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Leptospira spp. in the Oral Cavity of Urban Brown Rats (*Rattus norvegicus*) from Vancouver, Canada—Implications for Rat-Rat and Rat-Human Transmission

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ABSTRACT: We tested the urine and saliva of 137 wild rats (*Rattus norvegicus*) from Vancouver, Canada, for the presence of *Leptospira* spp. Only one saliva sample was found positive and two were suspect, all from urine-positive rats (n=81), indicating that active shedding of leptospires in saliva is unlikely to occur.

Leptospirosis is considered the most ubiquitous zoonotic disease today, with an estimated one million human cases occurring around the globe annually (Zilber et al. 2016). Brown rats (*Rattus norvegicus*) are the primary urban reservoir for the bacteria *Leptospira* spp., the etiological agent of human leptospirosis (Himsworth et al. 2013). The concurrent growth of urban centers and urban rat populations will likely lead to a rising incidence of rat-human interactions (Himsworth et al. 2014b). In response to the increasing risk to public health, several studies have sought to characterize the ecology of rat-borne zoonoses in cities (Costa et al. 2014; Himsworth et al. 2014a). However, there is still a major gap in our understanding of how *Leptospira* spp. is transmitted between rats in urban environments.

Leptospira spp. asymptomatically colonizes the kidneys and renal tubules of rats (Zilber et al. 2016). Leptospires shed in rat urine can survive in water and soil for days to months (Costa et al. 2015). Transmission between rats and to people can occur when leptospires from urine or the environment contact abraded mucous membranes, such as bite wounds, or orifices including the mouth, eyes, and nose (Zilber et al. 2016). If left untreated, leptospirosis can cause serious disease in people, leading to renal failure or pulmonary hemorrhage with 10% and 50% mortality rates, respectively (McBride et al. 2005).

Recent studies have identified associations between *Leptospira* spp. colonization in rats and increasing body mass, volume of internal fat, and number of bite wounds (Himsworth et al. 2013). Body mass is often considered a proxy for age, and several studies suggest that older rats show higher prevalence because of a longer period of exposure to environmental sources of *Leptospira* spp. (Easterbrook et al. 2007; Krøjgaard et al. 2009). However, if random contact with leptospires in the environment is the primary source of renal colonization, then young rats would become exposed shortly after leaving the nest, minimizing the difference in prevalence between age classes (Costa et al. 2015). Alternatively, body mass and the presence of bite wounds are characteristics related to dominance and social hierarchies within rat colonies (Himsworth et al. 2013), indicating that social interactions, such as biting, may play a key role in intraspecific transmission.

Biting may also be a mechanism for transmission of *Leptospira* spp. from rats to people, as several case studies have reported leptospirosis in human patients directly following a rat bite (Gollop et al. 1993; Søndergaard et al. 2016). The potential for salivary shedding of *Leptospira* spp. in urban rats has been suggested previously (Himsworth et al. 2013; Costa et al. 2015), but no published studies have tested the saliva of wild rats for the presence of *Leptospira* spp.

The objective of this study was to determine if *Leptospira* spp. were present in the saliva of wild brown rats (n=620) caught in the Downtown Eastside of Vancouver, Canada (49°17′N, 123°6′W), from June 2016 to January 2017. Saliva samples were collected by swabbing the oral cavity of each rat using sterile cotton swabs (Copan, Brescia, Italy). Urine samples were collected in cryovials after natural excretion into a disinfected container. Both saliva swabs and urine samples were stored at −80 C prior to testing.

All urine samples were tested for the presence of *Leptospira* spp. using PCR techniques as previously described by Stoddard et al. (2009). Samples were classified as negative (cycle threshold [Ct]$\geq$40), suspect positive (Ct=37–39.99), or positive (Ct$\leq$36.99). Any sample within the suspect range was retested three times. Of the 620 rats caught, 83 tested positive in their urine and 537 tested negative. All saliva samples that were acquired from urine-positive rats (n=81) were tested. To detect *L. interrogans* presence in saliva at a minimum prevalence of 5.0% (Cameron and Baldock 1998; Cameron 1999), we tested a random selection of 56 saliva samples from urine-negative rats.

All saliva samples from the urine-negative group tested negative. Two saliva samples from the urine-positive group were suspect positive and one was positive. However, the presence of *Leptospira* spp. could not be confirmed with sequencing. Given the low prevalence of saliva positive and suspect samples (3/81=4%) among urine-positive animals and our inability to confirm these samples through sequencing, it is possible that these were false positives.

Our results indicate that it is unlikely wild rats actively shed leptospires in their saliva. Instead, the presence of leptospires in the saliva of three rats from this study may be attributed to temporary contamination of the oral cavity. Given that all positive saliva samples came from urine-positive rats, it may be that grooming of the urogenital region led to temporary urinary contamination in the oral cavity (Costa et al. 2015). These results align with a previous experimental study that failed to detect *L. interrogans* in the salivary glands or saliva of artificially infected rats (Zilber et al. 2017).

Temporary oral contamination could result in transmission through biting, supporting the previously documented relationship between a greater number of bite wounds and *Leptospira* spp. colonization. Alternatively, bite wounds create a break in the skin through which leptospires can enter from contaminated urine or the environment, increasing chances of renal colonization (Costa et al. 2015; Zilber et al. 2016). Biting may also be part of a constellation of other behaviors, such as fighting, communal nesting, and urinary marking, which facilitate the transmission of *Leptospira* spp. among rats.

The results of this study help to resolve our understanding of the ecology of *Leptospira* spp. within rat populations and the pathways for rat-to-human transmission. Although rat saliva is unlikely to be an important mode of transmission, social behaviors involving biting and grooming may impact the prevalence of *Leptospira* spp. in rat populations. The cumulative evidence suggests that the spread of this pathogen is more complex than random contact with urine or environmental contamination. Future studies should seek to investigate how behavioral and environmental factors influence the prevalence of *Leptospira* spp. in order to effectively manage risks to people.

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