Leptospira spp. in Rodents and Shrews from Afghanistan

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ABSTRACT: Leptospirosis is an occupational risk for military personnel and many cases have been reported worldwide. Rodents are the most important maintenance hosts for Leptospira spp. and may infect both animals and humans. To determine the occurrence and identity of pathogenic Leptospira spp. in rodent and shrew populations in German military camps in Afghanistan, we examined 751 animals (Mus musculus, Cricetulus migratorius, Meriones libycus, Rattus tanezumi, Crocidura cf. suaveolens, and Suncus etruscus) from four military camps in Northern Afghanistan from 2009–12. Leptospiral DNA was found in 1.1% of the animals and only in Mus musculus. Partial secY sequencing identified Leptospira borgpetersenii and Leptospira kirschneri as infecting genomospecies. Multilocus sequence typing was successful in the L. borgpetersenii samples, which were identified as sequence type 155. The low prevalence we observed suggested that the exposure risk of military personnel to infectious Leptospira spp. in the region is low.

Key words: Diagnostics, emerging diseases, infectious diseases, leptospirosis, microbiology, molecular biology, multilocus sequence typing, zoonotic diseases.

Leptospirosis is a bacterial zoonosis of global distribution caused by spirochetes of the genus Leptospira. In the tropics and subtropics, leptospirosis remains a major public health threat, with incidences of 10–100 cases per 100,000 people (Torgerson et al. 2015). Leptospirosis has also been reported in arid, high-altitude, subarctic, and continental regions (Andersen-Ranberg et al. 2016). Leptospirosis is a known occupational risk for military personnel (Hadad et al. 2006; Lupi et al. 2013).

The ongoing crisis in Afghanistan has resulted in the deployment of tens of thousands of military personnel into the region. Afghanistan is an endemic region for a large variety of human pathogens, including Leptospira spp. (Wallace et al. 2002). Serologic studies in livestock detected antibodies in 16% of the tested animals with the most-frequently identified serogroups Hebdomadis, Tarassovi, Grippotyphosa, Pomona, Javanica, and Icterohaemorrhagiae (Sebek et al. 1978).

Although information about the occurrence of human infections in Afghanistan is limited, historical reports indicate exposure to Leptospira spp. Sebek et al. (1972) described a seroprevalence of 9% in a human study population from the city of Kabul and of 15% in persons from a village in Nangarhar Province, bordering Pakistan. In 2011, 20% of Czech soldiers returning from Afghanistan tested positive by enzyme-linked immunosor-
bent assay to *Leptospira* specific antibodies (Strakova et al. 2014).

We wanted to gain a better understanding of the occurrence and identity of pathogenic *Leptospira* in rodent and shrew populations in German military camps in Afghanistan. During January 2009 to October 2012, 751 small mammals were trapped in four military camps in Northern Afghanistan. Of these, 648 animals were captured in the German military base camp, Camp Marmal in Balkh Province, Northern Afghanistan (36°42′7″N, 67°13′41″E), and five in a camp in Hazrat-e-Sultan, Samangan province (36°26′58″N, 67°53′54″E), which borders the Balkh Province to the south. A further 27 animals were caught in Fayzabad in Badakhshan Province, North-Eastern Afghanistan (37°6′6″N, 70°33′18″E) and 61 in a camp in Kunduz, bordering Tajikistan (36°40′24″N, 68°54′12″E). The trapping location of 10 small mammals was not documented. Trapping was conducted as part of pest control measures using snap traps. Animal species collected for this study were the house mouse (*Mus musculus*, 629), grey dwarf hamster (*Cricetulus migratorius*, 96), jirds (*Meriones libycus*, 16), one rat (*Rattus tanezumi*), and nine shrews (*Crocidura suaveolens*, 8 and *Suncus etruscus*, 1). The house mouse (*Mus musculus*) comprised 84% of all trapped animals, ranging from 67–98% in the four locations (Gertler et al. 2017).

All collected small mammals were documented by the veterinary medical staff of the German Armed Forces, stored in aluminum containers at −20 C prior to transportation, and sent to the Friedrich-Loeffler-Institut, Germany for further analyses (Gertler et al. 2017). Here, species, weight, length (body and tail), and sex were determined. Tissues were removed as described before, placed in 1.5-mL reaction tubes, and stored at −20 C until further analysis (Schlegel et al. 2012). Molecular species identification of small mammals was conducted by a PCR-based cytochrome *b* sequence analysis as described (Gertler et al. 2017).

One kidney of each animal was removed under sterile conditions before it was sent to the German Federal Institute for Risk Assessment to investigate the presence of *Leptospira* spp. by molecular analysis. We extracted DNA from 30 mg of kidney tissue with the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). The samples were analyzed by a PCR targeting a 423-base pair sequence of the *lipl32* gene, which is highly conserved in all pathogenic *Leptospira* strains (Mayer-Scholl et al. 2011). The PCR-positive samples were further analyzed by single locus sequence typing of the *secY* gene (Victoria et al. 2008) and by multilocus sequence typing (MLST; Boonsilp et al. 2013).

For immunohistochemical detection of *Leptospira* spp., thawed tissues were fixed in 4% neutral buffered formaldehyde and then paraffin embedded before the avidin-biotin-peroxidase complex method (Teifke et al. 2007) was performed. In brief, a polyclonal serum pool against the serovars Grippotyphosa, Icterohaemorrhagiae, Saxkoebing, Hebdomadis, and Sejroe was diluted 1:4,000 in Tris-buffered saline and the avidin-biotin peroxidase complex method was done using a biotinylated goat anti-rabbit IgG1 (Vector, Burlingame, California) diluted 1:200 in Tris-buffered saline. Then amino-ethyl-carbazole substrate chromogen (DakoCytomation, Hamburg, Germany) was applied including positive- and negative-control sera. The sections were slightly counterstained with Mayer’s hematoxylin.

A total of 743 small mammals was analyzed by *lipl32*-PCR; for the remaining samples sufficient material was not available. Eight samples (1.1%) were *Leptospira* spp. positive, six of 541 (1.1%) house mice from Camp Marmal and two of 60 (3.3%) house mice from Kunduz. The quality of only three of the *lipl32* positive samples (two from Kunduz, one from Camp Marmal) was sufficient for analysis by partial *secY* sequencing. Both samples from Kunduz were identified as the genomospecies *Leptospira borgpetersenii* and the sample from Camp Marmal as *Leptospira kirschneri*. The *secY* sequences have been deposited in the European Nucleotide Archive (LS951294, LS951295, LS973877) and were used to construct a neighbor joining tree.
FIGURE 1. Tubulointerstitial nephritis associated with *Leptospira* infection observed in formaldehyde-fixed paraffin-embedded tissue of the kidney of one animal (*Mus musculus*). (A) Focal lymphoplasmacytic infiltration of the renal cortical interstitium. H&E stain. (B) Multifocal intratubular staining of clusters of slender spirochetes. Immunostaining, avidin-biotin complex method, polyclonal serum pool against *Leptospira* spp., Nomarski differential interference contrast.
together with published secY sequences from reference strains (Victoria et al. 2008) in MEGA6 (Tamura et al. 2013) using the Tamura-Nei model. Both L. borgpetersenii strains showed 100% sequence identity to each other and to strain 493 Poland belonging to serovar Sejroe (EU357987.1). The secY sequence of the L. kirschneri strain was 100% identical to 14 other strains belonging to seven different serogroups. Our MLST analysis was only successful in the two samples from Kunduz. The species L. borgpetersenii was represented for both samples by ST155, which corresponds to the serogroup Sejroe.

Histopathology of 743 small mammals revealed a slight to moderate lymphoplasmonic interstitial nephritis in 19 mice. In eight of these cases, intratubular protozoal microorganisms, most likely identified as Klossiella muris, were seen. In only one of these 19 animals the interstitial nephritis was associated with Leptospira infection as demonstrated by intratubular immunostaining of slender spirochetes (Fig. 1).

In a meta-analysis where more than 20,000 rodents (families Muridae and Cricetidae) and shrews (family Soricidae) from six continents and more than 40 countries were sampled, the mean prevalence ranged from 10.6–17.1% (Andersen-Ranberg et al. 2016). In contrast, our study observed a very low prevalence in Muridae, and Leptospira spp. were not detected in the shrew species. The small number of shrews analyzed in our study could explain why Leptospira were not detected in any sample. The low prevalence found in rodents could be due to the environmental conditions in the study region. Kunduz, Samanga, and Balkh have a cold, semiarid climate with an average annual temperature below 18 C and an average precipitation below 200 mm. Only Feyzabad in Badakhshan Province has a Mediterranean climate with moderate temperatures and changeable, rainy weather (Climate-Data.org 2017).

Leptospira spp. can survive for long periods of time in moist soil and natural bodies of water despite cold, nutrient-poor acidic conditions, but ordinarily do not survive drying (Faine et al. 1999). Andersen-Ranberg et al. (2016) observed that higher minimum ambient temperatures were associated with higher Leptospira prevalences in all examined rodents and shrews.

The low Leptospira prevalence observed in this study and the cold, semiarid nature of the climate in the majority of the study region suggest that the exposure risk of military personnel to infectious Leptospira spp. in the region is low. Precautional protection measures such as rodent control in encampments should be implemented to further reduce the exposure risk to Leptospira spp. but also to all other relevant rodent-borne, zoonotic pathogens.

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LITERATURE CITED


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