The South Gobi Desert supports a large number of Cashmere goats, as well as sheep, horses and camels. Herders keep dogs to help protect their flocks from predators such as snow leopards and wolves. There is significant interaction between the domestic animals, wild animals and people allowing for exchange of zoonotic pathogens as is seen with other herding communities (Zinsstag et al. 2006, Tomley and Shirley 2009). The interaction between people and animals can occur at times of slaughter, birthing and production harvesting such as shearing/combing. This enables increased exposure to pathogens via aerosolization and direct contact with excrement and blood. Indirect interaction can also occur via common sources of water and soil.

In addition to current burdens of disease, changes to global climate and agricultural practices can provide conditions favourable for the spread of vector-borne diseases (Semenza & Menne 2009). Milder and prolonged winters and hotter summers can improve survival times for parasitic vectors such as ticks, fleas and mosquitoes, enabling them to increase their distribution and thus spread pathogens into previously naïve areas (Gray et al. 2009, Semenza & Menne 2009, Hornok et al. 2014). Therefore even where health prevention is currently adequate the rapid pace of global change can threaten health systems (Sherman 2010).

Because of the lack of knowledge of zoonoses in the South Gobi Desert I aimed to provide baseline knowledge about important zoonotic pathogens prevalent within the region. To detect zoonotic pathogens that can circulate between, domestic animals, wildlife and people in the South Gobi Desert, I sampled the major domesticated animals within the region, local dogs and goats for zoonotic pathogens. The results of this study will provide new knowledge about endemic zoonotic pathogens that will enable improved management of herd health, human health and wildlife conservation in this remote region and other similar areas.

## **5.4 Methods**

### **5.4.1 Study area**

The study area comprised 1700 km<sup>2</sup> in the South Gobi Desert of Mongolia (43<sup>0</sup> N, 100<sup>0</sup>E) (Fig. 5.1). Three field trips were run in May/June 2012, June 2013 and March/April 2015, lasting four to five weeks each. Collection of samples was performed under ethics approval A1919 from James Cook University, Australia and with permission of the local herders.

### **5.4.2 Sampling and physical examinations**

#### *5.4.2.1 Goats*

Goatherds ranged from 125-500 goats. We sampled 270 goats from 27 herds widely distributed throughout the study area.

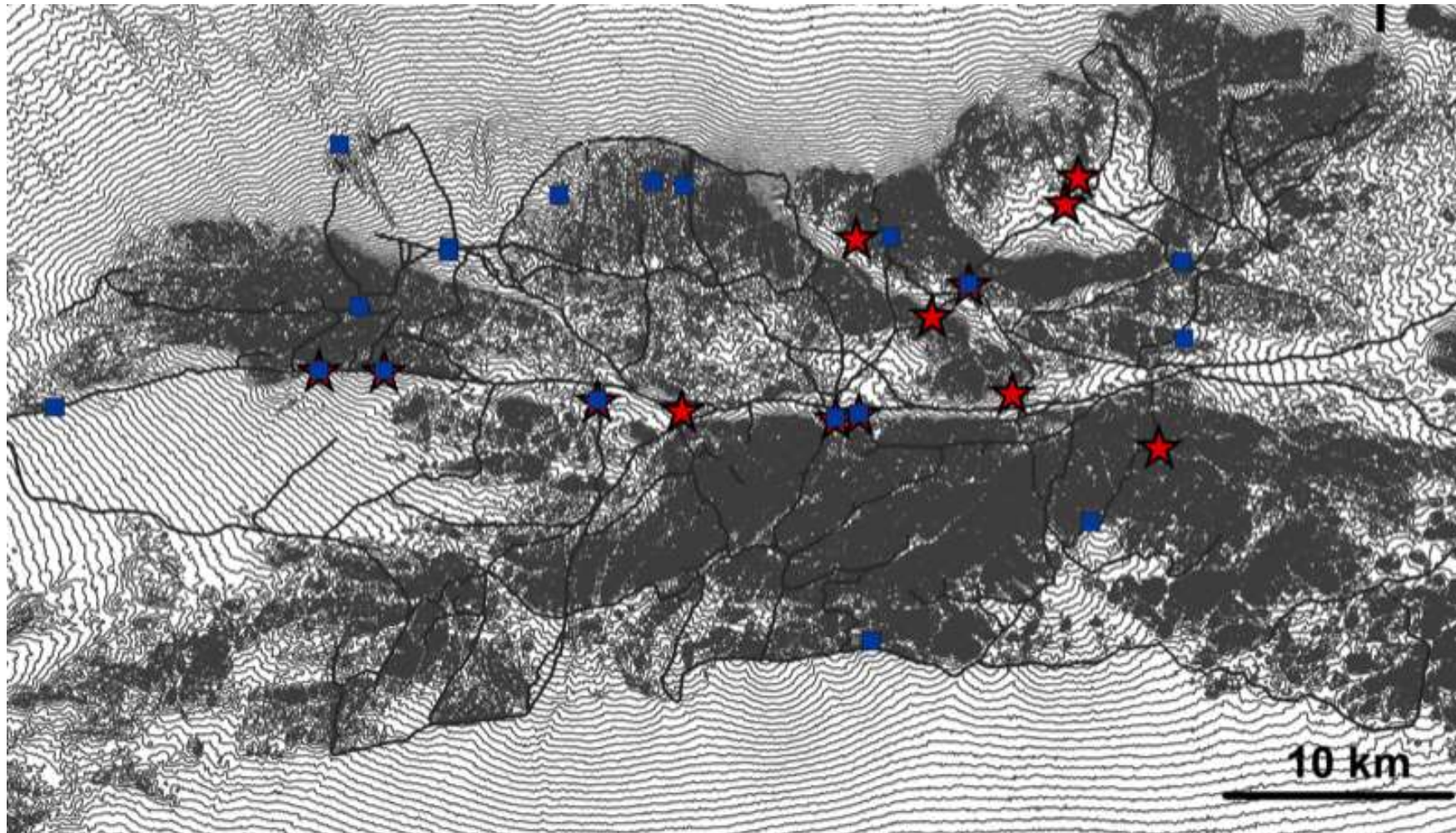
Goats are taken out to graze daily, so we sampled early morning or afternoon at the gers (huts). Some farmers locked their goats in corrals at night to help protect them from attack by snow leopards and wolves. The goats were free ranging through the day but calm enough for the herders to catch and restrain by hand, with 5% of each herd sampled including a mix of sex and ages. Goats were examined for body condition, coat condition, reproductive status, the presence of external parasites and any external injuries or hoof problems.

#### 5.4.2.2

#### 5.4.2.3 *Dogs*

Each herder had between one to three dogs ranging in age from puppies to old dogs (>10 years). I sampled all dogs that could be caught, resulting in forty-one animals sampled.

As the dogs were not used to being handled they were difficult to catch and examine, and most had to be muzzled. Dogs were examined for general physical health, body condition, external wounds and external parasites.



**Figure 5.1 Study area in the Tost Mountains of the South Gobi Desert.** Dark areas are ridgelines. Blue squares represent sites of dog sampling and red stars goat locations. (Map courtesy of Gustaf Samelius, Snow Leopard Trust).

### 5.4.3 Blood sample collection and storage

#### 5.4.3.1 Goats

While restrained, 4 ml of blood was collected from the jugular vein of each goat. Two ml was placed into a serum clotting tube and 0.5 ml into a lithium heparin tube.

Duplicate blood smears were made in the field. The remaining blood was placed on Advantec Nobuto strips (Toyo Roshi Kaisha. Ltd 2007, Japan).



#### Plate 5.1 Collection of blood samples from goats.

Serum tubes were allowed to stand overnight for separation of cells from serum, as there was no large centrifuge. The serum was then decanted into sterile cryovials, stored at the base camp at  $-18^{\circ}\text{C}$ , until transport back to the Zoonosis Science Centre (ZSC) at the Department of Medical Biochemistry and Microbiology, Uppsala University, in Sweden. Here the samples were stored at  $-80^{\circ}\text{C}$  until analyses. The blood from the lithium heparin tubes (Zebra Vet, Australia) was run for biochemistry and haematology using a hand-held Abaxis I-Stat analyser (REM Systems Pty, Ltd, North Ryde, Australia) in the field. Blood smears were air-dried and fixed in Diff Kwik fixative (Fronine Laboratory Supplies, Riverstone, NSW Australia), for transport and then were stained with eosin and methylene blue for microscopic examination in the laboratory. Nobuto strips were air dried and then placed in paper envelopes for storage and transport.

### 5.4.3.2 Dogs

Two ml of blood was collected from the cephalic vein and placed in 2.0 ml serum tubes. Blood was processed and stored as for the goat samples. Only Nobuto strips were used if <0.5 ml of blood was collected.

## 5.4.4 Faecal collection and external parasites

### 5.4.4.1 Goats

Fresh samples of goat faeces were collected from each farm. Where possible, faeces were collected from the goats that had been sampled but this was not always feasible. The faeces on the ground were in such high density it was not difficult to find fresh samples that had not touched the soil. The faecal samples were either placed in paper bags with silica gel for drying or in RNAlater. External parasites collected from the goats were placed in 70% alcohol.



**Plate 5.2 Taking blood samples from dogs.**

#### 5.4.4.2 Dogs

Dog faecal samples were hard to find therefore rectal swabs of faeces were collected from each dog using a sterile cotton tip swab. Rectal smears were placed into RNAlater and stored at ambient temperature.

#### 5.4.5 Laboratory analyses

Goat serum samples were tested for antibodies against *Brucella* sp., *Coxiella burnetii* and *Toxoplasma gondii*. Dog serum samples were tested for antibodies against *C. burnetii*, *T. gondii*, *Leptospira* spp. and canine distemper virus (CDV). Goat faecal samples and dog rectal smears were screened for *Giardia*, *Coccidia*, *Cryptosporidia*, *Echinococcus* and other enteric pathogens, using PCR or NGS.

##### 5.4.5.1 *Brucella* sp.

Antibodies against *Brucella* were tested for using an Svanovir Brucella I-ELISA Antibody test kit (Article number 10-2701-02, Boehringer Ingelheim Svanova, Uppsala, Sweden) for small ruminants, following the instructions in the kit. The optical density of plates was read using a Multiskan FC microplate photometer, (Thermo Scientific) at 450nm.

##### 5.4.5.2 *Coxiella burnetii*

*Coxiella burnetii* antibodies were tested for using an Innovative Diagnostics Q Fever Indirect Multi-species ELISA kit (IDvet, Grabels, France.FQS-MS ver 0514). The ELISA was performed following the protocol in the kit. The plate results were read at 450nm

##### 5.4.5.3 *Toxoplasma gondii*

*Toxoplasma gondii* antibodies were tested for using an ABNOVA IgG antibody ELISA kit (Abnova Taipei City, Taiwan, KA0225). As this was a human kit, the enzyme conjugates were changed to an alkaline phosphatase-conjugated affinipure rabbit anti-goat IgG (H+L) and to a rabbit anti-dog IgG (H+L) for the dog samples (305-005-003, 304-005-003 Jackson Immuno Research Laboratories INC.PA. USA). The

plates were read on a Multiskan FC microplate photometer, (Thermo Scientific) at 405nm.

#### *5.4.5.4 Leptospira spp.*

The one dog sera collected in 2013 was tested using a qualitative rat *Leptospira* IgG ELISA Kit. The kit protocol was followed, replacing the rat conjugate with the rabbit anti-dog conjugate. The remainder of the dog samples were collected the following field season and were analysed for *Leptospira* antibodies using a Microscopic Agglutination Test (MAT) at the National Veterinary Institute in Uppsala, Sweden. Serovars tested were *Leptospira* : Australis, Hebdomadis, Icterohaemorrhagiae, Pomona, Canicola and Grippityphosa as previously described (Boqvist 2012). The change in protocol was decided as it reduced handling the samples twice and using MAT specific serovars could be identified.

#### *5.4.5.5 Canine distemper virus (CDV)*

Dog sera were tested at the National Veterinary Institute Uppsala for CDV antibodies. The test was a serum neutralization test in Vero puppy cells against canine distemper virus strain Bussel (in-house test).

#### *5.4.5.6 Faeces and tick analyses*

Dog and goat faeces were tested using polymerase chain reaction (PCR) to detect the parasites *Echinococcus*, *Giardia* and *Cryptosporidium*. Next-generation sequencing (NGS) was used to identify bacteria in the faecal samples of both species and bacteria in ticks using the methods of Gillet et al. (2015).





**Plate 5.3 Natural water source in the South Gobi Desert used by people, domestic animals and wildlife.**

#### **5.4.6 Water collection**

Both natural water sources and man-made wells were sampled. There were 11 natural water sources and four man-made wells.

Water was collected in sterile 20 ml syringes, from ten different sites within each water source, totalling 200 mls from each water source. Water was then filtered through Sterivex filters (Sigma-Aldrich Pty. Ltd. Sydney, Australia) with a 7 $\mu$ m cellulose filter paper (Plate 5.5). The paper was air dried, before removing from the holder, rolled using sterile forceps and placed into a sterile cryovial. The cryovials were stored at ambient temperature in the field, before being sent back to the Zoonosis Science Centre (ZSC) at the Department of Medical Biochemistry and Microbiology Uppsala University, in Sweden for storage at -80°C. Each cellulose filter paper was analysed separately at the University of Liège Belgium for a suite of pathogens using NGS.



**Plate 5.4** Man-made well with trough attached.



**Plate 5.5** Filtering of water through a sterivex filter.

## **5.5 Results - Goats**

### **5.5.1 Goats: clinical examination**

The goats were lacking body condition on physical examination with spine and ribs palpable, however, all females appeared to be producing kids. Some of the goats also had dry coughs but there was no discharge from the nose and the lungs sounded clear on auscultation.

### **5.5.2 Serology**

Antibodies to *C. burnetii* and *T. gondii* were identified in each herd tested and in all years of the study. All samples were negative for antibodies to *Brucella* spp. (Table 5.1).

Table 5.1 Prevalence of antibodies to each pathogen in goat sera over the three years of study in the Gobi Desert of Mongolia.

Year	<i>Coxiella burnetii</i>		<i>Toxoplasma gondii</i>		<i>Brucella</i> spp.
2012	8/59	13.6%	74/80	92.5%	0/79
2013	4/78	5.1%	85/90	94.4%	0/91
2015	4/35	11.4%	61/74	82.4%	0/66
<b>Total</b>	16/172	9.3%	220/244	90.2%	0

### 5.5.3 PCR and NGS

DNA in the faecal samples was too degraded to identify any parasites in the goat samples but one hundred genera of bacteria were identified. Fifteen of these included species that were potentially zoonotic (Table 5.3). The tapeworm, *Taenia hydatigena* was identified in the dog rectal smears.

### Haematology and biochemistry

Ten of the 14 goats that underwent haematological testing were anaemic with haematocrits below 20%. However, when serum tubes were allowed to stand some samples had little serum and were composed mostly of a blood clot and others had

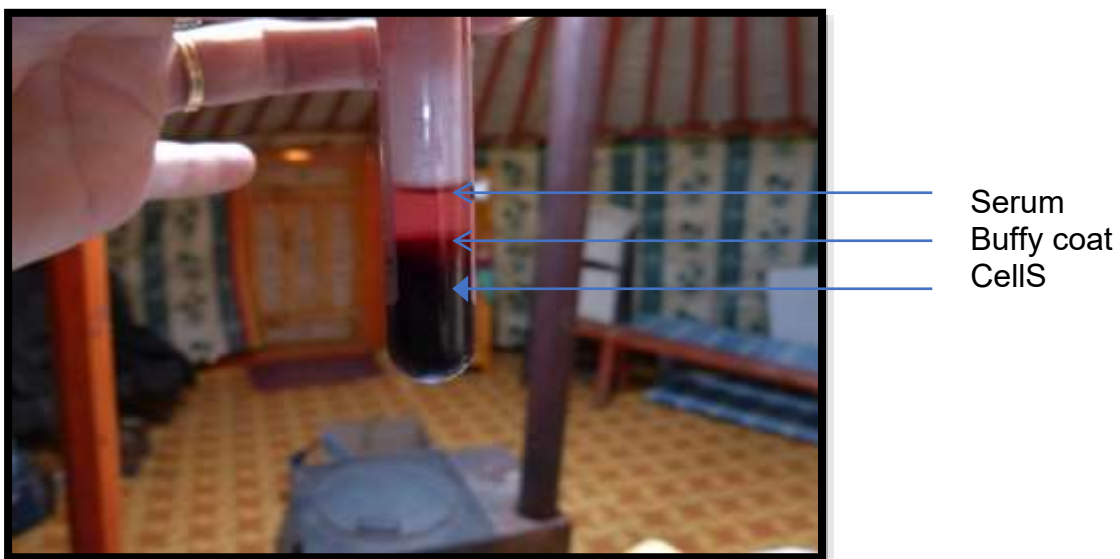


large buffy coats (

Plate 5.6). There were no other significant findings from haematology and biochemistry, which were within reference ranges for these species (Table 5.2).

Goats that were seropositive for antibodies to pathogens did not have correlating inflammatory markers.

Blood smears from all the goats sampled, including those samples with the large buffy coat, appeared normal under microscopic examination, both at the 40X and 100X magnification. Although the buffy coat layer contains the white cells, these goats did not have a corresponding increase in white cell numbers in their blood smears from a manual count. Several had unidentified inclusion bodies in the red blood cells.



**Plate 5.6 Blood sample from goat showing the blood constituents. Cells look like they take up a significant component, however, most goats were border line anaemic.**

Table 5.2 Haematology and biochemistry of goats from Farm 1, 4 and 7 in 2015 and Farms 4,5,6 and 10 in 2013 (n=14), in the Tost Mountains of Mongolia

	<b>Na</b> mmol/L	<b>K</b> mmol/L	<b>iCa</b> mmol/L	<b>TCO<sub>2</sub></b>	<b>Glu</b> mmol/L	<b>pH</b>	<b>PCO<sub>2</sub></b>	<b>PO<sub>2</sub></b>	<b>Hct %</b> <b>PCV</b>	<b>Hb*</b> g/dL	<b>SO<sub>2</sub></b>	<b>HCO<sub>3</sub></b>	<b>BE</b>
Mean	140.5	4.5	1.325	25	3.2	7.45	36.55	81.5	23	7.85	89.5	23.9	-0.5
STDEV	3.5	0.14	0.09	2.82	0.42	0.07	8.27	57.27	1.41	0.49	13.4	2.8	2.12
STEM	0.71	0.16	0.02	0.74	0.19	0.02	1.68	12	0.79	0.27	2.8	0.6	0.61
Range	138-145	4-5.7	1.2-1.39	22-29	1.4-3.5	7.4-7.5	30.7-32.4	38-134	16-24	5.4-8.2	79-89	21.9-27.4	-2-+3

Na-Sodium, K-Potassium, Ca-Calcium, TCO<sub>2</sub>-Total carbon dioxide, Glu-Glucose, PCO<sub>2</sub>-Percent carbon dioxide saturation, PO<sub>2</sub> Percentage oxygen concentration, Hct-Haematocrit, Hb-Haemoglobin content, HCO<sub>3</sub>-Bicarbonate, BE-Base excess. (See (Weiss and Wardrop 2011))

### 5.5.4 Tick analyses

Only five ticks were found on the 270 goats, however, collection of ticks was hindered by the high density of the goat hair. The species of ticks were not identified. Through NGS, one hundred and twenty-three genera of bacteria were identified in the ticks and sixteen of these were potentially zoonotic bacterial genera (Table 5.3).

Table 5.3 Potential zoonotic bacteria identified by NGS in ticks and faeces carried by goats in the Tost Mountains of Mongolia.

Potentially Zoonotic Bacterial Genera	Prevalence in Ticks		Prevalence in Faeces	
<i>Aeromonas</i>	40%	2/5	-	0/11
<i>Anaplasma</i>	20%	1/5	9.1%	1/11
<i>Bacillus</i>	100%	5/5	9.1%	1/11
<i>Bacteroides</i>	-	0/5	36.4%	4/11
<i>Bartonella</i>	-	0/5	27.3%	3/11
<i>Campylobacter</i>	-	0/5	9.1%	1/11
<i>Clostridia</i>	100 %	5/5	72.7%	8/11
<i>Corynebacterium</i>	-	0/5	27.3%	3/11
<i>Coxiella</i>	100%	5/5	45.5%	5/11
<i>Enterococcus</i>	-	0/5	27.3%	3/11
<i>Escheria/Shigella</i>	-	0/5	27.3%	3/11
<i>Francisella</i>	20%	1/5	18.2%	2/11
<i>Rickettsia</i>	100%	5/5	100%	11/11
<i>Staphylococcus</i>	-	0/5	27.3%	3/11
<i>Streptococcus</i>	-	0/5	27.3%	3/11
<i>Treponema</i>	-	0/5	45.5%	5/11

### 5.5.5 Dogs: clinical examination

The majority of dogs were in poor body condition but had no apparent signs of disease. One had a large cut on its carpus causing it to limp. Two older (>7 years) and two young dogs (<2 years), had lipaemic serum which normally indicates a high

fat-meal and can also indicate pancreatitis. However, none showed signs of abdominal pain or illness during sampling.

### **5.5.6 Serology**

Dogs were positive for antibodies to *C. burnetii* 4/38 (10.5%), *T. gondii* 25/38 (66%) and *Leptospira* sp. 2/38 (5.3%). The serovar identified via MAT was *Leptospira interrogans* Icterohaemorrhagiae.

All dogs tested for canine distemper virus were negative serologically for antibodies to CDV (N =33).

### **5.5.7 PCR and NGS**

After amplification of DNA from the dog rectal smears, bands were present for possible *Echinococcus* but these could not be identified further to species. *Taenia hydatigena* was identified in Dog 1 from Farm 3 (which was also positive for *T. gondii*) and Dog 1 from Farm 10, which was also positive for *C. burnetii*.

### **5.5.8 Haematology and biochemistry**

Haematology and biochemistry results are presented in Table 5.4. Haematocrit varied the most, ranging from 29% to 58%. Parameters for the four dogs with the lipaemic serum did not appear to differ from the other dogs. Their blood smears appeared normal when viewed microscopically, with no indicators of inflammation.

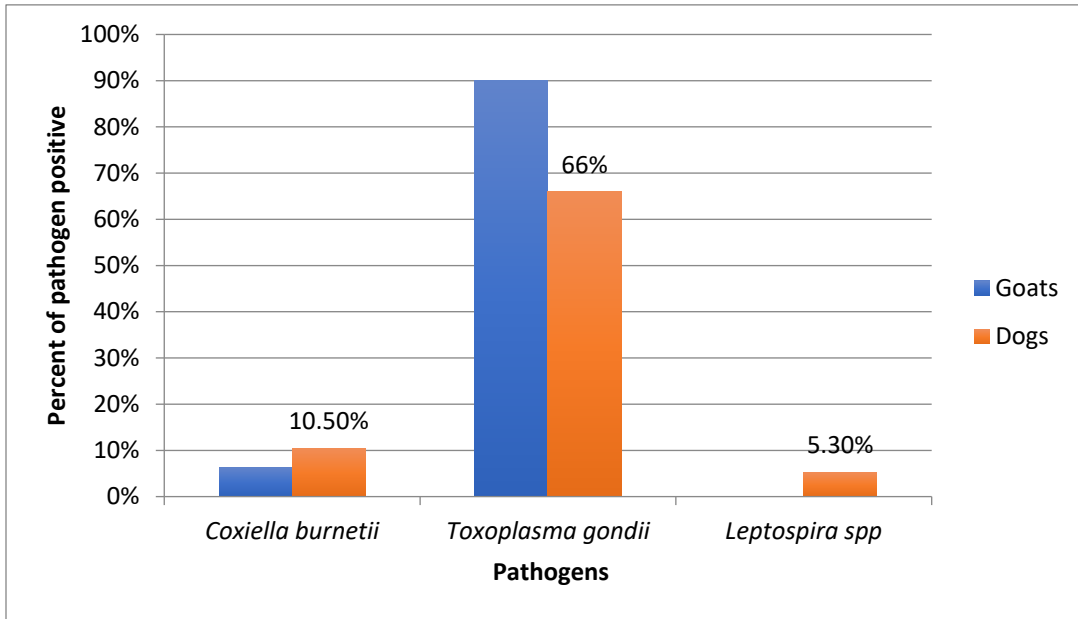
Table 5.4 Haematology and biochemistry values for dogs in the South Gobi Desert of Mongolia.

	<b>Urea mmol/L</b>	<b>Creatinine umol/L</b>	<b>Anion Gap mmol/Ld</b>	<b>TCO2 mmol/L</b>	<b>Na mmol/L</b>	<b>K mmol/L</b>	<b>Ca mmol/L</b>	<b>Glucose mmol/L</b>	<b>Hct %</b>	<b>Hb g/dL</b>	<b>Cl mmol/L</b>
Mean	8.34	61.71	16.64	19.07	141.79	5.13	1.16	4.03	41.43	14.09	112.43
STDEV	3.07	20.31	3.48	1.86	5.09	0.74	0.17	1.04	10.37	3.51	4.03
Range	3.4-13.5	29-100	10-21	17-23	129-149	4-6.4	0.87-1.35	2.2-5.3	29-58	9.9-19.7	105-117
SE	0.82	5.42	0.93	0.5	1.36	0.2	0.05	0.28	2.8	0.94	1.08

N = 16. Mean, standard deviation (STDEV), range and standard error (SE) of mean.

Na-Sodium, K-Potassium, Ca-Calcium, TCO<sub>2</sub>-Total carbon dioxide, Glu-Glucose, PCO<sub>2</sub>-Percent carbon dioxide saturation, PO<sub>2</sub> Percentage oxygen concentration, Hct-Haematocrit, Hb-Haemoglobin content, HCO<sub>3</sub>-Bicarbonate, BE-Base excess. (See Weiss and Wardrop 2011)





**Figure 5.2 Total percentages of goats and dogs positive to *Coxiella burnetii*, *Toxoplasma gondii* and *Leptospira spp.* over all years of the study.**

### 5.5.9 Water analyses

No significant pathogens were identified in the water samples

## 5.6 Discussion

### 5.6.1 Pathogens identified

Goats and dogs had a high prevalence of exposure to zoonotic pathogens, particularly *T. gondii* with lower levels of *C. burnetii*. *Leptospira spp.* were also detected in the dogs, including serovar Icterohaemorrhagiae and two dogs were positive for *T. hydatigena*. These infections are serious public health concerns and reduce livestock productivity. However, these infections were not associated with ill health in the livestock or dogs based on physical examinations and clinical pathology. However, due to the lack of records, the rates of abortion and morbidity from Q fever and toxoplasmosis are unknown in the goats and humans in this region.

Several of the goats at one farm were observed to be coughing. The coughs may have been due to the dry and dusty conditions or to parasites such as lungworms (Radostits et al. 2006). There was no nasal discharge or other signs of upper respiratory infection observed.

### 5.6.1.1 *Coxiella burnetii*

Antibodies to *C. burnetii*, the causative organism of Q-fever in humans, were identified in goats (10.5%) in all years of the study and in dogs (6.3%). *Coxiella* sp. was also present in goat faeces and in the ticks, which are vectors for *C. burnetii* (Špitalská and Kocianová 2003, Stuermer et al. 2011, Dantas-Torres 2012). The bacteria are also transmitted via excrement, aerosolized particles from aborted fetuses and placenta and environmental contaminants (Schelling et al. 2003). *Coxiella burnetii* can cause serious illness and abortion in humans, goats and other domestic and wild ruminants such as ibex (Arricau Bouvery et al 2003, Raoult et al. 2005, Candela et al 2015). This disease can have a significant impact on the community via the impact on livestock productivity (Paris et al. 2006). Effects on abundance of wild herbivores could have flow-on effects to endangered apex predators such as snow leopards, by causing a decrease in their native prey, possibly leading to increase in predation of the goats (Bagchi & Mishra 2006). The four dogs positive for prior exposure to *C. burnetii* were not from the farms with positive goats. It is possible we missed goat infections at these farms as only 5% of each herd was tested. Alternatively, as the dogs are free-ranging, they could have been exposed to infection from wildlife, such as native rodents, of which 13% in this area were seropositive (see Chapter 3) by consuming carcasses or faeces, or via ticks. The 2015 field trip occurred during kidding time and dogs were observed eating the goat placentas, allowing for a direct transfer of pathogens. Dogs can be intermediate hosts for *C. burnetii* and have been reported as reservoirs of human infection in other regions (Buhariwalla et al. 1996, Shapiro et al. 2016).

### 5.6.1.2 *Toxoplasma gondii*

Antibodies to *T. gondii* were identified at high prevalence in the goats (90%) and dogs (66%) spanning all years of the study. This has significant public health implications, as goat meat is one of the major protein sources for the indigenous people. *Toxoplasma gondii* can cause congenital malformation in the foetus and ocular lesions in immunocompromised people (Tomley 2009).

Dogs and goats from the same farms both showed prior exposure, which may indicate transmission between species or infection from a common source. A

common outcome of infection in sheep and goats is abortion and neonatal death (Dubey 2009), as well as foetal resorption and mummification (Abu-Dalbou and Ababneh 2010). Worldwide, toxoplasmosis is the main cause of ovine and caprine abortion and hence major economic loss (Buxton et al. 2007). A vaccine is available to reduce these losses in goats and sheep (Abu-Dalbou and Ababneh 2010), but cost may be preventing its use in the Tost region.

In dogs, *T. gondii* can cause acute or chronic infection, with disease occurring mainly in younger or immunocompromised animals. It can affect the gastrointestinal or neurological systems (Dubey et al. 2009). Dogs can act as intermediate hosts for *T. gondii* and sporulated oocysts can pass through their gut, hence their faeces and are therefore another source of infection for people and other animals (Lindsay et al 1997 and Frenkel and Parker 1996),

There are several wild felid species in the area that can be definitive hosts for *T. gondii* including snow leopards (*Panthera uncia*), lynx (*Lynx lynx*) and pallas cats (*Otocolobus manul*) as well as the few domestic cats observed. In a concurrent study 4/20 (20%) snow leopards had antibodies to *T. gondii*, in areas that overlapped where goats grazed (see Chapter 2) and snow leopards occasionally prey on goats (Johansson et al. 2015). With the lack of reporting for both humans and animals, it is difficult to determine the numbers of abortions occurring in the area due to toxoplasmosis or Q fever.

### 5.6.1.3 *Brucella* spp.

*Brucella* spp. were not identified in the goats, which was unexpected considering Mongolia has the highest reported prevalence of *Brucella* in the world (Odontsetseg et al. 2005a). There was a mass nation-wide vaccination program in the 1970s and in 2000-2001, which significantly reduced the incidence of disease in humans and stock. However, vaccination rates then declined due to withdrawal of the Russian regime and reduced government support. During the Russian period the animals in the study area were not vaccinated (Erdenebaatar et al. 2004). It is possible the Tost area was protected from infection by its isolation and effectively is a closed system as herders only transfer stock among themselves (herders pers com). As there is little regulation or reporting of illness among animals and people it is unknown if

brucellosis has previously occurred in the study area (Odontsetseg et al. 2005a). *Brucella mellitensis*, which is more common in goats, has caused serious health issues in other parts of the country (Foggin et al. 2000). However, given its prevalence elsewhere in the country and its serious production, economic and health impacts, it is worth monitoring for outbreaks in the study area in the Tost Mountains to ensure that the area remains brucellosis-free (Roth et al. 2003).

#### 5.6.1.4 *Leptospira*

Two apparently healthy adult dogs tested positive for *Leptospira* in different years and different areas of the study site. Depending on the serovar the dogs are infected with, determines whether the dogs show any clinical signs of illness and can act as maintenance hosts for this zoonotic pathogen (Goldstein et al. 2006, Rojas et al. 2010, Ward et al. 2004) Only one *Leptospira* serovar, *L. interrogans* Icterohaemorrhagiae, a pathogenic serovar, was identified which is the first time detected in a Mongolian dog. Therefore if the pathogen is transmitted to people or other animals the dogs come in contact with, this could lead to serious illness in those subjects. Serovars previously detected in dogs in Mongolia in two different regions were *L. jananika* *L. cynopteri*, *L. copehnhageni* and *L. saxkoebing* (Odontsetseg et al. 2005c). Cattle in other areas of Mongolia have been identified with *L. hardjo* and goats with *L. pomona* (Anan'ina et al. 2010). Infected regions included desert and dry steppe areas that are not optimal for waterborne transmission of leptospirosis, which suggests it circulates via another transmission pathway, possibly wildlife. In concurrent studies in the area, snow leopards tested positively to *L. interrogans* Australis and several species of rodents tested positive for *L. interrogans* Australis and *L. interrogans* Icterohaemorrhagiae (see Chapters 2 & 3). These results suggest *Leptospira* may transmit between the rodents and dogs in the study area with the identification of the same serovar in both species. Rodents are natural carriers of the pathogen with bacteria residing in kidney tubules so they could provide a pathway for transmission to dogs when dogs prey upon the rodents. Future surveys should include also testing goats to assess any role in human exposure.

### 5.6.1.5 Ticks

Ticks were collected from the goats contained genera of potentially zoonotic bacteria including *Anaplasma*, *Bacillus*, *Bartonella*, *Campylobacter*, *Coxiella*, *Francisella* and *Yersinia*. Ticks are well-known vectors for bacterial and protozoal pathogens (Smith 2003, Špitalská and Kocianová 2003, Gray et al. 2009) and these results should inform future disease surveillance. However, tick burdens on the goats were low and we only collected five from 270 goats. The collection of ticks however, was hindered by the high density of the goat hair, so it is quite possible ticks were missed.

### 5.6.1.6 Faeces

Faeces from the goats contained many of the same pathogens as were in the ticks plus enteric bacteria as would be expected. The dog rectal smears only provided minimal quantities of DNA for limited testing for detecting bacteria or parasites, however, we were able to identify the intestinal cestode *Taenia hydatigena*. The intermediate metacestode stage is found in ruminants and pigs (Singh et al. 2015), while various canids (dogs, foxes, wolves) are definitive hosts and become infected by scavenging on carcasses or eating offal (Braae et al. 2015). Effects on dogs are usually minimal except if there is a substantial burden, which causes ill thrift. However, in young intermediate hosts such as goats can have severe liver disease or brain infection contributing to stock loss (Braae et al. 2015). There is also the risk of human infection, although *T. hydatigena* do not usually reside in people (Singh et al. 2015). Further work should determine infection rates and impacts on livestock in the area.

## 5.6.2 Haematology and biochemistry

Haematology and biochemistry results for the goats and dogs were mostly within normal published ranges (Njidda, et al. 2013, Khan et al. 2005), however, ten out of the 14 goats tested had low (<23%) haematocrit, indicating anaemia. The anaemia could be due to haemorrhage during the recent birthing season, debilitation and blood loss due to high burdens of intestinal parasites, such as *Haemonchus contortus* (Ahmad and Ansari 1989, Waller and Chandrawathani 2005). On examination of the serum tubes there were a number of tubes that had a larger volume of clot compared to the serum layer and a large buffy coat. A decrease in serum volume can indicate

dehydration and may reflect high milk production during the kidding period. The buffy coat contains the white blood cell fraction and can indicate an inflammatory response to infection although white cell differential count on smears was within normal ranges (Egbe-Nwiyi et al. 2000). More blood samples need to be examined to see if this is a regular trend in the goats, combined with faecal examination for specific parasites. When goats are slaughtered examination of gastrointestinal contents would enable improved detection of endoparasites to inform preventative treatment protocols. Parasite removal would help increase production and quality of Cashmere produced and hence income for herders (Githigia et al. 2001, Krecek and Waller 2006).

Haematology results for the dogs were mainly within the normal published range, (Khan et al. 2005), however, three of the older dogs had haematocrits above 55%, which suggests dehydration. Four dogs from three farms had very lipaemic serum. As the goats were kidding, dogs often ate placentas, which may have caused the lipaemia. Lipaemia is often associated with a high-fat meal (Xenoulis and Steiner 2010). Those dogs that had tested positive for the three pathogens had no increase in inflammatory markers - white cell counts were normal, indicating past exposure or infection rather than active infection. All blood smears were normal in appearance, with the occasional Howell Jolly body or target cell, especially in the younger dogs. There were no increases in eosinophils to indicate a high parasite burden.

### **5.6.3 Water analyses**

No significant zoonotic bacteria or protozoans were identified in water samples from natural springs or wells that provide drinking water. This was an unexpected result as camera trap photos showed waterholes were visited by all species in the area and faeces from various animals were present in waterholes and the wells.

Contamination of water sources with protozoa such as *Giardia*, *Cryptosporidium* and *Toxoplasma* and enteric bacteria have been well documented (Iijima et al. 2001, Shaw et al. 2001, Briancesco and Bonadonna 2005, Savioli et al. 2006). It is possible that the volume of water collected for filtering was insufficient to provide detectable concentrations of bacteria and parasites. Alternatively, as several of the water sources were still partially frozen from the sub zero winter temperatures, the sampling time was not ideal. It would be prudent to repeat the water sampling to collect and filter larger volumes and from each season.

## 5.7 Summary and Future Recommendations

As both the dogs and goats were positive to antibodies for *C. burnetii* and *T. gondii* and are in close contact with wildlife, other domestic animals and humans, there is significant potential for transmission of these zoonotic diseases. Vaccinating goats against *C. burnetii* may reduce the public health risks of pathogen transfer from the goats to the herders during periods of working in proximity to their stock. Health education is key to improving hygiene practices during combing, kidding, slaughter and food preparation (Macpherson 2005, Zinsstag et al. 2006). Wearing of face-masks during combing of the goats would decrease transmission via aerosolized pathogens. During slaughter and assisting births, wearing disposable gloves would reduce transmission of both *C. burnetii* and *T. gondii*. During slaughter, intestinal contents were milked out by hand allowing small parasites and ova to be trapped under fingernails and enabling bacteria to infect small wounds on the hands. Improved parasite control, both endo and ectoparasites, for dogs and goats, would also increase herd productivity and income.

Future studies could use strain genotyping of pathogens from goats, dogs, humans and wildlife to elucidate transmission cycles and indicate risks. Longer-term studies are recommended to monitor the health of the local people, their animals and wildlife, so that appropriate health interventions can be made.

### Acknowledgements

I thank the local herders for allowing sampling of their goats and dogs.

Thank you to Midjii Avush for his excellent local knowledge and logistical support and Lkhagvasumberel Tomorsukh (Sumbee) for translating, animal restraint and photography.

Many thanks to the Snow Leopard Trust for funding and logistical support in the field especially Gustaf Samelius and Orjan Johansson.

We thank Nicollette Newell from the Abaxis company for donating the Abaxis I-Stat machine and cartridges and Zebra vet for donating veterinary consumables. We are grateful to Per Eriksson, Françoise Gillet and Irene Dergel for their diagnostic expertise and assistance.

Many thanks to Karen Gerber for her help with examination of blood smears.

# **Chapter. 6 Questionnaire Surveys Concerning Wildlife, Livestock and Human Health in the South Gobi Desert of Mongolia**

## **6.1 Introduction and Premise of Chapter 6**

Chapter 6 looks at the human component of my One Health study on snow leopard disease. Taking blood samples from people and testing them for the same suite of pathogens, which we tested the snow leopards, rodents, dogs and goats for, was outside the scope of this study. So herders and their families were presented with two questionnaire surveys to complete. The first survey asked questions regarding their health, and the second inquired about the health and management of their stock, primary cause of stock loss and observations of wildlife health/death. I wanted to know if the local people had seen any sick or dead snow leopards apart from the four that were originally reported.

I wanted to determine if there were epidemics in the area that could be due to the spread of zoonotic disease, if herders had observed illness personally after handling ill domestic stock or after interaction with ill wildlife. Questions were asked to determine whether the herders were aware that pathogens could pass from animals to people and vice versa. I also wanted to find out attitudes of the herders to stock loss, if they were biased to immediately assuming it was from predation or if they had considered it might have been due to other reasons such as disease.

The surveys were composed mainly of multiple choice and yes/no questions in an attempt to reduce the risk of ambiguity due to the language barrier. Surveys were designed in English, translated to Mongolian and then the answers translated back to English. With open-ended questions, there would have been an increased risk of misinterpretation due to the limitations of translation.

This Chapter has been written as a stand-alone manuscript for the purpose of publication. It will be submitted to Plos 1.



*Article: Questionnaire Surveys Concerning Wildlife, Livestock and Human Health in the South Gobi Desert of Mongolia*

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## **6.2 Abstract**

Determining health concerns of remote, rural nomadic people, their domestic animals and sympatric wildlife is challenging and frequently neglected. In the South Gobi Desert of Mongolia, nomadic herders live in close proximity to their animals and wildlife, making them vulnerable to zoonotic disease. We conducted questionnaire surveys to obtain broad and preliminary information on how people in the South Gobi Desert perceive their health, that of their animals and sympatric wildlife to assess potential links. This study also aimed to evaluate herders' attitudes towards wildlife, especially predators of their stock. Responses of the herders indicated the majority believed illness in people was mainly due to the common cold and they were unaware of zoonotic risks from livestock or wildlife. There was little concern regarding illness in domestic animals and routine animal health care was rare. However, most herders identified predation by wolves (70%) and snow leopards (50%) as the primary cause of livestock loss followed by extreme climatic conditions (20%). Annual livestock losses, from all causes, ranged from 1% to 6% (average 4%) across herds.

Although herders were highly concerned about predation, improved productivity could be achieved by implementing routine animal health measures, such as worming and vaccination. These perceptions need to be addressed to improve human and animal health in the area and help mitigate human wildlife-conflict.

### 6.3 Introduction

In Africa, Central Asia, Middle East and India, nomadic communities live in close association with their domestic stock and wildlife as they have done for hundreds of years (Macpherson 1994, Ostrowski 2009, Njeru et al. 2016). This interaction leads to issues concerning zoonotic disease spread and livestock predation by wild carnivores (Macpherson 1994, Ostrowski 2009, Njeru et al. 2016).

There are increasing numbers of studies into the health of nomadic pastoralists but most of these are based on Africa, India and a few Central Asian countries, with the health of pastoralists in countries such as Mongolia remaining poorly documented (Mocellin and Foggin 2008). Determinants of public health in remote, rural areas include environmental factors such as climate, diet, drinking water availability and proximity to domestic animals and wildlife. Globally, most emerging diseases are zoonotic (75%) originating in wildlife, with their emergence often driven by anthropogenic land changes (Rabozzi et al. 2012, Pfäffle et al. 2013). However, there is little background information available on pathogens in wildlife populations. This lack of information is especially true in Mongolia. Globally the major zoonotic diseases that pose a significant risk to nomadic herders from illness and loss of livestock productivity include brucellosis, Q fever, rabies, anthrax and echinococcosis (Tomley and Shirley 2009, Thompson 2013, Tompkins et al. 2015). Arthropod vectors such as fleas and ticks can also carry zoonotic pathogens. As climatic conditions change they are expanding into new geographical niches (Semenza & Menne 2009) and thus spreading disease (Thompson 2013).

Traditional Mongolian herders, like other nomadic people, live in close proximity to their livestock and wildlife, facilitating the possible spread of zoonotic pathogens. Serious zoonoses occur in humans, livestock and wildlife in Mongolia, including anthrax, (McFadden et al. 2015), plague (Riehm et al. 2011), leptospirosis (Chapters 2,3 and 5, Odontsetseg et al. 2005c), toxoplasmosis (Chapters 2,3 and 5, Brown et al. 2005), tick borne encephalitis (Ebright, Altantsetseg, & Oyungerel 2003) and Q fever (Chapters 2,3 and 5). These diseases are known to transfer between wildlife, domestic stock and people (Ostrowski 2009) and many have wildlife as reservoirs

(Thompson 2013). However, assessing risks and frequency of transmission is challenging.

The One Health doctrine approaches the study of these zoonotic health risks to nomadic people in an integrated fashion, bringing expertise from medical, veterinary and biological fields (Tomley and Shirley 2009, Zinsstag et al. 2011). Despite this acknowledgement of the importance of an integrated approach, wildlife is often overlooked in One Health programs, as the emphasis is placed on the relationship between domestic animals and people (Karesh and Cook 2009). However, linkages between human and wildlife health and as sentinels of environmental health are clear (Zinsstag et al. 2006).

In many nomadic communities in developing countries, predation of stock by large carnivores poses a threat to human lives and livelihoods resulting in significant human/wildlife conflict (Bagchi and Mishra 2006, Namgail et al. 2007). For nomadic pastoralists in Mongolia, predation of their livestock by wolves and snow leopards is perceived as a major threat to their food and income, but the magnitude and cost of stock predation has not been quantified. Predation by snow leopards on domestic stock commonly leads to retribution killing of the predator (Jackson et al. 1996). Retribution killing may have serious impacts for the leopard population and could affect the ecosystem due to the removal of the apex predator (Brook et al. 2008, Colman et al. 2014, Ripple et al. 2014).

It is only from understanding the issues contributing to the complexities of herder/wildlife conflict can we develop targeted interventions. In Machiara National Park, Pakistan, 90.6% of stock killed was attributed to leopards and resulted in negative herder attitudes to carnivores (Dar et al. 2008). However, upon further investigation, stock loss from disease was a more significant financial loss (72.7%) compared to that of predation (27.8%). When results were revealed to herders, attitudes switched from retribution killing of predators to improving herd health (Dar et al. 2008).

Serious zoonotic diseases occur in Mongolia that could negatively impact on nomadic herders their domestic stock and sympatric wildlife. This study was conducted in the Tost Mountains of the South Gobi Desert in rural Mongolia, home to

indigenous nomadic herders, their stock as well as the endangered snow leopard *Panthera uncia* and other wildlife. The herders live in close proximity to their stock and wildlife providing opportunities for pathogen transmission. We investigated herder perceptions of disease and predation, through questionnaire surveys (Babbie 1990). We asked herders questions about human health, stock and wildlife health and any perceived links. We also investigated perceptions of livestock loss and its cause, including predation,

We aimed to:

- 1) Assess the relative importance of predation and disease in stock loss to enable interventions that optimise productivity and conservation of snow leopards;
- 2) Identify which diseases herders thought were present in stock and/or wildlife
- 3) Determine which illnesses were common in the local people;
- 4) Elucidate gaps in areas of herd health that can be addressed to improve herd health and hence productivity; and
- 5) Identify where farming processes can be improved to reduce risks to herders from zoonotic diseases.

## 6.4 Methods

Study design- A cross-sectional survey was implemented in this study.

Two questionnaires were developed to investigate:

- 1) The health of the local herders and their families; and
- 2) The health of stock and wildlife in the area.

The surveys included questions on herders' attitudes to illness and if they were aware of possible linkages between people domestic animals and wildlife for pathogen transfer (see Appendix 1 for survey questions). Questions were designed to gain both qualitative and quantitative data. Questions were either yes/no or multiple choice to minimize ambiguity with opportunity to elaborate via further text. Surveys were translated into Mongolian and answers were translated into English by

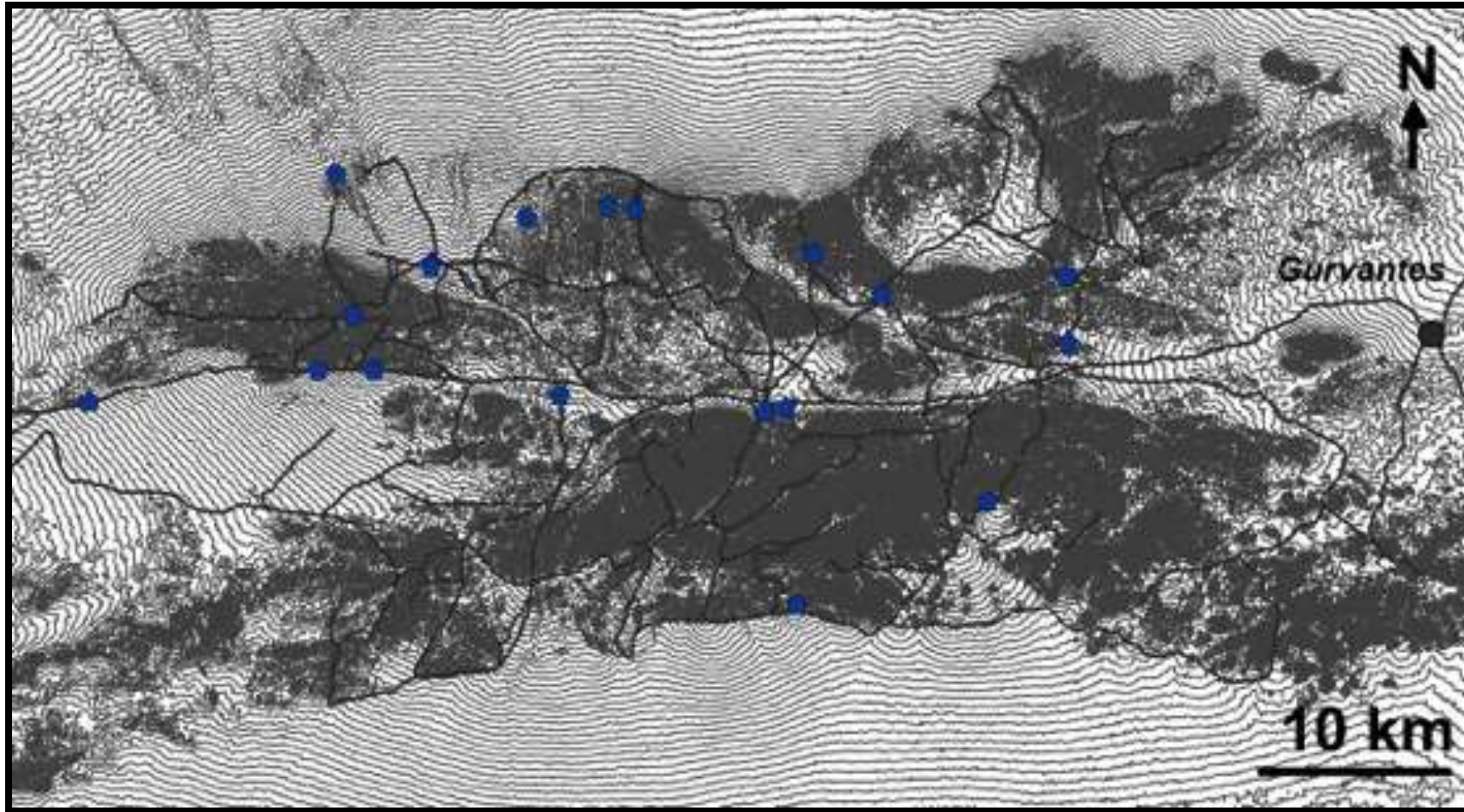
staff of the Snow leopard conservation foundation in Ulaanbataar. Participation in these surveys were voluntary and the survey complied with human ethics approval from James Cook University, number H6044 A.

#### **6.4.1 The study site**

The study area covers approximately 1700km<sup>2</sup> in the Tost Mountains of the South Gobi Desert of Mongolia (see Figure 6.1). The region is comprised of steppe areas surrounded by craggy steep mountains, with limited vegetation of grasses and shallow bushes. There are several natural watering spots and also man-made wells. Approximately 90 nomadic pastoralists and their families are distributed over the region. The dominant domestic animals kept by the herders are Cashmere goats for their wool and meat. Other domestic stock includes sheep, camels and horses. Each family owns one to five dogs to help look after the herds.

#### **6.4.2 Recruitment of participants and data collection**

The surveys were distributed by hand to 45 families across the study area, as well as to the closest doctor and veterinarian (Figure 6.1). When visiting the herders to collect samples from their goats and dogs for a concurrent disease study, the herders were asked to participate in the questionnaire surveys. The aims of the surveys were explained to the herders by a translator and were also provided in writing. All participants signed a consent form and questionnaires were completed during the visit, with help from the translator as needed.



**Figure 6.1** Distribution of surveys to the herders in the Tost Mountains over the study area. Blue dots represent herder locations.

### 6.4.3 Questionnaire data

The human health survey was composed of 19 questions aimed at gaining information on health issues within herder families or their neighbours. The animal survey had 23 questions regarding numbers of and perceived cause of stock loss, abortions, herd and wildlife illness and preventative herd health programmes, such as vaccination or drenching for parasites. Questions regarding wildlife covered observed mortalities of wildlife, scavenging by domestic dogs and predation. The local vet, who lived 50 km away and had worked for 30 years in the area, commented on the most common stock and wildlife diseases that he had observed. The local doctor also contributed extra comments as to human health in the region.

### 6.4.4 Data analyses and management

The surveys were translated back into English and the responses analysed using Excel and SPSS.



**Plate 6.1** Member of the SLCF in blue, explaining how to fill out the surveys to one of the local herders.

## 6.5 Results

### 6.5.1 Herders survey response to human health questions

Of the 45 questionnaires distributed, 27 (60%) were completed.

For questions 1) -3) Most family units were comprised of grandparents, parents and children living in one or two gers (local dwellings), with up to eight people sharing the same ger.



**Plate 6.2 Families occupying the moveable gers.**

Q4) 47% stayed in the one spot irrespective of season and 53% moved around the study area.

Q 5) Up to 48% of children boarded in town for schooling and returned home on weekends or holidays.

Q 6) 71% reported that there was no illness affecting more than one member of the family at a time.

Q 7,8, 9, 10.) The main illnesses reported were colds and flu of one-week duration. Two families reported chickenpox and measles in children and one family reporting



mumps. Two unknown illnesses were reported in two adults: one affected kidneys and limbs for 1-2 weeks and another unknown illness caused lameness and fever for greater than two weeks.

Q11). The severity of illness was mild for most of the colds and people could continue their daily routine. The two children with measles/chickenpox and the adult with lameness and fever were bed ridden. There were no reports of serious illnesses causing death or hospitalization.

Q 12) There were no reports of epidemics in the district

Q 13-17) There were only two respondents to these questions. One said there was illness in the area with high temperature being the symptom. The other respondent did not mention symptoms but thought the illness was from food.

Q 18) Up to 40% of families reported having their children immunized, but did not know for which diseases.

### **6.5.2 Response from the local doctor on the human health questionnaire**

The common illnesses among locals were hepatitis, toothache, blood pressure and heart problems (local doctor, Dr Oyuntseren, pers com). She blamed this on eating camel meat and drinking salty tea. She said the rural people got brucellosis but did not describe its effects or frequency. She stated there was 100% vaccination of babies and of a few adults. The babies are vaccinated against rubella, influenza, tetanus, hepatitis and “paralysis”, however it was unclear as to what was meant by paralysis or its cause.

### **6.5.3 Survey response by herders on domestic animal and wildlife health**

Twenty-five of forty-five (55.5%) of surveys distributed to herders were returned, however not all questions were answered.

Q1) Herds were comprised of 125-500 goats.

Q2) The average proportion of goats lost per herd per year was 4% (range 1% to 11%).

Q3) In total, 83% of herders reported losses due to predation, 13% due to mortality giving birth, 4% due to eating poisonous plants, 8% due to unknown illness and 13% due to unknown causes. Questions about climate were not on the surveys but many respondents reported mortalities due to reduced availability of fodder during severe winters or when there was no snowfall leading to reduced vegetation in spring (Plate 6.3).



**Plate 6.3 Often little grazing was available.**

Q4-6) The tables within the survey were largely unanswered, however, comments included reports of a poisonous plant (*Ephedra* sp.), eaten by goats during times of harsh weather and low food availability which caused sickness and death. Ingestion of another *Ephedra* species, *E. sinica*, made the goats burp and their breath smell like onions but did not result in illness (Nadia Mijiddorj pers com.)

Q7) A total of 75% of respondents said they used Ivomec (Formulation and manufacturer details were not known) but did not say for what purpose or on a routine basis.

Q 8) No response

Q 9) With regards to birthing issues with their goats, the responses gave slightly different answers to similar questions above concerning mortality of goats. Herders reported 21% abortion in goats, but this was not included in response to cause of death of kids.

Q 10) 4% of herders reported that does were sick at the time of birthing.

Q 11) One herder replied that they lost a kid at birthing.

Q 12) 87% reported that they saw predation of their goats.

Q 13) & 14) 70% of herders reported wolf and 50% reported snow leopard predated on their livestock, with 20% of herders reporting both wolf and snow leopard as the main predator. Other predators (8% raptor and 4% fox) were reported to cause less than 10% of the overall depredation of livestock.

None of the participants reported seeking or implementing, routine healthcare interventions such as vaccination or anti-parasitic drenching of their herds. However, one herder reported that he gave penicillin to the goats when they had “goat fever” which occurred rarely. He described “goat fever” as occurring when the placenta was retained after kidding with clinical signs including shivering and drooling.

Q15) The number of dogs owned by the herders ranged from 1-5

Q16-17) 75% of herders said they treated their dogs with unknown medication

Q 18) 30% of respondents said they saw their dogs scavenge on carcasses

Q 19) 10% of herders reported seeing sick wildlife: 1 wolf, 1 fox and 1 Argali sheep.

Q 20) There were no respondents to this question.

Q 21) 6% of respondents said they saw dead wildlife.

There were reports of a fox in the area with rabies (the regional veterinarian pers. com). He said the main problem with the stock, were intestinal parasites. He also reported there was no brucellosis in the area.

The surveys indicated that people did not recognize any connection between their health and that of their animals. It was difficult to determine if they saw a connection between sick animals being more vulnerable to predation. People were not forthcoming about their attitudes towards snow leopards or other predators, however wolves tended to be viewed as the main cause of predation.



**Plate 6.4 Dogs remain with the herds throughout the day.**

## **6.6 Discussion**

Serious human health issues were not reported and herders appeared unaware of zoonotic risks from livestock or wildlife. There was little concern regarding illness in domestic animals and routine animal health care was rare. Most herders identified predation by wolves and snow leopards as the major cause of livestock loss followed by extreme climatic conditions. Although herders were highly concerned about predation, improved productivity could be achieved by implementing routine animal health measures, such as worming and vaccination.

### 6.6.1 Human health and health care

Herders were reticent to discuss their health issues and responses to most open-ended questions were limited, possibly due to distrust of outsiders as seen with other surveys and responses of indigenous people (Edwards et al. 2005).

There were no reports of epidemics affecting the region as a whole, although children who boarded in town for school, are a potential pathway for transfer of pathogens from the town to the rural communities. The main illnesses reported by the local people were colds and flu and two cases of mumps/measles. The response by herders that 48% of people had their babies immunized was likely an under representation than the 100% reported by the local doctor, which may have referred to the town inhabitants. There were unknown illnesses in two people that caused kidney problems, lameness and fever and the doctor had reported hepatitis. Since 9.3% of goats in the study area had tested positive for Q fever (*Coxiella burnetii*) (Chapter 5), it is possible that flu-like cases were actually due to infection with this serious zoonosis (Woldehiwet 2004). Leptospirosis can result in chronic kidney issues in people and affect the liver causing jaundice (Vijayachari et al. 2008). Since 34% of rodents, 20% of snow leopards and 5% of dogs in the study area had antibodies to *Leptospira* spp, exposure from wild or domestic animals is likely and may have caused the renal disease that was reported (Chapters 2,3 and 5).



**Plate 6.5 Orphaned kids are cared for by family members.**

The herders did not appear concerned about risks of acquiring infections from their animals despite their close contact. During kidding time, the orphaned or abandoned kids were housed in the gers with the people. Slaughter of the goats was performed inside away from the dust and wind, likely exposing families to aerosolized pathogens. Intestinal contents were stripped by hand, providing an opportunity for transfer of parasites if hand washing afterwards was not rigorous (Esson personal obs). Helminth parasites occur in high prevalence in most wild and domestic ruminants distributed over Mongolia and slaughter represents a high exposure risk due to the methods employed as outlined above (Sharhuu and Sharkhuu 2004).

Herders did not mention any of the illnesses the doctor said were the most common, these being hepatitis, toothache and brucellosis. Furthermore, the report of brucellosis is inconsistent with the knowledge provided by the local veterinarian and a recent survey of goats from the study area (Chapter 5 in thesis), which were negative for *Brucella*. In a report by Zolzaya et al. (2014) on the prevalence of Brucellosis in herders and livestock in adjacent provinces in Mongolia, reported 27.3% in humans, 34.6% in dogs and 5.2% in goats, which differs to the results obtained here. Zolzaya et al. (2014) also concluded that there was a 15-fold under reporting of brucellosis in Mongolia and also theorized this discrepancy could be due to lack of consultation with the doctor by the local people and lack of access to adequate brucellosis testing, which appears a common problem in developing countries (Abdulraheem et al. 2012).

Health education in schools and the community needs to promote awareness of services available, although costs are a likely issue. The lack of routine health care services available and lack of integrated health information in other nomadic communities appears to contribute to low immunization rates (Zinsstag et al. 2006). Different institutions without strong coordination (World Health Organization have carried out activities directed at environmental health protection in the region. Western Pacific separately, Region 2005) which could reduce effective public communication. There is also a distrust issue between the pastoralists and government services (Zinsstag et al. 2006). The Mongolian Organization for Health (MOH) is considering the development of a geographic information system of collection and analysis of health and environmental data to support policy-making

and interventions. However, from their distribution maps, the Tost region appears to have been overlooked, likely due to the remoteness of the area and low population density (Cohen 2005).

### **6.6.2 Herd health and wildlife observations**

According to participating herders, on average, 4% of goats across all herds are lost annually. The main cause of stock death was attributed to predation, mostly by wolves and then snow leopards. However, it was not recorded whether the goats taken were healthy or sick. In a concurrent study in the area on snow leopard diet composition, it was observed the goats that lagged behind the main herd were those predated upon (Johansson et al. 2015). Hence its possible sick animals were targeted for predation and that illness may play a higher role in stock loss than the herders realize. Furthermore, snow leopards prey mostly on native ungulates- ibex and argali sheep- rather than domestic goats (Johansson et al. 2015). In a study on stock loss in Pakistani villages near Machiara National Park, it was found that although leopards were perceived as the main threat and reason for stock losses, more losses were from diseases due to lack of herd health programs (Dar and Linkie 2009).

Adverse climatic conditions were identified as a problem for stock health (Rao et al. 2015). During long periods of dry weather or harsh winters, vegetation is scarce, increasing grazing competition between domestic stock and native ungulates. When other pasture was scarce, goats reportedly ate *Ephedra sinica*, which contains high amounts of alkaloids that are metabolized to amphetamine-type substances (Wang et al. 2010). The effects of these metabolites in goats are unknown and require further investigation. Reductions in fodder also concentrate parasite exposure for domestic and wild ungulates and opportunities for pathogen exchange as grazing areas increasingly overlap (Chirichella et al. 2014). Increased grazing pressures can have diverse ecological impacts including loss of biodiversity and changes in ecosystems and population densities of numerous taxa (Chirichella et al. 2014). Clinical problems were not described that reflect the reported high levels of exposure to *C. burnetii*, *T. gondii* or *Leptospira* spp. (Chapter 5).

### **6.6.3 Livestock treatments**

Vaccination of goats was not standard. Of the herders that responded 75% said they treated goats with ivermectin anthelmintic but appeared unsure of the purpose.

However, a recent article in the Ulaanbaatar Post stated that most farmers used Ivomec not according to regulations for treatment of internal parasites but believed it to be a fattening medication (Khaliun 2017). It was unclear if herders complied with the guidelines to withhold meat from human consumption for 30 days after administration (Lanusse and Prichard 1993).

In a cost-benefit model of vaccination for brucellosis in sheep, goats and cattle in other regions of Mongolia, it was estimated that the savings to the community would be about \$18.3 million US by decreasing health issues and hence increasing productivity by people. Other studies aimed at improving stock health through the reduction of zoonotic diseases, via preventative medicine, had flow on beneficial outcomes to the nomadic people (Schelling et al. 2003, Schelling et al. 2005, Mantur and Amarnath 2008, Montavon et al. 2013).

Observations during our field-work revealed the occurrence of health problems in goats that were not recorded in questionnaire responses but required veterinary attention (Esson pers obs). Health issues observed in goats included poor body condition, kidding problems, retained placenta, urinary tract infection, rectal stricture and a discussion of coughing and “goat fever”. We were involved in opportunistic veterinary care of many of these cases, as routine health visits by the local veterinarian were infrequent due to costs and remoteness.

### **6.6.4 Wildlife health**

Only 10% of herders reported seeing sick wildlife and 6% reported seeing dead wildlife. The herders traverse large areas of the study site daily so are a unique source of surveillance information that could be documented within a database of disease incidents. Although the chance of observing dead rodents is low, carcasses from larger animals are likely to persist and be visible for a couple of days.





**Plate 6.6** ibex carcass.

## **6.7 Study Limitations**

The study was hindered due to low overall response rate and limited answers, mainly due to difficulties in translation and the reticence of participants to discuss health issues. More information could be gained from verbal interviews and further visits now that relationships and trust have been established. Later visits where previous serosurvey data were shared resulted in more open and discursive interactions (Esson pers.obs).

## **6.8 Conclusions and Recommendations**

Despite the limited responses to the questionnaires we gained useful information on health issues of humans, domestic animals and wildlife in this remote region.

The surveys indicated the herders lacked knowledge of their health and health services available. We received conflicting information from the people, the local veterinarian and the local doctor as to disease occurrence (such as brucellosis) and health management. The herders perceived the main threat to their stock was predation but had not considered loss from illness. They were unaware of diseases their stock could carry. Little routine health care was applied to the domestic stock, either due to lack of awareness or possibly due to the costs and availability of basic vaccinations and worming regimes. Wildlife health went either largely unnoticed or

not reported in the surveys. These issues should be addressed using One Health approaches involving vets and public health experts.

Therefore, we recommend:

- 1) Further surveys involving verbal interviews to gain a deeper understanding of the health issues in people and their animals.
- 2) Health education on identifying and reducing exposure to the common zoonotic diseases in the area.
- 3) Instigating routine vaccination and parasite control in livestock to improve productivity and reducing zoonoses.
- 4) With herder co-operation instigate recording of numbers of stock loss a) due to predation and b) illness so that appropriate management plans can be defined for stock productivity and snow leopard conservation.
- 5) With herder co-operation instigate recordings of sick and dead wild life, including species, location and any symptoms observed.

### **Acknowledgements**

We thank the herders who participated in these surveys, Mijid Ayush for his excellent driving, navigation and establishing contacts among herders. We are indebted to Lkhagvasumberel Tomorsukh (Sumbee) for his company, translating and explaining the survey to the herders and to Chimgee for translating answers.

## 6.9 Appendix

### 6.9.1 Human health surveys

#### *Survey for Human Health Assessment in the Gobi Desert in Mongolia*

This survey is intended to gather information on local perceptions of what diseases have been present in the past.

You will be answering a number of questions that will require you to circle the answer or write a response to the question.

- 1) How many people are in your family?
  - 2) How many adults?            Men            Women
  - 3) How many children?        Boys            Girls
  - 4) Do you always live in this area or do you move around?
  
  - 5) Do the children go away to school? Yes    No
  
  - 6) Has there ever been sickness in your family that affected more than one person at a time?    a) Yes    b) No
- If Yes:
- 7) Name the illness if you know:
  
  - 8) Who in your family did it affect?  
a) Men    b) Women    c) Children    d) Everyone
  - 9) What health problems/symptoms did they have?  
a) Fever    b) Vomiting    c) Diarrhoea    d) Cough    e) Other – Please describe
  
  - 10) How long did the illness last?  
a) 0-3 days    b) 4-7days    c) 1 – 2 weeks    d) Longer

- 11) How severe was the illness?
- a) Mild – could keep working, going to school
  - b) Moderate – Stayed at home, could not work or go to school but could get out of bed
  - c) Severe – Had to stay in bed, couldn't eat
  - d) Extreme - Died
- 12) Has there ever been sickness in the district that has affected more than one family at a time? a) Yes b) No
- 13) Name the illness if you know:
- 14) What health problems /symptoms did they have?
- a) Fever b) Vomiting c) Diarrhoea d) Cough e) Other – Please describe
- 15) Where do you think the sickness came from -
- a) Food b) water c) other people d) do not know e) other
- 16) Do any human sicknesses occur regularly? a) Yes b) No
- If yes
- a) How often?
    - a. Monthly b)Yearly c) Seasonally d) Other
- 17) What are the symptoms?
- a) Fever b) Vomiting c) Diarrhoea d) Cough e) Other – Please describe

18) Do you or anyone in your family ever receive any immunizations from the Doctor?

- a) Yes    b) No

If yes:

a) What are they for?

b) At what age do you have them?

c) How often do you have them?

19) Is there any other information about health or disease in the community or your family that you would like to comment on?

Thank you for participating in this survey it has been greatly appreciated. The results will be sent to you once all the data has been collated and analyzed.

## 6.9.2 Wildlife and Domestic Animal Health Survey

### Wildlife and Domestic Animal Health Survey

I am asking these questions to find out about your animals and how well they are.

- 1) How many animals are in your goatherd?
- 2) How many goats would you usually lose in a year?
- 3) What do you think are the causes of these losses?
  - a) predation
  - b) illness
  - c) unknown causes
  - d) any other reason?

- 4) Have you seen any animals in your area suffering from illness in the last 5 years?

Place an "X" in each space that is applicable

Animal	Yes	No	Juvenile/Mature	Season/year?	Number
Goat					
Dog					
Horse					
Camel					
Snow Leopard					
Wolf					
Ibex					
Rodent					
Other					

- b) Any other comments you would like to make?

- 5) IF any of the animals in question 4) were sick, what were their symptoms?

Animal	Cough	Sneeze	Thin	Bloated	Not eating	Dead
Goat						
Dog						

15) How many dogs do you own?

16) Are the dogs ever treated with any routine medication? Yes No

17) If yes, can you show me the bottles/packaging?

18) Do you ever see the dogs hunt/scavenge on or eat any wildlife such as rodents or ibex or anything else? Yes No

18a) If yes, what animals do you see them eat?

19) Do you ever see any sick wildlife? Yes No

a) What sick wildlife do you see if any?

20) How often do you see sick wildlife? Weekly monthly yearly other

b) At any particular time of the year?

21) Do you ever see any dead wildlife? Yes No

22) What wildlife do you see?

23) Are there any other comments you would like to make or information you would like to give?

15) How many dogs do you own?

16) Are the dogs ever treated with any routine medication? Yes No

17) If yes, can you show me the bottles/packaging?

18) Do you ever see the dogs hunt/scavenge on or eat any wildlife such as rodents or ibex or anything else? Yes No

18a) If yes, what animals do you see them eat?

19) Do you ever see any sick wildlife? Yes No

a) What sick wildlife do you see if any?

20) How often do you see sick wildlife? Weekly monthly yearly other

b) At any particular time of the year?

21) Do you ever see any dead wildlife? Yes No

22) What wildlife do you see?

23) Are there any other comments you would like to make or information you would like to give?



Thank you very much for answering my questions. I will be collating and organizing the results and then sending them to you for your information.

# **Chapter. 7 A Review of Disease and Conservation Threats to the Endangered Snow Leopard *Panthera uncia* in the South Gobi Desert of Mongolia, Utilizing a One Health Approach**

## **7.1 General introduction and Premise of Chapter 7**

In this chapter I collate the results of the previous chapters to demonstrate the efficacy of a One Health approach when investigating disease in an endangered species.

By dividing the study into sections that satisfy the One Health criteria, I was able to gain a greater understanding of threats to snow leopards than by just sampling from them alone. As they are usually solitary animals except when coming together for mating, territorial disputes or mothers travelling with young, the risks of them passing only felid pathogens that normally rely on close proximity for transfer seemed limited, especially in such a small population. The holistic One Health approach looking at zoonoses, which can be just as threatening or more so to snow leopard health than purely feline related illnesses, seemed to be the most logical study design and one that would accumulate the most significant amount of relevant data.

Each study component selected was based on the relevance and overlap with snow leopards in the area. Rodents are found in all environmental niches including human dwellings, overlapping in habitat, as food sources and reservoirs for pathogens. Goats as they can be a prey item, hence direct transfer of pathogens to snow leopards, overlap with the native ungulates establishing links between wildlife and domestic animals and have daily contact with people. Dogs interact directly with the goats, roam over the entire study area, interact with the rodents and people. Water sources as the environmental component are an obvious place for pathogen exchange as with many desert communities are the focal meeting places.

I found serious zoonotic pathogens common to each species and widespread across the study area, which may be linked from one species to the next. Further work using molecular epidemiology would help elucidate if these were the same strain of pathogen moving between species. By looking at pathogen transfer on a more

holistic level, I was able to detect areas where interventions could be introduced that may reduce the spread of pathogens. Improving herd health would decrease transfer of pathogens, especially those linked to parasites, between domestic stock and wildlife. As with the previous chapters, this chapter has been written in a format for publication so there is some repetition from the previous chapters with regards to the study area and the samples collected. This paper will be submitted to the Journal of One Health.

*Article: A Review of Disease and Conservation threats to the endangered Snow Leopard Panthera uncia in the South Gobi Desert of Mongolia utilizing a One Health approach*

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Key words: One Health, Snow Leopards, Disease, Mongolia

## **7.2 Abstract**

Snow leopard populations are continuing to decline across their range in the mountainous regions of Central Asia. To reverse this trend, reasons for their decline, besides those already identified, need to be examined – this includes disease threats. As snow leopards exist alongside nomadic herders, their domestic stock and sympatric wildlife, a One Health approach was utilized to collate information on zoonotic pathogens that may circulate among wildlife, domestic animals and humans and how these pathogens may impact on snow leopards. This review chapter aims to bring separate studies of different hosts together (previous chapters), to link the pathogens identified, their impacts and host associations to understand the threat they may pose to the population of snow leopards and ecological community including people in the Tost Mountains of the South Gobi Desert of Mongolia.

Sero-surveys of snow leopards, native rodents, domestic goats and domestic dogs living in the study area were performed and results collated. Ectoparasites and

faeces from all species were collected and environmental samples from water sources were taken. Analyses of the samples included, Enzyme Linked Immunosorbent Assay (ELISA), Polymerase Chain Reaction (PCR) and Next Generation Sequencing (NGS) for a suite of pathogens, focussing on zoonoses. We also review key points from questionnaire surveys of the local people, collecting information on health management and perceptions of human health, livestock and wildlife health and livestock predation. Results revealed antibodies to *Coxiella burnetii* and *Toxoplasma gondii*, occurred across all species tested, suggesting cross species transmission. *Leptospira* spp. were identified in snow leopards, dogs and rodents and Puumala and Seoul Hantavirus occurred in the rodents. Ticks and faeces from all species contained numerous genera of potentially pathogenic bacteria, including, *Anaplasma*, *Babesia*, *Bacillus*, *Borrelia*, *Coxiella*, *Clostridia*, *Rickettsia* and *Yersinia* spp. However, rodent fleas, the primary vector for *Yersinia pestis*, were negative for the pathogen. No zoonotic pathogens were identified in the water sources. Questionnaire surveys of the herders revealed gaps in essential health management for humans and livestock. The high prevalence of zoonotic disease identified in animals in the area may impact on public health, agricultural productivity and conservation. Simple improvements to infection control could reduce pathogen transmission among wildlife, humans and domestic animals. Risks of pathogen transmission to the endangered snow leopards could be reduced indirectly by improvements to key interventions including implementing: 1) vaccination regimes for the domestic stock which would have flow on effects to reduce the reservoir and intermediate hosts for pathogens such as *C. burnetii*. 2) hygiene practices relating to animal handling and slaughter and 3) exclusion of rodents from homes.

## **7.3 Introduction**

### **7.3.1 Snow leopard conservation and health**

Snow leopards (*Panthera uncia*) are a rare and threatened but iconic species across the mountain system of Central Asia. Their numbers were thought to have declined by 20% during the 16 years before 2008 when the last population census was performed (Jackson et al. 2008). However, during 2017, a review of population studies across their range suggested the decline to be 10% and their current status be altered to vulnerable rather than endangered (IUCN 2018). There appears to be a

significant amount of controversy around this decision. Determining the causes for the continual decline of an endangered species, so that practical interventions can be initiated to reduce the trend, is complex. Snow leopards in the Tost Mountains of Mongolia have been part of an ongoing ecological study since 2009, investigating their life history strategy and threats to their existence (Johansson et al. 2015, 2016, Sharma et al. 2014). Alongside similar ecological studies on snow leopards in different parts of their range and on other endangered apex predators, the main threats identified in the Tost region are anthropogenic causes such as retribution killing and loss of native prey due to competition with domestic stock (Oli et al. 1994, Wegge et al. 2012, Li and Lu 2014, Ripple et al. 2014). However, the numbers lost due to poaching for pelts and body parts or trophy hunting, in the study area are unknown. Until recently the Tost region, as with other areas of Mongolia, has been under threat from mining due to the rich ore reserves with 12 mining leases and two active mining sites (SLT 2016). However, due to the hard work of the Snow Leopard Trust (SLT) and the Snow Leopard Conservation Foundation (SLCF) in the Tost region, an area covering 8163 square kilometres became a protected nature reserve in 2016 (SLT, 2016). Snow leopards are thus a protected species, however, implementation of conservation policy is hard to enforce in these remote and sparsely populated regions (Wingard and Zahler 2006).

Another threat to the snow leopards in this area that has not been investigated is the unknown risk of disease. Many factors affect disease threats to endangered species. Drivers of disease emergence can relate to altered land use and other environmental pressures that affect pathogen prevalence in sympatric hosts as well as affecting contact rates between species (Smith et al. 2006).

During 2011, four snow leopards were found dead within the Snow Leopard Trust's long-term ecological study site (2009-2015). Three in the Tost Mountains and one in the neighbouring mountains of Nemegt in separate incidents, within months of each other (see Figure 2.1 Ch 2). Causes of mortality were not established but disease was a possible scenario. The last snow leopard found had blood coming from the nose and anus, which can be a sign of anthrax (Beyer and Turnbull 2009). Direct observation of disease is unusual in wild snow leopards due to their cryptic nature and their remote habitat. Only 10-14 adult snow leopards were present in the region

during our study (Sharma et al. 2014). The death of even a few individuals from pathogens can lead to complete population extinction in endangered species (Roelke-Parker et al. 1996). These mortalities fuelled the impetus for this study. If pathogens were present that caused death in the snow leopards, they could also pose a threat to sympatric wildlife, domestic animals and people that live in the area if these pathogens could cross species. Information regarding possible sources of pathogens in most cases is absent but other wild animals, domestic stock and humans can be pathogen reservoirs for wildlife causing mass mortalities (Roelke-Parker et al. 1996).

Emerging wildlife diseases are a growing concern across the world as they have wide-spread and diverse ramifications, including effects on endangered species, production animals, human health and livelihoods (Inskip and Zimmermann 2009). Improved disease control requires transcending the traditional boundaries between conservation, human health and agricultural departments to achieve integrated disease assessment and monitoring and control programs. For these reasons, a One Health approach was decided upon to investigate disease in this rare species, the snow leopard. This method seeks to meet the need of, “utilising multiple disciplines, drawing on knowledge from natural, social and health sciences and the humanities, working locally, nationally and globally to obtain optimal health for people, animals and the environment” (Inskip and Zimmermann 2009).

In rural and remote communities there is a clear need for such work, where humans and animals interact closely. Previous studies have mainly looked at transfer of pathogens between domestic animals and humans without considering environmental factors and the role of wildlife either as reservoirs or transmitters of disease (Rabinowitz et al. 2013, Peston et al. 2013). Similarly, there are few studies on the converse issue of humans and domestic animals contributing to disease in wildlife (Buttke et al. 2014).

### **7.3.2 Zoonoses and emerging wildlife diseases affecting biodiversity and human health**

Worldwide, zoonoses are one of the primary sources of emerging disease that cause illness and death of both wildlife and people, with 71.8% of zoonotic diseases originating in wildlife (Jones et al. 2008, Katare and Kumar 2010). For this reason, I

decided to survey for zoonotic pathogens and increase the likelihood of obtaining positive results for pathogens by sampling numerous taxa.

Along with newly emerging zoonotic diseases, there is the threat of previously known zoonoses recurring under favourable environmental conditions. These include diseases such as anthrax, rabies, plague, leptospirosis and toxoplasmosis, which can occur in a variety of hosts including wildlife and domestic animals (Williams et al. 2002, Rostal et al. 2013). Over 20 bacterial and viral zoonotic diseases and 18 parasitic zoonoses have been identified in Mongolia, with the most prevalent including anthrax, rabies, tularaemia, echinococcosis, brucellosis, leptospirosis and plague (Ebright, Altantsetseg, & Oyungerel 2003, WHAID 2013). These endemic zoonoses occur regularly and presumably are under-reported due to a lack of public awareness. According to the WAHID (World Animal Health Information Database, 2013), there have been recent outbreaks of these diseases across many continents. With the sporadic emergence of these zoonoses under changing environments, including changing climate, changing population dynamics and densities (human, domestic animal and wildlife populations), predictions and preparations should be made for new outbreaks.

Disease transmission and effects on hosts are influenced via a number of factors including population density and interaction, vectors for transmission and environmental conditions, particularly if favourable to the pathogen (Daszak et al. 2000, Dobson and Foufopoulos 2001, Dobson et al. 2013). Emerging diseases can also threaten species otherwise thought to have secure conservation status (Jones et al. 2007). In addition, emergence can occur when pathogens adapt to new species, such as canine distemper virus decimating a population of lions in the Serengeti, (Cleaveland et al. 2000).

The intensification of herding and increasing pressure on fragile rangelands has caused a reduction of vegetation standing crop. This results in declines in natural prey abundance of wild ungulates such as ibex (*Capra ibex*) and argali sheep (*Ovis ammon*) that snow leopards depend on (Berger et al. 2013, Johansson et al. 2015). Competition for grazing with domestic species in an already degraded landscape further exacerbates domestic livestock depredation by snow leopards (Jackson 2013). This increases the frequency and intensity of interactions between leopards,

natural prey, livestock and people that may lead to pathogen transfer between them. The increase of interactions may also lead to retribution killing by the herders (Mishra et al. 2003, Jackson and Wangchuk 2004).

The Tost and Tosonbumba Mountains in the South Gobi Desert of Mongolia have hitherto been overlooked concerning the investigation of zoonotic diseases, both established, re-emerging and newly emerging, making it challenging to formulate management plans for both the wildlife and domestic animals in the event of an emerging epidemic. Here we collate a number of studies on wildlife, domestic animals and humans in the Tost and Tosonbumba Mountains to try and identify and assess the effects of infectious pathogens on the snow leopard and the other hosts that share their environment. Combined with data on snow leopard livestock predation, this will be used to inform management plans for conservation of the endangered snow leopard, manage domestic animal herd health, improve public health and mitigate human/wildlife conflict.

### **7.3.3 Field sampling**

The study area lies in the remote South Gobi Desert of Mongolia in the Tost and Tosonbumba Mountains, covering an area of 1700 km<sup>2</sup> (43°N, 100°E). In the study area, it is estimated that there are currently 8-13 (Confidence Interval 95%) individual snow leopards (Sharma et al. 2014). To collate health issues within this threatened population in the Gobi Desert we used a One Health approach as this was deemed a more holistic approach for this complex situation.

The study was divided into several sections for ease of sampling and analysis. These have been written and will be published separately (see previous results chapters) but their findings are collated here.

- 1) Trapping, examining and sampling of snow leopards for blood and faeces in conjunction with a long-term ecological study (June 2009-May 2015).
- 2) Trapping, examining and sampling of rodents for blood and faeces. Rodents were trapped over the study area, being chosen as a sentinel species for disease (May 2012- April 2015). They were selected as a representative of another wildlife species as they interact either directly as



prey or indirectly via excrement with all other constituents of the study including the local people.

- 3) Sampling of domestic goats for blood and faeces. Goats were selected as a representative of domestic species as they were distributed over the study area, can be a predation source for snow leopards, interact with other domestic animals, overlap grazing areas with native ungulates and are in close contact with the herders (May 2012 - April 2015).
- 4) Sampling of domestic dogs distributed over the study area for blood and rectal smears. Dogs were chosen as a second domestic species as they interact with other domestic animals, wildlife and humans and also scavenge and prey on rodents, thus providing a possible pathogen pathway (June 2013- April 2015).
- 5) Sampling wild ibex as they are a native prey of snow leopards and overlap with domestic species for grazing and other wildlife. Faecal samples were collected from them.
- 6) Collection of fleas and ticks from all species of animals as arthropod vectors for pathogen spread.
- 7) Sampling of water sources, both natural and man-made wells, distributed over the study area, as an environmental representative, providing a communal interaction site for pathogen exchange.
- 8) Conducting human and animal health questionnaire surveys with the herders to determine perceived health risks to people and their animals and find out the common illnesses in the region.

Field trips were conducted from May 2012 to April 2015 during annual four to five week trips. Snow leopard data was collected from trapping, examining and sampling of snow leopards for blood and faeces in conjunction with a long-term ecological study (June 2009- April 2015) (Johansson et al. 2014, Esson et al. submitted, Chapter 2). For detailed trapping and sampling protocols for the snow leopards see Johansson et al. (2014) and Chapter 2. Rodents were also trapped, physically examined and sampled for blood, faeces and external parasites. Goats and dogs were restrained by hand and blood collected, faeces or rectal smears in the case of the dogs and external parasites were also collected. Water samples were collected

from 11 natural and man-made wells. For rodent, dog, goat, water, flea and tick protocols see Chapters 3 and 5.

Blood samples were either stored on Nobuto strips or serum was separated and frozen as per Chapter 2. Faecal samples were stored in RNAlater and then underwent NGS to identify pathogens and parasites (Gillet et al. 2015). External parasites were placed in 70% ethanol.

Fresh ibex faeces were collected opportunistically when ibex were seen. The droppings were allowed to air dry and stored in paper bags with silica gel and also in RNAlater. These were DNA tested to ascertain they were from ibex and then underwent NGS to identify pathogens and parasites (Gillet et al. 2015).

Water was collected in sterile 20ml syringes, totalling 200mls from several sites in the water source. It was then filtered through sterivex filters with a 7µm cellulose filter paper. The paper was allowed to air dry, before removing from the holder, rolled using sterile forceps and placed into a sterile cryovial. Samples were stored at ambient temperature.

Two surveys were distributed to the herders, one asking about human health and the second regarding herd health and wildlife health.

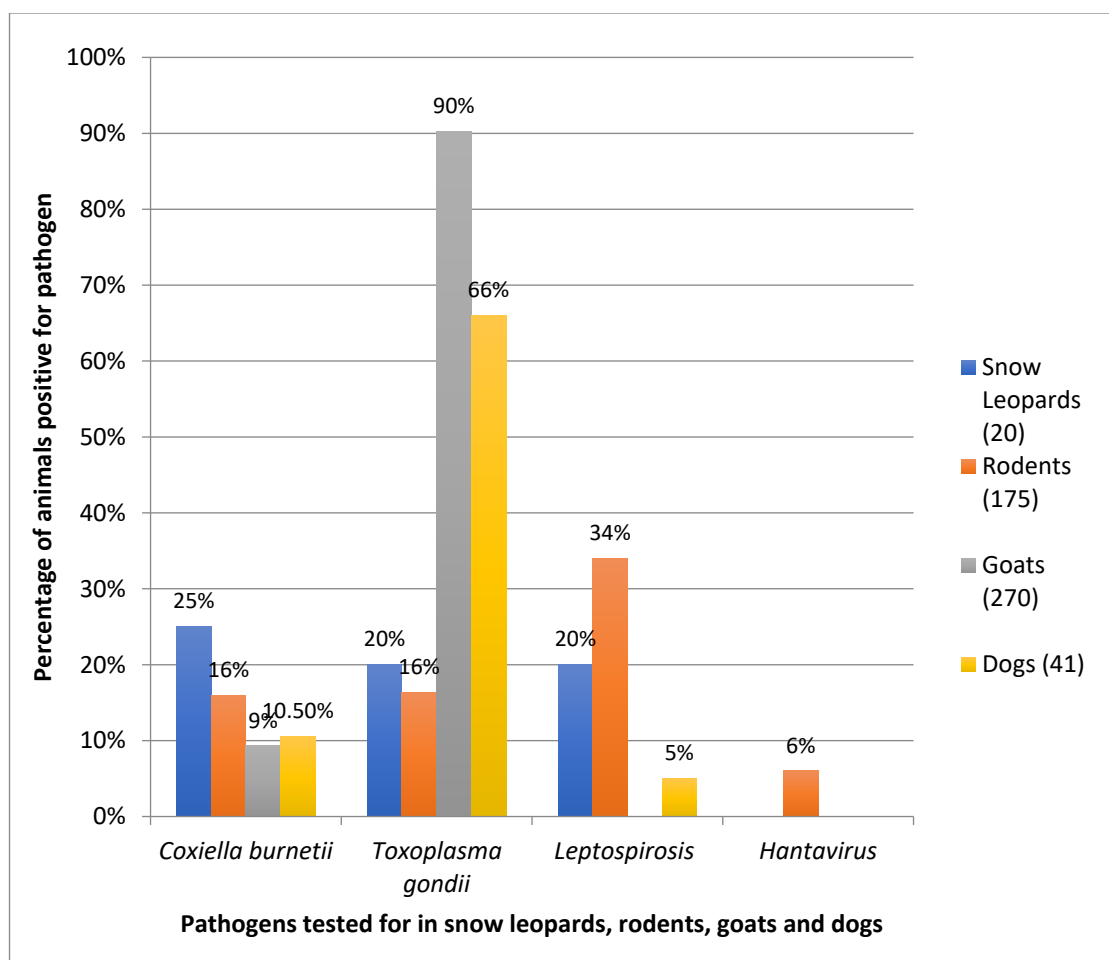
## **7.4 Results**

### **7.4.1 Health and pathogens detected in wildlife and domestic animals**

Serology results showed each of the host species tested were positive for exposure to several pathogens, these being *Coxiella burnetii*, *Toxoplasma gondii* and *Leptospira* spp., which included serovars *Leptospira interrogans* Australis in rodents and snow leopards and *L. interrogans* Icterohaemorrhagiae in rodents and dogs. The rodents were also positive for antibodies of Seoul and Puumala hantavirus. The ibex faecal samples and ticks were positive for *Coxiella* but could not be analyzed to species. All faecal samples and rectal smears were negative for *Echinococcus*, *Giardia* and *Cryptosporidium*, however, two dogs were positive for *Taenia hydatigena*. This is a species of tapeworm whose definitive host is the dog but the

intermediate host can be most mammals but especially pigs, goats and sheep (Braae et al. 2015b).

The findings suggest there can be multiple hosts for numerous pathogens, with the pathogens distributed over the whole study area (Figure 7.2). Since snow leopards prey on rodents, goats and ibex and dogs scavenge on carcasses from kills of predators, it is highly likely that these pathogens could spread between these hosts. Goats and ibex overlap in grazing areas, so are liable to share pathogens. There could also be a common source of infection from water or soil.



**Figure 7.1 Graph showing the prevalence of antibodies of *C. burnetii*, *T. gondii*, *Leptospira* spp and Hantavirus in each species. Goats were not tested for *Leptospira*.**

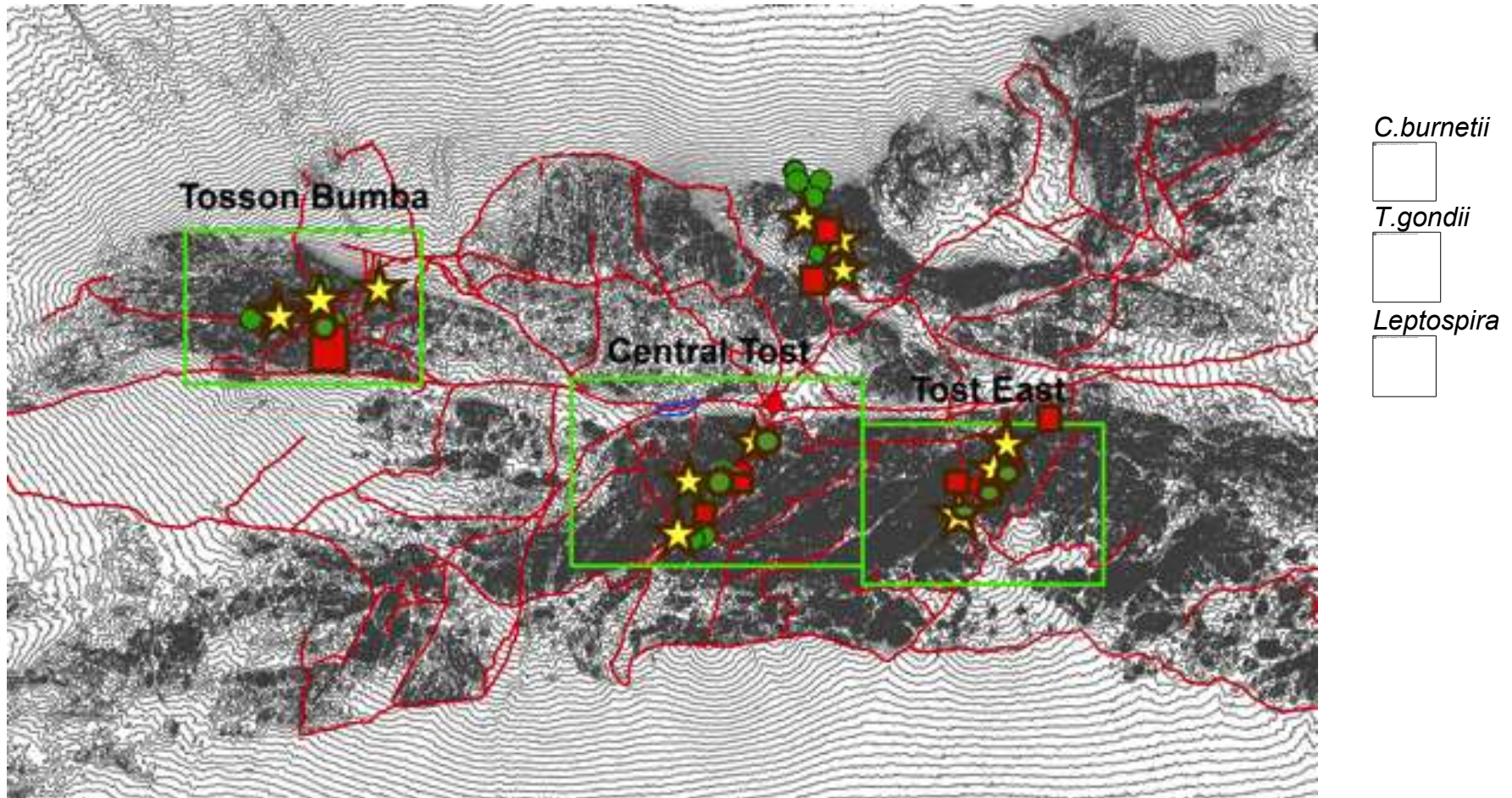


Figure 7.2 Distribution of pathogens over the study area. Snow leopard trapping areas outlined by green rectangles.

### 7.4.2 Bacteria in faeces

Two hundred and fifty genera of bacteria were identified in total from all faeces. All species of animals tested had genera of potentially zoonotic bacteria in their faeces including *Rickettsia* sp, *Bacillus* sp, *Bartonella* sp, *Streptococcus* sp, *Staphylococcus* sp and *Francisella*. Some of the ibex faeces also contained *Bordetella*, which is the genus of bacteria found in upper respiratory infections in dogs, yet this pathogen was not identified in the dogs sampled (Table 7.2). Interestingly the highest number of genera was found in the goat faeces (highlighted column in table 7.2).

Table 7.1 Genera of potentially zoonotic bacteria found in faeces from snow leopards, rodents, goats, dogs and Ibex. Highlighted columns were those genera of bacteria that were also identified in the ticks.

<b>Genera of Bacteria</b>	<b>Snow leopard</b>	<b>Rodent</b>	<b>Goat</b>	<b>Ibex</b>
<i>Anaplasma</i>			+	
<i>Bacillus</i>		+	+	
<i>Bacteroides</i>		+	+	
<i>Bartonella</i>		+	+	
<i>Bordetella</i>				+
<i>Campylobacter</i>		+	+	+
<i>Clostridium</i>		+	+	+
<i>Corynebacterium</i>			+	+
<i>Coxiella</i>			+	+
<i>Enterococcus</i>		+	+	
<i>Escheria/Shigella</i>		+	+	
<i>Francisella</i>		+	+	
<i>Rickettsia</i>		+	+	
<i>Staphylococcus</i>	+		+	
<i>Streptococcus</i>	+		+	
<i>Treponema</i>		+	+	

### 7.4.3 Tick and flea analyses

Fleas were negative for *Y. pestis*. There were 216 genera of bacteria identified in all ticks combined. Ticks were positive for numerous zoonotic bacterial genera, the most significant being *Aeromonas*, *Anaplasma*, *Bacillus*, *Bacteroides*, *Bartonella*, *Corynebacteria*, *Clostridia*, *Coxiella*, *Echinococcus*, *Francisella*, *Mycobacteria*, *Mycoplasma*, *Pseudomonas*, *Rickettsia*, *Salmonella*, *Staphylococcus*, *Streptococcus*, *Yersinia*. Only the potentially zoonotic ones are listed (Table 7.2). *Bacillus*, *Coxiella*, *Rickettsia* and *Yersinia* were genera identified in ticks that came from a snow leopard, goat, rodent and person.

Table 7.2 Genera of potentially zoonotic bacteria found in ticks collected from snow leopard, rodents, goats and people. Highlighted genera indicate pathogens that were common in all ticks analysed from each species.

Genus of bacteria	Snow leopards		Rodents		Goats		Humans	
Aeromonas	50%	2/4	-	-	40%	2/5	100%	3/3
Anaplasma	-	-	-	-	20%	1/5		
Bacillus	100%	4/4	21%	1/8	100%	5/5	100%	3/3
Bacteroides	100%	4/4	38%	3/8		-	67%	2/3
Bartonella		-	12%	1/8		-		
Bordetella	25%	1/4	12%	1/8		-	33%	1/3
Burkholderia		-	100%	8/8		-	100%	3/3
Clostridia	100%	4/4	100%	8/8	100%	5/5	100%	3/3
Corynebacterium	75%	3/4	100%	8/8		-	67%	2/3
Coxiella	75%	3/4	75%	6/8	100%	5/5	100%	3/3
Enterococcus		-	12%	1/8		-	-	
Escherichia/		-	50%	4/8		-	-	
Francisella		-	12%	1/8	20%	1/5	-	
Helicobacter		-	12%	1/8		-	-	
Legionella	75%	3/4	50%	4/8		-	67%	2/3



Mycobacteria		-	25%	2/8		-	33%	1/3
Pandoraea	100%	4/4	100%	8/8		-	100%	3/3
Rhodococcus		-	38%	3/8		-	33%	1/3
Rickettsia	100%	4/4	100%	8/8	100%	5/5	100%	3/3
Staphylococcus	100%	4/4	100%	8/8		-	33%	1/3
Streptococcus	100%	4/4	63%	5/8		-	100%	3/3
Yersinia		-	12%	1/8		-	-	

#### **7.4.4 Water sample analyses**

Water samples were clear of serious zoonotic pathogens. More samples and larger volumes need to be collected across seasons.

#### **7.4.5 Human health, disease management and perceptions**

We found no reports of *C. burnetii* (Q fever), *T. gondii* or *Leptospira* occurring in people in this area. Questionnaire surveys used in Chapter 6 were unable to ascertain confirmation from any personal communication from herders or their doctor that illness caused by these pathogens existed. The lack of records could be due to under reporting, under testing, or the local people not seeking medical attention. There was 40% of vaccination of babies against common childhood diseases such as rubella and mumps. However, there appeared little perception of the possibility of transfer of pathogens from animals to people.

#### **7.4.6 Qualitative assessment of domestic animal health care**

According to the questionnaire surveys (Esson et al. 2017, Chapter 5) there was little routine healthcare for the herds. Some herders treated their animals with ivermectin (Ivomec). There were no routine parasite control or vaccination measures in place by most owners. This could be due to lack of awareness or lack of funds to buy medication/vaccinations. People did not appear aware that pathogens could be obtained from their animals or drinking water or wildlife. They reported most stock losses were from predation rather than illness. Few observations were made regarding the health of wildlife but this could be due to lack of reporting. Repetition of the surveys with personal interviews would possibly improve the results

### **7.5 Discussion**

#### **7.5.1 Major disease issues across species**

Snow leopards, rodents, goats and dogs were positive for exposure to *C. burnetii*, *T. gondii* and *Leptospira* (Figure 7.1). As the tests were based on previous exposure, it is hard to determine if the same strains of pathogens were transmitted between species. Molecular epidemiology would be a significant aid in defining if these

pathogens were the same strain in future studies. This would aid in indicating transmission pathways of the pathogens between species and identify areas where intervention could be applied. It is highly likely that humans are exposed to these zoonotic pathogens, as they interact with all hosts to various degrees. Unfortunately, due to snow leopards preying on their stock there is retribution killing and this results in close contact between herders and snow leopards. Dogs are in proximity to people, although with little direct contact, as they are not regarded as pets but working animals. Goats are often handled especially during times of combing and kidding, with orphaned kids often housed inside gers. Goats are also slaughtered inside gers due to the windy, dusty environment outside. Native rodents are often found inside gers, so food, cooking and eating utensils are exposed to rodent excrement. Rodents especially have been incriminated as reservoirs for all three pathogens and serve as links between the sylvatic and domestic transmission cycles (Dubey 1996, Meerburg and Reusken 2011, Schmidt et al. 2014). These pathogens occur across many genera and species of rodent with infectivity being affected by habitat, host density, climate and association with other species (Tenter et al. 2000, Desvars et al. 2012)

Surprisingly, snow leopards had a higher prevalence of *C burnetii* compared with goats, as one of the major carriers of this pathogen are cloven hoofed animals. However, dogs and goats had the highest prevalence of *T. gondii*, interestingly as snow leopards are the definitive host and rodents are one of the main intermediate hosts. As would be expected rodents had the highest prevalence of *Leptospira* being one of the main reservoir hosts of this pathogen, the other being pigs (Singleton and Krebs 2003). Dogs too can be a reservoir host depending on the serovar they carry (Rojas et al. 2010).

#### 7.5.1.1 *Coxiella burnetii*

*Coxiella burnetii* was identified for the first time in snow leopards by this study, not having been identified in wild or captive animals previously. The pathogen test was based on prior exposure so we were unable to determine if active infection was present. However, none of the snow leopards were showing signs of ill-health at the time of sampling. *Coxiella burnetii* has been identified to occur at high prevalence in domestic cats and thus cats act as a potential reservoir host for the pathogen

(Meredith et al. 2015). It is worth continuing monitoring *C. burnetii* in the snow leopards to determine impacts on this species. *Coxiella burnetii* was also present across the study area in all goatherds tested. Ruminants, especially sheep and goats are the main carriers of *C. burnetii* and can cause abortion and premature births in these hosts (Rousset et al. 2009). The questionnaire survey revealed that herders reported 24% of goats aborted across herds, which is likely to be due to *C. burnetii*. *Coxiella* was also identified in the ibex faeces analyzed, so it may be causing the same issues in the native ungulates, which will have a flow on effect for recruitment of prey for the snow leopards (López-Olvera et al. 2009). As the goats and ibex graze on the same pastures, there is a significant opportunity for the passage of pathogens between the domestic and wild ungulates. It also is highly suggestive as to why we found *C. burnetii* for the first time in wild snow leopards as the ibex and goats are both prey and snow leopards maybe exposed when opening the carcass. Dogs in our study were also positive for *C. burnetii* at a slightly higher prevalence of antibodies (10.5%) compared with goats (9%) but were not as high as snow leopards (20%). Dogs who live in close association with sheep and goats have a higher prevalence of Q fever than those that are not associated with sheep and goats (Shapiro et al. 2016). Dogs are free to roam over the study area and scavenge from wildlife such as ibex carcasses. They possibly consume rodents when they can therefore having the opportunity for contracting the pathogen from several sources. Rodents were also positive for antibodies to *C. burnetii* (16%) and were distributed widely over the study area overlapping with the other species and thus providing an opportunity for pathogen exchange including with people. No definitive data is available on the presence of *C. burnetii* in humans in Mongolia but it has been ranked as of moderate importance based on expert opinion (McFadden et al. 2015). Symptoms of Q fever range from mild to moderate febrile illnesses with flu like components, to endocarditis (Woldehiwet 2004, Vanderburg et al. 2014). The major illnesses reported in the human surveys were colds and flu. It is possible undiagnosed Q fever as well as other upper respiratory tract viruses caused these illnesses.

#### 7.5.1.2 *Leptospira*

Sero-surveys for *Leptospira* spp. detected positive cases in 20% of the snow leopards in the Tost Mountains. Rodents had the highest prevalence of *Leptospira*

out of the animal groups tested (34%), with positive cases in all rodent species. Only 5% of the dogs tested positive for *Leptospira* antibodies. Serovars were identified as *L. interrogans* Australis and *L. interrogans* Ictohaemorrhagiae. Rodents carried both these serovars. This differed to the only other study on rodents from another site in Mongolia, where long tailed ground squirrels were found to be positive for *L. Pomona*. This is the first time *L. interrogans* Australis has been identified in Mongolia. Snow leopards were also positive for *L. interrogans* Australis only and dogs for *L. interrogans* Ictohaemorrhagiae. These results suggest there may have been pathogen transmission between the snow leopards, rodents and dogs. Captive, other non-domestic felids that contract leptospirosis showed interstitial nephritis. This is a significant finding as it can lead to death due to kidney failure (Ullmann et al. 2012, Azócar-Aedo. 2014, Schuller et al. 2015). This may have ramifications for the future health of the snow leopards that were positive to previous exposure for *Leptospira* spp. Continued monitoring of this population is thus important to determine if this pathogen causes any long-term issues to the health of the snow leopards.

Although *Leptospira* is not reported to have any ill effects on rodents, other host species including humans can become critically ill. Disease in people can include hepatitis (Thornley et al. 2002, Bharti et al. 2003). Even though dogs have been reported as being reservoir hosts, they can also become acutely or chronically ill showing similar signs to people (Odontsetseg et al. 2005c, Rojas et al. 2010). The impact of *Leptospira* on other wildlife in the region is unknown.

#### 7.5.1.3 *Toxoplasma gondii*

The high prevalence of *Toxoplasma* across species, although probably not a concern for the health of the snow leopards, is a grave concern for public health. Goats had the highest prevalence of *T. gondii* at 90%, followed by the dogs at 60%, ironically higher than that of the definitive host, the snow leopard and some intermediate hosts, the rodents. As goat meat forms a large part of the local diet, there is a high risk of pathogen transfer to people from all stages of food preparation and ingestion from slaughtering the goats to consumption of the meat (Hill & Dubey 2002, Kijlstra et al. 2008). There are several felid species in the study area that can act as definitive hosts. Apart from the snow leopard, these include the Lynx (*Lynx lynx*) and Pallas cats (*Otocolobus manul*). Pallas cats are extremely susceptible to *T. gondii* because

their immune reactions are deficient, which may be due to not co-evolving with the parasite (Brown et al. 2005, Hill et al. 2005, Parameswaran et al. 2010).

Rodents are an important intermediate host. When infected, they alter their behaviour to be less fearful of predators and hence enable the parasite lifecycle to be completed (McAllister 2005, Meerburg et al. 2009). Dogs as well can be an intermediate host in a sylvatic cycle for *Toxoplasma* (Overgaauw et al. 2009). The dogs in the study possibly became infected from eating goat meat, placentas or carcasses, or rodents. *Toxoplasma* has been found to be fatal in dogs and can transfer to humans, therefore providing another source of infection for the herders (Shahzad et al. 2006). In humans, symptoms can range from no signs to severe, infection being associated with schizophrenia and is of major concern for pregnant women because the parasite can cross the placenta and cause neurological problems in the foetus (Tenter et al. 2000).

#### *7.5.1.4 Canine distemper virus*

All dogs tested were negative for canine distemper virus (CDV) despite never having been vaccinated. Although this is not a zoonosis, I tested for CDV as it could pose a threat to the snow leopards if it arrives in this area. The snow leopards in the study all tested negative to CDV in a concurrent study (Wiseman pers com 2015). Two snow leopards in a zoo in Iowa died from CDV (Fix et al. 1989b), showing that it can cross to this species. CDV is a morbillivirus with a propensity for species switching (Viana et al. 2015). It has been identified in out breaks in other wild felids including lions and tigers, posing a threat to these populations (Roelke-Parker et al. 1996, Munson et al. 2008, Gilbert et al. 2014).

#### *7.5.1.5 Brucella*

Brucellosis was not identified in the study area despite it being one of the most highly ranked zoonotic diseases in Mongolia affecting both people and domestic stock, namely sheep, goats and cattle (Zinsstag et al. 2005, McFadden et al. 2015). Similarly, although brucellosis was recently found in other regions of the Umnogovi province, it was not detected in adjacent areas of the study site in the South Gobi (McFadden and Muellner 2013). There was a nationwide vaccination programme

instigated in 2000 to control the disease in regions outside the study area (Batsukh et al. 2012). The goats and sheep in the Tost Mountains are effectively in a closed system as no animals from outside the region are brought in from markets, with the herders swapping animals between themselves. There is no information available on the wildlife, such as ibex and argali sheep that overlap with the domestic goats for grazing, carrying *Brucella*. Testing argali and ibex is an avenue for further research.

### **7.5.2 Arthropod vectors for pathogen transmission**

All the ticks collected in these studies contained numerous zoonotic bacteria, including those from genera *Rickettsia*, *Bacillus*, *Aeromonas*, *Anaplasma*, *Coxiella*, *Clostridia* and *Francisella*. Three of the ticks were collected from people, so there is potential for the ticks to pass on these bacteria to people. As the herders have direct contact with their goats when combing them, there is ample opportunity for the ticks to transfer between people and the goats. Ticks generally leave one host after feeding from that host and then attach to another host as they pass through developmental stages, therefore providing a means for the spread of pathogens they carry between hosts (Durden 2006). According to Durden (2006), ticks transmit more pathogens than any other parasite worldwide. Through environmental and climatic changes ticks are being able to extend their niches and activity timeframes (Pfäffle et al. 2013). Thereby increasing the risk of transmitting their pathogens to places that to date have been naïve to certain diseases (Semenza & Menne 2009, Pfäffle et al. 2013).

The fleas were all negative for *Yersinia pestis*, the causative bacteria of plague. One tick from a ground squirrel was positive for *Yersinia* but I was unable to identify the bacterium to species level. Mongolia has several endemic areas of plague with rodents and their fleas playing a significant role as reservoirs (Riehm et al. 2011). Gerbils and jerboas were reportedly the main carriers in areas around the study site, however, most human cases are associated with contact with marmots and their ectoparasites (Riehm et al. 2011). No human cases have been reported in the study area, however this again could be due to lack of reporting rather than lack of disease presence. Fleas have been found to maintain active bacteria in their guts for up to 15 months depending on the temperature and can, therefore, overwinter leading to

infection of new cohorts of rodents when they emerge after hibernation (Adjemian et al. 2008).

### **7.5.3 Water as a potential pathogen source**

Serious zoonotic pathogens were not detected in the water samples. As natural water holes were visited by all species in the study area as confirmed by camera trap photos and faeces, this was an unexpected outcome. Further sampling should involve larger volumes of water being filtered and collected across all seasons.

### **7.5.4 Link to human health**

Via questionnaire surveys, Chapter 6, we attempted to collate information on the occurrence of disease in the herders in this study area and gaps in current health management. I did this because medical records and data were unavailable. Hence, I largely relied on the questionnaire surveys, which revealed qualitative and anecdotal information from herders and the local doctor. Not surprisingly we found no reports of *C. burnetii*, *T. gondii* or *Leptospira* occurring in people in this area. The questionnaire surveys were unable to obtain confirmation from any personal communication on these illnesses from herders or their doctor. This could be due to under reporting, under testing, or the local people not seeking medical attention. For the nomadic herders in the study area they are reticent to go to town to the doctor especially if symptoms are mild. Therefore, there is probably significant under reporting of illness.

Human survey results indicated a greater need for medical information, increasing awareness of diseases, and medical help to be provided to the local people. Many illnesses did not appear to be either identified or recorded. Family units were comprised of grandparents, parents and children all living in the one ger or two adjacent gers but there could be up to eight people sharing the same accommodation. Living in such close proximity to each other and their domestic animals provided ample opportunity for the transmission of pathogens.



## 7.6 Summary of Pathogen Impacts

Using a One Health approach to investigate disease in the threatened snow leopard, we examined wildlife health, domestic animal health, human health and the surrounding environment in the south Gobi Desert of Mongolia. We found zoonotic pathogens including *C. burnetii*, *T. gondii* and *Leptospira* spp were present across all species indicating a common pathway for infection, with rodents either acting as the intermediate host or possible reservoir for pathogen replication. These three pathogens can have a significant impact on the health of the local herding community by affecting their ability to work. *Coxiella burnetii* and *T. gondii* can cause abortion in the domestic stock, decreasing the productivity of the herds and hence income. They can also affect the native ibex and argali sheep decreasing their numbers and putting pressure on the snow leopards by decreasing their prey availability. This in turn can lead to increased predation on the domestic goats and sheep, leading to further conflict between the herders, snow leopards and wolves that were named as the most common predators in the area.

## 7.7 Recommendations for Improving Health, Livestock Production and Conservation

Previous actions initiated by the Snow Leopard Trust intending to conserve snow leopards have concentrated on human/wildlife conflict, including insurance schemes to compensate for leopard kills of goats and to enhance protection of livestock with electric fences (Mishra et al. 2003, Dar et al. 2009, Rosen et al. 2012). We are suggesting that instigating routine herd health and improving the productivity of herds will indirectly aid in conserving snow leopards. Stragglers in the herd are found to have the highest predation (Johansson et al. 2015). If this is due to illness, improved herd health may lead to decreased predation, which would lead to less retribution killing. This scenario was observed in Machiara National Park in Pakistan, where leopard predation caused significant herd loss, but disease caused the highest proportion of financial loss (Dar and Linkie 2009).

Education on zoonotic health risks to the herders and approaches to minimize exposure is key in aiding the reduction of pathogen transmission. We will provide our survey results to the local people with recommendations for improving human, herd

and wildlife health. According to our survey results up to 48% of the children from the study area board in town for school. Instigating lessons on health and the way pathogens are spread would benefit both town and rural children. Combined veterinary and medical doctor visits, sharing transport costs and equipment for keeping vaccines cold, have been suggested in other remote developing regions as a means of minimizing costs and increasing efficiency. Visits by medical officials could also be an educational opportunity for health information to be passed to the local people at these times. Costs and payments for these services would have to be negotiated with the relevant authorities (Schelling et al. 2005). Public health campaigns in developing countries are challenging and sensitive, having to take into account the different cultural, social, environmental and economic values and needs (Govender 2005). Many rely on mass media, including social media, while others involve distribution of pamphlets and hard copy information (Nutbeam 2000, Abdullah and Husten 2004, Rice and Atkin 2012). In the study area, many families did have television, but personal contact and communication would have a greater impact.

Providing data on the financial benefits of vaccinating herds against *C. burnetii* and regular parasite drenching regimes to improve the health of the herd may be an encouragement for herders to initiate and continue such practices. Vaccinations against *C. burnetii* would lead to fewer abortions and therefore increase in herd productivity as seen in Tanzania (Swai et al. 2010, Angelakis and Raoult 2010). Flow on effects could help decrease the occurrence of these pathogens in the wild ungulates, although pathogen effects on these species have not been quantified.

Many herders wear facemasks when outside due to the windy, dusty conditions. Encouraging this practice when slaughtering goats and while aiding birthing would reduce exposure to aerosolized particles. Wearing of disposable gloves during slaughter would also decrease the risk of pathogen and parasite transfer.

With the high prevalence of *T. gondii* in goats (90%), it is imperative to raise awareness of adequate cooking for all goat products for consumption. Rodent control within gers would also improve health and decrease the risk of the spread of toxoplasmosis and leptospirosis to humans.

As the dogs were negative for CDV and only a low percentage positive for leptospirosis a vaccination regime for them would not be economically viable. However, regular internal parasite control would benefit the dogs due to their scavenging behaviour and presence of *T. hydatigenia*. This would prevent contamination of pasture by faeces containing proglotids and therefore reduce uptake of tapeworm eggs by goats and other ungulates, which act as intermediate hosts.

## 7.8 Future Research

Continuing surveillance for those pathogens already identified and for others yet to be identified should be an ongoing long-term process to detect temporal changes and gain a deeper understanding of disease factors in this region. Molecular epidemiology to characterise strains among host species would provide evidence for transmission pathways.

Sample collection from other wildlife and domestic stock species within the study area would identify other reservoir sources for pathogens and perhaps further targets for controlling disease transmission. Continued monitoring is also necessary to assess the efficacy of interventions such as vaccination and parasite treatment. Closer and long-term interactions with the herders would increase trust and promote sharing of information and uptake of recommendations, which would be beneficial to all.

The linkages discussed above clearly indicate that wildlife health is intricately tied to the health of people, domestic animals and the environment. Wildlife health surveillance can be used to enhance a greater understanding of the pool of pathogens that may spillover into people or domestic animals; it can also be used to track the spread of wildlife diseases through populations. This surveillance can be used to investigate the ecology of the pathogen and hosts, which in turn can facilitate the prevention and control of important diseases (Rostal et al. 2013).

## 7.9 Conclusions

The One Health approach allowed me to investigate the health of wild snow leopards and the pathogens to which they are exposed through studying the other host species that share their environment. Using this approach thus allowed me to collect considerably more information on this species than a narrow research focus on snow leopard health alone would allow. By collecting information on sympatric wildlife, domestic animals, people and the environment in which snow leopards live, I was able to view the threats to snow leopards in a more holistic manner. Although snow leopards did not exhibit any detrimental effects from pathogens, under situations of duress, or a mixed infection of pathogens or other negative environmental conditions, infectious diseases may have greater implications for this population. Now that a baseline has been established, further investigation into the diseases and health status of this snow leopard population and the ecosystem that it shares can only lead to a greater understanding and hence better conservation outcomes in the future.

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