A Cross-Sectional Study of Small Mammals for Tickborne Pathogen Infection in Northern Mongolia

by

Laura Ann Pulscher

Duke Global Health Institute
Duke University

Date:_______________________
Approved:

____
Gregory Gray, Supervisor

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Charles Nunn

____
Chris Woods

Thesis submitted in partial fulfillment of
the requirements for the degree of
Master of Science in the Duke Global Health Institute
in the Graduate School of Duke University

2016

ABSTRACT

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Abstract

Introduction: Extensive studies on tickborne pathogens (TBPs) have been conducted in developed nations, relatively less has been done in developing nations leaving a large gap of knowledge. Mongolia, a country built upon nomadic culture and pastoralism is an ideal system to study TBPs as the population is at an increased risk due to increased time spent outside herding livestock. Discoveries of TBPs in Mongolia include *Babesia spp., Anaplasma spp., Borrelia spp., Rickettsia spp.* and tick-borne encephalitis virus. While research has focused on TBPs in humans and ticks in Mongolia, little research has assessed animal reservoirs, specifically small mammal species, as reservoirs for TBPs. This project aimed to 1) identify the role of small mammal species in the ecology of TBPs in Mongolia, specifically *Rickettsia spp.*, *Anaplasma spp.*, and *Borrelia spp*. using serological and molecular analysis and 2) identify risk factors associated with the prevalence of TBPs in small mammal populations in Mongolia.

Methods: From June to July 2016, rodents were live-trapped, and whole blood, serum and ear biopsy samples were collected. Sixty-four rodents were trapped in three aimags (provinces) in northern Mongolia. Whole blood samples were tested by PCR to detect the presence of *Rickettsia spp.*, *Anaplasma spp.*, and *Borrelia spp.*. In addition, ear biopsy samples were tested by PCR to detect the presence of *Borrelia spp.*. All rodents were serologically tested for antibodies to *Anaplasma phagocytophilum* and *Rickettsia rickettsii*. A

multivariate model was used to assess risk factors for the presence of tickborne pathogens. Risk factors examined included species and sex of animal, location and presence of ticks.

Results: 56.0%, 39.0% and 0.0% of animals were positive by PCR for *Borrelia spp.*, *Rickettsia spp.* and *Anaplasma spp.*, respectively. 41.9% and 24.2% of animals were seropositive for *A. phagocytophilum* and *Rickettsia rickettsii*, respectively. Risk factors found to be important predictors of *Borrelia spp.* molecular detection included aimag, small mammal species and sex of small mammals. After multivariate analysis only aimag and small mammal species remained statistically significant. Risk factors found to be important predictors of *Rickettsia spp.* molecular detection were small mammal species and presence of ticks. After multivariate analysis only small mammal species remained statistically significant. Risk factors found to be important predictors of *A. phagocytophilum* antibody detection included small mammal species and presence of ticks. No risk factors were identified as being important predictors of antibody detection of *R. Rickettsii.*

Conclusion: The results of this study provide considerable evidence of TBPs circulating in small mammal populations in northern Mongolia. These data suggest that further

TBP research is merited in Mongolia. Such research will be necessary to guide Mongolian public health interventions.

Dedication

I dedicate this thesis to my friends, to my family and especially to my husband Greg, who all have frequently urged me to follow my passion. This thesis would not be possible without their love and unqualified support.

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1. Introduction

During the last three decades, notable increases of tickborne pathogens (TBPs) have been identified across the world (Fang et al., 2015). In developed nations, extensive studies on TBPs have often previously been conducted, however, TBP research has been relatively sparse in developing nations. We recognize that, in both developed and developing countries TBP transmission to humans have a tendency to be sustained where ticks and animal hosts mix with humans. Such is the case in Mongolia where a large proportion of the human population relies on pastoralism and herding for their survival and livelihood. People and their livestock frequent the Mongolian countryside where populations of ticks have been identified, creating an ideal scenario for sustaining transmission of TBPs to humans and animals. For this reason, Mongolia is an ideal location for TBP research.

While both China and Russia have identified a number of TBPs along the Mongolian border, it wasn't until recently that TBPs have become an area of focused research in Mongolia. The discovery of TBPs in Mongolia include *Babesia spp.* (Karnath et al., 2016; Robert et al., 2005; Battsetseg et al., 2002), *Anaplasma spp.* (Karnath et al., 2016; Masuzawa et al., 2014; Javkhlan et al. 2014; Walder et al., 2006), *Borrelia spp.* (Masuzawa et al., 2014; Scholz et al., 2013; Walder et al., 2006), tick-borne encephalitis virus (Walder et al., 2006) and *Rickettsia spp.* (Narantsatsral et al., 2012; Speck et al., 2012). As an increasing public health concern, research focused on TBPs in Mongolia

will provide necessary information regarding the ecology of TBPs in the Mongolian ecosystem and increase possibilities for public health intervention efforts within the country. While research has focused on TBPs in human and tick species, little research has assessed animal reservoirs, specifically small mammal species for TBPs in Mongolia.

Small mammals are known to serve as reservoirs for a variety of TBPs worldwide. Pathogens including *Borrelia burgdefori* sensu lato, *Borrelia garinii*, *Borrelia afzelii*, *Rickettsia spp.*, specifically spotted fever group *Rickettsia* (SFGR), and *Anaplasma phagocytophilum* are found in Mongolia, affect both human and domestic livestock species and are known to replicate in small mammal reservoirs. Research based out of China has identified various TBPs in rodent species along the southern Mongolian border yet these pathogens have not been well-studied in small mammal reservoirs in Mongolia (Fang et al., 2015; Liu et al., 2015; Zhang et al., 2010; Zhan et al., 2009; Chu et al., 2008; Swanson et al., 2006). Hence in this study we sought to: 1) identify the role of small mammal species in the ecology of TBPs in Mongolia, specifically *Rickettsia spp.*, *Anaplasma spp.*, and *Borrelia spp.* using serological and molecular analysis and 2) identify risk factors associated with the prevalence of TBPs in small mammal populations in Mongolia.

2. Methods

2.1 Site Description

Using a cross-sectional study design, rodent sampling was performed in seven sums (districts) within three aimags (provinces) in Mongolia, from June 20th – July 23rd, 2016 (Table 1).

Latitude and longitude of each sampling site were determined using a global positioning system (GPS) handheld device (Juno Trimble Positions System, Sunnyvale, CA).

Table 1: Trap locations and ecosystem types.

Aimag	*Sum*	*Ecosystem Type*
Darkhan-Uul	Khongor	Grassland steppe
	Orkhon	Mixed forest grassland
	Khötol	Grassland steppe
Selenge	Bayangol	Grassland steppe
	Javkhlant	Mixed forest grassland
	Yeröö	Mixed forest grassland and taiga forest
Tov	Batsumber	Mixed forest grassland

2.2 Rodent Sampling

Trapping and handling procedures were approved by the Duke University Institutional Animal Care and Use Committee (#A086-16-04). Animal traps were set at each site for 1-2 nights, for a total of 17 trap-nights. Traps were placed near rodent burrows that had clear indications of recent rodent activity including fresh scratched

dirt and droppings in front of burrows. Live Tomahawk traps or Sherman traps were baited with a mix of oat, grain, potato and peanut butter. Traps were set between 7:00 PM and 10:00 PM and checked every 4 – 6 hours for rodents.

Rodents captured were anesthetized with ketamine (50 mg/Kg) and assessed for sex and species. Samples collected from small mammals included: 1) one 2 x 2 mm ear biopsy placed in 70% EtOH, 2) one serum sample placed onto blood sampling paper (Toyo Roshi Kaisha, Ltd., Tokyo, Japan), and 3) approximately 100 uL of whole blood preserved in 20 uL 10 mM EDTA/100 uL. Whole blood samples were kept refrigerated at 4˚C and ear biopsy and FTA cards were kept at room temperature until further analysis at the Institute of Veterinary Medicine in Ulaanbaatar, Mongolia.

2.3 DNA Extraction and Quantitative PCR

Nucleic acid was extracted from whole blood and ear biopsy samples using the TIANamp Genomic DNA Kit (Tiangen Biotech (Beijing) Co., LTD, Beijing, China) in accordance with the manual provided. PCR assays were performed using the Amplicon ThermoEx 500 ver 1.2. All amplified PCR products were loaded on a 2% gel for electrophoresis and stained with ethidium bromide before visualizing through an ultra violet trans-illuminator (ENDURO™ GDS, Labnet International, Edison, NJ, USA). Table 2 summarizes assays performed on each sample.

Table 2: Description of molecular and serological assays by sample and pathogen type.

	Molecular		IFA Serology
Pathogen Type	**Whole Blood**	**Ear Biopsy**	**Serum**
Anaplasma spp.	X		X
Borrelia spp.	X	X	X
Rickettsia spp.	X		X

2.3.1 *Borrelia spp.*

Nucleic acid extracted from whole blood and ear biopsy samples were screened for *Borrelia spp.* using the *rrs-rr1A* IGS gene as previously described (Bunikis et al., 2004) (Table 3). DNA fragments were amplified using a nested PCR protocol with 35 cycles for the first reaction and 40 cycles for the second reaction at 94˚C for 30 sec, 56˚C for the first reaction and 60˚C for the second reaction for 30 sec, and 74˚C for 60 sec.

2.3.2 *Rickettsia spp.*

Nucleic acid extracted from whole blood samples were screened for *Rickettsia spp.* using the *gltA* gene (citrate synthase gene) as previously described (Mediannikov et al, 2004) (Table 3). DNA fragments were amplified with 35 cycles using a nested PCR protocol at 95˚C for 30 sec, 50˚C for the first reaction and 54˚C for the second reaction for 30 sec, and 72˚C for 60 sec.

2.3.3 *Anaplasma spp.*

Nucleic acid extracted from whole blood samples were screened for *Anaplasma spp.* using the 16S rRNA gene as previously described (Rar et al., 2008; Rar et al., 2010) (Table 3). DNA fragments were amplified using a nested PCR protocol under the following conditions: 95˚C for 30 sec, 57˚C for the first reaction and 60˚C for the second reaction for 30 sec, and 72˚C for 60 sec for 35 cycles.

Table 3: List of primers used to conduct molecular analysis for the three pathogen species.

Target Gene	*Primers*	*Sequence (5′ - 3′)*	*Fragment size (bp)*
rrs-rr1A IGS for *Borrelia spp.*	BF1	GTATGTTTAGTGAGGGGGGTG	Various
	BR1	GGATCATAGCTCAGGTGGTTAG	
	BF2	AGGGGGGTGAAGTCGTAACAAG	
	BR2	GTCTGATAAACCTGAGGTCGGA	
gltA for Rickettsia spp.	CS2d	ATGACCAATGAAAATAATAAT	381 bp
	CSEndr	CTTATACTCTCTATGTACA	
	RpCS877p	GGGGACCTGCTCACGGCGG	
	RpCS1258n	ATTGCAAAAAGTACAGTGAACA	
16S rRNA for *Anaplasma spp.*	Ehr1	AACGAACGCTGGCGGCAAGC	524 bp
	Ehr2	AGTAYCGRACCAGATAGCCGC	
	Ehr3	TGCATAGGAATCTACCTAGTAG	
	Ehr4	CTAGGAATTCCGCTATCCTCT	

2.3.4 Sequencing

Positive PCR products from *rrs-rr1A* IGS, *gltA* and 16S rRNA genes were outsourced to a sequencing company (Invitrogen, Beijing, China) and analyzed using BLAST software (http://www.ncbi.nlm.nih.gov/BLAST/).

2.3.5 Indirect Fluorescent Assay

Serum samples were studied for antibodies against *Anaplasma phagocytophilum*, *Rickettsia rickettsii* and *Borrelia burgdefori* using indirect fluorescent assays (IFA). Blood sampling paper was cut into small pieces and soaked in 0.4 mL of PBS for 60 minutes at room temperature. Serum was diluted at 1:50 for *A. phagocytophilum* and *R. rickettsii* and 1:100 for *B. burgdefori*, applied to antigen slides (Protatek International, Inc., Minnesota, USA), and incubated with a wet paper towel at 37˚C for 45 minutes. Slides were then washed twice in PBS on a shaker for three minutes and incubated with A/G FITC secondary conjugate (BioVision, California, USA) diluted 1:100 for *R. rickettsii*, 1:200 for *A. phagocytophilum*, and 1:40 for *B. burgdefori* at 37˚C for 45 minutes. Slides were washed for 3 minutes in PBS on a shaker and stained with three drops of Erichrome T-Black for 3 minutes. Slides were next dried and evaluated with a fluorescent microscope. Due to difficulties reading *B. burgdefori* slides, the seroprevalence data regarding *B. burgdefori* were not included in the final results.

2.4 Data Analysis

2.4.1 Climactic Variable Analysis

GPS data points were downloaded into ArcGIS 10.4 (ESRI, Redlands, CA) for analysis. Locations were grouped into four distinct clusters: Darkhan-Uul aimag, Selenge aimag (north), Selenge aimag (south), and Tov aimag. A 10 km buffer was then created for each cluster to assess normal density vegetation index (NDVI), land surface temperature (LST) and elevation. Maximum NDVI, minimum LST and elevation data were collected in accordance to previously described procedures (Hay et al., 2011), aimag maps were downloaded from the Mongolian Environmental Health Geodatabase (http://www.eic.mn/). Descriptive spatial statistics for NDVI, LST and elevation were calculated using the zonal statistics tool in ArcGIS (Appendix A). Maximum NDVI, minimum LST and mean elevation were chosen as comparison statistics among sites. Maximum NDVI was selected as it accounts for the highest vegetation density at each site, and minimum LST was chosen because it records the coldest temperatures for each site.

2.4.2 PCR and Serology Analysis

Data were entered into Microsoft Excel and verified by three separate reviewers. A multivariate model was used to assess risk factors for the presence of tickborne pathogens. Outcome variables included 1) active infection of *Anaplasma spp.*, *Rickettsia*

spp. or *Borrelia spp.* in animals by PCR and 2) the presence of *A. phagocytophilum* or *R. rickettsii* antibodies by serology. Animals were determined to be actively infected for *Anaplasma spp.* and *Rickettsia spp.* if whole blood samples were positive by PCR. Animals were determined positive for *Borrelia spp.* if whole blood or ear biopsy samples were positive by PCR. In regards to serology, animals were identified as having a positive reading if florescence was present on the slides for *R. rickettsii* or *A. phagocytophilum*. Risk factors examined included species and sex of animal, collection site, and presence of ticks on the small mammal.

To assess potential risk factors using multivariate analysis, risk factors were first examined for association with the outcome variable by a Chi-squared (X^2) test or Fisher's exact test when sample sizes were low. Variables determined as being possibly statistically associated with the outcome ($p<0.25$) were then included into a saturated, backwards-elimination, unconditional logistic regression model. Logistic regression was chosen for analysis because variables were categorical and the outcomes were coded as binary. Backward elimination was performed to remove variables with a p-value greater than 0.10 in order to obtain a final model where variables were only retained if significant ($p<0.10$). Unadjusted odds ratios were calculated using unconditional logistic regression models or unconditional exact logistic regression models if sample sizes were small. All statistical analyses were conducted in STATA 14.1 (StataCorp, College Station, TX). A model was created for each outcome variable. Given there was no molecular

detection of *Anaplasma spp.*, and we ran into difficulties reading *B. burgdefori* IFA slides, models for these outcomes were not included in the final analysis.

3. Results

Rodents were captured from 12 different locations in the three aimags (Figure 1). Locations were similar with one another in regards to temperature at the time of sampling. Vegetation differed slightly among the sites with lower vegetation in Darkhan-Uul sites in comparison to the other two aimags which were relatively similar to each other. Elevation varied the most among aimags with the highest elevation in Tov aimag followed by Selenge aimag (south), Selenge aimag (north) and Darkhan-Uul aimag (Table 4).

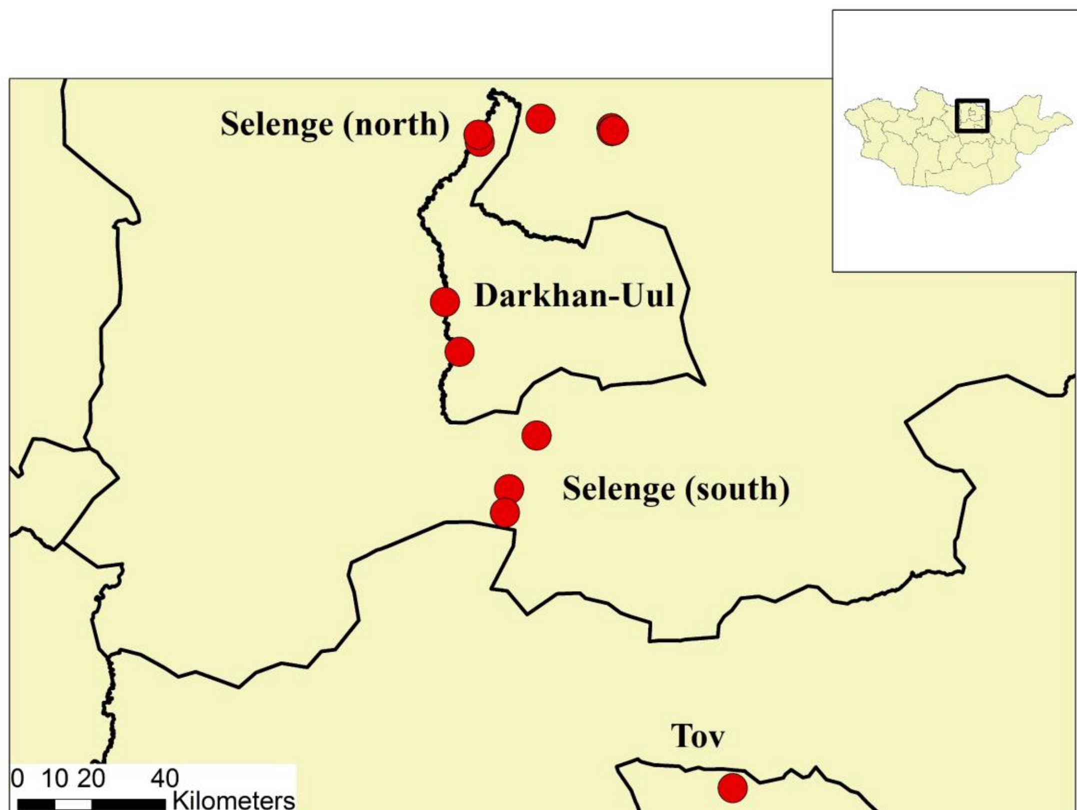

Figure 1: Map of trapping locations in northern Mongolia.

Table 4: Landscape variables by aimag.

Aimag	*Mean Elevation Above Sea Level*	*Minimum LST*	*Maximum NDVI*
Darkhan-Uul	788.14	2515	1637
Selenge (north)	832.97	2474	1799
Selenge (south)	942.98	2509	1827
Tov	1343.72	2543	1730

LST: Land surface temperature; NDVI: Normalized density vegetation index.

Over the study period a total of 64 rodents were captured. Rodents captured included 19 ground squirrels (*Spermophilus spp.*), 2 Daurain pika (*Ochotona dauurica*), 1 field mouse (*Apodemus spp.*), 21 Mongolian gerbils (*Meriones unguiculatus*), 4 Siberian chipmunks (*Tamius sibiricus*), and 17 striped dwarf hamsters (*Cricetulus barabensis*).

From the 64 rodents, 49 whole blood and 61 ear biopsy samples were collected. Whole blood samples were collected from 17 ground squirrels, 18 Mongolian gerbils, 3 Siberian chipmunks, and 11 striped dwarf hamsters. Ear biopsies were collected from all rodents except for 1 ground squirrel and 2 Mongolian gerbils. 56.0% and 39.0% of all animals were positive by PCR for *Borrelia spp.* and *Rickettsia spp.*, respectively. No samples had molecular detection of *Anaplasma spp.* (Table 5). Twenty point four percent (10/49 individual animals) of animals were coinfected with *Borrelia spp.* and *Rickettsia spp.*. Forty one point nine percent and 24.2% of animals were seropositive for *A. phagocytophilum* and *Rickettsia rickettsii*, respectively (Table 7).

3.1 PCR Active Infections in Rodents

3.1.1 *Borrelia spp.* Infection in Rodents

Of the 49 whole blood and 61 ear biopsy samples tested, 11 whole blood samples and 29 ear biopsy samples were positive for *Borrelia spp.* by PCR. Five animals tested positive for *Borrelia spp.* in both whole blood and ear biopsy samples. Of the 62 animals assessed for *Borrelia spp.* by whole blood or ear biopsies, 56% of all animals were PCR-positive. Ground squirrels had the highest prevalence of *Borrelia spp.* (74%, 14/19 individual animals), followed by striped dwarf hamsters (59%, 10/17 individual animals), Mongolian gerbils (50%, 10/20 individual animals), and Siberian chipmunks (25%, 1/4 individual animals) (Table 6). *Borrelia spp.* was not detected in Daurian pika or field mice.

In regards to location, animals in Tov aimag had the highest prevalence of *Borrelia spp.* at 75% (12/16 individual animals), followed by Darkhan-Uul aimag (64% in 7/11 individual animals) and Selenge aimag (46% in 16/35 individual animals) (Table 5).

3.1.2 *Rickettsia spp.* Active Infections in Rodents

Of the 49 whole blood samples tested, 19 animals (39%) were PCR-positive for *Rickettsia spp*.. Mongolian gerbils had the highest prevalence (94.4%, 17/18 individual animals) followed by striped dwarf hamsters (9.1%, 1/11 individual animals) and ground squirrels (5.9%, 1/17 individual animals) (Table 6). *Rickettsia spp.* was not

detected in Siberian chipmunks. No whole blood samples were available for PCR testing of *Rickettsia spp.* in Daurian pikas or field mice.

Animals in Tov aimag had the highest prevalence of *Rickettsia spp.* (60%, 6/10 individual animals), followed by Selenge aimag (34%, 10/29 individual animals) and Darkhan-Uul aimag (30%, 3/10 individual animals) (Table 5).

3.1.3 Anaplasma spp. Active Infections in Rodents

Of the 49 whole blood samples tested no animals were PCR positive for *Anaplasma spp.* (Table 5 and Table 6).

Table 5: PCR results for *Borrelia spp.*, *Rickettsia spp.*, and *Anaplasma spp.* by location and small mammal species.

			PCR Positive		
Aimag	**Sum**	**Species**	***Borrelia spp.****	***Rickettsia spp.***	***Anaplasma spp.***
Darkhan-Uul	**Orkhon**	Ground squirrel	83.3% (5/6)	0.0% (0/6)	0.0% (0/6)
		Striped dwarf hamster	100% (1/1)	-	-
	Khongar	Mongolian gerbil	0.0% (0/2)	100% (2/2)	0.0% (0/2)
	Xotol	Mongolian gerbil	50.0% (1/2)	50.0% (1/2)	0.0% (0/2)
		Total Darkhan-Uul	63.6%	30.0%	0.0%
Selenge	**Bayangol**	Mongolian gerbil	25.0% (2/8)	100.0% (8/8)	0.0% (0/8)
		Ground squirrel	66.7% (2/3)	0.0% (0/3)	0.0% (0/3)
	Eruu	Striped dwarf hamster	50.0% (7/14)	9.1% (1/11)	0.0% (0/11)
		Ground squirrel	33.3% (1/3)	0.0% (0/1)	0.0% (0/1)
	Yavkhlant	Ground squirrel	100.0% (3/3)	33.3% (1/3)	0.0% (0/3)

		Siberian chipmunk	25.0% (1/4)	0.0% (0/3)	0.0% (0/3)
		Total Selenge	45.7%	34.5%	0.0%
Tov	**Batsumber**	Ground squirrel	75.0% (3/4)	0.0% (0/4)	0.0% (0/4)
		Daurian pika	0.0% (0/1)	-	-
		Field mouse	0.0% (0/1)	-	-
		Mongolian gerbil	87.5% (7/8)	100.0% (6/6)	0.0% (0/6)
		Striped dwarf hamster	100.0% (2/2)	-	-
		Total Tov	75.0%	60.0%	0.0%
			Borrelia spp.*	***Rickettsia spp.***	***Anaplasma spp.***
		Total	56.4% (35/62)	38.8% (19/49)	0.0% (0/49)

*Indicates molecular detection in whole blood or ear biopsy samples.

Table 6: PCR prevalence of *Borrelia spp., Rickettsia spp.* and *Anaplasma spp.* by small mammal species.

	Prevalence		
Species	***Borrelia spp.****	***Rickettsia spp.***	***Anaplasma spp.***
Ground squirrel	0.74	0.06	0.00
Daurian pika	0.00	-	-
Field mouse	0.00	-	-
Mongolian gerbil	0.50	0.94	0.00
Siberian chipmunk	0.25	0.00	0.00
Striped dwarf hamster	0.59	0.09	0.00

* Indicates molecular detection in whole blood or ear biopsy samples.

3.2 Sequencing of PCR products

Due to miscommunication regarding the purification process, only two of the 59 positive samples were successfully sequenced. Both samples were identified as having *Bartonella spp.*. Both of these samples were from Mongolian gerbils, one from Selenge aimag and one from Darkhan-Uul aimag.

3.3 Seropositivity of Tickborne Pathogens in Rodents

3.3.1 Seropositivity of *Anaplasma phagocytophilum* in Rodents

Of the 62 serum samples tested, 26 animals (41.9%) were detected as having antibodies present for *A. phagocytophilum*. Striped dwarf hamsters had the highest seroprevalence (58.8%, 10/17 individual animals), followed by Siberian chipmunks (50%, 2/4 individual animals), ground squirrels (47.4%, 9/19 individual animals) and Mongolian gerbils (25.0%, 5/20 individual animals) (Table 8). No antibodies to *A. phagocytophilum* was detected in Daurian pikas or field mice.

Animals captured in Darkhan-Uul aimag had the highest seroprevalence of *A. phagocytophilum* (64.0%, 7/11 individual animals), followed by Tov aimag (38.0%, 6/16 individual animals) and Selenge aimag (37.0%, 13/35 individual animals) (Table 7).

3.3.2 Seropositivity of *Rickettsia rickettsii* in Rodents

Of 62 serum samples assessed, 15 animals (24.2%) were seropositive for *R. rickettsii*. Mongolian gerbils had the highest seroprevalence (30.0%, 6/20 individual animals), followed by Siberian chipmunks (25.0%, ¼ individual animals), striped dwarf hamsters (23.5%, 4/17 individual animals) and ground squirrels (21.0%, 4/19 individual animals) (Table 8). No antibodies to *R. rickettsii* were detected in serum of Daurian pika or field mice.

Animals in Selenge aimag had the highest seroprevalence of *R. rickettsii* (26.0%, 9/35 individual animals), followed by animals collected in Tov aimag (25.0%, 4/16 individual animals) and animals captured in Darkhan-Uul aimag (18.0%, 2/11 individual animals) (Table 7).

Table 7: Seropositivity of *R. rickettsii* and *A. phagocytophilum* by location and small mammal species.

			Seropositivity	
Aimag	**Sum**	**Species**	***R. rickettsii***	***A. phagocytophilum***
Darkhan-Uul	**Orkhon**	ground squirrel	0.0% (0/6)	66.7% (4/6)
		striped dwarf hamster	0.0% (0/1)	100.0% (1/1)
	Khongar	Mongolian gerbil	50.0% (1/2)	0.0% (0/2)
	Xotol	Mongolian gerbil	50.0% (1/2)	100.0% (2/2)
		Total Darkhan-Uul	18.2%	63.6%
Selenge	**Bayangol**	Mongolian gerbil	25.0% (2/8)	25.0% (2/8)
		ground squirrel	0.0% (0/3)	33.3% (1/3)
	Eruu	striped dwarf hamster	28.6% (4/14)	50.0% (7/14)
		ground squirrel	33.3% (1/3)	33.3% (1/3)
	Yavkhlant	ground squirrel	33.3% (1/3)	0.0% (0/3)

		Siberian chipmunk	25.0% (1/4)	50.0% (2/4)
		Total Selenge	25.7%	37.1%
Tov	**Batsumber**	ground squirrel	50.0% (2/4)	75.0% (3/4)
		Daurian pika	0.0% (0/1)	0.0% (0/1)
		field mouse	0.0% (0/1)	0.0% (0/1)
		Mongolian gerbil	25.0% (2/8)	12.5% (1/8)
		striped dwarf hamster	0.0% (0/2)	100.0% (2/2)
		Total Tov	25.0%	37.5%
			R. rickettsii	***A. phagocytophilum***
		Total	24.2% (15/62)	41.9% (26/62)

Table 8: Seroprevalence of *R. rickettsii* and *A. phagocytophilum* by small mammal species.

	Seroprevalence	
Species	***R. rickettsii***	***A. phagocytophilum***
Ground squirrel	0.21	0.47
Daurian pika	0.00	0.00
Field mouse	0.00	0.00
Mongolian gerbil	0.30	0.25
Siberian chipmunk	0.25	0.50
Striped dwarf hamster	0.24	0.59

3.4 Univariate and Multivariate Analysis

3.4.1 Active Infections of Tickborne Pathogens

Variables found to be important predictors of molecular detection of *Borrelia spp.* included aimag, species of small mammal, and sex of small mammals. Using multivariate analysis, predictor variables including aimag and species of small mammal

remained statistically significant (Table 9). As compared to Selenge aimag, small animals in Tov aimag (OR, 6.4; 95% CI, 1.30 – 31.91) and Darkhan-Uul aimag (OR, 1.8; 95% CI, 0.38 – 8.67) had a high odds of *Borrelia spp.* infection. As compared to other small mammal species ground squirrels (OR, 21.4; 95% CI, 1.50 – 304.83), striped dwarf hamsters (OR, 14.2; 95% CI, 1.01 – 199.15) and Mongolian gerbils (OR, 5.16; CI, 0.42 – 63.95) had a high odds of *Borrelia spp.* infections.

Variables found to be important predictors of molecular detection of *Rickettsia spp.* included having ticks present and aimag. After attempting to adjust for the presence of ticks using multivariate analysis, only the covariate small mammal species remained significant with Mongolian gerbils (OR, 246.5; 95% CI, 20.77 – 2925.88) having the highest odds of *Rickettsia spp.* infections followed by striped dwarf hamster (OR, 1.6; CI, 0.90 – 28.57) (Table 9).

Table 9: Unadjusted and adjusted odds ratios for risk factors associated with molecular detection of *Borrelia spp.* and *Rickettsia spp.*

	Molecular Detection of *Borrelia spp.*				Molecular Detection of *Rickettsia spp.*			
Risk Factor	**Total N**	**No. (%)**	**Unadjusted OR (95% CI)**	**Adjusted OR (95% CI)**	**Total N**	**No. (%)**	**Unadjusted OR (95% CI)**	**Adjusted OR (95% CI)**
Aimag								
Tov	16	12 (75.0)	3.6 (1.0 - 12.2)	**6.4 (1.3 - 31.9)**	10	6 (60.0)		
Darkhan-Uul	11	7 (63.6)	2.08 (0.5 - 8.4)	**1.8 (0.4 - 8.7)**	10	3 (30.0)	----	
Selenge	35	16 (45.7)	Ref.	Ref.	29	10 (34.5)		
Animal Species								
Ground squirrel	19	14 (73.7)	14.0 (1.3 - 150.9)	**21.4 (1.5 - 304.8)**	17	1 (5.9)	Ref.	
Dwarf Hamster	17	10 (58.8)	7.1 (0.7 - 75.2)	**14.2 (1.0 - 199.2)**	11	1 (9.1)	**1.6 (0.9 - 28.6)**	----
Mongolian gerbil	20	10 (50.0)	5.0 (0.5 - 50.8)	**5.16 (0.4 - 64.0)**	18	17 (94.4)	**272 (15.7, 4724.2)**	
Other	6	1 (16.7)	Ref.	Ref.	N/A	N/A	N/A	N/A
Sex of Animal								
Female	28	12 (42.8)	Ref.	----	23	11 (47.8)	----	
Male	33	22 (66.7)	2.7 (0.9 - 7.6)		25	8 (32.0)		
Presence of Ticks								
Present	50	27 (54.0)	----		37	9 (24.3)	0.1 (0.0, 0.3)	----
Absent	12	6 (50.0)			12	8 (66.7)	Ref.	Ref.

3.4.2 Serological Detection of Tick Borne Pathogens

Variables found to be important predictors of serological detection for *A. phagocytophilum* included small mammal species and having a tick present. No variables remained statistically significant after multivariate analysis (Table 10). No variables were identified as being significantly associated with *R. ricketsii* serological detection (Table 10).

Table 10: Unadjusted and adjusted odds ratios for risk factors associated with serological detection of *A. phagocytophilum* and *R. rickettsii*

	Serological Detection of *A. phagocytophilum*				Serological Detection of *R. rickettsii*			
Risk Factor	**Total N**	**No. (%)**	**Unadjusted OR (95% CI)**	**Adjusted OR (95% CI)**	**Total N**	**No. (%)**	**Unadjusted OR (95% CI)**	**Adjusted OR (95% CI)**
Aimag								
Tov	16	6 (37.5)			16	4 (25.0)	----	
Darkhan-Uul	11	7 (63.6)	----		11	2 (18.2)		
Selenge	35	13 (37.1)			35	9 (25.7)		
Species of Animal								
Ground squirrel	19	9 (47.4)	2.7 (0.70 – 10.5)		19	4 (21.1)		
Dwarf hamster	17	10 (58.8)	4.3 (1.1 – 17.4)	**----**	17	4 (23.5)	----	
Mongolian gerbil	20	5 (25.0)	Ref.		20	6 (30.0)		
Other	6	2 (33.3)	4.6 (0.9 – 23.2)		6	1 (16.7)		
Sex of Animal								
Female	28	10 (35.7)	----		28	8 (28.6)	----	
Male	33	16 (48.5)			33	6 (18.2)		
Presence of Ticks								
Present	50	24 (48.0)	4.6 (0.9, 23.2)	----	50	13 (26.0)	----	
Absent	12	2 (16.7)	Ref.		12	2 (16.7)		

4. Discussion

As far as we know, this is the first report of the detection of tickborne pathogens in small mammal reservoirs in Mongolia. A high prevalence of *Borrelia spp.* (56.4%) and *Rickettsia spp.* (38.8%) was seen in a number of small mammals. Seroprevalence of *R. rickettsii* (24.2%) was slightly lower than active infections in various host species. There was no molecular detection of *Anaplasma spp.* in any host species, however a high seroprevelance of *A. phagocytophilum* (41.9%) was identified. There was also high geographic distribution of infection of *Borrelia spp.* (46.0% - 75%) and *Rickettsia spp.* (30% - 60%).

4.1 PCR Detection of Borrelia spp.

Differences in the prevalence of infection of *Borrelia spp.* was observed between the different aimags. Although little literature exists assessing Tov and Darkhan-Uul aimags for *Borrelia spp.*, previous literature in Mongolia found a similar prevalence of *Borrelia burgdorferi* sensu lato in *Ixodes persulcatus* ticks in Selenge aimag ranging from 24.5% - 55.0% (Masuzawa et al., 2014; Scholz et al., 2013). In regards to Selenge aimag, the majority of animals infected with *Borrelia spp.* were located in the northern sums of Selenge aimag. Taiga forest comprises most of this region which is where *I. persulcatus* ticks are commonly found which would suggest the reason for a higher prevalence in

this area. The difference in prevalence could also be due to a larger sample size as more animals were captured in the northern part of Selenge aimag.

Darkhan-Uul and Tov aimags had a higher prevalence of infection in comparison to Selenge aimag. Sites in Darkhan-Uul aimag had a lower elevation in comparison to Selenge aimag which could indicate a possible reason for the higher prevalence of *Borrelia spp.* in this area. The sum sampled in Tov aimag was just outside the capitol of Mongolia, Ulaanbaatar, and therefore might have an influx of people and herders moving in and out of this area which could lead to a higher prevalence of TBPs. Additionally, prevalence between aimags could indicate differences due to host preferences in these regions.

Dermacentor spp. were the only tick species identified on rodents, suggesting that *Borrelia spp.* may differ in comparison to previous literature in Mongolia which has only identified *Borrelia spp.* in *I. persulcatus*. However, studies in China have identified multiple *Borrelia spp.* including *Borrelia garinii*, *Borrelia afzelii*, *Borrelia sinica* and *Borrelia burgdorferi* sensu stricto in *Dermacentor silvarum* and *Dermacentor nuttalli* ticks (Fang et al., 2015; Wan et al., 1998; Wang et al., 2015). Ticks are highly seasonal and the time which we were collecting our samples coincided with the *Dermacentor spp.* tick season. *I. persculcatus* ticks are found in forested regions in northern Mongolia (Swanson et al.,

2006) and although we did not identify this tick species on hosts at the time, they could still be present in the area.

Striped dwarf hamsters, ground squirrels, Mongolian gerbils and Siberian chipmunks were often infected with *Borrelia spp.*. This is similar to other literature reporting *Borrelia spp.* infections in various hamster species (Chu et al., 2008; Fang et al., 2015; Takada et al., 2001) and Siberian chipmunks (Marsot et al., 2011; Zhan et al., 2009) in China. Although ground squirrels have not been identified as competent reservoirs for *Borrelia spp.* in countries bordering Mongolia, research in the United States has identified gray squirrels in California as competent reservoir species (Lane et al., 2005). While not identified in wild reservoirs, experimental studies have historically used Mongolian gerbils as reservoirs for *Borrelia spp.* (Matuschka et al., 2000; Gray et al., 1996). Our prevalence of 46% is higher than infection of rodents identified in China which ranged from 2.3% - 25% (Chu et al., 2008; Zhan et al., 2009; Zhang et al., 2010). However, many of these studies focused only on the species of *Borrelia* which cause human disease. As we were unable to determine specific *Borrelia spp.* in our samples, our prevalence could be higher because our assay is likely to pick up other non-pathogenic *Borrelia spp.*. Previous studies in Russia found that a high prevalence of *Borrelia spp.* was more commonly found in unfed nymphs suggesting infection likely takes place during feeding of larvae (Korenberg et al., 2002). It should be noted that most of the ticks found

on the rodents captured were larvae; this could explain why our prevalence data were so high among rodents (due to the life stage of ticks).

In regards to the logistic regression data, aimag and small mammal species remained important risk factors in multivariate analysis. In comparison to Selenge aimag, small mammals in Tov and Darkhan-Uul aimag were at higher odds of having *Borrelia spp.* infections. The high odds of *Borrelia spp.* infections in Tov aimag could be due to a warmer climate in this region allowing for a longer lifespan of ticks, therefore increasing the season for tickborne pathogens. Additionally, as Tov aimag is surround by Ulaanbaatar, there may be a higher population of herders and animals in this region which may contribute to the life cycle of the tick species. In comparison to Tov aimag, small mammals captured in Darkhan-Uul had a lower odds of *Borrelia spp.* infections. This would make sense as Darkhan-Uul aimag is surrounded by Selenge aimag and likely has a similar prevalence of *Borrelia spp.* in small mammal species. As there are large confidence intervals for these variables, future studies to assess these regions in more detail using a larger sample size is necessary. In comparison to other species of small mammals, ground squirrels and dwarf hamsters had a high odds of *Borrelia spp.* infections. Potentially these small mammal species are more competent reservoirs due to behaviors such as spending more time outside of burrows or more vegetated areas.

4.2 PCR and Serology Detection of Rickettsia spp.

Previous research in Mongolia has identified *Rickettsia spp.* in ticks in Mongolia. Our data showed that Tov aimag had a high prevalence of 60%, however, this data only stems from one region in Tov. On the other hand, Darkhan-Uul and Selenge aimag had a prevalence of 30%-34% of *Rickettsia spp.* identified in various host species. Southern sums in Selenge aimag had a higher prevalence of *Rickettsia spp.* infections in comparison to northern sums. Seroprevalence data showed slightly lower antibody detection in Darkhan-Uul aimag (26.0%), Tov aimag (25.0%) and Selenge aimag (18.0%) in comparison to PCR data. This would be expected as our PCR assay is targeting the gltA gene which is found in many different tickborne pathogens and does not allow speciation of tickborne rickettsiosis such as *R. rickettsii*. Seroprevalence of small mammal species was similar to previous literature in Mongolia which found a 12.5% and 22.9% prevalence of *Rickettsia spp.* in *D. nuttalli* and *I. persulcatus* ticks, respectively (Narantsatsral et al., 2014). Additional literature in Mongolia has identified a high prevalence of 70.0 – 97.0% of *Rickettsia spp.* in *D. nuttallii* ticks (Speck et al., 2012). Along the Russian and Mongolian boarder in China, ticks had a similar prevalence of 53.4% of *Rickettsia raoultii* in *D. nuttalli* and *I. persulcatus* ticks (Liu et al., 2015).

In our study, Mongolian gerbils had the highest prevalence of *Rickettsia spp.* (94.4%), followed by striped dwarf hamsters (9.1%) and ground squirrels (5.9%). Similar

to our molecular detection, seroprevalence of *R. rickettsii* identified Mongolian gerbils as having the highest seroprevalence (30%), followed by Siberian chipmunks (25%), striped dwarf hamsters (23.5%) and ground squirrels (21.0%). The total seroprevalence identified in this study is on par with studies in China which have identified SFRG ranging from 9.1 – 21.6% in rodents along the Mongolian border (Zhan et al., 2009). Literature in China did not have supporting evidence for SFGR in Mongolian gerbils, striped dwarf hamsters, Siberian chipmunks or ground squirrels (Fang et al., 2015; Zhan et al., 2009). In other parts of the world, studies have identified gerbils (Shoukry et al., 1991), ground squirrels (Adjemian et al., 2008; Fleer et al., 2011) and chipmunks (Fleer et al., 2011) as competent reservoirs for SFGR.

In regards to the logistic regression data, a high odds of *Rickettsia spp.* was identified in Mongolian gerbils. Many of the Mongolian gerbils captured were located in areas with high densities of livestock and therefore could serve as more competent reservoirs for various vector-borne pathogens due to their close proximity to livestock. There is also a potential that Mongolian gerbils are highly competent due to behaviors such as spending less time grooming thus making them more susceptible to tick infestations. As our PCR assay is not specific to only *Rickettsial spp.* infections it is likely that our assay is reflecting other Rickettsial pathogens many of which can also be transmitted by fleas.

Interestingly, we sequenced two samples of *Bartonella spp.* from the gltA gene. *Bartonella spp.* have been identified in ticks, however, it is still unknown whether or not ticks are able to transmit *Bartonella spp.* to humans and other animals. It is more commonly thought that Bartonella is transmitted by fleas, body louse, sandflies or through infected cats (Centers for Disease Control and Prevention [CDC], 2016). Bartonella has not yet been identified in Mongolia, however antibodies to Bartonella have commonly been detected in rodents in China (Li et al., 2015; Liu et al., 2010; Mediannik et al., 2006; Rao et al., 2015). Additionally, *Bartonella spp.* have been identified in humans and domestic animals in China (Chai et al., 2010) and *I. persculcatus* ticks in western Siberia (Rar et al., 2005). As the sequence was identified as an uncultured *Bartonella spp.* there is a possibility that this *Bartonella spp.* is not infectious to humans or livestock. Therefore, more assays need to be conducted on these samples to assess the prevalence of *Bartonella spp.*. Further studies should be performed to assess humans and livestock in Mongolia for Bartonella infections.

4.3 Seroprevelance of Anaplasma spp.

We did not identify any molecular evidence of *Anaplasma spp.* in our reservoir hosts. However, we did find that 41.9% of their serum samples had antibodies for *Anaplasma phagocytophilum*. The seroprevalence we identified in small mammal species is

similar to a study on *A. phagocytophilum* in northern Mongolia which found a seroprevalence of 35.8% in domestic livestock (Sophia et al., 2012). Interestingly, studies on ticks in Mongolia have found a much lower prevalence of *Anaplasma spp.* in *I. persulcatus* (6.0%) and *D. nuttalli* (0-2.0%) ticks (Javkhlan et al., 2014; Karnath et al., 2016; Masuzawa et al., 2014). Research in Siberia and China have identified *A. phagocytophilum* in 1.0 – 5.1% of ticks (Rar et al., 2008; Rar et al., 2010; Swanson et al., 2006), suggesting that *Anaplasma spp.* may not be highly prevalent in ticks in this area or that Anaplasma is highly seasonal. Similar to findings in ticks, studies in China found that 5.5% of rodents captured were infected with *A. phagocytophilum* (Zhan et al., 2009).

Siberian chipmunks, striped dwarf hamsters, Mongolian gerbils, and ground squirrels had antibodies for *A. phagocytophilum*. Studies in China found that gerbils and Siberian chipmunks were likely to harbor *A. phagocytophilum* (Fang et al., 2015; Zhan et al., 2009). Additional studies in the United States have identified various *Spermophilus spp.* as competent reservoir hosts for *A. phagocytophilum* (Adjemian et al., 2008). Previous studies found that rodents are significantly more likely to harbor infections during peak nymphal or adult tick seasons and that infections have short durations (Bown et al., 2003; Chastagner et al., 2016). The majority of ticks found on the rodents captured were larvae, therefore it would make sense that we did not see any active infections in captured rodents but did see antibodies to *A. Phagocytophilum* from previous infections.

Potentially, small mammal reservoirs may not serve as important reservoir host species for *Anaplasma spp.* in comparison to domestic livestock or larger wildlife species.

4.4 Implications of Tickborne Pathogens on Humans and Domestic Animals and Implications for Public Policy and Practice

A recent review in China identified the emergence of many tickborne pathogens throughout mainland China, especially in the northern provinces along the Mongolian and Russian borders (Fang et al., 2015). As the climate and environment are similar in Mongolia, many of the pathogens found in nearby China likely also exist in Mongolia. The few studies which have assessed tickborne pathogen prevalence among people in Mongolia have identified seroprevalences for *A. phagocytophilum* ranging from 2.3% - 5.6% and a seroprevalence of *Borrelia spp.* ranging from 1.9% - 13.9% (Walder et al., 2006). Selenge aimag had the highest incidence of Lyme disease at 7.8 cases per 100,000 persons per year (Scholz et al., 2013). This is higher than in Russia which reported an incidence of 3.1 cases per 100,000 persons per year (Postic et al.,1997). Less is known regarding the prevalence of *Rickettsia spp.* in humans in Mongolia, however case studies have identified infections of *Rickettsia siberica* in people traveling in Mongolia (Lankester & Davey, 2008; Lewin et al., 2003), suggesting that SFGR is present in the area and should be a focus of future studies.

As domestic animals can 1) contract tickborne pathogens (Barbour et al., 2009; Fang et al., 2015; Littman et al., 2006; Steere et al., 2016) and 2) serve as a vector for the transfer of infected ticks to humans, more studies should be conducted in Mongolia assessing the impact of tickborne pathogens on domestic animals. Forty percent of the Mongolian population relies on herding for employment (Batsukh et al., 2013), therefore, the country would have much to gain by assessing tickborne pathogens in areas where domestic animals, humans and small mammal reservoirs overlap. A recent study in Mongolia identified a seroprevalence of 11.3%, 35.8% and 21.6% for *B. burgdorferi*, *A. phagocytophilum* and SFGR, respectively, in domestic livestock in northern Mongolia (Sophia et al., 2012). These findings are similar to the seroprevalences of *R. rickettsii* (24.2%) and *A. phagocytophilum* (41.9%) we found in small mammal reservoirs.

Studying the ecology of tickborne pathogens in Mongolia will be important to further public health policy and practice throughout the country. As discussed previously, tickborne pathogens affect both humans and animals. As herding is an important livelihood and source of revenue in Mongolia, the use of a One Health-based approach will be vital in establishing future public policy and practice in Mongolia. As suggested by others, the need for continued research and surveillance of tickborne pathogens will be necessary in future efforts guiding policies surrounding infectious diseases in Mongolia. Such collaborative efforts have begun taking place in Mongolia,

for example the Korean International Cooperation Agency funds vector surveillance, climactic monitoring and education (Batsukh et al., 2013). Similar collaborative efforts should continue in Mongolia focusing upon research of infectious disease threats in the country.

4.5 Implications for Further Research

Future research should aim to further understand the complexities of tickborne pathogens in humans, animals (domestic and wild) and ticks in Mongolia. Research should focus on the prevalence of tickborne pathogens in humans and animals including what types of animals and ticks are impacted by these pathogens. The seasonality and distribution of ticks also needs to be further parsed out to understand environmental conditions which may influence the distribution of tickborne pathogens. Research in the social sciences is needed to address human movement patterns, behavior and education of communities in Mongolia to better focus public health interventions. Additionally, studies assessing climactic fluctuations worldwide have identified increased expansion of tick populations contributing to the emergence of tickborne diseases (Fang et al., 2015; Ogden et al., 2013; Pfäffle et al., 2013). Over the last 65 years, the average temperature in Mongolia increased by 1.94˚C (Batsukh et al., 2013). Research on the distribution of ticks

in Mongolia is lacking and thus there is a need to monitor the distribution of tick species throughout the country.

4.3 Study Strengths and Limitations

This study is unique in that, as far as it is know, this is the first description of the evidence of tickborne pathogens in small mammal species in Mongolia. Strengths of this study include the variation in animal species collected and the numerous sites in northern Mongolia. As in any study, there are limitations to this study. As a cross-sectional study design, it is likely that we missed infections among captured animals as bacterial infections are short lived in hosts, and animals were only caught once during the duration of the study. Therefore, we were unable to look differences in infection rates over time. While 12 locations were sampled, we were only able to spend 1 to 2 days at each site, therefore it was difficult to get a full sample of all animals in each area. Sampling of aimags was limited to the locations where we had permission to sample and cannot be generalized to the aimag as a whole. We had a relatively small sample size in this study and further studies should assess more animals in this area to get a better idea of the prevalence of tickborne pathogens among small mammal species.

Although PCR is highly sensitive, there is a possibility that some infections were missed due to limited amounts of sample, specifically whole blood, collected from

animals. Additionally, with the general assays performed, it was not possible to parse out specific species of *Rickettsia spp.*, *Anaplasma spp.* and *Borrelia spp.* by PCR alone. This also makes it difficult to assess if the pathogens identified in small mammal reservoirs are pathogenic in humans or domestic animal species. We attempted to sequence samples but there was a miscommunication with the sequencing company, resulting in a limited number of fully sequenced samples. Lastly, while IFA's can be useful in distinguishing exposure to pathogens there are problems with cross reactivity when using IFA's and thus these results are not as useful without molecular or sequencing evidence of the pathogen.

5. Conclusion

In conclusion, this study identified *Borrelia spp.*, *Rickettsia spp.* and *Anaplasma spp.* circulating in small mammal reservoirs in northern Mongolia. Additional studies are needed to further assess tickborne diseases to the species level in small mammal reservoirs. There is also a strong need to assess pathogens in areas where small mammal reservoirs, domestic animals, humans and ticks overlap. Understanding the ecology and distribution of tickborne pathogens in Mongolia will help provide better diagnosis and treatment for people living in these regions. With multiple pathogens circulating in small mammal species there is also potential for coinfections in humans, animals and ticks. Further assessment will be important to help provide better diagnosis and treatment of tickborne pathogens in Mongolia. Lastly, studies on occupation, time spent outdoors, perceptions and knowledge of tickborne pathogens in Mongolian communities will be necessary to tailor public health interventions.

Appendix A

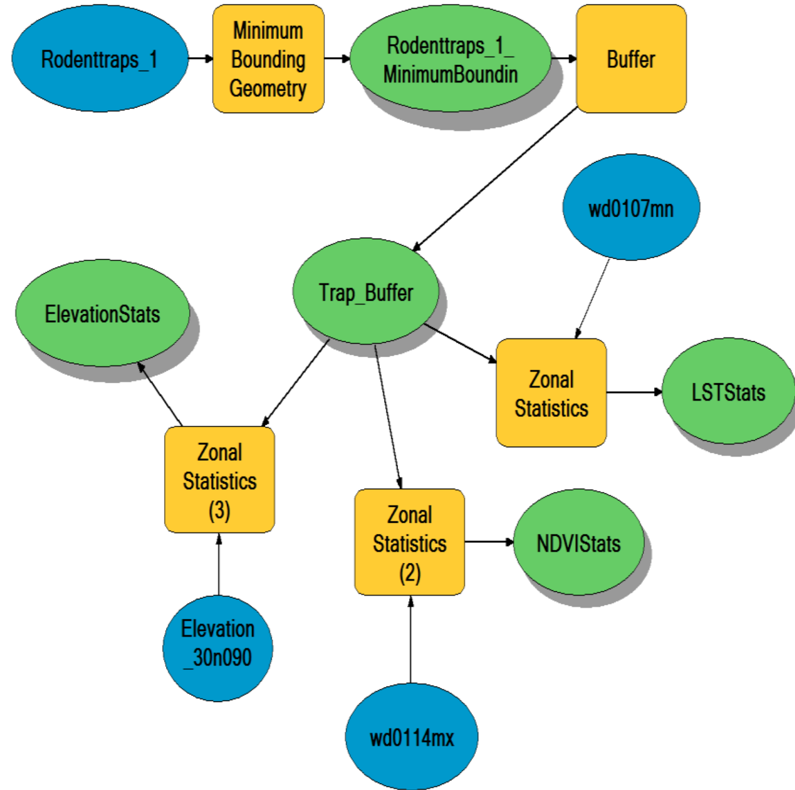

Figure 2: ArcGIS model describing landscape variable statistical analysis.

References

1. Adjemian, J. Z., Adjemian, M. K., Foley, P., Chomel, B. B., Kasten, R. W., & Foley, J. E. (2008). Evidence of multiple zoonotic agents in a wild rodent community in the eastern Sierra Nevada. *J Wildl Dis, 44*(3), 737-742. doi:10.7589/0090-3558-44.3.737

2. Barbour, A. G., Bunikis, J., Travinsky, B., Hoen, A. G., Diuk-Wasser, M. A., Fish, D., & Tsao, J. I. (2009). Niche partitioning of Borrelia burgdorferi and Borrelia miyamotoi in the same tick vector and mammalian reservoir species. *Am J Trop Med Hyg, 81*(6), 1120-1131. doi:10.4269/ajtmh.2009.09-0208

3. Barbour, A. G., Maupin, G. O., Teltow, G. J., Carter, C. J., & Piesman, J. (1996). Identification of an uncultivable Borrelia species in the hard tick Amblyomma americanum: possible agent of a Lyme disease-like illness. *J Infect Dis, 173*(2), 403-409.

4. Batsukh, Z., Tsolmon, B., Otgonbaatar, D., Undraa, B., Dolgorkhand, A., Ariuntuya, O. (2013). One Health in Mongolia. *Curr Top Microbiol Immunol,* 366, 123-137.

5. Battsetseg, B., Lucero, S., Xuan, X., Claveria, F., Byambaa, B., Battur, B., . . . Fujisaki, K. (2002). Detection of Equine Babesia spp. Gene Fragments in Dermacentor nuttalli Infesting Mongolian Horses, and Their Amplification in Egg and Larval Progenies. *Journal of Veterinary Medical Science, 64*(8), 727 - 730.

6. Bown, K. J., Begon, M., Bennett, M., Woldehiwet, Z., & Ogden, N. H. (2003). Seasonal dynamics of Anaplasma phagocytophila in a rodent-tick (Ixodes trianguliceps) system, United Kingdom. *Emerg Infect Dis, 9*(1), 63-70. doi:10.3201/eid0901.020169

7. Bunikis, J., Garpmo, U., Tsao, J., Berglund, J., Fish, D., & Barbour, A. G. (2004). Sequence typing reveals extensive strain diversity of the Lyme borreliosis agents Borrelia burgdorferi in North America and Borrelia afzelii in Europe. *Microbiology, 150*(Pt 6), 1741-1755. doi:10.1099/mic.0.26944-0

8. Center for Disease Control and Prevention. (2016). Bartonella Infection (Cat Scratch Disease, Trench Fever, and Carrión's Disease). Retrieved from http://www.cdc.gov/bartonella/

9. Chai, C. L., Lu, Q. Y., Sun, J. M., Jiang, L. P., Ling, F., Zhang, L. J., . . . Ge, J. H. (2010). Sero-epidemiologic investigation on tick-borne diseases of humans and domestic animals in Zhejiang province. *Zhonghua Liu Xing Bing Xue Za Zhi, 31*(10), 1144-1147.

10. Chastagner, A., Moinet, M., Perez, G., Roy, E., McCoy, K. D., Plantard, O., . . . Vourc'h, G. (2016). Prevalence of Anaplasma phagocytophilum in small rodents in France. *Ticks Tick Borne Dis, 7*(5), 988-991. doi:10.1016/j.ttbdis.2016.05.005

11. Chu, C. Y., Jiang, B. G., Liu, W., Zhao, Q. M., Wu, X. M., Zhang, P. H., . . . Cao, W. C. (2008). Presence of pathogenic Borrelia burgdorferi sensu lato in ticks and rodents in Zhejiang, south-east China. *J Med Microbiol, 57*(Pt 8), 980-985. doi:10.1099/jmm.0.47663-0

12. Fang, L.-Q., Liu, K., Li, X.-L., Liang, S., Yang, Y., Yao, H.-W., . . . Cao, W.-C. (2015). Emerging tick-borne infections in mainland China: an increasing public health threat. *The Lancet Infectious Diseases, 15*(12), 1467-1479. doi:10.1016/s1473-3099(15)00177-2

13. Fleer, K. A., Foley, P., Calder, L., & Foley, J. E. (2011). Arthropod Vectors and Vector-Borne Bacterial Pathogens in Yosemite National Park. *Journal of Medical Entomology, 48*(1), 101-110. doi:10.1603/me10040

14. Gray, J. S., Schonberg, A., Postic, D., Belfaiza, J., & Saint-Girons, I. (1996). First isolation and characterisation of Borrelia garinii, agent of Lyme borreliosis, from Irish ticks. *Ir J Med Sci, 165*(1), 24-26.

15. Hay, S.I., Tatem, A.J., Graham, A.J., Goetz, S.J. & Rogers, D.J. (2011). Global Environmental Data for Mapping Infectious Disease Distribution. *Adv. Parasitol.*, 62, 37-77. Doi:10.1016/S0065-308X(05)62002-7

16. Javkhlan, G., Enkhtaivan, B., Baigal, B., Myagmarsuren, P., Battur, B., & Battsetseg, B. (2014). Natural Anaplasma phagocytophilum infection in ticks from a forest area of Selenge province, Mongolia. *Western Pac Surveill Response J, 5*(1), 21-24. doi:10.5365/WPSAR.2013.4.3.001

17. Karnath, C., Obiegala, A., Speck, S., Essbauer, S., Derschum, H., Scholz, H., . . . Pfeffer, M. (2016). Detection of Babesia venatorum, Anaplasma phagocytophilum and Candidatus Neoehrlichia mikurensis in Ixodes persulcatus ticks from Mongolia. *Ticks Tick Borne Dis, 7*(2), 357-360. doi:10.1016/j.ttbdis.2015.12.007

18. Korenberg, E. I., Kovalevskii, Y. V., & Gorelova, N. B. (2002). Tick-host-Borrelia population interactions: long-term records in Eastern Europe. *Exp Appl Acarol, 28*(1-4), 225-229.

19. Lane, R. S., Mun, J., Eisen, R. J., & Eisen, L. (2005). Western gray squirrel (Rodentia: Sciuridae): a primary reservoir host of Borrelia burgdorferi in Californian oak woodlands? *J Med Entomol, 42*(3), 388-396.

20. Lankester, T., & Davey, G. (1997). A lump on the head from Mongolia. *Lancet, 349*(9052), 656. doi:10.1016/s0140-6736(05)61613-3

21. Lewin, M. R., Bouyer, D. H., Walker, D. H., & Musher, D. M. (2003). Rickettsia sibirica infection in members of scientific expeditions to northern Asia. *Lancet, 362*(9391), 1201-1202. doi:10.1016/s0140-6736(03)14515-1

22. Li, D. M., Hou, Y., Song, X. P., Fu, Y. Q., Li, G. C., Li, M., . . . Liu, Q. Y. (2015). High prevalence and genetic heterogeneity of rodent-borne Bartonella species on Heixiazi Island, China. *Appl Environ Microbiol, 81*(23), 7981-7992. doi:10.1128/AEM.02041-15

23. Littman, M. P., Goldstein, R. E., Labato, M. A., Lappin, M. R., & Moore, G. E. (2006). ACVIM small animal consensus statement on Lyme disease in dogs: diagnosis, treatment, and prevention. *J Vet Intern Med, 20*(2), 422-434.

24. Liu, L., Chen, Q., Yang, Y., Wang, J., Cao, X., Zhang, S., . . . Xu, B. (2015). Investigations on Rickettsia in Ticks at the Sino-Russian and Sino-Mongolian Borders, China. *Vector Borne Zoonotic Dis, 15*(12), 785-789. doi:10.1089/vbz.2014.1732

25. Marsot, M., Sigaud, M., Chapuis, J. L., Ferquel, E., Cornet, M., & Vourc'h, G. (2011). Introduced Siberian chipmunks (Tamias sibiricus barberi) harbor more-diverse Borrelia burgdorferi sensu lato genospecies than native bank voles (Myodes glareolus). *Appl Environ Microbiol, 77*(16), 5716-5721. doi:10.1128/AEM.01846-10

26. Masuzawa, T., Masuda, S., Fukui, T., Okamoto, Y., Bataa, J., Oikawa, Y., . . . Takada, N. (2014). PCR Detection of Anaplasma phagocytophilum and Borrelia burgdorferi in Ixodes persulcatus Ticks in Mongolia. *Japan Journal of Infectious Disease, 67*, 47-49.

27. Matuschka, F. R., Schinkel, T. W., Klug, B., Spielman, A., & Richter, D. (2000). Relative incompetence of european rabbits for Lyme disease spirochaetes. *Parasitology, 121 (Pt 3)*, 297-302.

28. Mediannikov, O., & Melikian, A. L. (2006). A case of cat scratch disease in a patient with diabetes mellitus and chronic renal failure. *Klin Med (Mosk), 84*(7), 61-63.

29. Mediannikov, O. Y., Sidelnikov, Y., Ivanov, L., Mokretsova, E., Fournier, P. E., Tarasevich, I., & Raoult, D. (2004). Acute tick-borne rickettsiosis caused by Rickettsia heilongjiangensis in Russian Far East. *Emerg Infect Dis, 10*(5), 810-817. doi:10.3201/eid1005.030437

30. Narantsatsral, S., Myagmarsuren, P., Davaasuren, P., Enkhtaivan, B., Battsetseg, B., & Battur, B. (2014). Molecular Biological Detection of Emerging Tick-Borne Zoonotic Pathogens in Ixodid Tick Species. *Mongolian Journal of Agricultural Sciences, 13*(2), 3-7.

31. Ogden, N. H., Mechai, S., & Margos, G. (2013). Changing geographic ranges of ticks and tick-borne pathogens: drivers, mechanisms and consequences for pathogen diversity. *Front Cell Infect Microbiol, 3*, 46. doi:10.3389/fcimb.2013.00046

32. Pfaffle, M., Littwin, N., Muders, S. V., & Petney, T. N. (2013). The ecology of tick-borne diseases. *Int J Parasitol, 43*(12-13), 1059-1077. doi:10.1016/j.ijpara.2013.06.009

33. Postic, D., Korenberg, E., Gorelova, N., Kovalevski, Y. V., Bellenger, E., & Baranton, G. (1997). Borrelia burgdorferi sensu lato in Russia and neighbouring countries: high incidence of mixed isolates. *Res Microbiol, 148*(8), 691-702. doi:10.1016/s0923-2508(99)80068-0

34. Rao, H. X., Yu, J., Guo, P., Ma, Y. C., Liu, Q. Y., Jiao, M., . . . Li, D. M. (2015). Bartonella Species Detected in the Plateau Pikas (Ochotona curzoiae) from

Qinghai Plateau in China. *Biomed Environ Sci, 28*(9), 674-678. doi:10.3967/bes2015.094

35. Rar, V. A., Fomenko, N. V., Dobrotvorsky, A. K., Livanova, N. N., Rudakova, S. A., Fedorov, E. G., . . . Morozova, O. V. (2005). Tickborne pathogen detection, Western Siberia, Russia. *Emerg Infect Dis, 11*(11), 1708-1715. doi:10.3201/eid1111.041195

36. Rar, V. A., Livanova, N. N., Panov, V. V., Doroschenko, E. K., Pukhovskaya, N. M., Vysochina, N. P., & Ivanov, L. I. (2010). Genetic diversity of Anaplasma and Ehrlichia in the Asian part of Russia. *Ticks Tick Borne Dis, 1*(1), 57-65. doi:10.1016/j.ttbdis.2010.01.002

37. Rar, V. A., Livanova, N. N., Panov, V. V., Kozlova, I. V., Pukhovskaya, N. M., Vysochina, N. P., . . . Ivanov, L. I. (2008). Prevalence of Anaplasma and Ehrlichia species in Ixodes persulcatus ticks and small mammals from different regions of the Asian part of Russia. *International Journal of Medical Microbiology, 298*, 222-230. doi:10.1016/j.ijmm.2008.01.001

38. Robert, N., Walzer, C., Ruegg, S. R., Kaczensky, P., Ganbaatar, O., & Stauffer, C. (2005). Pathologic findings in reintroduced Przewalski's horses (Equus caballus przewalskii) in southwestern Mongolia. *J Zoo Wildl Med, 36*(2), 273-285. doi:10.1638/03-035.1

39. Scholz, H. C., Margos, G., Derschum, H., Speck, S., Tserennorov, D., Erdenebat, N., . . . Fingerle, V. (2013). High prevalence of genetically diverse Borrelia bavariensis-like strains in Ixodes persulcatus from Selenge Aimag, Mongolia. *Ticks Tick Borne Dis, 4*(1-2), 89-92. doi:10.1016/j.ttbdis.2012.08.004

40. Shoukry, A., Merdan, A. I., & el-Kady, G. A. (1991). The role of rodents as reservoirs for some diseases in new reclaimed areas in north Sinai. *J Egypt Soc Parasitol, 21*(2), 513-519.

41. Sophia, P. (2013). Detection and Epidemiology of Tick-Borne Pathogens in Free-Ranging Livestock in Mongolia. *Journal of Clinical & Experimental Pathology, 01*(S3). doi:10.4172/2161-0681.s3-006

42. Speck, S., Derschum, H., Damdindorj, T., Dashdavaa, O., Jiang, J., Kaysser, P., . . . Essbauer, S. (2012). Rickettsia raoultii, the predominant Rickettsia found in Mongolian Dermacentor nuttalli. *Ticks Tick Borne Dis, 3*(4), 227-231. doi:10.1016/j.ttbdis.2012.04.001

43. Steere, A. C., Coburn, J., & Glickstein, L. (2004). The emergence of Lyme disease. *J Clin Invest, 113*(8), 1093-1101. doi:10.1172/JCI21681

44. Swanson, S. J., Neitzel, D., Reed, K. D., & Belongia, E. A. (2006). Coinfections acquired from ixodes ticks. *Clin Microbiol Rev, 19*(4), 708-727. doi:10.1128/CMR.00011-06

45. Takada, N., Masuzawa, T., Ishiguro, F., Fujita, H., Kudeken, M., Mitani, H., . . . Ma, X. H. (2001). Lyme disease Borrelia spp. in ticks and rodents from northwestern China. *Appl Environ Microbiol, 67*(11), 5161-5165. doi:10.1128/AEM.67.11.5161-5165.2001

46. Walder, G., Lkhamsuren, E., Shagdar, A., Bataa, J., Batmunkh, T., Orth, D., . . . Dierich, M. P. (2006). Serological evidence for tick-borne encephalitis, borreliosis, and human granulocytic anaplasmosis in Mongolia. *Int J Med Microbiol, 296 Suppl 40*, 69-75. doi:10.1016/j.ijmm.2006.01.031

47. Wan, K., Zhang, Z., & Dou, G. (1998). Investigation on primary vectors of Borrelia burgdorferi in China. *Zhonghua Liu Xing Bing Xue Za Zhi, 19*(5), 263-266.

48. Wang, Y. Z., Mu, L. M., Zhang, K., Yang, M. H., Zhang, L., Du, J. Y., . . . Shao, R. F. (2015). A broad-range survey of ticks from livestock in Northern Xinjiang: changes in tick distribution and the isolation of Borrelia burgdorferi sensu stricto. *Parasit Vectors, 8*, 449. doi:10.1186/s13071-015-1021-0

49. Zhan, L., Cao, W. C., Chu, C. Y., Jiang, B. G., Zhang, F., Liu, W., . . . Habbema, J. D. (2009). Tick-borne agents in rodents, China, 2004-2006. *Emerg Infect Dis, 15*(12), 1904-1908. doi:10.3201/eid1512.081141

50. Zhang, F., Gong, Z., Zhang, J., & Liu, Z. (2010). Prevalence of Borrelia burgdorferi sensu lato in rodents from Gansu, northwestern China. *BMC Microbiol, 10*, 157. doi:10.1186/1471-2180-10-157