Estimated seroprevalence of *Anaplasma spp.* and spotted fever group *Rickettsia* exposure among herders and livestock in Mongolia

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A B S T R A C T

Background: To better understand the epidemiology of tick-borne disease in Mongolia, a comprehensive seroprevalence study was conducted investigating exposure to *Anaplasma spp.* and spotted fever group (SFG) *Rickettsia* spp. in nomadic herders and their livestock across three provinces from 2014 to 2015.

Methods: Blood was collected from 397 herders and 2370 livestock, including sheep, goats, cattle, horses and camels. Antibodies against *Anaplasma spp.* and SFG *Rickettsia* were determined by indirect immunofluorescence using commercially available slides coated with *Anaplasma phagocytophilum* and *Rickettsia rickettsii* antigens. Logistic regression was used to determine if the odds of previous exposure differed by gender, location, and species, with or without adjustment for age. To examine the association between seroprevalence and environmental variables we used ArcGIS to circumscribe the five major clusters where human and animal data were collected.

Results: *Anaplasma spp.* exposure was detected in 37.3% (136/365) of humans and 47.3% (1120/2370) of livestock; SFG *Rickettsia* exposure was detected in 19.5% (73/374) humans and 20.4% (478/2342) livestock. Compared to the southern province (aimag) of Dornogovi, located in the Gobi Desert, humans were significantly more likely to be exposed to *Anaplasma spp.* and SFG *Rickettsia* in the northern provinces of Tov (OR = 7.3, 95% CI: 3.5, 15.1; OR = 3.3, 95% CI: 1.7, 7.5), and Selenge (OR = 6.9, 95% CI: 3.4, 14.0; OR = 2.2, 95% CI: 1.1, 4.8).

Conclusion: The high seroprevalence of *Anaplasma spp.* and SFG *Rickettsia* in humans and livestock suggests that exposure to tick-borne pathogens may be common in herders and livestock in Mongolia, particularly in the more northern regions of the country. Until more is known about these pathogens in Mongolia, physicians and veterinarians in the countryside should consider testing for *Anaplasma* and SFG *Rickettsia* infections and treating clinically compatible cases, while public health authorities should expand surveillance efforts for these emerging infections.

1. Background

Mongolia, a vast landlocked country between Russia and China, maintains traditional ties to nomadic-pastoral lifestyles. Roughly one third of Mongolia’s population of 3 million is pastoral, with livestock playing a critical role in Mongolia’s culture and economy. (Papageorgiou et al., 2012) This close proximity to animals and working outdoors increases the potential for exposure to ticks and the...
pathogens they harbor. This can directly impact the lives of Mongolians by causing illness in humans and indirectly through economic losses incurred from illness in livestock. Several epidemiological studies have documented spotted fever group (SFG) Rickettsia, (Lewin et al., 2003; Papageorgiou et al., 2012; Speck et al., 2012) Borrelia burgdorferi, (Masuzawa et al., 2014; Papageorgiou et al., 2012; Scholz et al., 2013; Walder et al., 2006) Anaplasma spp., (Haigh et al., 2008; Javkhlan et al., 2014; Masuzawa et al., 2014; Papageorgiou et al., 2012; Walder et al., 2006; Ybanez et al., 2013) and tick-borne encephalitis virus (TBEV) (Frey et al., 2012; Muto et al., 2015) in Mongolia, but most were limited by their sample sizes and small geographic coverage. A recent molecular study of Anaplasma species detected in Mongolian cattle, yaks, goats, and sheep found 33.2% – 44.5% of livestock screened, tested positive, suggesting a high burden of disease in the capital city of Ulaanbaatar (Ochirkhuu et al., 2017). However, this study targeted A. marginale and A. ovis which do not infect humans.

A previous serosurvey of Anaplasma and SFG Rickettsia in free-ranging livestock located in the northern provinces of Khuvsgul and Khentii found that 35.8% were seropositive for Anaplasma and 21.6% for SFG Rickettsia. (Papageorgiou et al., 2012) The high seroprevalence is concerning since A. phagocytophilum can cause potentially lethal human granulocytic anaplasmosis (HGA), and spontaneous abortion, tick-borne fever (TBF), and lethargy, in livestock. (Atif, 2015; Haigh et al., 2008; Renneker et al., 2013) Additionally, documentation of A. phagocytophilum in ticks(Jiang et al., 2011) as well as clinical cases of anaplasmosis not far from the Mongolian border, (Shchuchinova, 2013; Zhang et al., 2013) makes a case for further investigations in Mongolia. Cases of rickettsiosis caused by R. sibirica mongolitimonae, R. raoulti, R. helongiangeriensis, and possibly Candidatus R. tarasevichiae, which can cause severe, sometimes fatal disease in humans, have been documented in the surrounding region. (Jia et al., 2013a; Jia et al., 2014; Jia et al., 2013b; Liu et al., 1990; Medianikov et al., 2006; Medianikov et al., 2004) To investigate previous exposure to SFG Rickettsia spp. and Anaplasma spp., we conducted a large cross-sectional study of herders and livestock from across the north-south transect of central Mongolia.

2. Methods

2.1. Sample collection and study location

Between August 2014 and October 2015, 2747 serum samples were collected from five districts (soums) located across central Mongolia (Fig. 1). These included the soums of Tushig and Eroo located in Selenge aimag, Terejli soum located in Tov aimag, and Dalandjargalan and Sainshand soums located in Dornogovi aimag. Samples were collected from 397 healthy nomadic herders and 2370 livestock, including cattle, goats, sheep, horses, and camels, owned by participating herders living in the Mongolian countryside. Demographic characteristics of enrolled herders can be found in Table 1. Approximately 5 mL of venous blood was collected in serum separator tubes and transported to the National Center for Zoonotic Diseases (Ulaanbaatar, Mongolia) laboratory, where aliquots of serum from each sample were stored at −80 °C for serological testing by indirect immunofluorescence assay (IFA). For each sample we also recorded the age of the human or animal, biological sex, and geographic coordinates.

2.2. Serological methods

Indirect immunofluorescence (IFA) to detect IgG antibodies against Rickettsia spp. and Anaplasma spp. was performed using commercially prepared slides coated with whole organism R. rickettsii and A. phagocytophilum (ProtaTek International, Inc., St. Paul, MN) as recommended (http://www.protatek.com/IFA Slides/IFA Procedures.pdf) with minor modifications. Briefly, 20 µL of serum, at a 1:50 dilution with 1X phosphate buffered saline (PBS), was applied onto antigen coated slide wells. The slides were then incubated at 37 °C for 45 min in a humidified chamber. After incubation, slides were gently washed using 1X PBS for five minutes. Ten microliters of A/G- fluorescein isothiocyanate (FITC) conjugate, which can be used to detect IgG antibodies from a wide range of mammalian hosts, (BioVision, Inc., Milpitas, CA) were added to slide wells at a 1:100 dilution in 1X PBS for R. rickettsii and a 1:200 dilution for A. phagocytophilum. Following the addition of the conjugate, slides were incubated again at 37 °C for 45 min and washed twice (first wash with 1X PBS for 2 min and then subsequent washings with eriochrome T-Black (∼ 100 µL for 3 min). The slides were air dried, mounted with glycerol, and observed under a fluorescent microscope. The IFA was optimized using commercially available positive controls (ProtaTek International, Inc.). Sera demonstrating shiny green-yellow cytoplasmic inclusion bodies, or a ‘starry night’ array, were considered positive. (Papageorgiou et al., 2012) Sera with equivocal immunofluorescence were repeated in duplicate, with appropriate positive and negative controls. To avoid potential scoring bias, the laboratory technicians were blinded with regard to the location and animal species of the samples being read.

2.3. Statistical analysis and spatial data management

The seroprevalence of IgG antibodies against Anaplasma spp. and SFG Rickettsia in humans and animals was calculated by species, gender, soum (district), and aimag (province). Logistic regression was used to determine if the odds of previous exposure differed by gender, location, and species, with or without adjustment for age. All data were analyzed using STATA v 14.1 (StataCorp, College Station, TX). Spatial data sources included normalized differential vegetation index (NDVI) and land surface temperature (LST) captured by Moderate Resolution Imaging Spectroradiometer (MODIS) operated by the National Aeronautics and Space Administration (https://modis-land.gsfc.nasa.gov/index.html). Classified land cover data (GlobCover Version 2009 300m) was obtained from the European Space Agency (http://due.esrin.esa.int/page/globcover.php). ArcGIS 10.3.1 (ESRI, Redlands, CA) was used for geospatial operations, including joining datasets with shared geography, preparation of geospatial data layers, and map production. To examine the association between seroprevalence and environmental variables we used ArcGIS to circumscribe the five major clusters where human and animal data were collected. Briefly, a convex hull (minimum convex bounding geometry) was created around each of the five sampling sites with a 10 km buffer zone to accommodate the mobility among the nomadic people and animals being sampled (Fig. 1). Counts of seropositive and seronegative humans and animals were calculated within each of these sampling clusters along with the mean, maximum, and minimum values of NDVI and LST, and percent area of each land cover class. As there were only five sampling clusters, we graphically examined the relationships between these environmental variables and seroprevalence data, but did not perform statistical models.

3. Results

3.1. Seroprevalence of anaplasma

The IFA results for Anaplasma spp. are presented in tabular form by sample collection site (soum) and species in Table 2. Anaplasma spp. exposure as measured by IgG antibodies was detected in 37.3% of humans (95% CI: 32.3, 42.2) and 47.3% of livestock (95% CI: 45.2, 49.3). Livestock were significantly more likely to have Anaplasma IgG antibodies compared to humans (OR = 1.50; 95% CI: 1.2, 1.8), even after adjustment for age and soum (OR = 1.90; 95% CI: 1.2, 3.0). Of the livestock samples, 51.1% of cattle (95% CI: 45.9, 56.3), 44.4% of goats (95% CI: 41.0, 47.7), 49.4% of sheep (95% CI: 46.0, 52.7), 42.1% of horses (95% CI: 35.5, 48.8), and 53.8% of camels (95% CI: 39.8, 67.9) were seropositive by IgG (Fig. 2). The seroprevalence in livestock between soums was relatively homogenous within species, likely due to
the wide distribution of A. ovis throughout Mongolia, whereas the human seroprevalence was more heterogeneous, which could be due to A. phagocytophilum primarily being transmitted in northern Mongolia. The IgG seroprevalence in herders from the northern and central aimags of Selenge and Tov (soums Tu, Er, and Te) was significantly higher (P < 0.001) than the southern aimag of Dornogovi (soums Da, Sa). Compared to humans in Dornogovi, those living in Selenge and Tov were 7.1 times (OR = 7.1; 95% CI: 3.6, 13.8) more likely to have IgG Anaplasma antibodies. No significant differences were observed by gender, with age a significant predictor of previous exposure in humans.

### 3.2. Seroprevalence of rickettsia

The IFA results for *Rickettsia* spp. are presented in tabular form by sample collection site (soum) and animal species in Table 3. *Rickettsia* spp. exposure as measured by IgG was detected in 19.5% of humans (95% CI: 15.5, 23.6) and 20.5% of livestock (95% CI: 18.8, 22.0). Of the livestock, 30.2% of cattle (95% CI: 25.5, 35.0), 13.2% of goats (95% CI: 10.9, 15.5), 20.7% sheep (95% CI: 18.0, 23.4), 35.2% of horses (95% CI: 28.8, 41.6), and 1.9% of camels (95% CI: 0.01, 57.8) were seropositive (Fig. 3). The IgG seroprevalence of livestock and humans was relatively homogenous with respect to soum. The human seroprevalence in the northern and central aimags of Selenge and Tov was significantly higher than the southern aimag of Dornogovi. Compared to humans in Dornogovi, those living in Selenge and Tov were 2.8 times more likely (OR = 2.8; 95% CI OR: 1.4, 5.6) to have *Rickettsia* antibodies. No significant differences were observed by gender, with age a significant predictor of previous exposure in humans.
also a significant predictor of previous exposure in humans.

Fig. 4 illustrates the regional human seroprevalence to both *Rickettsia* and *Anaplasma*. Information on NDVI and LST, with regard to human seroprevalence can be found in supplemental files, with the two soums located in the Gobi Desert having markedly lower maximum NDVI and higher land surface temperature than the three northern soums (supplements 1, 2, 3). The abundance of vegetation as measured by a normalized vegetative index, decreases in a gradient from the northern to southern sampling locations, along with an increase in the minimum annual temperatures.

4. Discussion

This is among the largest studies to date of previous exposure to tick-borne diseases ever conducted in Mongolia. In general, we observed a higher seroprevalence of *Anaplasma* compared to *Rickettsia* in both humans and their livestock, and a higher seroprevalence in livestock compared to humans. The significant association between age and previous exposure, as measured by IgG antibody, is expected, since IgG antibody reflects previous exposure at any time and older individuals have had a longer time to be exposed at some point during their lifetime. Similarly, the higher seroprevalence in livestock compared with humans is plausible due to the high likelihood of exposure to tick-borne pathogens while grazing in tick-infested pastures and grass fields (no time indoors). In our study, the regions with the highest seroprevalence in humans did not have the highest seroprevalence in animals. A prospective cohort study with serial sampling to identify incident infections in herders and their animals could aid understanding of when and where infections are occurring.

Previous reports describing SFG *Rickettsia* in Mongolia ticks documented *R. raoultii* and *R. sibirica mongolitimonae* as probable causative agents of exposure, (Lankester and Davey, 1997; Lewin et al., 2003; Speck et al., 2012) and possibly *Candidatus R. tarasevichiae* in ticks collected from Selenge (Boldbaatar et al., 2017), although antibody response for *Candidatus R. tarasevichiae* is poorly understood at this time. The known vectors of Anaplasmosis in this region are, in most cases, *Ixodes persulcatus* ticks and vectors of SFG *Rickettsia* are *Dermacentor* and *Haemaphysalis* ticks. (Fang et al., 2015; Javkhlan et al., 2014; Liu et al., 2015) Variations in ecological niches for ticks between the northern and southern aimags could be in part responsible for the geographic differences in seroprevalence observed with both *Anaplasma* spp. and *Rickettsia* spp. The burden of tick-borne disease in Selenge has been previously documented, (Javkhlan et al., 2014; Scholz et al., 2013; Walder et al., 2006) but the high prevalence of exposure in Terelj National Park (Tov) is of concern due to frequent foot traffic from local...
Fig. 3. The seroprevalence of antibodies toward Rickettsia spp. is presented with 95% confidence intervals for the seroprevalence (spikes) by species for the following sample clusters: Tushig (Tu), Eroo (Er), Terelj (Te), Dalanjargalan (Da), and Sainshand (Sa).

Fig. 4. The pie charts depict the cluster specific seroprevalence among herders for Rickettsia spp. and Anaplasma spp., with clusters going from north to south: Tushig, Eroo, Terelj, Dalanjargalan, and Sainshand.
and international tourists hiking through this region. We suspect that the lower human seroprevalence of both pathogens in the locations closest to the Gobi Desert is the result of reduced grassland. It is important to note that our findings are consistent with previous work from other aims, (Papageorgiou et al., 2012) but are likely an overestimation of seroprevalence due to our inability to titrate out positive samples. Furthermore, Walder et al., found similarly high rates of Anaplasma exposure in northern Mongolia using IFA methodology, but when western-blot confirmation was used, only 18% of IFA positive samples were confirmed positive (Walder et al., 2006). It is possible that additional novel Anaplasma spp. and cross-reactivity from Ehrlichia spp in the region are partially responsible for the high IFA signals, as suggested by Walder et al. (Walder et al., 2006) Regardless, the ORs comparing likelihood of exposure between herders from the northern and southern sample sites for Anaplasma spp. (OR = 7.1) and Rickettsia spp (OR = 2.8) are valuable metrics of regional proportionate risk, warranting further efforts to understand TBDs in northern Mongolia.

4.1 Limitations

We chose to test sera for IgG to Anaplasma and SFG Rickettsia using IFA, since it is the reference standard for diagnosis of rickettsial infections. However, it is important to note that IFA can be labor-intensive and subjective in how fluorescence is scored. Although R. rickettsii has not been found in the region, use of R. rickettsii as antigen allowed us to assess the seroprevalence of SFG Rickettsia, since broad cross-reactivity within SFG Rickettsia is expected. However, we are unable to differentiate between exposure to Rickettsia species possibly transmitted by other vectors like fleas, lice and mites, which may be contributing to the observed SFG seroprevalence. Similarly, although Anaplasma phagocytophilum is present in Asia, serology cannot definitively confirm that Anaplasma phagocytophilum is the agent responsible for detected antibody fluorescence signals, as cross-reactivity to other Anaplasma species has been reported (Dreher et al., 2005). Thus, antibodies detected in screened humans may not be due to the same agents causing antibody responses in ruminants. Given these limitations, we therefore only report an estimate of the seroprevalence of anaplasmosis and SFG rickettsioses, since screening at a higher dilution would be expected to yield a lower seroprevalence and using the specific species present if known (homologous species) would also be expected to yield a relatively lower overall seroprevalence. Owing to the large number of samples tested, limited resources, and lab constraints we were unable to titrate sera to endpoint. Without culture or specific PCR, we are unable to determine exactly what species exposure is directly responsible for IFA positivity. Thus, we would interpret our findings as a compelling case for further study of the epidemiology of Rickettsia and Anaplasma in humans and animals in Mongolia, with the recommendation that future studies include full titer and/or other confirmatory assays as part of their approach.

5. Conclusion

This report, to our knowledge, represents the largest serosurvey of Anaplasma and SFG Rickettsia in humans and animals in Mongolia. A high proportion of both herders and livestock had serological evidence of previous exposure to Anaplasma and SFG Rickettsia, with variations observed by region. Our findings suggest that further studies are needed to investigate the epidemiology of anaplasmosis and rickettsiosis in both humans and livestock, the specific species responsible, and their vectors and reservoir hosts. Additionally, population-based and clinical studies including humans and animals are needed to determine the clinical importance of these agents and their spectrum of disease. Until more is known about these pathogens in Mongolia, physicians and veterinarians in the countryside should consider testing for Anaplasma and SFG Rickettsia infections and treating clinically compatible cases, while public health authorities should expand surveillance efforts for these emerging infections.

Conflicts of interest

None

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Ethics approval and consent to participate

Informed consent was obtained from humans prior to sample collection and all animals were treated with standard practices of animal care. Human research protocols used in this study were approved by Duke University’s Institutional Review Board (IRB Protocol #: Pro00056687) and by Mongolian Ministry of Health’s Monitoring Committee of Medical Ethics on July 29, 2014. Animal research protocols were approved by Duke University Institutional Animal Care and Use Committee (IACUC Protocol #: A217-15-08).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.actatropica.2017.10.015.

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