Leptospira and Leptospirosis

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Abstract

Leptospira spp. are morphologically and phylogenetically unique Bacteria that are ubiquitous in the environment. Pathogenic species are the agents of leptospirosis, an emerging zoonotic disease transmitted to both human and animals from the environment contaminated with the urine of reservoir animals. The taxonomy of *Leptospira* has recently undergone extensive revisions with the use of whole-genome sequences, thus expanding considerably the number of species.

1. General features of *Leptospira*, an atypical bacteria

Leptospira spp. are long, thin, flexible rods, 0.1 µm in diameter and 6-12 µm in length, with a helical cell shape and periplasmic flagella. The envelope structure is similar to Gram-negative bacteria and the cell surface is covered with lipopolysaccharides (LPS) which determine the antigenic diversity within the genus. Leptospires are not stained by Gram staining and dark-field microscopy is required for visualization of cells. *Leptospira* spp. are highly motile aerobic or microaerophilic bacteria. They use long-chain fatty acids as primary carbon and energy sources. In Ellinghausen-McCullough-Johnson-Harris (EMJH) culture medium, the optimum growth temperature is 28-30°C, with a generation time of 6 to 20 h. Growth on solid agar medium results in the formation of subsurface colonies. *Leptospira* spp. comprise free-living saprophytes that are well adapted to soils and aquatic, but not marine, environments and pathogens that cause the disease leptospirosis in both human and animals. Animal reservoirs of pathogens consist of domesticated and wild mammalian hosts. Long term survival of pathogens in water has also been described [1, 2].

2. The genus *Leptospira*, a blooming tree

The genus *Leptospira* forms a deep unique branch of spirochetes and is classified in the family Leptospiraceae which is currently divided into the genera *Leptospira*, *Turneriella*, and *Leptonema*. The genera *Turneriella* and *Leptonema* each contain a single species, and relative to *Leptospira* very little information is available for these species [3]. The genus *Leptospira* is highly diverse and comprises 64 different species, which have been identified since the isolation of *L. interrogans* in 1915 [4, 5]. The availability of a new selective medium

[6], the advent of relatively cheap whole genome sequencing (WGS) and increased interest in metagenomic studies and soil microbial communities have resulted in expanding the number of *Leptospira* species from 22 in 2018 [7] to 64 in 2019 [4].

Phylogenetic analysis, initially based on 16S rRNA gene sequencing but now on whole-genome sequences showed that the genus is separated in two clades: “Saprophytes” containing species isolated in the natural environment and not responsible for infections and “Pathogens” containing all the species responsible for infections in humans and/or animals, plus environmental species for which the pathogenicity remains unclear [4]. The two clades are further subdivided in four subclades called P1, P2, S1 and S2 [4]. The subclade P1 (formerly described as the pathogen group) comprises 17 species (*L. mayottensis, L. alexanderi, L. kirschneri, L. kmetyi, L. alstonii, L. adleri, L. barantonii, L. ellisii, L. dzianensis, L. gomenensis, L. putramalaysiae, L. tipperaryensis, L. borgpetersenii, L. interrogans, L. noguchii, L. santarosai, L. weilii*). Some species, such as *L. kmetyi*, *L. adleri*, *L. ellisii*, *L. gomenensis*, *L. barantonii*, *L. dzianensis*, and *L. putramalaysiae*, first identified in the environment, have never been isolated from infected animals or patients, suggesting that they are not true pathogens [4, 8]. The sub-clade S2, also called intermediate species, form a group of 21 species distinct from the pathogens (*L. broomii, L. licerasiae, L. fainei, L. venezuelensis, L. wolffii, L. haakeii, L. hartskeerlii, L. saintgironsiae, L. neocaledonica, L. perolatii, L. dzoumogneensis, L. fletcheri, L. fluminis, L. johnsonii, L. koniamboensis, L. langatensis, L. sarikeiensis, L. selangorensis, L. semungkisensis, L. andrefontaineae, L. inadai*). Most of these species have been isolated from the environment and their virulence status has not been proven in animal models. The saprophytes are then subdivided in subclades S1 (*L. terpstrae, L. vanthielii, L. yanagawae, L. brenneri, L. harrisiae, L. levettii, L. kemamanensis, L. bandrabouensis, L. bourretii, L. bouyouniensis, L. congkakensis, L. ellinghausenii, L. jelokensis, L. kanakyensis, L. montravelensis, L. mtsangambouensis, L. noumeaensis, L. perdikensis, L. biflexa, L. meyeri, L. wolbachii, L. idonii*) and S2 (*L. ilyithenensis, L. kobayashii, L. ognonii, L. ryugenii*).

3. Leptospirosis and virulence factors

Pathogenic *Leptospira* species such as *L. interrogans* are the agents of leptospirosis, which is a zoonotic disease responsible for more than 1 million cases and 60,000 deaths per year worldwide [9]. These are likely underestimates because of misdiagnosis and under-reporting, particularly in regions where other diseases with similar non-specific presentations, such as dengue and malaria, are prevalent. Leptospirosis is also emerging due to global climate changes resulting in more frequent and severe flooding events. Although leptospirosis has

high burden and mortality and is treatable, it is not acknowledged as a neglected tropical disease; thus, efforts to raise awareness at the local and international levels are needed [10].

Transmission to humans usually occurs through contact with soils or surface waters contaminated with the urine of reservoir animals such as rats that are asymptomatic carriers of the pathogens. The severity of the disease appears to be mainly determined by the interaction of the virulence characteristics of the infecting strain, infecting inoculum size during environmental exposure and host susceptibility factors. However, the basic biology and virulence factors of leptospires remain poorly characterized. This gap in our knowledge is largely due to the fact that this research area has been unattractive to both funders and researchers in the past decades [10]. As a consequence, for example, genetic manipulation of *Leptospira* remains relatively inefficient in comparison to other Bacteria [11].

Pathogenic *Leptospira* spp. have developed different strategies such as rapid dissemination and ability to escape or hijack the host immune system to successfully establish and maintain an infection. The presence of endoflagella (or periplasmic flagella) enable the pathogens to rapidly cross the mammalian cell barriers, disseminate hematogenously, establish infection in target organs and avoid flagellin recognition from the innate immune system, thus enabling the spirochetes to escape the immune attack during the infection [11, 12]. Leptospires produce several adhesins for binding to several components of host cells, including the extracellular matrix [13]. Under normal *in vitro* growth, the most abundant proteins are the lipoproteins LipL32, LipL41, LipL36, and Loa22 [14]. These lipoproteins could potentially interact with host cells and the immune system (complement, etc) during the hematogenous dissemination of leptospires in the host. In addition, it has been shown that the leptospiral LPS, in contrast to other gram-negative bacteria, escapes human TLR4 recognition [15], and is recognized by TLR2 in human cells [16].

The first leptospiral genome sequence to be determined in 2003, that of *L. interrogans* serovar Lai, consists of a 4.33-megabase large chromosome and a 359-kilobase small chromosome [17]. Today, the genome sequences of hundreds of *Leptospira* strains have been determined, including representative of each of the 64 *Leptospira* species [4]. The genome of *Leptospira* is always composed of two chromosomes and some plasmids have been recently identified [18-21]. Access to large sets of genome sequences has significantly improved our understanding of the emergence of virulence in *Leptospira*. Genomic comparisons between pathogenic and non-pathogenic species have revealed a number of important differences, suggesting that pathogens evolved from free-living ancestral species by successive gain and loss of genes/functions associated with the adaptation to new hosts

[4, 8, 22]. For instance, it is possible to observe a gradient in the repertoire of genes encoding proteins (hemolysins, etc) or protein domains (Leucin-Rich Repeat, peptidases, etc) known to be associated with virulence, with the most virulent species of subclade P1 having the most genes encoding these virulence factors [4].

4. Conclusion

Leptospira was identified as the causative agents of leptospirosis 100 years ago [5]. Despite some recent progress, although the burden of leptospirosis is comparable or even higher than diseases that are much better known, such as dengue or rabies [23], there is a considerable deficit in the understanding of basic aspects of the epidemiology of the disease and the biology of the bacterium responsible.

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