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To cite this article: Per M. Jensen & Eydfinn Magnussen (2016) Is it too cold for Leptospira interrogans transmission on the Faroese Islands?, Infectious Diseases, 48:2, 156-160, DOI: 10.3109/23744235.2015.1092579

To link to this article: http://dx.doi.org/10.3109/23744235.2015.1092579

Published online: 07 Oct 2015.
Is it too cold for *Leptospira interrogans* transmission on the Faroese Islands?

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**ABSTRACT**

*Leptospira interrogans* is a bacterium that can infect most mammal species. Brown rats are considered to be one of the most important reservoirs of *Leptospira* because they frequently are infected and live in close proximity to humans. Past studies of prevalence of *Leptospira* in brown rats indicate that temperature – both high and low – may negatively affect the prevalence rate in rats, so that *Leptospira* is rare or even absent at temperatures below 7–8°C. Here we investigated the prevalence of infection in brown rats on the Faroese Islands (mean temperature of 6.5°C) and did not find any infected animals in a sample of 95 animals. We propose that prevalence rates of *Leptospira* are very low in rural brown rats in the cooler Scandinavian regions, even though urban/sewer rats might be highly infected in the same regions.

**Introduction**

The brown rat (*Rattus norvegicus*) is a globally distributed species that is widespread in temperate regions. It also occurs sporadically in many seaports, cities, and islands, etc., where it has been accidentally introduced [1]. Rats are considered pests because they infest human housing and food stores, and carry infectious disease agents such as *Leptospira interrogans* [2]. *L. interrogans* is a spirochete that can infect many if not all mammal species. Animals are infected with *Leptospira* by direct contact or through contaminated water, whereafter they will carry the bacteria in the renal distal tubules and continuously or intermittently shed bacteria to the environment through the urine [3]. Animal hosts are considered to be either maintenance hosts or incidental hosts, depending on their ability to serve as a reservoir for the bacteria. Particular *Leptospira* serotypes are adapted to particular host taxa, e.g. serovar Hardjo in cattle, serovar Copenhageni, Ballum, and icterohaemorrhagiae in rats, and serovar Canicola in dogs [4]. When infected with the appropriate serotype, these animals will have a long-lasting or life-long asymptomatic infection. In contrast, an incidental host, being infected with a non-corresponding serotype, may become seriously ill and possibly die. In humans *Leptospira* infections usually cause self-limiting febrile illness that rarely progresses to jaundice and renal failure [3].

The persistent infection of *Leptospira* in maintenance hosts is likely one of the main reasons why prevalence of infection is high in many species, for example, in brown rat populations prevalence rates are frequently reported to be more than 25%, but little information is available on how the prevalence of infection is influenced by factors such as environmental condition, population structure, and interaction between individuals. Nevertheless, experimental studies are strongly indicative of several factors influencing *Leptospira* survival in the environment, and through this its probability of transmission. The survival of *Leptospira* in water and soil depends on the acidity (pH) of the soil and salinity of the water [5,6]. Ultraviolet light may also limit survival [7] and it has been reported that survival is reduced at temperatures above 27°C [6]. The survival may vary from a few to more than 100 days [5] and thus varying environmental conditions can in theory greatly influence the probability of transmission. Direct transmission is influenced by contact between infected and uninfected individuals. This includes direct urine transfer, possibly in connection with sexual activity, and through aggressive interactions where bite wounds may be infected or increase the risk of infection as result of impairment of the dermal barrier [8,9]. Since the factors that affect the survival of *Leptospira* in the environment also affect host densities and environmental adaptation, then it is challenging to identify causes and mechanisms that define the prevalence of *Leptospira* in any host species under natural conditions.

*Leptospira* infection is often referred to as a tropical disease, because most cases of human leptospirosis occur in the tropical regions [10]. However, Chang et al. [5] summarized their experimental findings on the effect of temperature on *Leptospira* survival by stating that: “At low temperatures multiplication was retarded, but the life span of individual organisms was increased; at higher temperatures, multiplication was favored, but the life span of individual organisms was shortened; and increase in temperature in the presence of (other) bacteria shortened the survival of the Leptospira by favoring the bacterial activities” – which indicates that transmission may be limited by both too high and too low
temperatures. While the ambient temperatures that rats and bacteria are subjected to in the vegetation and burrows are unknown, published studies on the prevalence of *Leptospira* infection in brown rats may inspire the same idea, since prevalence rates for a subsection of studies appear to increase with increasing (30-year average) temperatures, peak at around 22°C, and decline as temperatures increase towards 30°C (Figure 1). However, it is also clear that the prevalence rates are too uncertain to clearly support such a deduction and that in addition to temperature there must be other factors influencing prevalence of infection, since *Leptospira* prevalence rates in a few studies on urban rats at high latitude have been reported to exceed 50% [9,11].

It should be noted that the published records on the prevalence of *Leptospira* infection (Figure 1) mostly have been sampled at locations where the 30-year average maximum daily temperature is above ca. 20°C and where the 30-year average daily minimum temperature typically is above 0°C. The five studies with higher than 50% prevalence rates deviate from the remaining studies by being performed in urban environments at low daily minimum temperatures (<–2°C in four studies). It could thus at first be speculated that synurbization is a relevant factor to consider, in particular because urban and rural settings under warmer temperatures in Thailand also show a two-to threefold difference: urban, 8.0 [12], rural, 3.0 [13]. Second, we would suggest that there are two main factors influencing prevalence rates: one relating to temperatures and one to synurbization, which also show an interaction resulting in very high prevalence rates in cooler regions (Figure 1). However, founder effects might also be relevant to consider, not only in our study on the Faroe Islands, but also at smaller geographical scale, since two urban studies [9,11] reported that some subpopulations were uninfected, even though other nearby subpopulations were approaching a 100% prevalence rate. However, such differences could also arise from varying degree of synurbization (smaller home range, aggressive behaviors, altered reproductive patterns, etc.) [14,15] across rat subpopulations, which means that we cannot effectively identify what caused such marked differences. Finally, uncertainties also arise from the uniqueness of brown rat ecology. Eusynanthphopic species have particular ecological history and we do not normally discuss synurbization in such species [14], even though corresponding principles of adjustment and adaptation in urban environments should apply for these mammals.

Here we bypassed the complexities of synurbization and its possible impact on *Leptospira* transmission by simply hypothesizing that the prevalence rates in non-urban rats do peak at an
post-mortem autolysis from the texture of the tissue before DNA analysis. Evidence of tissue degradation was noted in four cases. DNA was extracted from approximately 15–25 mg of tissue originating from a cross-section of the kidney (Qiamp® Minikit, Qiagen, Hilden, Germany). As positive controls five samples were spiked with Leptospira bacteria before DNA extraction, and Leptospira were added to five DNA extracts after extraction. Leptospira bacteria for positive controls (L. Interrogans serovar Icterohaemorrhagiae strain M695) were donated by Dr K. Krogfeldt, State Serum Laboratory, Denmark. For detection of Leptospira spp. a conventional PCR was used, based on the primers G1: 5k-CTG AATCGC TGT ATA AAA GT-3k and G2: 5k-GGA AAA CAA ATG GTC GGA AG-3k [20]. The primers amplify DNA from at least six pathogenic Leptospira spp.: L. interrogans, L. noguchii, L. santarosai, L. meyeri, L. weilii, and L. borgpetersenii. PCR reactions were performed in a total volume of 25 μl, including 17.5 μl MilliQ, 2.5 μl Extabuffer (1×) (VWR, Denmark), 2.5 μl template, and 2.5 μl PCR mix (dNTP, Taq polymerase and primers), providing final concentrations of dNTP, 200 μM; Taq (5 U/μl), 0.04 U; G1, 0.4 μM; and G2, 0.4 μM. The cycling reactions were performed on a Biometra Thermocycler PCR system (Biometra GmbH, Göttingen, Germany), using an initial hot start at 95 °C for 2 min followed by 40 cycles with denaturation at 95 °C for 30 s, annealing at 50 °C for 15 s, and elongation at 72 °C for 30 s, finalized by 5 min at 72 °C. A touchdown PCR procedure as described by Krøjgaard et al. [11] was also performed on 61 samples. PCR products were visualized by EZ-Vision®. One staining (Amresco, Ohio, USA) followed by electrophoresis on a 1.5% agarose gel. Fragment sizes were estimated using a 50 bp ladder (Generel; Thermo-Scientific, Waltham, Massachusetts, USA). Gene products were sequenced at Beckman Coulter Genomics (Beckman Coulter Danmark ApS, Copenhagen, Denmark, UK) and for identification, gene products were BLAST-ed using National Center for Biotechnology Information (NCBI) nucleotide BLAST.

Results and Discussion

We only found positive samples when they were spiked with Leptospira bacteria before or after DNA extraction. Sequencing of positive samples resulted in 99% homology with L. interrogans while other BLAST analyses yielded no results and in one case showed 94% homology with Mus musculus BAC klon RP23-398K14. Thus there was no evidence of natural Leptospira infection among the 95 specimens, but an indication that the primers in some cases align with host DNA. The prevalence rate of Leptospira infection can thus be stated as being 0–3.8% [21], which appears to be among the lowest reported prevalence of Leptospira in brown rats (Figure 1). The only other study reporting comparable prevalence rates was performed in rural rats in Oxford, UK (0–3%, n = 296 [22]), which also is located in the cooler North-European Atlantic climate. The indication of low prevalence rate or absence of Leptospira in Faroese brown rats is corroborated by the lack of any known confirmed cases of leptospirosis. Leptospirosis is a notifiable disease for both veterinary and medical doctors in the Faroe Islands. Reports are so far isolated to one case of suspected canine leptospirosis in 2013, which later was retracted due to failure of confirmation in secondary samples.

Material and Methods

We collected brown rats on the Faroese Islands (62°N and 7°W) over a period of 3.5 years (March 2011 to August 2014). Topographically the western and northern shorelines of the islands are characterized by spectacular, precipitous cliffs. Inland the terrain consists of valleys, mountain ridges, and upland hills; the northern islands being the most mountainous (highest peak 882 m a.s.l.). The climate is pronounced oceanic with a mean temperature of 4 and 11 °C during the coldest (January–February) and warmest (July) months, respectively. Mean annual precipitation varies locally (823–3261 mm) and is highest during the wettest months (June–July) and lowest during the driest months (January–February). Inland the terrain consists of valleys, mountain ridges, and upland hills; the northern islands being the most mountainous. The climate is pronounced oceanic with a mean temperature of 4 and 11 °C during the coldest (January–February) and warmest (July) months, respectively. Mean annual precipitation varies locally (823–3261 mm) and is highest during the wettest months (June–July) and lowest during the driest months (January–February).

One kidney for each of the 95 animals was extracted and kept frozen until analysis. Defrosted kidneys were assessed for...
This view is supported by staff at the national hospital and the two veterinary clinics on the islands. The medical staff do request serological analyses when relevant, which are evaluated under a certified serological analysis at Statens Serum Institut, Denmark [23], but all tested samples have so far been negative.

There is no particular reason to believe that *Leptospira* is limited by the isolated location of the Faroe Islands because brown rats on many other islands are infected throughout the world [24]. Certainly it is impossible to fully exclude founder effects as possible cause, but this type of argument is perhaps more relevant for parasites and infections that have a naturally low prevalence rate, have short duration of infection or require additional host species for transmission [19]. In the case of brown rats we would strongly suspect that it has been introduced numerous times by numerous individuals, having a prevalence rate of ca. 20–30%, which makes it unlikely to have escaped introduction at some point in time in the last century. We therefore believe that the likely reason for the low prevalence of *Leptospira* must be linked to environmental factors leading to the conclusion that – in the absence of environmental factors that are conducive to phenological and behavioral adjustment or adaptation corresponding to synurbanization – it is either too cold for *Leptospira* transmission or

**Figure 2.** Locations (red dots) where brown rats (*Rattus norvegicus*) were collected for investigations of leptospirosis. The green colour indicates islands where the brown rat occurs. Names written in capital letters are island names. The map in the lower left corner shows the location of the Faroe Islands in the North Atlantic (red circle).

**Figure 3.** Size distribution of brown rats used for investigation of *Leptospira* spp. in the Faroe Islands.
too cold for adequate densities of brown rats to be maintained. The latter could also be connected to rat populations effectively being separated in a mountainous and barren landscape and each of the populations being below the critical community size for Leptospira maintenance [25]. If that indeed is the case then Leptospira prevalence rates may be low in many rural localities in Northern European countries with Atlantic climates, such as the Shetland Islands, Iceland, and Norway, as to our knowledge no reports of Leptospira infection in rural brown rats have been published for these areas. Studies of rodent populations in these countries are required to firmly establish that Leptospira are negatively affected by low temperatures and to identify possible threshold temperatures for Leptospira maintenance in brown rats. It should be underlined that even though this can be confirmed then it cannot be excluded that urban rats in the same climatic regions may have very high Leptospira prevalence rates (Figure 1). Resolving the potential effect of urbanization on leptospirosis transmission and its possible variation with climate will, however, require a number of carefully designed studies that allow for assessing differences in home range, aggression, reproduction across populations, and their effects on transmission of Leptospira.

Acknowledgments

Acknowledgements are given to Chief Veterinary Officer, B. Mørkøre, and K. Hansen.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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