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

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ABSTRACT

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

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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. raoultii, R. heilongjiangensis,* 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; Mediannikov et al., 2006; Mediannikov 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, Terelj soum located in Tov aimag, and Dalanjargalan 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